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Dietary analysis of the emerald shiner (Notropis atherinoides) in the Upper Niagara River using fatty acids, stomach contents, and stable isotopes

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by

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An Abstract of a Thesis

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ABSTRACT OF THESIS

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Describing ontogenetic and temporal shifts in diet is a fundamental step in understanding food web structure in any ecological community. I conducted a dietary analysis of the emerald shiner (*Notropis atherinoides*), a keystone species in the Niagara River (NY), using a detailed analysis of fatty acids combined with data on stomach contents and stable isotopes. For both 2014 and 2015, oleic acid and DHA were important fatty acids responsible for the majority of the differences among groups. There was a seasonal shift in fatty acids from relatively high levels of 22:5n-6 and DHA early in the season to increased levels of EPA and 18:3n-3 later in the season. Smaller shiners had lower values of EPA, DHA, and oleic acid and higher values of 18:2n-6 compared to large shiners. Multivariate statistical analysis showed that emerald shiners had approximately 80% similarity in fatty acid composition across all size classes and seasons; despite this similarity, the analysis was able to differentiate among most groups of shiners. In general, the fatty acid results were consistent with data from stomach contents, which indicated that copepods were more important later in the season and were more common in the stomachs of large shiners. Stable isotope ratios of carbon and nitrogen indicated that all emerald shiners were eating at a similar trophic level. These results establish an important dietary baseline for the emerald shiner which will be useful in the future given the ongoing anthropogenic influences and habitat alterations that are occurring in the upper Niagara River.

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LIST OF ABBREVIATIONS

Fatty Acids	
Common name	Short hand/Abbreviation
Palmitic acid	16:0
Palmitoleic acid	16:1n-7
Oleic acid	18:1n-9
Linoleic acid	18:2n-6 (ω6)
α Linolenic Acid	ALA/ 18:3n-3 (ω3)
Eicosatetraenoic acid	ARA/ 20:4n-6 (ω6)
Eicosapentaenoic acid	EPA/ 20:5n-3 (ω3)
Docosatetraenoic acid	22:4
Docosapentaenoic acid	DPA/ 22:5n-3 (ω3)
Docosapentaenoic acid	DPAn-6/ 22:5n-6 (ω6)
Docosahexaenoic acid	DHA/ 22:6n-3 (ω3)
Polyunsaturated Fatty Acids	PUFAs
Highly Unstaturated Fatty Acids	HUFAs
PRIMER	
Non-Metric Multidimensional Scaling Plots	nMDS
Similarity of Percentages Analysis	SIMPER
Analysis of Similarities	ANOSIM

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Figure 16. Average percent composition of major prey items found in the stomach contents of emerald shiners collected in 2014.

INTRODUCTION

Fish communities in the upper Niagara River have been strongly impacted by anthropogenic effects and introduced species. In the early 1970's, the Niagara River was listed as an Area of Concern (AOC) due to an increase in development, industrial growth on the river, and problems related to toxic waste and superfund sites in the watershed, leading to an influx in contaminants in the river including PCBs, oil, chlordane, phenols, and dioxins (Milani et al. 2013). The increase in contaminants led to an alteration of fish communities and an overall degradation of fish and wildlife health, resulting in the loss of several beneficial uses. This increase in development also led to the alteration of shorelines, replacing natural aquatic vegetation with sheet metal and rock boulder shorelines (Milani et al. 2013). This led to dramatic declines in natural fish habitat and an increase in the overall water velocity in the Niagara River (Allen 2015). It was as a result of these activities that over 80 fish species have been observed with high levels of PCBs in their system. In addition, several fish species have also gone into decline, one being lake sturgeon, that was hit the hardest as a result of development that destroyed their spawning habitat.

In addition to the AOC listing, the introduction of several non-native species has altered fish communities and drastically altered energy flows in pelagic and benthic pathways. The Niagara River is directly connected to the eastern basin of Lake Erie and it is because of this connection that any changes that occur in the lake also have the potential to impact the Niagara River. As a result, several non-native species that have become established in Lake Erie have also found their way into the Niagara River, including alewives (*Alosa pseudoharengus*), dreissenid mussels, round gobies (*Neogobius melanostomus*), and common rudd (*Scardinius erythrophthalmus*) (Kapuscinski et al. 2014). Non-native alewives and dreissenid mussels

consume large amounts of zooplankton and phytoplankton in both nearshore and offshore areas (Kao et al. 2016). Filter-feeding efficiencies of both species have altered zooplankton community composition and size while improving water clarity, resulting in increased algae blooms and growth in both Lake Erie and the Niagara River (Kao et al. 2016). Furthermore, the round goby have established dense populations in coastal and riverine systems, including the Niagara River, consuming a wide variety of benthic prey and further shifting energy flow toward the benthos. The rudd, which is a relatively new invader, is also becoming abundant in the Niagara River and its impacts on the food web are currently unknown (Kapuscinski et al. 2014). Despite an increased understanding of changes in the food webs found in Lake Erie, trophic interactions and roles held by nearshore and riverine species of fish are understudied and any changes in food webs and their reaction to changing conditions are based primarily on assumptions and data collected from lake habitats (Atkinson et al. 2015).

Planktivorous fishes are essential in the energy transfer from lower to upper trophic levels by converting nutrients from phytoplankton, zooplankton, and aquatic insects into biomass and then channeling these nutrients to the broader food web (Hartman et al. 1992). One of the most important native planktivorous fishes in the Great Lakes, including the upper Niagara River, is the emerald shiner (*Notropis atherinoides).* The emerald shiner serves both as a top predator on plankton communities and as a key prey item for valuable sport fish and New York state threatened resident birds. Being such an important prey item has made the emerald shiner not only vital to the local piscivorous fishes in the upper Niagara River but also to the fishing industry as a bait fish. The emerald shiner is harvested locally for bait and exploited commercially, giving it value to the local economy.

Although the emerald shiner has the ability to impact invertebrate communities and are consumed by many planktivorous fishes, few studies have examined the diet of emerald shiners in the Great Lakes and their tributaries. Previous research has indicated that emerald shiners in central Lake Erie consume a variety of invertebrate prey. Young-of-the-year (YOY) emerald shiners consume mainly chironomid pupae, bosminids, sididae, and some *Bythotrephes*, while adult emerald shiners consume more *Daphnia*, *Leptodora*, and *Bythotrephes* (Atkinson et al. 2015, Hartman et al. 1992, Pothoven et al. 2009). Beyond these cited studies, information on seasonal and ontogenetic changes in the diet of emerald shiners in the Great Lakes is scarce, and this information is vital to fully assess the role that the emerald shiner plays in the food web and in energy transfer. Moreover, previous diet studies of the emerald shiner in the Great Lakes have not included major tributaries or connecting channels such as the Niagara River.

Diet analysis of fishes has typically focused on identifying and enumerating stomach contents. This approach has aided ecologists in understanding diets by providing details on specific prey items consumed, potential resource overlap, and anthropogenic and management actions on fish communities. However, there can be a great deal of variability in stomach content data as a result of human error in identification, digestion rates within the fish, ontogeny, and rates of digestion of different types of prey (Atkinson et al. 2015). These factors may decrease the reliability of stomach content data as the sole dietary assessment method and has resulted in the use of more modern diet research techniques. It is now more common in diet studies to employ indirect methodologies based on biomarkers that accumulate in a predictive way, paired with traditional stomach content data, to provide both long- and short-term foraging information (Beaudoin et al. 1999, Hooker et al. 2001, Vinson and Budy 2011).

The use of stable isotope ratios in trophic ecology dates back to the 1970's (DeNiro and

Epstein 1978, 1981). Stable isotope analysis provides an accurate description of an organism's diet and its trophic interactions within a community while providing a two-dimensional dietary space for consumers relative to other organisms. Since stable carbon (δ13 C) ratios remain relatively constant through trophic transfer and vary between primary production sources, stable isotope analysis can be used to identify biomarkers that indicate differences in the diets of various fish species while also inferring foraging habits (Alfaro et al. 2006, Deither et al. 2013, France 1995). Stable isotopes can be used to provide a good description of carbon flow through a food web and can highlight possible trophic pathways. Consumers are enriched in nitrogen (δ15 N) relative to their prey source and consequently these values can serve as indicators of the trophic levels that are represented in the diet. Although specific prey species cannot be identified and quantified in complex systems, stable isotopes do provide a method of determining inter- and intra-specific relations within communities (Happel 2013).

Fatty acid analysis is another indirect approach to diet analysis based on biomarkers, and this method is becoming more and more common in studies of natural fish populations and communities (Haubert et al. 2011, Irisarri et al. 2014, Iverson 2009). Fatty acids are released from lipid molecules (e.g. phospholipids and triglycerides) during digestion and are generally not degraded. These compounds are often incorporated into tissues in their basic form, making it possible in many instances to trace fatty acids back to certain types of food sources (Iverson 2009). Fatty acids are essential in fish for a variety of reasons, including growth, reproduction, cell integrity, and temperature acclimation. Certain fatty acids are also considered essential because fish cannot synthesize them on their own, instead they must obtain them from their diet (Happel et al. 2015). Some essential fatty acids in fish include EPA, DHA, and DPA. Fatty acids are considered a valuable tool in ecological studies because of the large amount of unique

structures that can be synthesized and linked back to specific diet items (Parrish et al. 2015). The unique signature provided by fatty acids are increasingly being used to delineate the transfer of dietary material through freshwater, marine, and terrestrial systems (Parrish et al. 2015). Moreover, fatty acids can show more than the "snapshot" that gut contents can provide since long chain fatty acids can be stored in predator tissues in patterns reflective of prey consumed over a 4-12 week period (Czesny et al. 2011, Happel et al. 2015). For example, palmitoleic acid and EPA are associated with a benthos-dominated diet while prominent levels of DHA are more commonly found in certain zooplankton species (Czesny et al. 2011, Kelly and Scheibling 2012). However, fatty acid signatures cannot be used to identify and estimate specific prey species within a sample without prior controlled experimentation (Iverson 2009). Thus, using multiple techniques for dietary analysis may offer stronger insights into trophic interactions than any one technique alone.

In this study I conducted a detailed analysis of fatty acids in emerald shiners from the upper Niagara River and combined the results with new information from stomach contents and stable isotope analysis. The purpose was to use information from these three sources to provide a thorough assessment of the diet of the emerald shiner in the upper Niagara River and to determine the importance of the emerald shiner to some top resident piscivorous fish species in the river. The emerald shiner was selected as a model species due to its economic and ecological importance within the Great Lakes and the lack of current information on its diet within the upper Niagara River. Specifically, the objectives of this study were to 1) examine the fatty acid composition, stomach contents, and stable isotope profiles of the emerald shiner during different times of the year and using different fish size classes, 2) evaluate any temporal or ontogenetic

shifts that may have occurred in the diet of the emerald shiner, and 3) evaluate the potential role of emerald shiners in the diet of key piscivorous fishes in the upper Niagara River.

METHODOLOGY

Fatty Acid Analysis

Emerald shiners were collected from June – October in 2014 and 2015 using electroshocking and seining at various locations in the upper Niagara River (Figure 1). Three size classes were used for this research: small (50-59 mm), medium (60-74.9 mm), and large (>75 mm), which corresponded to three different age classes (young of the year or YOY, age-1, and age-2, respectively). I attempted to analyze 20 YOY, 20 age-1, and 20 age-2 shiners from early in the season, mid-season, and late in the season for both 2014 and 2015. In some instances, suitable fish were not available for each sampling event and these exceptions are noted in Table 1. All emerald shiners from 2014 and 2015 were frozen in water and stored until either stable isotope or fatty acid analysis could be conducted.

Prior to fatty acid analysis, samples were partially thawed and 0.50 ± 0.10 g of fish muscle tissue was homogenized. Fatty acids were processed using a direct methylation technique according to Parrish et al. (2014) and the fatty acid methyl esters (FAME) produced were re-suspended in hexane with a known concentration of C23:0 as an internal standard (details are provided in Appendix A). The FAME were analyzed using an HP 5890 Series II gas chromatograph equipped with a flame ionization detector, HP 6890 series auto-injector, and Chemstation software for peak identification and quantification. Helium was used as the carrier gas and injector and detector temperatures were 220^0C and 230^0C, respectively. After an initial setting of 130^0C, oven temperatures were increased at a rate of 6^0C per min until a final temperature of 225^0C was reached. Individual fatty acids were identified by comparing their

retention times to that of known standards, and fatty acids were quantified by comparing the areas under each peak with that of the C23:0 internal standard (Snyder et al. 2012). Fatty acids are expressed as percent of total identified FAME.

Predator Species

For fatty acid analysis of the predatory fish the same direct methylation technique was used. The predatory fish used were smallmouth bass (*Micropterus dolomieu*) and white bass (*Morone chrysops*) from 2015 since they are resident fish species in the upper Niagara River and are known to prey upon emerald shiners. Ten individuals were processed from each predator species, five from early in the season and five from late in the season. The sample size was smaller than the emerald shiner sample size because the rate at which the predators were caught was much lower than the emerald shiners.

Stable Isotopes

The samples for the stable isotope analysis were performed by the Colorado Plateau Stable Isotope Laboratory (CPSIL). Initially, the emerald shiner samples were dried via a drying oven at 50 to 60 C for 24 to 48 hours. Once the samples were dried they were ground up using a standard mortar and pestle. The grinding step helped improve sample homogeneity and created a consistent particle size in the sample. Once the drying and grinding steps were completed, the samples were weighed in a small tin capsule. These capsules varied depending on sample weight. The samples had to be weighed using a tared micro-analytical balance and were determined to the third decimal place on a milligram scale. Once the appropriate amount of sample had been weighed and placed in the tin capsule, the capsule was crushed into a small ball by rolling the sample gently in between the thumb and index finger. Once the sample was crushed and it was

confirmed that none was lost, the tin capsule was reweighed to confirm the final mass, which was then recorded. The samples were packed using 96-well polystyrene plates and sent to CPSIL for further analysis (http://www.isotope.nau.edu/).

Stomach Contents

Stomach contents were collected from emerald shiners from the upper Niagara River from May to October of 2014. A subsample of 150 shiner guts were evaluated to evenly represent fish size class, sampling sites and sampling dates. Gut contents were removed with a probe and diluted with 10 mL deionized water. The dilution was thoroughly mixed by hand with a Henson-stempel pipette, and then added to a zooplankton counting chamber in 1 ml aliquots. All organisms were identified to the lowest possible taxa using a dissection microscope and total length was measured using an Olympus DP21 digital system. All organisms were identified one aliquot at a time until 100 were encountered, or the entirety of the solution if there were less than 100. Any organisms with a head, but not full body were counted as partials. Partial zooplankton was counted but lengths were not measured. Cladocerans were identified to family if possible; copepods were identified as either calanoids or cyclopoids. Chironomids were identified by life stage (larvae, pupae, or adult). Algae, diatoms and detritus were noted but not quantified. Spine barbs from *Bythotrephes* were counted but were not included in the organism count.

Data Processing and Statistical Analysis

Means and standard errors (SE) were calculated for all fatty acids and fatty acid indices (e.g. percent saturated fatty acids, percent monounsaturated fatty acids, etc.) used in the analysis. Ontogenetic differences (i.e. differences between small, medium, and large emerald shiners) and seasonal differences (i.e. differences between shiners sampled in early, mid-, and late season) in

key fatty acids and the fatty acid indices were compared using either t-tests (for two-sample comparisons) or ANOVAs with *post-hoc* tests (for three-sample comparisons).

Fatty acid composition for each sample was also analyzed and compared using Plymouth Routines in Multivariate Ecological Research (PRIMER), a software package used for the statistical analysis of complex ecological data sets. The analysis of similarities procedure (ANOSIM) in PRIMER is a multivariate nonparametric analog of univariate ANOVA tests, and it was used to describe the degree of separation among groups based on fatty acid composition. The ANOSIM analysis provides R values between 0 and 1 at a fixed significance level of 0.01. An ANOSIM R value of "0" would indicate that groups are identical and cannot be separated, whereas an ANOSIM R value of "1" would represent widely divergent groups with no significant similarity. I interpreted ANOSIM R values following Pethybridge et al. (2011): values > 0.75 indicated "well-separated" groups, values between 0.25 – 0.75 indicated "separated" groups, and values < 0.25 indicated "barely separated" groups. The similarity of percentages analysis (SIMPER) in PRIMER was used to identify key fatty acids that were most responsible for differences among groups. SIMPER and ANOSIM procedures were performed on untransformed percentage composition data using a nonparametric Bray–Curtis similarity matrix; these non-parametric procedures do not require that percentage data be transformed (Parrish et al. 2014).

Non-Metric Multidimensional Scaling plots (nMDS) were used to statistically separate and visualize differences among groups based on fatty acid composition. The nMDS plots illustrate dispersion within and between groups. The degree of dispersion indicates how similar the samples are to one another, with more tightly-clustered sample points having a similar fatty acid profile and more dispersed sample points having different fatty acid profiles. The nMDS

procedure generates “2-dimensional stress values” which provide a description of the potential accuracy of the representation of differences among groups. Stress values that are below 0.05 indicate an “excellent representation” of differences among samples, while values between 0.10 and 0.20 portray a “potentially useful” 2-dimensional picture (Clarke and Warwick 2001). The nMDS plots also provide boundaries around subsets of data points that reflect overall similarities in fatty acid composition ranging from 80 – 90%.

Analysis of the stable isotope composition for the emerald shiners and predatory fish was done using an ANOVA (Stowasser et al. 2009). Bi-plots of stable isotope 95% confidence intervals around the mean were created using the statistical program SIAR located in R (R Development Core Team 2010). Bi-plots allow for the plotting of δ15N versus δ13C and illustrate potential diet niche overlap among the different age and size classes of emerald shiners. Any overlaps between carbon intervals were considered to have similar prey sources, while nitrogen values greater than 3-4 ‰ were considered to be of a different trophic status altogether (Happel et al. 2013).

RESULTS

Fatty Acids

For both 2014 and 2015 and across all seasons and size classes, broad patterns in fatty acids and indices were very similar. The four most common fatty acids observed were palmitic acid (16:0), oleic acid (18:1n-9), EPA (20:5n-3), and DHA (22:6n-3) (Table 2, 3). For both 2014 and 2015, oleic acid and DHA were key fatty acids responsible for the majority of differences among groups (Table 4). In 2014, EPA and 22:5n-6 were also important in differentiating groups, whereas in 2015 16:1n-7 and 22:4n-6 accounted for more variation among samples

(Table 4). For example, in 2014, DHA was higher in small shiners when compared to large ones in both the early and late season. While for both 2014 and 2015, lower oleic acid values and higher DHA values were observed in small shiners when compared to medium ones (Figure 2).

For the fatty acid indices, saturated fatty acids represented approximately 25% of the total fatty acids, monounsaturated fatty acids approximately 20%, n-3 polyunsaturates about 35%, and n-6 polyunsaturates about 15% for both 2014 and 2015 (Table 2, 3). Fatty acid indices values were more similar across all size classes late in the season in comparison to early and mid-seasons (Figure 3). For both years the biggest differences were observed between small and medium shiners, where small shiners were lower in monounsaturates in mid-season 2014 and early season 2015, and higher in the poly n-3 fatty acids in mid-season 2014 (Figure 3). Across all size classes and seasons, the overall differences in the fatty acid composition of the emerald shiners were statistically significant according to the ANOSIM and SIMPER analyses ($p = 0.01$, $R = 0.687$).

Ontogenetic differences

Comparison of the three size classes was restricted to the 2014 sampling season since no large emerald shiners were collected in 2015 (see Table 1). Comparison of all three size classes was further restricted to only early and late sampling seasons since no large emerald shiners were collected in the mid-season. For the early season in 2014 the clustering of the small emerald shiners was much tighter in comparison to the large, indicating a greater variation in diet in the large shiners (Figure 4). The small and large shiners are also well separated ($R=0.809$, $p=0.01$) with each group being roughly 85-90% similar (Figure 4). The SIMPER analysis specifies that

DHA, oleic acid, and 22:5n-6 were the three fatty acids responsible for the differences in the nMDS plots and fatty acid profiles between large and small shiners in early 2014 (Table 4).

Small and medium shiners were collected in 2014 in the mid-season, with the two groups exhibiting distinct separation between them ($R=0.921$, $p=0.01$). The medium emerald shiners were closely clustered with the group having a 90% similarity amongst themselves, indicating similar fatty acid profiles (Figure 5). For the small emerald shiners the samples were relatively dispersed which resulted in two subgroups being formed that had a 90% similarity amongst each group (Figure 5). The two groups of small emerald shiners were roughly 85% similar to each other, indicating a greater variation in fatty acid content than the medium shiners examined at this time. Oleic acid, EPA, and DHA were the three primary fatty acids responsible for the differences examined between these two groups (Table 4).

Late season 2014 is the only season in which all three size classes (small, medium, and large) were collected. For the late season in 2014 the individual data points for the small emerald shiners were the most dispersed while the medium and large emerald shiners were more tightly clustered yet overlapped greatly with one another (Figure 6). The dispersion and overlap indicates relatively high variation in fatty acid content within and between small, medium, and large shiners. It was observed that the similarities within each of the size classes was no greater than 85%, while the overall similarity between all the groups was roughly 80% (Figure 6). There were some subgroups that formed, with the most notable one being within the small emerald shiners that created a subgroup that was 90% similar and encompassed all the small emerald shiners except one (Figure 6). The variation between the small and large versus the medium and large shiners was due to differences in oleic acid, 22:4, and DHA, while oleic acid, EPA, and

DHA were the three fatty acids that were responsible for the differences observed between small and medium shiners (Table 4).

In 2015 only small and medium size fish were collected during the early and mid-points of the sampling season. For the early season the two groups were separated from one another but there was a high amount of dispersion in each group that resulted in several outliers (R=0.361, p=0.01) (Figure 7). There were several subgroups that were 90% similar, but the overall similarity for all samples was 80% (Figure 7). There was a large amount of variation in the fatty acids between each group, with palmitic acid, oleic acid, and DHA being the three main fatty acids responsible for the differences observed (Table 3, 4).

For the mid-season in 2015, the small and medium fish were barely separated with a high amount of dispersion within the two groups (R=0.225, p=0.01). There were multiple subgroups that formed that were 90% similar but it was a mix of both small and medium shiners, indicating a large amount of variation in the fatty acids within and between the groups (Figure 8). Overall all the samples were 80% similar to one another with oleic acid, 22:4, and DHA being the three main fatty acids that explained any differences observed between the two groups (Table 4).

Seasonal Shifts

Across both years, small emerald shiners were the group that was collected across all seasons, allowing for seasonal changes in fatty acid indices and individual fatty acids to be examined. Overall, seasonal changes in the indices were more pronounced in 2014 in comparison to 2015, with both years following the same trends. For example, a distinct increase in monounsaturates from early to late season in small shiners was observed in 2014, with the same trend being observed in 2015 but to a lesser degree (Figure 9). The biggest difference

between the two years was in the poly n-6 fatty acid indices, where there was an overall decrease in poly n-6 from early to late season in 2014, but a small increase in the same time period in 2015 (Figure 9).

Seasonal changes were observed in the small shiners in their individual fatty acids as well. In 2014, the four main fatty acids in the small shiners were oleic acid, EPA, DHA, and 22:5n-6, while in 2015 they were oleic acid, 16:1n-7, DHA, and 20:4n-6. In 2014 and 2015, oleic acid increased from early to late season with similar increases occurring in both years (Figure 10). The biggest difference between the two years other than the different fatty acids, was the pattern observed for DHA. In 2014, DHA decreased by roughly 5% from the early season to the mid-season and then maintained that percentage into the late season (Figure 10). In 2015, DHA experienced a slight dip in the mid-season followed by a small increase in the late season (Figure 10). The other fatty acids in 2014 and 2015 only experienced minor increases and decreases from early to late season and were at a much lower percentage than oleic acid and DHA.

Seasonal shifts were also observed amongst the small size class of emerald shiners in 2014 and to a lesser extent in 2015. Both years did have separate groups with more separation occurring in the 2014 small shiners ($R=0.745$, $p=0.01$) than the 2015 fish ($R=0.490$, $p=0.01$). In 2014, even though the groups were separated, some dispersion led to the creation of subgroups within the mid-season and late season fish (Figure 11). Overall the small emerald shiners in 2014 were well separated from one another and had an overall similarity of 85% across all seasons. The key fatty acids responsible for the differences between the mid-season and late season fish as well as the early season and late season fish were palmitic acid, oleic acid, and DHA, while the separation of the early and mid-season fish were due to differences in oleic acid, 22:5n-6, and DHA (Table 4).

In 2015, small emerald shiners were collected at the beginning, middle, and end of the sampling season and although the differences in diet were significant ($p=0.01$, $R=0.49$), all of the samples were very scattered, indicating large variation in the fatty acids (Figure 11). An overall 80% similarity was observed in 2015 with fatty acids like palmitic acid, oleic acid, 22:4, and DHA explaining the differences between the groups (Table 4).

Medium sized emerald shiners were collected in 2014 and 2015, allowing for an examination of seasonal changes in this size class as well. In 2014, fish used were from the mid-season and the late season (Table 1). These two groups were barely separated ($R=0.119$) and were much dispersed, creating overlap between the two groups (Figure 12). Even though the groups were mixed together, the high amount of dispersion resulted in an 80% similarity value. Any differences between the two groups were as a result of differences in oleic acid, 22:4, and DHA concentrations (Table 4).

Medium emerald shiners from 2015 were collected at the start and in the middle of sampling season. Figure 12 shows that the samples within each time period are much dispersed but are still separated from one another, indicating that the shiners have variation in their fatty acids ($R=0.442$, $p=0.01$). The similarities within both the early and mid-season medium shiners are roughly 85%, while the similarity between each group is 80%. The fatty acids responsible for the differences between the two groups are palmitic acid, oleic acid, and DHA (Table 4).

Seasonal shifts in fatty acids and diet were the greatest and easiest to detect in the large emerald shiners collected in the early and late season in 2014. The early and late large shiners are well separated from one another ($R=0.807$, $p=0.01$). The late season fish are more tightly clustered amongst themselves while the early season fish are more dispersed with two outliers,

one of which ends up being 90% similar to one subgroup of late large shiners (Figure 13). Although there are subgroups within some of the larger groups, overall there is an 85% similarity within the groups and an 80% similarity overall (Figure 13). The SIMPER analysis indicates that DHA, 22:5n-6, and oleic acid are the key fatty acids that can explain the differences between the two groups (Table 4).

Predator fatty acids

The fatty acid composition of all groups of emerald shiners and the two predators (smallmouth bass and white bass) were 80% similar, with similarities within most groups being around 85% (Figure 14). Overall, the shiners tend to cluster around one another and are relatively distinct from the predators ($R=0.719$, $p=0.01$). The one exception to this is the early large emerald shiners from 2014 that cluster more with the predators and are in an 85% similarity subgroup with the white bass (Figure 14).

Stable Isotopes

Stable isotope data for the emerald shiner was highly variable and overlap between the three size classes was high (Figure 15). In general, nitrogen ratios were between 13.5 and 14.5‰, suggesting that fish were feeding at similar trophic positions. Carbon ratios varied little and were generally between -23 and -19‰. There was little separation between the three size classes (Figure 15).

Stable isotope data for three resident predators (largemouth bass, smallmouth bass, and white bass) was highly variable, but had little overlap within themselves and the emerald shiners that had been tested (Figure 15). The one exception to this is the large shiners and white bass. Even though their vectors do not overlap, they do contain some of the same nitrogen and carbon

ratio values (Figure 15). Stable isotope values amongst the predators had larger nitrogen ratios compared to the shiners, indicating that they were potentially eating at a higher trophic level. Using stable isotope data alone, the carbon and nitrogen ratios tend to be more enriched in largemouth bass. In general, white bass carbon values were around -22‰, smallmouth bass were around -19‰, and largemouth bass were around -18‰, with nitrogen ratios of all three species clustering around 15-17‰, indicating similar trophic levels.

Stomach Contents

Major prey categories of the emerald shiners in 2014 included cladocerans, copepods, and chironomids (Figure 16), although a wide variety of other types of food items were consumed as well (see Table C1 and Figure C1 in the Appendix). Early in the season, small and medium shiners had similar diets, with cladocerans and chironomids being the most common food items; large shiners, however, consumed mainly copepods during the early season (Figure 16). In the late season samples, large shiners were still consuming mainly copepods while small shiners were eating mostly cladocerans; medium-sized shiners were consuming approximately equal proportions of cladocerans and copepods (Figure 16). Chironomids were relatively common in the diet of all size classes of shiners early in the season but were much less common late in the season (Figure 16).

DISCUSSION

One of the most important native planktivorous fishes in the Niagara River, and in the Great Lakes as a whole, is the emerald shiner. Emerald shiners serve both as a top predator on mostly plankton communities and as a key prey item for valuable sport fish and endangered migratory birds. In this study I examined seasonal dietary trends in three different size classes of

emerald shiners collected from two different years (2014 and 2015). I focused mainly on the use of fatty acids as biomarkers to determine similarities and differences among diets, and I also used data from stomach content and stable isotope analyses to evaluate any links between the three methods.

The use of fatty acids as biomarkers has become increasingly common in aquatic food web studies (Rude et al. 2016). Fatty acid biomarkers are a useful tool in identifying energy sources consumed by fish because fish lack the ability to synthesize certain fatty acids, like long chain polyunsaturated fatty acids (PUFAs), and therefore must get them from their diet. Thus, differences in consumption of prey items of fish can often be detected by using biomarkers if the forage bases they are consuming differ in fatty acid composition (Happel et al. 2015, Rude et al. 2016). Fatty acids are also good biomarkers because of how they are absorbed into fish tissue. When a prey item is consumed and digested, their lipids are broken down into simpler compounds and individual fatty acids. These fatty acids, which are major components of energy storage molecules (triacylglycerides), are often deposited into the tissues in patterns reflective of the fatty acids present in the diet (Happel et al. 2015). Fatty acids make ideal biomarkers because they are generally absorbed into the storage lipids relatively unchanged, making it easier to link any given fatty acid profile back to a certain diet item (Budge et al. 2006, Elsdon 2010, Sargent et al. 2002).

Ontogenetic changes in fatty acids

In my study, the most common fatty acids found in emerald shiners were palmitic acid (16:0), oleic acid (18:1n-9), EPA, and DHA. Palmitic acid did not show ontogenetic changes whereas oleic acid, EPA, and DHA did differ among shiners of different sizes. Oleic acid, EPA,

and DHA all had lower percentages in the smaller emerald shiners and greater percentages in the larger ones. Other minor fatty acids like 22:5n-3 followed a similar pattern, whereas levels of 18:2n-6 were higher in smaller fish and lower in larger fish (Tables 2-3). These differences in fatty acid composition most likely indicate differences in prey items or amounts of those prey items being consumed (Stowasser et al. 2009). As discussed further below, the increases in fatty acids like oleic, EPA, and DHA may suggest a shift to a more copepod or chironomid based diet as the shiners grow in size (Stowasser et al. 2009).

In contrast to the ontogenetic differences in individual fatty acids discussed above, there were fewer differences in fatty acid indices among size classes of emerald shiners. For the four major fatty acid indices examined (saturated fatty acids, monounsaturated fatty acids, n-3 polyunsaturated fatty acids, and n-6 polyunsaturated fatty acids), the only differences were higher monounsaturated fatty acids in medium shiners compared to small shiners in mid-season 2014 and early season 2015, and higher n-3 polyunsaturated fatty acids in small shiners compared to medium shiners in mid-season 2014 (Figure 3). Overall, only a few fatty acids were responsible for differences among the various groups of shiners that I compared in this study (see Table 4). Since many fatty acid values are combined to produce each index and each individual fatty acid therefore has less impact, it is not surprising that the indices do not reflect as many differences among groups as the analysis based on individual fatty acids.

The multivariate analysis of ontogenetic differences indicates that smaller emerald shiners tend to have more varied fatty acid profiles than larger ones. This is illustrated by more dispersion or variation in the MDS plots comparing the fatty acid composition of emerald shiners of different size classes (Figures 4-8). According to optimal foraging theory, smaller emerald shiners may consume a wider variety of diet items due to the fact that they are trying to consume

as much as they can as quickly as possible so that they can get larger and potentially escape predation (Pyke 1984, Townsend and Winfield 1985). Larger-sized fish can specialize or pick one diet item over another because the threat of predation is less and therefore they can afford to wait for a more nutritious prey item that will be more beneficial for survival and reproductive development (Townsend and Winfield 1985). Although smaller shiners generally had more variable fatty acid composition (and presumably more variable diets), an exception is seen in the comparison of early season large and small emerald shiners from 2014 (Figure 4). In this instance, there is more dispersion among large emerald shiners than small. According to stomach content data, invertebrate eggs composed a much higher proportion of the diet of these large emerald shiners than any other size class (see Table C1 and Figure C1 in the Appendix). There is a possibility that the large emerald shiners were eating invertebrate eggs from a variety of invertebrate species that differed in fatty acid composition, which could have contributed to the large amount of variation seen in the nMDS plot for this particular sampling period.

Seasonal changes in fatty acids

Through the use of fatty acid biomarkers, it is possible to detect changes in diet in a relatively short period of time. In a study conducted by Haubert et al. (2011), fatty acid biomarkers were used to examine changes in a controlled diet study before and during starvation. It was determined that the biomarkers derived from the diet could be detected as soon as one day after the diet changed and up to 14 days after starvation (Haubert et al. 2011). In another study, Antonio and Richoux (2016) examined fatty acid turnover rates and found that depending on the organism being examined, changes in diets can be observed anywhere from 1.5 – 4 days after a change in diet occurred. This response is relatively fast in comparison to other diet techniques like stable isotopes, which can take up to 8 months before full turnover occurs that is reflective

of changes in diet (Antonio and Richoux 2016). These results indicate that fatty acid biomarkers can be useful for comparing diets during different seasons, since fatty acid content is likely to reflect dietary changes within days or weeks.

My results indicate that seasonal changes occurred in the fatty acid content of emerald shiners from the upper Niagara River (Table 2-3; Figures 9-13). In 2014, fatty acids including oleic acid, EPA, and 18:3n-3 all expressed smaller percentages in the early season samples and increased significantly late in the season. The opposite trend was observed for 22:5n-6 and DHA, where the percentages in the early season were almost double that of what they were late in the season (Table 2). Fatty acids like EPA and 18:3n-3 are often associated with diatoms and copepods, while elevated levels of DHA and 22:5n-6 are more common in non-diatom plankton and some cladocerans (Czesny et al. 2011, Happel et al. 2015). The changes in fatty acid composition I observed suggest that emerald shiners shifted their diet from more cladocerans early in the season to more copepods late in the season. This trend is generally supported by the change in the percent composition of these prey items in the diet of the shiners, as discussed further below. Previous studies indicate that the changes in stomach contents and fatty acids are often directly linked to changes in plankton communities. For example, studies conducted by Turschak et al. (2011) and Pothoven et al. (2009) in the Great Lakes found that *Bythotrephes* and *Daphnia* make up a large part of the spring and summer cladoceran community while declining temperatures in the end of summer and fall coincided with a shift to a community dominated more by copepods. Overall, the changes in fatty acid composition from spring to fall that I observed, as well as the changes in the stomach contents, support these previous findings.

Stomach contents

Stomach content data are characterized by a large amount of variability during all sampling periods, suggesting that emerald shiners are generalists and will consume a wide variety of prey types. For opportunistic feeders such as the emerald shiner, stomach items or biomass averages for any given sampling period may not be representative of overall consumption patterns. On the other hand, stomach contents can be used to give a general understanding of prey composition and can be used in a percent biomass context to understand the nutritional and caloric importance of the prey items to the predator (Happel et al. 2015).

In the upper Niagara River in 2014, emerald shiners consumed more cladocerans early in the season and a higher proportion of copepods late in the season. Also, the abundance of chironomids in the diet declined from early to late in the season (Figure 16). This is consistent with stomach content data collected for the emerald shiner in central Lake Erie that indicated that emerald shiners were consuming primarily *Daphnia*, *Leptodora*, and *Bythotrephes* (Pothoven et al. 2009). These results are also similar to descriptions of emerald shiner diets in the literature, which generally list food items such as cladocerans (including *Bosmina* and *Daphnia*), copepods including *Cyclops*, and insects (Diptera) as common food items (Scott and Crossman 1973).

Although cladocerans, copepods, and chironomids were important prey categories for the emerald shiners examined in this study, eggs were also present in large numbers in shiners from all size classes and across all seasons (see Table C1 and Figure C1 in the Appendix). However, since the taxonomic identity and fatty acid composition of the eggs found in the stomach contents are unknown, it is not possible to assess the potential impact of these eggs on my conclusions with respect to fatty acid biomarkers or stable isotopes.

A combined approach: fatty acids, stable isotopes, and stomach contents

By pairing the long term data collected by biomarkers and stable isotopes with the short term data reflected in stomach contents, a more conclusive idea about trophic interactions can be reached. In a study conducted by Happel et al. (2015), all three techniques were used to examine the diets of yellow perch. Although the fatty acid biomarkers they used changed across habitat types, in most cases they correlated well with the items found in the stomach contents and lined up with the results from stable isotopes. Not only did the fatty acid biomarkers corroborate what the other techniques found, but the fatty acids also highlighted diet shifts across habitat types better than the other techniques.

In my study, all three methods of diet analysis depicted a seasonal shift in diet as well as differences in diet among size classes, and each method offered insight into these changes that the others did not. Stable isotope ratios of carbon and nitrogen indicated that emerald shiners are all eating at a similar trophic level, while stomach contents and fatty acids suggested more specific differences in diet composition. When stomach contents and fatty acid data are combined, the results suggest heavy foraging on cladocerans early in the season and a more diverse diet later in the season. Stomach contents alone indicated that large shiners were consuming a copepod-dominated diet, yet certain fatty acids present in the shiners may be associated with chironomids eating diatoms and benthic invertebrates, suggesting that there was a larger variety of food items being consumed. The combined data set suggests that small emerald shiners have a much more variable diet in all seasons compared to medium and large size classes, with cladoceran and copepod fatty acids including DHA and 22:5n-6 being common in the small fish (Gladyshev et al. 2010). Overall, fatty acid signatures varied strongly between individuals of different sizes and sampling seasons with small variations occurring within each

group, suggesting ontogenetic and seasonal difference that persist over a certain period of time that were not picked up by stomach contents or stable isotopes.

Physiological implications of variation in fatty acid content

Polyunsaturated fatty acids (PUFAs) have two or more double bonds. PUFAs that derive from α-linolenic acid or ALA (18:3n-3) are considered to be in the n-3 series, and those that have linoleic acid or LA (18:2n-6) as their precursor are in the n-6 series. In all of the emerald shiners samples, polyunsaturated fatty acids (PUFAs) in the n-3 series were the group that had the highest overall values (Figure 3). PUFAs are important for regulating cell membrane properties like temperature acclimation (Brett and Muller-Navarra 1997, Haubert et al. 2011, Snyder et al. 2012). Temperature acclimation is key for fish in temperate climates like the Great Lakes due to the large seasonal temperature changes that these aquatic habitats experience. For the emerald shiner, eating a diet high in PUFAs may help regulate cell membrane processes and aid them in adjusting to the river when the temperature drops (Haubert et al. 2011, Snyder et al. 2012). Saturated fatty acids, which had the third highest concentration in all shiners, have been linked to helping with temperature regulation at higher temperatures. Saturated fatty acids can generally be synthesized by most animals, however, so it is not necessary for aquatic organisms to obtain these fatty acids from the diet.

Elevated levels of highly unsaturated fatty acids (HUFAs) in the n-3 series were also found in the tissues of the emerald shiners I examined from the upper Niagara River. HUFAs are a subset of PUFAs that contain 20 or more carbon atoms. These fatty acids have been found to be critical for maintaining growth, survival, and reproduction (Brett and Muller-Navarra 1997). HUFAs are essential because they cannot be synthesized by most animals and thus must be

obtained from the diet, making them a good indicator of dietary change. High HUFA content has been linked to herbivorous zooplankton production and when high HUFA-content phytoplankton is present, it leads to a higher zooplankton biomass (Brett and Muller-Navarra 1997). Since key HUFA fatty acids like EPA were found in high concentrations in all emerald shiners, it suggests that the shiners were eating a diet of zooplankton which consumed high HUFA-content phytoplankton. This conclusion is consistent with the stomach contents of the shiners I examined, since many copepods and cladocerans are known to consume an herbivorous diet.

In comparison to other forage species that occupy a similar niche and prey on the same type of food, like spottail shiners and alewives, the HUFA content of the emerald shiner is higher (Czesny et al. 2011). This gives the emerald shiner a higher nutritional value since it has a greater concentration of essential fatty acids and therefore makes them a more desirable prey item to top predators. With the benefits of HUFAs and PUFAs being supported by previous research and their current importance in the diets of the emerald shiner, it is essential to maintain the emerald shiner population throughout the upper Niagara River to ensure the successful development of other fish species and piscivorous birds.

The relationship between dietary lipids and fatty acid composition can be complicated due to factors such as innate metabolism and fatty acid chain length. Depending on a consumers' metabolism, fatty acid signatures can become altered relative to the diet, preventing exact matching of predator fatty acid signatures to prey even when fed a single diet (Budge et al. 2011, Copeman et al. 2013, Taipale et al. 2011). Also, several aquatic species, like *Daphnia* and various copepods, have been shown to preferentially accumulate long-chain fatty acids over others (Kainz et al. 2004, Masclaux et al. 2012, Taipale et al. 2011). Variation in consumers'

metabolism and the preferential assimilation of fatty acids have been modeled in controlled feeding studies, so that calibrations derived from them can be used to estimate and understand the fatty acid composition of wild caught fish (Happel et al. 2015). For example a study conducted by Happel et al. (2015) examined the fatty acid profiles of three species, lake trout, yellow perch, and round gobies, after feeding them different controlled diets they would encounter in their natural environment. By examining how the fatty acids from the controlled diets were assimilated into the tissues of these fishes, this study was able to provide a model that could help identify certain fatty acids found in wild caught fish of the same species.

Despite the fact that both PUFAs and HUFAs are fatty acids that are essential for temperature acclimation in fishes, temperature changes are probably not responsible for the fatty acid differences I found in this study. Temperature does have the ability to impact fatty acids within fish, but the impacts are limited mainly to their polar lipids or phospholipids. Phospholipids are located in the cell membrane and it is these lipids that become more or less fluid with changes in temperature (Snyder et al. 2012). Although some of the fatty acids consumed by the shiners do go into the maintenance of cell membranes, the fatty acids that were examined in my study were taken from the storage lipids due to the extraction technique used. Direct methylation is an extraction technique that is known to primarily extract storage lipids and fatty acids out of the tissues of any given organism (Parrish et al. 2014). Since this technique was used and storage fatty acids were analyzed, dietary lipids most likely had strongest influence on the fatty acids within the emerald shiners that I examined.

Resident predators

In the examination of the resident predators in the upper Niagara River, both stable isotopes and fatty acids were employed to understand their potential relationship to the emerald

shiners. The stable isotope results for the emerald shiners on average showed nitrogen ratios at 14‰ and carbon ratios at -23‰. For the top predators the nitrogen ratios ranged from 15 to 17‰, while carbon ratios ranged from -21 to -18‰. For the emerald shiners, the very negative δ13C indicates pelagic feeding, while the low δ15N indicates mid-trophic position. Both large and medium sized emerald shiners had wide nitrogen ranges (NR), suggesting high variability in prey trophic levels, while small emerald shiners had wide NR and carbon ranges (CR), indicating a potential generalist diet (Giraldo et al. 2016). These data are in agreement with the stomach content data that contained a wide variety of pelagic zooplankton species and fatty acid data that suggested a zooplankton diet as indicated by high percentages of fatty acids like DHA and EPA. For the resident predators, the δ13C values are consistent with benthic feeding and use of slower moving water, while the relatively high δ15N suggests higher trophic prey (Giraldo et al. 2016). The large CR values exhibited by all predators further suggests they are consuming prey from a range of carbon sources, both pelagic and benthic in origin, while the large NR observed in the white bass indicates variability in prey trophic levels. The fatty acid analysis for the predators showed roughly an 80% similarity in fatty acid composition between the predators and the emerald shiners, suggesting that they may be consuming a variable diet that also includes emerald shiners. The fatty acid results are consistent with the stable isotope data for the resident predators in the upper Niagara River.

CONCLUSION

Utilizing a data set that was collected from the Niagara River during the 2014 and 2015 intensive sampling seasons, I explored the diet of the emerald shiner and resident predators using multiple dietary assessments. Employing stomach contents, fatty acid signatures, and stable isotope ratios, I was able to demonstrate the value of combining all three techniques in

identifying seasonal and ontogenetic shifts in diet. I also compared trends in fatty acids and stable isotopes to describe links between the diets of resident predators and the emerald shiner. In general, the fatty acid results were consistent with data from stomach contents, which indicated that copepods were more important later in the season and were more common in the stomachs of large shiners. I also found that stable isotopes were not sensitive to ontogenetic and seasonal shifts in diet of the emerald shiners but did highlight different trophic positions between the shiners and the predators. With an increasing number of studies being conducted on freshwater species, more fatty acid biomarker libraries and quantification of diets could lead to a greater understanding of how growth and seasonal changes can impact trophic interactions and food web structures. Future dietary assessments should be conducted using a multiple technique approach in order to strengthen our knowledge of changing food web structures in understudied freshwater fish species like the emerald shiner.

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Table 1. Sample sizes for each season in which emerald shiners were collected in 2014 and 2015.

	2014		
Size Class	Early Season	Mid-Season	Late Season
Small	N=20	N=20	N=20
Medium	N/A	N=20	N=20
Large	N=13	N/A	N=20
	2015		
Small	N=20	N=20	N=20
Medium	N=20	N=20	N/A
Large	N/A	N/A	N/A

Table 2. Fatty acid content (Mean ± SE) of emerald shiners by season and size class in the upper Niagara River for 2014. Fatty acid content is expressed as the percentage of total fatty acids. The most common fatty acids (N = 24) found across all samples are included.

	Early Season		**Mid-Season**		**Late Season**		
	Small	**Large**	**Small**	**Medium**	**Small**	**Medium**	**Large**
Saturated							
14:0	2.9 ± 0.07	1.2 ± 0.10	2.0 ± 0.06	2.9 ± 0.11	2.8 ± 0.10	3.5 ± 0.13	2.8 ± 0.16
16:0	17.6 ± 0.20	18.3 ± 0.32	19.4 ± 0.13	17.7 ± 0.47	17.2 ± 0.25	18.0 ± 0.48	17.7 ± 0.14
17:0	0.6 ± 0.01	0.5 ± 0.04	0.6 ± 0.01	0.4 ± 0.01	0.5 ± 0.01	0.4 ± 0.01	0.4 ± 0.01
18:0	4.9 ± 0.10	5.4 ± 0.11	5.5 ± 0.10	4.6 ± 0.13	4.2 ± 0.08	4.8 ± 0.14	4.9 ± 0.08
20:0	0.3 ± 0.02	0.3 ± 0.05	0.3 ± 0.03	0.3 ± 0.02	0.3 ± 0.03	0.4 ± 0.03	0.3 ± 0.02
22:0	0.3 ± 0.04	0.1 ± 0.04	0.1 ± 0.02	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.03	0.1 ± 0.02
Subtotal	**26.9 ± 2.49**	**26.1 ± 2.67**	**28.2 ± 2.79**	**26.2 ± 2.52**	**25.4 ± 2.45**	**27.4 ± 2.56**	**26.4 ± 2.53**
Monounsaturated							
14:1	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.01	0.0 ± 0.02	0.0 ± 0.01	0.0 ± 0.02
16:1n-7	3.8 ± 0.13	3.6 ± 0.23	4.4 ± 0.12	5.9 ± 0.19	7.2 ± 0.12	5.8 ± 0.21	5.9± 0.16
18:1n-9	7.7 ± 0.22	8.7 ± 0.35	10.2 ± 0.20	14.5 ± 0.43	13.3 ± 0.30	15.0 ± 0.45	14.8 ± 0.33
18:1n-7	2.4 ± 0.04	3.4 ± 0.09	3.1 ± 0.04	2.5 ± 0.09	2.7 ± 0.06	2.1 ± 0.06	2.7 ± 0.05
20:1	0.3 ± 0.02	0.7 ± 0.03	0.5 ± 0.02	0.6 ± 0.02	0.2 ± 0.02	0.4 ± 0.05	0.6 ± 0.04
22:1	0.2 ± 0.04	0.2 ± 0.08	0.1 ± 0.04	0.1 ± 0.04	0.2 ± 0.03	0.1 ± 0.03	0.1 ± 0.02
Subtotal	**14.4 ± 1.12**	**16.8 ± 1.24**	**18.4 ± 1.48**	**23.8 ± 2.11**	**23.8 ± 2.00**	**23.6 ± 2.19**	**24.1 ± 2.13**

Table 2. Continued.

	Early Season		Mid-Season		Late Season		
	Small	**Large**	**Small**	**Medium**	**Small**	**Medium**	**Large**
Polyunsaturated (n-3)							
18: 3n-3 gamma	0.2 ± 0.02	0.0 ± 0.02	0.14 ± 0.02	0.2 ± 0.02	0.2 ± 0.01	0.1 ± 0.02	0.1 ± 0.02
18:3n-3 alpha	3.2 ± 0.07	1.9 ± 0.14	5.2 ± 0.13	4.6 ± 0.14	4.7 ± 0.24	4.9 ± 0.14	4.7 ± 0.12
18:4n-3	2.1 ± 0.05	0.4 ± 0.07	1.3 ± 0.06	0.9 ± 0.04	0.9 ± 0.05	0.7 ± 0.03	0.7 ± 0.03
20:3n-3*	0.8 ± 0.03	0.8 ± 0.02	1.0 ± 0.03	0.9 ± 0.03	0.9 ± 0.05	0.9 ± 0.03	1.11 ± 0.03
20:5n-3*	8.4 ± 0.11	9.6 ± 0.23	8.8 ± 0.15	10.8 ± 0.30	9.0 ± 0.18	10.9 ± 0.31	10.2 ± 0.15
22:5n-3*	1.8 ± 0.07	3.1 ± 0.09	2.0 ± 0.07	2.5 ± 0.09	1.8 ± 0.05	2.5 ± 0.08	2.6 ± 0.07
22:6n-3*	19.8 ± 0.30	17.4 ± 0.43	15.1 ± 0.38	9.6 ± 0.34	14.9 ± 0.23	9.4 ± 0.32	12.1 ± 0.34
Subtotal	**36.2 ± 2.45**	**33.3 ± 2.26**	**33.4 ± 1.90**	**29.4 ± 1.52**	**32.4 ± 1.92**	**29.3 ± 1.52**	**31.5 ± 1.68**
Polyunsaturated (n-6)							
18:2n-6	3.7 ± 0.08	2.8 ± 0.22	4.4 ± 0.10	3.6 ± 0.12	4.0 ± 0.18	3.5 ± 0.14	3.5 ± 0.09
20:2n-6	0.8 ± 0.02	0.8 ± 0.04	0.7 ± 0.02	0.6 ± 0.02	0.6 ± 0.01	0.5 ± 0.03	0.7 ± 0.03
20:3n-6*	0.5 ± 0.02	0.4 ± 0.06	0.5 ± 0.01	0.5 ± 0.02	0.4 ± 0.02	0.4 ± 0.02	0.4 ± 0.02
20:4n-6*	5.5 ± 0.01	9.4 ± 0.33	5.4 ± 0.11	6.8 ± 0.20	4.2 ± 0.09	6.4 ± 0.19	6.5 ± 0.12
22:5n-6*	4.9 ± 0.57	4.4 ± 0.93	2.5 ± 0.15	1.3 ± 0.10	2.5 ± 0.09	1.2 ± 0.13	2.0 ± 0.19
Subtotal	**15.4 ± 0.92**	**17.8 ± 1.45**	**13.4 ± 0.87**	**12.7 ± 1.08**	**11.7 ± 0.72**	**12.1 ± 1.02**	**13.1 ± 1.00**
*** HUFAs**							

Table 3. Fatty acid content (Mean ± SE) of emerald shiners by season and size class in the upper Niagara River for 2015. Fatty acid content is expressed as the percentage of total fatty acids. The most common fatty acids (N = 24) found across all samples are included.

	Early Season		**Mid-Season**		**Late Season**
	Small	**Medium**	**Small**	**Medium**	**Small**
Saturated					
14:0	2.6 ± 0.09	3.1 ± 0.11	2.1 ± 0.09	2.2 ± 0.06	2.03 ± 0.06
16:0	17.4 ± 0.16	17.9 ± 0.15	18.5 ± 0.16	18.7 ± 0.11	18.2 ± 0.10
17:0	0.6 ± 0.02	0.4 ± 0.01	0.7 ± 0.03	0.6 ± 0.03	0.6 ± 0.01
18:0	4.8 ± 0.09	4.7 ± 0.06	6.0 ± 0.15	5.4 ± 0.08	4.8 ± 0.05
20:0	0.4 ± 0.02	0.3 ± 0.03	0.4 ± 0.03	0.3 ± 0.02	0.2 ± 0.02
22:0	0.1 ± 0.03	0.4 ± 0.21	0.2 ± 0.03	0.2 ± 0.02	0.1 ± 0.02
Subtotal	**25.8 ± 2.48**	**26.9 ± 2.54**	**27.9 ± 2.65**	**27.3 ± 2.68**	**25.9 ± 2.61**
Monounsaturated					
14:1	0.0 ± 0.01	0.0 ± 0.01	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
16:1n-7	6.3 ± 0.36	7.5 ± 0.20	5.1 ± 0.26	5.2 ± 0.17	4.8 ± 0.09
18:1n-9	6.8 ± 0.15	9.3 ± 0.034	8.5 ± 0.16	10.5 ± 0.33	11.4 ± 0.26
18:1n-7	3.0 ± 0.05	3.4 ± 0.22	3.2 ± 0.07	3.4 ± 0.05	2.7 ± 0.04
20:1	0.4 ± 0.02	0.5 ± 0.02	0.5 ± 0.01	0.5 ± 0.03	0.4 ± 0.04
22:1	0.2 ± 0.03	0.1 ± 0.02	0.2 ± 0.05	0.0 ± 0.01	0.0 ± 0.01
Subtotal	**16.6 ± 1.16**	**20.7 ± 1.51**	**17.4 ± 1.26**	**19.7 ± 1.54**	**19.4 ± 1.65**

Table 3. Continued.

	Early Season		Mid-Season		Late Season
	Small	**Medium**	**Small**	**Medium**	**Small**
Polyunsaturated (n-3)					
18: 3n-3 gamma	0.2 ± 0.02	0.4 ± 0.13	0.1 ± 0.02	0.2 ± 0.02	0.0 ± 0.01
18:3n-3 alpha	3.1 ± 0.06	3.2 ± 0.13	3.9 ± 0.17	4.5 ± 0.11	2.6± 0.09
18:4n-3	1.8 ± 0.06	1.9 ± 0.12	1.2 ± 0.05	1.3 ± 0.03	0.9 ± 0.03
20:3n-3*	0.7 ± 0.02	0.8 ± 0.11	0.8 ± 0.03	0.9 ± 0.02	0.6 ± 0.02
20:5n-3*	10.7 ± 0.18	11.3 ± 0.32	10.5 ± 0.15	10.9 ± 0.16	10.3 ± 0.17
22:5n-3*	2.2 ± 0.06	2.3 ± 0.06	2.2 ± 0.05	2.5 ± 0.05	1.9 ± 0.04
22:6n-3*	17.8 ± 0.39	14.1 ± 0.58	16.8 ± 0.42	14.0 ± 0.56	19.5 ± 0.31
Subtotal	**36.5 ± 2.30**	**33.9 ± 1.93**	**35.5 ± 2.18**	**34.4 ± 1.90**	**35.9 ± 2.53**
Polyunsaturated (n-6)					
18:2n-6	4.4 ± 0.13	4.7 ± 0.13	3.9 ± 0.13	4.0 ± 0.15	2.7 ± 0.13
20:2n-6	0.9 ± 0.02	0.7 ± 0.01	0.7 ± 0.02	0.8 ± 0.02	0.7 ± 0.02
20:3n-6*	0.5 ± 0.02	0.4 ± 0.02	0.5 ± 0.02	0.5 ± 0.02	0.4 ± 0.01
20:4n-6*	5.2 ± 0.11	4.5 ± 0.14	5.3 ± 0.14	6.0 ± 0.09	6.1 ± 0.11
22:5n-6*	2.6 ± 0.13	2.3 ± 0.25	2.3 ± 0.13	2.0 ± 0.15	3.7 ± 0.11
Subtotal	**13.6 ± 0.84**	**12.5 ± 0.80**	**12.6 ± 0.82**	**13.3 ± 0.93**	**13.5 ± 0.94**
*** HUFAs**					

Table 4. Key fatty acids responsible for differences among groups of emerald shiners identified via the SIMPER procedure in PRIMER. For each comparison, the three fatty acids that are most responsible for differences between groups are indicated with an "X". For each sampling year, the total number of times a particular fatty acid (FA) was identified as a key fatty acid separating groups and the percentage of comparisons that this represents is provided.

	16:1	Oleic	EPA	22:4	22:5n-6	DHA
2014						
Early small vs. Mid small		X			X	X
Early small vs. Late small	X	X				X
Mid small vs Late small	X	X				X
Early small vs Mid medium		X			X	X
Mid small vs Mid medium		X	X			X
Late small vs Mid medium		X	X			X
Early small vs Late medium		X			X	X
Mid small vs Late medium		X	X			X
Late small vs Late medium		X	X			X
Mid medium vs Late medium		X		X		X
Early small vs Early large		X			X	X
Mid small vs Early large		X			X	X
Late small vs Early large	X	X				X

	16:1	Oleic	EPA	22:4	22:5n-6	DHA
Mid medium vs Early large		X			X	X
Late medium vs Early large		X			X	X
Early small vs Late large		X			X	X
Mid small vs Late large		X	X			X
Late small vs Late large		X		X		X
Mid medium vs Late large		X		X		X
Late medium vs Late large		X		X		X
Early large vs Late large		X			X	X
# of comparisons as key FA: (21 possible)	3	21	5	4	9	21
% comparisons as key FA	14%	100%	24%	19%	43%	100%

Table 4 (cont.)

	16:1	Oleic	EPA	22:4	22:5n-6	DHA
2015						
Early small vs Mid small	X			X		X
Early small vs Late small	X	X				X
Mid small vs Late small		X		X		X
Early small vs Early medium	X	X				X
Mid small vs Early medium	X	X				X
Late small vs Early medium	X	X				X
Early small vs Mid medium	X	X				X
Mid small vs Mid medium		X		X		X
Late small vs Mid medium		X			X	X
Early medium vs Mid medium	X	X				X
# of comparisons as key FA: (10 possible)	7	9	0	3	1	10
% comparisons as key FA	70%	90%	0%	30%	10%	100%

Figure 1. Map of sampling locations in the upper Niagara River. Orange stars indicate the places from which the samples used in this analysis were collected; this includes Big Six Mile Creek, Beaver Island, Vacant Marine, La Salle Yacht Club, Strawberry Island, Gun Creek, and Motor Island.

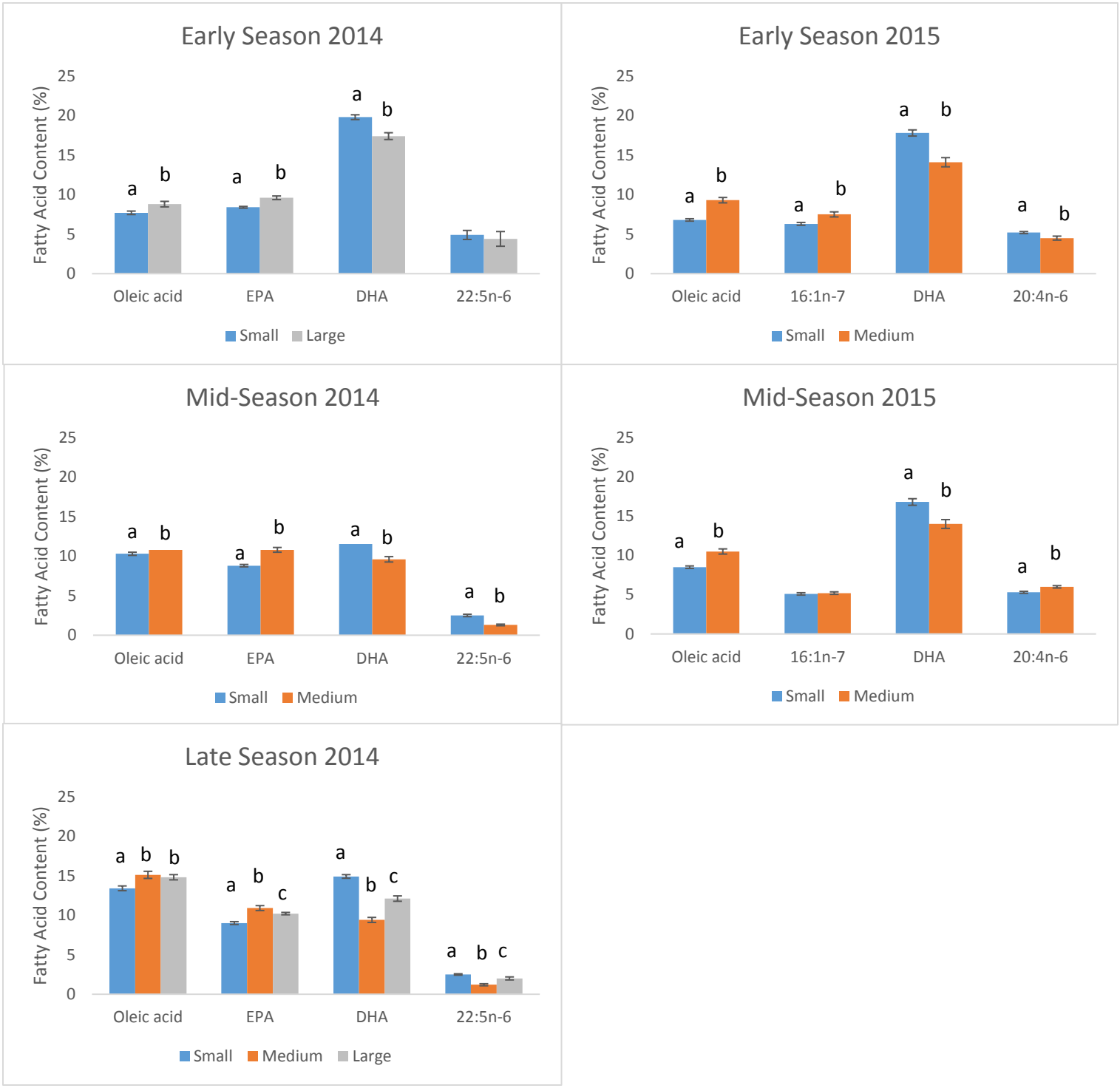

Figure 2. Ontogenetic differences in key fatty acids for emerald shiners from 2014 and 2015. The four fatty acids presented in each year are those identified via the SIMPER procedure in PRIMER as being most responsible for differences among groups in that year (see Table 4). The late season 2015 sample consisted of only one size class (small) and is not presented. Groups with letters indicate groups that are significantly different, while groups with no letters are not significantly different.

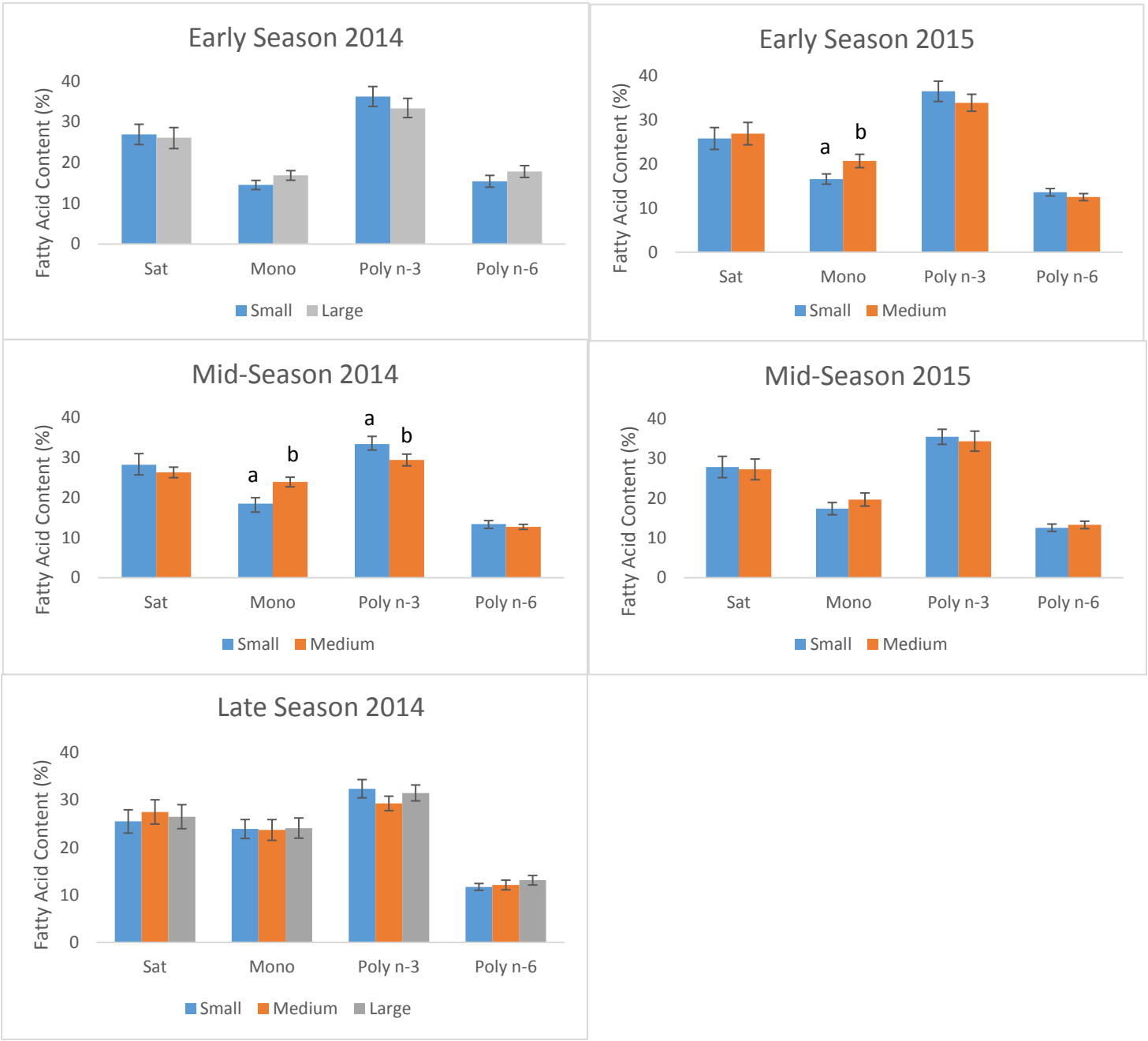

Figure 3. Ontogenetic differences in fatty acid indices for emerald shiners collected in 2014 and 2015. The late season 2015 sample consisted of only one size class (small) and is not presented. Sat = saturated fatty acids, Mono = monounsaturated fatty acids, Poly n-3 = polyunsaturated fatty acids in the n-3 series, and Poly n-6 = polyunsaturated fatty acids in the n-6 series. Groups with letters indicate groups that are significantly different, while groups with no letters are not significantly different.

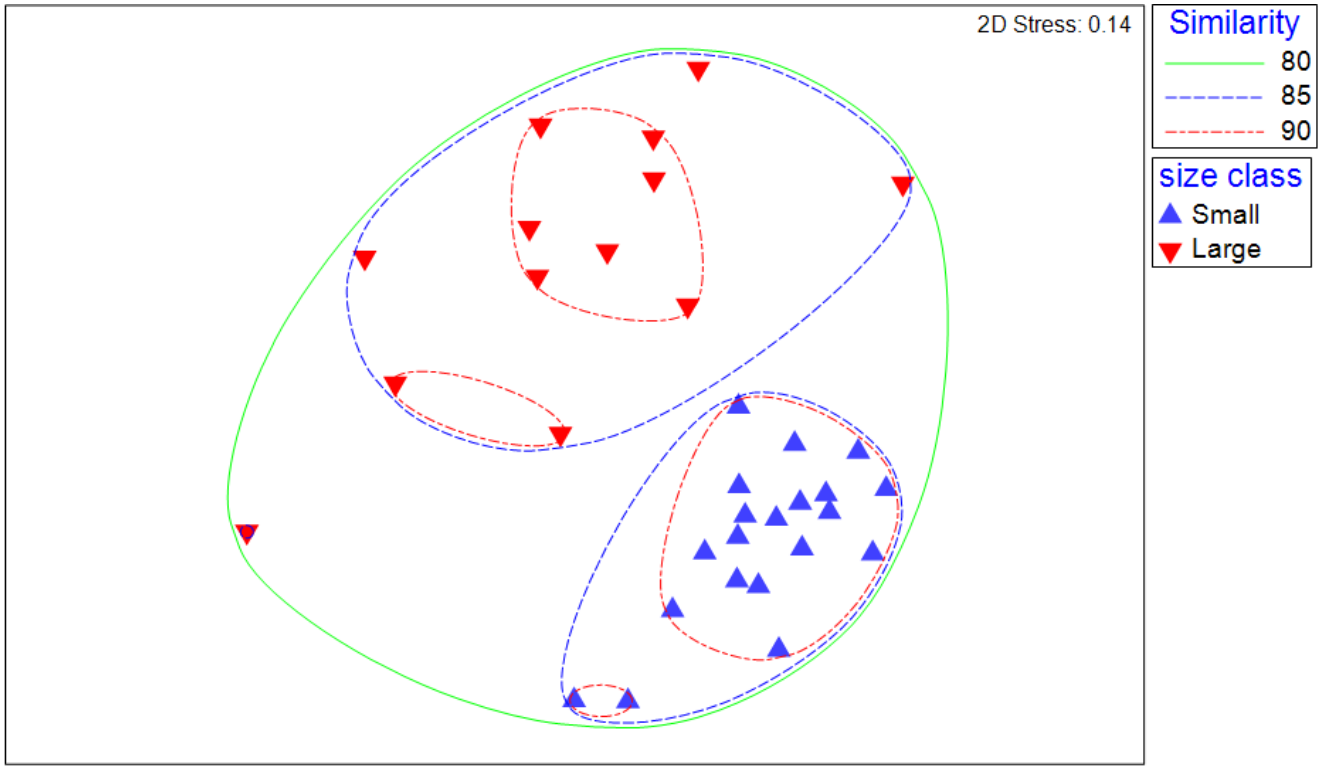

Figure 4. Non-Metric Multidimensional Scaling plot (nMDS) of ontogenetic differences in fatty acid composition of small and large emerald shiners collected in early season 2014. The ANOSIM R value = 0.809, indicating "well-separated" groups.

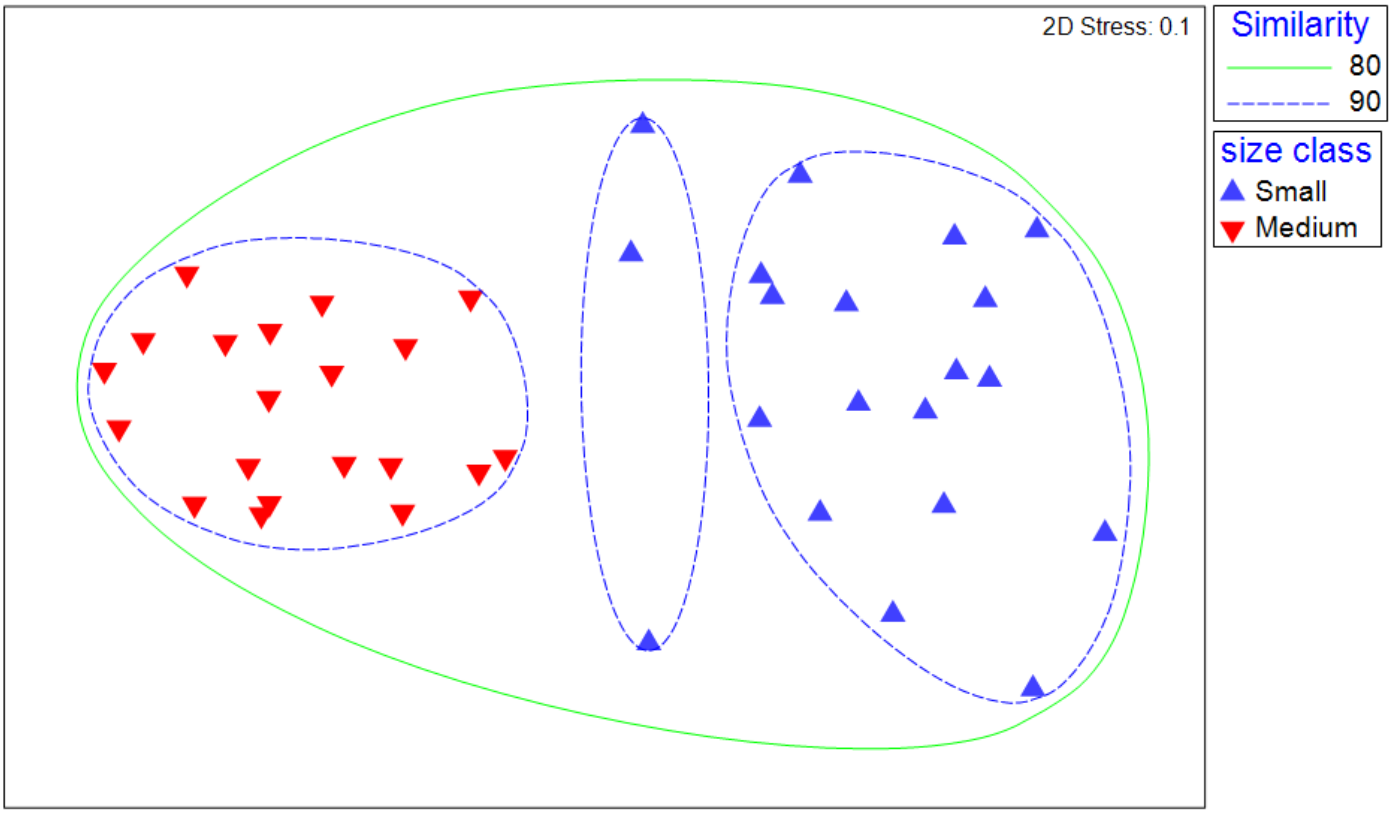

Figure 5. Non-Metric Multidimensional Scaling plot (nMDS) of ontogenetic differences in fatty acid composition of small and medium emerald shiners collected in mid-season 2014. The ANOSIM R value = 0.921, indicating "well-separated" groups.

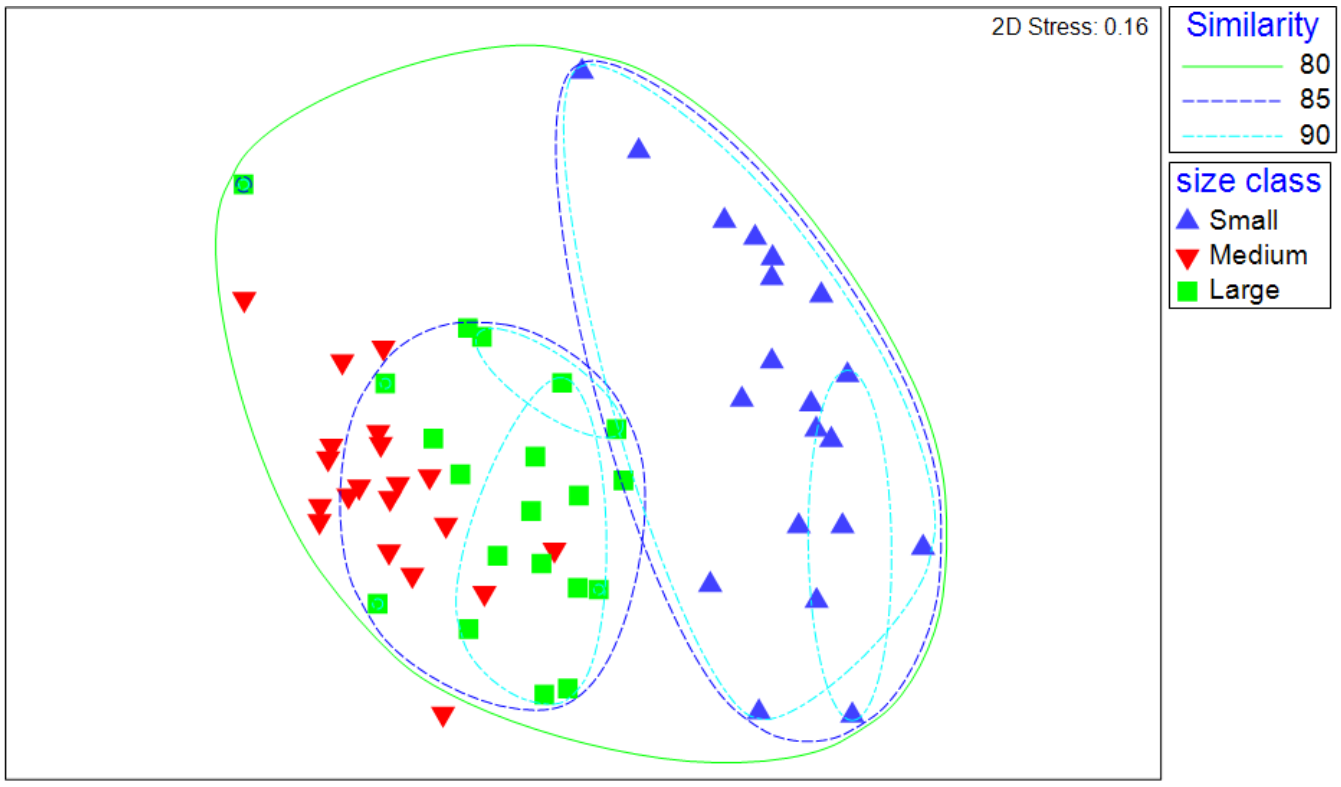

Figure 6. Non-Metric Multidimensional Scaling plot (nMDS) of ontogenetic differences in fatty acid composition of small, medium, and large emerald shiners collected in late season 2014. The ANOSIM R value = 0.562, indicating "separated" groups.

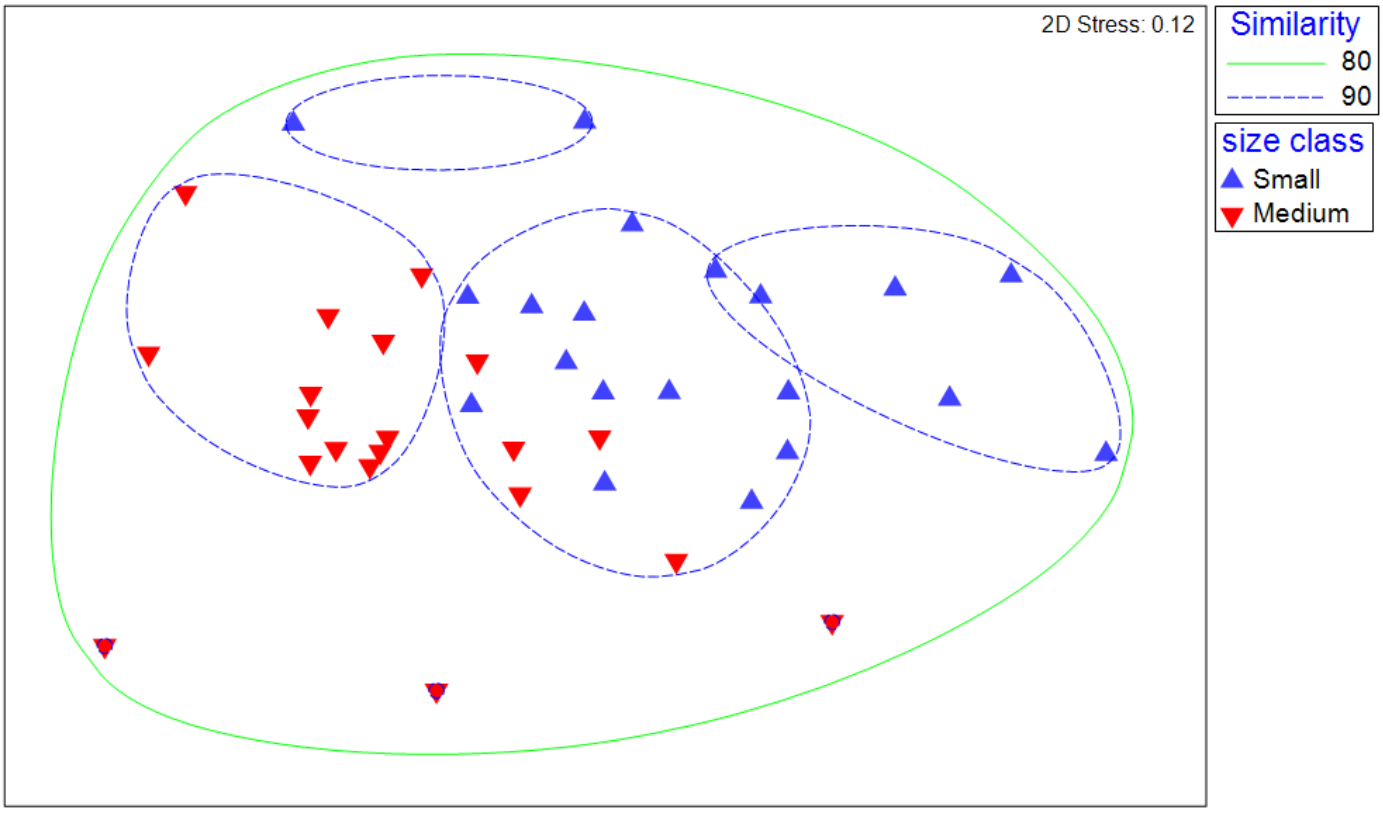

Figure 7. Non-Metric Multidimensional Scaling plot (nMDS) of ontogenetic differences in fatty acid composition of small and medium emerald shiners collected in early season 2015. The ANOSIM R value = 0.361, indicating "separated" groups.

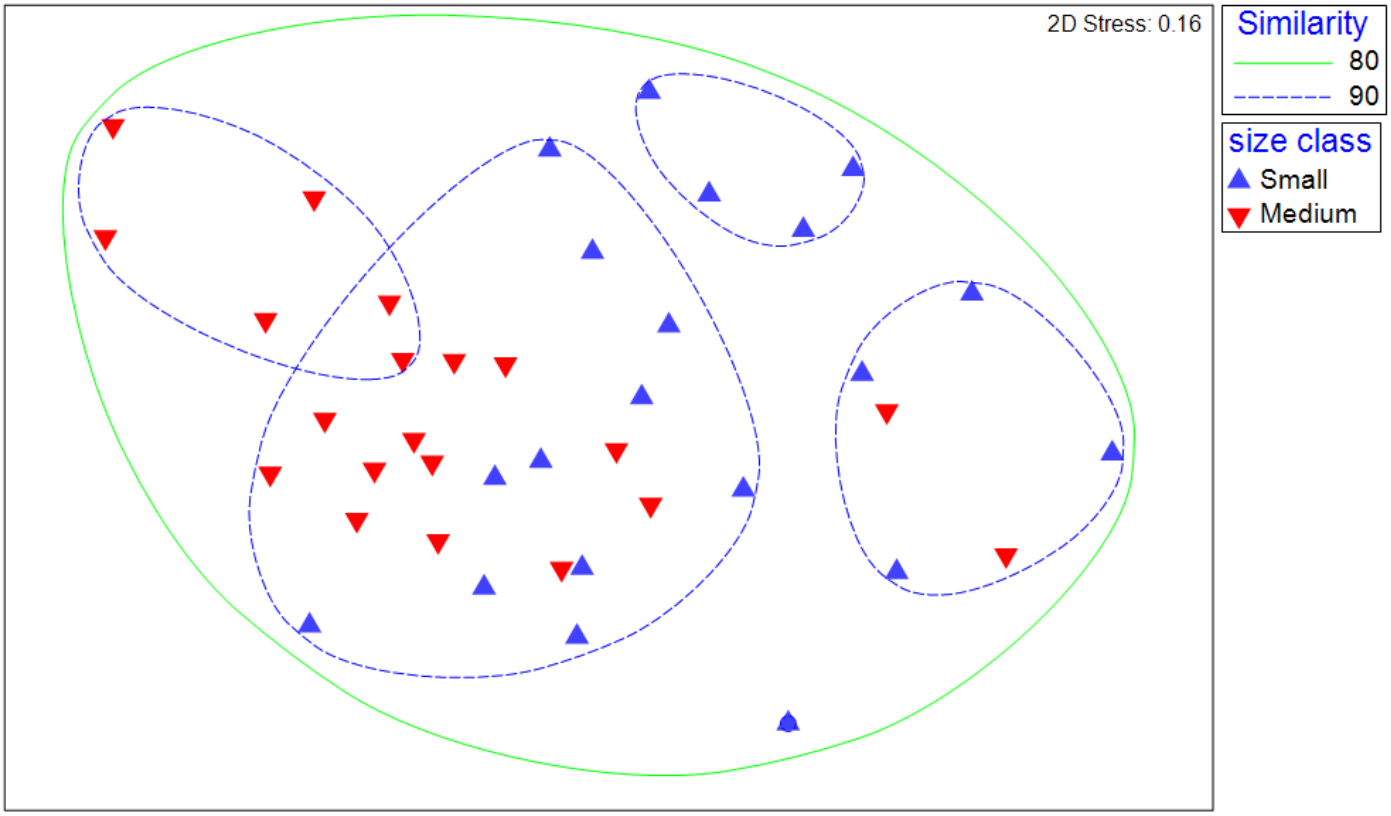

Figure 8. Non-Metric Multidimensional Scaling plot (nMDS) of ontogenetic differences in fatty acid composition of small and medium emerald shiners collected in mid-season 2015. The ANOSIM R value = 0.225, indicating "barely-separated" groups.

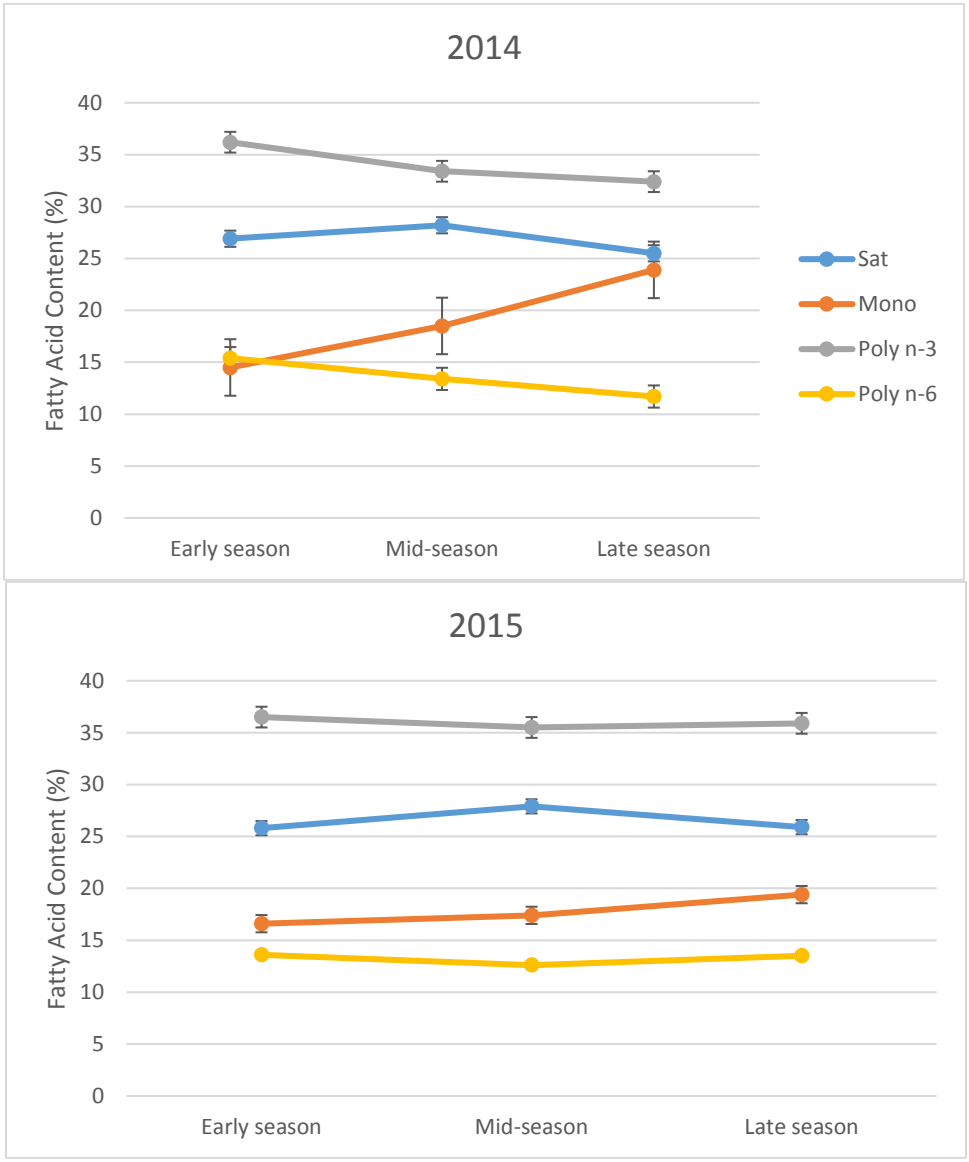

Figure 9. Seasonal changes in fatty acid indices in emerald shiners from 2014 and 2015. Data are presented for shiners in the small size class (50-59 mm) since this size class is represented in all three sampling periods in both years. Sat = saturated fatty acids, Mono = monounsaturated fatty acids, Poly n-3 = polyunsaturated fatty acids in the n-3 series, and Poly n-6 = polyunsaturated fatty acids in the n-6 series.

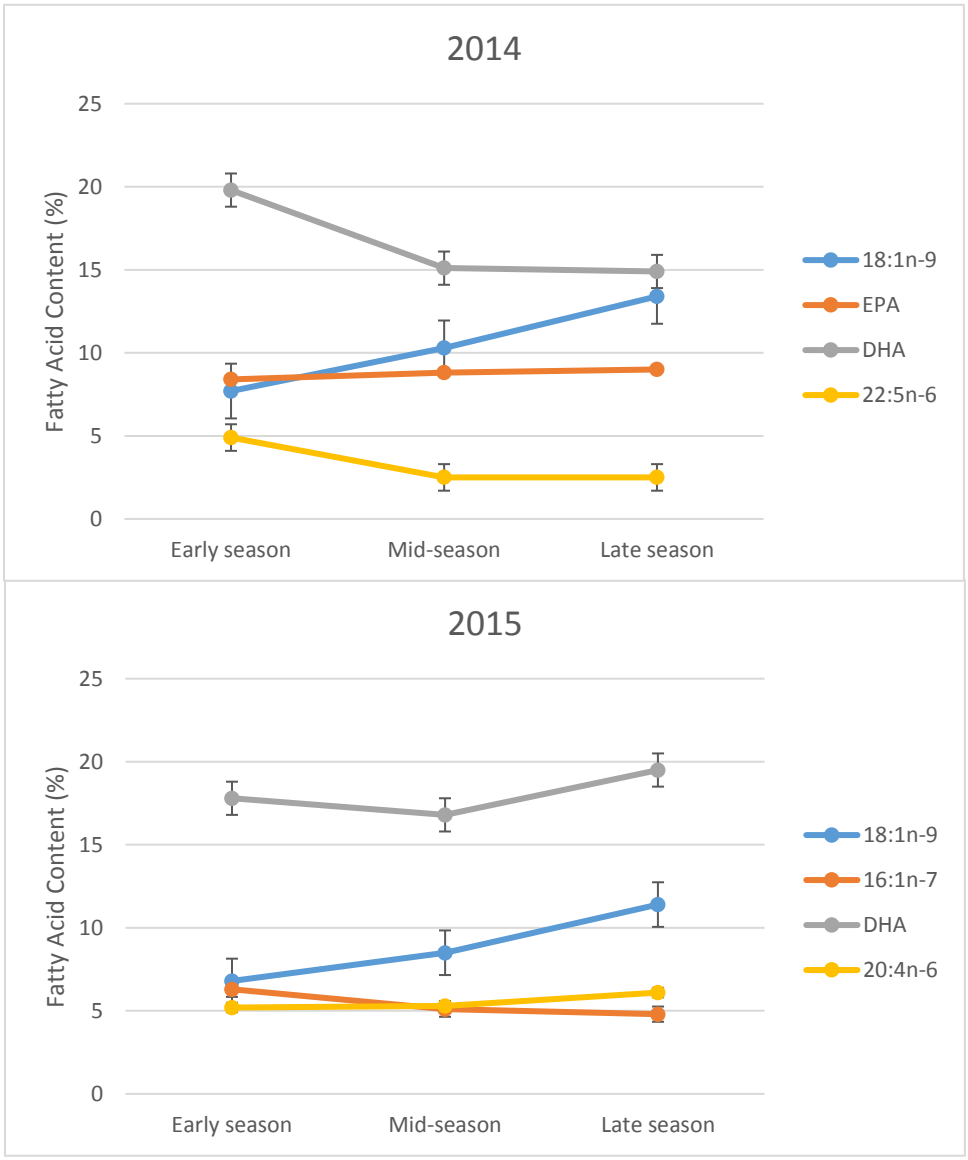

Figure 10. Seasonal changes in key fatty acids in emerald shiners from 2014 and 2015. Data are presented for shiners in the small size class (50-59 mm) since this size class is represented in all three sampling periods in both years. The four fatty acids presented in each year are those identified via the SIMPER procedure in PRIMER as being most responsible for differences among groups in that year (see Table 4).

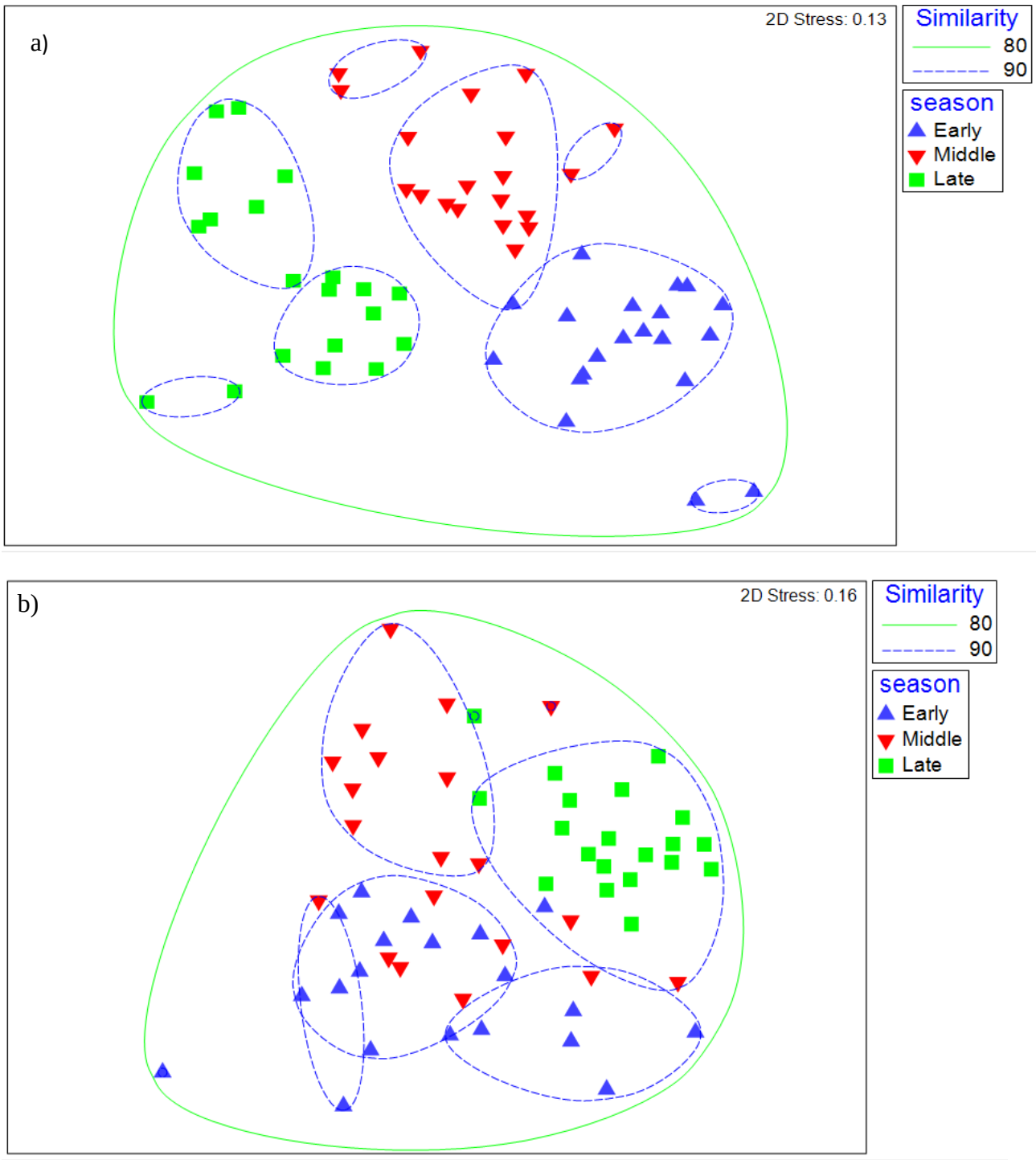

Figure 11. Non-Metric Multidimensional Scaling plots (nMDS) of seasonal differences in fatty acid composition of small emerald shiners collected from the upper Niagara River in 2014 (a) (p=0.01, R= 0.745) and 2015 (b) (p=0.01, R=0.49).

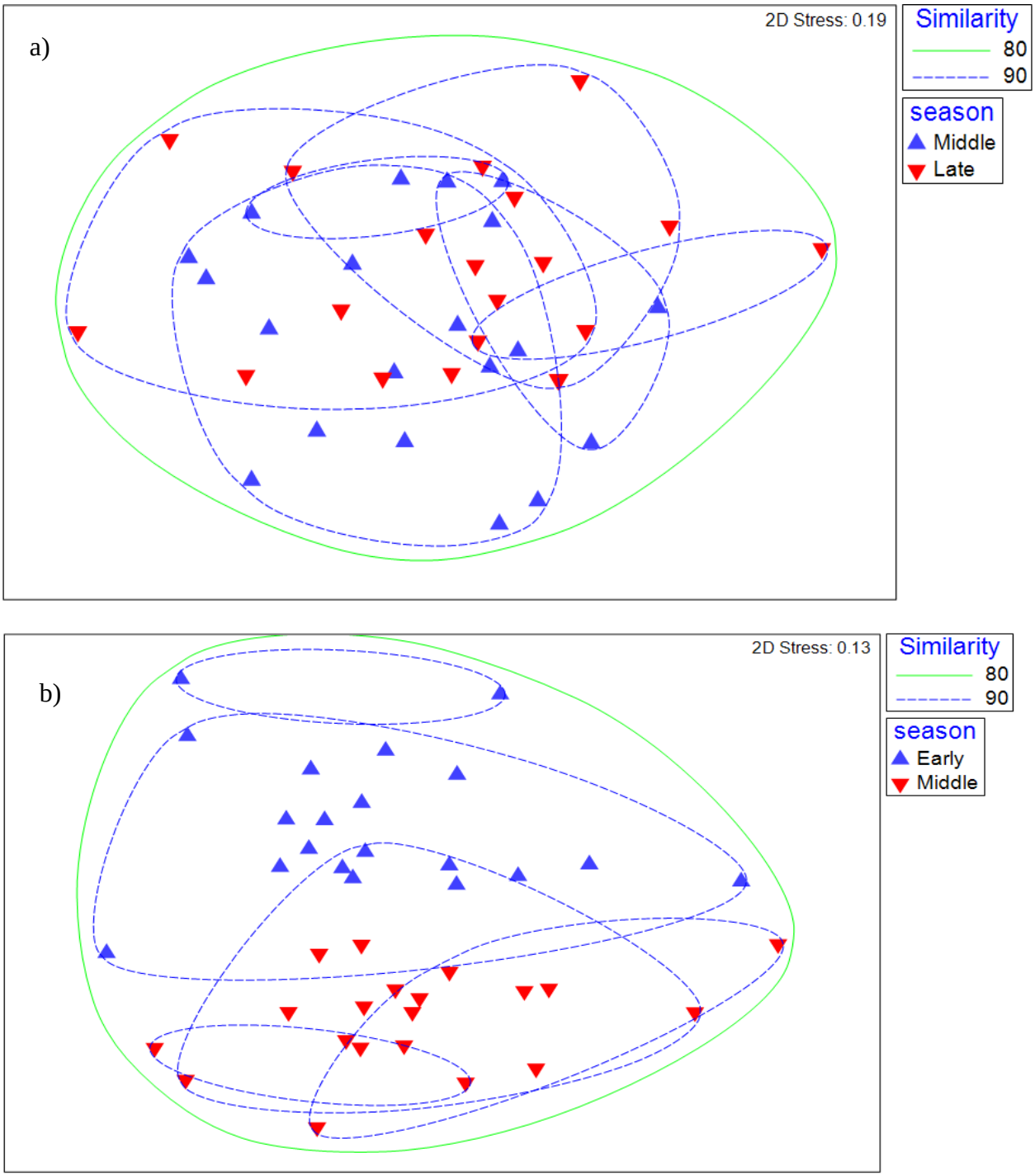

Figure 12. Non-Metric Multidimensional Scaling plots (nMDS) of seasonal differences in fatty acid composition of medium emerald shiners collected from the upper Niagara River in 2014 (a) (p=0.04, R=0.119) and 2015 (b) (p=0.01, R=0.442).

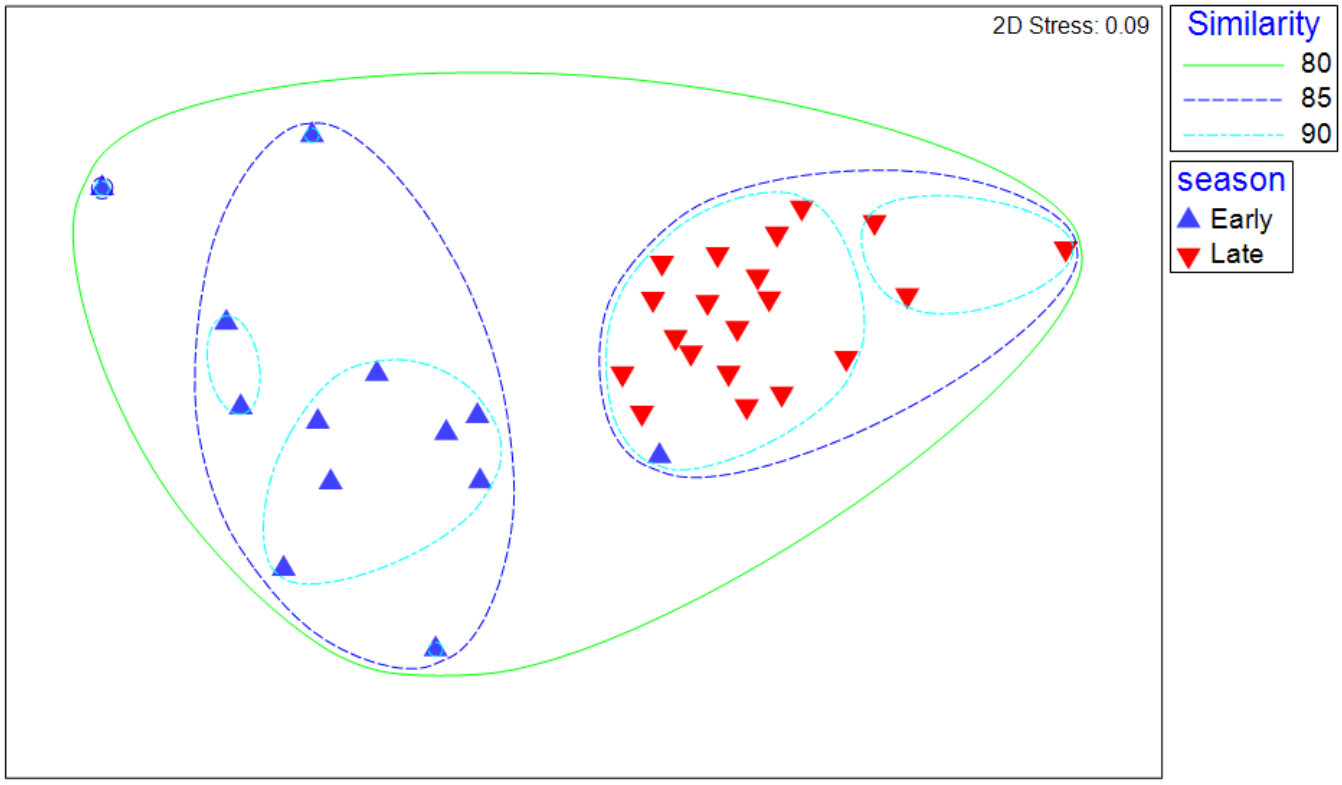

Figure 13. Non-Metric Multidimensional Scaling plot (nMDS) of seasonal differences in fatty acid composition of large emerald shiners collected from the upper Niagara River in 2014 (p=0.01, R=0.807)

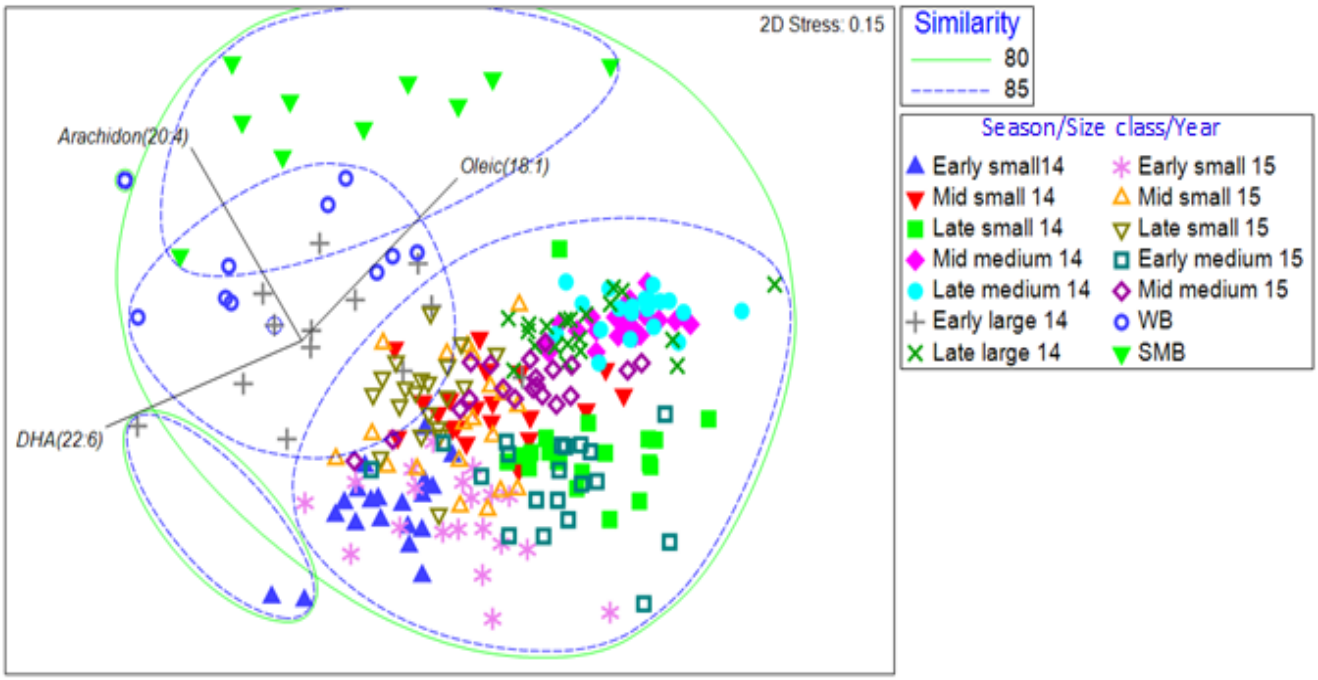

Figure 14. Nonmetric Multidimensional scaling plot of fatty acid signatures of emerald shiners and two top predators (smallmouth bass and white bass) captured in the upper Niagara River. (p=0.01, R=0.719/ 636 for 2015). The vectors were created using an 0.85 pearson correlation value. The fatty acid values that were indicated by the vectors are the ones that are the most related between the sampling points and determined the similarity values within and between groups. The distance of the vectors represent how correlated the values of the fatty acids are between the sampling points, with longer vectors having a higher value and similar fatty acid values between the samples than shorter ones.

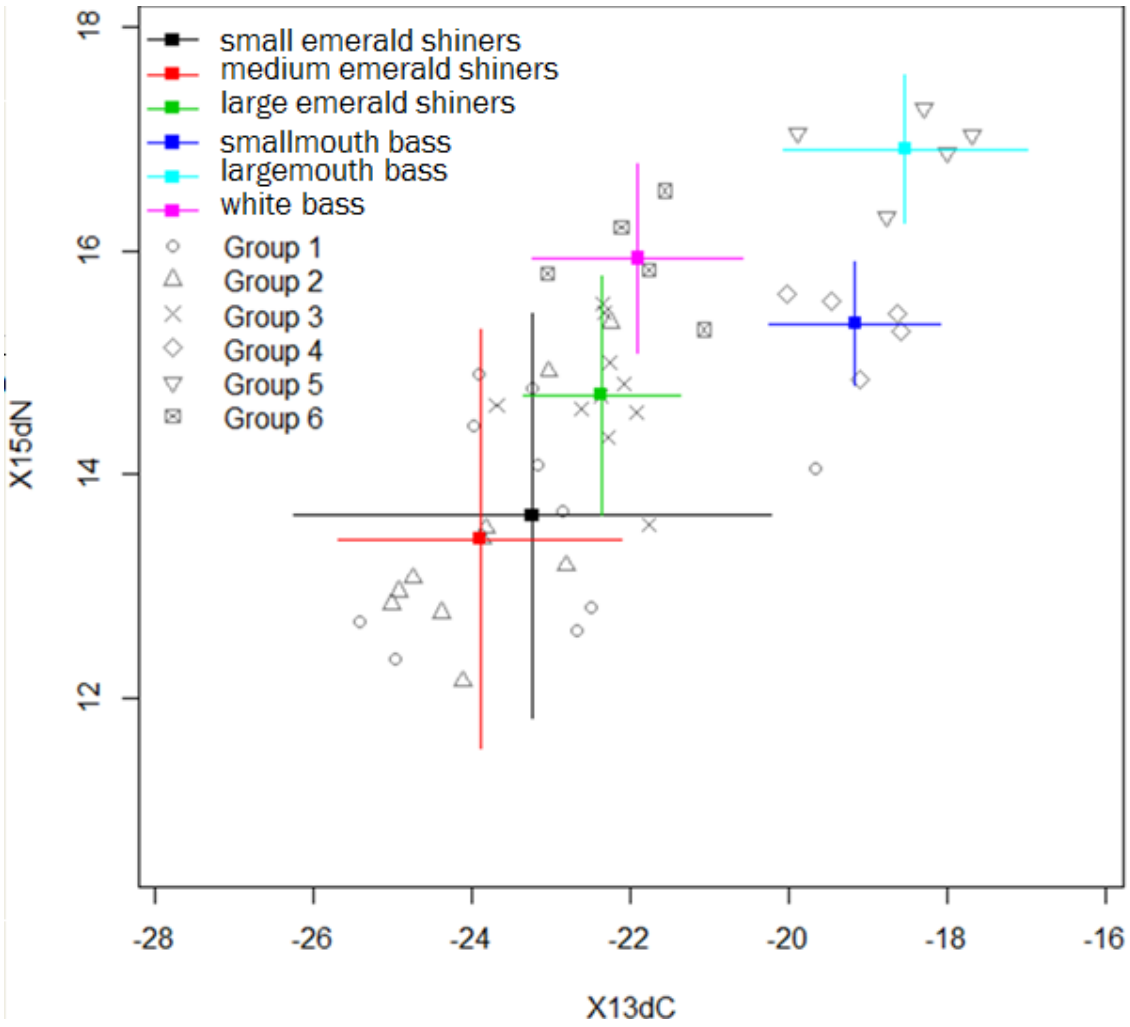

Figure 15. Bi-plot of stable isotope ratio withstandard errors around the mean, depicting size class trends between emerald shiners and potential diet overlap with three top predators.

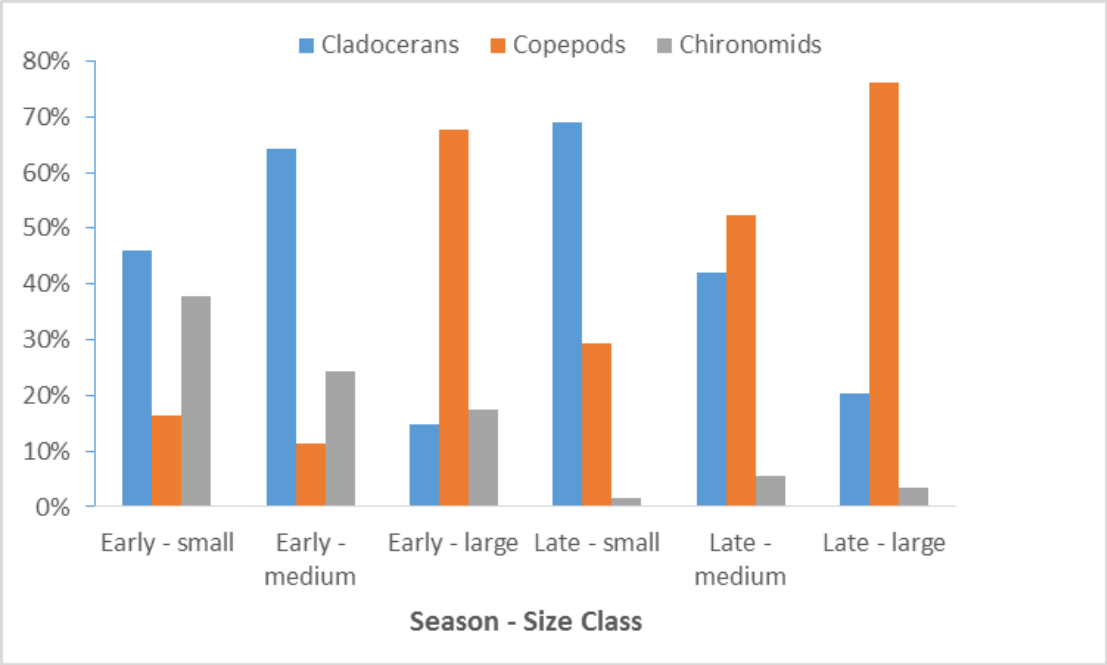

Figure 16. Average percent composition of major prey categories found in the stomach contents of emerald shiners collected in 2014. The total number of prey items in each category listed above were summed for each season – size class, and each category was then expressed as a percentage of the total. Chironomids includes all life history stages (pupae, larvae, and adults). Other prey items found in the stomach contents were not included in this summary because they either occurred in low numbers or it was not possible to infer likely fatty acid compositions (see Table C1 and Figure C1 in the Appendix).

Appendix A: Fatty Acid Analysis

Procedures largely followed the methods provided in Parrish et al. (2014). Direct methylation of emerald shiner tissues was conducted by first removing the digestive tract and taking a muscle tissue subsample of 0.50 ± 0.10 g. The subsample was homogenized in a test tube with 3 mL of a methylating solution containing a 10:1:1 ratio of methanol: dichloromethane: concentrated hydrochloric acid to aid in the breakdown of the fish tissue and the extraction of the fatty acids. Test tubes were capped, vortexed, and placed in a heating block for 2 hours at 80 ^{0}C; after which they were allowed to cool. 1 ml of pure water was then added along with 1.8 ml of a 4:1 hexane: dichloromethane extraction solution. The test tubes were vortexed for 10 seconds and placed in a centrifuge for 5 minutes at moderate speed. After centrifugation, the contents in the tube separated into two layers. The upper layer was the organic layer that contained the fatty acids and the lower layer contained any excess water and chemicals. The upper organic layer was transferred to another vial and blown down under a nitrogen stream until dry. The 1.8 ml extraction process was repeated twice more, blowing down with nitrogen in between to ensure that the greatest amount of fatty acids were collected. Each sample was subsequently ran through a gas chromatograph (GC). Each GC run lasted about 30 min and resulted in a chromatogram that indicated what fatty acids were in each sample and the quantity of each. This process was sped up through the use of an auto-sampler which allowed for samples to be injected automatically. Fatty acids were identified using retention times compared to known standards. A known standard containing the exact same concentration and amount of several common fatty acids was also run through the GC to provide reference peaks and to create a calibration table for the experimental runs.

Appendix B: Detailed Statistical Results

Table B1. SIMPER values for fatty acid composition in emerald shiners collected in the upper Niagara River in 2014 and 2015. These values represent percent similarities for each pairwise comparison represented in the table.

	Early small 2014	Mid small 2014	Late small 2014	Mid Medium 2014	Late medium 2014	Early large 2014	Late large 2014	Early small 2015	Mid small 2015	Late small 2015	Early medium 2015	Mid medium 2015
Early small 2014	91.54											
Mid small 2014	86.70	91.30										
Late small 2014	83.81	87.19	90.02									
Mid Medium 2014	80.42	85.89	86.65	93.23								
Late medium 2014	79.47	85.11	85.99	92.76	93.04							
Early large 2014	84.02	84.17	80.27	80.34	79.08	87.48						
Late large 2014	81.98	87.02	87.72	91.39	90.85	81.81	91.12					
Early small 2015	88.53	86.52	85.12	82.48	81.25	83.35	83.34	90.06				
Mid small 2015	87.57	89.36	85.96	84.66	83.77	85.18	85.71	88.55	89.93			
Late small 2015	88.39	87.75	86.26	84.93	83.88	86.18	86.40	87.35	88.41	92.64		
Early medium 2015	84.90	86.82	87.09	86.00	84.97	81.41	86.19	87.95	87.67	86.22	90.66	
Mid medium 2015	85.01	89.58	86.56	88.50	87.57	84.14	88.67	86.71	89.17	87.68	88.34	90.81

Table B2. Percentages of key fatty acids responsible for differences among groups of emerald shiners identified via the SIMPER procedure in PRIMER.

	16:0	16:1	Oleic acid	18:3	EPA	22:4	22:5n-6	DHA
Early small 14 vs. Mid small 14	7.27	3.85	10.48	7.81	3.5	6.38	9.51	18.12
Early small 14 vs. Late small 14	4.71	10.78	17.47	5.2	N/A	5.57	7.48	15.19
Mid small 14 vs Late small 14	9.68	11.08	12.59	5.9	4.79	7.72	N/A	8.71
Early small 14 vs Mid medium 14	N/A	5.91	18.71	3.96	6.71	N/A	8.97	25.53
Mid small 14 vs Mid medium 14	5.1	5.97	16.86	N/A	8.13	5.99	4.27	18.78
Late small 14 vs Mid medium 14	6.11	4.77	8.86	5.03	7.66	6.65	4.49	19.07
Early small 14 vs Late medium 14	N/A	5.61	19.02	4.47	6.7	3.62	8.77	24.86
Mid small 14 vs Late medium 14	N/A	5.72	17.6	N/A	8.09	5.87	4.34	18.42
Late small 14 vs Late medium 14	6.43	5.13	9.56	4.97	7.73	6.52	4.58	18.92
Mid medium 14 vs Late medium 14	5.82	6.94	10.88	N/A	5.74	10.18	4.14	10.91
Early small 14 vs Early large 14	5.22	N/A	6.19	4.25	4.74	4.84	11.73	9.47
Mid small 14 vs Early large 14	4.88	N/A	6.67	10.64	4.42	5.96	9.21	9.84
Late small 14 vs Early large 14	5.15	9.24	11.71	7.28	N/A	5.22	7.27	7.35
Mid medium 14 vs Early large 14	N/A	6.53	15.94	7.23	N/A	4.11	8.15	19.29

Table B2 Continued.

Late medium 14 vs Early large 14	N/A	6.13	16.13	7.48	N/A	4.02	7.86	18.62
Early small 14 vs Late large 14	N/A	6.02	19.59	4.35	5.12	3.62	8.39	21.47
Mid small 14 vs Late large 14	6.83	6.13	17.45	N/A	6.26	5.98	4.58	13.82
Late small 14 vs Late large 14	6.08	6.17	10.13	5.62	6.16	6.96	4.67	12.68
Mid medium 14 vs Late large 14	5.33	5.83	11.37	4.32	6.57	7.02	5.92	16.51
Late medium 14 vs Late large 14	5.76	6.13	11.45	N/A	6.7	7.22	5.93	15.98
Early large 14 vs Late large 14	4.31	6.7	16.51	7.85	N/A	N/A	8.67	14.69
Early small 15 vs Mid small 15	5.91	10.27	8.12	4.71	5.16	9.37	N/A	13.09
Early small 15 vs Late small 15	4.39	8.14	18.38	N/A	5.02	7.52	5.03	11.30
Mid small 15 vs Late small 15	N/A	5.60	13.25	6.12	4.82	6.66	6.32	15.31
Early small 15 vs Early medium 15	4.59	9.89	12.11	N/A	6.27	8.28	4.96	18.62
Mid small 15 vs Early medium 15	N/A	11.43	7.22	4.51	6.19	6.79	4.48	15.68
Late small 15 vs Early medium 15	N/A	9.97	8.81	3.51	6.04	N/A	6.23	20.51
Mid small 15 vs Mid medium 15	4.00	7.32	11.09	5.40	5.38	7.54	N/A	19.59
Late small 15 vs Mid medium 15	3.35	4.43	9.34	7.80	5.21	4.29	7.18	24.76
Early medium 15 vs Mid medium 15	4.53	10.27	9.42	5.71	5.97	4.43	4.75	15.04

Appendix C: Detailed Stomach Analysis Data

Table C1. Total number of prey items found in the stomach contents of emerald shiners collected in 2014 by sampling periods.

	Bosminidae	Daphnia	Bythotrephes	Cyclopoida	Calanoida	Chironomidae Pupae	Chironomidae Larvae	Chironomidae Adult	Dipteran	Unknown Insect	Eggs	Ant	Chydoridae	Cladocera	Mite	Dreissenid veligers
Early small	29	354	31	3	145	111	48	183	17	3	2978	0	0	2	1	0
Late small	3662	70	53	150	1472	42	3	38	0	156	666	0	33	31	0	3
Early medium	34	269	188	0	110	160	5	69	3	0	1321	2	0	127	0	0
Late medium	1407	115	134	229	1833	89	81	51	1	2239	1778	0	0	0	2	0
Early Large	292	671	35	1903	2666	558	141	485	0	25	37956	0	0	0	0	0
Late large	5	82	15	3	477	0	4	17	0	266	3184	0	0	27	0	0

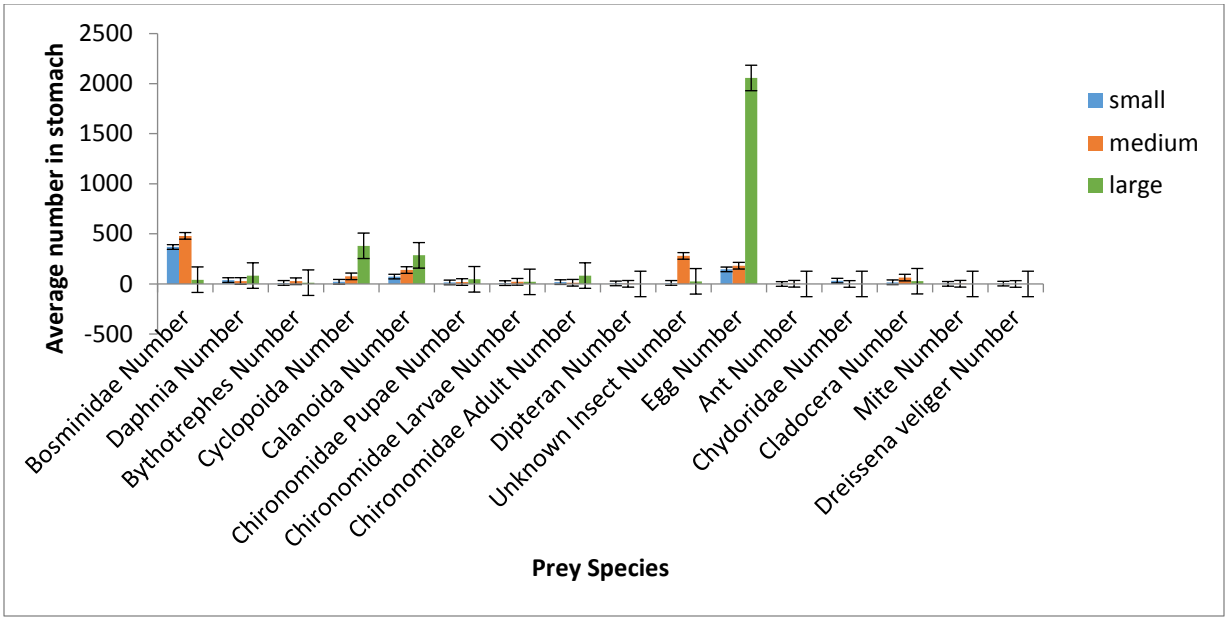

Figure C1. Average number of prey items with standard errors found in the stomachs of the three separate size classes of emerald shiners collected during early and late sampling season 2014.