Abstract

A STUDY OF COMPOSITION, ABUNDANCE, AND FATTY ACID PROFILES OF ZOOPLANKTON IN ALBEMARLE SOUND AND CHOWAN RIVER, NORTH CAROLINA DURING SPRING AND EARLY SUMMER

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In the Albemarle Sound and Chowan River, North Carolina, river herring (alewife and blueback herring) once comprised a commercially important fishery; however, this fishery has since collapsed and a moratorium on river herring harvest is currently in place. River herring stocks have not recovered despite this moratorium. These rivers are critical nursery habitat for larval river herring and one potential reason for the lack of river herring recovery may be related to poor water quality that could affect the zooplankton nutrition and therefore larval river herring nutrition. The goal of this thesis was to examine the species composition, abundance, and nutritional quality of zooplankton, measured using fatty acid profiles, to determine if the zooplankton prey available to larval river herring are of sufficient quality to support the nutritional needs of larval fish.

In the western Albemarle Sound and Chowan River, the zooplankton fatty acid profiles and community structure changed over time and space. In April, the zooplankton composition for 200 µm mesh size was comprised of freshwater species, mainly Cyclopoids and *Bosmina* spp. The most noticeable change in the zooplankton species composition occurred during the month of May when precipitation was very low. This resulted in a salt intrusion that reached

midway up on the Chowan River. The salt intrusion caused a decline in the freshwater species, and an increase in brackish water species in the middle to lower estuary. The upper river sites were dominated by *Leptodora* spp., a freshwater, predatory zooplankter. This was followed by a wet June, which led to an influx of freshwater, returning the salinity to zero. The zooplankton species composition then returned to one dominated by freshwater species as an increase in water flow moved this community down river, resulting in higher overall abundances. The results demonstrated that there are two distinct size classes of prey for larval river herring, as evidenced by the distinct communities represented by the two mesh sizes. The rotifers, a small bodied zooplankton that have high reproductive rates, were abundant in the 60 µm mesh size samples. In contrast, the 200 µm mesh size samples showed variability in the dominant species, suggesting that a wide range of potential prey for larger herring larvae exists.

The May saltwater intrusion also changed the fatty acid profiles of the zooplankton. The amount of DHA in the system increased due to the higher abundance of a dominant brackish water copepod species, *Acartia* spp. Overall, zooplankton fatty acid profiles during the salinity increase in May were higher in PUFAs, DHA and EPA. Salinity played the most important role in structuring the zooplankton community which, in turn, explained the fatty acid profiles seen. This change in the overall fatty acid composition over the spring period suggests that larval river herring may experience a range of prey items that vary considerably in fatty acid composition. Therefore, the fatty acid profiles of the zooplankton prey field likely have considerable influence over the growth and development of larval river herring. At first feeding, larval river herring consume rotifers and smaller bodied cladoceran which have lower PUFAs compared to larger bodied zooplankton. This study suggests that adequate prey abundance and prey types exist for

larval river herring; however, more work is needed to determine the influence of the fatty acid profiles of the zooplankton community on larval herring growth and survival.

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Introduction

Estuaries are dynamic systems that are the connection between the land and oceans. These systems can pose challenges to researchers because of the highly dynamic nature of environmental conditions, habitat gradients, and carbon inputs from both ocean and terrestrial sources (Alfaro et al. 2006). One type of estuary is a lagoon and is characterized by limited connection with ocean, resulting in little to no tidal movement and an increasing impact of wind on water movement. A salinity gradient comprised of freshwater (0 to 0.4), oligohaline (0.5 to 5), and mesohaline (5 to 18) is often present and has a strong effect on species distribution, including distinct zooplankton communities (Day et al. 1989, Johnson and Allen 2012). The salinity transition zone has high turbidity because of the increase in nutrient levels and highest species abundances because of a shift in nutrient availability between fresh and brackish water (Telesh and Khlebovich 2010). These differences can lead to changes in the food web due to shifts in the relative carbon source (allochthonous versus authocthonous) thereby affecting the zooplankton community composition.

In order to understand food webs in estuarine systems, it is necessary to focus on the interaction between the prey and predator, i.e. the pathway of energy transfer (Moderan et al. 2012). Estuaries exhibit high rates of primary production and are characterized by organic detrital sources (Moderan et al. 2012). River flow brings terrestrial inputs of nutrients that are used by the main functional group of autotrophs, the phytoplankton. Phytoplankton growth is limited by light, grazing by secondary consumers, nutrients, and temperature (Day et al. 1989). Microbes are also very important constituents of estuarine food webs because a large amount of

carbon processing is done by bacteria. Smaller protists consume bacteria, recycling nutrients back into the system from decomposing phytoplankton and plant material. Microzooplankton are primary consumers of phytoplankton, and important connection between the autotrophic and higher trophic level organisms.

The Albemarle Sound is considered one of the largest lagoonal systems in North America, and two major tributaries (Roanoke and Chowan River) supply 75% of the freshwater (Gray and Copeland 1989). The water movement in the sound is dominated by wind since there is very little tidal influence as the closest inlet, Oregon Inlet, is some distance away (Figure 1.1). The lack of a nearby inlet restricts the influence of tidal flow and oceanic waters seldom reach the Albemarle Sound. The sound is known for low surface salinity levels (<5), but in recent years higher salinities have been recorded throughout the sound, and into the Chowan River, thought to be caused by sea level rise and intrusion of saline groundwater (Copeland et al. 1985, Leech et al. 2009, Horton et al. 2009, and Sallenger et al. 2012). Salt wedges have been observed that increase the salinity on the bottom to higher levels (>7) (Leech et al. 2009). Human impacts on the Albemarle Sound have increased as more land is used agriculturally. This has led to increase nutrients from farm and livestock production, placing the waters on the impaired water list (NCDENR 2006). The Albemarle Sound has been affected by increased nutrient levels from anthropogenic sources that can lead to increased likelihood of cyanobacteria blooms (Winslow et al. 1985).

Zooplankton, are divided into different categories by size. Microzooplankton (60-200 µm) are the smallest individuals consisting of protists, rotifers, and copepod nauplii. Rotifers are the main consumer of phytoplankton, and are an important connection between autotrophic and higher level heterotrophic food webs (Park and Marshall 2000). Rotifers achieve high

abundances during summer because of their short development period, parthenogenetic reproduction, and the influence of warmer temperatures on growth (Park and Marshall 2000). The main food sources for rotifers and copepod nauplii are small phytoplankton, nanoplankton, and some species also ingest bacteria directly (Park and Marshall 2000). Mesozooplankton (200-500 µm) consist of herbivorous cladocerans and omnivorous Calanoid and Cyclopoid copepods. Macrozooplankton (>500 µm) consist of large body cladocerans (*Leptodora* spp.) that are predatory and consume other cladocerans and copepods (nauplii and adults). During the pre and post phytoplankton bloom period, many zooplankton species also ingest particulate organic matter originating from terrestrial sources (Napolitano et al. 1997).

The freshwater component of estuarine systems may experience cyanobacterial blooms due to human activities that add nutrients to the system (Anderson et al. 2008 and Heisler et al. 2008). The Chowan River and Albemarle Sound experienced cyanobacteria blooms in the 1980s due to increased nutrients (Winslow et al. 1985). Even though no bloom conditions were detected, cyanobacteria constituted greater than 70% of the phytoplankton population in the summer 2012 in the western Albemarle Sound and mouth of the Chowan River (Michelle Moorman, personal communication). One result of the cyanobacteria blooms is the increased production of extracellular polysaccharides and/or cellular toxins that decrease zooplankton grazing and growth (Goleski et al. 2010 and Lehman et al. 2010). In Florida Bay, the microzooplankton grazing rates on the picoplankton were undetectable during cyanobacteria bloom events (Goleski et al. 2010). The survival of two zooplankton species, *Pseudiaptomus forbesii* and *Eurytemora affinis,* was reduced when their diet exceeded 10% *Microcystis* in laboratory feeding studies (Ger et al. 2009). The larger cladoceran species like *Daphnia* spp. are more sensitive to *Microcystis* compared to smaller cladocera (Lehman et al. 2010).

Another factor that can play a role in determining species composition is abiotic factors (e.g., temperature and salinity) in a system thereby affecting the food web at the lower trophic levels. Salinity and water temperature play a role in determining the dominant zooplankton species found in different areas of the shallow brackish lakes in the Limfjord Fiord (Jensen et al. 2010). The lowest species richness of zooplankton in estuarine systems is when salinity ranges from 5 to 7; however the largest bodied cladocerans can only tolerate salinity less than 3.5 before disappearing from the species community (Jensen et al. 2010). Small bodied cladocerans (*Bosmina* spp. and *Ceriodaphnia* spp.) and rotifers are dominant species in estuaries because of a higher tolerance for salinity (Jensen et al. 2010). Cyclopoid and Calanoid species occur in both fresh and brackish water, but the species composition changes depend on the salinity levels. The dominant brackish water Calanoida species is *Acartia* spp. (Johnson and Allen 2012). Copepods can overwinter in systems as juveniles and adults, and during cooler temperatures, the systems are dominated by copepods especially smaller bodied Cyclopoid. Copepod nauplii are also present in higher quantities during time periods of warmer water, especially when a adult copepods are found. Cladoceran species are not able to overwinter, and as temperature increases, dormant eggs begin to hatch (Farkas 1979).

During the spring and summer, estuaries are used by fish as spawning and nursery grounds. Both diadromous fish use estuaries; however, the anadromous fish are most prevalent during the spring and early summer. Zooplankton are considered an important food source for first feeding fish larvae. The zooplankton community present in a system can therefore affect the survival and growth of larval fish directly. The Albemarle Sound and two tributaries (Roanoke and Chowan Rivers) are important nursery habitat for river herring (alewife (*Alosa pseudoharengus*) and blueback herring (*Alosa aestivalis*)) (NCDMF 2007). The larval and

juvenile river herring remain in the tributaries and migrate to the ocean between July and October. River herring are planktivores, and during their time in the tributaries the zooplankton population supports the growing larval and juvenile stages (Mullen et al. 1986, Rulifson et al. 1993, Riley 2012). The river herring begin feeding at 6 mm total length, and their diet consists of small cladoceran species, rotifers and smaller size Cyclopoida and copepod nauplii (Mullen et. al 1986 and Riley 2012). As river herring grow larger, the size of consumed zooplankton increases and consists of larger bodied cladocerans and Calanoids. As alewife grow, their diets move to more benthic invertebrates, but blueback herring appear to keep a filter feeding diet, and continue to consume zooplankton throughout their life (Mullen et al. 1986).

The Albemarle Sound was once home to a very productive river herring fishery (NCDMF 2007) that has since collapsed. In 2007, a moratorium was placed on fishing of river herring because the stocks had drastically decreased (NCDMF 2007). Since the moratorium, the stock of river herring has not recovered and a number of causes have been cited: overfishing, dam construction, declining habitat area, water quality, and lack of sufficient food resources (NCDMF 2007). Over the years, studies have been conducted to determine phytoplankton and zooplankton abundances in the western Albemarle Sound and two main tributaries (Roanoke and Chowan Rivers) (Rulifson et al. 1993, Coggins 2005, Winslow et al. 1985, Binon 2012, Leech et al. 2009). No research before this study has been conducted in the lower Chowan River (Figure 1.2). This study will be summarized below and provide the framework for this chapter.

A study was conducted between 1982-88 on the Roanoke River, the delta and sound to determine phytoplankton and zooplankton levels available for larval finfish. The numbers of phytoplankton were higher in spring compared to late spring and early summer, which was related to zooplankton consumption and growth. The most common densities for zooplankton

were 0.06-0.10 individuals L^{-1}. Rulifson et al. (1993) found that copepods dominated the river, delta and western section of the sound with cladoceran species, *Bosmina* and *Daphnia* more abundant in the river and *Leptodora* more abundant in the sound. The larval and juvenile river herring diets by biomass were *Bosmina* (10%) and other cladocerans (14%), rotifers (9%), and copepodites (2%). The presence of rotifers in river herring diets, but not in the zooplankton samples, was likely a function of sampling net size as rotifers are <100 µm and the zooplankton net used had a mesh size of 250 µm. Coggins (2005) using a 250 µm mesh net found that zooplankton densities in the lower Roanoke River were also very low with the average abundance being 0.0096 zooplankton L^{-1} and the dominant species being *Daphnia*, rotifers, and copepods (Coggins 2005).

In 1982-83, the zooplankton population on the Chowan River was studied to determine the abundance and species composition and whether cyanobacteria blooms had an effect on the zooplankton population. The overall densities of zooplankton were 15 individuals L^{-1}, excluding rotifers, compared to James River, VA were the range was 50 to 200 individuals L^{-1} (Winslow et al. 1985). Rotifer abundances ranged from 30 to 50 individuals L^{-1} in the Chowan River during the late summer and were an important food source for river herring (Winslow et al. 1985). Winslow et al. (1985) showed an increase in abundance of larger zooplankton during cyanobacteria blooms, contrary to what was expected. Winslow et al. (1985) concluded that a possible toxic, growth-suppressing, or other direct effect of the bloom could be having an impact on the river herring because the herring were smaller in size compared to other Atlantic estuaries.

In 2008 and 2009, the zooplankton population was again examined to determine if enough food for larval river herring was present. Binion (2012) used a 90 µm mesh net and collected zooplankton from March until June in the river, delta, and sound. She found

zooplankton abundance was higher in the sound than the river and delta. The cladoceran species were composed of Daphniidae, Bosminiidae, Sididae, Chydoridae, and Leptodoridae. Rotifers dominated all systems in 2009. In 2008, the river was dominated by rotifers in March, May and June, and cladoceran in April. The delta in 2008 was dominated by calanoid copepod in March, copepod nauplii in April and May, and rotifers in April, May and June. The sound was dominated by copepod nauplii in 2008. Binion (2012) found zooplankton abundances in the study to be two to seven times higher than the Rulifson et al. (1993) study even when excluding copepod nauplii and rotifers. Binion (2012) also examined the diet of first feeding river herring and found it consisted of copepod nauplii and Bosminidae in the river, copepod nauplii and rotifers in the delta, and copepod nauplii and cyclopoid copepods in the sound (Riley 2012). The larger river herring (7-12mm total length) diets consisted mainly of copepod nauplii in all locations (Riley 2012). The condition for blueback herring was not different in the three areas, but the condition of alewife was higher in the river than the delta or sound (Riley 2012).

Leech et al. (2009) also conducted a similar study in 2008 and 2009 on the Chowan River to determine abundance of zooplankton. In 2008, zooplankton were collected with a 63 µm mesh net for March and April, but then in June 2008 and through 2009, a bilge pump was used to collect zooplankton by filtering water over a 63 µm mesh filter. The dominant zooplankton in the summer months were rotifers, followed by copepod nauplii, and cladocerans were the rarest. Leech et al. (2009) also investigated the juvenile river herring diets and found that copepods dominated the diet. My study investigated the zooplankton population in the Chowan River and western Albemarle Sound. The locations were chosen to expand understanding of the zooplankton by moving downstream from where most sampling has taken place to include the Albemarle Sound. Downstream locations also represent potentially important nursery habitat for

larval herring and this area has not been sampled for zooplankton abundance and community composition.

Objectives

Objective One: Characterize water quality parameters for April, May and June at multiple locations in the Chowan River and western Albemarle Sound

Objective Two: Characterized zooplankton species composition and abundance for April, May, and June at multiple locations in the Chowan River and western Albemarle Sound

Hypotheses

Hypothesis One: There is no difference for water quality between the four spatial locations (3 river locations and 1 sound location) or three months (April, May and June)

From April to June, water quality parameters (temperature and salinity) were recorded at each site bimonthly. The data were analyzed by multivariate procedures in PRIMER.

Hypothesis Two: There is no difference between the abundance and species composition of zooplankton between four spatial locations (3 river locations and 1 sound location) or three months (April, May, and June)

From March until June, zooplankton samples were collected twice a month by a two minute oblique tow at each site using a 60 and 200 μm mesh zooplankton net. The samples were preserved in 4% sugar formalin, and brought back to the lab for identification and counting. Using PRIMER, the species compositions were compared by multivariate procedures between seasons and locations. Using SAS, the abundance was compared by location using a repeated measure ANOVA.

Methods

Study Site

Albemarle Sound is part of the Albemarle-Pamlico Estuarine System (APES) and is bordered by the Outer Banks, which is the barrier to the Atlantic Ocean (Figure 1.1). The only saltwater connection is Oregon Inlet (Figure 1.1). The system is a lagoonal estuary, with only one inlet and high volume of freshwater input, thus Albemarle Sound has salinity levels <5 (Copeland et al. 1983). The two main tributaries that empty into the Albemarle Sound are the Roanoke and Chowan Rivers (Figure 1.1). The Chowan River originates in the Virginia coastal plain and is the 12th largest river basin in North Carolina (NCDENR 2006). Overall water quality is poor with low dissolved oxygen levels (<3.0mg L^{-1}) with the first large scale algae bloom in 1972 classifying the Chowan River as "nutrient sensitive waters" in 1979 (NCDENR 2006). The Chowan River is considered a critical habitat for larval and juvenile river herring, and zooplankton research has been conducted on both of these rivers (NCDMF 2007). The sites on the river will allow for comparison from up river to the mouth (Figure 1.2). The sound was chosen to compare to the three locations on the Chowan River (Figure 1.2). These results were used to determine if the zooplankton communities in these areas were suitable for river herring growth and development.

Field Work

Zooplankton samples were collected from 1 April 2013 to 25 June 2013 at ten locations in the western Albemarle Sound and Chowan River (Figure 1.2), twice per month, excluding April where a third sampling period occurred. The second sampling period in June sampled the river locations, but did not include the western Albemarle Sound because of a strong wind event. The water depths ranged from 3.84 meters to 7.56 meters. The sampling took place on the south

of Holiday Island on a 34 kilometer transect of Chowan River with each site being 3.22 kilometers apart. A zooplankton net with a 50 cm mouth, mesh and cod end of 200 or 60 µm, with a weight was towed obliquely through the water for one minute with an exception in April at the third sampling period were the nets were towed vertically through the water column to reduce clogging from a green algae bloom. The horizontal sampling depth consisted of 1 meter above the bottom, and the vertical tow had the net lowered to 0.5 meters above the bottom. During the oblique tows, a flowmeter was attached to the net mouth to later determine water volume. The timing of zooplankton sampling corresponded to the critical feeding period for larval river herring (Winslow et al. 1985, Leech et al. 2009, Binion 2011). Each sample was filtered through a 200 or 60 µm filter, and zooplankton were preserved in 120 ml glass jar with 10 ml of 10% buffered formaldehyde with sucrose and filtered laboratory water. The addition of sucrose to the formalin helps to reduce ballooning of cladoceran bodies and inflation of their carapace (Haney and Hall 1973). The 60µm sample had a half a tablet of Alka Seltzer added to keep rotifers from pulling in critical body parts (legs and arms) that ease identification (Chick et al. 2010).

Lab Processing

In the laboratory, the samples were filtered through a sieve (200 or 60 µm) to remove the sugar formalin solution. Each sample was diluted to a known volume of water (100 to 1400 ml) and a subsample was taken with a 2 or 5 ml Hensen-Stempel pipette for a total of three subsamples. The organisms were identified on an Olympus SZX10 dissecting scope and 1810 Ward counting wheel. The zooplankton were identified to genus except for the freshwater copepods that were identified to order. Copepod nauplii were grouped together because identification can be difficult at this stage (Johnson and Allen 2012). If a species in a subsample

comprised greater than 500, then that species was not counted for the other two subsamples. The goal was to count 100 individuals of zooplankton per sample. Overall abundances per species was determined using the equation A = # of individuals in the subsample (Total Water volume/ subsample volume).

Temperature and salinity were measured with a YSI Castaway Handheld CTD at the surface of each site and sampling period until end of May. During the second May sampling period, a top to bottom profile for salinity and temperature was also taken to determine if a salt wedge had occurred and if so, at what depth.

Statistical Analysis

Multivariate statistics were conducted in PRIMER 6 and PERMANOVA + (Clarke and Gorley 2006). The zooplankton percent composition data were transformed with a square root and a Bray-Curtis Similarity matrix was constructed. The square root was used to reduce the influence of the more dominate species throughout the months. The species percent composition data by month, site, and zooplankton net mesh size were plotted using nonparametric multidimensional scaling (n-MDS) to determine visual similarities in the data and a hierarchical cluster analysis was conducted to determine the level of similarity. The cluster analysis included the SIMPROF to determine the clusters that are significant at a 0.05 Clarke and Gorley 2006). The cluster analysis was used to determine the similarities among groups compared to between groups (Clarke and Gorley 2006). The group average was used to determine nodes (Clarke and Gorley 2006).

A PERMANOVA test was used to determine significance differences by month and location. Pairwise comparisons were used for post-hoc tests. Models were considered significant at $\alpha = 0.05$ level. The mesh size was analyzed because other studies (see

introduction) used different mesh sizes to collect zooplankton and this likely resulted in different dominant species determinations. The collection of both sizes allows the impact of mesh size on species composition to be determined. The locations were chosen to provide comparisons of percent species composition across different gradients in the river and sound. Month was used to determine how zooplankton species composition differs over time during the critical nursery period of larval river herring.

To analyze the average abundance of zooplankton over time, a Repeated Measure Analysis of Variance (ANOVA) was run in SAS 9.2 to determine a significant difference among the three months at each location combining mesh sizes. Models were considered significant at $\alpha = 0.05$. Repeated measured analysis of variance was used because sampling was repeated at the same location for each month.

Results

Zooplankton species composition and abundance comparisons for the entire sampling period

The zooplankton species compositions changed over month and location. There was a significant interaction between the month and location for the zooplankton composition (200 µm) (Table 1.1). In the sound and upper river, the zooplankton composition (200 µm) was different for April compared to May, April compared to June, and May compared to June (Table 1.2). In the lower and middle river, the zooplankton composition (200 µm) differed for April compared to May and April compared to June (Table 1.2). In April, the zooplankton composition (200 µm) differed between the sound and lower, middle and upper river (Table 1.3). The zooplankton composition (200 µm) in April was different between the upper river and lower and middle river (Table 1.3). In May, the size zooplankton composition (200 µm) differed

between the sound and the middle and upper river (Table 1.3). Between the lower and upper river in May, the zooplankton composition (200 µm) differed (Table 1.3). In June, the zooplankton composition (200 µm) differed between the sound and middle and upper river (Table 1.3).

There was a significant interaction between month and location for the zooplankton composition (60 µm) (Table 1.4). In the sound, the zooplankton composition (60 µm) was different when comparing April and May, and April and June (Table 1.5). In the middle and upper river, the zooplankton composition (60 µm) differed between April and May (Table 1.5). In April, there were no differences for the zooplankton composition at 60 µm mesh size for all locations (Table 1.6). In May, the zooplankton composition (60 µm) differed between the sound and middle and upper river, as well as between the lower river and middle and upper river (Table 1.6). In June, the zooplankton composition (60 µm) differed between the sound and middle and upper river (Table 1.6). The average abundances did not differ for the interaction of location and month, month or location (Table 1.7).

Monthly comparisons of water quality, zooplankton community composition, and abundance

April

Water Quality

The mean water temperature ranges from 10.2-11.4°C during the first sampling period for all locations (Table 1.8). Over the next two sampling periods the mean water temperature increased with ranges from 14-18.4°C with a similar temperatures measured at all locations (Table 1.8). Mean salinity was less than 0.05 for April except on the second sampling period with the sound having a higher mean level of 0.14 (Table 1.8).

Zooplankton Abundance and Composition

The average abundance for the zooplankton (200 µm) from the sound was 1.54 to 22.97 individuals L^{-1} (Figure 1.3, Appendix Table 1.1). By the third sampling period (April 26), there was an increase in zooplankton abundance at three locations (Figure 1.3, Appendix Table 1.1). The zooplankton (60 µm) in the sound ranged from 2.98 to 67.00 individuals L^{-1} (Figure 1.4, Appendix Table 1.2). The lower river had a spike in zooplankton (60 µm) during the third sampling period with an abundance of 101.52 individuals L^{-1} and the middle river experienced similar peaks in zooplankton abundance (60 µm) individuals during the second and third sampling period (Figure 1.4, Appendix Table 1.2). The upper river had the lowest average abundances at 1.20 to 44.62 individuals L^{-1} (Figure 1.4, Appendix Table 1.2).

The similarities between the 4 locations are portrayed in an n-MDS plot with cluster analysis similarity contour overlays for the zooplankton (200 µm) and zooplankton (60 µm) samples for species percent composition. The similarity between the zooplankton (200 µm) percent composition for all locations except the sound for sampling period two and three was at 60% (Figure 1.5). The zooplankton (200 µm) samples from the sound during sampling periods two and three were clustered together with the zooplankton (60 µm) samples because the dominant species were rotifers and copepod nauplii (Figure 1.5). All zooplankton samples (60 µm) had a similarity of 60% for species composition (Figure 1.5).

The sound had a large green algae bloom from the beginning of April, which had moved into the lower river by the third sampling period. The zooplankton composition (200 µm) in the sound was dominated by Calanoida during the first sampling period, and then was replaced with rotifers and copepod nauplii with the increase in green algae (Figure 1.6). The zooplankton (200

μm) in the lower and middle river were dominated by Cyclopoida and *Bosmina* with a decrease in the percent of Calanoida (Figure 1.6). The lower river zooplankton (200 μm) had an increase in rotifers once the green algae bloom moved up river (Figure 1.6). The upper river started with Chydoridae being the dominant present zooplankton (200 μm), and then had a switch to being similar to the rest of the river with Cyclopoida, Calanoida, and *Bosmina* spp. (Figure 1.6). Zooplankton (60 μm) at all locations were dominated by rotifers (> 70%) over two sampling periods (Figure 1.7). Copepod nauplii increased in percent composition during the third sampling period for zooplankton (60 μm) at all locations (Figure 1.7).

May

Water Quality

The two sampling periods occurred from the middle to end of May. The temperature increased from April, but was steady between to two sampling periods at all locations with a range of 22.6 to 25.0°C (Table 1.9). The salinity in the sound started to increase following the first sampling period (Table 1.9). During the second sampling period, the salinity in the sound increased to an average of 2.59 with the bottom 3 meters having levels up to 7 (Table 1.9). The brackish water moved into the lower river during the second sampling period with a level of 1.07 (Table 1.9). The middle river had had low levels of salinity (0.26, upper range), and the upper river had zero over all sampling periods (Table 1.9).

Zooplankton Abundance and Composition

The overall zooplankton abundance (200 μm) for the sound and lower river was 0.22 to 2.06 individuals L^{-1}, and was higher than the abundances found in the middle and upper river over the two sampling periods (Figure 1.3, Appendix Table 1.1). The zooplankton abundance

(200 µm) ranged 0.05 to 0.23 individuals L^{-1} in the middle and upper river (Figure 1.3, Appendix Table 1.1). The zooplankton (60 µm) had average abundances ranging 7.48 to 67.57 individuals L^{-1} during the first sampling period, with the peak at the sound site of 67.57 individuals L^{-1} (Figure 1.4, Appendix Table 1.2). The zooplankton (60 µm) from the second sampling period abundance ranged from 3.64 to 16.60 individuals L^{-1} with the peak in the sound with an abundance of 16.60 individual L^{-1} (Figure 1.4, Appendix Table 1.2). The lowest abundances for zooplankton (60 µm) occurred in the upper river sites, with an abundance of 3.64 individuals L^{-1} (Figure 1.4, Appendix Table 1.2).

The similarities between the 4 locations are displayed in n-MDS plot with similarity contour overlays for the zooplankton (200 µm) and zooplankton (60 µm) samples for species percent composition. There were three distinct groupings of zooplankton percent composition for zooplankton (200 µm) and zooplankton (60 µm). The zooplankton (60 µm) from both sampling periods cluster together with a 50% similarity (Figure 1.8). The zooplankton (200 µm) from the sound, lower river, and middle river (only the second sampling) clustered together with a 50% similarity (Figure 1.8). The middle (only first sampling) and upper rivers sites clustered together when lower to zero salinity was found at the 50% similarity (Figure 1.8).

The zooplankton (200 µm) in the sound and lower river were dominated by *Acartia* spp. (>75% of the sample), a common brackish water species (Figure 1.9). There was a decrease in the presence of *Bosmina* spp. from the first sampling period to the second in the lower river. The middle river was dominated by *Bosmina* spp. (55% of the composition) near zero salinity levels, but once the brackish water moved up river the presence of *Acartia* spp. was found to comprise of 70% of the composition (Figure 1.9). The upper river remained at 0.0 salinity levels, and the zooplankton (200 µm) had *Ergasilus caeruleus*, a parasitic copepod, and *Bosmina* spp. as the

dominate species for the first sampling period, but then an increase in Calanoida during the second sampling period (Figure 1.). The zooplankton (60 µm) in the sound and river were dominated by rotifers and copepod nauplii (>80%) (Figure1.10). The middle and upper river zooplankton (60 µm) had a decrease in copepod nauplii, but were still dominated by rotifers (Figure 1.10).

June

Water Quality

The water temperatures were similar (26°C) at all four locations for both sampling periods (Table 1.10). The water experienced some mixing prior to the first sampling as a result of Tropical Storm Andrea. This storm decreased the salinity in the sound to 1.85, but increased the salinity at the lower river to 1.12, and at the middle river sites to 0.55 (Table 1.10). The upper river sites remained at near zero salinity (Table 1.10). From the first to second sampling period, heavy rainfall continuously fell over North Carolina. There was a record rainfall with 15.2 to 19.05 cm in June making it the second wettest June since 1895 (Hiatt 2013). The salinity returned to zero at all locations on the river with a range of 0.05 to 0.08 (Table 1.10).

Zooplankton Abundance and Composition

Zooplankton abundance (200 µm) was lowest for all months at <0.43 individuals L^{-1} in the sound (Figure 1.3, Appendix Table 1.1). The average abundance of zooplankton (200 µm) was steady with a range of 0.12 to 0.15 individuals L^{-1} at the river locations during the first sampling period (Figure 1.3, Appendix Table 1.1). Those abundances were similar to the first sampling period in April. For the second sampling period, the lower river zooplankton abundance (200 µm) was 0.62 individuals L^{-1} (Figure 1.3, Appendix Table 1.1). The middle

river zooplankton abundance (200 µm) was 0.40 individual L^{-1}, and the upper river zooplankton abundance (200 µm) was 0.09 individuals L^{-1} (Figure 1.3, Appendix Table 1.1). Zooplankton (200 µm) abundance from the first to second sampling period for the lower and middle river increased, but abundance remained similar at the upper river. For the zooplankton (60 µm) from the first sampling period, the upper river had the lowest overall abundances with 4.66 individuals L^{-1}, and the highest overall abundances being at the middle river sites with 10.52 individuals L^{-1} (Figure 1.4, Appendix Table 1.2). For the second sampling period, the highest overall abundance of zooplankton (60 µm) was at the lower river with 42.96 individuals L^{-1}, and the lowest overall abundance at the upper river with 4.93 individuals L^{-1} (Figure 1.4, Appendix Table 1.2).

The similarities between the 4 locations are displayed in n-MDS plot with similarity contour overlays for the zooplankton (200 µm) and zooplankton (60 µm) samples for species percent composition. The zooplankton (200 µm) composition had a separation between the first and second sampling period for all locations, except the upper river, because of salinity (Figure 1.11). The three separate groups had similarities of 50% for the percent composition of zooplankton (Figure 1.11). The zooplankton (60 µm) clustered together at the 50% similarity for both sampling periods (Figure 1.11).

The zooplankton (200 µm) during the first sampling period were dominated by *Acartia* spp. in the sound, and lower and middle river sites (Figure 1.12). The middle river zooplankton (200 µm) for the first sampling period had *Bosmina* spp. present in higher proportions compared to the rest of the locations (Figure 1.12). For the upper river, the zooplankton (200 µm) were dominated by the predator zooplankton *Leptodora* spp. (Figure 1.12). After heavy rain over next two weeks, the zooplankton (200 µm) composition drastically changed with an influx of

freshwater. All locations had an increase in larger body rotifers in the zooplankton (200 µm) (Figure 1.12). The lower river zooplankton (200 µm) was composed of *Leptodora* spp., Calanoida, and *Diaphanosoma* spp. (Figure 1.12). The zooplankton (200 µm) at the middle river sites were dominated by *Leptodora* spp., and an increase in smaller body cladoceran species (*Bosmina* spp. and *Diaphanosoma* spp.) was observed (Figure 1.12). The upper river was dominated *Bosmina* spp. and *Bosminopsis* spp. (Figure 1.12). The zooplankton (60 µm) were dominated by rotifers and copepod nauplii at all locations (Figure 1.13). The copepod nauplii increased in percent at all river locations during the second sampling period (Figure 1.13).

Discussion

The zooplankton community composition showed changes over time and space. The most noticeable change in the zooplankton species composition occurred during the month of May when precipitation was very low. This resulted in a salt intrusion that reached the middle of the Chowan River (Table 1.9). The salt intrusion was associated with decline in the presence of freshwater species, but brackish water species became prevalent in the middle to lower estuary (Figure 1.6 and 1.9). This was followed by a wet June, which led to an influx of freshwater, returning the salinity to zero. The zooplankton species composition then returned to one dominated by freshwater species as an increase in water flow moved this community down river, resulting in higher overall abundances (Figure 1.12). Therefore, I suggest that salinity played the most important role in structuring the zooplankton community. In addition, the two mesh sizes effectively sampled two zooplankton communities. My results demonstrate that there are two distinct size classes of prey for larval river herring, as evidence by the distinct communities represent by the two mesh sizes. The rotifers, a small bodied zooplankton that have high reproductive rates, were highly abundant in the 60 µm mesh size samples. In contrast, the 200

μm mesh size samples showed variability in the dominate species, suggesting that a wider range of potential prey for larger herring larvae.

The transitional areas and gradients seen in estuaries are driven by wind movement and freshwater input which affect salinity, and are difficult to define. Copeland et al. (1983) and Pearsall et al. (2005) found that due to limited saltwater intrusion and high freshwater input, the salinity was <5 in the Albemarle Sound because the only connection to the Atlantic Ocean is at Oregon Inlet. The Albemarle Sound is a shallow body of water with depths <5 meters, and strong mixing that is primarily wind driven (Copeland et al. 1983). In the western part of the Albemarle Sound and the stretch of the Chowan River studied, the water depth was >5 meters, and even with stronger wind, the system was stratified during the salinity intrusion. In May and early June 2013, the salinity in the bottom 3 meters had increased to 7 in the sound, and lower to middle Chowan River had salinities of 1 – 3. Leech et al. (2009) found salinities of 2 – 7 in the Chowan River in October to December 2008. The increase in salinity over the sampling period and the stratification of the system were cause by reduced precipitation and southerly winds. By the end of May 2013, the northeastern part of North Carolina was abnormally dry (Hiatt 2013). The data showed that systems may change because of drought conditions and a reduction in freshwater input from the rivers (Flemer and Champ 2005). In June 2013, a tropical storm brought heavy rains for a two week period and the Chowan River and Albemarle Sound returned to salinities of 0.05 – 1. North Carolina saw the second wettest June since 1895 with a rain fall of 15.2 to 19.05 cm (Hiatt 2013). This dramatic shift in precipitation during the sampling period was reflected in the zooplankton community, indicating the strong influence salinity has on community composition.

Another factor that can affect the zooplankton composition is the mesh size of the sampling nets. Over the years, researchers with the Albemarle Sound region have used different mesh sizes to collect zooplankton, and determine abundance and species composition (Binon 2011, Leech et al. 2009, Rulfison et al. 1993, and Winslow et al. 1985). My research used 60 and 200 µm mesh size to compare species composition and abundances for the Chowan River and Albemarle Sound. Binon (2011) reported that river herring at 6 mm notochord length had a maximum gape width of 400 µm, and estimated maximum prey size of 200 µm. The 60 µm mesh size was important to determine the food resources available for river herring 6 mm and less which consisted of Bosminidae, rotifers, and copepod nauplii (Binon 2011). For American shad at 9 mm notochord length, the mouth gape was 420 µm for an estimate of prey size of 410 µm (Binon 2011). The 200 µm mesh size net would collect prey within this size range and the community consisted of Calanoida and Cyclopoida copepods, and larger body cladoceran (e.g. Daphniidae) (Binon 2011). For the Chowan River and Albemarle Sound in 2013, zooplankton were dominated by high overall abundances of zooplankton (60 µm) from 1.20 to 101.52 individuals L^{-1}. Zooplankton abundances (60 µm) from 2013 upper river sites by Holiday Island were similar to abundances from the early 1980s, 2008 and 2009 during late spring and early summer when collected with a 60 to 64 µm mesh size (Winslow et al. 1985, Leech et al. 2009). Even the 64 µm mesh size does not collect all rotifers, and averages abundances would be higher with smaller mesh size (Chick et al. 2010). In the Roanoke River, Binon (2011) used a 90 µm mesh size net compared to the 250 µm mesh size net used in the 1980s study (Rulfison et al. 1993). The overall abundances from the 250 µm mesh size net were similar to the overall abundances found in 200 µm mesh size from the Chowan River and Albemarle Sound in 2013 except for April when the Albemarle Sound samples had abundances affected by a large, green

algae bloom. The algae bloom clogged the net, but among the algae, microzooplankton were present. The abundances from the 60 µm mesh size in the Chowan River and Albemarle Sound in 2013 were higher (9.97 to 28.87 individuals L^{-1}) compared to those determined by Binon (2009), 4.65 to 16.55 individuals L^{-1} in the Roanoke River. For investigating species composition in the tributaries and sounds, a 60 µm mesh size or smaller should be used to allow for a representative composition of zooplankton species to be collected when determining the food resources for river herring of 6 mm or less. The 200 µm mesh size net allows for the collection of larger zooplankton, but does underestimate or exclude the smaller zooplankton community. The main drawback from the 60 µm mesh size is it easily clogs from algae blooms and suspended sediment and can underestimate the population, but the 200 µm mesh size does not represent the full zooplankton community in the systems.

Microzooplankton, in general, are an important connection between the phytoplankton and secondary consumers and larval fish (Park and Marshall 2000). Microzooplankton abundances were lowest in early spring when water temperatures were close to 11°C and later in the season (early summer) when overall zooplankton abundances decreased. The microzooplankton species composition was dominated by rotifers and to a lesser extent, copepod nauplii. Rotifers are an important component to aquatic environments due to their short development period, and parthenogenetic reproduction (Herzig 1983). Rotifers can populate vacant niches and produce 30% of the total plankton biomass, and convert primary production to a usable source for secondary consumers (Park and Marshall 2000). Rotifers are a major grazer of algae and small ciliates (Haven 1991, Arndt 1993, and Gilbert and Jack 1993). Larger mesozooplankton and larval fish prey upon rotifers (Burbidge 1974,Crecco and Blake 1983, Willianson 1983, Mullen et al. 1986, Dolan and Gallegos 1992, Rulfison et al. 1993, Gabe 1996,

Conde-Porcuna and Declerck 1998, Binon 2011, and Riley 2012). Rotifer abundances from 2013 upper river sites by Holiday Island, NC were similar to abundances from the early 1980s, 2008 and 2009 (Winslow et al. 1985, Leech et al. 2009). The studies from the 1980s, 2008 and 2009 did not collect zooplankton below the Holiday Island site or in the Albemarle Sound. Binion et al. (2011) reported that rotifers and copepod nauplii were dominant in the Albemarle Sound, and Roanoke River for 2008 and 2009. The same was found for the Rhode River, Maryland where rotifers exceeded the copepods in abundance (Allan et al. 1976, Dolan and Gallegos 1992). The microzooplankton were dominated by rotifers over all months and overall abundance of rotifers did not appear to be related to salinity changes in the river. Rotifers were not identified down to species; however, it appears that both freshwater and brackish species were prevalent in the samples (Figure 1.5, 1.8, and 1.11).

The mesozooplankton were dominated by Cyclopoid copepods and *Bosmina* spp. throughout the river in April. Calanoid and Cyclopoid copepods can overwinter, thus these species are present early in the spring. As water temperatures warmed, cladoceran species numbers, especially *Bosmina* spp., began to increase. Leech et al. (2009) found sites above Holiday Island to have the same dominant species in early spring, Cyclopoid and *Bosmina* spp.. In the sound, April estimates of zooplankton (200 µm) were likely underestimated due to clogging of nets with a filamentous green algae bloom or in low abundance. Webster and Peters (1978) reported that larger cladoceran species could not filter filamentous algae and rejected it as a food source, but microzooplankton (rotifers and copepod nauplii) could still filter some filamentous algae. Rotifers and copepod nauplii had higher abundances in the zooplankton samples (200 µm) from the sound. In May, there was a change in species composition in the sound and lower and middle river because of the intrusion of salt water. The brackish water

species *Acartia* spp. was found to dominate the system excluded the extreme upper river that remained fresh. In the delta of the Roanoke River in March of 2008 and 2009, Calanoida increased in number similar to rotifer numbers when an increase in salinity was seen in the sound and delta (Binion 2011). Binion (2011) did not identify the species any further than Order, however it is highly likely these were *Acartia* spp. When salinities increased above 3, freshwater copepods are replaced with brackish species, especially *Acartia* spp., a cosmopolitan species of temperate estuaries and coastal regions (Telesh et al. 2010). *Bosmina* spp. were still found at the higher salinities because these small bodied cladocerans can tolerate salinities of 7 or lower (Jensen et al. 2010). Many larger bodied cladocerans are less tolerant of salinities <3.5 (Jensen et al. 2010). In lower salinity ranges of 5 – 8, zooplankton have the lowest species richness since a few species from the freshwater and one or two dominant brackish water species can survive (Jensen et al. 2010 and Telesh and Khlebovich 2010). In the upper river, the zooplankton were dominated by *Leptodora* spp., a larger, predatory cladoceran.

Two important anadromous fish species in the Chowan River and Albemarle Sound are the river herring (alewife and blueback herring), with the freshwater reaches being important nursery habitat (Winslow 1985 and Rulfison et al. 1993). The alewife grows larger than the blueback herring and over time begins to selectively feed where blueback herring continues to filter feed (Mullen et al. 1986). After mouth development, which is completed when the fish are approximately 6mm total length, alewife and blueback herring start feeding on smaller cladocerans and copepods (Mullen et al. 1986). In the Roanoke and Chowan Rivers, the river herring diets consisted of Bosminidae and rotifers for river herring in the size class of 10-26 mm total length (Rulfison et al. 1993 and Winslow et al. 1985). In 2008 and 2009, the river herring in the Roanoke River had a similar diet of rotifers, copepod nauplii, and Bosmindiae (Riley

2012). A similar diet for blueback herring was seen in the Connecticut River with Bosminidae being dominant for fish 5-16+ mm total length, rotifers found in the diets of fish 5-12 mm and 16+mm total length and Cyclopoida being present in fish 16+mm total length (Crecco and Blake 1983). In the James River, Virginia, blueback herring (37 mm fork length) had diets dominated by adult copepods (Burbidge 1974). In the Hudson River, the juvenile alewife (mean fork length 64.8 mm) had a switch to larger prey items consisting of Amphipoda, but blueback herring (mean total length 56.3 mm) continued to consume zooplankton with the dominance being adult copepods (Gabe 1996). The zooplankton abundance over all systems was variable, but a comparison of the Chowan River to the other systems suggest that the community composition and abundance observed would support larval river herring populations (Table 1.11) (Burbidge 1974, Winslow et al. 1985, Rulfison et al. 1993, Leech et. al. 2009, and Binon 2011) In the Chowan River and Albemarle Sound in 2013, the zooplankton composition comprised the appropriate species that are needed during the critical time period of feeding for the larval river herring. Therefore, zooplankton community composition and ambient abundances are not likely factors influencing the lack of a herring recovery.

Table 1.1: PermANOVA for square root of zooplankton percent composition by month and location for 200 µm mesh size. * indicates significant results.

Source	DF	Sum of Squares	Pseudo-F- ratio	Prob > F
Month	2	38238	24.707	0.001*
Location	3	26374	11.361	0.001*
Month x Location	6	23467	5.0542	0.001*
Residual	54	41787	-	-
Total	65	1.28e5	-	-

Table 1.2: Pairwise comparison of the Month x Location of zooplankton percent composition by month for 200 µm mesh size. * indicates significant results.

Location	Comparison	t-value	P(perm)
Sound	April vs. May	5.3876	0.001*
	April vs. June	4.7949	0.006*
	May vs. June	2.4871	0.027*
Lower River	April vs. May	4.2106	0.004*
	April vs. June	2.3354	0.003*
	May vs. June	1.724	0.179
Middle River	April vs. May	3.1072	0.001*
	April vs. June	3.45	0.001*
	May vs. June	1.3984	0.17
Upper River	April vs. May	3.208	0.006*
	April vs. June	3.2465	0.004*
	May vs. June	2.0061	0.031*

Table 1.3: Pairwise comparison of the Month x Location of zooplankton percent composition by location for 200 µm mesh size. * indicates significant results.

Month	Comparison	t-value	P(perm)
April	Sound vs. Lower River	2.4942	0.019*
	Sound vs. Middle River	4.0889	0.001*
	Sound vs. Upper River	3.3486	0.002*
	Lower River vs. Middle River	1.3942	0.058
	Lower River vs. Upper River	1.8182	0.005*
	Middle River vs. Upper River	1.7265	0.027*
May	Sound vs. Lower River	1.3096	0.199
	Sound vs. Middle River	2.6669	0.012*
	Sound vs. Upper River	5.5942	0.005*
	Lower River vs. Middle River	1.5437	0.134
	Lower River vs. Upper River	4.0876	0.033*
	Middle River vs. Upper River	1.436	0.107
June	Sound vs. Lower River	1.668	0.176
	Sound vs. Middle River	3.3904	0.015*
	Sound vs. Upper River	5.4467	0.018*
	Lower River vs. Middle River	0.99434	0.302
	Lower River vs. Upper River	1.5449	0.14
	Middle River vs. Upper River	1.0703	0.351

Table 1.4: PermANOVA for square root of zooplankton percent composition by month and location for 60 µm. * indicates significant results.

Source	DF	Sum of Squares	Pseudo-F- ratio	Prob > F
Month	2	3712	7.9172	0.001*
Location	3	2210.6	3.1432	0.009*
Month x Location	6	2856.6	2.0309	0.027*
Residual	55	12893	-	-
Total	66	21124	-	-

Table 1.5: Pairwise comparison of the location of zooplankton percent composition by month for 60 µm. * indicates significant results.

Location	Comparison	t-value	P(perm)
Sound	April vs. May	1.9304	0.006*
	April vs. June	2.724	0.005*
	May vs. June	1.1683	0.242
Lower River	April vs. May	1.6325	0.087
	April vs. June	1.028	0.358
	May vs. June	1.1555	0.332
Middle River	April vs. May	2.1936	0.028*
	April vs. June	1.3734	0.163
	May vs. June	1.5057	0.164
Upper River	April vs. May	2.7203	0.018*
	April vs. June	2.1187	0.049
	May vs. June	1.2113	0.307

Table 1.6: Pairwise comparison for month vs. location of zooplankton percent composition by location for 60 µm. * indicates significant results.

Month	Comparison	t-value	P(perm)
April	Sound vs. Lower River	1.3208	0.197
	Sound vs. Middle River	0.84905	0.492
	Sound vs. Upper River	1.1509	0.251
	Lower River vs. Middle River	0.64403	0.551
	Lower River vs. Upper River	1.2611	0.218
	Middle River vs. Upper River	0.79108	0.443
May	Sound vs. Lower River	0.96334	0.428
	Sound vs. Middle River	2.1753	0.009*
	Sound vs. Upper River	2.4056	0.004*
	Lower River vs. Middle River	2.5974	0.003*
	Lower River vs. Upper River	2.7503	0.02*
	Middle River vs. Upper River	1.4076	0.155
June	Sound vs. Lower River	1.4806	0.247
	Sound vs. Middle River	2.4087	0.017*
	Sound vs. Upper River	3.2576	0.019*
	Lower River vs. Middle River	0.61447	0.66
	Lower River vs. Upper River	1.2066	0.235
	Middle River vs. Upper River	0.91171	0.442

Table 1.7: Repeated Measured ANOVA for average abundance by month and location. * indicates significant results.

Effects	Numerator DF	Denominator DF	F Value	Prob > F
Location	3	9	0.59	0.6394
Month	2	4	0.70	0.5490
Location*Month	6	9	0.25	0.9492

Table 1.8: Mean temperature (±SD) in degree Celsius and mean salinity (±SD) for the four locations from sampling period one (a), two (b) and three (c) in April.

a)	First Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	10.7 ± 0.51	0.02 ± 0.03	3
Lower River	10.2	0.04	1
Middle River	11.4 ± 1.15	0.03 ± 0.02	3
Upper River	11.22	0.03	1

b)	Second Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	16.9 ± 0.22	0.14 ± 0.08	3
Lower River	14.0 ± 0.78	0.02 ± 0.03	2
Middle River	15.5 ± 0.09	0.04 ± 0.00	3
Upper River	16.1	0.04	1

c)	Third Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	14.5 ± 0.50	0.05 ± 0.01	3
Lower River	17.4 ± 0.06	0.05 ± 0.01	2
Middle River	17.8 ± 0.13	0.04 ± 0.00	3
Upper River	18.4 ± 0.61	0.04 ± 0.00	2

Table 1.9: Mean temperature (±SD) in degree Celsius and mean salinity (±SD) for the four locations from the sampling period one (a), and two (b) in May.

a)	First Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	22.6 ± 0.54	0.83 ± 0.45	3
Lower River	22.7 ± 0.06	0.33 ± 0.11	2
Middle River	23.2 ± 0.26	0.09 ± 0.04	3
Upper River	23.6 ± 0.33	0.05 ± 0.01	2

b)	Second Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	22.7 ± 0.22	2.59 ± 1.86	3
Lower River	23.8 ± 0.05	1.07 ± 0.10	2
Middle River	24.7 ± 0.39	0.26 ± 0.13	3
Upper River	25.0 ± 0.16	0.07 ± 0.03	2

Table 1.10: Mean temperature (±SD) in degree Celsius and mean salinity (±SD) for the four locations from the two sampling periods in June.

a)	First Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	25.4 ± 0.31	1.85 ± 0.32	3
Lower River	26.0 ± 0.34	1.12 ± 0.44	2
Middle River	26.5 ± 0.31	0.55 ± 0.24	3
Upper River	26.5 ± 0.04	0.13 ± 0.06	2

b)	Second Sampling Period		
	Temperature ± SD	**Salinity ± SD**	**N**
Sound	------	--------	--
Lower River	26.1 ± 0.1	0.08 ± 0.02	2
Middle River	26.3 ± 0.58	0.05 ± 0.00	3
Upper River	26.2 ± 0.18	0.04 ± 0.01	2

Table 1.11: Abundance (mean number of individuals L^{-1} ± Standard deviation) of zooplankton during different months from nursery locations of larval river herring.

Location	Sites	Year	Mesh Size (µm)	Abundance	Sampling Period	References
St. James River, VA.	N/A	1974	76	5 – 210	June	Burbidge 1974
Chowan River	Holiday Island	1982-1983	64	3.59 ± 11.15	Yearly	Winslow et al. 1985
Roanoke River, NC	River	1984-88	250	0.327	Yearly	Rulifson et al. 1993
	Delta	1984-88	250	0.696	Yearly	Rulifson et al. 1993
Albemarle Sound		1984-88	250	0.532	Yearly	Rulifson et al. 1993
Chowan River	Holiday Island	2008-09	64	16.63 ± 28.41	Yearly	Leech et al. 2009
Roanoke River	River	2008-09	90	4.93 ± 3.81	March – June	Binon 2011
	Delta	2008-09	90	4.65 ± 2.85	March – June	Binon 2011
Albemarle Sound		2008-09	90	16.55 ± 14.68	March - June	Binon 2011
Albemarle Sound		2013	60	27.28 ± 43.75	April –June	This study
Albemarle Sound		2013	200	5.34 ± 8.88	April – June	This study
Chowan River	Lower	2013	60	28.87 ± 36.92	April - June	This study
	Middle	2013	60	17.49 ± 16.88	April – June	This study
	Upper	2013	60	9.97 ± 14.98	April – June	This study
Chowan River	Lower	2013	200	2.89 ± 7.41	April – June	This study
	Middle	2013	200	1.34 ± 2.68	April – June	This study
	Upper	2013	200	0.90 ± 2.08	April – June	This study

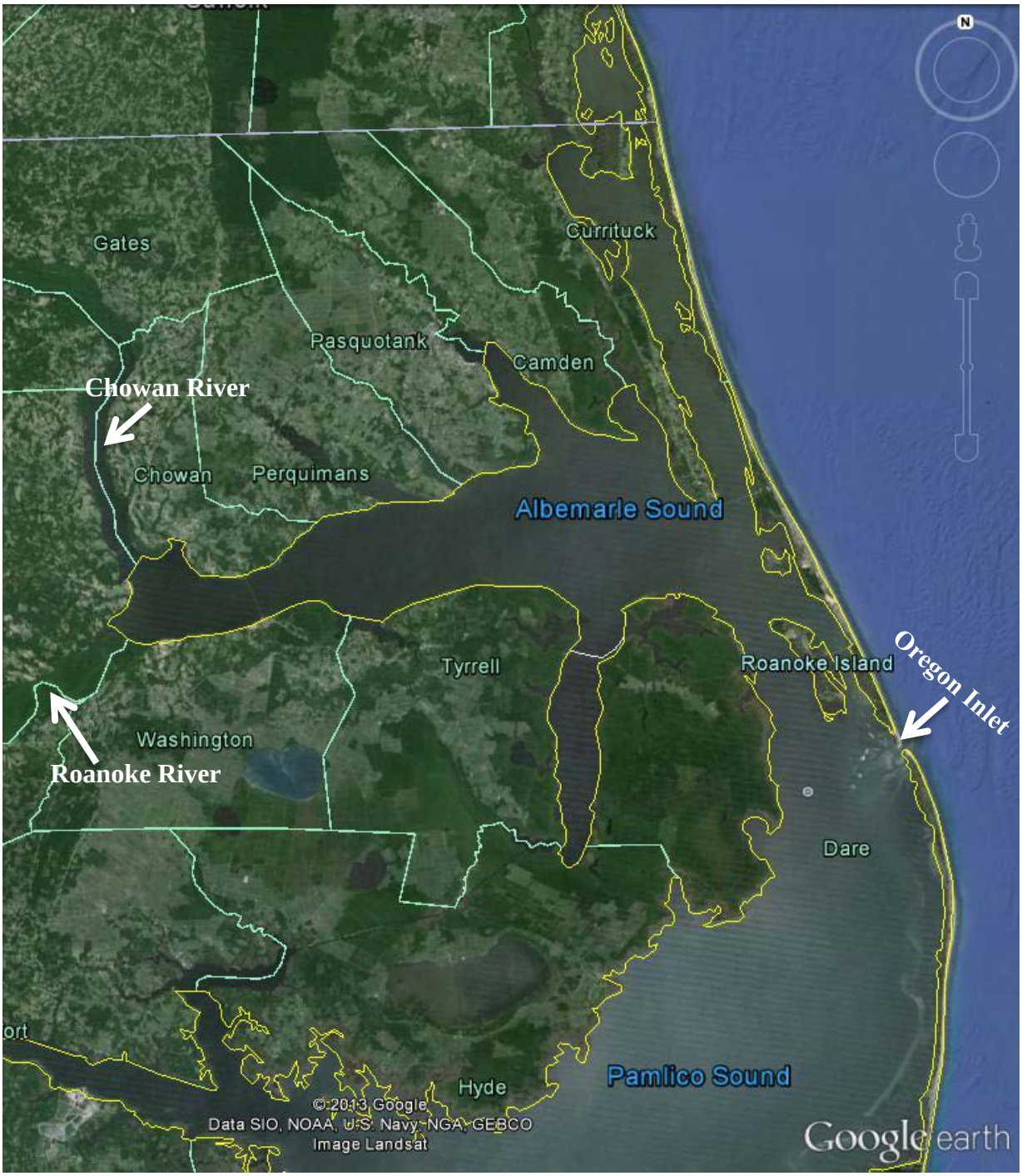

Figure 1.1: The overview map of the North Carolina northern Albemarle and Pamlico Sound systems.

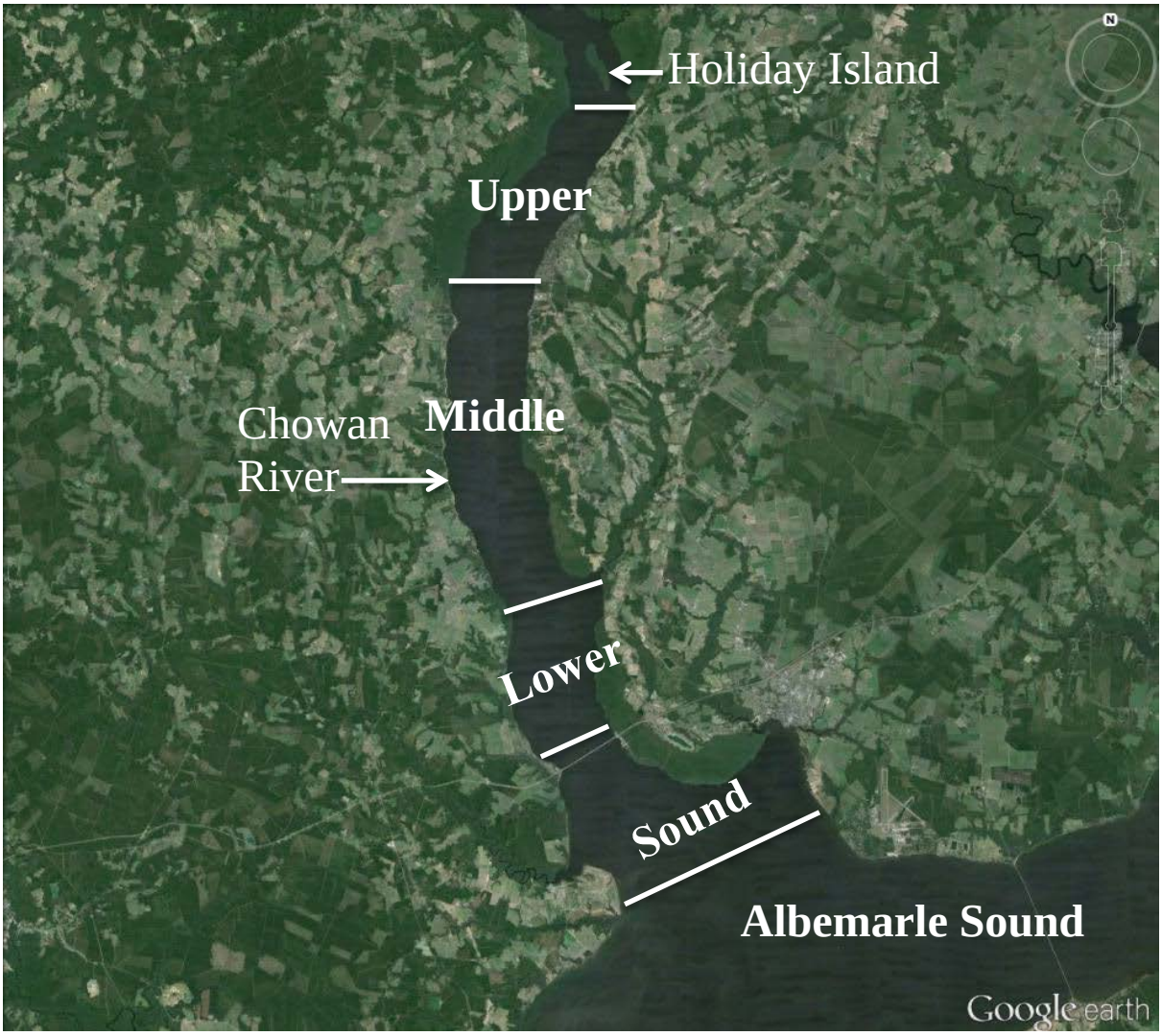

Figure 1.2: The western Albemarle Sound and Chowan River field sites for zooplankton samples collection.

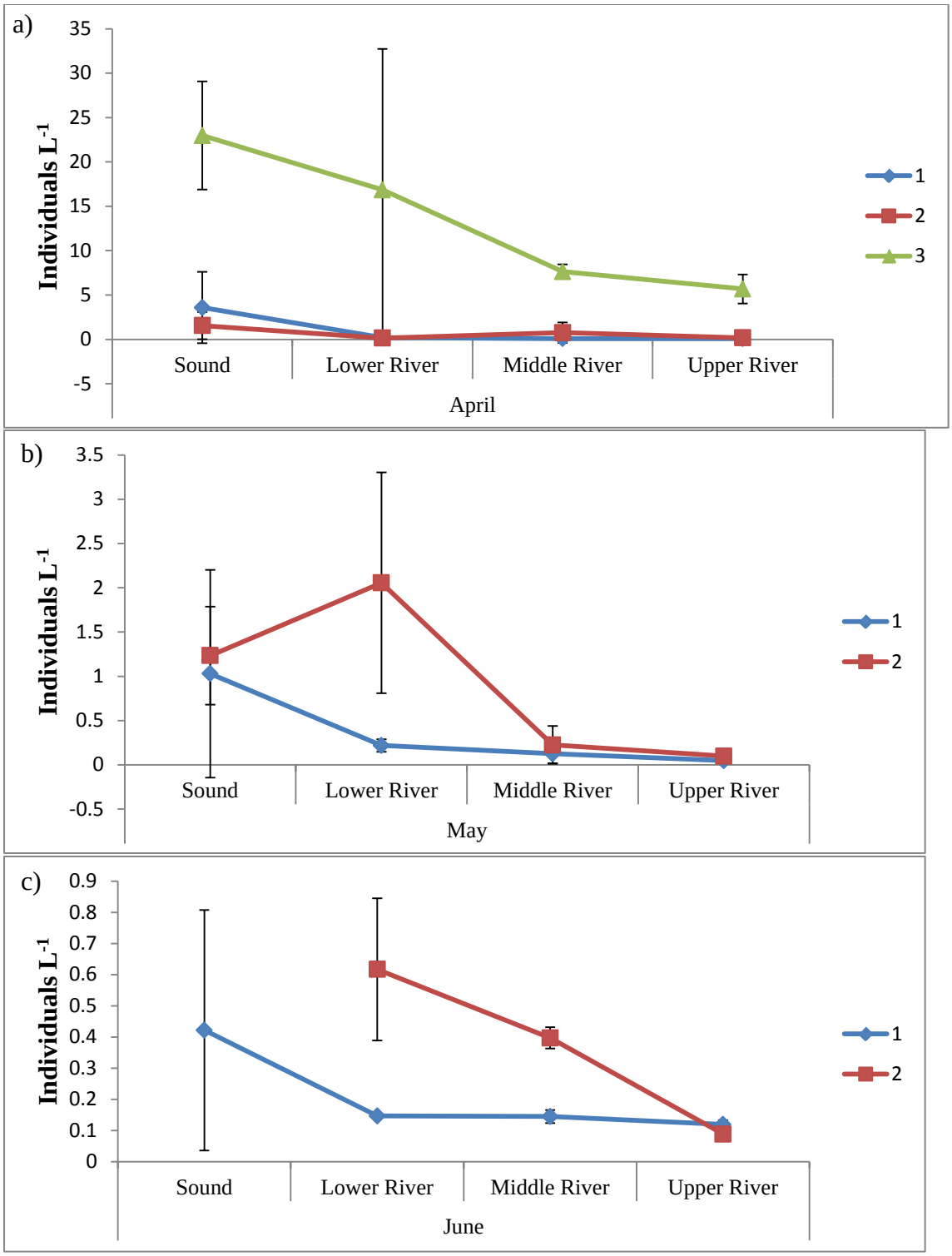

Figure 1.3: Average zooplankton abundance during April (a), May (b), and June (c) for the four locations by sampling period from the samples collected using 200 µm mesh nests. Each line represents a sampling period. Error bars represent standard deviation. The scale on the y-axis was not standardized.

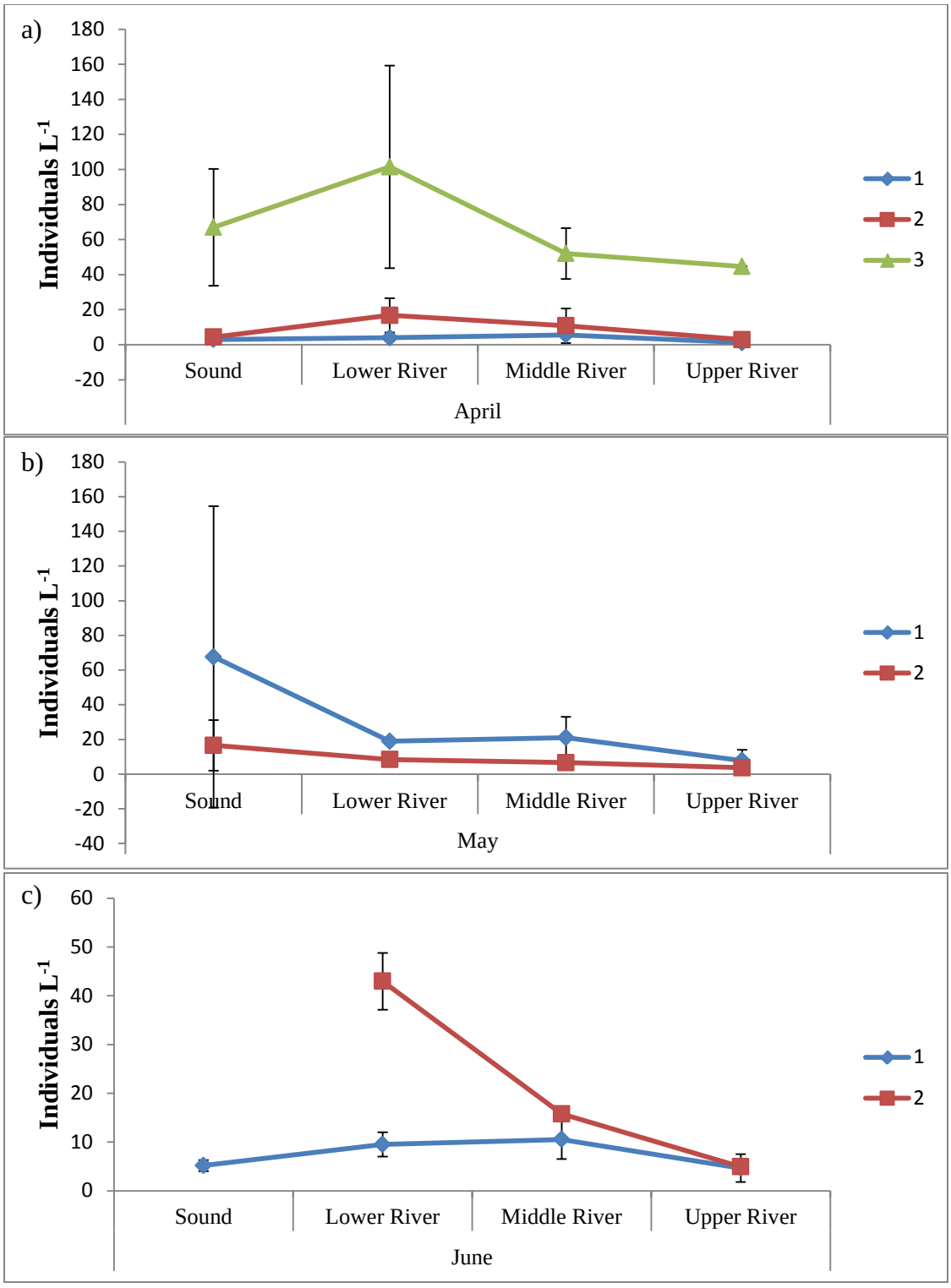

Figure 1.4: Average zooplankton abundance during April (a), May (b), and June (c) for the four locations by sampling period from the samples collected using 60 µm mesh nests. Each line represents a sampling period during the month. Error bars represent standard deviation. The scale on the y-axis was not standardized.

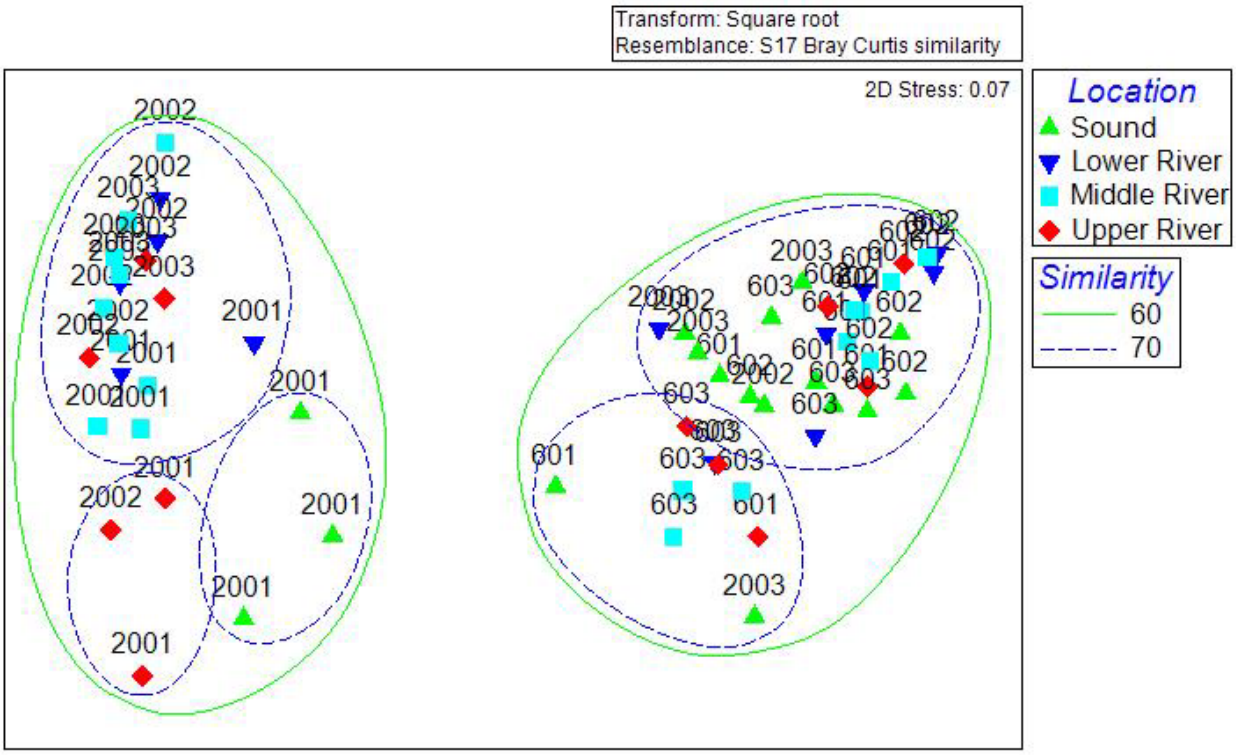

Figure 1.5: n-MDS plot showing similarity in percent composition of zooplankton at two mesh sizes (60 & 200 µm) for all four locations in April. Label for each point represent the mesh size and site number. Solid and dash lines show clusters at 60% and 70%.

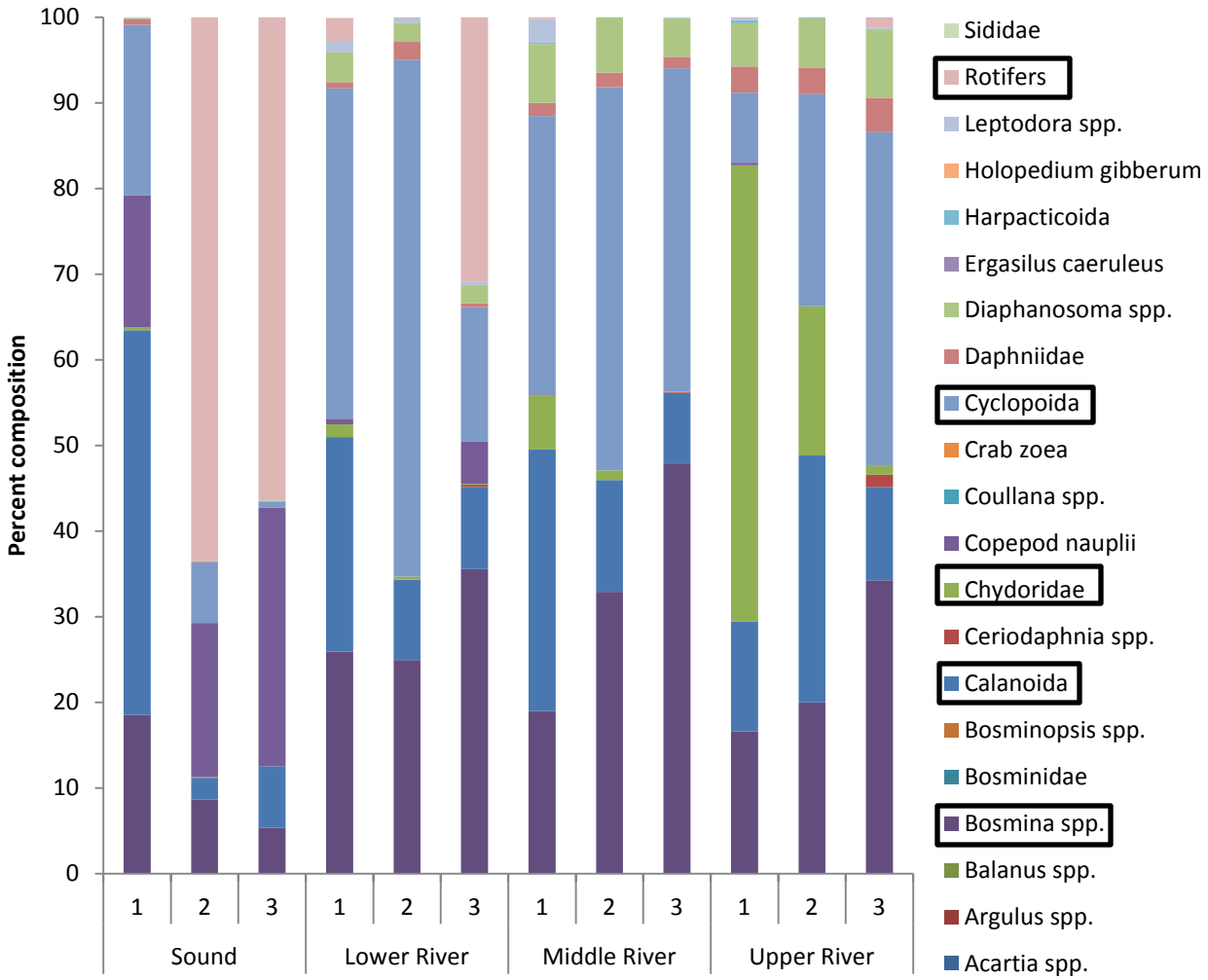

Figure 1.6: Percentage of samples composed of observed taxonomic groups in 200 µm mesh nets by location for the three sampling periods in April. The dominant species have a box around them.

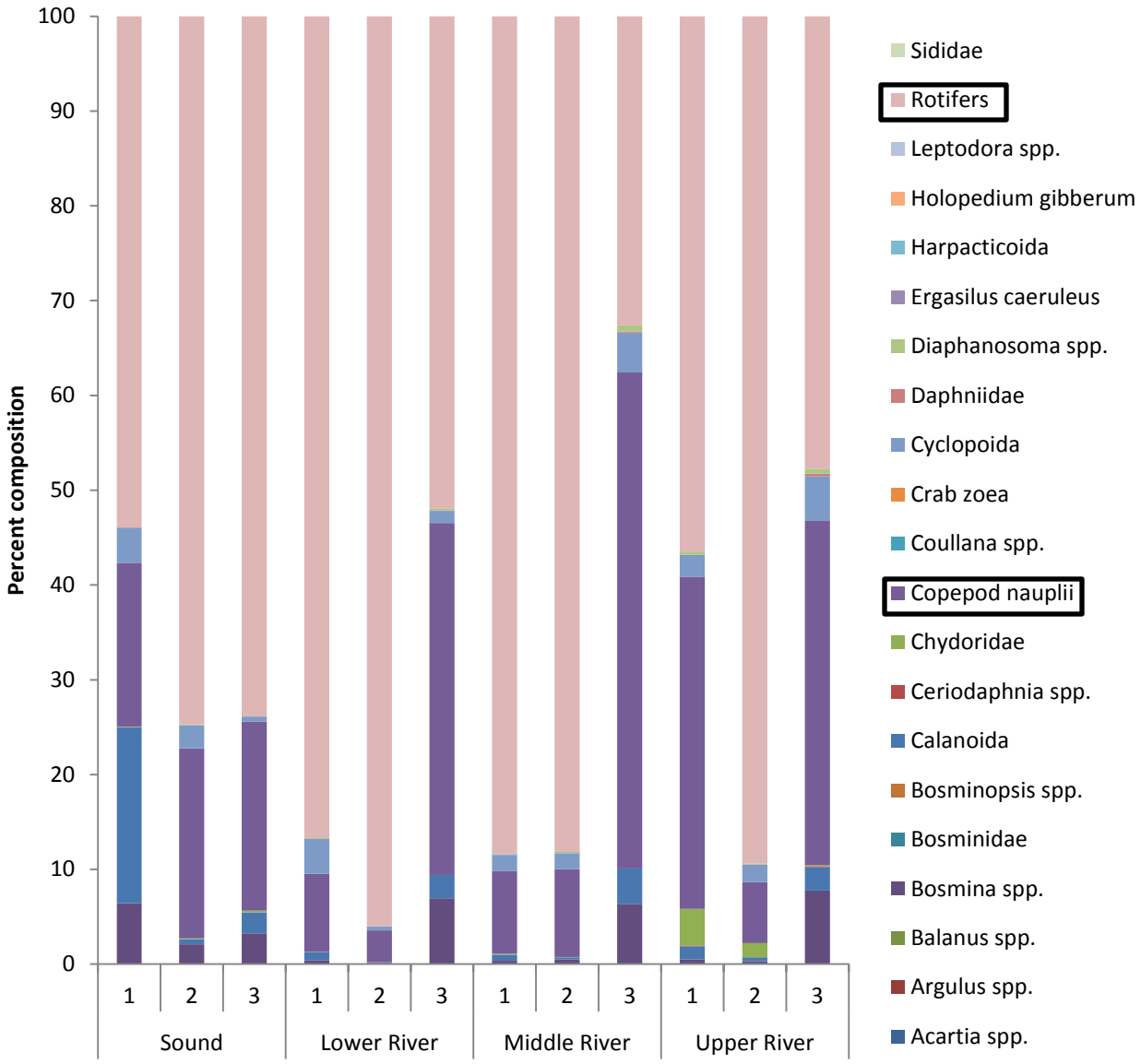

Figure.1.7: Percentage of samples composed of observed taxonomic groups in 60 µm mesh nets by location for the three sampling periods in April. The dominant species have a box around them.

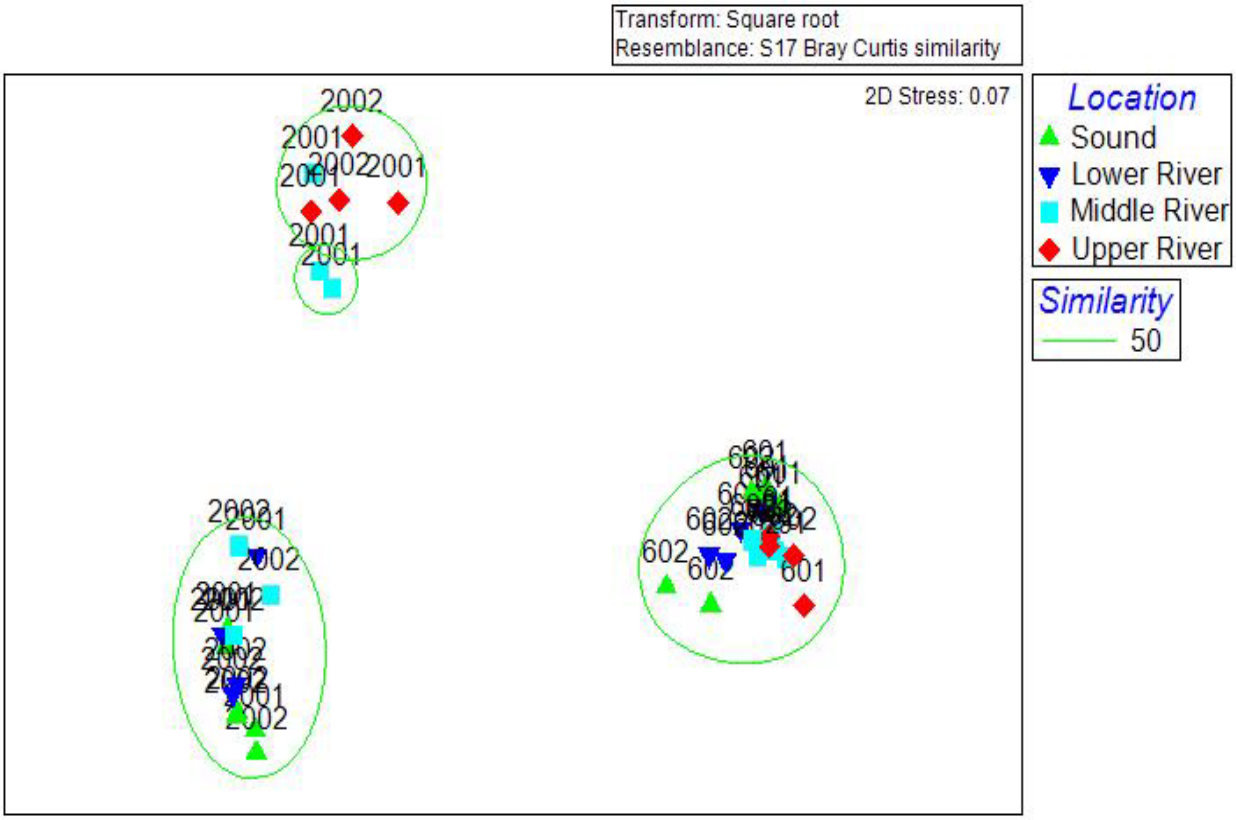

Figure 1.8: n-MDS plot showing similarity in percent composition of zooplankton at two mesh sizes (60 & 200 µm) for all four locations in May. Label for each point represent the mesh size and site number. Solid line shows clusters at 50%.

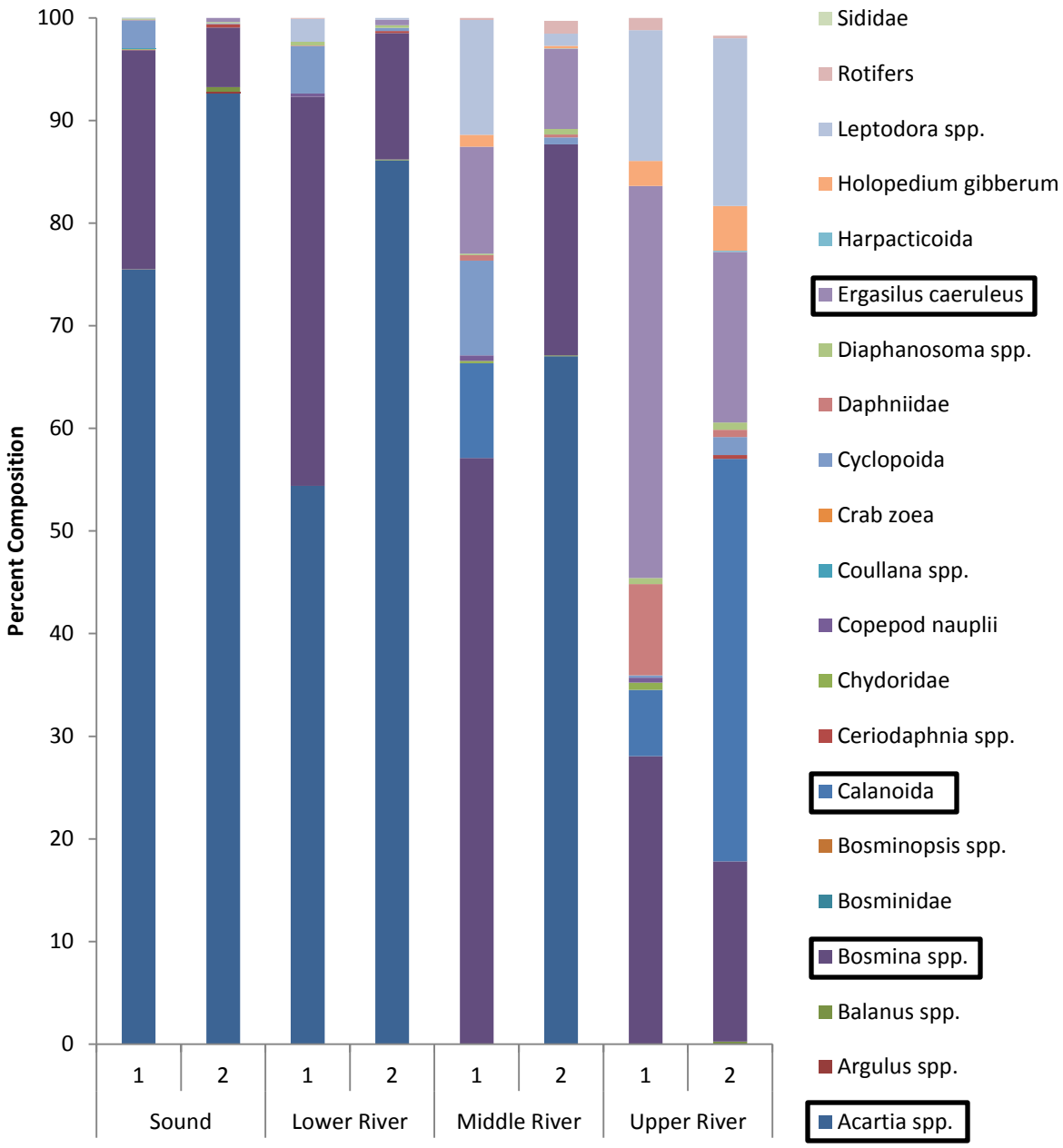

Figure 1.9: Percentage of samples composed of observed taxonomic groups in 200 µm mesh nets by location for the two sampling periods in May. The dominant species have a box around them.

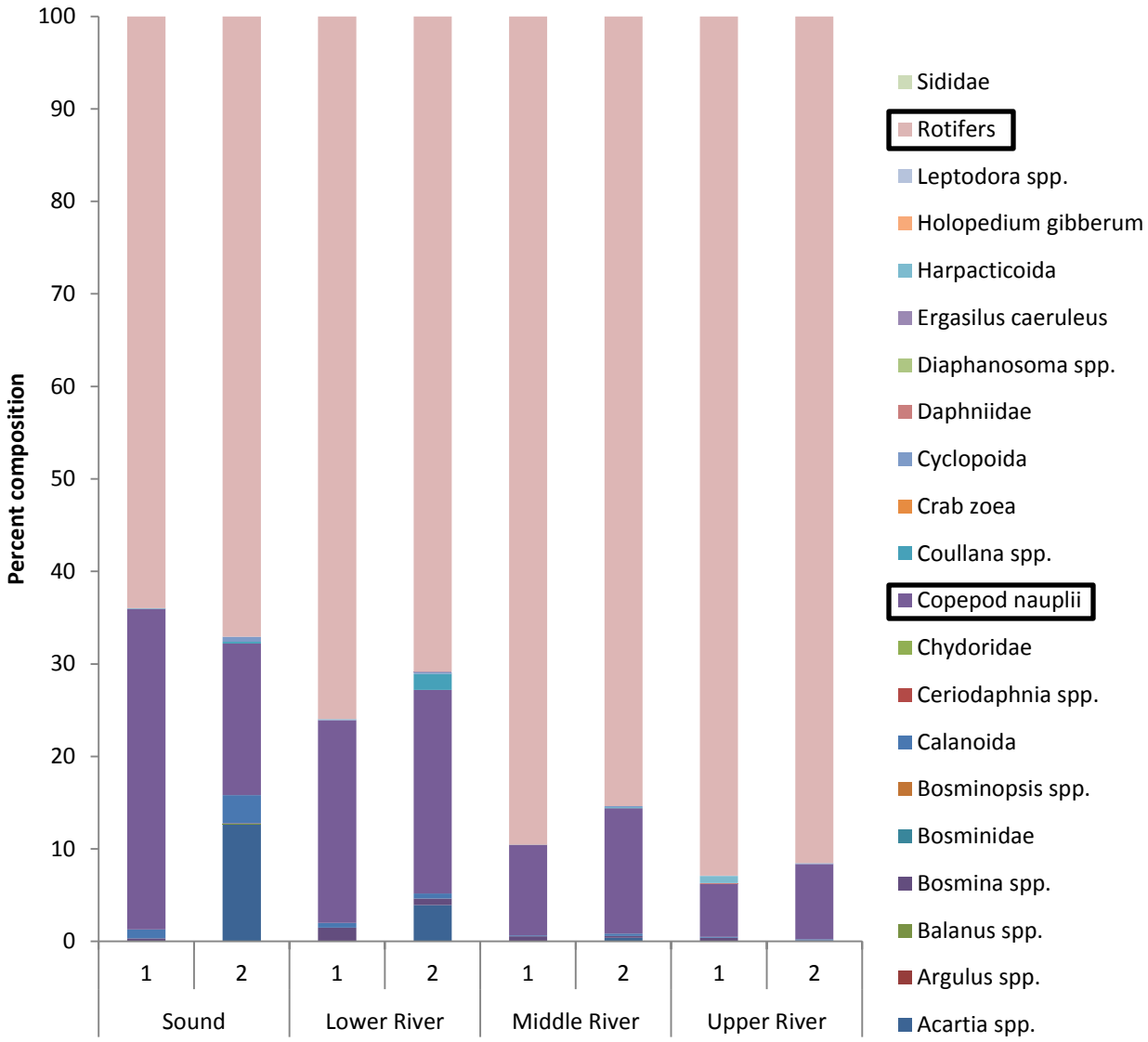

Figure 1.10: Percentage of samples composed of observed taxonomic groups in 60 µm mesh nets by location for the two sampling periods in May. The dominant species have a box around them.

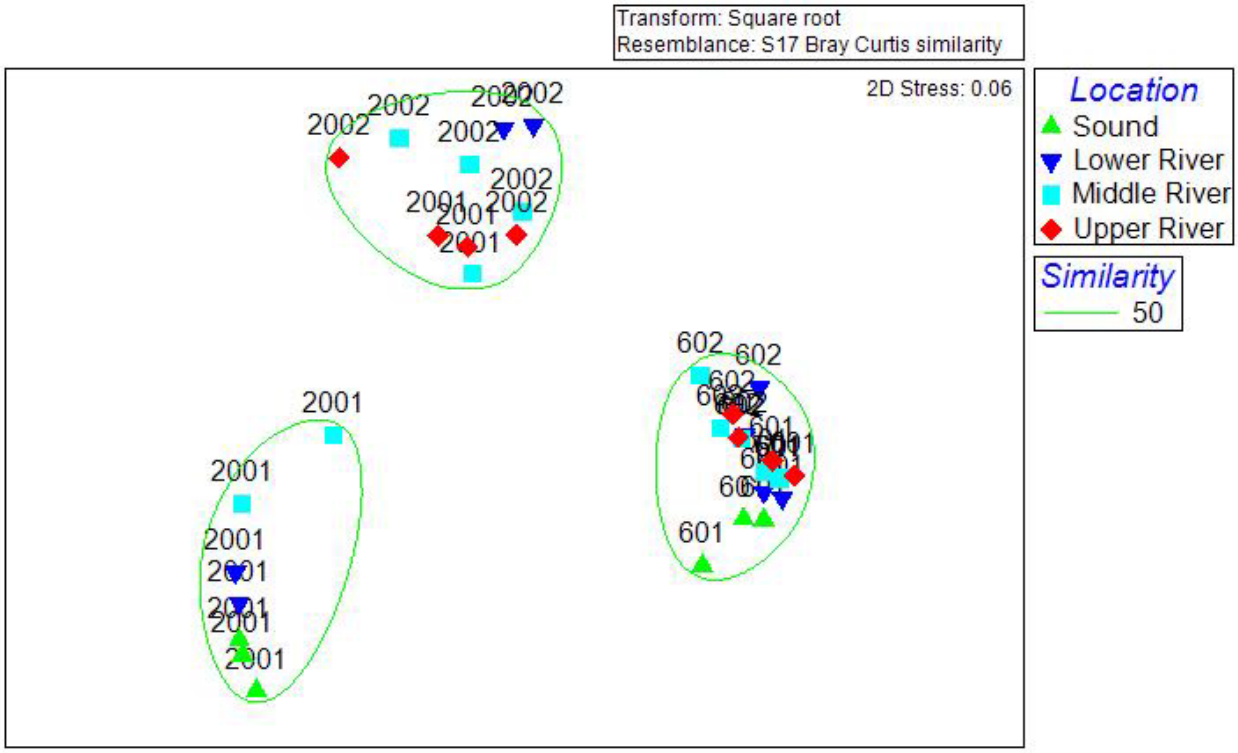

Figure 1.11: n-MDS plot showing similarity in percent composition of zooplankton at two mesh sizes (60 & 200 µm) for all four locations in June. Label for each point represent the mesh size and site number. Solid line shows clusters at 50%.

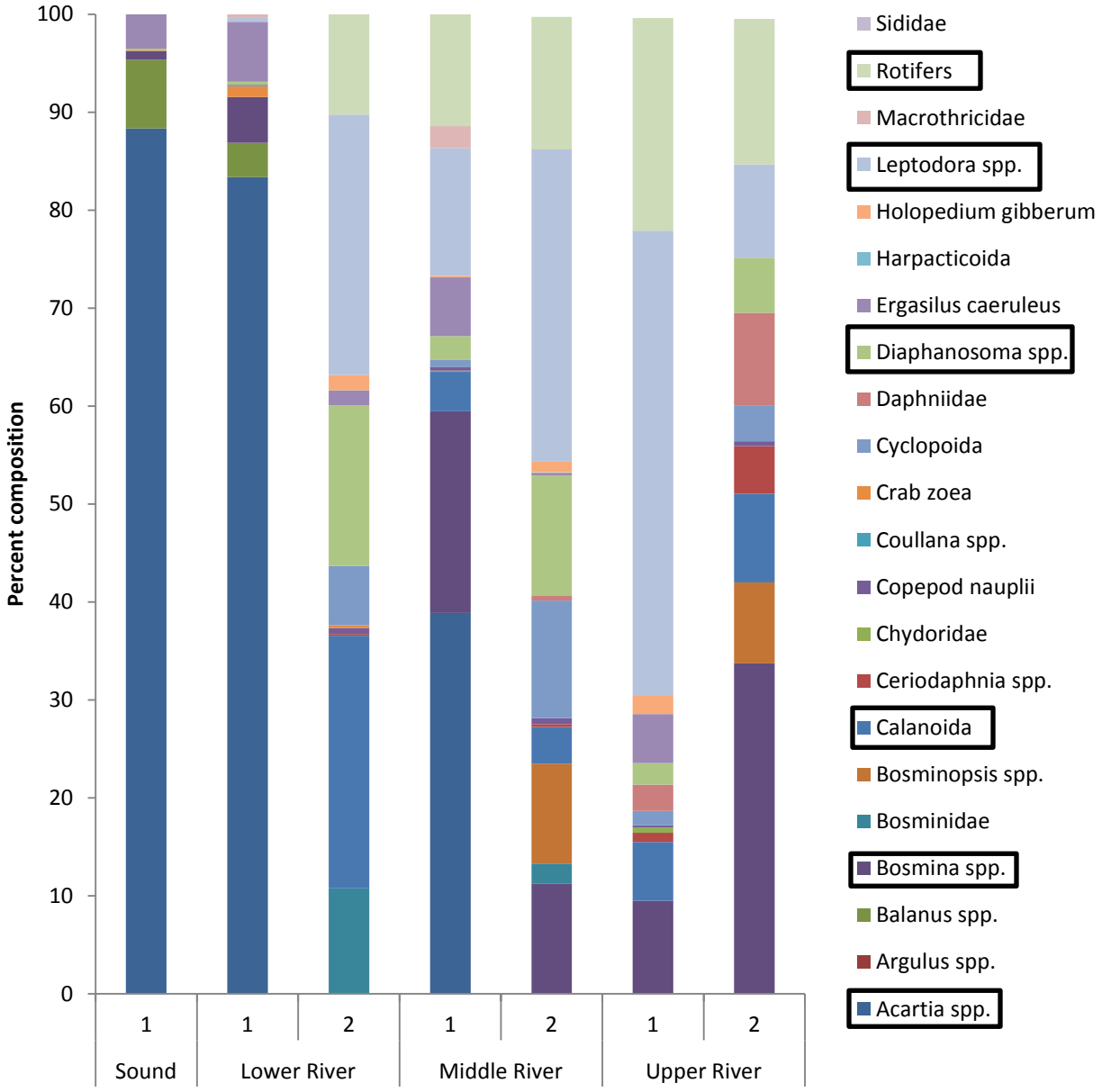

Figure 1.12: Percentage of samples composed of observed taxonomic groups in 200 µm mesh nets by location for the two sampling periods in June. The dominant species have a box around them.

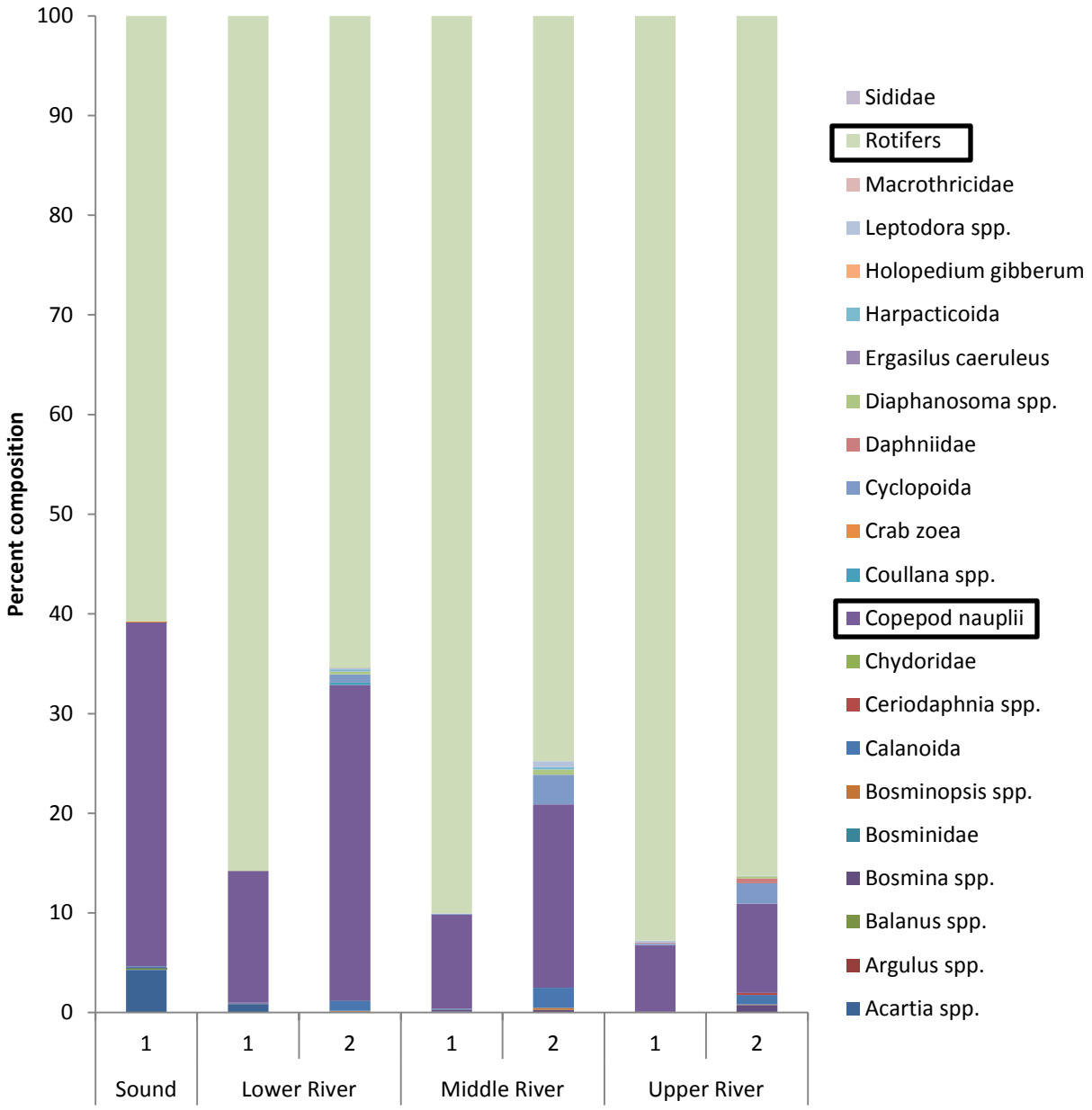

Figure 1.13: Percentage of samples composed of observed taxonomic groups in 60 µm mesh nets by location for the two sampling periods in June. The dominant species have a box around them.

Appendix

Table A1.1: Average zooplankton abundance (± Standard deviation) during April (a), May (b), and June (c) for the four locations by sampling period from the samples collected using 200 µm mesh nests. – means no sample was taken.

a)	Average Abundance (Individuals L^{-1} ± S.D.) Sampling Period			
	One	**Two**	**Three**	**N**
Sound	3.58 ± 4.02	1.54 ± 1.52	22.97 ± 6.09	3
Lower River	0.18 ± 0.06	0.15 ± 0.02	16.84 ± 15.90	2
Middle River	0.09 ± 0.01	0.76 ± 1.15	7.64 ± 0.82	3
Upper River	0.08 ± 0.01	0.17 ± 0.05	5.68 ± 1.63	2

b)	Average Abundance (Individuals L^{-1} ± S.D.) Sampling Period		
	One	**Two**	**N**
Sound	1.03 ± 1.17	1.23 ± 0.55	3
Lower River	0.22 ± 0.07	2.06 ± 1.25	2
Middle River	0.13 ± 0.11	0.23 ± 0.21	3
Upper River	0.05 ± 0.03	0.10 ± 0.07	2

c)	Average Abundance (Individuals L^{-1} ± S.D.) Sampling Period		
	One	**Two**	**N**
Sound	0.42 ± 0.39	----	3
Lower River	0.15 ± 0.00	0.62 ± 0.16	2
Middle River	0.14 ± 0.02	0.40 ± 0.23	3
Upper River	0.12 ± 0.01	0.09 ± 0.03	2

Table A1.2: Average zooplankton abundance (± Standard deviation) during April (a), May (b), and June (c) for the four locations by sampling period from the samples collected using 60 µm mesh nests. – means no sample was taken.

a)

Average Abundance (Individuals L^{-1} ± S.D.)				
	Sampling Period			
	One	**Two**	**Three**	**N**
Sound	2.98 ± 0.26	4.33 ± 1.73	67.00 ± 33.35	3
Lower River	3.99 ± 1.82	16.77 ± 9.92	101.52 ± 57.78	2
Middle River	5.62 ± 1.32	10.82 ± 9.92	52.02 ± 14.54	3
Upper River	1.20 ± 1.05	2.87 ± 3.26	44.62 ± 0.24	2

b)

Average Abundance (Individuals L^{-1} ± S.D.)			
Sampling Period			
	One	**Two**	**N**
Sound	67.57 ± 86.93	16.60 ± 14.56	3
Lower River	18.96 ± 0.05	8.43 ± 1.14	2
Middle River	21.04 ± 11.95	6.65 ± 2.50	3
Upper River	7.84 ± 6.18	3.64 ± 2.10	2

c)

Average Abundance (Individuals L^{-1} ± S.D.)			
Sampling Period			
	One	**Two**	**N**
Sound	5.19 ± 1.13	----	3
Lower River	9.51 ± 2.50	42.96 ± 3.25	2
Middle River	10.52 ± 4.02	15.78 ± 5.84	3
Upper River	4.66 ± 2.86	4.93 ± 0.31	2

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Chapter 2: Fatty Acid profiles of the zooplankton community of the Chowan River and western Albemarle Sound, North Carolina

Introduction

Food web studies are important for understanding the effects of both abiotic and biotic changes on trophic interactions (Goncalves et al. 2012). The analysis of gut contents is less reliable among smaller taxa in general and zooplankton in particular because of high gut evacuation rates and differential rates of prey assimilation in the gut (Arts et al. 2009). The use of lipids and fatty acid as biomarkers is therefore a promising way to study diet composition of zooplankton. Interaction among the lower food web can be traced using fatty acid composition and it is possible to compare what is available to what is being transferred through the food web. The principle of "you are what you eat" can be determined from the fatty acid signatures of the organisms, but also relies on the type of food available for the organisms (Goncalves et al. 2012). Another use of lipids and fatty acids is to determine the nutritional quality of organisms for higher trophic levels and how food quality can affect growth and development of the organisms (Gulati et al. 1997, Kainz et al. 2004, and Masclaux et al. 2012). Aquatic organisms, especially consumers, need essential dietary compounds for somatic development and fitness (Masclaux et al. 2012). These two reasons have led an increased interest in investigating organism fatty acid and lipid profiles within aquatic food webs.

Lipids play an important role in organisms by affecting fitness, energy and essential nutrients for general metabolic function, somatic growth, and reproduction (Muller-Navarra et al. 2000). Storage lipids contain triacylglycerol and are high energy sources; while phospholipids and other structural lipids are essential building blocks for cell membranes (Arts et al. 2009). Many investigations into lipid signatures focus on the fatty acids. Fatty acids are chains of

carbons with no double bonds (saturated, SFA) or double bonds (unsaturated)). Fatty acids having one double bound are considered monounsaturated (MUFA) while two or more double bonds per molecule are considered polyunsaturated (PUFA) (Arts et al. 2009). Fatty acids that contain greater than 20 carbon atoms are considered highly unsaturated fatty acids (HUFA). The two essential fatty acids from the omega-3 (n-3, nomenclature), and omega-6 (n-6, nomenclature) families are linolenic (18:3n-3) and linoleic acids (18:2n-6). These forms arise because plants can only insert the double bond in third or sixth position from the terminal methyl group (Arts et al. 2009).

The change from freshwater to marine systems has an impact on the fatty acid profiles in zooplankton and other organisms (Arts et al. 2009). Salinity affects the food source and species of zooplankton present, which in turn leads to changes in the fatty acid composition for higher trophic levels (Arts et al. 2009). The highly unsaturated fatty acids (i.e., 20:5n-3, EPA, eicosapentaenoic acid, 22:6n-3, DHA, docosahexaenoic acid, and 20:4n-6, ARA, arachidonic acid) are important for all organisms and play a role in health and cell function (Arts et al. 2009). The zooplankton from marine systems have higher levels of the PUFAs from diet alone compared to freshwater water zooplankton and most species of zooplankton cannot synthesize the precursor fatty acids into higher chain fatty acids (Arts et al. 2009). For the longer chain fatty acids such as EPA, DHA, and ARA, many freshwater organisms can synthesize them if they are provided with the precursors (Brett and Muller-Navarra 1997 and Arts et al. 2009). However, most marine fish and zooplankton cannot synthesize them, thus they need to obtain them in their diet (Brett and Muller-Navarra 1997 and Arts et al. 2009). The movement of highly unsaturated fatty acids across trophic levels is the only way higher trophic level organisms accumulate these fatty acids.

The fatty acid profiles of zooplankton vary among species and are also influenced by diet. The cladoceran species that have high reproductive potential (*Bosmina* spp. and *Leptodora* spp.) have higher levels of EPA and ARA (Persson and Vrede 2006). In contrast, copepods have higher relative DHA levels because this fatty acid is critical for nervous system development. Copepods feature more developed nervous systems compared to cladocerans and this is a function of the active hunting of prey, mate location, and predator avoidance (Arts et al. 2009). Carnivorous zooplankton have shown to be richer in PUFAs and this is thought to be related to their food source (rotifers and smaller bodied cladocerans/copepods compared to phytoplankton) (Arts et al. 2009). Therefore, the potential impact of eutrophication (i.e., a shift to less nutritious phytoplankton, such as cyanobacteria) on the phytoplankton community may resonate in the broader food web through the quantity and quality of fatty acids.

Phytoplankton, one of the main dietary components for secondary consumers, play a role in growth and development of zooplankton. Muller-Navarra et al. (2000) demonstrated that even with a high Chl-a/Carbon ratio, *Daphnia* spp. growth was affected by phytoplankton composition. The highest *Daphnia* growth was observed during the winter/spring when diatoms and cryptophytes dominated the phytoplankton compared to summer assemblages comprised of cyanobacteria (Muller-Navarra et al. 2000). Muller-Navarra et al. (2000) showed that the higher amounts of 20:5n-3 (EPA) increased growth for *Daphnia* spp. in a natural enviornment. The *Daphnia* spp. that consumed a diet of diatoms and cryptophytes in winter and spring had an increased growth rate compared to *Daphnia* spp. that consumed cyanobacteria during the summer, even though the amount of carbon available was greater in the summer. Cyanobacteria usually have high levels of alpha-linolenic acid (18:3n-3), but do not contain high levels of greater than 20 carbon fatty acids (DHA and EPA) (Muller-Navarra et al. 2000). The alpha-

linolenic acid (18:3n-3) is high in algal populations undertaking rapid cell division (Napolitano et al. 1997).

In estuarine and costal systems, the zooplankton fatty acid signatures often correspond to the dominant phytoplankton species or detritus (Napoitano et al. 1997, Rossi et al. 2006, Goncalves et al. 2012). The zooplankton in the fresh and brackish water of the Mondego estuary showed a higher amount of fatty acids in the mono- and poly-unsaturated fatty acid signatures compared to the ones living in saltwater and this was thought to be related to less abundant or diverse prey composition in the saltwater (Goncalves et al. 2012). In winter, *Daphnia longispina* was found at all locations in the Mondego estuary, but the fatty acid signatures showed a consumption of different dominant phytoplankton species at different locations within the estuary (Goncalves et al. 2012). Zooplankton consumed the phytoplankton >5 μm in size (diatoms and dinoflagellates) near the Catalan coast because they are easier to capture, even though the dominant phytoplankton was *Prymnesiophyceae* (Rossi et al. 2006). During a phytoplankton bloom dominated by diatom species, the zooplankton had an increase of two polyunsaturated fatty acids (20:5n-3 and 22:6n-3) which can be traced to the phytoplankton fatty acids (Napolitano et al. 1997). During the pre and post phytoplankton bloom, zooplankton had high concentrations of linoleic acid (18:2n-6) which can be related to particulate organic matter with an originating from terrestrial sources (Napolitano et al. 1997). Thus, spatial and temporal variability in the zooplankton diets is clearly linked to the fatty acid profile of the zooplankton.

Larval fish are a primary consumer of zooplankton in the food web, and their growth and development can be affected by the fatty acid composition of the zooplankton (Rossi et al. 2006). Near the Catalan coast, Rossi et al. (2006) compared the fatty acid signatures of small and large anchovy, as well as their zooplankton prey, to determine if prey affected their growth. The

larger larval anchovy fatty acid signatures correlated with the zooplankton signature and this demonstrated the consumption of copepod nauplii and copepodites (Rossi et al. 2006). The smaller larval fish gut analysis showed that nauplii were the main food source, but after analyzing the fatty acids, the smaller larvae actually feed on ciliates and flagellates because of the high concentration of 18:1(n-9) and 18:4(n-3), which corresponded with the high abundance of *Prymnesiophyceae* (Rossi et al. 2006). The smaller larvae were more efficient at consuming the ciliates and flagellates than the larger larval anchovy, which can be inferred through fatty acid analysis (Rossi et al. 2006). Jeffries (1975) studied the fatty acid signature of the gut contents of larval menhaden and determined the existence of two different feeding mechanisms (filter feeding or predation), in three distinct estuarine habitats (river, marsh, open water). The amount and type of fatty acids found in the larval fish can relate to recruitment (Bell & Sargent 1996). Atlantic herring that had a deficiency in DHA did not produce as many retinal rods, which reduced their ability to find prey in low light environments (Bell et al. 1995). Since Atlantic herring are visual predators and must be able to escape from predators as larvae, the reduction in rods would decrease the efficiency of these tasks in low light environments (Bell et al. 1995).

Fatty acids are also susceptible to anthropogenic stressors in the environment, in particular eutrophication. Eutrophication in freshwater systems may result in cyanobacteria blooms, a group that contains very low concentrations of highly unsaturated fatty acids (Arts et al. 2009). The cyanobacteria blooms can decrease the foraging of zooplankton, which can lead to decreased growth (Lehman et al. 2010). Since the river herring decline in North Carolina, reduced water quality has been designated as one of the main reasons for the river herring demise (Moser and Patrick 2000). The zooplankton populations have been studied in recent years and

the abundance of zooplankton appears adequate to sustain the larval river herring (Binon 2012 and Leech et al. 2009 and this study See chapter 1). Up until now, there has been no research conducted on the fatty acid profiles for the zooplankton in the Chowan River and the Albemarle Sound. The aim of this chapter is determine the nutritional quality of the zooplankton in the Chowan River and the western Albemarle Sound.

Objective

Objective: Determine fatty acid composition of zooplankton collected using two different mesh sizes for April and May, and three mesh sizes for June for seven locations in the Chowan River and three locations in western Albemarle Sound

Hypothesis

Hypothesis: The fatty acid nutrient quality of zooplankton for larval and juvenile river herring is not different in the Chowan River and the western section of Albemarle Sound and over three months (April, May and June).

In April, May, and June, zooplankton samples were collected using a two minute, oblique tow at each site with a 60 and 200 µm mesh zooplankton net. The zooplankton were brought back to the laboratory alive, and the samples were separated by up to three mesh sizes (60, 200 and 500 µm). Fatty acid methyl esters (FAME) were separated by gas chromatography using a 7693 mass spectrometer detector, a capillary column and a 7890A autoinjector. The percent fatty acid composition data by mesh size were analyzed using multivariate procedures to compare between locations and months using PRIMER.

Method

Study Site

Albemarle Sound is part of the Albemarle-Pamlico Estuarine System (APES) and is bordered by the Outer Banks, which is the barrier to the Atlantic Ocean (See Chapter One Figure 1.1). The only saltwater connection is Oregon Inlet and is at the southern end of the sound. The system is a lagoonal estuary, with only one inlet and high volume of freshwater input, thus Albemarle Sound has salinity levels <5 (Copeland et al. 1983). The two main tributaries that empty into the Albemarle Sound are the Roanoke and Chowan Rivers (See Chapter One Figure 1.1). The Chowan River originates in the Virginia coastal plains and is the 12th largest river basin in North Carolina (NCDENR 2006). Overall water quality is poor with low dissolved oxygen levels (<3.0 mg L^{-1}) with the first large scale algae bloom in 1972 classifying the Chowan River as "nutrient sensitive waters" in 1979 (NCDENR 2006). The Chowan River is considered critical habitat for larval and juvenile river herring, and zooplankton research has been conducted on both of these rivers (NCDMF 2007). The sites on the river will allow for comparison from up river to the mouth (Figure 2.1). In the Albemarle Sound, three sites where chosen to compare to the seven sites on the Chowan River (Figure 2.1). The three sites are near the mouth of the river, and would allow comparisons between the river and sound especially if larval or juvenile river herring could move out into this area to determine if those areas are suitable for river herring growth and development.

Field Work

The zooplankton sampling occurred on 10 and 11 April 2013, 31 May 2013, and 25 June 2013. The samples were collected at the seven locations on the Chowan River for April and June, and at locations 1, 4, 7 and 10 in May. The three sound sites in April were not sampled

because of a large green algae bloom, and in June because of strong wind event. The water depths ranged from 5.27 meters to 7.56 meters. Each location was 3.22 kilometers apart, and ended right below Holiday Island in the Chowan River. The zooplankton net with a 50 cm mouth and mesh and cod end of 200 and 60 µm with a weight were towed through the water for two minutes. The horizontal sampling depth consisted of 1 to 3 meters below the surface. The timing of zooplankton sampling corresponded to the critical feeding period for larval river herring. The zooplankton collected were washed into a 200 or 60 µm mesh filter depending on the net mesh size, and placed together in a 1000 ml plastic container and then completely filled with water. The samples were place into a cooler with ice until back at the laboratory for processing.

Laboratory Processing

The zooplankton samples were filtered through 200 and 60 µm sieves for April and May, and in June a 500 µm filter was used to remove the larger predatory freshwater zooplankton. Each sample was visual identified to determine the dominant species with the Olympus SZX10 dissecting scope. The samples were concentrated on a GF/F filter (25mm diameter) by mesh size, and placed on paper towel to remove excess water. All samples were kept at -80°C until ready to process.

The fatty acid analysis took place in Dr. Jacques Rinchard's laboratory on the campus of the College of Brockport - State University of New York. Lipids were extracted using the gravimetric method developed by Folch et al. (1957). The zooplankton sample including the paper filter was placed into a glass test tube and weighted to determine a total sample weight. The filter weight was subtracted from the total weight for a weight of just zooplankton material. Then 20 mL of chloroform/methanol/0.01% of butylated hydroxytoluene (BHT) solvent mixture

was added to the test tube, and the sample homogenized for one minute using a Power Gen 500 homogenizer (Fisher Scientific, Pittsburgh, PA). After each sample, the probe was rinsed twice with deionized water, twice with solvent, and wiped dry. Homogenized samples were capped and kept on ice. Samples were then vacuum-filtered. The homogenized sample was then filtered under vacuum through a GF/F filter (Whatman, Piscataway, NJ) to remove all filter material and debris. The filtered extract was transferred to a clean test tube with 4 mL of 6% magnesium chloride $MgCl_26H_20$. The test tubes were then filled with nitrogen gas and vortexed for one minute, and then additional nitrogen gas was added. The samples were then left for 24 hours to allow the lipids to separate from water. Nitrogen gas is used to keep oxygen out of the sample, reducing the oxidation of lipids. The bottom layer was extracted using a Pasteur pipette and transferred to a clean glass tube. Samples were placed in a warm water bath and put under nitrogen to evaporate solvent. Once samples had a small amount of solvent left, they were transferred to pre-weighed test tubes. Evaporation under nitrogen continued and samples were weighed until a stable weight was reached. A small amount of chloroform was added and samples were capped under nitrogen before storage at -80˚C. Percent of lipid content ((weight of lipid/weight of tissue)*100) was then calculated.

Transmethylation of fatty acids was done according to the method described by Metcalfe and Schmitz (1969). Chloroform from samples was evaporated under nitrogen. A known amount of nonadecanoate acid dissolved in hexane at a concentration of 8 mg/ml (19:0, Nu Check Prep Inc., Elysian, MN) was added as internal standard. The sample was then evaporated under nitrogen. After the sample was evaporated, 1.5 mL of 0.5M sodium hydroxide in methanol was added, and then incubated for one hour at 80°C. This step is known as saponification, which cleaves the fatty acid from the glycerol and adds a hydroxyl group. After the sample had cooled

2 mL of borontrifluoride methanol (Sigma-Aldrich Company, St. Louis, MO) was added. This cleaves the hydroxyl group from the fatty acids and replaces it with a methyl group, which is detectable by the gas chromatography/mass spectroscopy (GC/MS). Samples were capped under nitrogen, incubated at 80˚C for 0.5 h, and cooled to room temperature. One mL of hexane was added to the samples, which were then capped and vortexed. This was repeated with one mL of water. The hexane layer was transferred using a Pasteur pipette to a clean test tube containing a small spoonful of anhydrous sodium sulfate to remove any excess water. Another one mL of hexane was added to the sample and was capped and vortexed. This hexane layer was also transferred to the vial with sodium sulfate, which was capped and vortexed. Samples were transferred to a 4-mL vial, filled with nitrogen, and capped. Samples were stored at -80˚C until being injected into the GC/MS. The fatty acid methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc., Santa Clara, CA) using a 7693 mass spectrometer detector (Agilent Technologies, Inc.), a capillary column (Omegawax^{TM} 250 fused silica capillary column, 30 m x 0.25 mm and 0.25 mm film thickness, Supleco®, Bellefonte, PA), and a 7890A autoinjector (Agilent Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.8 mL min^{-1} and the injection volume was 2 ml. Initial temperature of the oven was 175^{o}C for 26 min, which was increased to 205^{o}C by increments of 2^{o}C/min, then held at 205^{o}C for 24 min. The source and analyzer for the mass spectrometer was set at 230^{o}C. The individual fatty acid methyl esters were identified by comparing the retention times of authentic standard mixtures (FAME mix 37 components, Supleco, Bellefonte, PA) and quantified by comparing their peak areas with that of the internal standard (Czesny and Dabrowski 1998). The results of individual fatty acid composition are expressed in percentage of total identified FAME.

Statistical Analysis

Multivariate statistics were conducted in PRIMER 6 with PERMANOVA + (Clarke and Gorley 2006). The percentages of total identified fatty acid data were transformed with a square root to reduce weighting of dominant species, and a Bray-Curtis Similarity matrix was constructed. The fatty acid profiles were analyzed by month for site and zooplankton mesh size through a nonparametric multidimensional scaling (n-MDS) to determine visual similarities in the data. The n-MDS plot places point together until the lowest stress is achieved. With a stress < 0.1, there is an underlying factor causing the distribution of data points, as opposed to a random assortment of data within the plot (Clarke and Gorley 2006). Cluster analysis with a SIMPROF was used to determine the highest level of similarity, and used in the n-MDS to cluster mesh sizes and locations. The SIMPER test was used to determine the greatest differences by month and mesh size for the locations to investigate which fatty acids drove the differences seen in the n-MDS plot. The SIMPER test was set to a 50% cumulative contribution for the similarities and dissimilarities.

PERMANOVA was used to determine differences in fatty acid signatures over all locations between mesh sizes (60, 200 and 500 µm) and months (April, May, and June). Pairwise comparisons were used for post-hoc statistical testing. Models were considered significant at the $\alpha = 0.05$ level. The mesh sizes were used to determine if different species composition by size affected the fatty acid percentage composition during the month. PERMDisp was performed to determine homogeneity of the fatty acid profiles around the centroid or mean value. The different mesh sizes represent the size classes of zooplankton fatty acid sampled and were divided among the different species sampled, thus the mesh size represents the zooplankton community. The dominant species of zooplankton were determined

and results shown in Chapter One. Even with dominant species being different between the two mesh sizes, the fatty acid profiles needed to be compared by mesh size to determine how fatty acids compared between different zoopolankton size classes.

Results

Summary of zooplankton fatty acid profiles for the entire sampling period

A total of 24 fatty acids were found in all samples for both mesh sizes (Appendix Tables 2.1- 2.4). There were six dominant fatty acids found in all the samples, but the concentration varied among samples (Figure 2.2). The dominant saturated fatty acid was palmitic acid 16:0 and the dominant monounsaturated fatty acids were palmitoleic acid 16:1n-7, and oleic acid 18:1n-9. The polyunsaturated (PUFA) fatty acids were dominated by 18:3n-3, 20:5n-3, and 22:6n-3 (Figure 2.2). The n-MDS with cluster analysis similarity contours had two distinct groupings at 80% similarity (Figure 2.3). The April 60 µm mesh size separated from the rest of the samples (Figure 2.3) PERMANOVA explained that there was a significant difference found in the fatty acid profiles and there was an interaction between mesh size and month (Table 2.1). The 500 µm mesh is only shown for June when predatory zooplankton species were collected; however, April, May and June had 60 and 200 µm mesh for comparison with PERMANOVA. The pairwise comparisons for the interaction with the factor of mesh size (60, 200 and 500 µm) showed differences in April between 60 and 200 µm, and June between 60 and 200 µm, and 60 and 500 µm, and 200 and 500 µm (Table 2.2). For the zooplankton species fatty acid profiles collected with the 200 µm, there were differences between April and May, April and June, and May and June (Table 2.3). The differences found for the zooplankton fatty acid profiles from 60 µm mesh were April and May, and April and June (Table 2.3). The PERMDisp showed that variation of the zooplankton fatty acid profiles from the centroid for within group differences by

month x mesh size was not significant. PERMANOVA showed that an interaction for month and mesh size when comparing percent composition of SFA, MUFA and PUFA was significant (Table 2.4). For the 60 µm mesh size, April and June had different significantly different concentrations of SFA, MUFA, and PUFA (Table 2.5). In April, there was a significance difference for SFA, MUFA, and PUFA between 60 and 200 µm mesh size (Table 2.6). The specific FA profiles driving these differences are presented across three months below.

April

Water Quality and Zooplankton composition

The salinity was 0.04 (± 0.01 S.D.) for the river sites (Figure 2.4a). The sound was not included in the fatty acid analysis because of a green algal bloom. Cyclopoida and *Bosmina* spp. comprised 70% of the zooplankton (200 µm) for all sites except site 01 which was dominated by Calanoida and Chydoridae (Figure 2.5). Rotifers and copepod nauplii comprised 90% of zooplankton (60 µm) abundance for all sites (Figure 2.6). Zooplankton species composition was different from Chapter One because these results are per site, whereas location was used for Chapter 1.

Fatty Acids

The similarities in fatty acid profiles between the 7 sites on the river are portrayed in n-MDS plot with similarity contour overlays from a hierarchical cluster analysis for the zooplankton (200 µm) and zooplankton (60 µm) samples for fatty acid profiles. The similarity between the zooplankton (200 µm) fatty acid profiles for all sites was at 80%, and sites 6 and 7 were separated from sites 1 to 5 at 90% (Figure 2.7). The zooplankton (60 µm) samples from sites 3 and 7 had a fatty acid profile 80% similar to the mesozooplankton (200 µm). The fatty acid profiles for zooplankton (60 µm) samples from sites 1, 2 and 4-6 are 80% similar with sites

1 and 2 separating from sites 4-6 at the 90% level (Figure 2.7). Each group had similarities of greater than 90% for the fatty acids. The SIMPER results for fatty acids that grouped sites 6 and 7 for the 200 µm mesh size were SFA (14:0 16:0, 18:0), MUFA (18:1n-9), and PUFA (22:6n-3, 20:5n-3) with similar percent composition (Appendix Table 2.1). The fatty acids that grouped sites 1-5 from the zooplankton (200 µm) were SFA (16:0), PUFA (20:5n-3, 18:3n-3, 22:6n-4, 18:4n-3) and MUFA (18:1n-9) with similar percent composition (Appendix Table 2.1). The fatty acids that drove the grouping of sites 3 and 7 for the zooplankton (60 µm) were SFA (16:0), MUFA (18:1n-9), and PUFAs (20:5n-3, 18:4n-3, 18:3n-3, 18:2n-6) with similar percent composition (Appendix Table 2.1). The fatty acids that grouped sites 4 to 6 for the zooplankton (60 µm) were SFA (16:0, 18:0), MUFA (18:1n-9), and PUFA (18:2n-6, 20:5n-3) (Appendix Table 2.1). The fatty acids that grouped 1 and 2 at the zooplankton (60 µm) were MUFA (18:1n-9), SFA (16:0, 18:0) and PUFA (18:2n-6) with similar percent composition (Appendix 2.1).

The zooplankton (200 µm) MUFA had the lowest percentage at all sites when compared to the percentage of SFA and PUFA. At sites 6 and 7, the zooplankton (200 µm) had a higher percentage of SFA, and a lower percentage of PUFA (Figure 2.8a). The zooplankton (200 µm) at sites 1 to 5 had a higher percentage of PUFAs, and a lower percentage of SFA (Figure 2.8a). The zooplankton (60 µm) at sites 3 and 7 had a similar composition for SFA, MUFA, and PUFA to the zooplankton samples (200 µm) from sites 1 to 5. For sites 4-6, the zooplankton (60 µm) had equal percentages of SFA, MUFA and PUFA (Figure 2.8b). The zooplankton (200 µm) for sites 1 and 2 had a higher percentage of SFA and MUFA (Figure 2.8b).

The fatty acid profiles from the river were evaluated with SIMPER to determine the largest dissimilarities between the fatty acids that were driving the site differences. Zooplankton (200 µm) at sites 1-5 had high levels of 18:3n-3, 18:4n-3 and 20:5n-3 (PUFA) whereas the

zooplankton (200 µm) at sites 6 & 7 had a high concentration of 18:1n-9 (MUFA) and 18:0 (SFA) (Appendix Tables 2.2 and 2.3). Fatty acids of zooplankton (60 µm) from sites 3 and 7 were similar to the mesozooplankton from sites 01 to 07. Zooplankton (60 µm) from sites 1, 2, and 4 to 6 had high levels of 18:1n-9 (MUFA) and 18:2n-6 (PUFA) compared to sites 3 to 7 (Appendix Table 2.2 and 2.3). Zooplankton (60 µm) from sites 4 to 6 had higher 18:1n-7 (MUFA), 22:6n-3, 20:5n-3 and 18:4n-3 (PUFA) compared to site 1 and 2 (Appendix Tables 2.2 and 2.3). The zooplankton (200 µm) at all sites and the zooplankton (60 µm) at sites 3 and 7 had higher amounts of 20:5n-3, 22:6n-3, and 18:3n-3 (PUFA) then the rest of the zooplankton (60 µm) samples (Appendix Tables 2.2 and 2.3).

May

Water Quality and Zooplankton Composition

In May, the salinity was averaged between surface and bottom for each site because of a salt wedge that formed at 3 meters. The highest salinity was 7.68 for the bottom reading at site 9. The average salinity in the sound was 2.59 (± 1.86 S.D.) (Figure 2.4b). The average salinity near the mouth of the river was 1.07 (± 0.10 S.D.), and the rest of the river was 0.19 (± 0.14 S.D.) (Figure 2.4b). By site 2, the salinity had returned to zero. The zooplankton composition changed over locations from dominant brackish water species to freshwater species. *Acartia* spp. comprised >70% of the zooplankton (200 µm) for sites 3 to10. *Bosmina* spp., *Calanoida,* and *Eragasilus caeruleus* dominated sites 1 and 2 (Figure 2.9). *Leptodora* spp. dominated site 1 (Figure 2.7). Rotifers and copepod nauplii comprised 90% of abundance for the zooplankton (60 µm) at all sites (Figure 2.10). Zooplankton species composition was different from Chapter One because these results are per site, compared to by location.

Fatty Acids

The zooplankton samples for fatty acid analysis were only taken at four sites in May. The similarities between the 4 sites on the river are portrayed in n-MDS plot with similarity contour overlays from the cluster analysis for the zooplankton (200 µm) and zooplankton (60 µm) samples for fatty acid profiles. The similarity among the zooplankton (200 µm) fatty acid profiles for sites 4, 7, and 10 was at 87% (Figure 2.11). The fatty acid profiles for zooplankton (60 µm) samples from sites 4 and 10, and zooplankton (200 µm) for site 1 are 87% similar (Figure 2.11). The SIMPER results found similar fatty acid profiles at sites 4, 7, and 10 from zooplankton (200 µm) and the dominant fatty acids were SFA (16:0, 18:0), PUFA (22:6n-3, 20:5n-3), and MUFA (16:1n-7) (Appendix Table 2.4). Site 1 zooplankton (200 µm) and sites 4 and 10 zooplankton (60 µm) had similar percentages of SFA (14:0, 16:0,18:0) PUFA (20:5n-3), and MUFA (16:1n-7, 18:1n-9) (Appendix Table 2.4). The zooplankton (200 µm) had a low percentage of MUFA at all sites and higher percentage of PUFA at sites 7 and 10 (Figure 2.12a). The zooplankton (200 µm) at sites 1 and 4 had a lower percentage of PUFAs that equaled the percentage of SFA (Figure 2.12a). The zooplankton (60 µm) had a higher percentage of SFA at site 10 and an equal percentage of PUFA and SFA at site 4 (Figure 2.12b).

The fatty acid profiles from the river and sound were evaluated with SIMPER to determine the largest dissimilarities between the fatty acids that were driving the site differences. Zooplankton (200 µm) at sites 4, 7, and 10 had higher levels of 22:6n-3 and 20:5n-3 (PUFA), whereas zooplankton (200 µm) at site 1 had a higher concentration of 18:1n-9 (MUFA) and 18:2n-6 (PUFA) (Appendix Tables 2.5 and 2.6). Zooplankton (60 µm) at site 4 had high concentration of 18:1n-9 (MUFA), whereas zooplankton (60 µm) at site 10 had high levels of 16:1n-7 (MUFA) (Appendix Tables 2.5 and 2.6).

June

Water Quality and Zooplankton Composition

The salinity was 0.06 (± 0.02 S.D.) for the seven river sites (Figure 2.4c). The sound was not included in sampling because of strong winds. The salinity returned to zero after Tropical Storm Andrea, and two weeks of rainfall. There was a record rainfall with 15.2 to 19.05 cm in June making it the second wettest June since 1895 (Hiatt 2013). The heavy precipitation caused the zooplankton community to change over a two week period. *Leptodora* spp. dominated the zooplankton (500 µm) and a mixed assemblage of copepods and cladocerans dominated the zooplankton (200 µm), depending on site. The dominant zooplankton (200 µm) was Calanoida, at site 7, but site 6 and 5 were dominated by *Diaphanosoma* spp. and Cyclopoida (Figure 2.13). Rotifers were dominant at site 4 and sites 1, 2, and 3 were dominated by *Bosmina* spp. and *Bosminopsis* spp. (Figure 2.13). Rotifers and copepod nauplii comprised the zooplankton (60 µm) at all sites (Figure 2.14). Zooplankton species composition was different from Chapter One because these results are per site, compared to by location.

Fatty Acids

The similarities of fatty acid percent composition among the 7 sites on the river are portrayed in n-MDS plot with similarity contours from cluster analysis overlays for the zooplankton (500 µm), zooplankton (200 µm) and zooplankton (60 µm) samples for fatty acid profiles. The similarity of fatty acid profiles between the zooplankton (500 µm) was 90% (Figure 2.14). The zooplankton (200µm) from site 3 was included in the zooplankton (500 µm) 90 similarity contour (Figure 2.15). The fatty acids that grouped the zooplankton (500 µm) and zooplankton (200 µm) from site 3 were SFA (16:0, 18:0) MUFA (16:1n-7, 18:1n-9) and PUFA

(20:5n-3, 18:3n-3) (Appendix Table 2.7). The zooplankton (200 µm) for sites 4, 5, and 6, and the zooplankton (60 µm) from sites 3-7 fatty acid profiles were 90% similar (Figure 2.15). The fatty acids that grouped zooplankton (200 µm) from sites 4-6 and zooplankton (60 µm) from sites 3-7 using SIMPER were SFA (14:0, 16:0, 18:0), PUFAs (22:6n-3, 20:5n-3, 18:3n-3) and MUFA (16:1n-7) (Appendix Table 2.7). The zooplankton (200 µm) and zooplankton (60 µm) fatty acid profiles from sites 1 and 2 were clustered together at 90% similarity (Figure 2.15). The fatty acids that grouped zooplankton (200 µm) and zooplankton (60 µm) from sites 1 and 2 were SFA (14:0, 16:0, 18:0), MUFA (16:1n-7) and PUFAs (20:5n-3, 18:3n-3) (Appendix Table 2.7).

Overall for the zooplankton (500 µm), zooplankton (200 µm) and zooplankton (60 µm), the monounsaturated fatty acids (MUFA) had the lowest percentage at all sites. The zooplankton (500 µm) had similar percentages of SFA, MUFA, and PUFA at all the sites (Figure 2.16a). The zooplankton (200 µm) had a higher percentage of PUFA and a lower percent of SFA at sites 4-6 (Figure 2.16b). The zooplankton (200 µm) had similar percentages of SFA, MUFA, and PUFA at site 3 (Figure 2.16b). The zooplankton (200 µm) at sites 1 and 2 had a higher percentage of SFA, and a lower percentage of PUFA (Figure 2.16b). The zooplankton (60 µm) had higher percentage of PUFAs at sites 3-7 and a higher percentage of SFA at sites 1 and 2 (Figure 2.16c).

The fatty acid profiles from the river were evaluated with SIMPER to determine which fatty acids were causing the largest differences. Zooplankton (500 µm) at sites 2 to 7 and site 3 for zooplankton (200 µm) had a higher percentage of 18:1n-9 (MUFA) and 20:5n-3 (PUFA) compared to zooplankton (200 µm) at sites 4 to 6 and zooplankton (60 µm) at sites 3-7 that had a higher percentage of 22:6n-3, 22:5n-6 and 22:5n-3 (PUFAs) (Appendix Tables 2.8 and 2.9). Zooplankton (200 µm) at sites 4 to 6 and zooplankton (60 µm) at sites 3-7 had higher

percentages of 22:6n-3, 22:5n-6 and 22:5n-3 (PUFAs) compared to zooplankton 60 and 200 µm) at sites 1 and 2 that had higher percentages of 16:1n-7 (MUFA) and 16:0 (SFA) (Appendix Tables 2.8 and 2.9). Zooplankton (500 µm) at sites 2 to 7 and zooplankton (200 µm) at site 3 had higher percentages of 18:1n-9 (MUFA) and 20:5n-3 (PUFA) compared to zooplankton (60 and 200 µm) at sites 1 and 2 that had higher percentages of16:1n-7 (MUFA), 16:0 (SFA), and 22:6n-3 (PUFA) (Appendix Tables 2.8, 2.9 and 2.10).

Discussion

The fatty acid profiles of zooplankton varied considerably over time and space. As demonstrated in Chapter 1, the zooplankton species composition changed in response to environmental conditions and the fatty acids profiles of the zooplankton appear to mirror the community composition changes. There is no data at the present time to explain how this variable distribution of zooplankton nutritional quality may relate to larval river herring; however, by examining diets, some indication of the dietary fatty acid acquisition of a larval river herring can be attempted. At first feeding, larval river herring consume rotifers and smaller bodied cladoceran (microzooplankton) that have lower PUFAs compared to larger bodied zooplankton. The saltwater intrusion event of May resulted in a brackish water system that changed the composition and fatty acid profiles of the zooplankton. The amount of DHA in the system increased due to the presence of a dominant brackish water copepod species, *Acartia* spp. Overall, fatty acids that were higher in PUFAs (DHA and EPA) came to characterize the community during the salinity increase in May. In June, after two weeks of continuous precipitation and subsequent salinity decrease, the zooplankton in the areas of saltwater intrusion still had similar fatty acids found during the May intrusion event. This indicated that the impact of the salinity intrusion event on the fatty acid signatures of the zooplankton appeared to extend

beyond the intrusion event. The change in the overall fatty acid composition over the spring period suggests that larval river herring may experience a range of prey items that vary considerably in fatty acid composition. Therefore, the fatty acid profiles of the zooplankton prey field may have considerable influence of the growth and development of larval river herring.

A dry period and strong southern winds in May resulted in a salt intrusion into the western sound and Chowan River. The brackish water samples (sites 4, 7, and 10) had higher levels of PUFAs compared to freshwater samples. Arts et al. (2009) described that phytoplankton from marine systems have higher PUFAs than freshwater phytoplankton. Since very little is known about fatty acids in coastal systems, the extensive data from the marine system are used here to compare the freshwater zooplankton samples to brackish water. Many freshwater fish and zooplankton can synthesize longer chain fatty acids (such as EPA, DHA, and ARA) if they are provided with the precursors. However, most marine fish and zooplankton cannot synthesize these longer chain fatty acids, thus they need to obtain them in their diet (Arts et al. 2009). Since freshwater zooplankton have the ability to convert lower chain fatty acids to higher chains (Arts et al. 2009), presence of ALA (18:3n-3) could be converted to EPA (Kainz et al. 2004). The relative proportion of the shorter chain FA precursors and the longer chain FA varied over the sampling period and the consequences of this variation is explained below.

In April, the microzooplankton were dominated by rotifers had higher amounts of the monounsaturated 18:1n-9 and polyunsaturated fatty acid 18:2n-6 (LIN). The 18:1n-9 is a significant component of MUFAs in most algae classes except for the Chrysophyceae and Cryptophyceae (Desvilettes et al. 1997). The fatty acid 18:2n-6 (LIN) has been related to an increased consumption of terrestrial driven particulate organic matter before and after phytoplankton blooms (Napolitano et al. 1997). Ciliates possessing this FA can be distinguished

from particulate matter, indicating a dietary link between phytoplankton derived detritus and the microbial loop (Desvilettes et al. 1997). This finding indicates that early in the year, a detrital pathway for zooplankton fatty acid acquisition likely exists. Such a finding is not surprising given the large amount of terrestrial derived organic matter in coastal plain estuaries (Alfaro et al. 2006). There was an increase in EPA and a decrease in all other fatty acids in microzooplankton that dominanted sites 3 and 7. This suggests a diatom, dinoflagellates, and/or Cryptophyceae diet as the major PUFA in diatoms, dinoflagellates and Cryptophyceae is EPA (Desvilettes et al. 1997). During the freshwater period throughout April in the river, the dominant species in the mesozooplankton were Cyclopoida and *Bosmina* spp. having a high percent of EPA and DHA (Figure 2.2). Persson and Vrede (2006) speculated that the higher EPA found in cladocerans was related to the higher potential for reproduction compared to copepods, but more research is needed to clarify the function of EPA in cladocerans. The fatty acids ARA and EPA are precursors of eicosanoids which are locally acting hormones and have several functions in reproduction, ion and water transport and the immune system in invertebrates and veretebrates (Persson and Vrede 2006). The presence of DHA is representative of the copepod dominance in the zooplankton species composition since DHA is found in low levels in cladocerans (Arts et al. 2009).

In May, rotifers still dominated with a small increase in copepod nauplii. In the two sites that fatty acid profiles were analyzed during May, the PUFA concentrations had an overall increase compared to the April rotifer fatty acid profiles. Phytoplankton in marine systems have higher PUFA concentrations then those typically found in freshwater systems (Arts et al. 2009). This fact, combined with the increasing salinity, suggests that salt-tolerant rotifers were present and the zooplankton community was acquiring its fatty acids from phytoplankton sources, as

opposed to detrital pathways. The dominance of the brackish water copepod *Acartia* spp. also explains the increase in PUFA concentration. *Acartia* spp. adults and nauplii had high levels of EPA and DHA as demonstrated by laboratory studies conducted to determine the best phytoplankton for *Acartia* spp. growth and development (Stottrup et al. 1999). In May, the zooplankton species composition shifted because the majority of cladocerans were negatively affected by salinity except for *Bosmina* spp. which can be found in salinity levels of 3 or below (Chapter One). The dominant zooplankton species switched to a brackish water species, *Acartia* spp. in the mesozooplankton except site 1 which was not affected by the salt intrusion. DHA is known for being associated with neural tissue (Persson and Vrede 2006). Since copepods have a highly developed nervous system that supports prey capture, predator avoidance, food selection, and mate finding, DHA appears critical for copepod survival (Arts et al. 2009). The dominant macrozooplankton was *Leptodora* spp., predatory cladoceran, at site 1. The fatty acid profile for this site had a sharp increase in EPA, and a dramatic drop in DHA compared to the brackish water areas (sites 4, 7, and 10) (Figure 2.2). The carnivorous cladoceran *Leptodora* has been shown to possess higher levels of PUFAs compared to omnivorous copepods and herbivorous cladoceran since the food consumed is higher in PUFAs, especially EPA (Arts et al. 2009).

Leptodora spp. were dominant for the macrozooplankton at the all sites except site 1 in June and the fatty acid profiles reflected this, consisting of increased PUFAs (especially EPA) and a drop in DHA. DHA has been found to be absent in Daphniidae, a group of cladocerans that show low levels of DHA even when fed diets rich in DHA (Von Elert 2002, Persson and Vrede 2006, Kainz et al. 2009 and Masclaux et al. 2012). The microzooplankton and mesozooplankton for sites 3 -7 had an increase in copepod nauplii, Cyclopoida ,and Calanoida which resulted in an increase in DHA and it appeared that the impact from the salt water intrusion on the zooplankton

community in May persisted into June (Figure 2.2). Rotifers were still the dominant microzooplankton at all sites in June as indicated by the high percentage of 18:3n-3 and higher chain PUFAs (EPA and DHA). Kennari et al. (2008) reported that freshwater rotifers fed microalgae had 18:2n-6, 18:3n-3 and 18:1n-9 as dominant fatty acids, and this demonstrated that rotifers converted 18:3n-3 to 20:5n-3 from microalgae low in EPA. The fatty acids were dominated by EPA at sites 1 and 2 due to the presence of cladoceran species and this was also associated with a decline in the percentage of DHA (Arts et al. 2009). Since the salt intrusion never moved to the upper river sites, there were no remnants of the higher PUFAs in the upriver zooplankton population.

The present study shows that the zooplankton fatty acid profiles of a transition zone in an estuarine system are similar to other freshwater and estuarine systems. Goncalves et al (2009) determined that zooplankton fatty acids changed over seasons as the salinity gradient changed the potential food items of zooplankton. Their results showed that fresh and brackish water species had higher MUFAs, SFAs, and PUFAs compared to marine systems and this was related to the larger variety of phytoplankton and microorganisms compared to the marine systems (Goncalves et al. 2009). The data on fatty acid profiles for the different zooplankton species from the Mondego estuary were similar to the results from the Albemarle Sound and Chowan River. *Acartia* spp. had high levels of DHA and EPA compared to freshwater herbivorous cladoceran species (Goncalves et al. 2009).

Zooplankton in the Chowan River and Albemarle Sound have fatty acid profiles that allow for the growth and development of the zooplankton species present. More research is needed to determine if the fatty acid quantities were appropriate for zooplankton growth and development. The precursor fatty acids (18:3n-3 and 18:2n-6) are present and can be converted

to higher chain fatty acids in freshwater zooplankton, i.e. rotifers, cladocerans, and copepods. The zooplankton in the Albemarle Sound and Chowan River had higher chain fatty acids (EPA, DHA, and ARA) that can be passed on to predators, especially larval fish. Though the study cannot say definitively that the zooplankton community provides all of the essential fatty acid nutrition to larval river herring, my results suggest this to be the case.

The fitness and food source of larval fish can be determined by fatty acid analysis. An example from the Catalan Coast shows that larger, larval anchovy consumed zooplankton that consumed diatoms and dinoflagellates that were rich in 16:1(n-7) and 22:6(n-3) compared to the smaller, larval anchovy that consumed ciliates and flagellates that had high concentration of 18:1(n-9) and 18:4(n-3) (Rossi et al. 2006). The presence of PUFAs, especially DHA and EPA, have been shown to increase development and fitness in fish and PUFAs accumulate in brain, nervous system, and retina tissue (Rossi et al. 2006). If PUFAs are not present in the diet, then brain development can be affected, which in turn can lead to changes in schooling behaviors (Ishizaki et al. 2001). The amount and type of fatty acids found in the larval fish can relate to recruitment and determine if certain habitat areas show differences in larval survival and growth (Bell & Sargent 1996). Freshwater fish can synthesize long chain PUFA from precursor fatty acids, but marine fish have lost the ability to synthesize PUFAs, presumably because there were larger quantities in the food sources (Arts et al. 2009). The degree to which different fish species can accumulate particular fatty acids and if the fatty acids can be elongated or desaturated is a complex topic in research. Even though no fish were collected during the field sampling, the fatty acids present in the zooplankton, with both PUFAs and some of the precursors of longer chain fatty acids present, suggest that FA present appear adequate for the needs of larval fish. Research is needed to determine how the fatty acids from the zooplankton found in the Chowan

River and Albemarle Sound effect the larval river herring during the critical life stages of first feeding and migrating through the sound to the ocean (See Chapter 3).

Table 2.1: PermANOVA for square root of zooplankton fatty acid profiles by Mesh size and Month. * indicates significant results.

Source	DF	Sum of Squares	Pseudo-F- ratio	Prob > F
Mesh size	2	8.26.05	7.4214	0.001*
Month	2	1725.4	15.502	0.001*
Mesh size x Month	2	694.05	6.2355	0.001*
Residual	32	1780.9	-	-
Total	38	5496.9	-	-

Table 2.2: Pairwise comparison of the Mesh size x Month of zooplankton fatty acid profiles for the factor of mesh size. * indicates significant results.

Month	Comparison	t-value	P(perm)
April	200 µm vs. 60 µm	3.83	0.001*
May	200 µm vs. 60 µm	1.42	0.144
June	200 µm vs. 60 µm	2.10	0.011*
	200 µm vs. 500 µm	1.97	0.001*
	60 µm vs. 500 µm	3.33	0.001*

Table 2.3: Pairwise comparison of the Mesh size x Month of zooplankton fatty acid profiles for the factor of month. * indicates significant results.

Mesh Size	Comparison	t-value	P(perm)
200 µm	April vs. May	1.94	0.008*
	April vs. June	2.19	0.01*
	May vs. June	2.02	0.024*
60 µm	April vs. May	2.38	0.028*
	April vs. June	5.13	0.001*
	May vs. June	1.49	0.108

Table 2.4: PermANOVA for square root of zooplankton fatty acid sums of SFA, MUFA, and PUFA by Mesh size and Month. * indicates significant results.

Source	DF	Sum of Squares	Pseudo-F- ratio	Prob > F
Mesh size	2	42.0	1.0176	0.386
Month	2	80.6	1.9516	0.147
Mesh size x Month	2	151.1	3.6607	0.023*
Residual	32	660.4	-	-
Total	38	929.0	-	-

Table 2.5: Pairwise comparison of the Mesh size x Month of zooplankton fatty acid sums of SFA, MUFA and PUFA for the factor of mesh size. * indicates significant results.

Month	Comparison	t-value	P(perm)
April	200 µm vs. 60 µm	2.25	0.035*
May	200 µm vs. 60 µm	1.34	0.199
June	200 µm vs. 60 µm	0.94	0.391
	200 µm vs. 500 µm	0.89	0.461
	60 µm vs. 500 µm	1.18	0.300

Table 2.6: Pairwise comparison of the Mesh size x Month of zooplankton fatty acid profiles of SFA, MUFA, and PUFA for the factor of month. * indicates significant results.

Mesh Size	Comparison	t-value	P(perm)
200 µm	April vs. May	0.41	0.756
	April vs. June	1.13	0.288
	May vs. June	1.08	0.298
60 µm	April vs. May	0.87	0.569
	April vs. June	2.46	0.034*
	May vs. June	1.42	0.190

Figure 2.1: The ten sites used to compare fatty acid profiles of zooplankton for the Albemarle Sound and Chowan River.

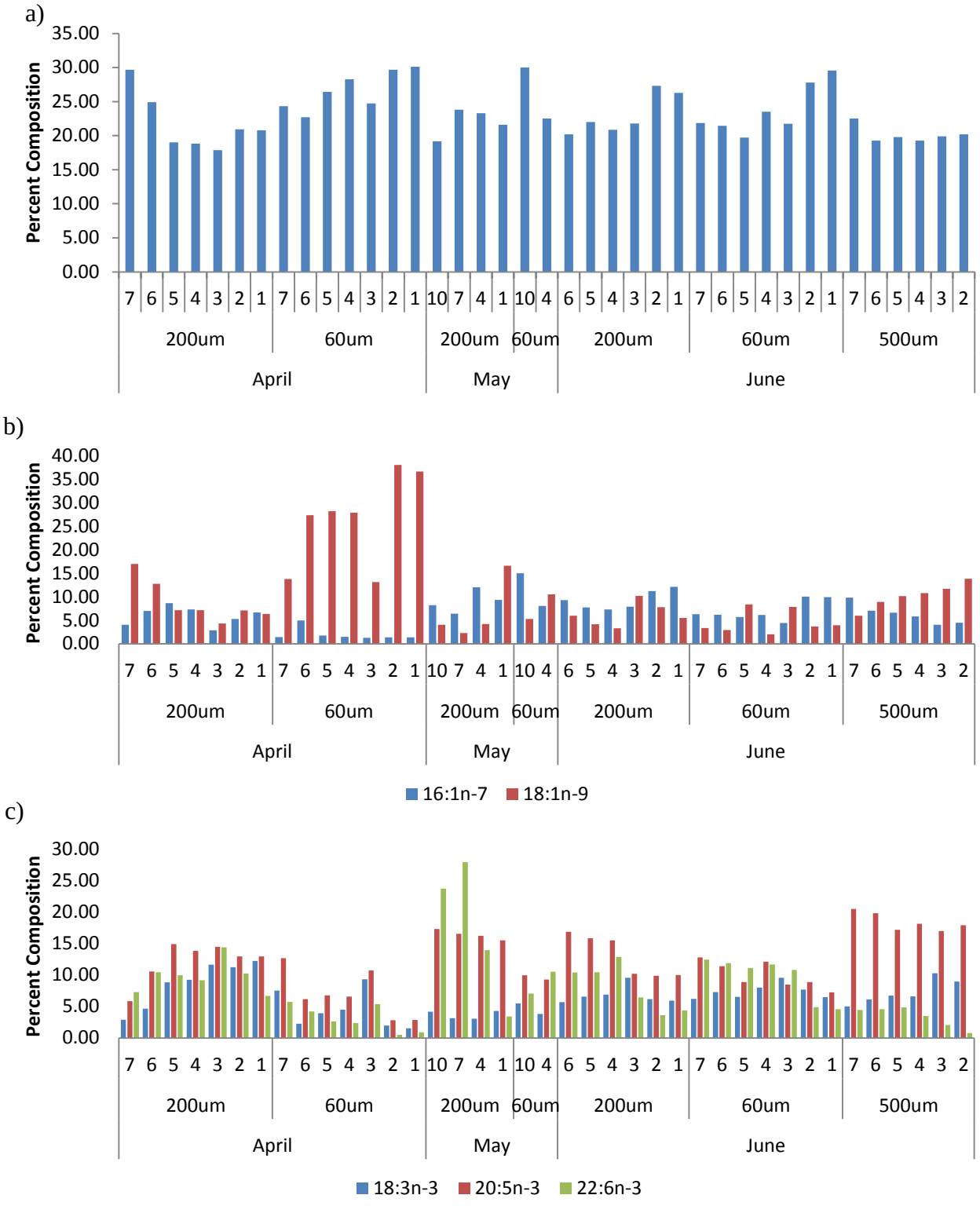


Figure 2.2: The dominant SUFA (16:0) (a), MUFA (b), and PUFA (c) by percent composition for the zooplankton by size class, month and site.

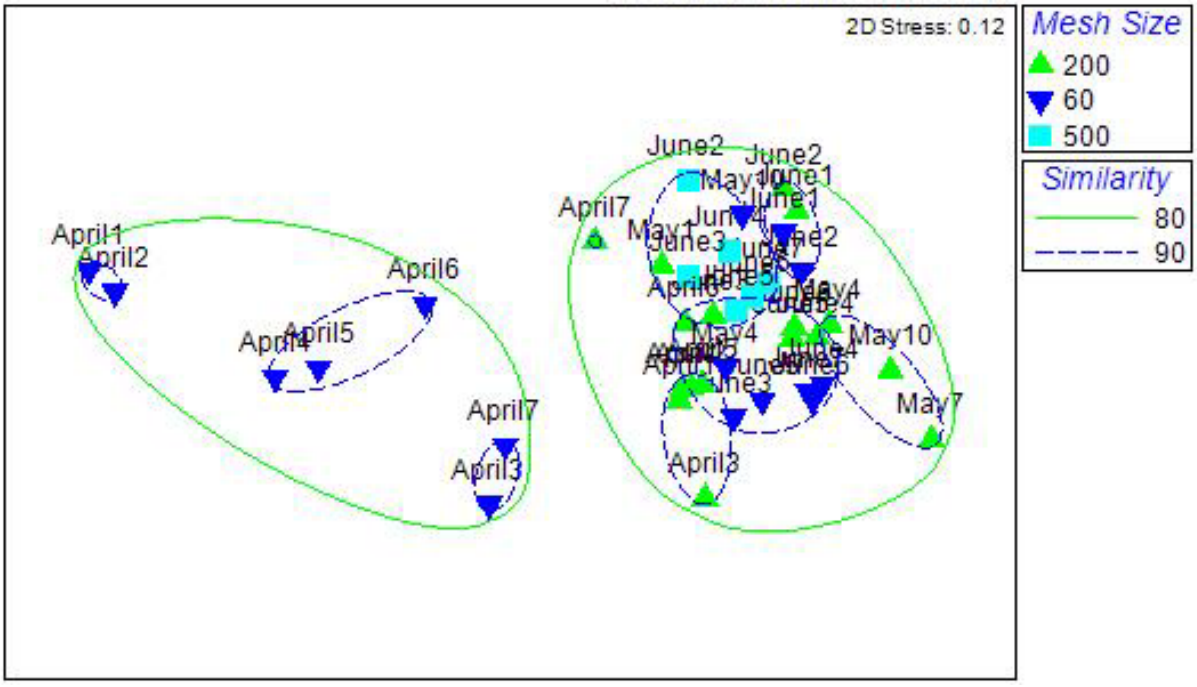


Figure 2.3: n-MDS plot showing similarity in fatty acid profiles of zooplankton at three mesh sizes (60, 200 & 500 µm) for all sites on the river from April, May, and June. Solid lines show clusters at 80%, and the dash lines show clusters at 90%.

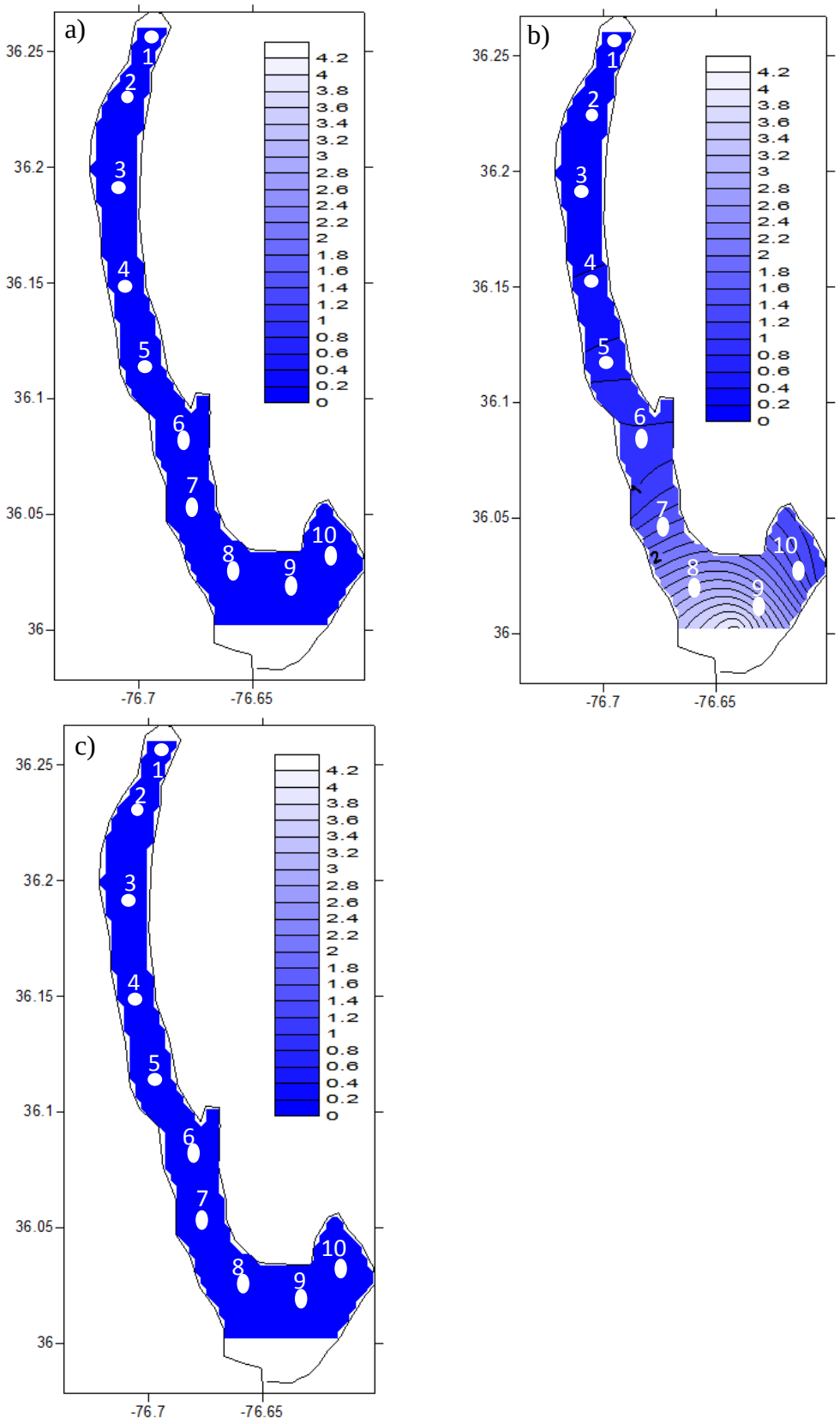


Figure 2.4: Average salinity readings for the western Albemarle Sound and Chowan River for April (a), May (b), and June (c) in 2013.

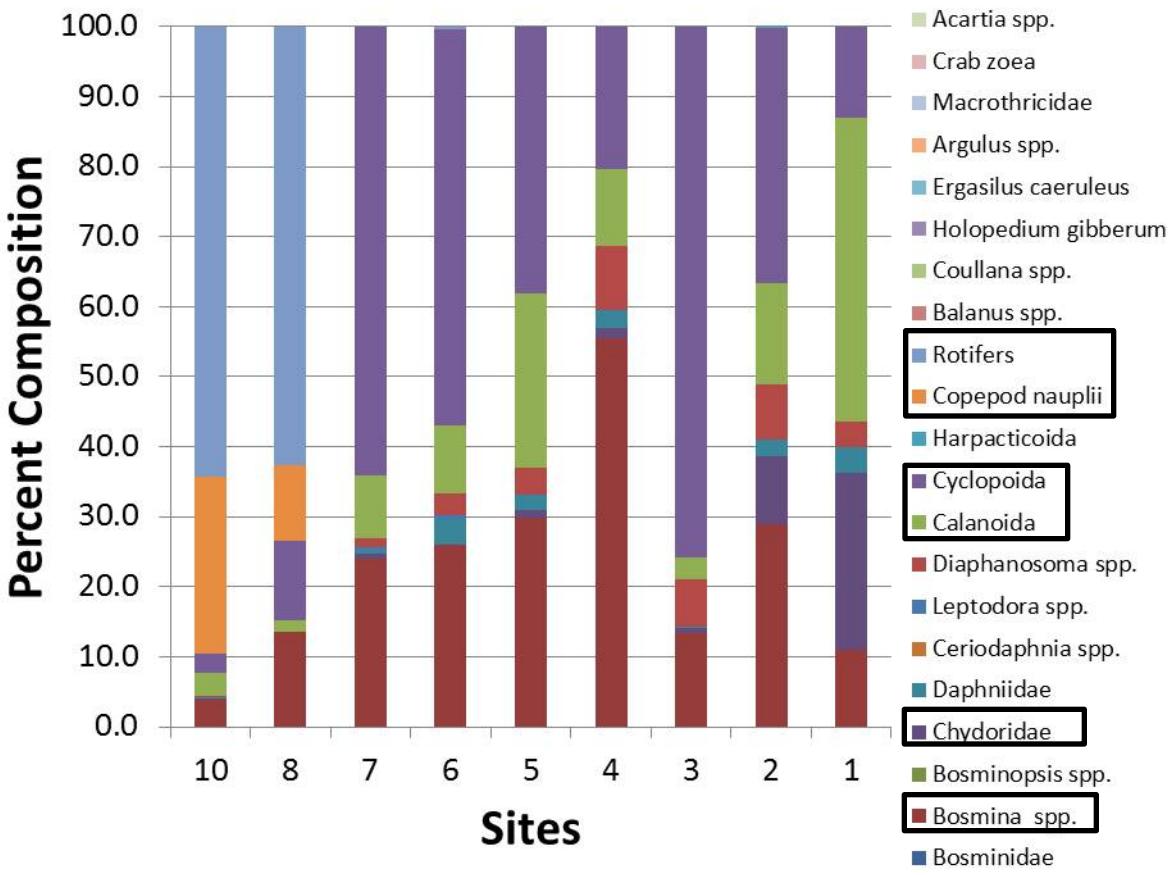


Figure 2.5: Percentage of samples composed of observed taxonomic groups in 200 µm mesh nets by sites for April. The dominant species have a box around them.

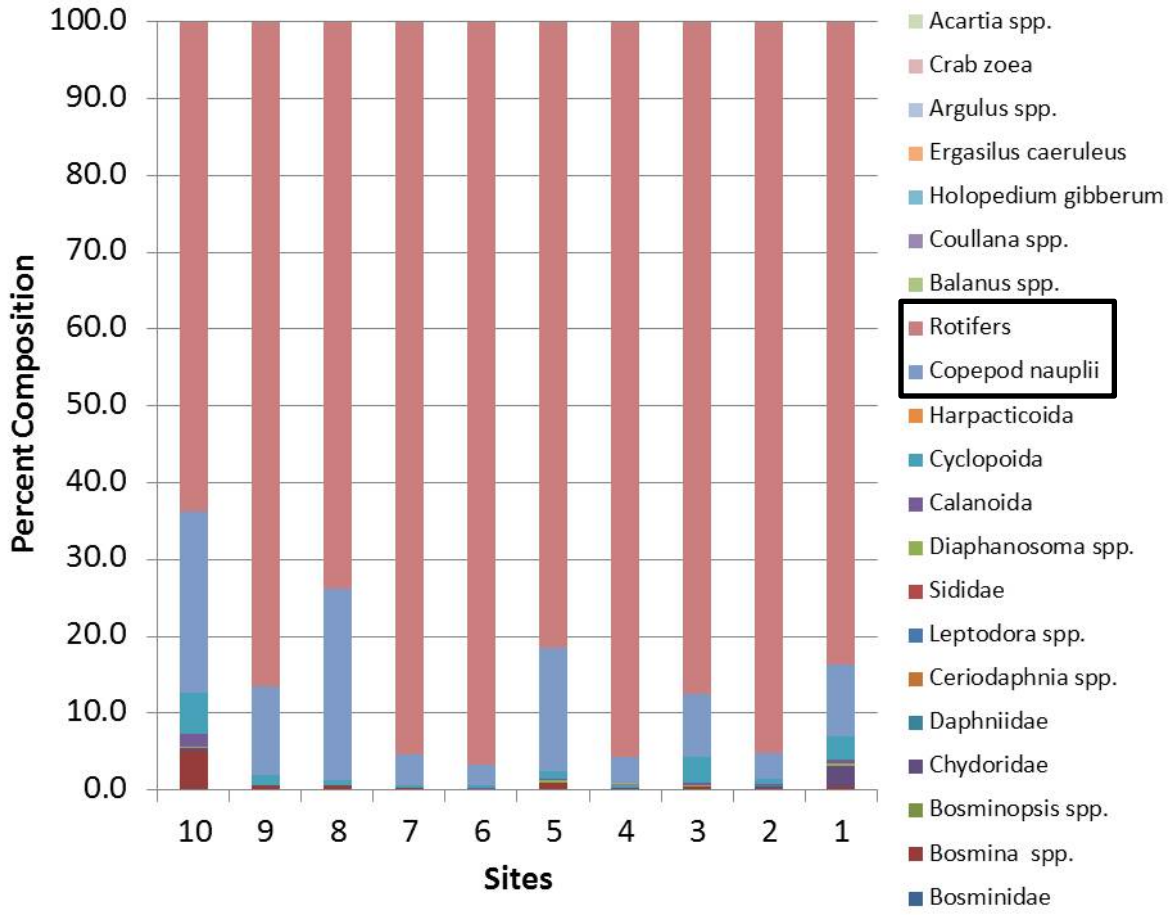


Figure 2.6: Percentage of samples composed of observed taxonomic groups in 60 µm mesh nets by sites for April. The dominant species have a box around them.

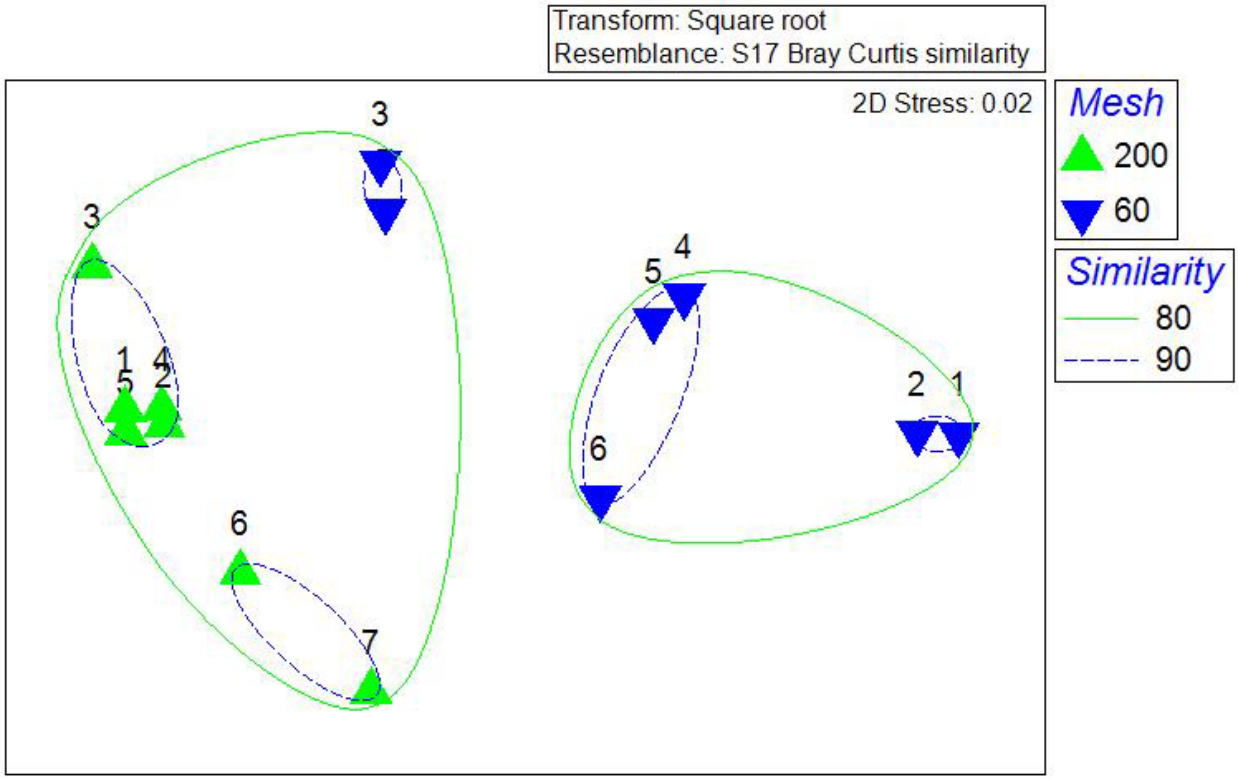
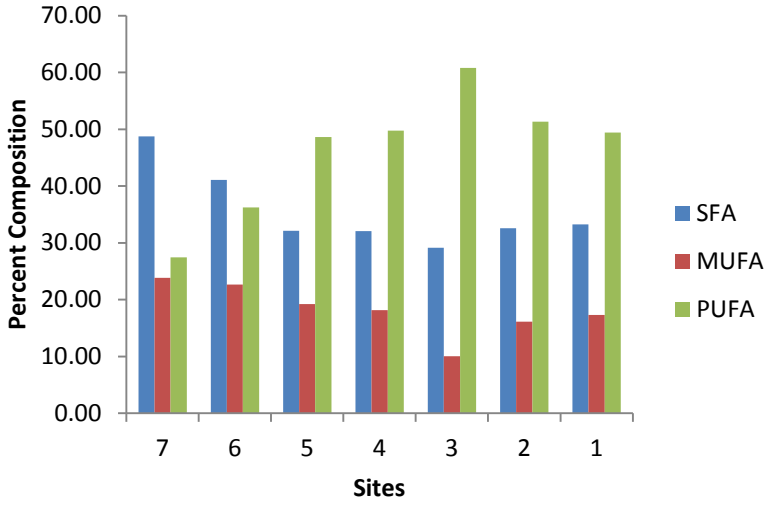


Figure 2.7: n-MDS plot showing similarity in fatty acid profiles of zooplankton at two mesh sizes (60 & 200 µm) for all sites on the river in April. Solid and dash lines show clusters at 80% and 90%.

a)



b)

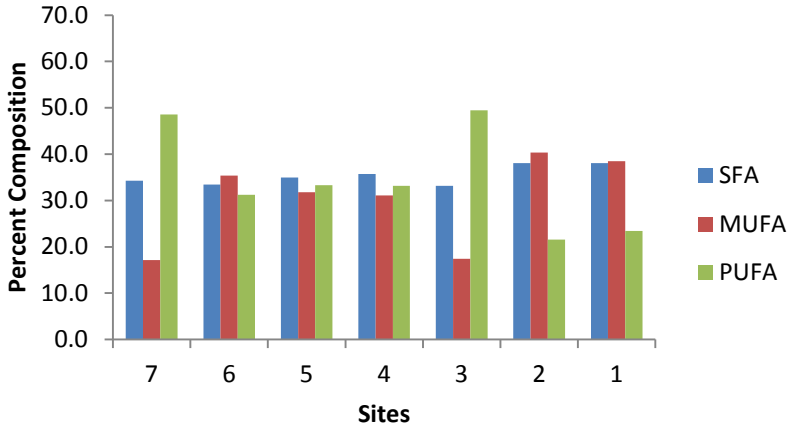


Figure 2.8: The percent composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid of zooplankton from April for the 200 µm mesh (a), and 60 µm mesh (b).

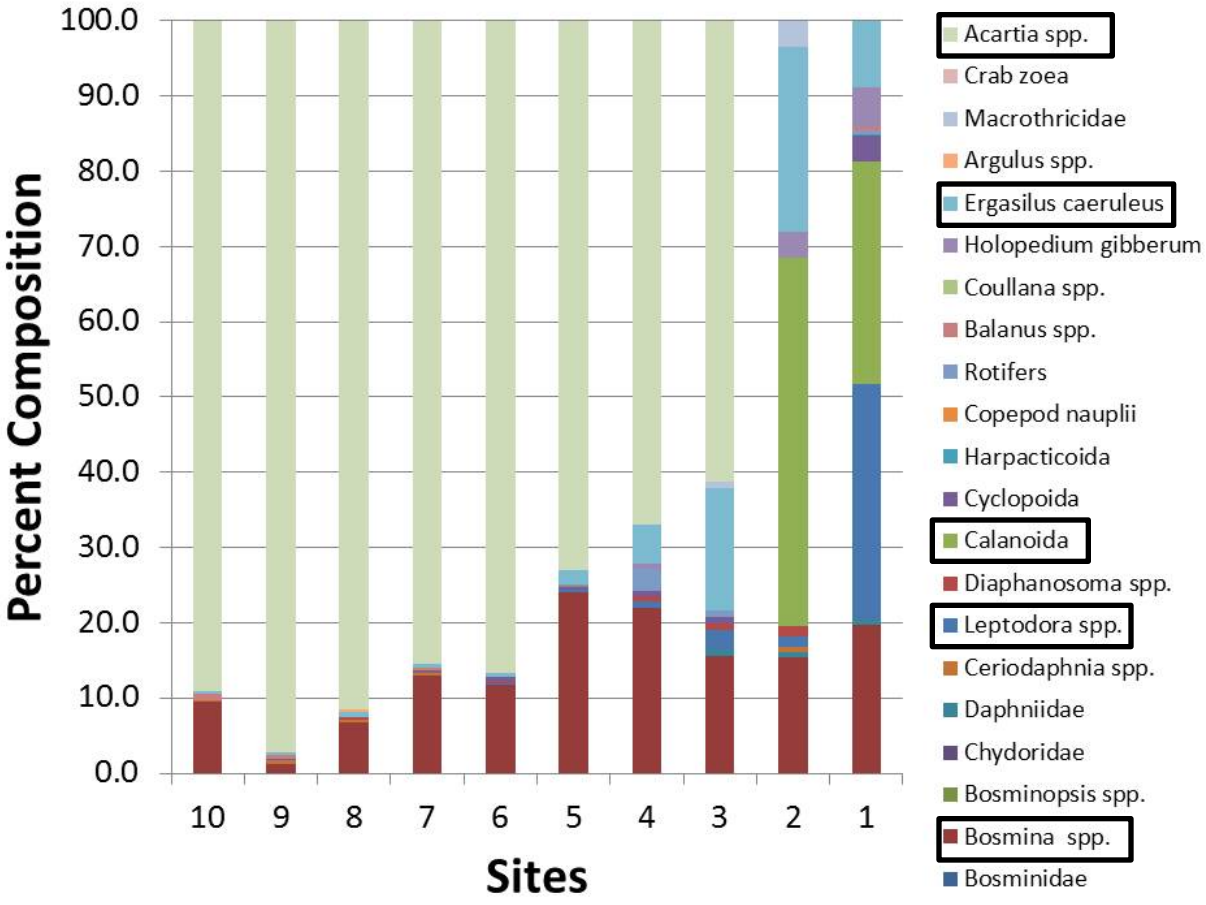


Figure 2.9: Percentage of samples composed of observed taxonomic groups in 200 µm mesh nets by sites for May. The dominant species have a box around them.

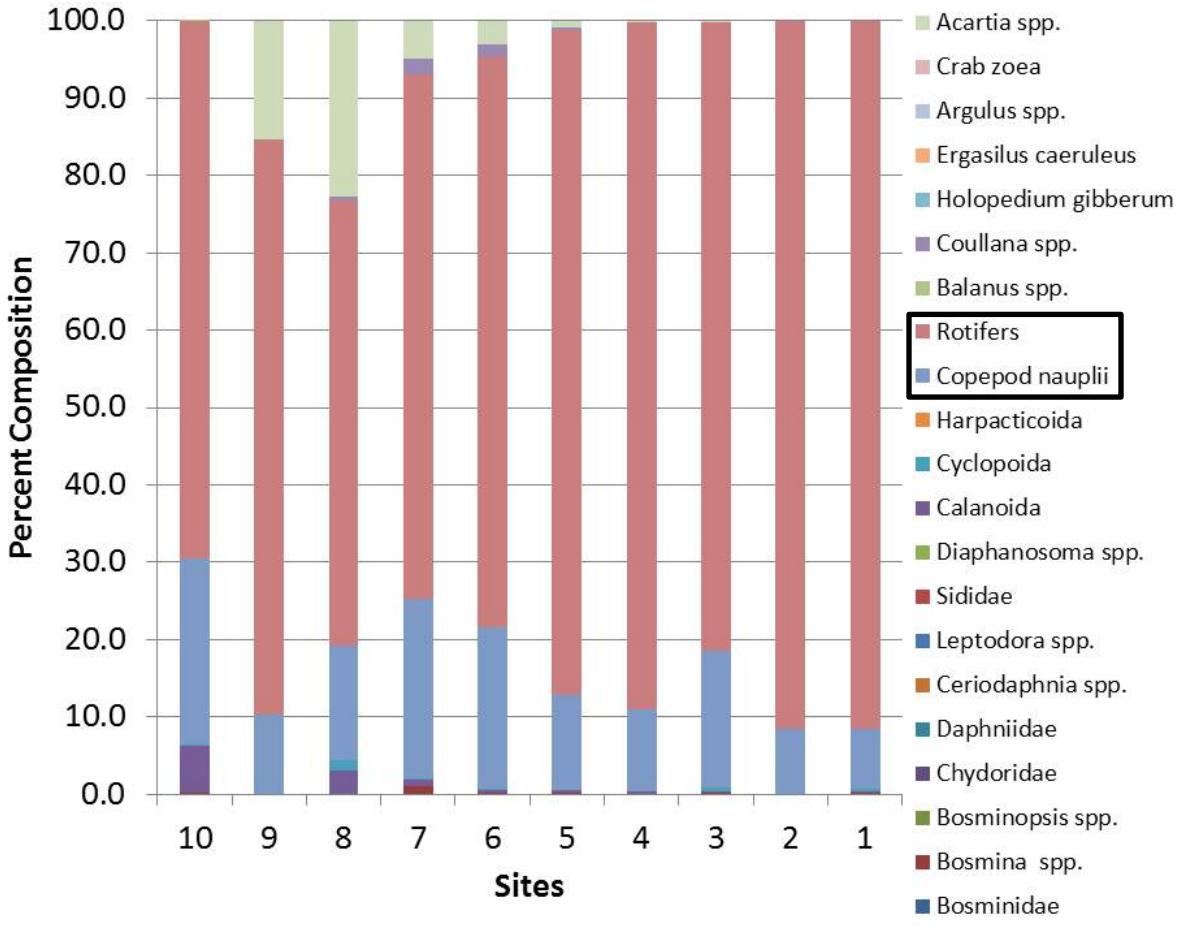


Figure 2.10: Percentage of samples composed of observed taxonomic groups in 60 µm mesh nets by sites for May. The dominant species have a box around them.

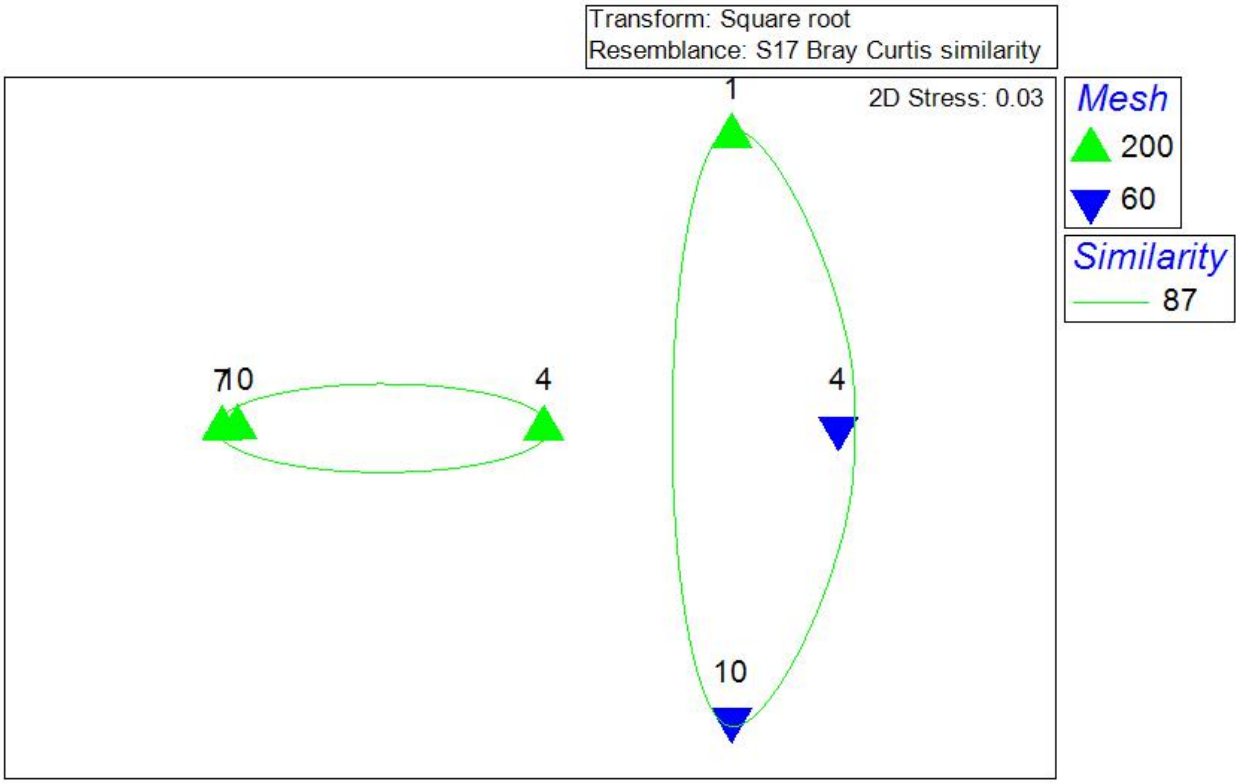


Figure 2.11: n-MDS plot showing similarity in fatty acid profiles of zooplankton at 200 µm mesh sizes (60 & 200 µm) for Sites 1, 4, 7 & 10 and 60 µm mesh size for Sites 4 and 10 on the river and sound. Solid lines show clusters at 87%.

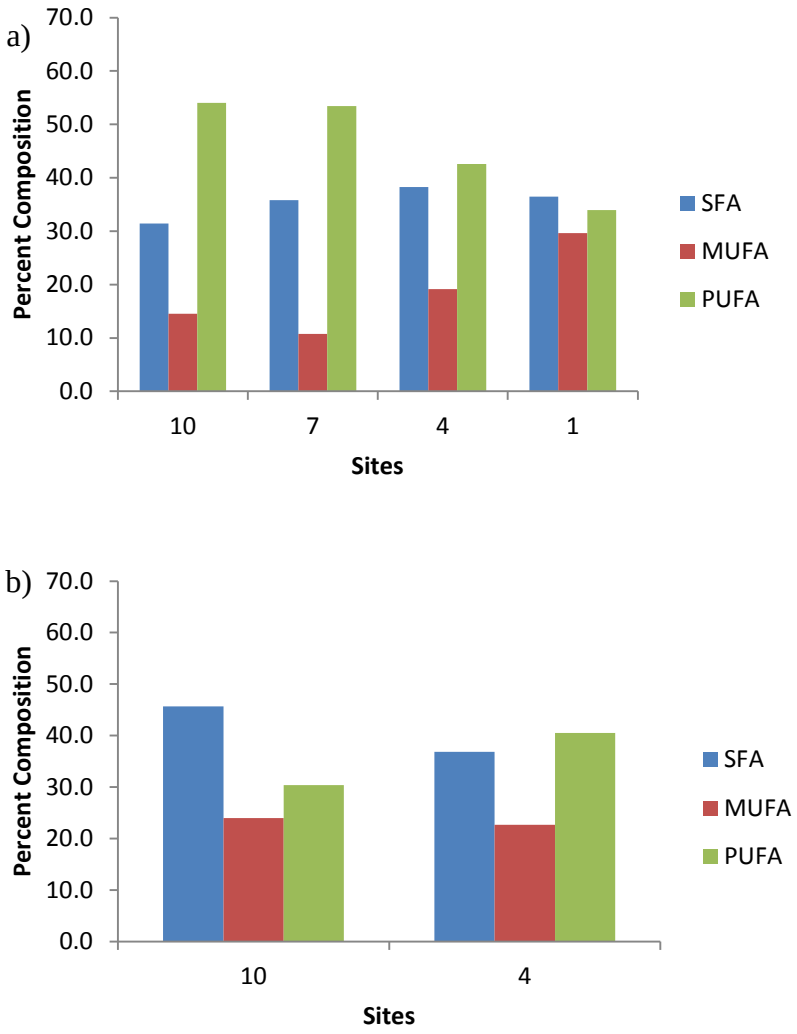


Figure 2.12: The percent composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid of zooplankton from May for the 200 µm mesh (a), and 60 µm mesh (b).

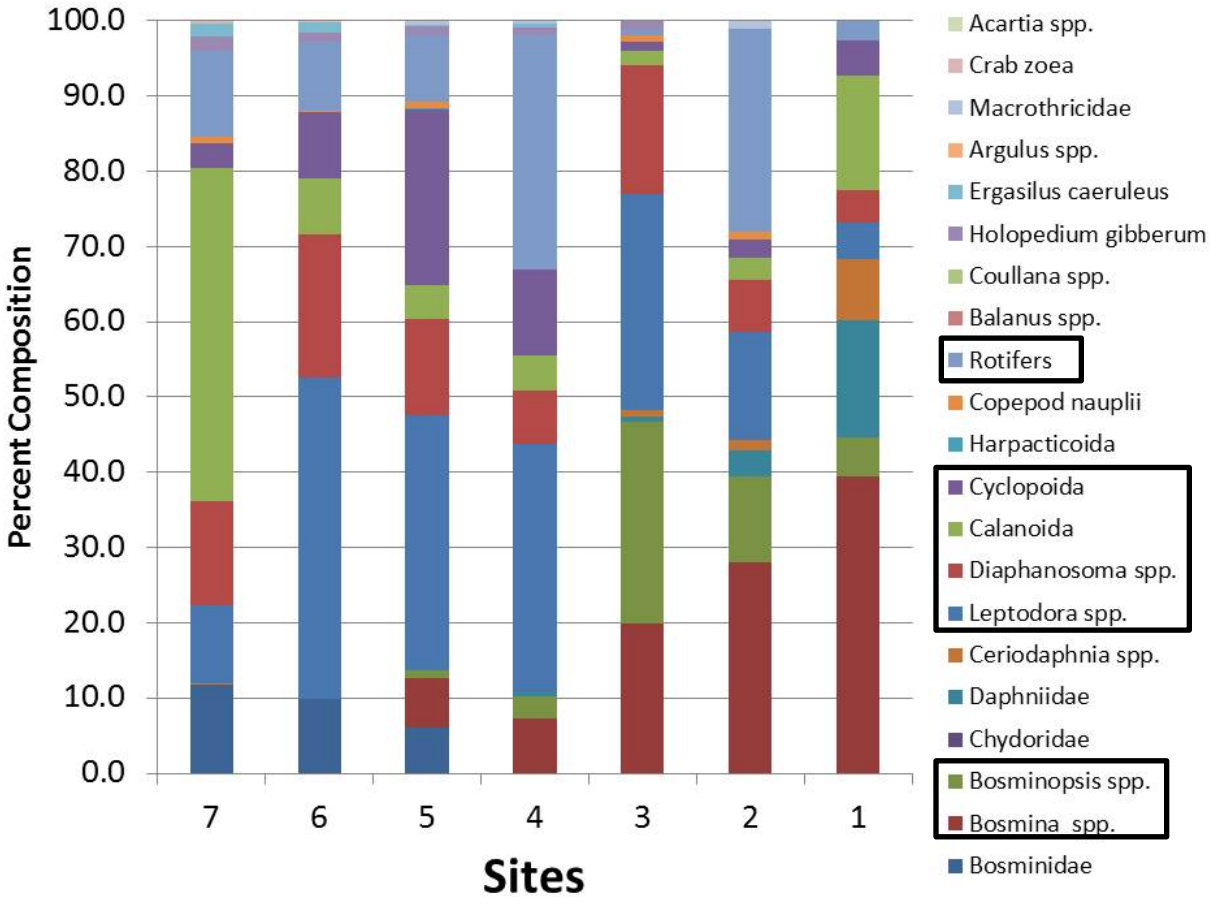


Figure 2.13: Percentage of samples composed of observed taxonomic groups in 200 µm mesh nets by sites for June. The dominant species have a box around them.

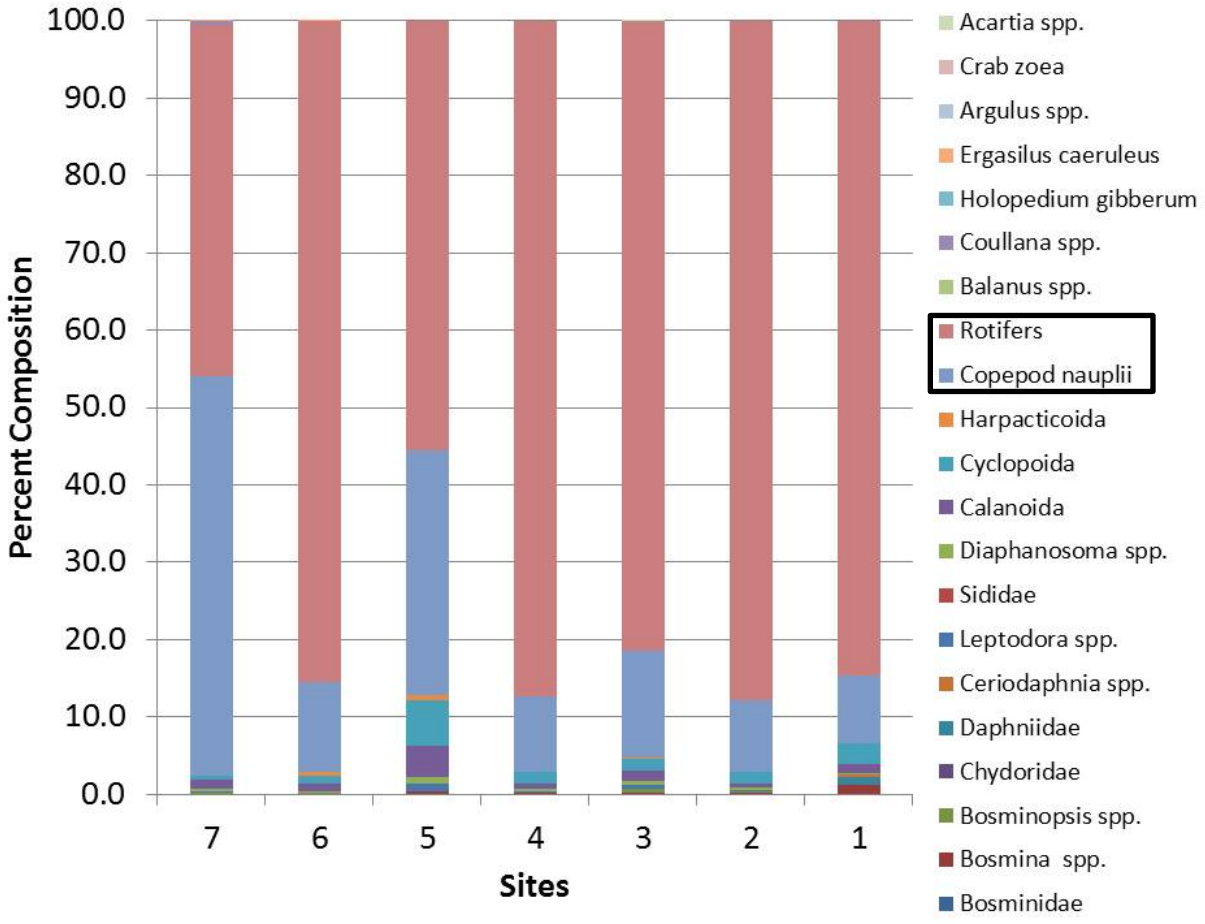


Figure 2.14: Percentage of samples composed of observed taxonomic groups in 60 µm mesh nets by sites for June. The dominant species have a box around them.

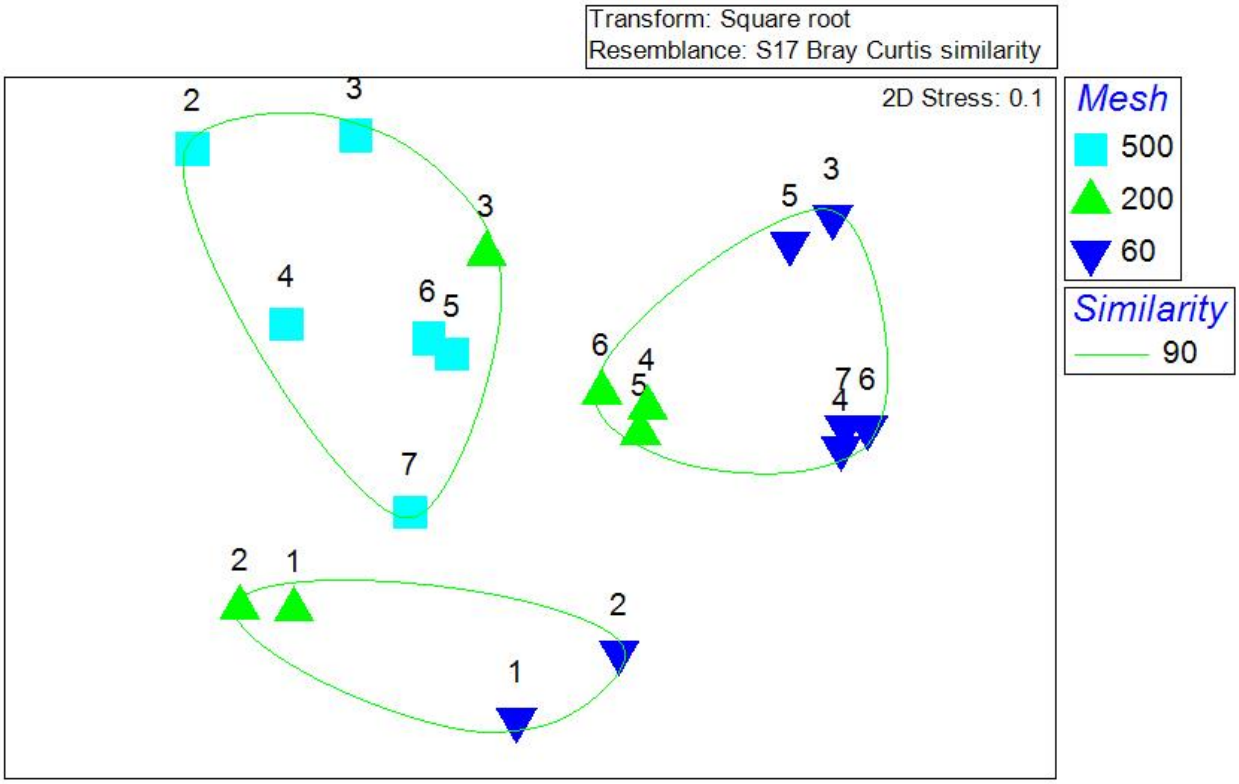


Figure 2.15: n-MDS plot showing similarity in fatty acid profiles of zooplankton at three mesh sizes (60, 200 & 500 µm) for all sites on the river in June. Solid lines show clusters at 90%.

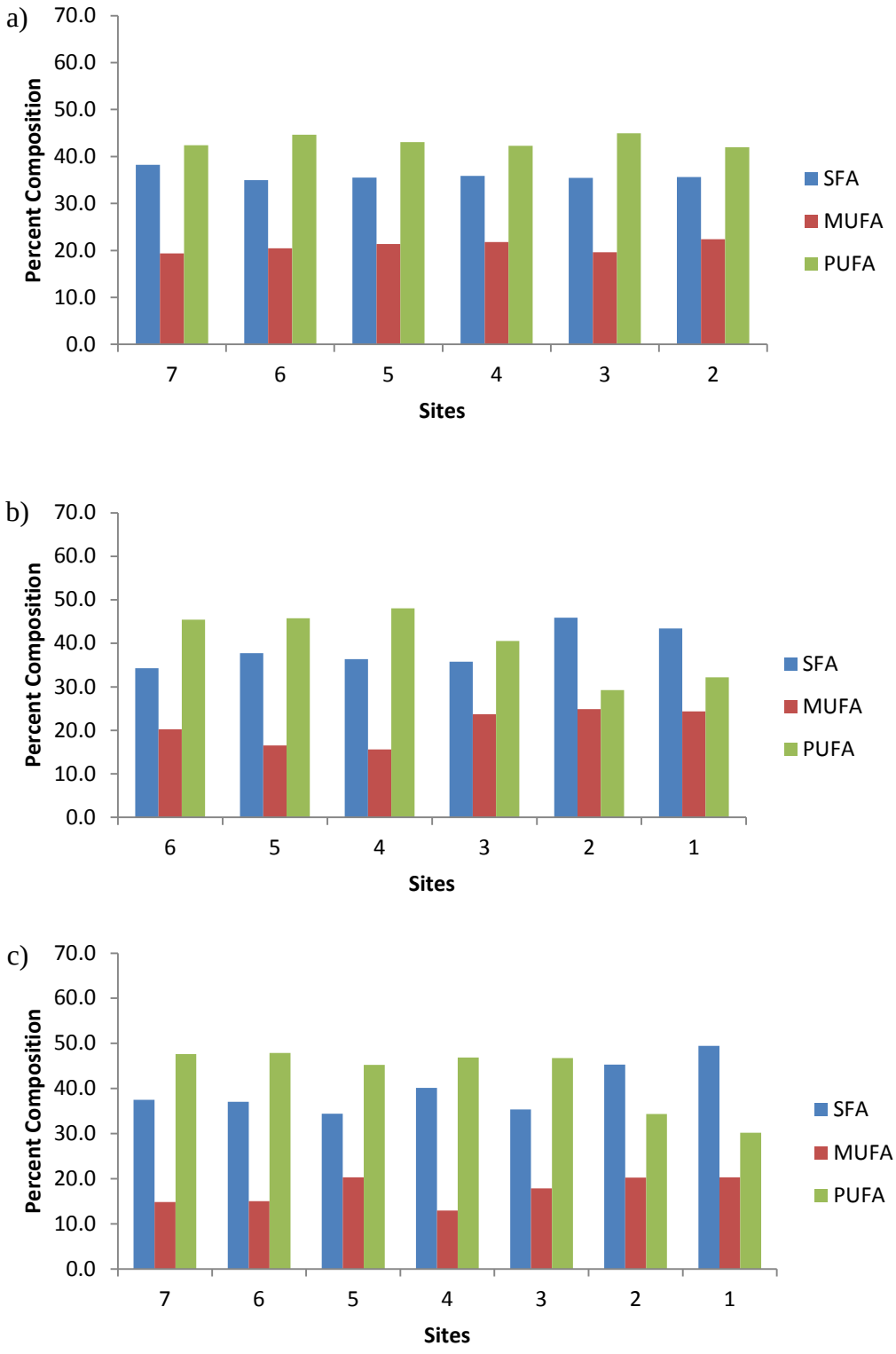


Figure 2.16: The percent composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid of zooplankton from June for the 500 µm mesh (a), and 200 µm mesh (b), and 60 µm mesh (c).

Appendix

Table A2.1: SIMPER analysis evaluating similarity between sites that are grouped by cluster analysis on the n-MDS plot for April. Abundances are squared root transformed. Average Bray-Curtis scores are listed as average similarity. Sim/SD identifies how consistently taxa contribute to similarity. Contribution percentage is the amount of dissimilarity that can be attributed to taxa. – means less than 2 groups.

Sites/ Mesh Size	Average Similarity	Fatty Acid	Average abundance	Average Similarity	Sim/SD	Contribution %
6 and 7 (200 µm)	91.3	16:0	5.22	12.83	--	14.1
		18:1n-9	3.85	9.18	--	10.1
		18:0	3.07	7.18	--	7.9
		22:6n-3	2.97	6.95	--	7.6
		20:5n-3	2.84	6.22	--	6.8
		14:0	2.28	5.47	--	6.0
1-5 (200 µm)	94.0	16:0	4.41	10.8	41.67	11.48
		20:5n-3	3.72	9.11	48.04	9.69
		18:3n-3	3.26	7.79	16.55	8.28
		22:6n-3	3.16	7.24	10.71	7.7
		18:4n-3	2.65	6.3	36.71	6.7
		18:1n-9	2.52	5.97	8.92	6.35
3 &7 (60 µm)	95.5%	16:0	4.95	12.57	--	13.16
		18:1n-9	3.67	9.23	--	9.66
		20:5n-3	3.42	8.34	--	8.74
		18:4n-3	3.2	7.73	--	8.1
		18:3n-3	2.9	6.99	--	7.32
		18:2n-6	2.63	6.38	--	6.68
4 -6 (60 µm)	92.3%	18:1n-9	5.27	14.21	46.29	15.39
		16:0	5.07	13.25	16.78	14.35
		18:2n-6	3.36	8.84	68.23	9.57
		20:5n-3	2.55	6.8	29.77	7.36
		18:0	1.9	4.73	74.19	5.12
1 & 2 (60 µm)	96.0%	18:1n-9	6.11	18.75	--	19.53
		16:0	5.47	16.87	--	17.57
		18:2n-6	3.67	10.86	--	11.31
		18:0	1.73	5.35	--	5.58

Table A2.2: SIMPER analysis evaluating dissimilarity between sites represented from the cluster analysis on the n-MDS plot for April. Abundances are squared root transformed. Average Bray-Curtis scores are listed as average dissimilarity. Diss/SD identifies how consistently fatty acids contribute to dissimilarity. Contribution percentage is the amount of dissimilarity that can be attributed to taxa. Group one is always the first sites in the comparison versus group two.

Comparison	Overall Average Dissimilarity (%)	Fatty Acid	Average abundance Group One	Average Abundance Group Two	Diss/ SD	Contribution %
6 and 7 (200 µm) vs. 1-5 (200 µm)	13.3	18:4n-3	1.29	2.65	5.48	12.94
		18:1n-9	3.85	2.52	3.45	12.63
		18:3n-3	1.93	3.26	4	12.62
		20:5n-3	2.84	3.72	1.94	8.43
		18:0	3.07	2.26	2.71	7.78
6 and 7 (200 µm) vs. 3 and 7 (60 µm)	17.7	18:4n-3	1.29	3.2	7.91	13.87
		18:0	3.07	1.85	3.76	8.88
		16:1n-7	2.33	1.17	3.23	8.39
		20:4n-6	1.78	0.65	6.12	8.17
		18:3n-3	1.93	2.9	3.02	7.01
		20:4n-3	0.93	1.68	13.29	5.43
6 and 7 (200 µm) vs. 4 to 6 (60 µm)	17.6	18:1n-9	3.85	5.27	4.77	10.69
		18:2n-6	1.96	3.36	10.43	10.48
		22:6n-3	2.97	1.74	3.19	9.26
		18:0	3.07	1.9	3.03	8.83
		20:4n-6	1.78	0.84	2.17	7.1
		16:1n-7	2.33	1.59	1.67	6.12
6 and 7 (200 µm) vs. 1 and 2 (60 µm)	25.1	18:1n-9	3.85	6.11	7.32	12.64
		22:6n-3	2.97	0.84	6.69	11.93
		18:3n-3	1.96	3.67	8.05	9.56
		20:5n-3	1.42	0	25.84	7.97
		18:2n-6	3.07	1.73	4.1	7.51
1-5 (200 µm) vs. 3 and 7	13.4	16:1n-7	2.45	1.17	2.82	12
		18:1n-9	2.52	3.67	4.61	10.78
		22:6n-3	3.16	2.36	1.94	7.51

(60 µm)						
		20:4n-6	1.43	0.65	3.92	7.36
		18:1n-7	1.55	0.91	4.48	6.05
		20:4n-3	1.05	1.68	2.3	5.99
		18:4n-3	2.65	3.2	2.02	5.22
1-5 (200 µm)	20.3	18:1n-9	2.52	5.27	10.95	17.64
vs.		22:6n-3	3.16	1.74	3	9.11
4-6 (60 µm)		18:3n-3	3.26	1.87	4.11	8.86
		20:5n-3	3.72	2.55	9.94	7.49
		18:2n-6	2.24	3.36	6.89	7.12
1-5 (200 µm)	31.7	18:1n-9	2.52	6.11	14.16	15.63
vs.		22:6n-3	3.16	0.84	5.44	10.11
1 and 2 (60 µm)		20:5n-3	3.72	1.69	18.08	8.85
		18:3n-3	3.26	1.33	7.94	8.4
		18:1n-7	1.55	0	14.56	6.77
		18:2n-6	2.24	3.67	6.57	6.2
3 and 7 (60 µm)	13.8	18:1n-9	3.67	5.27	21.79	15.33
vs.		18:4n-3	3.2	1.98	2.04	11.63
4-6 (60 µm)		18:3n-3	2.9	1.87	3.18	9.77
		20:5n-3	3.42	2.55	5.27	8.3
		18:2n-6	2.63	3.36	4.12	6.93
3 and 7 (60 µm)	23.2	18:1n-9	3.67	6.11	32.55	14.73
vs.		18:4n-3	3.2	1.24	7.49	11.84
1 and 2 (60 µm)		20:5n-3	3.42	1.69	10.39	10.45
		18:3n-3	2.9	1.33	7.7	9.47
		22:6n-3	2.36	0.84	11.07	9.18
4-6 (60 µm)	12.8	18:1n-7	1.05	0	3.99	11.86
vs.		22:6n-3	1.74	0.84	3.39	10.19
1 and 2 (60 µm)		20:5n-3	2.55	1.69	13.29	9.79
		18:1n-9	5.27	6.11	12.83	9.48
		18:4n-3	1.98	1.24	1.47	9

Table A2.3: Fatty acid composition (percentage of total fatty acids detected) of microzooplankton (<60µm) and mesozooplankton (>200 µm) from the Chowan River for April 2013. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	200µm Sites							**60µm Sites**						
	7	**6**	**5**	**4**	**3**	**2**	**1**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
12:0	0.5	0.4	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.2
14:0	4.5	5.9	6.1	6.0	3.7	4.4	5.8	4.4	3.3	3.0	2.3	3.1	2.3	2.1
15:0	1.1	0.8	0.7	0.7	0.7	0.6	0.7	0.6	0.4	0.3	0.2	0.3	0.3	0.2
16:0	29.7	24.9	19.0	18.8	17.9	21.0	20.8	24.3	22.7	26.4	28.3	24.7	29.7	30.1
17:0	1.3	1.0	1.0	1.1	1.2	1.1	0.9	0.6	0.5	0.5	0.4	0.6	0.3	0.4
18:0	11.2	7.8	5.0	5.0	5.5	5.2	4.7	3.4	4.8	3.2	3.0	3.4	3.0	3.0
20:0	0.4	0.2	0.2	0.3	0.1	0.2	0.2	0.7	1.7	1.5	1.5	0.8	2.3	2.1
∑SFA	**48.7**	**41.1**	**32.2**	**32.1**	**29.1**	**32.6**	**33.2**	**34.3**	**33.4**	**35.0**	**35.7**	**33.2**	**38.1**	**38.1**
16:1n-9	0.3	0.9	1.1	1.1	0.3	0.8	1.3	0.6	0.3	0.4	0.4	1.0	0.3	0.2
16:1n-7	4.1	7.0	8.7	7.3	2.9	5.3	6.7	1.5	5.0	1.7	1.5	1.3	1.4	1.4
18:1n-9	17.0	12.8	7.1	7.2	4.3	7.1	6.4	13.8	27.3	28.2	27.9	13.1	38.1	36.7
18:1n-7	2.1	1.9	2.1	2.2	2.2	2.7	2.9	0.7	2.0	0.8	0.7	1.0	0.0	0.0
20:1	0.3	0.2	0.2	0.4	0.4	0.2	0.1	0.6	0.8	0.6	0.6	1.0	0.6	0.3
∑MUFA	**23.8**	**22.7**	**19.3**	**18.2**	**10.1**	**16.1**	**17.3**	**17.1**	**35.4**	**31.8**	**31.1**	**17.4**	**40.3**	**38.5**
18:2n-6	4.2	3.5	4.3	5.1	5.0	4.8	6.0	7.6	12.2	11.3	10.4	6.3	12.3	14.7
18:3n-3	2.9	4.7	8.9	9.2	11.6	11.3	12.2	7.5	2.3	3.9	4.5	9.3	2.0	1.6
18:4n-3	1.4	2.0	6.2	7.4	9.1	6.3	6.4	9.2	1.5	4.8	6.3	11.4	1.9	1.2
20:2n-6	0.2	0.3	0.3	0.3	0.4	0.3	0.5	0.5	0.5	0.5	0.5	0.6	0.4	0.3
20:3n-6	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.2	0.1	0.0	0.0	0.1	0.1
20:4n-6	3.7	2.7	1.7	1.7	1.6	2.5	2.9	0.4	1.7	0.6	0.2	0.5	0.3	0.2
20:3n-3	0.2	0.4	0.4	0.5	0.9	0.4	0.1	0.9	0.3	0.5	0.4	1.1	0.2	0.2
20:4n-3	0.8	0.9	1.0	1.2	1.9	1.3	0.4	2.7	1.3	1.8	1.5	2.9	0.8	1.3
20:5n-3	5.9	10.6	14.9	13.9	14.5	13.0	13.0	12.7	6.2	6.8	6.6	10.7	2.8	2.9
22:5n-6	0.4	0.2	0.6	0.7	0.8	0.7	1.1	1.0	0.3	0.4	0.3	1.1	0.0	0.0
22:5n-3	0.4	0.4	0.4	0.5	0.7	0.4	0.2	0.3	0.6	0.1	0.1	0.2	0.2	0.1
22:6n-3	7.3	10.5	10.0	9.2	14.4	10.3	6.7	5.7	4.2	2.6	2.4	5.4	0.5	0.9
∑PUFA	**27.4**	**36.2**	**48.7**	**49.8**	**60.8**	**51.3**	**49.4**	**48.6**	**31.2**	**33.3**	**33.2**	**49.5**	**21.6**	**23.4**

Table A2.4: SIMPER analysis evaluating similarity between sites that are grouped by cluster analysis on the n-MDS plot for May. Abundances are squared root transformed. Average Bray-Curtis scores are listed as average similarity. Sim/SD identifies how consistently taxa contribute to similarity. Contribution percentage is the amount of dissimilarity that can be attributed to taxa.

Sites/ Mesh Size	Average Similarity	Fatty Acid	Average abundance	Average Similarity	Sim/SD	Contribution %
4, 7 & 10 (200 µm)	93.4%	16:0	4.69	12.26	16.12	13.27
		22:6n-3	4.63	11.17	5.65	12.09
		20:5n-3	4.09	10.95	40.06	11.86
		16:1n-7	2.96	7.15	17.84	7.74
		18:0	2.49	6.7	38.49	7.25
1 (200 µm) and 4 & 10 (60 µm)	87.6%	16:0	4.96	11.96	55.79	13.65
		20:5n-3	3.38	7.9	24.01	9.01
		16:1n-7	3.26	7.45	15.23	8.51
		18:1n-9	3.2	6.66	5.02	7.6
		14:0	2.55	6.13	11.37	6.99
		18:0	2.44	5.89	21.95	6.72

Table A2.5: SIMPER analysis evaluating dissimilarity between sites represented from the cluster analysis on the n-MDS plot for May. Abundances are squared root transformed. Average Bray-Curtis scores are listed as average dissimilarity. Diss/SD identifies how consistently fatty acids contribute to dissimilarity. Contribution percentage is the amount of dissimilarity that can be attributed to taxa. Group one is always the first sites in the comparison versus group two.

Comparison	Overall Average Dissimilarity (%)	Fatty Acid	Average abundance Group One	Average Abundance Group Two	Diss/ SD	Contribution %
4, 7, 10 (200 µm) vs. 1 (200 µm) and 4, 10 (60µm)	13.53	22:6n-3	4.63	2.58	2.15	20.09
		18:1n-9	1.86	3.2	1.61	13.05
		20:5n-3	4.09	3.38	1.68	6.82
		18:2n-6	1.46	2.07	1.59	5.91
		16:1n-7	2.96	3.26	1.29	5.3

Table A2.6: Fatty acid composition (percentage of total fatty acids detected) of microzooplankton (<60 µm) and mesozooplankton (>200 µm) from the western Albemarle Sound and Chowan River for May 2013. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	200µm Sites				**60µm Sites**	
	10	**7**	**4**	**1**	**10**	**4**
12:0	0.0	0.1	0.2	0.3	0.2	0.3
14:0	4.0	3.7	6.6	6.6	7.7	5.3
15:0	0.7	0.8	0.9	0.8	1.4	0.9
16:0	19.2	23.8	23.3	21.6	30.0	22.5
17:0	1.0	1.2	1.0	1.9	1.2	0.8
18:0	6.3	6.2	6.1	6.0	5.0	6.9
20:0	0.1	0.1	0.2	0.2	0.2	0.1
∑SFA	**31.4**	**35.8**	**38.3**	**36.4**	**45.7**	**36.8**
16:1n-9	0.1	0.1	0.9	1.3	0.4	0.2
16:1n-7	8.3	6.4	12.1	9.4	15.0	8.1
18:1n-9	4.1	2.3	4.2	16.6	5.3	10.5
18:1n-7	2.0	1.9	1.9	2.3	2.5	2.9
20:1	0.1	0.1	0.2	0.1	0.8	1.1
∑MUFA	**14.5**	**10.8**	**19.2**	**29.6**	**24.0**	**22.7**
18:2n-6	2.4	1.2	3.1	4.2	3.3	5.5
18:3n-3	4.2	3.2	3.1	4.3	5.5	3.8
18:4n-3	1.9	1.6	2.3	2.5	1.3	1.7
20:2n-6	0.2	0.2	0.2	0.1	0.3	0.3
20:3n-6	0.1	0.0	0.1	0.0	0.1	0.2
20:4n-6	1.6	1.1	1.5	2.8	1.1	3.5
20:3n-3	0.1	0.2	0.1	0.1	0.1	0.4
20:4n-3	0.5	0.3	0.9	0.5	1.2	1.7
20:5n-3	17.3	16.6	16.3	15.5	10.0	9.3
22:5n-6	1.7	1.1	0.8	0.4	0.4	1.4
22:5n-3	0.4	0.3	0.3	0.1	0.2	2.1
22:6n-3	23.8	27.9	14.0	3.4	7.1	10.5
∑PUFA	**54.0**	**53.4**	**42.6**	**33.9**	**30.4**	**40.5**

Table A2.7: SIMPER analysis evaluating similarity between sites that are grouped by cluster analysis on the n-MDS plot for June. Abundances are squared root transformed. Average Bray-Curtis scores are listed as average similarity. Sim/SD identifies how consistently a taxa contributes to similarity. Contribution percentage is the amount of dissimilarity that can be attributed to taxa.

Sites/ Mesh Size	Average Similarity	Fatty Acid	Average abundance	Average Similarity	Sim/SD	Contribution %
4-6 (200 µm) and 3-7 (60 µm)	93.4%	16:0	4.63	11.04	40.28	11.82
		22:6n-3	3.39	8.01	37.34	8.58
		20:5n-3	3.55	7.95	8.42	8.51
		18:0	2.67	6.38	54.83	6.83
		18:3n-3	2.66	6.14	24.22	6.57
		16:1n-7	2.56	5.82	11.05	6.23
		14:0	2.3	5.36	20.49	5.74
2-7 (500 µm) and 3 (200 µm)	93.0%	16:0	4.51	11.21	46.63	12.05
		20:5n-3	4.14	9.89	8.03	10.63
		18:1n-9	3.18	7.45	8.54	8.01
		18:0	2.73	6.83	54.48	7.35
		18:3n-3	2.74	6.38	9.82	6.86
		16:1n-7	2.53	5.79	9.06	6.23
1 & 2 (60 and 200 µm)	92.9%	16:0	5.27	12.98	64.4	13.98
		16:1n-7	3.29	8	25.04	8.61
		20:5n-3	3	7.18	12.24	7.73
		18:0	2.81	6.75	17.61	7.27
		18:3n-3	2.56	6.19	65.25	6.67
		14:0	2.5	5.85	16.55	6.3

Table A2.8: SIMPER analysis evaluating dissimilarity between sites represented from the cluster analysis on the n-MDS plot for June. Abundances are squared root transformed. Average Bray-Curtis scores are listed as average dissimilarity. Diss/SD identifies how consistently fatty acids contribute to dissimilarity. Contribution percentage is the amount of dissimilarity that can be attributed to taxa. Group one is always the first sites in the comparison versus group two.

Comparison	Overall Average Dissimilarity (%)	Fatty Acid	Average abundance Group One	Average Abundance Group Two	Diss/SD	Contribution %
4-6 (200 µm) and 3-7 (60 µm)	11.1	22:6n-3	3.39	1.89	2.75	16.78
vs.		18:1n-9	2.12	3.18	1.88	12.15
2-7 (500 µm) and 3 (200 µm)		22:5n-6	1.63	0.82	4.03	9.06
		20:5n-3	3.55	4.14	1.62	7.85
		22:5n-3	1.31	0.64	1.75	7.52
4-6 (200 µm) and 3-7 (60 µm)	11.9	22:6n-3	3.39	2.09	6.92	13.4
vs.		22:5n-3	1.31	0.48	2.21	8.47
1 and 2 (60 & 200 µm)		22:5n-6	1.63	0.87	2.88	7.94
		16:1n-7	2.56	3.29	2.41	7.5
		16:0	4.63	5.27	3.94	6.58
		20:1	0.99	0.52	1.59	6.28
2-7 (500 µm) and 3 (200 µm)	10.8	20:5n-3	4.14	3	2.51	13.27
vs.		18:1n-9	3.18	2.26	2.03	10.89
1 and 2 (60 & 200 µm)		16:1n-7	2.53	3.29	1.9	8.89
		16:0	4.51	5.27	4.46	8.75
		20:4n-3	1.26	0.99	1.48	5.23
		22:6n-3	1.89	2.09	0.99	4.71

Table A2.9: Fatty acid composition (percentage of total fatty acids detected) of microzooplankton (<60 µm) and mesozooplankton (200- 500 µm) from the Chowan River for June 2013. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	200µm Sites						**60µm Sites**						
	6	**5**	**4**	**3**	**2**	**1**	**7**	**6**	**5**	**4**	**3**	**2**	**1**
12:0	0.1	0.3	0.2	0.2	0.5	0.5	0.3	0.3	0.2	0.3	0.2	0.7	0.9
14:0	5.0	5.7	4.6	4.1	5.5	5.0	5.5	6.0	5.1	6.50	4.4	7.1	7.6
15:0	0.9	1.0	1.1	0.9	1.3	1.7	1.1	1.1	0.9	1.30	0.7	1.4	1.6
16:0	20.2	22.0	20.9	21.8	27.3	26.3	21.9	21.5	19.7	23.53	21.8	27.8	29.6
17:0	1.3	1.5	1.8	1.2	1.9	1.8	1.5	1.3	1.1	1.32	0.9	1.3	1.6
18:0	6.7	7.1	7.7	7.4	9.2	8.0	7.0	6.8	7.3	7.05	7.2	6.7	7.8
20:0	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.2	0.2	0.16	0.2	0.3	0.4
∑SFA	**34.3**	**37.7**	**36.4**	**35.8**	**45.9**	**43.4**	**37.5**	**37.0**	**34.4**	**40.16**	**35.4**	**45.3**	**49.5**
16:1n-9	0.8	1.2	0.8	0.7	0.9	0.9	1.2	0.9	0.5	0.67	0.5	1.6	2.0
16:1n-7	9.3	7.8	7.3	7.9	11.2	12.1	6.3	6.2	5.7	6.14	4.5	10.0	10.0
18:1n-9	6.0	4.2	3.3	10.2	7.8	5.5	3.4	2.9	8.4	2.00	7.9	3.7	4.0
18:1n-7	4.0	3.0	4.1	4.6	4.9	5.7	2.6	2.9	3.5	3.01	3.4	4.1	4.0
20:1	0.2	0.3	0.1	0.2	0.0	0.1	1.5	2.2	2.2	1.13	1.7	0.9	0.5
∑MUFA	**20.3**	**16.5**	**15.6**	**23.7**	**24.9**	**24.4**	**14.9**	**15.1**	**20.3**	**12.97**	**17.9**	**20.3**	**20.3**
18:2n-6	2.5	2.4	2.1	3.0	2.2	2.2	2.3	2.2	2.9	1.56	3.0	2.1	2.8
18:3n-3	5.7	6.6	6.9	9.6	6.2	5.9	6.2	7.3	6.6	8.01	9.6	7.7	6.5
18:4n-3	2.1	2.5	2.5	3.7	1.5	2.1	2.8	3.1	2.7	3.10	4.0	2.8	2.2
20:2n-6	0.2	0.2	0.2	0.2	0.1	0.1	0.3	0.3	0.4	0.13	0.3	0.2	0.5
20:3n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.17	0.2	0.1	0.3
20:4n-6	3.4	3.1	3.6	4.8	4.9	6.1	2.2	2.9	3.9	3.20	3.2	3.3	3.6
20:3n-3	0.2	0.2	0.3	0.3	0.1	0.1	0.3	0.3	0.5	0.22	0.6	0.3	0.2
20:4n-3	0.9	1.1	0.8	0.9	0.3	0.5	2.4	2.1	2.4	2.20	2.0	2.3	1.5
20:5n-3	16.9	15.9	15.5	10.2	9.9	10.0	12.8	11.4	8.9	12.12	8.5	8.9	7.3
22:5n-6	2.2	2.3	2.5	0.7	0.5	0.42	3.3	3.3	2.6	2.89	2.4	1.5	0.9
22:5n-3	1.0	0.9	0.6	0.6	0.1	0.23	2.4	2.9	3.1	1.56	2.2	0.5	0.3
22:6n-3	10.4	10.5	12.9	6.5	3.6	4.41	12.5	11.9	11.1	11.71	10.8	4.9	4.6
∑PUFA	**45.5**	**45.8**	**48.0**	**40.6**	**29.3**	**32.20**	**47.6**	**47.9**	**45.3**	**46.87**	**46.7**	**34.4**	**30.2**

Table A2.10: Fatty acid composition (percentage of total fatty acids detected) of macrozooplankton (>500 µm) from the Chowan River for June 2013. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	500µm Sites					
	7	**6**	**5**	**4**	**3**	**2**
12:0	0.1	0.1	0.1	0.2	0.2	0.2
14:0	6.0	5.3	5.3	4.9	5.2	4.6
15:0	1.0	1.2	1.1	1.3	1.0	1.1
16:0	22.5	19.3	19.8	19.3	19.9	20.2
17:0	1.2	1.9	1.6	2.1	1.7	1.9
18:0	7.3	7.1	7.5	7.9	7.5	7.6
20:0	0.2	0.1	0.1	0.1	0.1	0.1
∑SFA	**38.2**	**34.9**	**35.5**	**35.9**	**35.5**	**35.7**
16:1n-9	1.3	0.9	0.9	0.9	0.3	0.4
16:1n-7	9.8	7.0	6.6	5.8	4.1	4.5
18:1n-9	6.0	8.9	10.2	10.8	11.7	13.8
18:1n-7	2.2	3.3	3.3	4.2	3.2	3.6
20:1	0.1	0.3	0.4	0.1	0.3	0.1
∑MUFA	**19.4**	**20.4**	**21.4**	**21.8**	**19.6**	**22.4**
18:2n-6	2.9	2.8	2.8	3.0	2.8	2.7
18:3n-3	5.0	6.2	6.8	6.6	10.3	9.0
18:4n-3	2.4	2.1	2.6	1.7	3.3	2.2
20:2n-6	0.1	0.1	0.1	0.1	0.1	0.1
20:3n-6	0.1	0.1	0.1	0.1	0.1	0.1
20:4n-6	3.2	5.2	5.3	6.8	6.2	7.2
20:3n-3	0.1	0.2	0.2	0.1	0.3	0.2
20:4n-3	2.8	1.7	1.5	1.2	1.8	1.6
20:5n-3	20.5	19.8	17.2	18.2	17.0	17.9
22:5n-6	0.7	1.1	0.9	0.7	0.6	0.3
22:5n-3	0.2	0.8	0.8	0.3	0.5	0.1
22:6n-3	4.5	4.6	4.9	3.5	2.1	0.8
∑PUFA	**42.4**	**44.6**	**43.1**	**42.3**	**44.9**	**42.0**

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Chapter 3: Future research and perspective on impacts on the Albemarle Sound estuary and Chowan River

Introduction

Salinity appears to structure the zooplankton community in the western Albemarle Sound and Chowan River and the shifts in zooplankton community composition are directly related to the fatty acid profiles observed. The result is that salinity shifts have the potential to impact the nutritional value of prey items during the larval river herring residency period. For example, when freshwater zooplankton species dominate the system, the fatty acid profiles for mesozooplankton resemble the fatty acids found in freshwater species in lake systems. When the zooplankton species are dominated by brackish water species, especially copepods, the fatty acid profiles resemble a copepod dominant, marine system. This change in the overall fatty acid composition over the spring period suggests that larval river herring may experience a range of prey items that vary considerably in fatty acid composition. Therefore, the fatty acid profiles of the zooplankton prey field likely have considerable influence of the growth and development of larval river herring. However, more work is needed to make this connection empirically. However, estuarine systems within this region are currently undergoing significant changes due to several factors and the consequences of these changes with respect to the food web will be the focus of this chapter.

Since estuarine systems are important connections between land and the ocean, the systems will be affected by climate change impacts both on land and the ocean (Kennish 2002 and Flemer and Champ 2006). Climate change will lead to sea level rise, possibly stronger storms, and drought conditions (Kennish 2002). Sea level rise will affect estuaries by increasing salinity and in lagoonal systems the increased salinity would drastically change systems that are

usually fresh and lack a direct connection to the ocean (Day et al. 1989). Increasing frequency and intensity of trophic storms due to global warming could erode the barrier islands and create an inlet near Albemarle Sound. The system would shift to a brackish/marine system and the community of organisms found in the sound would change or be moved westerly, possibly into the Roanoke and Chowan rivers. These changes will have a large effect on the food web dynamics and could possibly lead to trophic disconnects before organisms can adapt to the new surroundings (Kennish 2002). Monitoring the organism populations and abiotic factors, including fatty acid profiles through field and laboratory studies, will help to understand the changes that are likely to occur between trophic levels.

Another concern is the ever increasing human population living in the coastal zone. In the United States, 60% of the population lived within 60 km of the coast in the 1990s (Flemer and Champ 2006) and this value is expected to increase. An increase in human population often leads to eutrophication, over fishing, land development, habitat alterations, freshwater diversion, and introduction of exotic species (Kennish 2002). The freshwater input from tributaries delivers increased nutrients, over-enriching the water bodies with nitrogen and phosphorous from increased farming activities, human wastewater treatment plants, and land conversion from forest to agriculture and urban centers (Bennett et al. 2001 and Flemer and Champ 2006). The increased nutrients can cause harmful algal blooms that can lead to a change in secondary consumers and higher trophic levels, potentially impacting commercially important fish populations Seventy percent of phytoplankton community of the western Albemarle Sound and mouth of the Chowan River in the summer of 2012 consisted of cyanobacteria. Even though these were not bloom conditions, the high proportion of cyanobacteria can lead to a change in fatty acids present. Therefore, the presence of excess nutrients that result in cyanobacterial

blooms can lead to a change in the zooplankton fatty acid structure with lower chains fatty acid present, reducing nutritional quality for zooplankton and higher trophic levels.

The final concern is the diversion of freshwater or drought conditions that will affect the freshwater input from the tributaries to the estuary. The diversion of freshwater is primarily caused by dam and reservoir construction (Kennish 2002 and Flemer and Champ 2006). Freshwater diversion reduces the amount of sediments and nutrients entering the estuary, which may have a number of different impacts. The concern is that freshwater systems have always replenished the estuaries with new nutrients, and allowed movement of sediment and matter between the bodies of water that enabled high productivity in the estuary (Flemer and Champ 2006). The reduction of nutrients would possibly clear the water, and change the primary production from phytoplankton to more dominant bottom macrophytes (Flemer and Champ 2006). Without freshwater input, the salinity will increase over areas that have low salinity level which would change the composition of different organisms, and decrease the suitable nursery habitat for larval fish, especially the river herring.

Future Research

Field Monitoring

The most important step is to continue monitoring the lower trophic levels, including zooplankton, to understand how spatially and temporally the population is changing throughout the year in Albemarle Sound, and the Chowan and Roanoke Rivers. The expansion of sampling would allow for comparisons between locations and further the understanding the dynamics of the abiotic factors that have the potential to drive food web changes. The sampling should continue with 60 and 200 µm mesh size net and include a 20 µm mesh net to fully identify the rotifer population. The collection should also include sampling the phytoplankton community

through chlorophyll a and phytoplankton pigments. The data can be supplemented with data collected by the North Carolina Department of Water Quality and the United States Geological Survey that have monitoring programs in place. Using the data collected to determine when cyanobacteria counts are high, or bloom formation, would be useful in determining how the phytoplankton is affecting the zooplankton populations.

The next step would be to understand the movement and feeding habits of the larval fish, especially river herring and ctenophores in the system. Using historical data from East Carolina University and the Division of Marine Fisheries, the river herring population and diet could be linked to the zooplankton abundance and community composition. The ctenophore population should be monitored because they are rarely seen in the Albemarle Sound and understanding their movement with wind and currents in the saltwater wedge is vital. Ctenophores are considered a veracious predator on zooplankton populations, and understanding the overlap of diet with larval fish is important. During the salinity intrusion event, the ctenophore *Mnemiopsis leidyi* was observed through the Chowan River and western Albemarle Sound.

The processing of larval river herring and other species for fatty acid profiles is important to begin understanding their connection to zooplankton. The river herring should be collected at different stages of their life to determine what fatty acids are present during development, and how that changes over time before juveniles return to the ocean. Including some benthic invertebrates in the processing would help to determine when and how selective feeding by alewife could affect the fatty acid profiles compared to blueback herring that continue to filter feeding. Zooplankton samples for fatty acid analysis should be continued throughout the year to determine how changes in the composition would affect what is present for the larval river herring, and other planktivorous organisms.

A field experiment for fatty acids would be to collect zooplankton and determine whether individual species separation, as compared to mesh size sampling, would produce different results. The next field experiment would be to collect zooplankton from high, medium, and low salinity to determine how total lipid and fatty acid profiles differ in total amount, as well as, dominant fatty acids. Finally, for field work, the zooplankton diet could be determined by sequencing DNA and amplification of the prey DNA from universal primers in a single polymerase chain reaction to allow the connection between trophic levels and gain a better understanding of the fatty acid data.

Laboratory Experiments

Laboratory experiments should focus on feeding different phytoplankton diets to different zooplankton species in order to determine the dietary effects on growth and reproduction. This research would help to determine whether certain food sources are better or worse for the zooplankton species found in the Albemarle Sound and tributaries. Then the zooplankton should be fed at different ratios of dominant fatty acids in order to determine how fatty acids affect growth, development, and reproduction. These laboratory results can then be directly compared to field measurements.

Similar experiments should be conducted for larval river herring to determine how the fatty acids effect growth and development. The first experiment should investigate how different food sources (microzooplankton, macrozooplankton, benthic invertebrates) affect growth and development. The next set of experiments should use different ratios and amounts of dominant fatty acids to determine appropriate levels for the highest growth and compare it to the field samples. It has been shown in yellow perch that more essential fatty acids are not better, but that a certain ratio is better for growth (Czesny and Rinchard, personal communication). Through

these experiments, data will be acquired that will allow a better understanding of whether larval river herring can convert low chain fatty acids to higher chains since most marine fish have lost this ability.

Conclusion

The snapshot of the zooplankton composition and fatty acid profiles from 2013 show that the Albemarle Sound and Chowan River are dynamic environments and the freshwater-saltwater interface has a dynamic zooplankton community. The main rationale for my research was to explore the variability in zooplankton abundance, community composition, and nutritional quality in the hopes of at least partially explaining why larval river herring have not recovered. My results suggest that the zooplankton abundance appears to be similar to other regions where larval river herring growth and survival is well documented. The zooplankton community composition I observed appears no different from that observed in the diets of larval river herring in NC and other systems. Finally, the fatty acid profiles of the zooplankton community are highly variable and larval river herring are likely to experience a wide range of nutrition, depending on diet. More research is need to determine if this variability plays a role in the lack of herring recovery, but I cannot rule out that such a possibility exists.

My results suggest that the Albemarle Sound and Chowan River are likely to change significantly with moderate changes in salinity that will accompany sea level rise. This will result in a different spatial arrangement of the zooplankton community composition and associated fatty acid profiles. In turn, the prey field available to larval river herring that return to spawn in the Chowan River is likely going to shift dramatically over the next 50-100 years. Such shifts may impact the recovery of this species within the Albemarle Sound system. Starting to continuously collect data is the best way to monitor the changes seen in zooplankton and higher

trophic levels to understand the exchange and accumulation of fatty acids. The laboratory experiments would help to determine how phytoplankton changes in the environment could affect the zooplankton, as well as higher trophic levels. The data collected can also be used to determine essential fatty acid levels that are lethal for development and growth in zooplankton and river herring. The information can be related to the field sampling, and allow the determination of possible food web disconnects. In conclusion, the field and laboratory experiments would help to begin understanding the food webs in coastal systems and estuaries, especially the Albemarle Sound and its tributaries and provide the necessary foundation to predict larval river herring population recovery potential.

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