ARE ALL FISH NURSERIES EQUAL? DETERMINING HOW FOOD WEB DYNAMICS AFFECT FISH NURSERY HABITAT

by

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Abstract

The fish nursery habitat concept has been used to define important habitat for larval and juvenile fish throughout coastal and estuarine areas. Nursery habitat has been defined as an area that produces more fish biomass, in the form of recruits to the adult population, compared to other habitat types. Management agencies and scientists have used this definition to identify nursery habitats; however, how nursery habitats function has remained a "black box." The complex mechanisms that make a particular habitat a nursery remain unknown. One such mechanism hypothesizes that food quantity and quality are linked to enhanced larval fish growth and survival. The objective of this dissertation was to investigate this particular hypothesis by characterizing the planktonic food web that supports larval and juvenile fish. More specifically, to examine abiotic and biotic factors that play a role in determining food quantity and quality and therefore help explain how a nursery habitat may function for larval fish feeding. In North Carolina, strategic habitat areas (SHAs) are defined as areas that contribute most to the integrity of the system and for fish as "locations of individual fish habitats or systems of habitats that have been identified to provide exceptional habitat functions or that are particularly at risk due to eminent threats, vulnerability or rarity", but did not incorporate river herring nursery habitat in designations. The quality of strategic habitat areas was explored for two rivers (Chowan and Tar/Pamlico Rivers) in North Carolina that have been designated strategic habitat areas and have spawning anadromous fish populations including river herring. River herring were an important commercial fishery in North Carolina and

throughout the eastern seaboard, but a decline in populations resulted in a moratorium on river herring harvest being implemented at the state level in 2007; yet, the population has not recovered. Chapter 2 examined the percent total lipids and fatty acid profiles of tissue and ovaries from river herring. The goal was to determine if maternal effects (ex. lipids and fatty acids composition) on the offspring are a potential contributor to the lack of population recovery. Results demonstrated that female river herring had increased percent total lipids compared to other river herring populations from different geographical regions and a fatty acid profile that represented both a marine and freshwater diet. The ovaries had increased percent of docosahexaenoic acid (DHA), similar to other herring species, a fatty acid critical for development and growth of larval fish. River herring female tissue and ovary total lipids and fatty acid profiles were found to be adequate for successful migration, spawning, and energy provisioning for larval river herring to survive to first feeding. The goals of Chapters 3 and 4 were to determine if plankton species and fatty acid composition of the lower food web (i.e., phytoplankton and zooplankton) varied in relation to abiotic factors within the estuarine fish nursery. In order to achieve this goal, the spatial and temporal variability of abiotic factors (e.g., temperature, dissolved oxygen, pH, salinity, and nutrients), phytoplankton pigments, zooplankton species composition, as well as the fatty acid composition of the seston, zooplankton, and larval fish were examined in two estuaries. The main findings were that phytoplankton biomass was correlated to changes in nutrient dynamics, and the overall phytoplankton pigment composition differed within and between the two river systems. This study identified that seston fatty acid profiles correlated to the phytoplankton pigments, but some caution needs to be taken because seston fatty acids can be indicators of detritus and/or other microplankton. The zooplankton in the Chowan River and tributaries was a mix of cladoceran and copepods in 2016, while communities were mainly composed of cladocerans, especially *Bosmina* spp. and *Daphnia* spp., in 2017. This change in zooplankton community composition resulted in decreased percent DHA and increased eicosapentaenoic acid (EPA) for the zooplankton fatty acid profiles. The zooplankton in the Tar/Pamlico River and tributaries was a 50/50 mix of cladoceran and copepods both years in the freshwater reaches, and *Acartia* spp. was the dominant genus in the brackish water reaches. The zooplankton fatty acid profiles in

freshwater had a similar percent of EPA and DHA, but the brackish water sites had an increase in percent DHA. The larval river herring from the Chowan River and tributaries had a similar fatty acid profiles with increased DHA over space and time when compared with the plankton community. This increased DHA could have been a result from bioaccumulation or bioconversion, elongating shorter chain fatty acids to longer chain fatty acids. This dissertation research resulted in an assessment of nursery habitat areas that included the important component of the lower trophic food web. The planktonic food web in both rivers had the critical fatty acids EPA and DHA present; and therefore, fatty acid limitation is not likely a factor in the lack of herring recovery. However, further investigation is needed to determine if the total amount of these fatty acids is limiting to river herring. At present, a quantitative assessment of the fatty acid requirements for river herring growth and survival remains unknown. All research sites are considered strategic habitat areas in North Carolina, and this research can inform and improve the model for defining important fish habitats that are not listed as primary nursery habitat. For example, fatty acids of the plankton could be monitored to determine if changes are occurring in the food quality for zooplankton and larval fish. The answer to the question "Are all fish nursery areas equal?" is no. This answer informs management and researchers because it incorporates more factors than physical habitat alone. For example, if a cyanobacteria bloom occurred, this major shift in phytoplankton composition would alter fatty acid profile of the food web, but abiotic habitat parameters would not change. These results then can help to better predict possible future effects on important nursery habitat that could relate to river herring recovery or the lack of recovery in the future.

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Chapter One: Nursery Habitat and Strategic Habitat Areas Overview

Nursery habitat is considered important for fishes and invertebrates as a place where juveniles are subjected to lower predation levels and can put on biomass prior to returning to the ocean (Beck et al. 2001). The concept that nursery habitat exists for aquatic species has been around since the early 1900's. In the early 2000s, Beck et al. (2001) redefined and clarified the nursery habitat concept to be described as the nursery habitat hypothesis and developed a number of testable hypotheses. For instance, nurseries were defined as areas where juvenile fishes or invertebrates have higher densities, areas that allow them to avoid predation, and where faster growth rates could be observed compared to other habitats (Beck et al. 2001) (Table 1). Furthermore, a nursery functions "for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur" (Beck et al. 2001). Both the nursery habitat concept and associated hypotheses do not directly measure other factors (e.g. abiotic and biotic factors) that affect fish. Further, they do not incorporate the nursery areas used by larval fishes since most juveniles are often not found in the same locations as larvae.

Researchers have recognized that the nursery habitat concept/hypothesis does not consider the complex dynamics occurring within the lower trophic food web in nursery areas (Sheaves et al. 2015). Management agencies need to reconsider nursery habitat quality not only by what emerges at the end in terms of fish biomass, but also focusing on the complex mechanisms that regulate the interactions between both the environment and other trophic levels (e.g. phytoplankton and zooplankton) (Sheaves et al. 2015). Coastal and estuarine areas are increasingly impacted by anthropogenic effects, such as eutrophication, climate change and fishing pressure. These external stressors and food web interactions occurring within the nursery

habitat are not incorporated into the current definition for nursery habitat (Sheaves et al. 2015). However, efforts are underway to incorporate three major aspects into the nursery habitat concept: connectivity/population dynamics, ecological/eco-physiological factors, and resource dynamics (Fig. 1) (Sheaves et al. 2015). My research aimed to address the importance of ecological/eco-physiological factors and resource dynamics by focusing on eco-physiological factors, food webs, and resource availability that impact larval fishes in nursery habitats (Sheaves et al. 2015, Fig. 1). Therefore, I can ask the question: "**Are all larval fish nursery habitat areas equal?**"

Management agencies throughout coastal and estuarine areas have adopted the nursery habitat concept when designating conservation areas for anadromous fish spawning and nurseries (Sheaves et al. 2015). In particular, managers of the North Carolina Division of Marine Fisheries (DMF) and Wildlife Resource Commission (WRC) use a similar definition of nursery areas to designate Anadromous Fish Spawning Areas (AFSA) (NCDMF 2015) (Table 1). In 2009, North Carolina DMF started to designate areas throughout the coastal habitat as strategic habitat areas (SHAs) (NCDMF 2015). These SHAs are defined as areas that contribute most to the integrity of the system and for fish as "locations of individual fish habitats or systems of habitats that have been identified to provide exceptional habitat functions or that are particularly at risk due to eminent threats, vulnerability or rarity" (Deaton et al. 2006) (Table 1). Two regions, the Chowan River (Albemarle Sound) and the Tar/Pamlico River (Pamlico Sound), in North Carolina have SHAs throughout the watershed (NCDMF 2015) (Fig. 2). The Chowan River was designated as SHAs because of its AFSA designation and its importance for spawning and migrating anadromous fishes (NCDMF 2015). The Tar/Pamlico River had SHAs area designated, but those areas were not based on nursery habitat for anadromous fishes or AFSA designation but on land

cover, water quality and other fish data present (NCDMF 2015). One group of vulnerable fish are the river herring, which are made up of two species of alosines, blueback herring (*Alosa aestivalis*) and alewife (*A. pseudoharengus*). Most of the monitoring for river herring occurs in the Albemarle Sound basin, which encompasses the Chowan River (NCDMF 2015). Other rivers in North Carolina have not been monitored since the 1980s for river herring because of a reduction in federal funding (NCDMF 2015).

River herring were an important commercial fishery in North Carolina and throughout the eastern seaboard, but a decline in populations resulted in a moratorium on river herring harvest implemented at the state level in 2007 (ASMFC 2012 & NCDMF 2015). The Atlantic State Marine Fisheries Commission (ASMFC) followed suit by implementing a fishing moratorium throughout the entire river herring range by 2012 (ASMFC 2012 & NCDMF 2015). River herring return to spawn throughout the SHAs and AFSA in North Carolina in early spring with the largest population returning to the Albemarle Sound basin (NCDMF 2015). Larval and juvenile river herring stay in the freshwater reaches until they migrate back to the ocean between June and October (NCDMF 2015). River herring have not returned with increased numbers and are still considered a depleted stock despite having a harvest moratorium in place for over 10 years (NCDMF 2015).

I chose two rivers that have SHA and AFSA designations to determine if and how the habitats that larval river herring experience differ in these two systems. The Chowan River and tributaries are managed as AFSA habitat for river herring and were monitored for river herring population since 1970s but only sporadically until a continuous monitoring program started in 2008 (NCDMF 2015, Fig. 2). The Tar/Pamlico River and tributaries are designated SHA and AFSA, but because of limited funding, have not been or very sporadically monitored since the

1980s for river herring populations (NCDMF 2015, Fig. 2). Most of the water areas are under the jurisdiction of the NCWRC, with a few sites and the main Chowan River also managed by the NCDMF. NCDMF does not allow any river herring to be caught or kept in coastal waters, but the WRC included a regulation that allows fishers to keep river herring less than 6 inches long if fished from boat or pier (NCDMF 2015).

The Chowan River originates in the Virginia coastal plain and is the 12th largest river basin in North Carolina (NCDENR 2006). The Chowan River and its tributaries are the main drainage basin into the Albemarle Sound. The river is mainly freshwater, with intermittent saltwater intrusion events during drought years and winter months. Throughout parts of the year, water quality is poor with low dissolved oxygen levels (<3.0 mg L^{-1}). The first large scale algae bloom occurred in the Chowan River in 1972, resulting in the classification of the Chowan River as "nutrient sensitive waters" in 1979 (NCDENR 2006 & NCDMF 2015). Increased nutrients from factories and agricultural runoff affected this system (NCDENR 2006 & NCDMF 2015). These increases in nutrients resulted in nuisance algal blooms (Paerl et al. 1995). The blooms have since returned in 2015 after an absence of 25 to 30 years (NCDWR 2017). The Chowan River is considered a critical habitat for larval and juvenile river herring (NCDMF 2007). Zooplankton research conducted in the Chowan River was used to determine if the zooplankton communities and abundance in these areas were suitable for river herring growth and development. Results showed that the quantity of zooplankton was enough to sustain the larval and juvenile river herring; however, prey quality was not considered (Leech et al. 2009).

The Tar/Pamlico River begins in the Piedmont region and is the 4th largest river basin in North Carolina (NCDWQ 2010). Tar/Pamlico River is a major freshwater source to the Pamlico Sound (NCDWQ 2010). The entire basin is classified as nutrient sensitive waters, and nutrient

enrichment and the nuisance algal blooms are the main water quality issues (Stanley 1992 & NCDWQ 2010). The Tar/Pamlico River flows into the Pamlico Sound and has a salinity gradient throughout the year in parts of the river. Historically, river herring have been found in some of the tributaries and upriver (NCDMF 2015).

The goal of this dissertation was to expand the nursery habitat concept, and apply this research to the SHAs (Table 1). Nursery habitat concept in a general definition and in North Carolina considers the juvenile stage of fishes and the physical habitat in the area (Beck et al. 2001 and NCDMF 2015). The SHAs consider more factors based on the available data from the state of North Carolina and can include any life stage of fish, physical habitat, land cover, and water quality (Deaton et al. 2006) (Table 1). I wanted to incorporate water quality, lower trophic food web (phytoplankton and zooplankton), and food "quality" through the use of fatty acid analysis into these models to allow more information to be used to define nursery habitat and manage these SHAs (Table 1). I explored how abiotic factors (temperature, salinity, dissolved oxygen, pH, and nutrients) would affect the phytoplankton, zooplankton, and larval fish. I suggest that the fatty acid composition would be determined from the "bottom-up", and I sought to determine this by characterizing the functional groups of phytoplankton and their fatty acids. Furthermore, I investigated zooplankton composition because these groups can have an effect on the fatty acid profiles moving up the food to the larval fish (Table 1). From the research, I hoped to gather the baseline data in the Chowan and Tar/Pamlico River that could be used to demonstrate that similar physical habitat would not be the same when incorporating these key elements.

My dissertation includes 5 chapters: a general and historical introduction of what defines nursery habitat and a description of my research locations (CH 1), a chapter that focuses on how

lipid and fatty acid composition of the spawning females impact larval herring prior to feeding (CH 2), nursery habitat conditions within the Chowan River and Tar/Pamlico River (CH 3 and 4, respectively), and concludes by comparing and contrasting the two rivers as nursery habitats and what might inform management decisions (CH 5). I examined how the quality of the female river herring affect larval river herring. Female river herring impart lipids via the yolk sac to their larvae, and these lipids are used for development and growth before the first feeding. These lipids are obtained during the time the female forages in the ocean and have the potential to impact larval growth and development prior to their first feeding independent of the local habitat. To test this effect, I investigated the ovaries and tissue from the females to determine percent total lipids and fatty acid profiles as indices of quality.

In the third chapter, I characterized the lower trophic level food web in five locations within the Chowan River (3 tributaries and 2 sites) to determine if there were differences in the phytoplankton composition (i.e. pigment), seston fatty acid, micro- and mesozooplankton community composition and fatty acid profiles, and larval fish fatty acid composition. I then examined whether these factors were related to differences to abiotic factors (temperature, dissolved oxygen, pH, salinity, and nutrients). These findings were used to assess habitat quality for larval river herring in areas designated as a strategic habitat.

In the fourth chapter, I characterized the lower trophic level food web in five locations within the Tar/Pamlico River (3 tributaries and 2 sites) to determine if there are differences in the phytoplankton composition (i.e. pigment), seston fatty acid, micro- and mesozooplankton community composition and fatty acid profiles. I then examined whether these factors were related to differences to abiotic factors (temperature, dissolved oxygen, pH, salinity, and

nutrients). These findings were used to assess habitat quality for larval river herring in an area not designated as a strategic habitat for river herring, but that contains larval river herring.

In the final chapter, I discussed how my findings may be used to inform the fish nursery concept as discussed in Sheaves et al. (2015). The goal was to populate the "black box" of what constitutes good nursery habitat with information that may be used to revisit the fish nursery concept. I also discussed how my findings fit into the SHA definitions for North Carolina and suggestions are given for potential changes in the management of these two rivers. The chapter ends by discussing the limitations of the data set and future research that needs to be conducted to expand the nursery habitat concept. My dissertation provides the first examination of the fatty acids available for larval fish for the lower trophic food web in the Chowan and Tar/Pamlico Rivers and discusses the next research steps to help place this research in management context.

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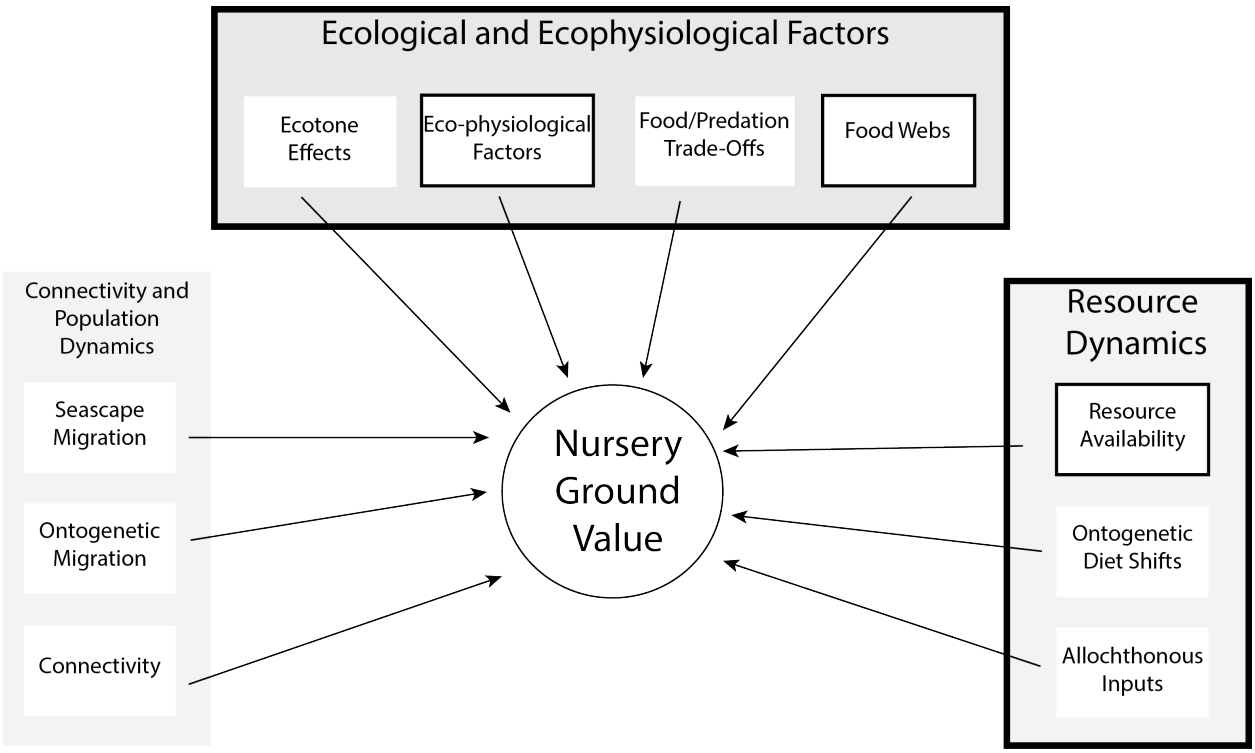

Fig. 1: Components of nursery ground value for successful nursery occupation. Image redrawn from Sheaves et al. (2015). Boxes outlined in black represent the components examined in this dissertation.

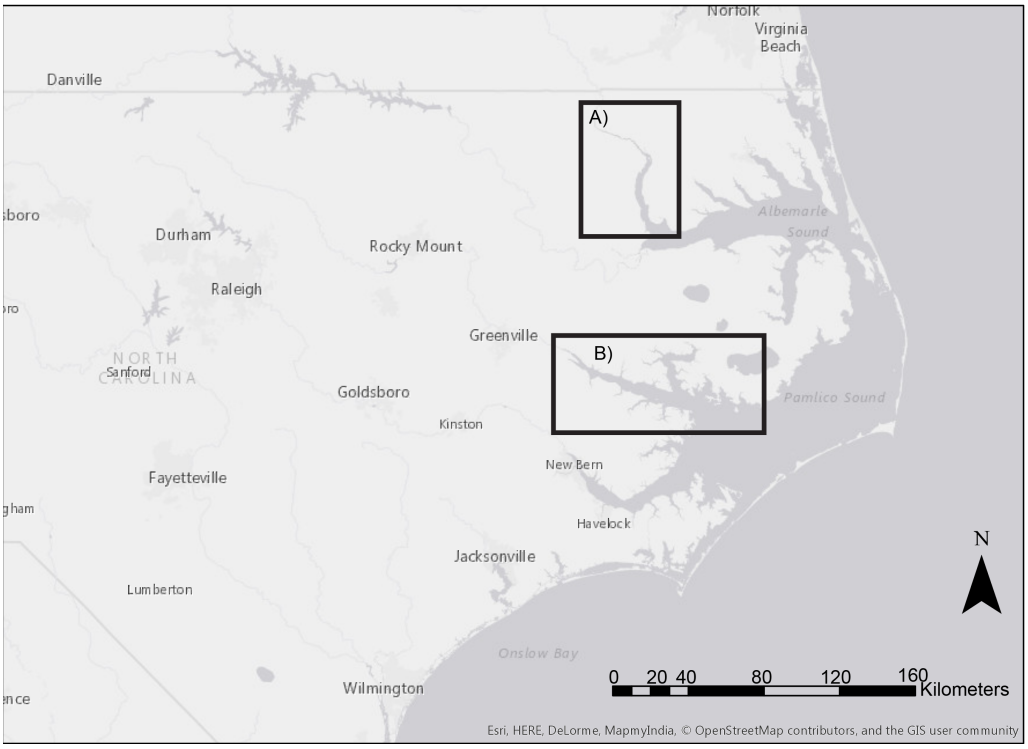

Fig. 2: Map of the location of the two rivers (A) Chowan River, and B) Tar/Pamlico River in North Carolina.

Table 1: Comparison of the nursery habitat concept in Beck et al. (2001), nursery habitat and strategic habitat areas in North Carolina by comparing the main categories used to determine classification. Smiley faces designate what will be included throughout this dissertation.

		North Carolina	
	Nursery Habitat	**Nursery Habitat**	**SHAs**
Fish Life Stage	Juvenile	Juvenile	Any stage
Habitat	✓	✓	✓ Land Cover
Nutrients	✗ ☺	✗	✓ ☺
Abiotic	✗ ☺	✗	✓ ☺
Fish	✓ ☺	✓	✓ ☺
Zooplankton	✗ ☺	✗	✗ ☺
Phytoplankton	✗ ☺	✗	✗ ☺

✓ = included ✗ = not included ☺ = my dissertation

Chapter 2: A comparison of tissue fatty acid profiles from pre-spawning river herring (*Alosa aestivalis* and *A. pseudoharengus*) in the Chowan River, North Carolina, USA.

Introduction

Two species of alosines, blueback herring (*Alosa aestivalis*), and alewife (*A. pseudoharengus*) comprise the group named river herring. River herring are anadromous fish species found on the eastern seaboard of North America from Newfoundland to Florida. Late-stage juveniles and adults live in the ocean, but return to natal streams after reaching sexual maturity between the ages of 3 and 6 to spawn (NCDMF 2015, Schmidt et al. 2003). River herring are iteroparous and return once a year to spawn (Walters et al. 2009). There has been documentation of river herring straying from their natal river during the spawning run in the Connecticut River and the Hudson River, which could result in population's dynamics being altered in natal habitat by reducing genetic adaptation to conditions in their natal river as well as greater population connectivity (Gahagen et al. 2012, Turner & Limburg 2014). Overtime, the river herring population has declined throughout the eastern seaboard.

River herring populations have declined historically (Fig. 3) and were listed as a species of concern by the National Marine Fisheries Service in 2006 (NOAA 2006). Fishing moratoria were put into place by 2012 throughout the eastern seaboard, yet the populations have not recovered (NCDMF 2015). In North Carolina, the largest population of river herring was found in the tributaries of the Albemarle Sound. This population experienced a similar decline to the eastern seaboard as a whole, and river herring are still considered a depleted stock despite the ongoing moratorium that began in 2007 (NCDMF 2015). Continued declines in female biomass

have been observed, and are only recently as of 2014 are beginning to show signs of potential population increase (Fig. 3) (NCDMF 2015).

There are many reasons for population declines occurring in the marine and freshwater environments. The main hypothesis in marine environments is that bycatch of river herring occurs during their resident time in the ocean and during migrations to feeding grounds in the mid-Atlantic region when river herring are school near cod (*Gadus morhua*), Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*) (Bethoney et al. 2013, Turner et al. 2016). The bycatch of river herring in 2007 for direct landings was double compared to the 2005 bycatch, which was only one-tenth to one half of commercial river herring landings (NEFMC 2012). Bycatch is harder to manage because data are not consistently recorded for monitoring purposes, and this may be due, in part, to the decline in river herring (Bethoney et al. 2013, Turner et al. 2016). Past overfishing, spawning habitat loss, pollution, and increasing predator populations are the reasons for the river herring decline during spawning and nursery area residency (ASMFC 2012). Most research regarding the population decline has occurred in the freshwater spawning and nursery habitats, where river herring reside for 3 to 9 months before migrating to the ocean (Limburg & Waldman 2009, ASMFC 2012, Hall et al. 2011, NCDMF 2015, Mattocks et al. 2017). Construction of dams and culverts throughout watersheds have reduced connectivity of spawning habitat and changed hydrologic flow regimes, which has led to spawning habitat loss (Limburg & Waldman 2009, Mattocks et al. 2017). Altering hydrology by river fragmentation started as early as the 1600s in the northeastern United States, and these activities still continue today although dam removal is becoming more common in some regions (Hall et al. 2011). In addition to physical changes to river ecosystems, humans have greatly increased nutrient pollution from non-point sources (e.g., agriculture, urban development).

Specifically, increases in nitrogen and phosphorus concentrations throughout the river systems have resulted in harmful algae blooms and increased incidences of hypoxia and anoxia (ASMFC 2012, NCDMF 2015). Humans have also altered aquatic food web through the management of other important fisheries especially striped bass, which prey on all stages of river herring. Therefore, increases in predator populations may have also contributed to river herring population declines (Hartman & Margraf 2003, Heimbuch 2008). In North Carolina, river herring spawning and nursery habitat are designated as SHAs and anadromous fish spawning areas to allow for the management of the population (NCDMF 2015). However, in order to effectively manage this fishery, the condition of the spawning females must be assessed prior to assessing the nursery habitat.

Maternal effects on offspring are not well documented in anadromous river herring, but should be considered as a potential contributor to population decline. When fish populations decline, there may be a shift in the population structure to smaller, and younger fish returning to the spawning grounds (Olsen et al. 2005, Hsieh et al. 2010). River herring populations in Connecticut had fewer repeat spawners, with smaller individuals ages between 3 and 4 years in 2005 and 2006 compared to 1966 because of population structure changes (Davis & Schultz 2009). There was a similar trend of fewer repeat spawners both throughout the eastern seaboard and in North Carolina (NCDMF 2015). Lower numbers of offspring may result since fecundity increases exponentially with female size and age. In addition, changes in food resources for river herring impact fish populations. Energy from food resources is allocated for gonad development after the females reach sexual maturity, and in migratory fish is used towards storage lipids (Crawford et al. 1986, Wiegand 1996, Huynh et al. 2007, McBride et al. 2010). One advantage anadromous fish have is their consumption of energy rich food in the marine environment, but

changes in those environments could also lead to reduction in spawning success (Gross et al. 1988, Lynch et al. 2015). Lipids, especially fatty acids, are an important diet component that river herring need from their food source, thus, could be used as a proxy to assess female condition.

Fishes in marine environments cannot convert lower chain fatty acids to higher chain fatty acids. but freshwater fish can bioconvert fatty acids (Arts et al. 2009). Therefore, these fish need to consume long chain fatty acids from the food sources to allow growth and gonad development (Dalsgaard et al. 2003). River herring are planktivores, and the zooplankton consumed are the main source of lipids and fatty acids. Percent total lipids can provide information on allocation of energy within the fish and are also used to determine the storage lipids in the adult fish returning to the spawning grounds (Crawford et al. 1986, Huynh et al. 2007). Fish total lipids will determine if they have enough energy for the spawning event. The yolk sac on larval fishes needs to provide nutrition for growth before the critical stage of first feeding (Hjort 1914, Wiegand 1996, Huynh et al. 2007). There should be the presence of omega-3 fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are necessary for cellular membrane, neural, and vision development and omega-6 fatty acids, in particular arachidonic acid (ARA) (Bell et al. 1995, Dalsgaard et al. 2003). If they do not have the proper nutrition in the yolk, then the larvae may not survive until the food is present for their first feeding.

When investigating spawning strength and reproductive success, one needs to investigate the maternal effects as well as nursery area characteristics. We quantified the total length, weight, GSI, and percent total lipids of female river herring returning to the Chowan River and analyzed the fatty acid profiles for the adult tissue and ovaries of these fish. I hypothesized that

1) female river herring would be similar to each other in size, weight, and percent total lipids during the spawning period; 2) gonadal somatic index would be high and thus indicate that these fish are pre-spawn females; 3) the ovaries would have increased omega-3s fatty acids, especially EPA and DHA compared to female river herring somatic tissue; and 4) female tissue fatty acid profiles would be different during the spawning run, but ovary fatty acid profiles would be similar during the spawning period. These hypotheses are based on the assumption that river herring returning from the ocean are consuming a diet of marine zooplankton, which have increased omega 3 fatty acids, particularly DHA, compared to freshwater zooplankton (Art et al. 2009).

Methods

Study Site

The Chowan River is one of the largest tributaries that drains into the Albemarle Sound and is the 12th largest river basin in North Carolina (NCDENR 2006) (Fig. 4). It is mainly a freshwater estuary that experiences intermittent salinity intrusion (Leech et al. 2009, Lichti et al. 2017). The Chowan River was classified as "nutrient sensitive waters" in 1979 (NCDENR 2006) mainly due to the fact that it has routinely experienced algal blooms and low dissolved oxygen levels (< 3.0 mg L^{-1}). The entire Chowan River and its tributaries are classified as a strategic habitat area because these areas are also designated as anadromous fish spawning areas (AFSA) and are monitored for river herring throughout the system (NCDMF 2015).

Sample collection

The North Carolina Division of Marine Fisheries (NCDMF) collected female river herring in March and February in 2016 and 2017 on the tributaries of the Chowan River. Gillnets were set overnight at culverts or bridge overpasses, and fished Tuesday through Friday (Fig. 4).

Gillnet length was 2.13 to 2.44 m, and the nets had two stretched mesh sizes of 6.35 cm for blueback herring and 6.99 cm for alewife. An aggregate sample of river herring was collected from various locations throughout the Chowan River. Those river herring were frozen and brought back to East Carolina University to process for lipid analysis. I processed a total of 24 river herring: 5 fish collected on February 17, 2016, 8 fish collected on March 17 in 2016, and 11 fish collected on February 24, 2017.

Laboratory Processing

I measured river herring for total length (mm), weight (g), and in 2017 the ovaries were weighed. A tissue sample from the belly flap and the ovary were removed, and stored at -80°C until lipid analysis.

Lipid and fatty acid samples

Total lipids were extracted with chloroform-methanol (2:1, *v*/*v*) containing 0.01% butylated hydroxytoluene as an antioxidant (Folch et al. 1957). The organic solvent was evaporated under a stream of nitrogen and lipid concentration determined gravimetrically. Transmethylation of fatty acids was done according to the method described by Metcalfe and Schmitz (1969). A known amount of nonadecanoate acid (19:0) dissolved in hexane at a concentration of 8 mg ml^{-1} (Nu Check Prep Inc.) was added as internal standard. The fatty acid methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc.) using a 7693 mass spectrometer detector (Agilent Technologies, Inc.), a capillary column (Omegawax^{TM} 250 fused silica capillary column, 30 mm x 0.25 mm and 0.25 mm film thickness, Supleco®), and a 7890A autoinjector (Agilent Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.3 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^{-1}, 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) and quantified by comparing their peak areas with that of the internal standard (Czesny and Dabrowski 1998). The results of individual fatty acid composition were expressed in percentage of total identified FAME.

Statistical Analysis

I performed a series of multivariate analyses to address my specific objectives using the R environment (R v3.4.3, R Core Development Team 2017). I excluded any specific fatty acids that had a percent less than one for all treatments from the analysis. To test for the interaction of tissue type and month for the fatty acid profiles in female river herring and ovaries, I conducted a permutational analysis of variance (PERMANOVA) using the *adonis* function in the vegan package (Oksanen et al. 2018). PERMANOVA is a non-parametric technique related to ANOVA, but uses permutations and is particularly well suited to multivariate data sets that violate the traditional assumptions of ANOVA and also have low sample sizes, as was my case (Anderson 2001). To visualize patterns of fatty acid composition in female river herring and ovaries, I generated an ordination plot based on Principle Coordinates Analysis (PCoA).

Results

Female and Ovary Characteristics

River herring had similar total length, weight, and GSI between months. Female river herring had a mean total length of 282.3 mm (± 9.7 S.D.) in February and 277.5 mm (± 11.2 S.D.) in March, and a mean total weight of 202.6 grams (± 31.9 S.D.) in February and 221.1

grams (± 30.7 S.D.) in March. Gonadal Somatic Index (GSI) ranged from 15.5 to 27.3% for the female river herring in 2017.

Total Lipids and Fatty Acid Profiles

Percent total lipids were similar between months with ovaries having lower percent total lipids compared to female belly tissue. Percent total lipids for the female river herring ranged from 4.1 to 20.2% with a mean of 12.0% (± 4.5 S.D.) in February, and ranged from 7.0 to 18.7% with a mean of 12.6% (± 4.4 S.D.) in March. Percent total lipids for the ovaries ranged from 3.7 to 5.8% with a mean of 4.7% (±0.7 S.D.) in February and ranged from 1.2 to 4.3% with a mean of 2.3% (±1.0 S.D.) in March.

A total of 24 specific fatty acids were measured in all samples, and 19 specific fatty acids were selected using the criteria that at least one sample had the specific fatty acid greater than one percent present in the samples for statistical analysis (Table 2). Female river herring tissue had a higher percent of monounsaturated fatty acids (MUFA), and similar percentages of saturated fatty acids (SFA) and polyunsaturated fatty acid (PUFA) (Fig. 5 & Table 2). Ovaries had a higher percent composition PUFA, and similar percentages of SFA and MUFA (Fig. 5 & Table 2).

Female river herring tissue and ovary fatty acid profiles were significantly different between their type and month collected (PERMANOVA, R^2=0.04, $p < 0.001$) (Fig. 6). Female river herring tissue fatty acid profiles had larger between month differences compared to the ovary fatty acid profiles. Female river herring tissue in March had the highest mean percent of 20:1 (17.3%) compared to river herring tissue in February (Fig. 7 & Table 1). Female river herring tissue in February had a higher mean percent of 18:1n-9 (31.1%) compared to river

herring tissue in March (Fig. 7 & Table 1). Female river herring tissue had an increased concentration of 18:2n-6, 18:3n-3 (ALA) and 18:4n-3 compared to the ovaries which had lowest percent (Fig. 7 & Table 1). Ovary fatty acid profiles had a higher percent of highly unsaturated omega-3 fatty acids (EPA and DHA) compared to the female river herring flesh (Fig. 7 & Table 1).

Discussion

The quality of the female river herring on the spawning grounds during February and March is likely to result in spawning events that would produce viable offspring. Differences in river herring fatty acid composition occurred between different months and tissue types, although the variance explained by these two factors was low. River herring were similar in length and weight, but gonadal somatic index (GSI) and percent total lipids varied between individual female river herring. The GSI differences could be due to different developmental stages of oocytes, but all females were considered pre-spawned individuals. The differences in females' percent total lipids can reflect varying time spent within the spawning grounds. GSI values increase as river herring are closer to spawning, and lipid depletion increases as fish decrease feeding during the spawning run (Netzel and Stanek 1966, Crawford et al. 1986, Huynh et al. 2007, Simonin et al. 2007, McBride et al. 2010). Female river herring fatty acid profiles were similar to marine planktivores with increased 20:1 during the time of spawning (Huynh et al. 2007, Tocher 2003). In addition, female river herring signatures showed an increase in particular fatty acids (18:2n-6, 18:3n-3 (ALA), 18:4n-3) that are essential fatty acids for all fish (Dalsgaard et al. 2003). In contrast, river herring ovary fatty acid composition was enriched in PUFAs, especially the omega-3 (DHA), which is needed for cell and neural development in

larval fish. Therefore, I conclude that the condition of female river herring are likely not related to the lack of population recovery.

River herring population recovery is ongoing, and more time is needed for the population to rebound. During the population rebound, river herring could return previous size class and older individuals. For my study, the age class for the river herring collected in the Chowan River was estimated to be between 4 to 6 years old based on the total length of the females, which is similar to the ages seen in the Albemarle Sound since 1972 (NCDMF 2015). Historically, river herring could be found up to 7 to 10 years of age spawning throughout the south (NCDMF 2015). This has been seen in other systems, as well. For example, in Connecticut, river herring populations were younger (4 to 5 years of age), and smaller in 2005 and 2006 compared to the river herring in 1966 (Davis & Schultz 2009). There was a reduction of fork length in river herring in 2011 (220 to 280 mm) compared to 1972 (240 to 310 mm) in North Carolina (NCDMF 2015). Larger sized river herring may start to return in larger numbers with the continued closure of the fishery, which had been closed in North Carolina for 9 to 10 years when these river herring were collected. After the closure of the Atlantic cod (*Gadus morhua*) fishery, cod were smaller and younger; but after 15 to 20 years, cod size increased and older fish were able to spawn again (Olsen et al. 2005). River herring might be experiencing a similar trend with increase age, and possibly as time continues, in size structure as well.

Anadromous fish migration and oocyte development result in lipid depletion from stored energy. Alewife store lipids during the fall season and deplete lipids when overwintering and during the spawning migration (Netzel and Stanek 1966). River herring in the Chowan River had the highest total lipids in February with a maximum of 20.2%. One female in February 2016 that was close to spawning, as indicated by hydrated oocytes, had the lowest percent total lipid at

4.1%. The females from March in the Chowan River ranged between 7% and 18.7% in total lipids. This range could represent the time spent on the spawning grounds and distance of migration. For example, river herring from a population in Nova Scotia had a mean range of total lipids from 5.4 to 8.7% with a 22 to 38% depletion of lipids from their migration for a site near the marine environment to a site farther way (Crawford et al. 1986). Similar lipid depletions have occurred for migrating alosines, such as shad (*A. sapidissima*), which depleted 40% of their lipid reserves during peak runs on the Connecticut River (137 km) (Glebe & Leggett 1981). Four explanations for the higher percentage of total lipids for the Chowan river herring compared to other population are: (1) possible feeding until reaching the freshwater reaches of the migration when actual feeding stops, (2) continued feeding in the freshwater reaches and (3) decreased time from overwintering to reaching the spawning grounds, and (4) multiple spawning sub-populations. In a previous study, blueback herring in the St. John River, FL actively fed on copepods, cladoceran, and amphipods during the migration and spawning run in the freshwater reaches (McBride et al. 2010). Such feeding strategies would result in changes in percent total lipids present in females on the spawning grounds. Feeding strategies could differ between river herring populations, and populations may vary in both lipid and fatty acid content. The main food source of river herring in the marine environment is zooplankton, which store lipids in the northern latitudes at increased rates compared to southern populations (Lee et al. 2006). River herring migration time results in different levels of percent total lipids. River herring spawning occurred in early January in Florida, February and March in the Chowan River, but in Nova Scotia spawning occurred from April to June (Crawford et al. 1986, McBride et al. 2010, NCDMF 2015). River herring could be consuming food during the migration to spawning

grounds, but also southern populations spawn earlier in the year compared to northern populations, which could result in reduction of lipid depletion.

The lipid composition tells a story about the female river herring beyond the total lipids. River hearing have similar SFA percent composition compared to other marine herring species, such as Pacific herring (*Clupea pallasii*) and Baltic herring (*C. harengus*) (Aro et al. 2000, Huynh et al. 2007, Linko et al. 1985). The most abundant SFA is 16:0 in most fishes because of the prominence it has in metabolic energy for growth and development of the roe (Henderson et al. 1984). The fatty acid 18:1n-9 had the highest percent (31.1%) in females in February but was depleted (13.7%) in March. The 18:1n-9 percent is similar in non-spawning fish, and usually found at these levels during summer feeding periods (Henderson et al. 1984, Huynh et al. 2007). This fatty acid is used for energy metabolism and gonad development (Wiegand 1996). River herring feeding during spawning could result in increases of 18:1n-9 compared to other fish species that see depleted amounts when feeding has stopped. Female river herring in March on the Chowan River had increased 20:1 MUFA. This MUFA has been linked to marine copepods (Huynh et al. 2007, Tocher 2003). River herring in the Chowan River had similar percent of 18:2n-6, ALA and 18:4n-3 to other marine species. Pacific herring that are a marine species have low concentrations of 18:2n-6 and ALA in their fatty acid profile before or during spawning (Huynh et al. 2007). Female river herring sampled in the Chowan River had similar fatty acid profiles to planktivorous marine fish.

Female river herring sampled were all pre-spawn individuals. The GSI index for the female river herring ranged from 15.5 to 27.3%, which is similar to other populations of river herring that are pre-spawned and/or close to spawning. For example, river herring in the St. John River had a GSI index of 26.8% for females with hydrated oocytes, but spent females had GSI as

low as 1.2% (McBride et al. 2010). River herring in the Hudson-Mohawk River had GSI greater than 25% during the middle of spawning (Simonin et al. 2007). Oocytes' total lipids are a small percent of the chemical composition, but are important for development of the embryo once fertilized (Huynh et al. 2007). In the present study, river herring had a similar total lipid content ranging from 1.2 to 5.8%. Pacific herring roe had a mean total lipid of 2.81% for spawning individuals (Huynh et al. 2007). In this study, river herring oocyte PUFAs ranged from 26.6% to 31.1%, which is similar to Pacific herring (Huynh et al. 2007). Oocytes have increased in PUFAs especially DHA because they are important component of membrane structural lipids (Arts et al 2009, Tocher & Harvie 1988). The oocytes' percent DHA for river herring was similar to Atlantic herring, cod, and Pacific herring (Huynh et al. 2007). There is selective transfer of DHA to the oocytes which results in higher percent in the oocytes compared to female tissue (Tocher 2003). There was lower percent of 20:1 because of non-selection transfer in the oocytes (Tocher 2003, Wiegand 1996). Overall, female river herring ovaries seem to compare to other marine spawning planktivorous fishes and have the fatty acids for producing viable offspring.

Conclusion

Female river herring sampled in this study had tissue and ovary total lipids and fatty acid profiles that would suggest they are able to successfully migrate, spawn, and provide egg lipids that allow larval river herring to survive to first feeding. However, we must qualify this statement by noting that the exact amount of these lipids and fatty acids required for migration, spawning, and larval provisioning remain unknown. Female river herring that had returned to the spawning ground in the Chowan River did not appear to be starving based on lipid composition and possessed particular indicator fatty acids (e.g., DHA) that are essential for viable offspring. The fatty acid composition of river herring oocytes indicated that a significant percentage of

DHA was present, thus river herring larvae would have been likely to be well provisioned prior to first-feeding. In 2014 and 2015, there was a slight increase in female abundance, which could result in more viable offspring (NCDMF 2015).

Continued monitoring of adult river herring populations is essential as additional stressors such as climate change could result in food web changes that may impact recovery. Moreover, as the ocean warms, the marine environment could experience a mismatch of food resources to predators, or reduction in lipid rich food (Litzow, et al. 2006). Taken together, changes in diet can impact river herring storage of lipids for the migration and oocyte development (Arts et al. 2009, Hoegh-Guldberg & Bruno 2010, Petchey et al. 1999). The decrease in preferred habitat for river herring has also been shown in ocean warming models (Lynch et al. 2015). Continued monitoring of female and ovary condition is as important tool to guide the management of nursery habitats during their recovery. Next, we will evaluate nursery habitat areas to determine how nutrient dynamics affect the lower trophic food web and therefore the survival and recruitment of larval and juvenile river herring.

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Figures:

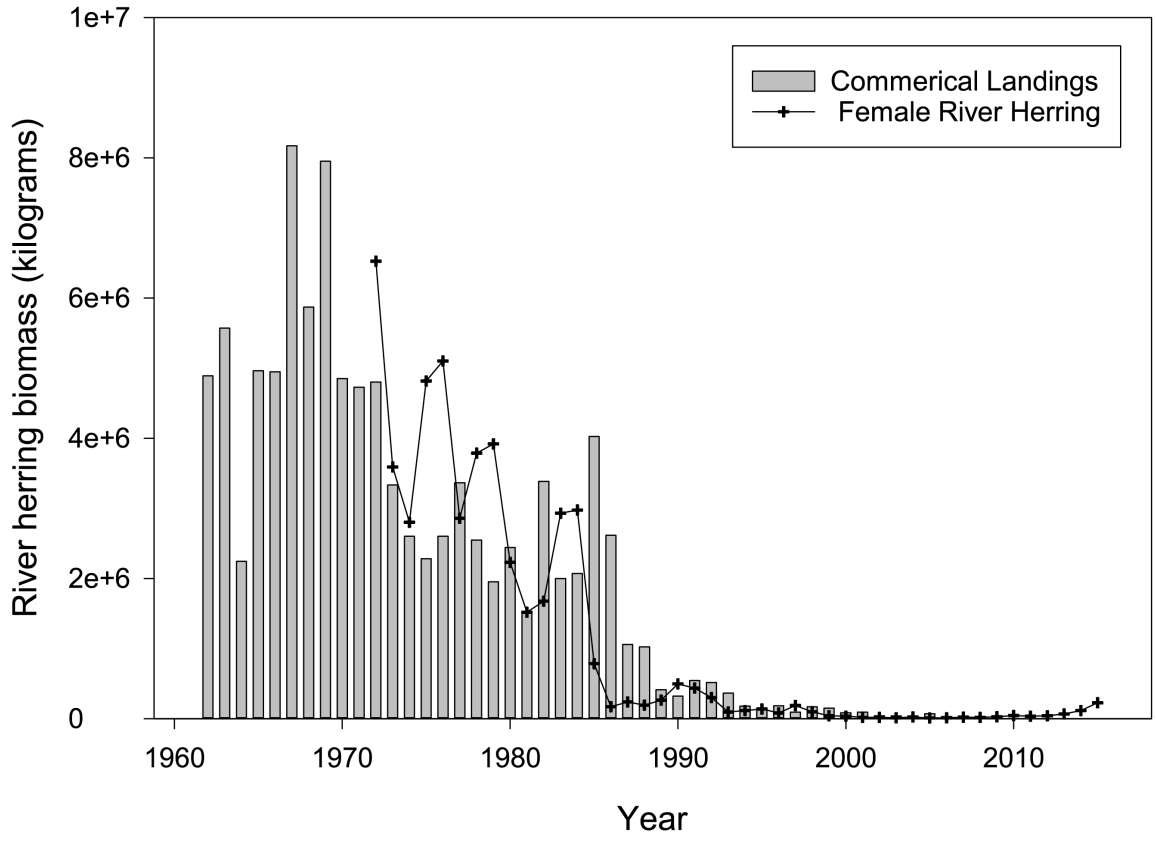

Fig. 3: Commercial landings (kilograms) and estimated total female biomass (kilograms) of river herring in the Chowan River, North Carolina. Data from the NCDMF (2015) Management Plan for River Herring.

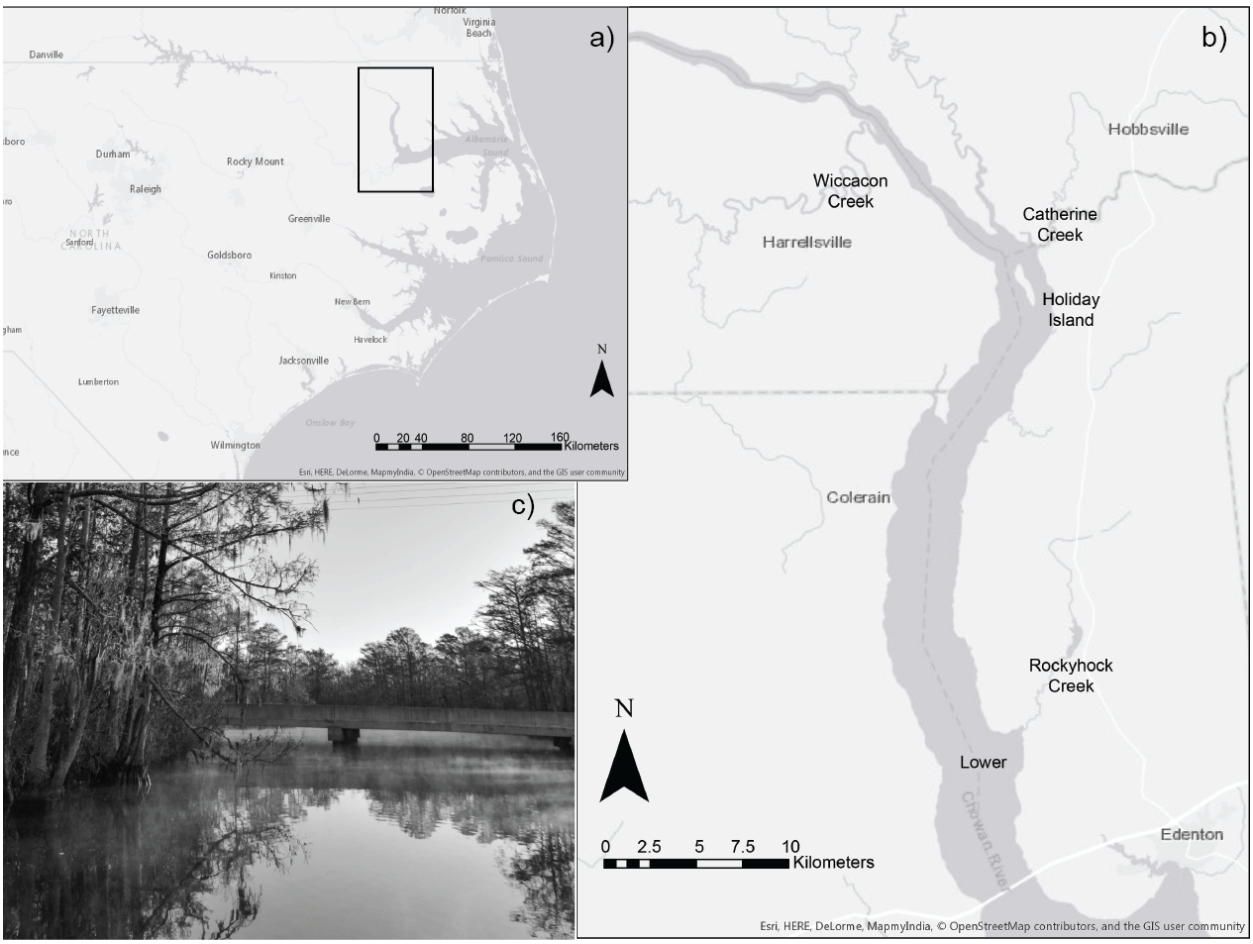

Fig. 4. The overview of North Carolina with the Chowan River (box) (a). The close-up view of the Chowan River and some of the tributaries that are considered spawning and nursery habitat (b). An example of a bridge overpass on Rockyhock Creek used by Division of Marine Fisheries for gillnet surveys to collect spawning river herring (c).

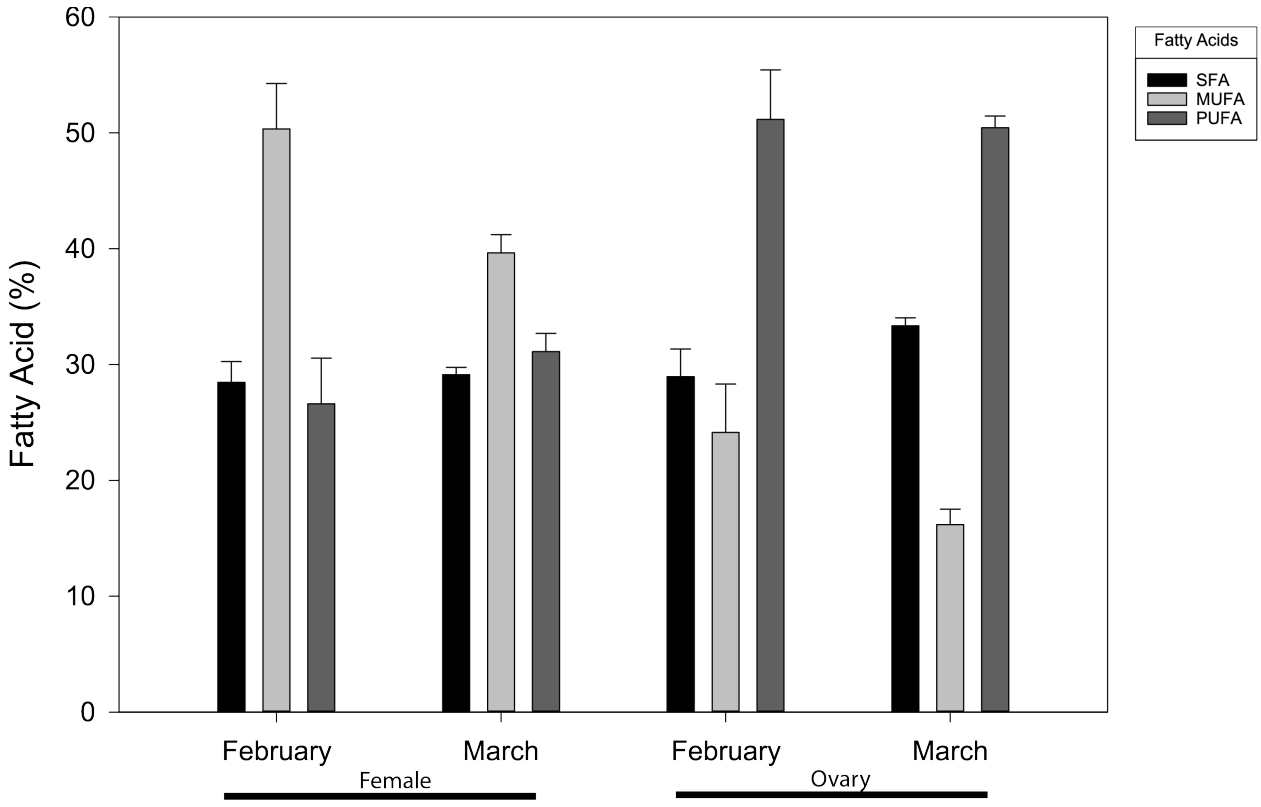

Fig. 5: Mean (%, + S.D.) of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid composition for female and ovaries over two months (February and March).

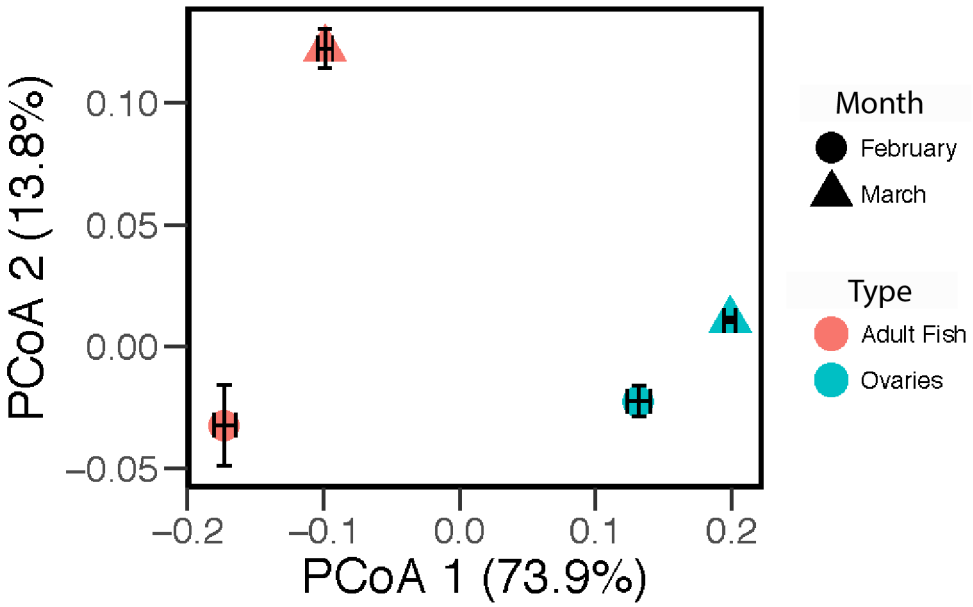

Fig. 6: Ordination from principal coordinates analysis depicting the fatty acid composition for female river herring and ovary over two months (February and March). Symbols are colored according to tissue type. The error bars are standard deviation.

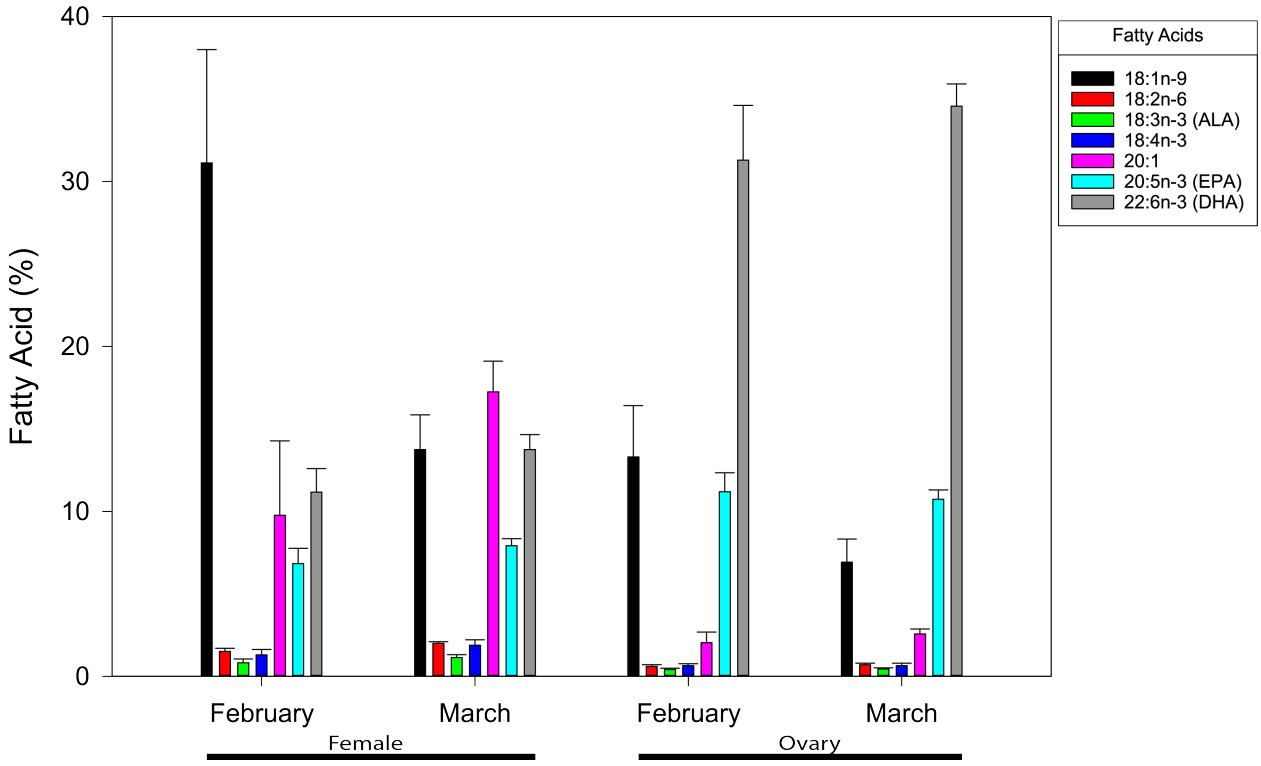

Fig. 7: The mean fatty acid composition (%, + S.D.) for female and ovary over two months (February and March). These fatty acids are the dominant PUFAs in the samples.

Table

Table 2: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of adult river herring tissue and ovaries from the Chowan River by month. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	Adult		**Ovaries**	
	February n = 16	**March n = 8**	**February n = 16**	**March n = 8**
14:0	5.4 ± 0.7	6.9 ± 0.3	2.9 ± 0.3	3.4 ± 0.5
15:0	0.4 ± 0.2	0.3 ± 0.0	0.3 ± 0.2	0.1 ± 0.0
16:0	17.9 ± 1.2	17.1 ± 0.5	21.1 ± 1.9	25.5 ± 0.6
17:0	0.4 ± 0.1	0.6 ± 0.0	0.4 ± 0.1	0.5 ± 0.0
18:0	3.7 ± 0.5	3.8 ± 0.4	4.1 ± 0.4	3.9 ± 0.6
20:0	0.7 ± 1.6	0.5 ± 0.1	0.1 ± 0.0	0.0 ± 0.0
∑SFA	**28.5 ± 1.8**	**29.1 ± 0.6**	**29.0 ± 2.4**	**33.4 ± 0.7**
16:1n-9	0.1 ± 0.2	0.3 ± 0.0	0.5 ± 0.4	0.3 ± 0.0
16:1n-7	6.2 ± 1.5	5.5 ± 0.5	4.5 ± 1.3	3.3 ± 0.5
18:1n-9	31.1 ± 6.9	13.7 ± 2.1	13.3 ± 3.1	6.9 ± 1.4
18:1n-7	3.1 ± 1.8	2.9 ± 0.2	3.8 ± 0.5	3.1 ± 0.2
20:1	9.8 ± 4.5	17.3 ± 1.9	2.0 ± 0.6	2.6 ± 0.3
∑MUFA	**50.3 ± 3.9**	**39.6 ± 1.6**	**24.1 ± 4.2**	**16.2 ± 0.7**
18:2n-6	1.5 ± 0.2	2.0 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
18:3n-3	0.8 ± 0.2	1.1 ± 0.2	0.4 ± 0.1	0.4 ± 0.1
18:4n-3	1.3 ± 0.3	1.9 ± 0.3	0.6 ± 0.1	0.6 ± 0.2
20:2n-6	0.6 ± 0.4	0.5 ± 0.0	0.5 ± 1.1	0.2 ± 0.0
20:3n-6	0.1 ± 0.1	0.1 ± 0.0	0.6 ± 0.9	0.1 ± 0.0
20:4n-6	0.7 ± 0.7	0.1 ± 0.0	1.4 ± 2.0	0.0 ± 0.0
20:3n-3	0.3 ± 0.3	0.2 ± 0.0	0.7 ± 1.0	0.1 ± 0.1
20:4n-3	1.3 ± 0.8	1.2 ± 0.1	1.8 ± 2.8	0.6 ± 0.1
20:5n-3	6.8 ± 0.9	7.9 ± 0.4	11.2 ± 1.2	10.7 ± 0.6
22:5n-6	0.3 ± 0.1	0.4 ± 0.0	0.2 ± 0.1	0.4 ± 0.1
22:5n-3	1.7 ± 0.3	2.0 ± 0.1	1.8 ± 0.2	2.1 ± 0.2
22:6n-3	11.2 ± 1.4	13.7 ± 0.9	31.3 ± 3.3	34.6 ± 1.3
∑PUFA	**26.6 ± 3.9**	**31.1 ± 1.6**	**51.2 ± 4.3**	**50.4 ± 1.0**

Chapter 3: Lower trophic food web dynamics and structure of nursery areas for anadromous fish in the Chowan River and three tributaries, North Carolina, U.S.A.

Introduction

Estuaries are important spawning and nursery areas for many anadromous fish (Beck et al. 2001). Nursery habitats are areas that produce high densities of juvenile fish, support faster growth rates, and are refugia from predation compared to other habitats (Beck et al. 2001). The nursery habitat concept leaves out multiple factors (e.g., abiotic and biotic factors) that affect fishes and does not include larval fish life stage (Sheaves et al. 2015). Three major aspects should be considered when investigating the nursery habitat concept: connectivity/population dynamics, ecological/eco-physiological factors, and resource dynamics (Sheaves et al. 2015). I examined two of these overall components (ecological/eco-physiological factors and resource dynamics) by investigating food webs, resource availability, and eco-physiological factors in this study.

An important component of a functional nursery habitat is the composition of the food source for fish, primarily zooplankton, which can be affected by abiotic conditions in the system (Sheaves et al. 2015). Since estuaries are important nursery habitats, nutrient dynamics in estuarine ecosystems that alter the phytoplankton composition result in changes in the quality of food for zooplankton (Müller-Navarra et al. 2000, Paerl et al. 2003). High growth rates of larval fishes are possible because zooplankton are present during their critical transition from yolk sac to free-living, feeding larvae (Hjort 1914; Mullen et al. 1986; Rulifson et al. 1993; Cooper et al. 1998; Martino and Houde 2010; Binon 2011). However, spatial and temporal overlap between predators and prey does not completely explain how fish nurseries function mechanistically. The

quality of prey also plays a major role in determining the effectiveness of a nursery for early stages of fish development (Fraser et al. 1989, Webster and Lovell 1990, Copeman et al. 2002, Rossi et al. 2006, Malzahn et al 2007, Paulsen et al. 2014). To better understand nursery habitat, I investigated how these dynamics systems in especially the food web resulted in changes for larval fish.

An organism's chemical composition (e.g., lipids) can be used to study how abiotic factors affect the different food sources for zooplankton and larval fishes (Fraser et al. 1989; Webster and Lovell 1990; Copeman et al. 2002; Rossi et al. 2006; Malzahn et al 2007, Paulsen et al. 2014). Fatty acids are chemically diverse, often incorporated into organisms unmodified, and different species have distinct profiles (Dalsgaard et al. 2003). Fatty acids are one class of lipids that are particularly important, impacting development in zooplankton and fish (Gulati et al. 1997; Müller-Navarra et al. 2000; Kainz et al. 2004; Masclaux et al. 2012). Fatty acids can act as both dietary tracers in the food web and indicators of overall food quality (Iverson et al. 2004). The majority of organisms need specific dietary fatty acids for somatic development and fitness (Masclaux et al. 2012). For example, 18:3n-3, α-linolenic acid (ALA), and 18:2n-6, linoleic acid (LA) are essential fatty acids because they cannot be directly synthesized by heterotrophic organisms and must come from the diet (Arts et al. 2009). Polyunsaturated fatty acids (i.e., 20:5n-3, eicosapentaenoic acid (EPA), 22:6n-3, docosahexaenoic acid (DHA), and 20:4n-6, arachidonic acid (ARA)) are required for all organisms and play a role in membrane development and cell function (Dalsgaard et al. 2003). Thus, an organisms' fatty acid signature can indicate dietary consumption and nutritional quality of its prey (Goncalves et al. 2012). Environmental factors can result in changes to the composition of the food web, especially among phytoplankton.

Phytoplankton can be an indicator of environmental change because they respond to fluctuations in nutrients, hydrology, sedimentation, irradiance, and temperature (Paerl et al. 2003). Nutrient concentrations have increased throughout estuaries from agricultural production, increased population growth in coastal areas, and retention time of nutrients recycled from storm events, which has resulted in increased phytoplankton biomass (Cloern 2001, Paerl et al. 2003, Flemer and Champ 2006, Walters et al. 2009). The increased nutrient loads and subsequent change in phytoplankton composition and abundance can be seen within hours and over years especially in freshwater areas of estuarine systems (Paerl et al. 2003). Even anadromous fishes returning to the freshwater environment will alter nutrient conditions. For example, alewife during peak spawning contribute 800-1500 g of nitrogen and 90-180 g of phosphorus to Bride Brooks in New London County, Connecticut (Walters et al. 2009).

Freshwater systems are affected by changes in nitrogen and phosphorus, which can result in algal blooms when excess nutrients are added to the system (Paerl et al. 2003). For example, when nitrate and phosphate concentrations were elevated in the Neuse River, chlorophyll *a* levels increased (Pinckney et al. 1999). While nutrients can impact overall chlorophyll *a* levels, less attention is often paid to the phytoplankton community composition. For example, different phytoplankton groups uptake nutrients at different rates. Diatoms use nitrate and are found in increased biomass when there is increased water clarity throughout estuaries (Paerl et al. 2003, Domingues et al. 2011). However, dinoflagellates respond to environmental perturbations differently and can consume dissolved organic matter by osmomixotrophy (Paerl et al. 2003, Wacker and Weithoff 2009). During the spring when temperatures increase in freshwater areas of estuaries, chlorophytes and cryptophytes outcompete other phytoplankton by having efficient growth rates and enhanced nutrient uptake rates, especially in acquiring ammonium and nitrate

(Pinckney et al. 1999, Paerl et al. 2003, Valdes-Weaver et al. 2006, Domingues et al. 2011). The changes in phytoplankton composition and biomass result in the differences seen in community wide phytoplankton lipid and fatty acid composition.

The origin of fatty acids in most aquatic food webs comes from phytoplankton (Farkas and Herodek 1964; Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). However, in estuaries, fatty acids may enter the system via detrital material (Farkas and Herodek 1964; Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). The two combined sources are often reflected in the composition of the seston, the inorganic and organic material in the pelagic environment (Postel et al. 2000). The fatty acid composition of the seston changes as a result of local conditions, such as temperature, nutrient concentration, and the degree of autotrophy or heterotrophy in the system (Farkas and Herodek 1964; Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). The detrital material of the seston would result in increased amounts of saturated fatty acids (SFA) (Persson and Vrede 2006, Gladyshev et al. 2010, Ravett, Brett, Arhonditsis 2010, Burns, Brett, and Schallenberg 2011, Gonclaves et al 2012).

The phytoplankton component of seston can be evaluated using pigments, which indicate phytoplankton functional groups because specific accessory pigments are found in particular groups (Paerl et al. 2003). Diatoms have the primary indicator pigment of fucoxanthin and have increased percent composition of 16:1n-7 and omega-3 fatty acids, especially EPA (Napolitano et al. 1997; Dalsgaard et al. 2003; Paerl et al. 2003, Boschker et al. 2005; Arts et al. 2009; Bec et al. 2010) (Table3). Photosynthetic dinoflagellates have a primary indicator pigment of peridinin and have increased DHA, an important omega-3 fatty acid in the fatty acid profile (Paerl et al. 2003, Art et al. 2009, Strandberg et al. 2015) (Table 3). Cryptophytes are a group of algae that

have a primary indicator pigment of alloxanthin, and had increased percent composition of omega-3, ALA and 18:4n-3 (Paerl et al. 2003, Strandburg et al. 2015) (Table 3). Zeaxanthin can be found in prochlorophytes, cyanobacteria, green algae, and chrysophytes, which could result in differences in the fatty acid composition, and the common fatty acid found throughout is ALA, a lower chain omega-3, and essential fatty acid throughout the food web (Paerl et al. 2003, Strandberg et al. 2015) (Table 3). Chlorophyll *b* is found as the primary indicator pigment in green algae or chlorophytes (Paerl et al. 2003) (Table 3). Chlorophytes have increased percent composition of omega-6 (18:2n-6), and omega-3 (ALA) (Ahlgren et al. 1990; Dalsgaard et al. 2003; Boschker et al. 2005; Masclaux et al. 2012; Strandberg et al. 2015) (Table 3). These changes in the seston fatty acid result in changes for the zooplankton from their diet.

Zooplankton fatty acid profiles are linked to species composition and the food sources in the system. Cladocerans, copepods, and rotifers are the dominant groups of zooplankton found in the freshwater areas of estuaries (Tackx et al. 2004, Marques et al. 2006, Winder and Jassby 2011, Chambord et al. 2016). Part of the cladocerans' and copepods' fatty acid profile does not result from the food source (Persson & Vrede 2006). Cladocerans have increased levels of EPA, but none to very low abundance of DHA (Persson & Vrede 2006), whereas, copepods have increased DHA and EPA (Arts et al. 2009). Cladocerans are characterized by higher levels of EPA and arachidonic acid (ARA) and this is thought to be related to a life history strategy focused on high rates of somatic growth and reproduction compared to copepods (Persson & Vrede 2006). In contrast, copepods have higher relative DHA levels because this fatty acid is critical for nervous system development (Arts et al. 2009). Rotifers are usually the dominant smaller bodied (>20 μm) zooplankton in freshwater areas (Park and Marshall 2000). Rotifer fatty acid profiles are closely related to their food source that usually is composed of lower chain fatty

acids (Gladyshev et al. 2010). A major food source for zooplankton is the phytoplankton component of seston (Brett et al 2006, Rossi et al. 2006). Zooplankton fatty acid profiles have been correlated to seston fatty acid profiles in some studies (Goulden and Place 1990, Desvilettes et al. 1997, Brett et al 2006, Rossi et al. 2006, Smyntek et al. 2008, Taipale et al. 2009, Gladyshev et al. 2010, Ravet, Brett, and Arhoditsis 2010). Zooplankton composition and changes in fatty acid profiles due to species or diet would impact the nutritional quality and food availability for larval fishes.

Most larval fishes are planktivores that consume smaller bodied zooplankton, therefore, the lower trophic food web is an important factor affecting growth and survival after first feeding (Hjort 1914; Mullen et al. 1986; Rulifson et al. 1993; Cooper et al. 1998; Martino and Houde 2010; Binon 2011). Most larval fishes in freshwater consume cladocerans, copepods, and rotifers, depending on the size of the fish (Ahlgren et al. 1992, Vuorio & Taipale 2017). Changes in the food consumed results in changes in the nutritional quality for the larval fishes (Bell et al. 1995, Arts et al. 2009, Taipale et al. 2018). Larval fish that reside in freshwater can convert lower chain omega-3 fatty acids (ALA) to higher chain omega-3 fatty acids (EPA and DHA), but the conversion may be inefficient because of the amount of energy (Taipale et al. 2018). Thus, larval fish grow better when consuming a diet rich in EPA and DHA (Brett and Müller-Navarra 1997, Arts et al. 2009). Larval fishes require DHA for growth and development, which results in bioaccumulation of DHA even when the food source may be lower in DHA levels (Arts et al. 2009, Taipale et al. 2018). When DHA is not present or low in dietary supply, this leads to growth and development issues. For example, Atlantic herring (*Clupea harengus*) had inferior vision when they did not consume enough DHA in their diet (Bell et al. 1995). In another study, juvenile rainbow trout (*Oncorhynchus mykiss*) had reduced growth and survival when fed a poor

quality diet of EPA and DHA (Taipale et al. 2018). Larval fish need to consume a diet that allows them appropriate growth and development. This is especially important when studying a fish population that declined and has not returned to pre-collapse numbers even with the closure of the fishery.

River herring are comprised of two alosine species, (blueback herring (*Alosa aestivalis*), and alewife (*A. pseudoharengus*)), and are anadromous fish that return to freshwater rivers during spring. River herring were an important fishery throughout the eastern United States, but the populations declined to very low levels (ASMFC 2012, NCDMF 2015). Moratoria have been in place in North Carolina since 2007, and throughout the eastern seaboard since 2012. However, the population has not returned, and populations are still considered depleted stocks (NCDMF 2015). Past overfishing, bycatch in marine environments, increased pollution, and loss of connectivity at the spawning grounds contributed to population declines throughout its range (ASMFC 2012). The Chowan River and its tributaries are an important spawning and nursery habitat for river herring in North Carolina, and a better understanding of the food web dynamics could be used to determine if the lack of river herring recovery may have something to do with quality of food available to larval fish (NCDMF 2015). Strategic habitat areas (SHAs) are defined as areas that contribute most to the integrity of the system and for fish as "locations of individual fishes habitats or systems of habitats that have been identified to provide exceptional habitat functions or that are particularly at risk due to eminent threats, vulnerability or rarity" (Deaton et al. 2006). Nutrient pollution is hypothesized to be one reason for the decline of river herring in the Chowan River as excess algae can lead to hypoxic zones and some algal blooms may contain toxic species (NCDMF 2015).

North Carolina Division of Marine Fisheries (DMF) has designated the Chowan River and its tributaries as important SHAs and as Anadromous Fish Spawning areas (AFSAs) and are monitored for river herring (NCDMF 2015). AFSAs were "established to protect those inland waters which as spawning areas for anadromous fishes, and provide the physical, biological, and chemical attributes necessary for spawning", but not all SHAs include spawning habitat (NCDMF 2015). The whole Chowan River was designated as SHA using the criteria that this area, and its tributaries, are an important spawning and nursery habitat for river herring. Therefore, the whole river was designated as SHA since this is the migration route to return to the spawning grounds (NCDMF 2015). These designations allow for management of these areas and research to better understand the populations.

The overall goal of my study was to investigate the lower food web dynamics within designated fish nursery habitat in the Chowan River. I determined if species and fatty acid composition of the lower food web varied in relation to abiotic factors of the sampling site in an estuarine fish nursery. In order to achieve this goal, I examined the spatial and temporal variability of abiotic factors, phytoplankton pigments, zooplankton species composition, as well as the fatty acid composition of the seston, zooplankton, and larval river herring during larval residency in the three tributaries (Wiccacon, Catherine and Rockyhock Creeks), and two sites (Lower and Holiday Island) on the Chowan River. I hypothesized that phytoplankton pigments would be related to nutrient dynamics and zooplankton species composition would be related to abiotic factors, especially temperature. I related abiotic factors (temperature, dissolved oxygen, pH, and nutrients) to patterns in phytoplankton pigments and zooplankton species composition. I hypothesized that the seston fatty acid would relate to the phytoplankton pigment and increased chlorophyll *a* would result in increased omega-3s. I determined the patterns in seston fatty acids

were related to phytoplankton pigments. I predicted that fatty acid profiles of microzooplankton would have increased EPA and DHA when dominated by copepods, and ALA when rotifers were dominated. I related the microzooplankton community composition to fatty acid profiles. I hypothesized that the fatty acid profiles of mesozooplankton would have increased EPA when cladoceran species were dominant and increased DHA when copepods were dominant. I related the mesozooplankton fatty acid profiles to mesozooplankton community composition. I hypothesized that larval river herring fatty acid profiles would reflect the zooplankton fatty acid profile, but not the seston fatty acid profile because most larval fish consume zooplankton. I did not expect the fatty acids of larval fish to match the seston because there is generally an accumulation of omega-3s up the food web (Persson and Verde 2006; Gladyshev et al. 2010; Ravett, Brett, Arhonditsis 2010; Burns, Brett, and Schallenberg 2011). If supported, this would suggest that the quality of the larval fish forage, based on fatty acid composition, could be used to assess fish nursery quality.

Materials and Methods

Study Site

The Chowan River is one of the largest tributaries that drains into the Albemarle Sound and is the 12th largest river basin in North Carolina (NCDENR 2006) (Fig. 8 a,b & c). It is mainly a freshwater estuary that experiences intermittent salinity intrusion (Leech et al. 2009, Lichti et al. 2017). The Chowan River was first classified as "nutrient sensitive waters" in 1979 (NCDENR 2006) and has routinely experienced algal blooms and low dissolved oxygen levels (< 3.0 mg L^{-1}).

Sampling occurred at 3 tributaries (Wiccacon, Catherine, Rockyhock Creeks) and 2 sites (Holiday Island and Lower Chowan near the mouth) on the Chowan River (Fig. 8c). The tributaries were chosen for their historically high river herring abundances. The two Chowan sites were selected for comparison to the main body of the Chowan River. Sampling occurred weekly from March through May, weather permitting. I had a total of 11 sampling trips in 2016, and 9 sampling trips in 2017. Water depths ranged from 1.45 to 6.56 m during all sampling trips.

Sample Collection

Water column properties

I measured vertical profiles of temperature (°C), salinity, dissolved oxygen (mg L^{-1}), and pH using a YSI Pro handheld multi-sensor reader (Yellow Springs Instruments) and a conductivity, temperature, and depth sensor (CTD, Yellow Springs Instrument, Castaway). Water samples were collected at a depth of 1 meter with a Niskin water sampler.

Zooplankton

Two horizontal net tows were done using 0.5 m diameter nets of two different mesh sizes (60 and 200 µm). Two mesh sizes were used in order to generate an adequate representation of the zooplankton for the size range > 60 µm. The zooplankton samples between 60 and 200 µm are designated microzooplankton and the > 200 µm zooplankton samples are designated mesozooplankton throughout this study. The zooplankton net was towed obliquely through the water for 2 minutes at an average boat speed of 1.06 m s^{-1}. Each zooplankton composition sample, depending on mesh size, was filtered through a 60 or 200 µm filter, and the zooplankton were preserved in a 120 mL glass jar with 10 ml of 10% buffered formaldehyde, sucrose, and filtered 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 60 μm sample had a half tablet of Alka Seltzer added to keep rotifers from pulling in critical body parts (legs and arms) to ease identification (Chick et al. 2010). The fatty acid zooplankton samples by mesh size were placed in individual 1000 mL brown plastic containers on ice, and processed in the laboratory.

Larval Fish

Larval fish were collected with a pushnet with a 1.0 m mouth opening with 500 μm mesh size. The pushnet was pushed horizontally through the water to a depth of 1 meter for 2 minutes at an average boat speed of 0.96 m s^{-1}. All larval fish samples were euthanized on the boat with 250 to 1000 mg L^{-1} of tricaine methanesulfonate (MS-222, Western Chemical Company). Larval fish for fatty acid analysis were placed in a 1000 mL plastic container on ice, and processed in the laboratory.

Laboratory Processing

Zooplankton Identification

Samples were filtered through a sieve (60 or 200 μm) to remove the sugar formalin solution, and then added to a beaker with a known volume of water. A total of three subsamples (2 mL per subsample for microzooplankton and 5 mL per subsample for mesozooplankton) were analyzed for community composition using a Hensen-Stempel pipette to subsample. If 1000 individuals of single species were counted in one subsample, then that individual was not counted in the other two subsamples. Organisms were identified using a dissecting microscope and enumerated using a Ward counting wheel. 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).

Phytoplankton Pigment Samples

The water samples (150 to 500 mL) were filtered on a 0.07 µm Whatman™ GF/F filter (47mm diameter) and stored at -80°C until further processing. Each filter was placed into 100% acetone and sonicated (Qsonica®) for 30 seconds. The filters were left for 24 hours in acetone in a -20°C freezer to extract the phytoplankton pigments, and then filtered into small brown vials. The samples were run on the Shimadzu high pressure liquid chromatograph (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) to separate the pigments over 52 minutes using the methods of Mantoura and Llewellyn (1983) and Van Heukelem and Thomas (2001). The samples were analyzed for six pigments (peridinin, fucoxanthin, alloxanthin, zeaxanthin, chlorophyll *a*, and chlorophyll *b*), and the areas under the peak were calculated. The areas of the peak were converted into µg L^{-1} per each pigment (Mantoura and Llewellyn 1983, Van Heukelem and Thomas 2001).

Nutrient Samples

Water that was filtered through a 0.07 µm Whatman GF/F filter (47 mm diameter) was placed in a 200 mL plastic bottle and frozen at -20°C for nutrient analysis. Ammonium (NH_4), nitrate and nitrite ($NO_3^- + NO_2^-$), and orthophosphate (PO_4^{3-}) were analyzed using an automated system on the *SmartChem200* discrete analyzer (Unity Scientific, Milford, MA, U.S.A.) in 2016, and a Seal Autoanalyzer 3 (SEAL Analytical, Mequon, WI, U.S.A.) in 2017. The methods used were EPA method 350.1 for ammonium EPA, method 353.2 for nitrate+nitrite, and EPA method 65.1 for orthophosphate (O'Dell 1993).

Dissolved organic carbon was measured on a Shimadzu TOC-V total carbon analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) in

2016, and a Teledyne Tekmar Torch analyzer with a total N module (Teledyne Tekmar Instruments, Mason, OH, U.S.A.) in 2017. The total dissolved phosphorus was analyzed using the manual persulfate method, and concentrations read on the spectrometer (APHA 1999).

Lipid and Fatty Acid Samples

The water samples (100 to 500 mL) were concentrated on three 0.7 μm Whatman™ GF/F filters (47 mm diameter), which constituted the seston material, and were stored at -80°C until lipid analysis. The zooplankton samples were filtered through stacked 60 and 200 μm sieves to collect species based on size. Each sample was visually analyzed to determine the dominant species using a dissecting microscope, and detritus and phytoplankton were removed. The samples were concentrated on a GF/F filter (47 mm diameter) by mesh size (60, 200 μm), and stored at -80°C until lipid analysis. Ten grams of larval river herring when available were weighed, placed in a small bag, and stored at -80°C until lipid analysis.

Total lipids were extracted with chloroform-methanol (2:1, *v*/*v*) containing 0.01% butylated hydroxytoluene as an antioxidant (Folch et al. 1957). The organic solvent was evaporated under a stream of nitrogen and lipid concentration determined gravimetrically. Transmethylation of fatty acids was done according to the method described by Metcalfe and Schmitz (1969). A known amount of nonadecanoate acid (19:0) dissolved in hexane at a concentration of 8 mg ml^{-1} (Nu Check Prep Inc.) was added as an internal standard. The fatty acid methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc.) using a 7693 mass spectrometer detector (Agilent Technologies, Inc.), a capillary column (Omegawax™ 250 fused silica capillary column, 30 mm x 0.25 mm and 0.25 mm film thickness, Supleco®), and a 7890A autoinjector (Agilent

Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.3 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^{-1}, 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) 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. I calculated the seston' omega-3 to omega-6 ratio allowing us to determine the dominant source of fatty acids.

Statistical Analysis

I performed a series of multivariate analyses to address my specific objectives using the R environment (R v3.4.3, R Core Development Team 2017). I determined if abiotic (temperature, dissolved oxygen, pH) and nutrient factors were related to the observed patterns in phytoplankton pigments and zooplankton community composition, and if the fatty acid profiles were related to the observed patterns in the phytoplankton pigment and zooplankton community composition using redundancy analysis (Legendre and Legendre 1998). The redundancy analysis was carried out using the *rda* function in the vegan package (Oksanen et al. 2017).

I performed permutational analysis of variance (PERMANOVA) using the *adonis* function in the vegan package (Oksanen et al. 2018) in the R environment (R v3.4.3, R Core Development Team 2017), to examine differences among sampling sites and years on the phytoplankton pigment composition, microzooplankton and mesozooplankton community composition. I also used the PERMANOVA to examine seston, zooplankton, and larval fish fatty

acid profiles to determine if there are differences among sampling sites and years. PERMANOVA is a non-parametric technique related to ANOVA, but this method uses permutations and fewer assumptions compared to the traditional ANOVA approach (Anderson 2001). As such, it is particularly well suited to multivariate data sets of low sample size that also violate the traditional assumptions of ANOVA, as was the case for this data set (Anderson 2001).

I generated ordination plots based on Principle Coordinates Analysis (PCoA) to visually show the results for site and year for the phytoplankton pigment composition, microzooplankton and mesozooplankton community composition, and interaction between seston, zooplankton, and larval river herring fatty acid profiles. Finally, I used a Mantel matrix comparison to correlate fatty acid profiles between the seston, microzooplankton, mesozooplankton, and larval river herring using *mantel.rtest* function in the ade4 package (Oksanen et al. 2017) in the R environment (R v3.4.3, R Core Development Team 2017).

Results

Abiotic Conditions

Salinities ranged from 0.02 to 0.10 (Table 4) along the Chowan River and three tributaries. Temperature increased from the start of sampling in March (10-12°C) to the end in May (23-26°C). One exception occurred during the March 2017 sampling when a cold snap occurred in the middle of the month. During this time, the water temperature decreased from 12°C to 7.1-9.8°C, and then began to warm again (Table 4). pH levels were similar throughout the sites and sampling periods with a range of 6.3 to 8.1 in 2016, and an increased range in 2017 of 6.8 to 8.5. The highest mean pH (8.0±0.4) was measured in the lower Chowan in 2017.

Dissolved organic carbon (DOC) had increased concentrations from March to May in 2016 for Catherine, Rockyhock, and Wiccacon Creeks compared to 2017 when the DOC concentrations were highest in April and May (Fig. 9 & Table 5). The Lower Chowan River site had the lowest DOC concentrations for 2016 and 2017, while the Holiday Island site had similar DOC concentrations in 2016 throughout the sampling period, and had an increase from March to May in 2017 (Fig 9 & Table 5).

Ammonium (NH_4^+) concentrations were similar in Catherine Creek and Holiday Island site for 2016 and 2017, but the lower Chowan River site decreased in ammonium concentrations in 2017 compared to 2016 (Fig. 10a & Table 5). Ammonium concentrations were similar during the sampling period in 2016 for Rockyhock and Wiccacon Creek. In 2017, there was an increase in ammonium levels during May for Rockyhock Creek, and from April to May in Wiccacon Creek (Fig. 10a & Table 5).

Nitrate and nitrite (NO_x) concentrations were similar across rivers and throughout sampling periods in 2016 except for the two spikes on March 8 in Catherine and Rockyhock Creeks (Fig. 10b and Table 5). The NO_x concentrations were similar in 2017 between sites, and sampling dates with higher concentrations measured during March at all sites except Wiccacon Creek. In addition, NO_x concentrations increased at Rockyhock Creek during May (Fig. 10b & Table 5).

Orthophosphate (PO_4^{3-}) concentrations were lower throughout the sampling period during March, and early April at all sites, except Rockyhock and Wiccacon in 2016 (Fig. 3c & Table 5). The PO_4^{3-} concentrations increased during May both years at all sites except at the lower Chowan River in 2017 when PO_4^{3-} concentrations decreased (Fig. 10c and Table 5). At the

Holiday Island site, PO_4^{3-} concentrations spiked in early March of 2017 compared to the other sites (Fig. 10c and Table 5).

Phytoplankton Pigments

Phytoplankton pigment composition differed among sites (PERMANOVA, R^2= 0.14, $p<0.001$) and between years (PERMANOVA, R^2= 0.12, $p<0.001$) (Fig. 11). Phytoplankton pigment composition was different for Catherine Creek, the Holiday Island site, and lower Chowan River site (Fig. 11). In 2017, phytoplankton pigment composition was similar for all creeks (Fig. 11). Catherine and Rockyhock Creeks had increased chlorophyll *a* levels, and the sites at Holiday Island, lower Chowan, and Wiccacon Creek had the lowest chlorophyll *a* levels (Fig. 12). Catherine Creek had spikes of chlorophyll *a* in March, and Rockyhock Creek had spikes of chlorophyll *a* in mid-April and end of May for 2016 in relation to blooms (Fig. 12). Chlorophyll *a* differed in 2017 with increased concentrations throughout the sampling period (Fig. 12). Chlorophyll *a* concentrations were higher on the first day of sampling for Catherine, Rockyhock and Wiccacon Creeks in 2017 compared to the other sites (Fig. 12).

Individual phytoplankton pigments varied over sampling period and sites during 2016 and 2017. Rockyhock and Catherine Creeks had the highest concentrations of peridinin and fucoxanthin in 2016, and increased concentrations of fucoxanthin, alloxanthin, and chlorophyll *b* in 2017 (Fig. 13). Wiccacon Creek had low concentrations of pigments with low amounts of peridinin, fucoxanthin, and zeaxanthin in 2016, and increased concentrations of peridinin, fucoxanthin, alloxanthin, and chlorophyll *b* were present in 2017 (Fig. 13). Holiday Island site, and lower Chowan River had increased concentrations of peridinin in 2016, and increased fucoxanthin, alloxanthin and chlorophyll *b* were present in 2017 (Fig. 13).

Abiotic factors and nutrients were correlated with the phytoplankton pigment composition (RDA, R^2=0.35, *p*= 0.001). The first two axes of the RDA shown in Figure 14 account for 86% of variance in the phytoplankton pigments. RDA1 was correlated with pH (65%), DOC (65%), NOx^- (46%), and PO_4^{3-} (44%), while RDA2 correlated to TDP (56%), NH_4^+ (42%), and temperature (19%) (Fig. 14). Alloxanthin and fucoxanthin were correlated with increasing pH and decreasing nutrients (Fig. 14). Chlorophyll *a* was correlated with TDP and NH_4^+ (Fig. 14). Peridinin and zeaxanthin were correlated with DOC, NOx^-, and PO_4^{3-} (Fig. 14). Chlorophyll *b* was correlated to temperature (Fig. 14).

Zooplankton Community Composition

Microzooplankton community composition was similar among sites (PERMANOVA, R^2= 0.04, p=0.48) and year (PERMANOVA, R^2= 0.02, p=0.10) (Fig. 15). Microzooplankton was composed of rotifers and copepod nauplii and was variable during the sampling period (Fig. 16). Catherine Creek had increased percent copepod nauplii in 2016 and 2017 (Fig. 16). Wiccacon Creek and Holiday Island site, had increased percent copepod nauplii in 2016, and increased percent composition of rotifers in 2017 (Fig. 16). Lower Chowan River site had increased percent composition of rotifers during both years, and higher percent of copepod nauplii in 2016 compared to 2017 (Fig. 16). Rockyhock Creek had similar percent of copepod nauplii and rotifers for both years (Fig. 16). Abiotic factors and the microzooplankton community composition were not correlated (RDA, R^2=0.02, p=0.668).

Mesozooplankton community composition was different by site (PERMANOVA, R^2= 0.15, p<0.001) and year (PERMANOVA, R^2= 0.07, p<0.001) though there was some overlap in zooplankton composition at all sites (Fig. 17). *Bosmina* spp. were dominant throughout all the

sites for both years except in 2017 when *Bosmina* spp. represented a lower percent at the lower Chowan River site (Fig. 18). Cyclopoida and Calanoida had increased in percent composition in 2016 at all sites especially at the lower Chowan River site compared to 2017 (Fig. 18). *Daphnia* spp. had increased percent composition in 2017 at all sites compared to 2016 (Fig. 18). Chydoridae had an increased presence during March and May in Catherine and Wiccacon Creeks for both years, and in 2016 at Holiday Island site during March and early April (Fig. 18). Larger bodied rotifers had higher percent composition in Rockyhock Creek during May in 2016, and during late March and April in 2017 (Fig. 18). Temperature and pH were correlated to the changes in the mesozooplankton community composition (RDA, R^2=0.23, p=0.001).

Fatty Acid Composition

A total of 22 specific fatty acids were measured in all samples (Tables 6-9). Fatty acids were first separated into broad categories: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Table 4-7). Seston had the highest percent SFA at all the sites and the lowest percent of MUFAs and PUFAs (Fig. 19). Larval fish had a higher percent PUFA. The zooplankton samples had increased PUFAs and MUFAs at all the sites compared to seston (Fig. 19). The most common SFA was palmitic acid (16:0), the most common MUFAs were palmitoleic acid (16:1n-7) and oleic acid (18:1n-9), and the most common PUFAs were 18:2n-6, ALA, 18:4n-3, EPA and DHA (Table 4-7).

Seston

Seston fatty acid percent composition varied between site and year. Seston decreased in mean percent of 18:2n-6 in 2017 at all sites compared to 2016 (Fig.20). Mean percent EPA increased in 2017 but were similar in percent of ALA and DHA (Fig. 20). Percent composition

for 16:1n-7 and 18:1n-9 during 2016 and 2017 was similar in the seston for all sites except for lower Chowan River which had an increased 18:1n-9 in 2016, and seston from Rockyhock Creek in 2016 had increased of both fatty acids (Fig. 20). Omega-3 to omega-6 ratio in seston gives a quick look to determine if the system has increased phytoplankton compared to detrital material. Seston fatty acid profiles varied in the omega-3 to omega-6 ratio with increased ratio throughout the sampling period in 2017 (Fig. 21). Seston from Catherine Creek had increased ratio compared to 2017 (Fig. 21). Seston from Wiccacon Creek and the Holiday Island site had increased omega-3 to omega-6 ratio in 2016 compared to 2017. Seston from Wiccacon Creek and Holiday Island site had an increase at the end of April in 2017 (Fig. 21). In 2017, the ratio increased during March for Catherine Creek, Holiday Island site, and lower Chowan River (Fig. 21).

Specific phytoplankton pigments were correlated to different fatty acids found in the seston profile (RDA, r^2=0.26, p= 0.001). The first two axes accounted for 92% of variance in the phytoplankton pigment. RDA1 correlated with DHA (58%), and EPA (45%), and LIN (18:2n-6) (58%) (Fig. 22). RDA2 positively correlated with ALA (63%) and SA (18:4n-3) (52%), and negatively correlated with PA (16:1n-7) (23%) and OA (18:1n-9) (27%) (Fig. 22). Chlorophyll *b*, alloxanthin, and fucoxanthin were correlated with increased omega-3s (ALA, 18:4n-3, DHA, EPA) (Fig. 22). Chlorophyll *a* was correlated with an increase in OA (18:1n-9), EPA, and DHA (Fig. 22). Peridinin correlated with increased LIN (18:2n-6), ALA, and SA (18: 4n-3), while zeaxanthin correlated with increased LIN (18:2n-6) and OA (18:1n-9) (Fig. 22).

Microzooplankton

Microzooplankton fatty acid profiles were similar during the two years. Microzooplankton had increased mean percent of ALA and EPA throughout the sites during both years (Fig. 23). Microzooplankton from Rockyhock and Catherine Creeks in 2016 were higher in mean percent composition of ALA compared to the other fatty acids (Fig. 23). In 2017, EPA was the dominant fatty acid for the microzooplankton at all sites except Wiccacon Creek, which had increased 18:4n-3 in the microzooplankton (Fig. 16). At all sites, microzooplankton decreased in mean percent of 18:2n-6, and ALA in 2017 compared to 2016 (Fig. 23). Microzooplankton fatty acids were correlated to the microzooplankton community composition, although this relationship was not statistically significant (Redundancy, R^2=0.30, p= 0.179). The first axes accounted for 29.6% of variance in the microzooplankton community composition, and RDA2 accounted for <1% of variance. RDA1 correlated with DHA (56%), EPA (48%), ALA (38%), and SFA (37%). Copepod nauplii are correlated to increased DHA (56%) and EPA (48%). Rotifers are correlated with increased ALA (38%) and SFA (37%).

Mesozooplankton

Mesozooplankton fatty acid profiles were similar among sites and between years except at the lower Chowan site (Fig. 24). The mesozooplankton from the lower Chowan River site had the highest decrease of DHA from 2016 to 2017, which decreased from 17% to 2% (Fig. 24). Mesozooplankton fatty acid profiles increased in mean percent of EPA and ALA across the sites and during both years (Fig. 24). Mesozooplankton had increased percent of DHA at Wiccacon Creek in 2017, and at Holiday Island, and the lower Chowan River in 2016 (Fig. 24). Mesozooplankton decreased in mean percent in LIN (18:2n-6) for 2017 throughout all sites (Fig. 24).

Mesozooplankton fatty acids were correlated to the mesozooplankton community composition (RDA, R^2=0.53, *p*= 0.001). The first two axes accounted for 77% of the variance in the mesozooplankton community composition. RDA1 was correlated with EPA (70%), SA (18:4n-3) (79%), LIN (18:2n-6) (61%) and SFA (78%) (Fig. 25). RDA2 correlated with DHA (51%), ALA (62%), OA (18:1n-9) (28%), and PA (16:1n-7) (27%) (Fig. 25). *Bosmina* spp. correlated to increased ALA (62%), OA (18:1n-9) (28%), and EPA (26%) (Fig. 25). *Daphnia* spp. was correlated to increased EPA (70%), and SA (18:4n-3) (79%) (Fig. 25). Chydoridae was correlated to increased LIN (18:2n-6) (61%), and SFA (78%) (Fig. 25). Calanoida and Cyclopoida were correlated with increased DHA (51%), and PA (16:1n-7) (27%) (Fig. 25).

Larval River Herring

Larval river herring fatty acid profiles were similar across sites and years (Fig. 26). DHA was the dominant fatty acid present in the larval fish (Fig. 26). Lower chain omega-3 (ALA and 18:4n-3), and omega-6 (18:2n-6) were present in the larval fish fatty acid profile (Fig. 26).

Relationships between trophic levels

Larval fish, zooplankton, and seston differed in fatty acid profiles (PERMANOVA, R^2=0.47, p<0.001) (Fig. 27). Both micro- and mesozooplankton had similar fatty acid profiles to the seston along PCoA axis 2, and similar fatty acid profiles to larval fish along PCoA axis 1 (Fig. 20). Larval river herring did not have a similar composition to seston (Fig. 27).

Seston fatty acid profiles were correlated to the microzooplankton (Mantel, r=0.18, p= 0.057) and to the mesozooplankton (Mantel, r=0.24, p= 0.015) fatty acid profiles. The microzooplankton fatty acid profiles were correlated to the mesozooplankton fatty acid profiles

(Mantel, r=0.30, p = 0.002). The larval river herring fatty acid profiles did not correlate to seston (Mantel, r=-0.20, p = 0.988) or mesozooplankton (Mantel, r=0.16, p = 0.183) fatty acid profiles.

Discussion

Across trophic levels, fatty acid and species composition changed temporally over the two years and spatially over all locations in the Chowan River fish nurseries. I followed these lower food web changes from seston to larval fish using fatty acid analyses. Phytoplankton pigments were related to patterns in nutrient concentrations and were related to the fatty acid composition of the seston. Chlorophyll *a* concentrations increased in 2017 for all sites compared to 2016. The increase in chlorophyll *a* was seen in the seston fatty acid composition through a higher ratio of omega-3 to omega-6. Zooplankton fatty acid composition was related to species composition and presumably diet. A freshwater assemblage of zooplankton was always present, but its composition differed across sites and years. *Bosmina* spp. were dominant across all sites except at the lower Chowan River in 2017 where copepods increased in 2016, but cladocerans were more dominant in 2017. Mesozooplankton fatty acid profile had similar levels of EPA both years at all sites, but decreased in DHA in 2017. At the lower Chowan River, DHA levels were lower when compared to other sites. Larval fish FA composition did not differ across sites and years, which could be from the conversion or bioaccumulation of fatty acids from the diet. Additionally, based on correlations between trophic levels, I observed that fatty acids appeared to be incorporated relatively unchanged in micro and mesozooplankton in terms of relative composition from seston; however, MUFA and PUFA percent compositions increased in zooplankton relative to seston, and larval fish had increased PUFAs compared to zooplankton. This suggests that MUFAs and PUFAs are bioaccumulating at higher trophic levels, as seen in

other studies (Persson and Verde 2006; Gladyshev et al. 2010; Ravett, Brett, Arhonditsis 2010; Burns, Brett, and Schallenberg 2011).

Chlorophyll *a* levels were negatively correlated with higher nutrient concentrations, particularly orthophosphate, and this was likely due to nutrient uptake. Increased nutrient loading releases the phytoplankton from nutrient limitation leading to algal blooms and resulting in increased chlorophyll *a* levels (Pinckney et al. 1999, Paerl et al. 2003, Valdes-Weaver et al. 2006). Phytoplankton composition was also related to nutrient dynamics. When there were increased DOC concentrations in 2016, the phytoplankton pigment was predominantly peridinin, which represents dinoflagellates (Paerl et al. 2003). Dinoflagellates can be mixotrophic, reside in areas of light limitation, and consume DOC through osmomixotrophy (Stoecker 1999, Keith et al. 2002, Klug 2002, Anderson et al. 2018, Taipale et al. 2018). All sites except Wiccacon had increased fucoxanthin in March of 2017 compared to 2016. This was correlated to increased pH, and negatively correlated with nitrate+nitrite concentrations. Fucoxanthin indicates diatoms are in the system (Paerl et al. 2003). Diatoms are better at nitrate uptake compared to other nutrients and are found in estuaries during spring blooms (Paerl et al. 2003, Valdes-Weaver et al. 2006). However, in 2017, alloxanthin and chlorophyll *b* were also present in all sites. These pigments are found in cryptophytes and chlorophytes, which can outcompete other phytoplankton because of their efficient growth rates and enhanced nutrient uptake rates (Valdes-Weaver et al. 2006). These shifts in nutrient concentrations and phytoplankton composition can change the fatty acid composition.

The seston fatty acid composition consisted of mainly SFAs. Seston from freshwater and estuarine systems typically have a larger percentage of SFA, and this fraction has been attributed to detrital input, as opposed to originating from phytoplankton (Persson and Vrede 2006,

Gladyshev et al. 2010, Ravett, Brett, Arhonditsis 2010, Burns, Brett, and Schallenberg 2011, Gonclaves et al 2012). Müller-Navarra et al. (2004) analyzed seston and found phytoplankton only explained 27% of variance in FA composition and concluded that detritus and heterotrophic organisms also needed to be considered. One difference I saw was the increase in omega-3 to omega-6 ratio throughout the sampling period, which resulted from an increase of phytoplankton relative to detritus, particularly in 2017. Omega-3s from phytoplankton originate in aquatic systems, and would be seen in greater percent with increased chlorophyll *a* (Dalsgaad et al. 2003, Arts et al. 2009, Twining et al. 2016). During the time and location that peridinin pigment concentrations increased, the seston fatty acid profile had increased 18:2n-6 at the sites. This fatty acid is not normally associated with dinoflagellates, but it may be the remnant of increased particulate organic matter (Dalsgaad et al. 2003, Arts et al. 2009, Twining et al. 2016). Omega-6, especially 18:2n-6, has been related to terrestrial plant material (Dalsgaad et al. 2003, Twining et al. 2016). In 2017, the increase in diatoms could have resulted in the fatty acid profile having increased 16:1n-7 and EPA (Dalsgaad et al. 2003, Arts et al. 2009, Strandberg et al. 2015), as was observed in my study. From the phytoplankton pigment, the increase in chlorophytes and cryptophytes would result in increased C16 and C18 PUFAs, and the cryptophytes would have increased EPA (Arts et al. 2009, Perga et al. 2009, Strandberg et al. 2015, Twining et al. 2016). Overall, phytoplankton pigments were correlated to seston fatty acid profiles, but caution needs to be taken because the fatty acid profiles could be related to detrital material and heterotrophic organisms, neither of which were directly quantified.

Microzooplankton fatty acid composition was correlated to dominance of copepod nauplii or rotifers and an overall increase in omega-3 compared to seston. Rotifers increased in percent of SFA, 18:2n-6, and 18:3n-3, which would relate to the food items available to the

rotifers (Kennari et al. 2008, Wacker and Weithoff 2009). Rotifer fatty acid profiles represent more of their diet as compared to copepod nauplii (Kennari et al. 2008, Wacker and Weithoff 2009). There is also the increase in the lower chain omega-3 because rotifers are filter feeders and consume smaller particles (phytoplankton and detritus) (Rothhaupt 1990). Rotifers in other studies have similar fatty acid profiles and are characterized by increased percent composition of ALA (Kennari et al. 2008, Wacker and Weithoff 2009). Copepod nauplii were correlated to increased DHA in the fatty acid profile since they are larvae of copepods, and copepods have increased DHA (Arts et al. 2009). Copepod nauplii would result in bioaccumulation of EPA/DHA to the next trophic level, but rotifers, depending on their diet, would result in lower omega-3s being transferred to the next trophic level.

Bosmina spp. were dominant species throughout the sites over the two years, and Chydoridae were dominant in 2016 throughout the tributaries. *Bosmina* spp. are found throughout river systems and can achieve high abundance due to quicker reproduction and smaller size compared to larger cladocerans and copepods (Bec et al. 2010). Cladocerans including *Bosmina* spp. and Chydoridae have increased EPA and little to no DHA, even when prey are high in DHA levels (Persson and Verde 2006). I saw similar trends throughout the Chowan River and tributaries. The increase in EPA for cladocerans is based on life history strategies of increased reproduction (Persson and Verde 2006). When Cyclopoida and Calanoida had an increased presence in the system, DHA increased in the fatty acid profiles. Copepods have increased DHA because this fatty acid is critical for nervous system development (Persson and Verde 2006, Arts et al. 2009). This could result in consuming higher quality food items compared to cladoceran species. For example, the Lower Chowan River site had a higher percent of Calanoida and Cyclopoida and a mean of 14% DHA in 2016, but in 2017 *Daphnia* sp. was the

dominant zooplankton, and the mean DHA decreased to 2.7%. For both sampling years, an increase in rotifers in Rockyhock Creek increased ALA in the system (Kennari et al. 2008, Wacker and Weithoff 2009). The rotifers were a larger size and collected in the mesozooplankton sample. These results are similar to the microzooplankton population but larger in size. Overall, mesozooplankton were variable in species and fatty acid composition and this variability changed the fatty acids available to larval fish.

Larval river herring were found throughout the system and had similar fatty acid profiles among the sites and years. Larval river herring had the highest percent of PUFAs, especially DHA. PUFAs (ALA, EPA, and DHA) are important fatty acids for brain and nervous system development, acuity, survival and maintaining cell membranes (Bell et al. 1995; Bell and Sargent 1996; Rainuzzo et al. 1997; Sargent et al. 1999; Rossi et al. 2006). Even though larval fish can bioconvert lower chain to higher chain fatty acids, bioconversion can be less effective in larval fish, and having a dietary source of DHA is also crucial (Muje 1989, Wirth et al. 1997). Under poor food conditions, rainbow trout (*Oncorhynchus mykiss*) were not able to convert C18 PUFAs to EPA and DHA (Taipale et al. 2018). The increased presence of 18:2n-6, ALA, and 18:4n-3 in the larval river herring fatty acid profile demonstrated that these fish are feeding since these fatty acids are not found in high percentage in the yolk-sac. Female river herring ovaries from the Chowan River had none to low percent of 18:2n-6, ALA and 18:4n-3 when on the spawning grounds (Chapter 2). Alewife and blueback herring start feeding on smaller cladocerans and copepods at about 6 mm total length (Mullen et al. 1986). In the Connecticut River, the diet for blueback herring were dominated by rotifers for fish 5-12 mm, Bosminidae for fish 12-16 mm, and cyclopoid copepods for fish >16mm in total length (Crecco and Blake 1983).

This study demonstrated that investigating the lower trophic food web using fatty acid analysis could be used in the future to assess the quality of fish nursery habitat. My study provides a baseline look at the fatty acids present in the planktonic food web that river herring prey upon during initial development and growth. Abiotic factors (temperature, dissolved oxygen, pH, and nutrients) correlated to changes in phytoplankton pigments and these are, in turn, correlated to the seston fatty acid profiles. I can then incorporate prey and fatty acid composition to assess the quality of the prey base available to larval fish to assess differences in nursery habitat. Furthermore, my results indicated that changes in nutrient dynamics can lead to differences in seston composition, and therefore quality, of the prey base available to larval fish. For example, Rockyhock Creek had a mesozooplankton population dominated by larger bodied rotifers, which changed the fatty acid profile of the prey for fish. Another example is the change from copepods to a *Daphnia* spp. at the lower Chowan River site in 2017, which resulted again a change in fatty acid profile of the prey for fish. These changes could affect larval fish, resulting in strong or weak year class strength at the beginning of life by altering larval fish growth, development, and survival (Takeuchi et al. 1997, Perga et al. 2009, Paulsen et al. 2014, Taipale et al. 2018). Interestingly, the fish I collected in each year had very similar fatty acids profiles, despite the observed differences in the prey characteristics across the two years as evident from changes in the fatty acid profiles at different trophic levels. This shows that larval fish possibly bioaccumulate critical fatty acids or bioconvert lower chain to higher chain fatty acids and that fish have both critical fatty acids present in significant percentages. This research cannot be directly used by the management agencies in SHAs at present because the exact amounts of fatty acids required for fish growth and survival remain unknown; however, the work strongly

suggests that shifts in nutrient levels and the phytoplankton community can impact habitat quality by changing the fatty acids present in the system.

SHAs in North Carolina were designated based on past information such as land cover, improved water quality, and fish data to determine the areas that should be managed (Deaton et al. 2006). Since the Chowan River already had an AFSAs designation, this extended to the new SHAs designation, and the whole river was designated SHA (NCDMF 2009). AFSAs do not include the nursery habitat, but since the SHAs cover the whole Chowan River and tributaries this includes the nursery habitat, which extends the protection and management. Using fatty acid profile analysis as a tool, we can determine whether or not essential fatty acids are present in the system and establish that DHA and EPA were present in the system. The next logical step is determining how food quality, e.g. the ratio of EPA and DHA required for optimum growth, affects larval river herring growth and survival, and this data could be used to further define nursery habitat. Furthermore, the realized diets of larval river herring from stomach analysis would determine if we measured non-preferred prey using net sampling. Despite these gaps, it is clear that fish nurseries in the Chowan River can be impacted by abiotic factors that are linked to the quality of the prey base available for larval fish. Incorporating fatty acids data from the lower trophic level can help further refine SHA designation suggest areas of further research to further refine the definition.

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Figures

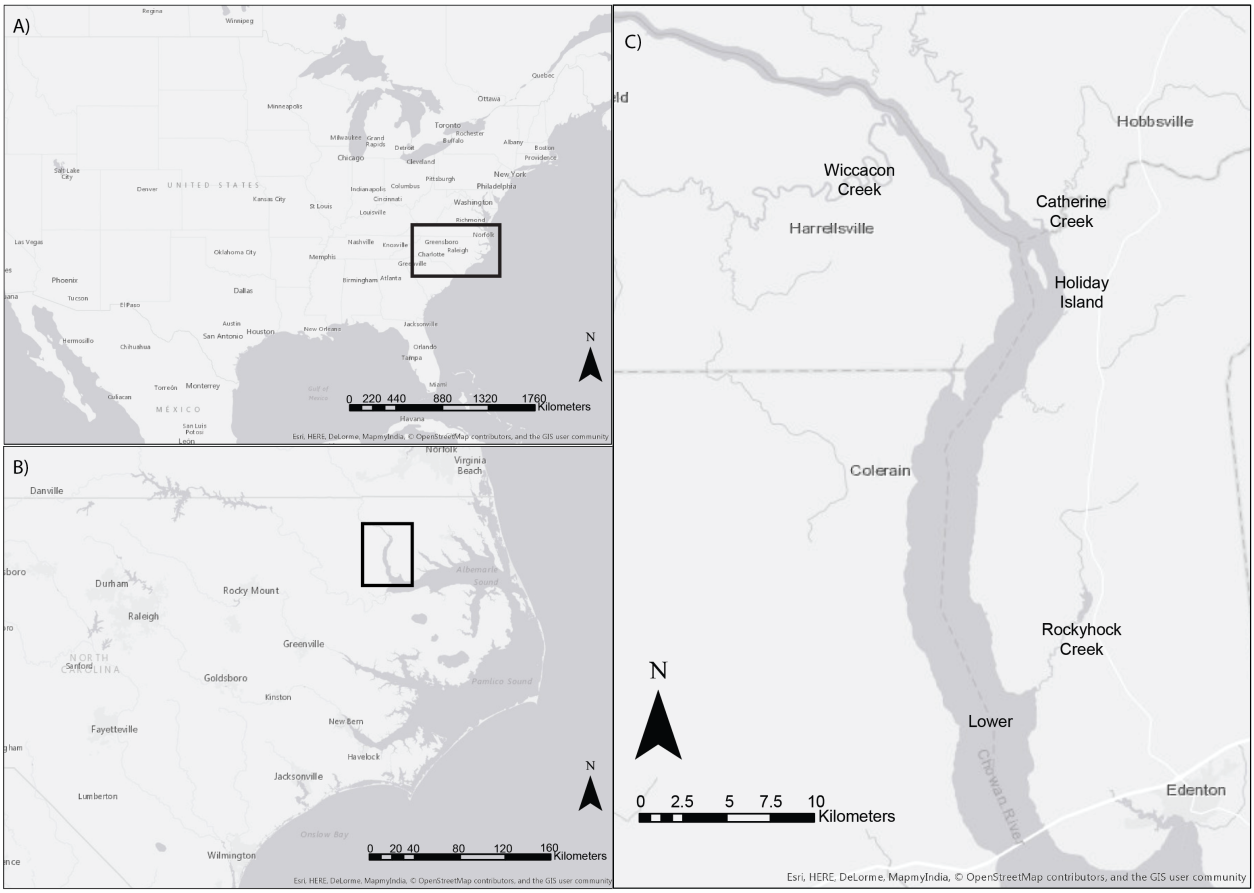


Fig. 8: The overview of North Carolina (A). The close-up view of the location for the Chowan River (B). The five sites for sampling on the Chowan River (C).

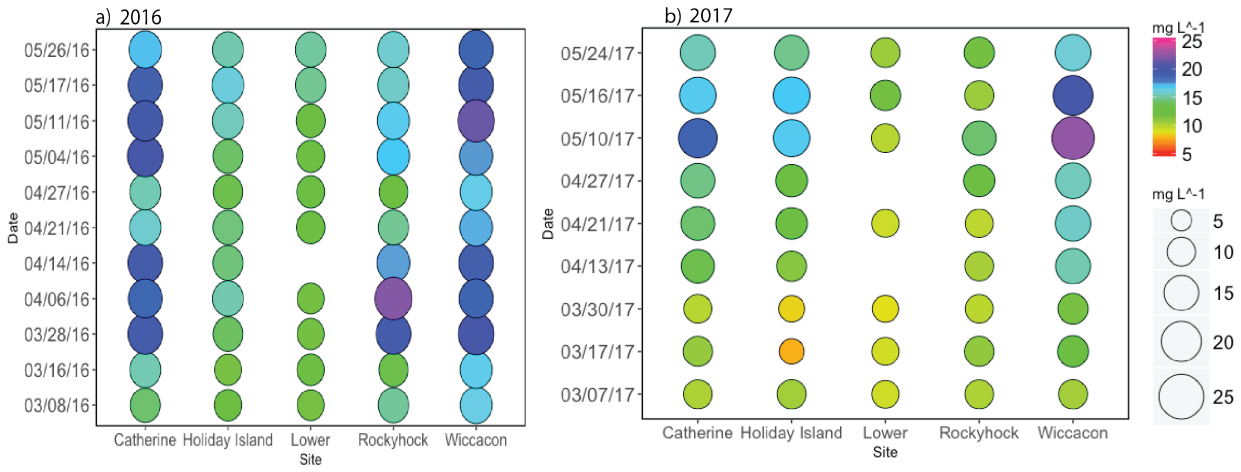


Fig. 9: Dissolved organic carbon (mg L^{-1}) across all the sampling dates comparing the five sites for a) 2016 and b) 2017.

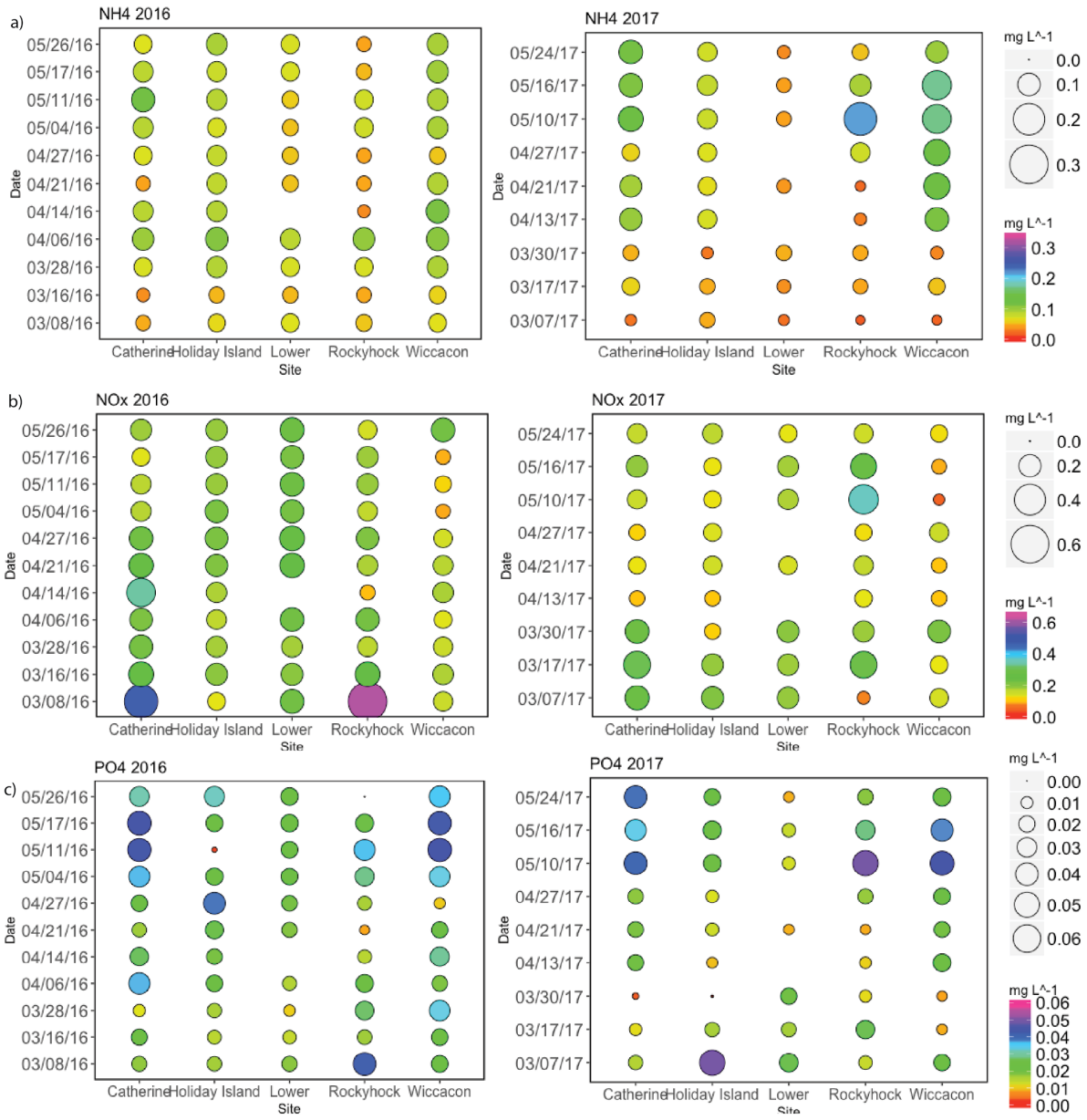


Fig. 10: Nutrient data from a) ammonium (NH_4, mg L^{-1}), b) nitrate and nitrite (NOx, mg L^{-1}), and c) orthophosphate (PO_4^{3-}, mg L^{-1}) for the five sites over the sampling dates for 2016 and 2017.

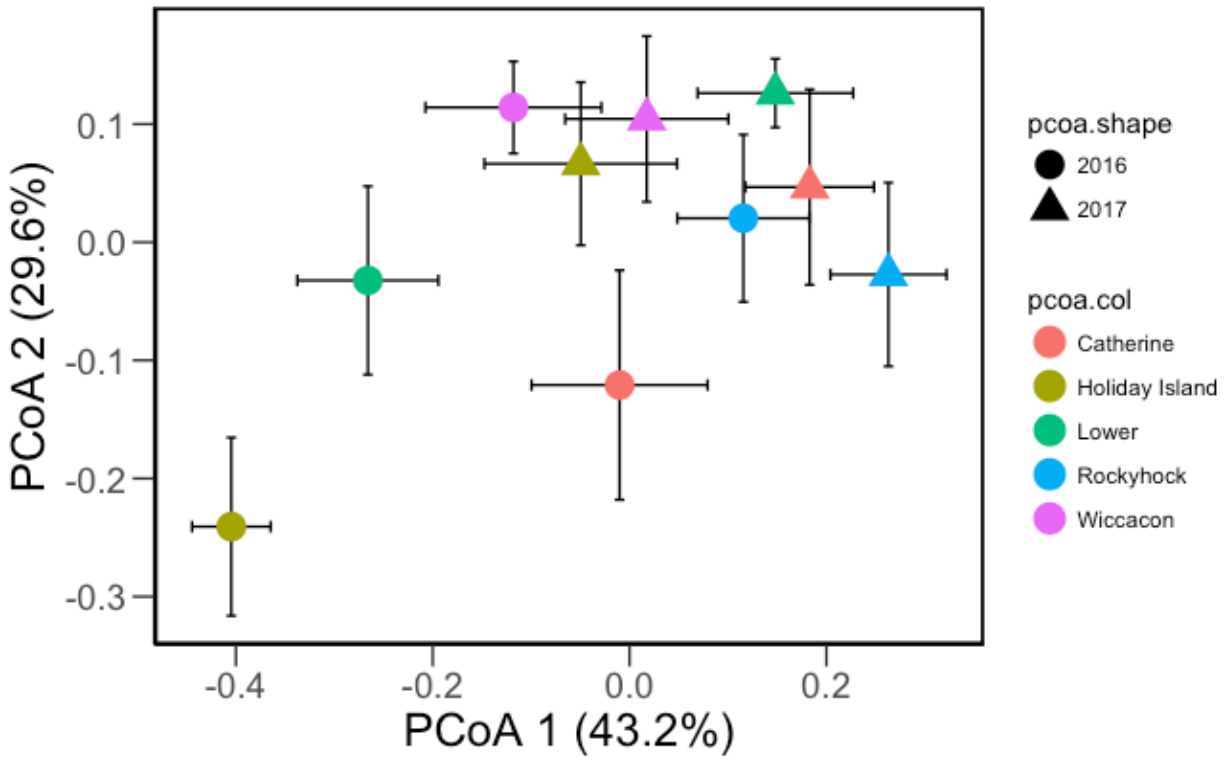


Fig. 11: Ordination from Principal Coordinates Analysis depicting the accessory phytoplankton pigment composition average per site. Symbols are colored according to sampling sites on the Chowan River.

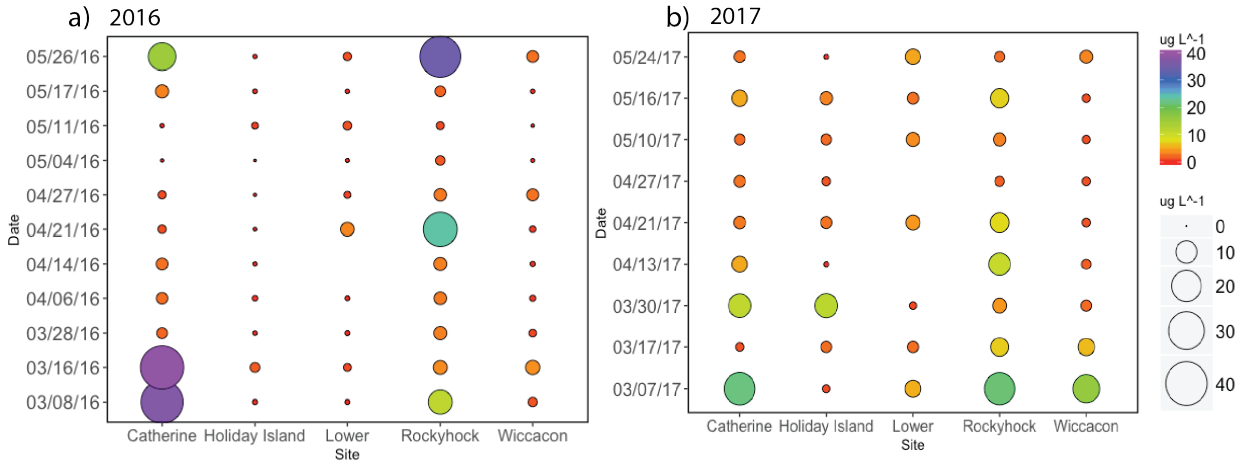
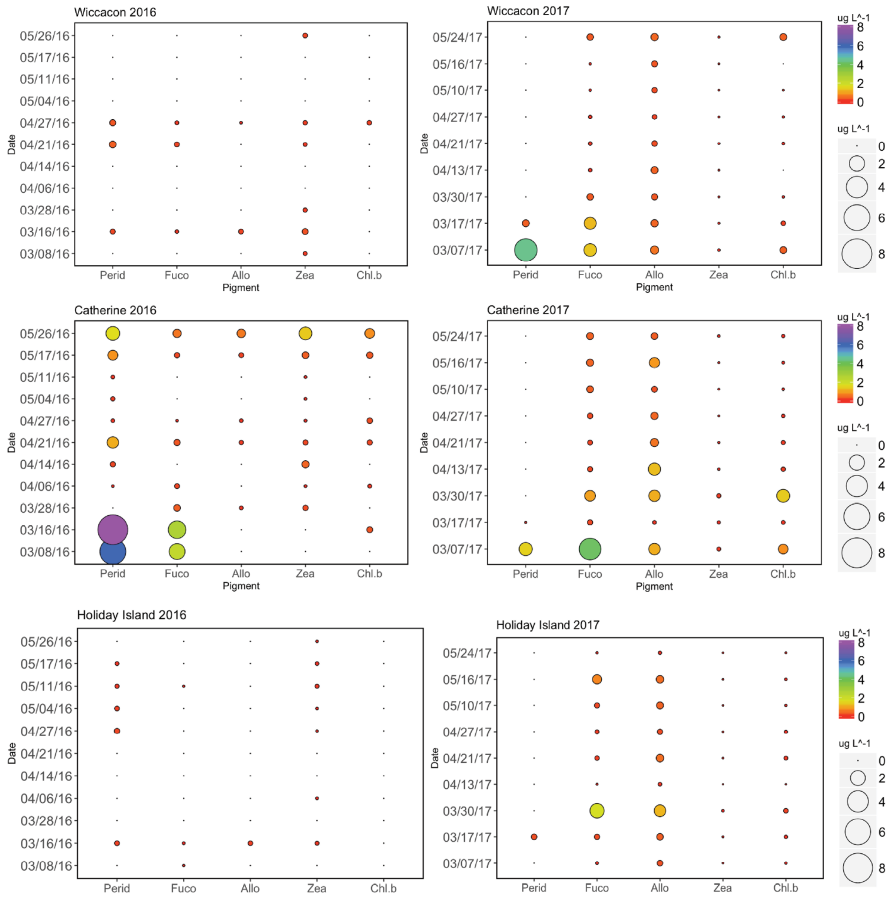
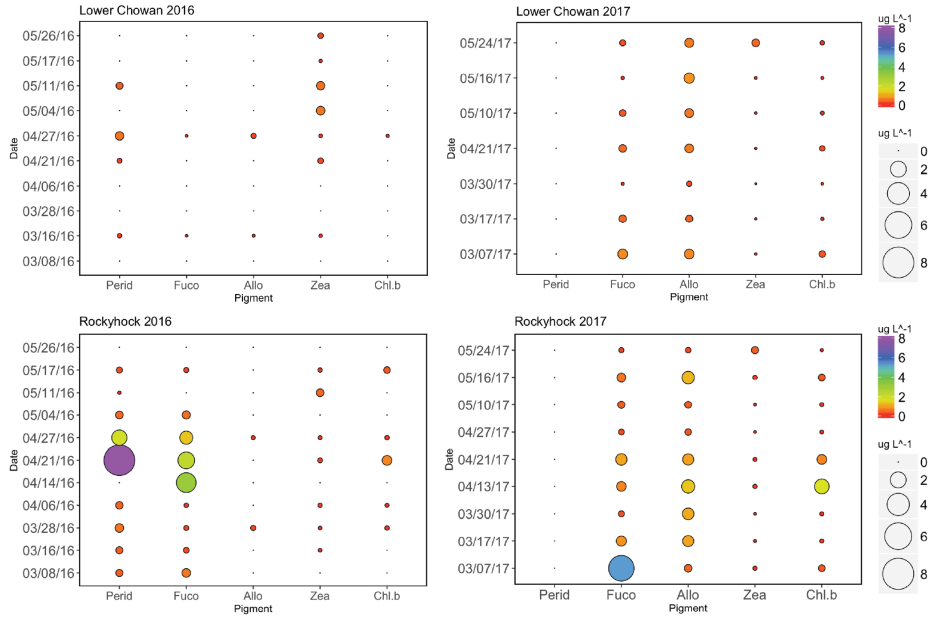


Fig. 12: Chlorophyll *a* concentrations (µg L^{-1}) for each sampling location over a) 2016 and b) 2017.



1



2

3 Fig. 13: Phytoplankton pigment concentration (ug L^{-1}) in 2016 and 2017 for Wiccacon Creek, Catherine
4 Creek, Holiday Island, Lower Chowan River, and Rockyhock Creek.

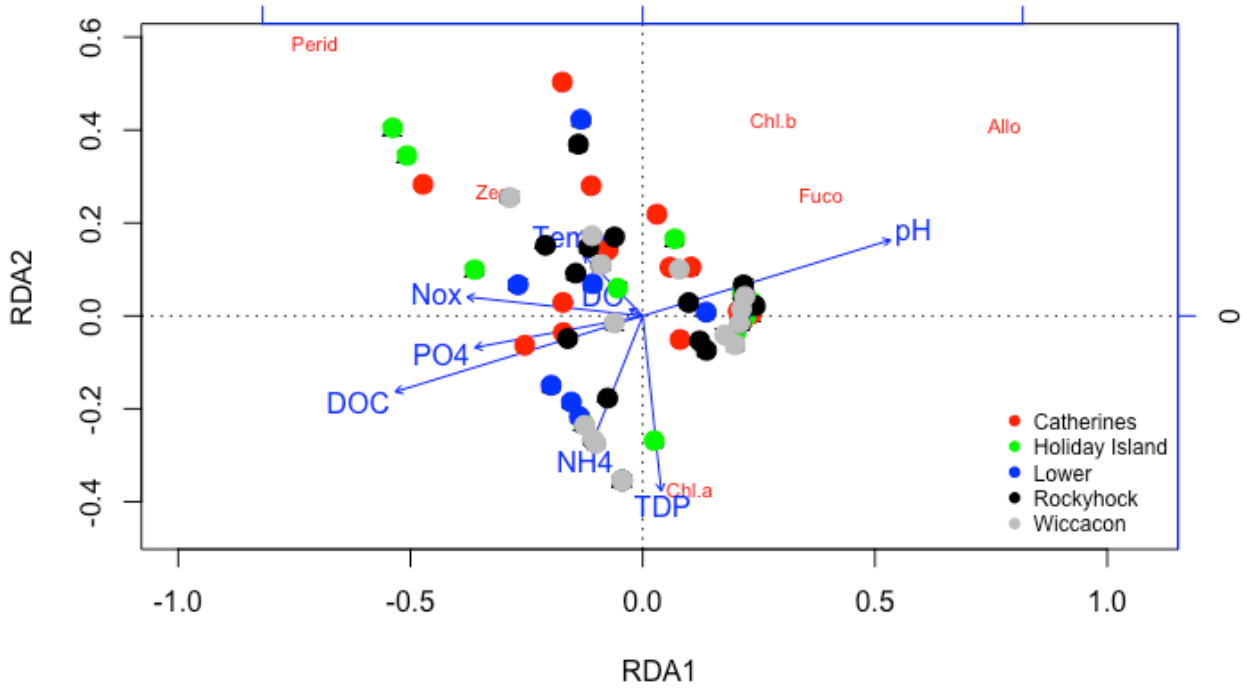


Fig. 14: Correlation plots of the redundancy analysis (RDA) for the phytoplankton pigment and environmental variables. Pigment Abbreviations: Perd= Peridinin, Fuco=Fucoxanthin, Allo= Alloxanthin, Chl a = Chlorophyll *a*; Chl.b = Chlorophyll *b*, Zea=Zeaxanthin. Abiotic Abbreviations: Temp = Temperature, DO= Dissolved Oxygen, DOC= Dissolved organic carbon, TDP= Total dissolved phosphorus, NH4= Ammonium, Nox= Nitrate+Nitrite, PO4= Orthophosphate

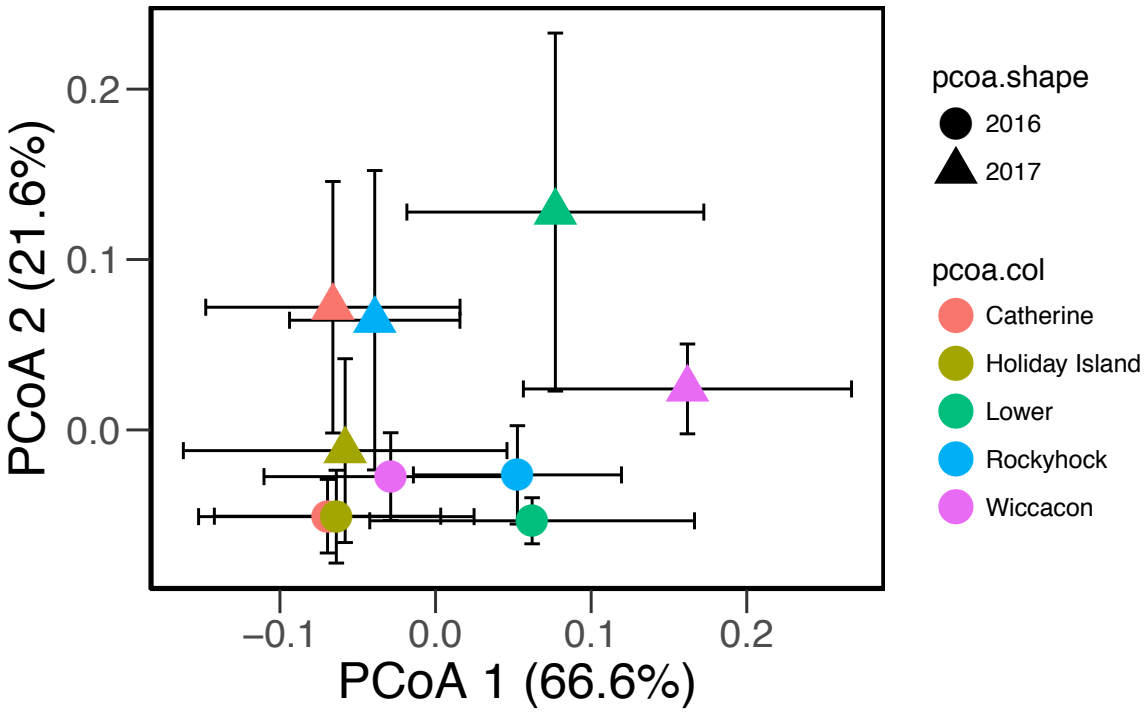


Fig. 15: Ordination from Principal Coordinates Analysis depicting the microzooplankton community composition for 2016 and 2017. Symbols are different to represent the year. Symbols are colored according to sampling sites average per site on the Chowan River.

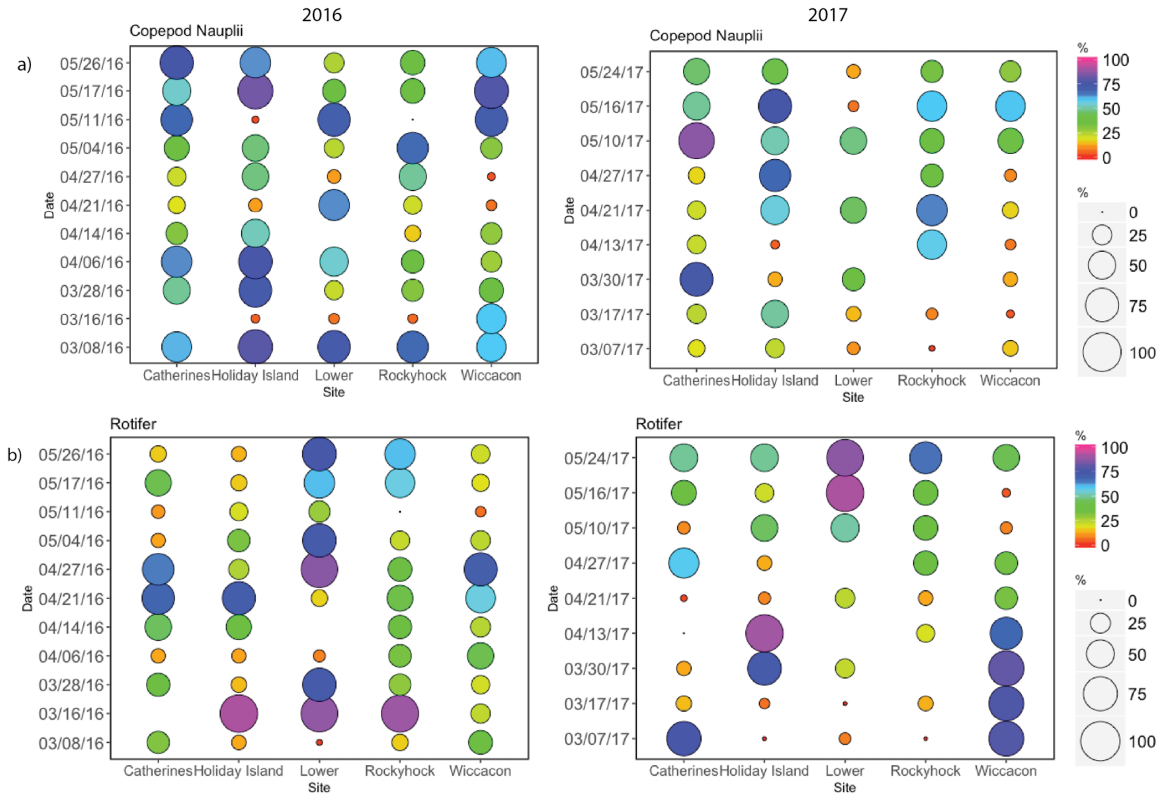


Fig. 16: Microzooplankton percent composition for rotifers and copepod nauplii by site for sampling period during 2016 and 2017.

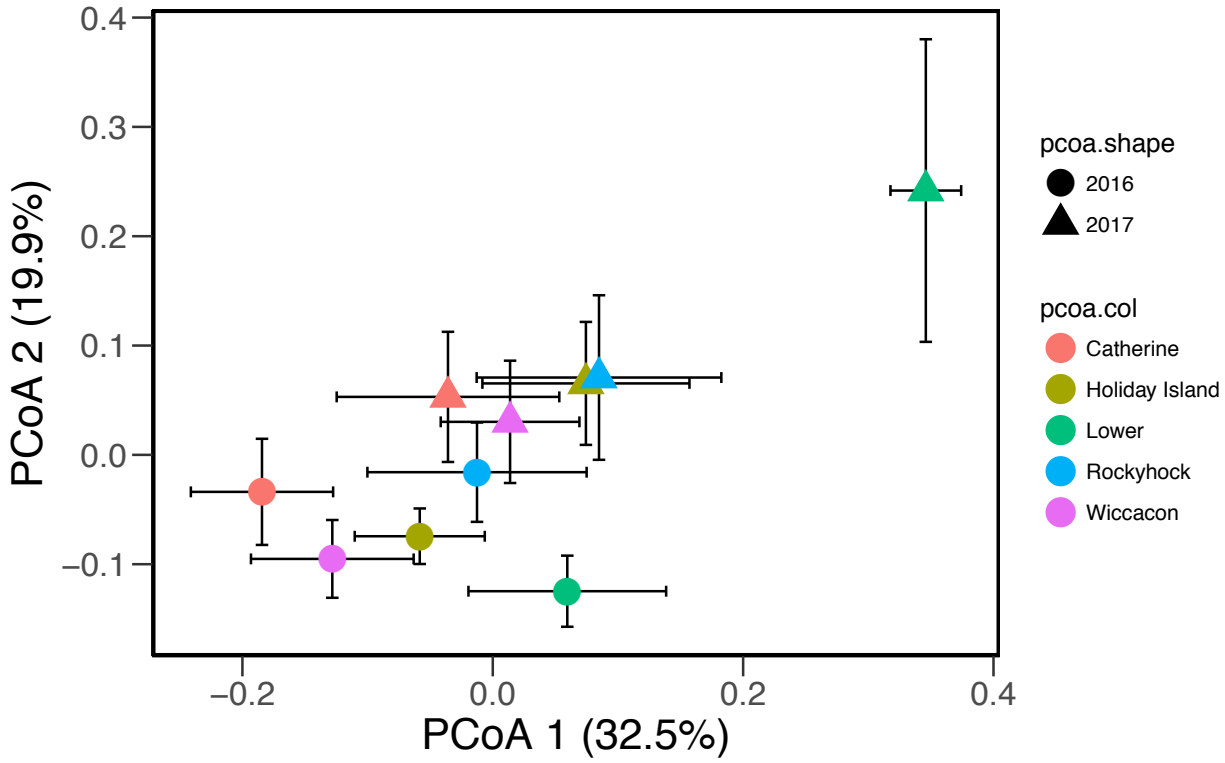


Fig. 17: Ordination from Principal Coordinates Analysis depicting the mesozooplankton community composition for 2016 and 2017. Symbols are different to represent the year. Symbols are colored according to sampling sites average per site on the Chowan River.

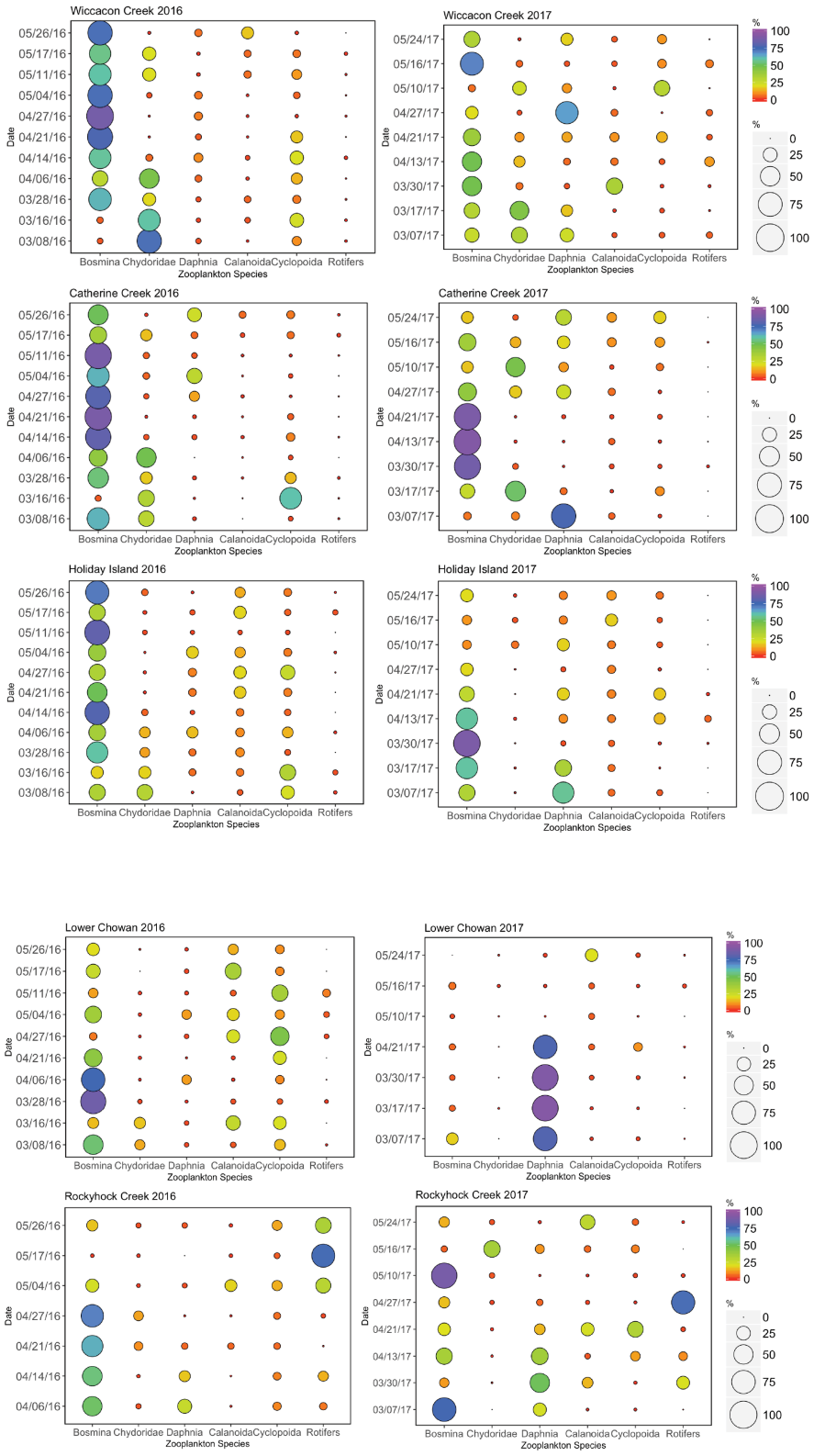


Fig. 18: Mesozooplankton percent composition for the six dominant species by site for sampling period during 2016 and 2017.

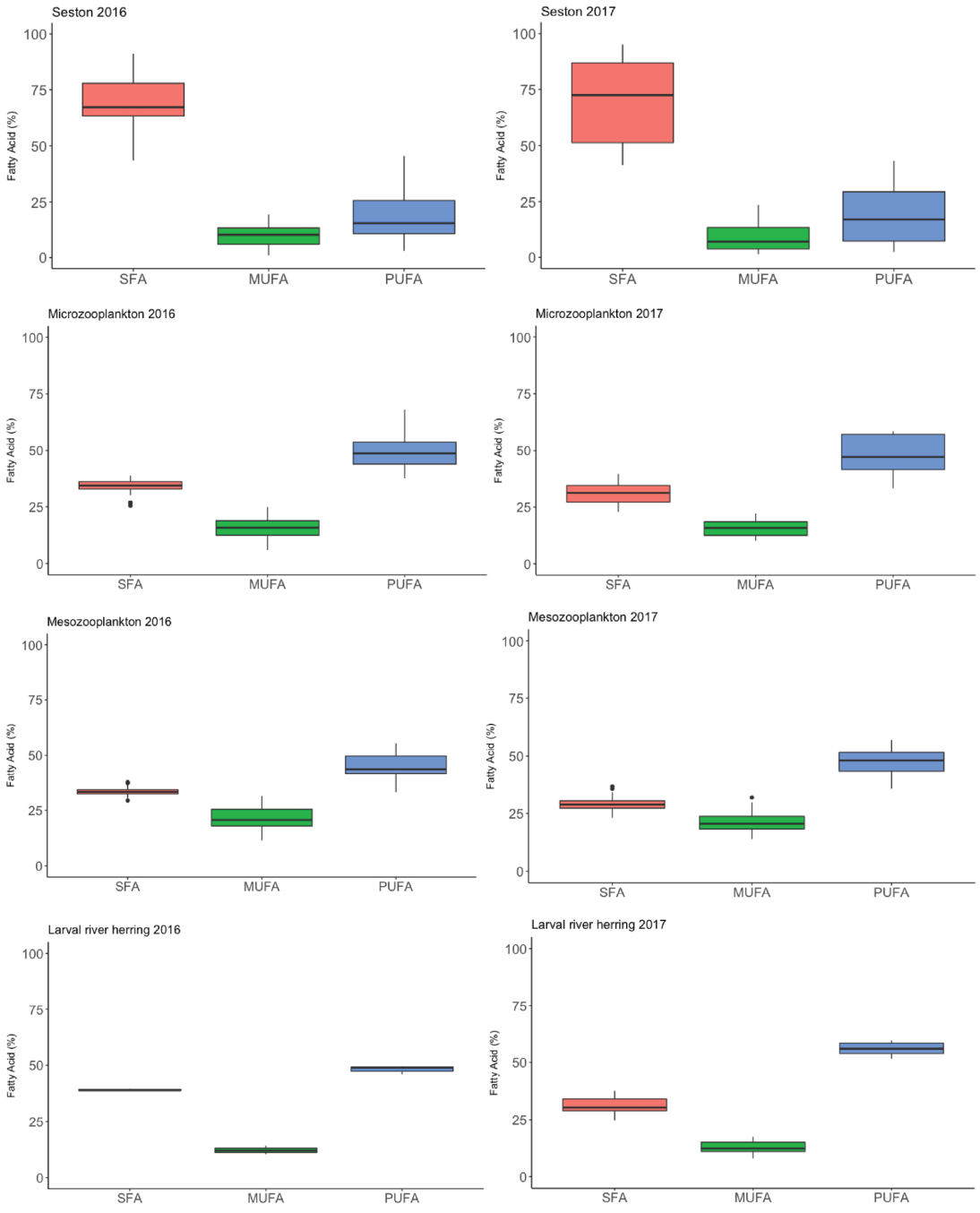


Fig. 19: Box plot for saturated fatty acid (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) for seston, microzooplankton, mesozooplankton, and larval river herring by site for 2016 and 2017.

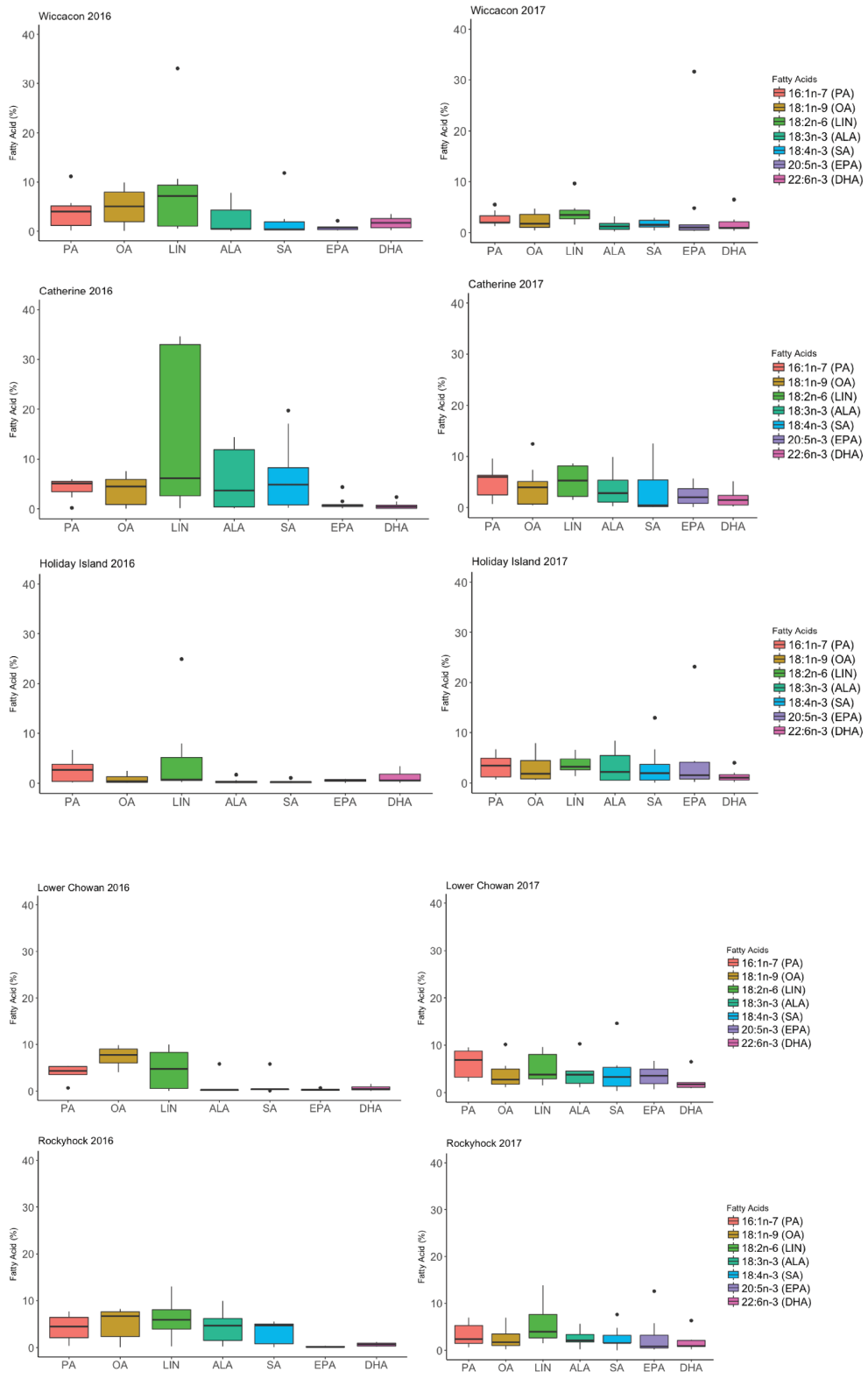


Fig. 20: Boxplot of seston fatty acid percent composition for five sites for 2016 and 2017.

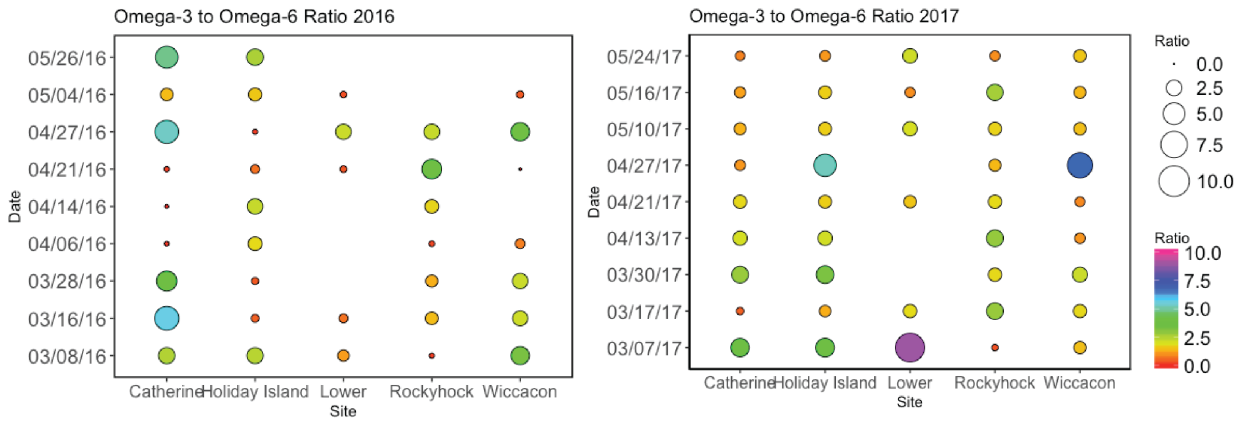


Fig. 21: Seston ratio of omega-3 to omega-6 for by site during the sampling period for 2016 and 2017.

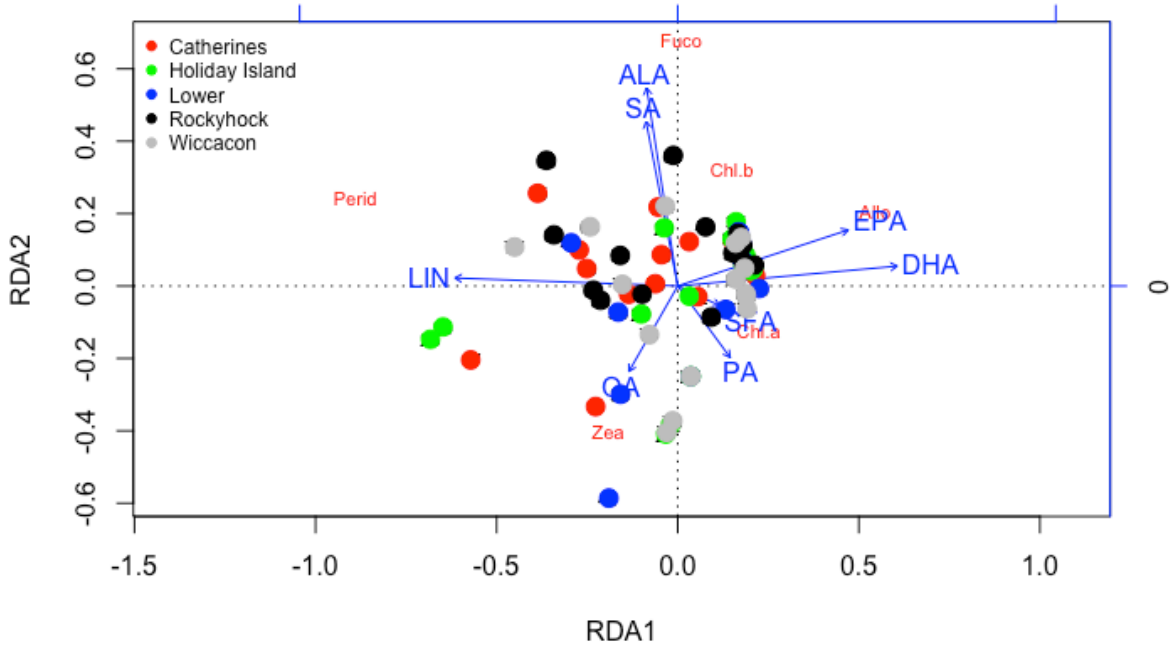


Fig. 22: Correlation plots of the redundancy analysis (RDA) for the seston fatty acids and phytoplankton pigment. Pigment Abbreviations: Perd= Peridinin, Fuco=Fucoxanthin, Allo= Alloxanthin, Chl a = Chlorophyll *a*; Chl.b = Chlorophyll *b*, Zea=Zeaxanthin. Fatty Acids: SFA=Saturated Fatty Acids, PA=16:1n-7, OA=18:1n-9, LIN=18:2n-6; ALA=18:3n-3, SA=18:4n-3, EPA=20:5n-3, DHA=22:6n-3

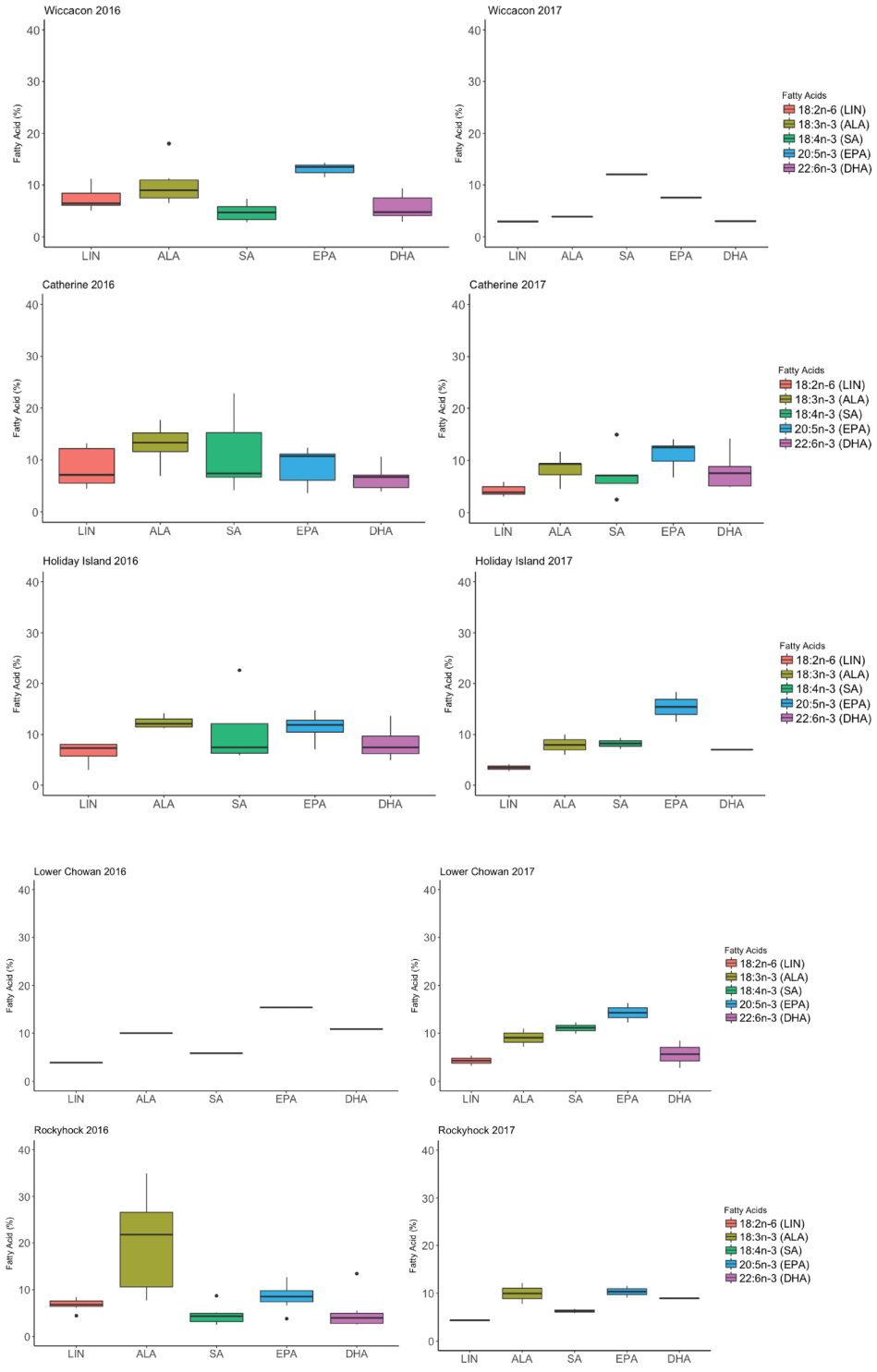


Fig 23: Boxplot of microzooplankton fatty acid percent composition for five sites in 2016 and 2017.

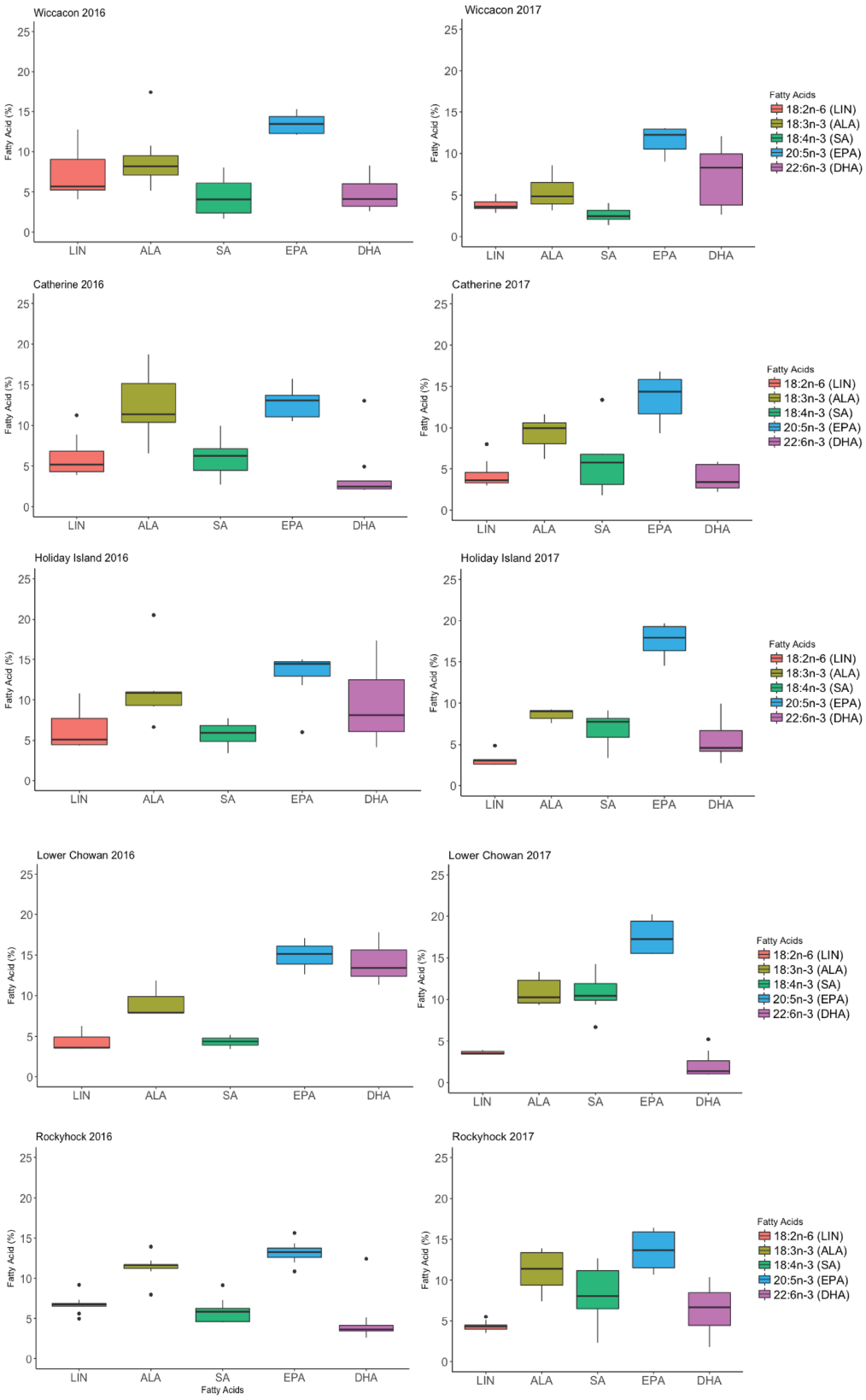


Fig. 24: Boxplot of mesozooplankton fatty acid percent composition for five sites in 2016 and 2017.

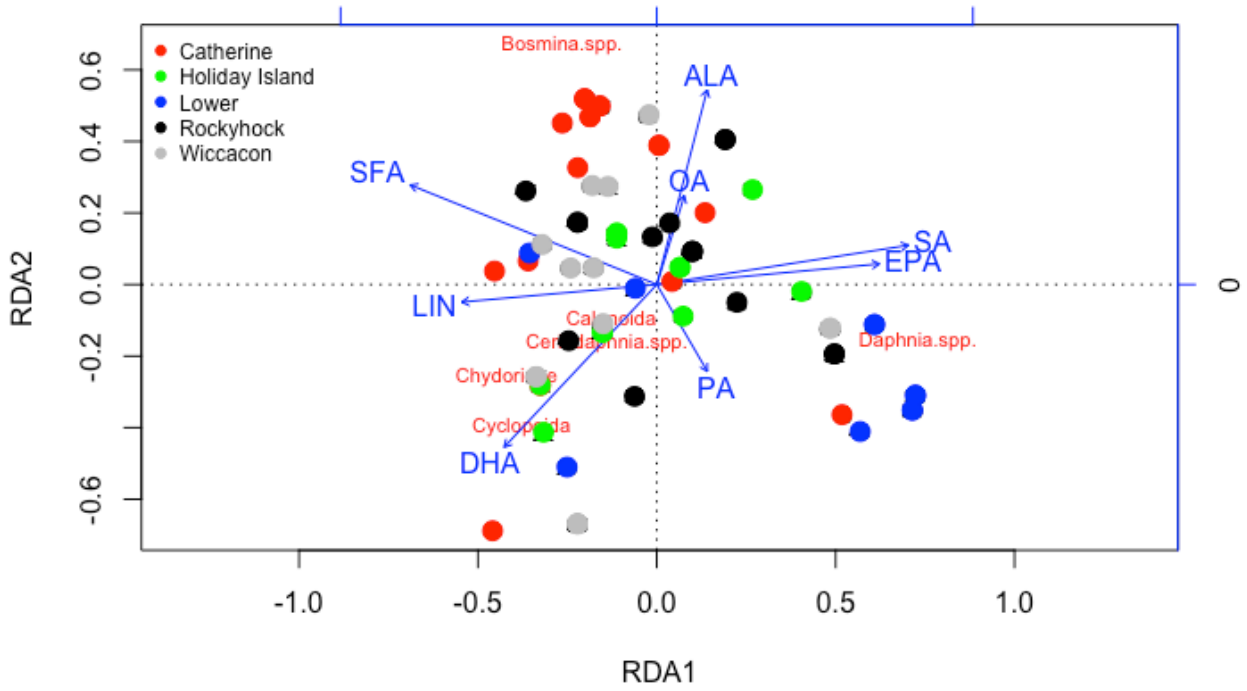


Fig. 25: Mesozooplankton correlation plots of the redundancy analysis (RDA) to the fatty acid composition. Fatty Acids: SFA=Saturated Fatty Acids, PA=16:1n-7, OA=18:1n-9, LIN=18:2n-6; ALA=18:3n-3, SA=18:4n-3, EPA=20:5n-3, DHA=22:6n-3

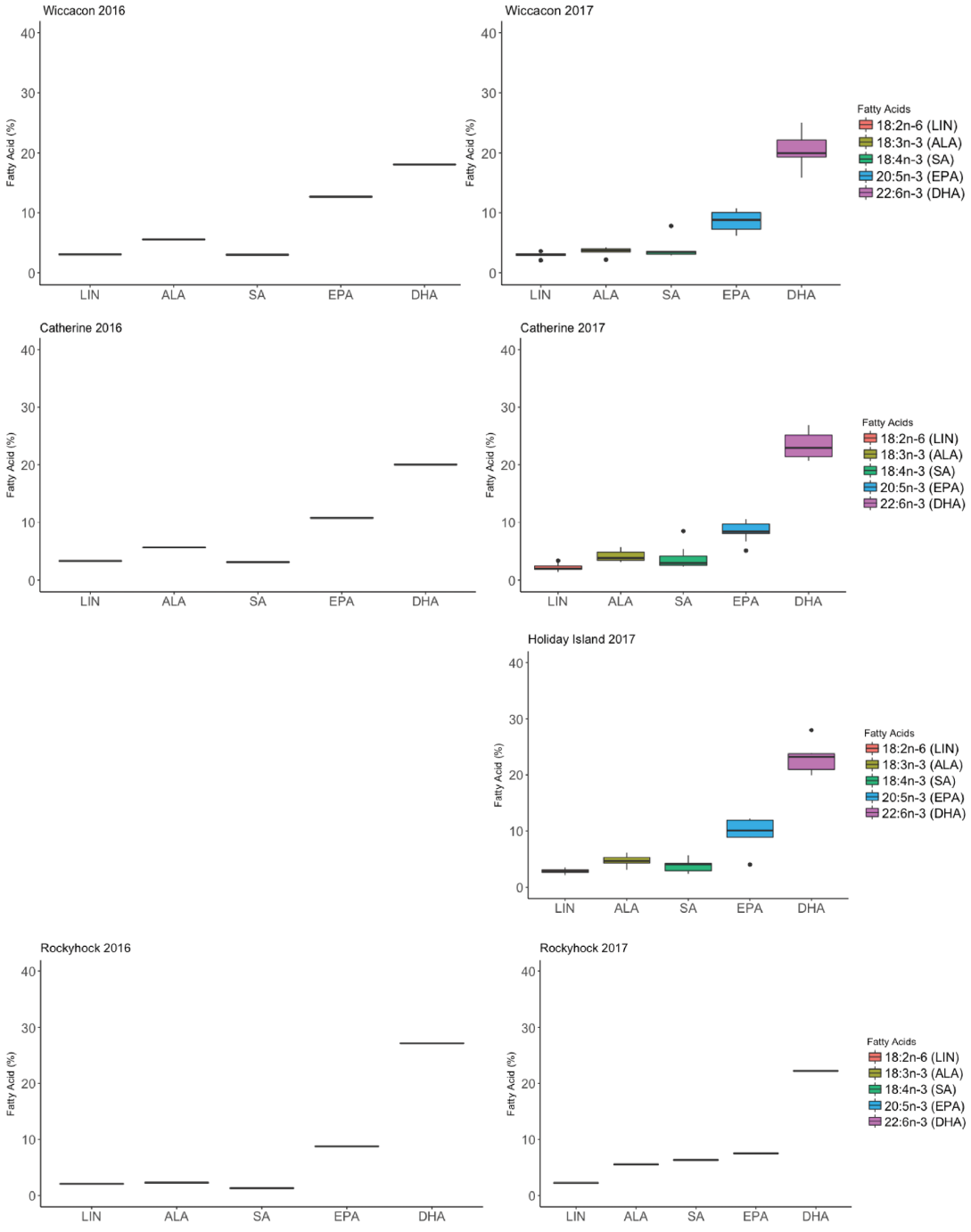


Fig. 26: Boxplot of larval river herring fatty acid percent composition by year and the four sites with the greatest abundance of river herring.

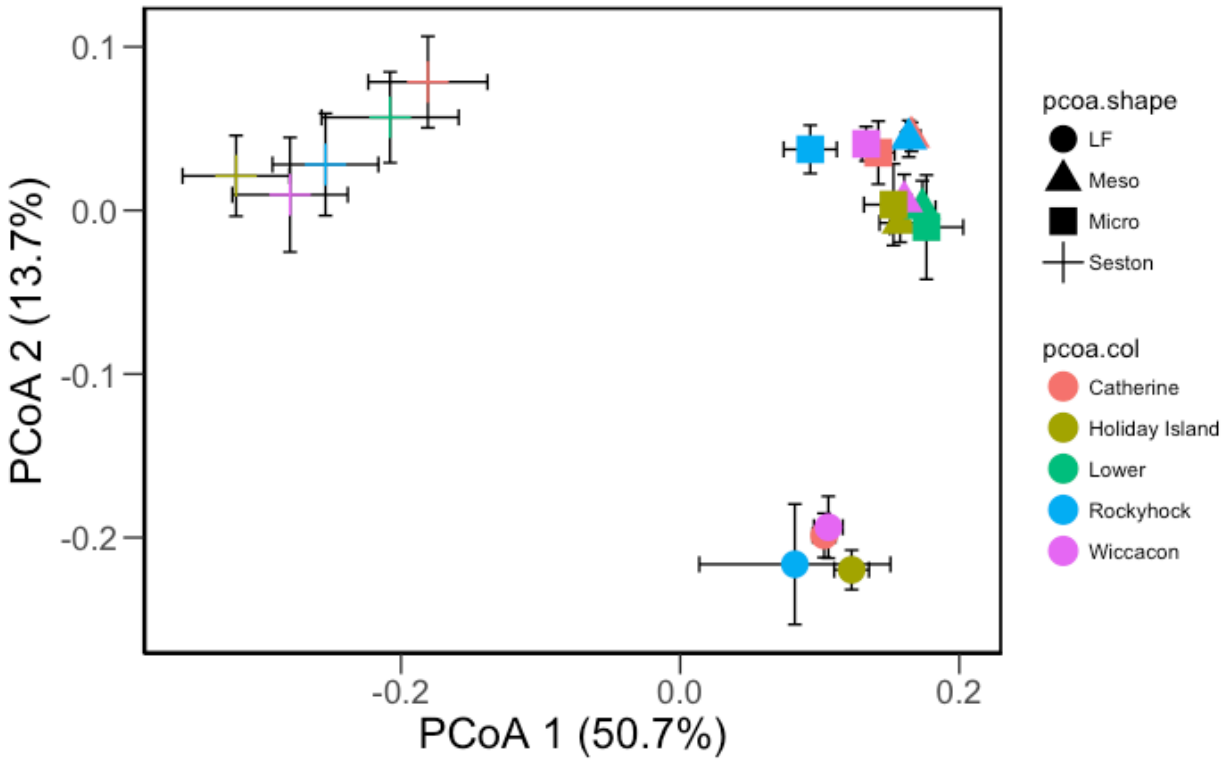


Fig. 27: Ordination from Principal Coordinates Analysis depicting the fatty acid composition for seston, microzooplankton, mesozooplankton, and larval river herring for 2016 and 2017. Symbols are different to represent the type of sample. Symbols are colored according to sampling sites on the Chowan River.

Tables:

Table 3: Phytoplankton functional groups with indicator pigments and fatty acids.

Phytoplankton Functional Group	Phytoplankton Pigment	Fatty Acids
Dinoflagellates	Peridinin	DHA
Diatoms	Fucoxanthin	16:1n-7, EPA
Crytomonad	Alloxanthin	ALA, 18:4n-3, EPA
Green Algae	Chlorophyll b	ALA, EPA, 18:2n-6
Cyanobacteria	Zeaxanthin	ALA, 18:4n-3

Table 4: Mean abiotic factor (±S.D.) by site and year in the Chowan River system. Range is shown on the line below the mean.

	n	Temperature (°C)	Salinity	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Specific Conductivity (µS/cm)	Conductivity (µS/cm)	pH
				2016				
Wiccacon Creek	9	17.6± 3.5	0.04±0.01	55.7±13.9	5.4±1.6	81.2±15.9	70.3±16.7	6.6±0.3
		11.3-22.0	0.03-0.05	38.4-74.3	3.4-7.8	55.1-97.5	41.7-88.0	6.3-7.2
Catherine Creek	9	18.1±3.6	0.07±0.02	65.5±25.6	6.2±2.8	155.7±49.8	133.0±37.1	6.8±0.4
		11.6-23.8	0.04-0.10	25.1-102.5	2.3-11.2	83.9-262.5	82.0-92.5	6.5-7.9
Holiday Island	9	17.7±3.6	0.04±0.01	83.4±7.7	8.0±1.1	83.6±11.5	73.4±12.7	7.1±0.3
		11.2-22.3	0.03-0.04	68.5-92.0	6.0-9.2	65.1-96.5	48.3-74.7	6.6-7.3
Lower Chowan	8	17.9±4.0	0.04±0.01	96.2±4.1	9.0±0.8	76.5±29.1	73.7±14.0	7.3±0.3
		10.1-22.5	0.03-0.04	90.0-101.4	7.7-10.1	7.1-96.1	51.1-89.1	7.0-7.7
Rockyhock Creek	9	18.8±4.4	0.07±0.02	89.4±24.4	8.3±2.2	151.8±52.4	130.9±35.6	7.3±0.4
		11.1-22.0	0.05-0.10	48.8-127.9	4.8-11.4	99.2-234.3	92.5-194.5	6.8-8.1
				2017				
Wiccacon Creek	7	17.2±4.6	0.04±0.01	52.6±21.1	5.2±2.5	88.1±17.4	74.0±9.6	7.5±0.5
		11.9-22.4	0.03-0.05	28.0-80.5	2.5-8.5	67.5-115.7	59.7-86.6	6.8-8.1
Catherine Creek	6	17.7±6.0	0.06±0.02	54.1±17.2	5.3±2.2	130.7±37.6	110.2±23.6	7.4±0.6
		7.6-22.4	0.05-0.10	27.5-73.2	2.3-8.2	100.1-200.2	92.5-155.7	6.8-8.2
Holiday Island	7	17.3±5.1	0.05±0.02	86.3±8.8	8.4±1.7	114.8±44.6	94.8±26.8	7.8±0.5
		9.8-22.9	0.04-0.10	74.5-98.6	6.4-10.7	83.6-200.2	74.7-151.0	7.2-8.5
Lower Chowan	5	15.6±6.0	0.06±0.02	96.6±3.0	9.7±1.3	136.3±30.1	109.4±15.0	8.0±0.4
		8.6-22.8	0.04-0.08	92.2-100.5	7.9-11.2	96.5-169.8	51.1-133.3	7.5-8.5
Rockyhock Creek	7	17.5±5.7	0.07±0.02	84.1±15.9	8.2±2.0	158.6±35.0	132.9±18.0	7.8±0.4
		8.3-22.6	0.05-0.10	58.1-99.2	5.1-10.4	101.7-210.9	97.1-148.3	7.3-8.4

Table 5: Mean nutrient factors (±S.D.) by site and year in the Chowan River system. Range is below the mean. NH4 = ammonium, NOx = nitrate and nitrite, PO4 = orthophosphate, TDP= Total dissolved phosphorus, and DOC= dissolved organic carbon

		2016				
	n	NH_4 (mg/L)	NOx (mg/L)	PO_4 (mg/L)	TDP (mg/L)	DOC (mg/L)
Wiccacon Creek	9	0.08±0.02	0.15±0.04	0.03±0.01	1.99±1.12	17.9±1.5
		0.06-0.11	0.08-0.23	0.01-0.04	0.27-3.14	16.3-20.5
Catherine Creek	9	0.06±0.02	0.25±0.10	0.03±0.01	1.74±0.87	17.3±2.2
		0.03-0.09	0.13-0.47	0.01-0.05	0.81-3.13	14.2-20.2
Holiday Island	9	0.08±0.01	0.19±0.03	0.02±0.01	2.12±1.99	13.9±1.5
		0.05-0.09	0.13-0.21	0.01-0.04	0.88-7.32	11.4-16.1
Lower Chowan	8	0.06±0.01	0.22±0.03	0.02±0.00	1.25±0.44	12.8±1.4
		0.05-0.07	0.18-0.26	0.01-0.02	0.49-1.77	11.5-15.0
Rockyhock Creek	9	0.05±0.01	0.22±0.16	0.02±0.01	1.95±2.42	15.7±1.9
		0.03-0.07	0.09-0.62	0.00-0.04	0.57-8.27	13.1-19.4
		2017				
Wiccacon Creek	7	0.08±0.05	0.13±0.04	0.02±0.01	1.86±0.78	13.7±2.4
		0.02-0.14	0.10-0.21	0.01-0.02	1.07-3.31	10.4-15.9
Catherine Creek	6	0.08±0.03	0.17±0.08	0.02±0.01	1.92±0.95	13.1±2.1
		0.03-0.11	0.10-0.30	0.01-0.04	0.79-3.67	10.2-15.4
Holiday Island	7	0.06±0.02	0.15±0.04	0.02±0.02	1.42±0.76	11.0±2.5
		0.03-0.07	0.10-0.20	0.00-0.05	0.50-2.62	7.7-14.7
Lower Chowan	5	0.04±0.01	0.17±0.03	0.02±0.01	1.28±0.59	9.4±0.7
		0.02-0.05	0.13-0.20	0.01-0.03	0.75-2.07	8.7-10.6
Rockyhock Creek	7	0.04±0.02	0.15±0.07	0.01±0.01	1.39±0.80	10.7±0.9
		0.02-0.07	0.06-0.29	0.01-0.03	0.67-2.98	9.7-12.3

Table 6: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of seston by site and year from the Chowan River by month. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	2016					2017				
	Wiccacon	**Catherine**	**Holiday Island**	**Lower**	**Rockyhock**	**Wiccacon**	**Catherine**	**Holiday Island**	**Lower**	**Rockyhock**
	n=7	**n=9**	**n=9**	**n=5**	**n=7**	**n=9**	**n=9**	**n=9**	**n=6**	**n=9**
14:0	6.0±2.3	8.7±3.9	5.4±3.1	5.8±1.3	12.2±7.0	1.9±1.1	3.0±2.3	3.8±3.7	4.4±3.4	2.6±1.3
15:0	1.4±1.5	2.6±1.4	1.2±0.9	1.1±0.4	1.3±1.0	1.2±0.6	1.9±1.0	1.4±0.6	2.0±0.8	1.0±0.9
16:0	33.9±15.4	31.7±10.0	45.6±7.6	41.8±2.5	35.9±7.2	63.8±25.1	48.5±30.0	58.4±27.4	40.8±22.2	59.3±25.0
17:0	3.1±3.0	2.1±1.1	3.9±1.7	2.3±1.7	1.9±0.6	0.3±0.5	0.4±0.8	0.2±0.2	0.7±0.4	0.3±0.4
18:0	19.3±6.4	11.7±3.4	19.6±2.3	22.5±9.8	13.4±7.4	5.1±2.7	8.5±6.5	6.1±5.3	7.8±2.1	5.5±4.3
20:0	0.6±0.4	0.4±0.3	2.5±5.9	0.4±0.2	4.5±4.2	3.7±4.9	4.0±4.4	1.3±1.6	5.0±4.7	2.5±3.2
∑SFA	64.2±7.8	57.3±12.0	78.2±9.5	73.7±11.2	69.2±5.4	76.1±17.6	66.4±20.3	71.0±20.3	60.7±16.9	72.1±17.9
16:1n-9	2.5±2.0	1.0±0.9	2.2±1.7	0.6±0.6	0.5±0.4	0.3±0.3	0.5±0.4	0.3±0.4	0.6±0.6	0.3±0.4
16:1n-7	4.0±3.8	4.1±1.9	2.6±2.3	3.9±1.9	4.2±2.9	2.6±1.4	4.8±2.9	3.4±2.2	6.2±3.2	3.3±2.3
18:1n-9	5.0±3.8	3.9±2.8	0.8±0.9	7.4±2.3	5.0±3.5	2.1±1.5	4.2±3.9	2.9±2.6	4.0±3.4	2.4±2.1
18:1n-7	0.7±0.7	0.6±1.1	0.3±0.2	0.1±0.1	0.4±0.3	1.1±0.7	1.8±1.4	1.3±1.0	1.6±1.2	1.3±0.9
∑MUFA	12.1±5.1	9.7±3.8	6.0±2.7	12.0±4.1	10.1±5.4	6.1±3.3	11.4±7.3	7.9±5.1	12.4±6.9	7.3±4.8
18:2n-6	8.8±11.4	14.0±15.1	4.7±8.1	4.7±4.5	6.2±4.1	4.0±2.4	5.1±3.0	3.6±1.7	5.2±3.4	5.4±4.0
18:3n-3	2.5±3.2	5.3±5.8	0.4±0.5	1.3±2.5	4.3±3.5	1.4±1.0	3.8±3.4	3.3±2.9	4.2±3.3	2.5±1.7
18:4n-3	2.4±4.2	7.0±7.1	0.3±0.3	1.4±2.5	3.2±2.4	1.6±0.9	3.3±4.4	3.3±4.2	4.7±5.3	2.5±2.4
20:2n-6	0.5±0.3	0.4±0.5	0.7±0.4	0.3±0.2	0.3±0.2	0.3±0.4	0.6±0.8	0.7±1.7	0.4±0.4	0.3±0.4
20:3n-6	0.3±0.2	0.3±0.3	0.4±0.3	0.5±0.2	0.5±0.5	0.1±0.2	0.3±0.3	0.3±0.6	0.3±0.4	0.2±0.2
20:4n-6	0.5±0.3	0.2±0.2	0.6±0.4	0.4±0.2	0.4±0.4	0.5±0.8	0.7±0.7	0.7±0.5	1.3±0.8	0.7±0.5
20:3n-3	0.6±0.4	0.3±0.3	1.5±1.3	0.3±0.1	0.5±0.3	0.4±0.6	0.8±0.8	1.1±0.9	0.8±0.8	0.8±1.0
20:4n-3	0.6±0.4	0.3±0.3	0.7±0.4	0.5±0.3	0.4±0.2	1.1±2.0	0.8±1.6	0.3±0.4	1.9±2.0	1.2±1.7
20:5n-3	0.7±0.6	1.0±1.3	0.6±0.3	0.3±0.2	0.2±0.1	4.6±10.2	2.4±2.0	4.4±7.2	3.6±2.3	3.0±4.0
22:5n-6	0.8±0.4	0.4±0.3	0.7±0.4	0.5±0.4	0.6±0.9	0.3±04	0.4±0.3	0.1±0.1	0.3±0.3	0.2±0.2
22:5n-3	0.7±0.5	0.3±0.2	0.6±0.4	0.4±0.3	0.4±0.4	0.1±0.1	0.2±0.1	0.3±0.5	0.2±0.2	0.1±0.1
22:6n-3	1.7±1.2	0.7±0.8	1.2±1.1	0.6±0.6	0.7±0.4	1.7±1.9	1.8±1.6	1.3±1.1	2.3±2.1	1.7±1.9
∑PUFA	19.9±9.6	30.4±14.1	12.2±8.8	11.3±6.8	17.7±4.6	16.1±13.8	20.1±13.6	19.5±14.4	25.2±11.9	18.7±12.7
Omega-6	10.8±11.0	15.4±15.2	7.0±8.5	6.4±4.5	8.1±3.6	5.1±3.7	7.0±4.0	5.4±3.4	7.4±3.3	6.8±4.7
Omega-3	9.1±6.5	15.0±12.5	5.2±2.5	4.9±45	9.6±5.5	11.0±11.6	13.1±10.3	14.1±11.9	17.8±11.5	11.9±8.7

Table 7: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of microzooplankton by site and year from the Chowan River by month. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	2016					2017				
	Wiccacon	**Catherine**	**Holiday Island**	**Lower**	**Rockyhock**	**Wiccacon**	**Catherine**	**Holiday Island**	**Lower**	**Rockyhock**
	n=6	**n=7**	**n=4**	**n=1**	**n=8**	**n=1**	**n=5**	**n=2**	**n=2**	**n=2**
14:0	5.1±1.1	9.1±4.0	6.9±4.0	4.5	6.1±3.2	7.4	7.7±2.7	5.2±1.4	4.8±0.2	7.0±1.6
15:0	3.0±0.8	1.8±1.0	1.0±0.4	0.7	2.0±1.1	1.5	1.7±0.7	1.8±0.0	2.0±0.2	1.6±1.1
16:0	19.8±0.8	16.9±5.6	18.6±6.0	19.8	22.5±3.3	16.3	16.8±3.5	16.4±6.1	14.5±2.8	18.3±5.6
17:0	0.8±0.7	0.3±0.4	0.7±0.7	1.1	0.6±0.5	1.2	0.8±0.5	1.0±0.2	0.7±0.1	0.5±0.6
18:0	5.9±0.5	4.5±1.7	4.3±1.8	5.9	4.3±0.9	6.1	5.1±2.3	4.2±0.8	3.9±1.6	5.5±1.0
20:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0±0.0	0.1	0.3±0.2	0.1±0.1	1.0±0.5	0.3±0.4
∑SFA	34.5±2.2	32.5±4.2	31.5±4.7	31.9	35.5±1.7	32.6	32.3±5.4	28.6±8.1	26.9±4.4	33.2±5.0
16:1n-9	0.9±0.9	0.7±0.7	0.5±0.3	0.4	0.9±0.3	0.0	0.8±0.5	0.2±0.3	0.2±0.3	0.5±0.7
16:1n-7	8.4±3.0	4.3±2.2	4.0±3.7	2.7	5.6±2.8	7.3	4.8±1.6	6.6±1.3	8.0±0.8	4.7±1.3
18:1n-9	6.6±1.2	6.1±1.1	5.6±2.8	5.8	5.3±1.7	4.2	4.2±1.3	9.0±4.3	8.7±3.8	5.6±0.3
18:1n-7	5.7±1.1	3.3±1.2	2.6±1.0	2.6	3.7±1.1	5.0	3.2±1.6	3.7±1.7	3.1±0.2	3.2±1.2
∑MUFA	21.6±3.2	14.5±3.5	12.7±7.3	11.5	15.5±3.8	16.5	13.0±3.6	19.5±1.0	20.1±3.0	14.0±1.5
18:2n-6	7.4±2.3	8.6±3.8	6.4±2.4	3.9	6.8±1.2	2.9	4.3±1.2	3.4±0.9	4.3±1.5	4.4±0.2
18:3n-3	10.2±4.2	13.1±3.6	12.4±1.3	10.0	20.2±10.3	3.9	8.4±2.7	8.0±2.8	9.1±2.7	10.0±3.1
18:4n-3	4.8±1.8	11.2±8.0	10.9±7.9	5.8	4.5±2.0	12.1	7.5±4.6	8.2±1.5	11.1±1.7	6.3±0.6
20:2n-6	0.2±0.5	0.1±0.1	0.0±0.0	0.0	0.0±0.0	0.0	0.3±0.4	0.2±0.2	0.2±0.3	0.2±0.3
20:3n-6	0.0±0.0	0.1±0.1	0.1±0.1	0.0	0.1±0.1	0.1	0.0±0.0	0.1±0.1	0.0±0.0	0.1±0.2
20:4n-6	0.0±0.0	0.1±0.1	0.1±0.1	0.0	0.0±0.1	5.8	4.9±2.1	4.5±4.1	2.9±0.9	3.3±2.4
20:3n-3	0.2±0.3	0.2±0.2	0.4±0.5	1.3	0.2±0.3	0.1	1.7±1.8	0.7±0.2	0.3±0.4	0.5±0.6
20:4n-3	1.1±0.5	1.8±0.6	2.1±0.6	2.5	0.9±0.8	6.5	1.9±1.2	1.3±0.2	2.6±1.5	2.6±1.2
20:5n-3	13.2±1.1	8.7±3.6	11.4±3.2	15.4	8.5±2.7	7.6	11.2±2.9	15.4±4.1	14.3±2.9	10.3±1.8
22:5n-6	0.3±0.6	2.2±1.7	2.7±3.0	4.2	2.1±2.3	0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22:5n-3	0.0±0.0	0.0±0.0	0.0±0.0	0.0	0.0±0.0	0.0	0.2±0.4	0.1±0.2	0.0±0.0	0.4±0.5
22:6n-3	5.7±2.6	6.4±2.3	8.4±3.8	10.9	5.0±3.6	3.0	8.1±3.8	7.0±0.0	5.6±4.0	8.9±0.1
∑PUFA	43.1±4.4	52.4±7.5	54.9±11.2	54.0	48.4±4.4	42.0	48.6±11.2	48.8±11.7	50.5±9.2	47.0±1.6
Omega-6	8.0±3.0	10.1±4.7	9.3±3.2	8.1	9.1±2.6	8.9	9.6±2.1	8.1±3.5	7.4±0.2	8.0±2.2
Omega-3	35.1±3.5	41.1±3.6	45.6±8.5	45.9	39.3±5.0	33.1	39.1±12.0	40.7±8.2	43.1±9.4	39.0±0.5

Table 8: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of mesozooplankton by site and year from the Chowan River by month. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	2016					2017				
	Wiccacon	**Catherine**	**Holiday Island**	**Lower**	**Rockyhock**	**Wiccacon**	**Catherine**	**Holiday Island**	**Lower**	**Rockyhock**
	n=7	**n=9**	**n=7**	**n=3**	**n=9**	**n=7**	**n=9**	**n=5**	**n=7**	**n=9**
14:0	4.4±1.1	4.9±1.3	4.5±1.2	3.4±1.1	6.9±1.5	3.5±0.8	4.8±0.9	4.5±0.8	3.4±0.7	5.7±2.1
15:0	3.7±1.4	3.5±1.2	1.2±0.3	0.8±0.2	2.4±0.6	3.0±1.0	2.7±0.7	2.0±0.2	1.5±0.4	2.0±0.7
16:0	17.7±1.4	17.4±1.6	19.9±3.3	20.1±0.6	17.5±1.3	14.8±0.7	15.6±1.7	15.2±1.7	15.3±1.6	16.5±2.5
17:0	14±0.7	1.5±0.4	1.2±0.8	1.5±0.2	1.6±0.2	1.8±0.4	1.3±0.4	1.3±0.4	1.0±0.2	1.0±0.2
18:0	6.1±0.4	5.9±0.4	6.4±1.3	7.4±1.2	5.9±0.6	6.2±1.0	5.3±1.4	5.1±0.9	4.8±1.0	4.6±0.9
20:0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.1	0.2±0.4	0.0±0.1	0.3±0.4	0.2±0.2
∑SFA	33.4±1.8	33.2±2.5	33.2±1.0	33.3±1.2	34.3±2.1	29.6±0.9	30.0±3.1	28.2±2.2	26.3±2.2	29.9±3.3
16:1n-9	1.0±0.6	1.4±1.0	0.7±0.3	0.7±0.4	1.2±0.7	0.6±0.8	0.5±0.5	0.3±0.3	0.2±0.1	0.3±0.2
16:1n-7	10.4±3.1	8.0±3.1	4.2±1.8	3.9±1.1	7.3±2.2	12.0±3.7	7.2±1.6	6.9±2.6	8.8±1.0	6.4±1.3
18:1n-9	6.9±1.2	7.3±1.4	7.9±4.0	5.5±0.8	7.4±1.1	5.7±1.0	8.1±2.3	7.3±1.8	7.8±1.1	6.8±1.0
18:1n-7	7.4±1.6	6.1±1.4	4.6±1.2	4.4±1.3	5.1±0.7	8.0±1.7	5.8±1.4	4.8±0.9	5.0±1.1	3.5±0.6
∑MUFA	25.7±4.2	22.8±4.9	17.4±4.9	14.5±3.0	20.9±3.6	26.3±5.1	21.7±2.6	19.3±2.7	21.9±2.2	17.1±2.4
18:2n-6	7.3±3.3	6.1±2.5	6.4±2.7	4.4±1.6	6.8±1.2	3.8±0.8	4.3±1.7	3.2±0.9	3.6±0.2	4.4±0.6
18:3n-3	9.1±4.1	12.8±4.1	11.2±4.4	9.2±2.3	11.4±1.6	5.3±2.1	9.6±1.8	8.6±0.7	11.0±2.5	11.2±2.5
18:4n-3	4.4±2.5	6.4±2.4	5.8±1.5	4.3±0.9	6.0±1.5	2.6±0.9	5.7±3.4	6.9±2.3	10.7±2.5	8.4±3.3
20:2n-6	0.0±0.1	0.1±0.1	0.1±0.1	0.3±0.2	0.0±0.0	0.2±0.2	0.2±0.2	0.2±0.2	0.2±0.1	0.3±0.3
20:3n-6	0.1±0.1	0.1±0.1	0.1±0.1	0.0±0.0	0.1±0.1	0.0±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.1
20:4n-6	0.1±0.1	0.1±0.1	0.0±0.0	0.0±0.0	0.1±0.1	9.0±1.8	7.2±2.0	6.2±1.8	3.0±0.3	3.5±1.2
20:3n-3	0.1±0.2	0.2±0.2	0.2±0.3	0.7±0.4	0.1±0.1	0.7±0.8	0.4±0.5	0.4±0.3	0.8±1.0	0.8±0.9
20:4n-3	0.5±0.3	0.9±0.3	0.9±0.4	1.2±0.0	0.8±0.3	0.9±0.6	0.7±0.4	1.0±0.7	1.0±0.3	1.4±0.4
20:5n-3	13.5±1.3	12.8±1.8	13.0±3.2	15.0±2.2	13.2±1.4	11.6±1.8	13.4±2.8	17.6±2.1	17.5±2.1	13.6±2.3
22:5n-6	0.6±0.5	0.5±0.4	1.8±1.4	1.9±1.8	1.4±1.2	0.0±0.0	0.0±0.0	0.7±1.6	0.0±0.0	0.0±0.0
22:5n-3	0.1±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.1	0.4±0.5	0.1±0.2	0.1±0.2	0.4±0.6	0.3±0.3
22:6n-3	4.8±2.1	3.9±3.6	9.5±5.1	14.2±3.3	4.7±3.0	7.2±38	3.9±1.5	5.6±2.8	2.1±1.7	6.1±3.0
∑PUFA	40.6±5.2	43.7±6.5	49.0±4.9	51.3±1.4	44.5±3.0	41.8±4.7	45.4±4.8	50.6±2.3	50.2±4.5	50.1±5.6
Omega-6	8.1±2.8	6.9±2.5	8.4±1.6	6.6±1.1	8.4±1.1	13.1±20	11.7±2.4	10.4±3.0	6.8±0.4	8.3±1.5
Omega-3	32.4±6.5	36.8±7.9	40.6±5.7	44.7±1.6	36.2±2.4	28.7±6.4	33.7±6.1	40.2±3.1	43.4±4.7	41.8±6.6

Table 9: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of larval river herring by site and year from the Chowan River by month. Combined zooplankton from 60 and 200 um mesh because of low volume to run lipids. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids

	2016			2017			
	Wiccacon	**Catherine**	**Rockyhock**	**Wiccacon**	**Catherine**	**Holiday Island**	**Rockyhock**
	n=1	**n=1**	**n=1**	**n=7**	**n=11**	**n=6**	**n=1**
14:0	2.9	2.5	1.9	1.7±0.3	1.5±0.3	1.7±0.4	1.8
15:0	0.0	0.4	0.4	0.6±0.3	0.5±0.2	0.5±0.2	0.3
16:0	27.1	26.4	25.9	17.9±2.9	19.2±2.4	19.0±1.4	16.7
17:0	1.1	1.2	1.1	1.3±0.3	1.3±0.5	1.2±0.2	0.7
18:0	8.3	8.0	8.3	8.0±2.5	8.4±2.6	6.6±1.5	5.1
20:0	0.0	0.0	0.0	1.7±2.0	1.1±1.7	1.3±0.8	4.8
∑SFA	39.4	38.6	38.9	31.2±4.3	32.1±3.6	30.3±1.9	29.3
16:1n-9	0.5	0.7	0.9	0.3±0.0	0.2±0.1	0.1±0.1	0.1
16:1n-7	0.0	2.0	2.6	3.3±1.0	3.0±1.0	2.1±0.4	1.7
18:1n-9	6.7	6.4	7.3	6.3±1.5	7.2±1.9	7.1±1.8	5.1
18:1n-7	3.1	3.1	3.4	3.2±0.7	3.2±0.6	2.6±0.4	2.0
∑MUFA	10.4	12.0	14.2	13.1±3.0	13.5±3.1	12.0±2.5	8.9
18:2n-6	3.0	3.3	2.1	2.9±0.5	2.2±0.6	2.8±0.5	2.2
18:3n-3	5.6	5.7	2.3	3.6±0.7	4.2±1.0	4.7±1.1	5.6
18:4n-3	3.0	3.1	1.3	3.9±1.8	3.8±1.8	3.8±1.2	6.4
20:2n-6	0.3	0.6	0.3	1.5±1.5	0.9±0.9	1.1±0.7	0.3
20:3n-6	0.0	0.0	0.0	0.2±0.2	0.5±0.8	0.1±0.1	0.0
20:4n-6	0.0	0.1	0.0	6.1±2.3	5.6±2.3	7.8±5.4	11.6
20:3n-3	0.0	0.1	0.0	1.7±1.1	0.8±0.8	0.7±0.9	0.0
20:4n-3	0.0	1.5	0.9	2.7±1.2	2.1±1.3	2.7±0.8	3.1
20:5n-3	12.7	10.8	8.8	8.6±1.8	8.5±1.7	9.6±3.1	7.6
22:5n-6	4.1	3.7	3.1	1.1±1.8	1.6±2.2	0.4±1.0	0.0
22:5n-3	2.8	0.1	0.0	2.1±0.6	1.8±0.7	0.9±0.7	0.0
22:6n-3	18.0	20.0	27.2	20.5±3.1	23.4±2.3	23.1±2.9	22.2
∑PUFA	49.5	48.9	46.0	55.0±2.9	55.2±2.7	57.8±1.7	59.0
Omega-6	7.5	7.6	5.6	11.8±2.0	10.7±1.7	12.3±4.7	14.2
Omega-3	42.1	41.2	40.4	43.2±2.2	44.5±3.1	45.5±4.4	44.8

Chapter 4: Lower trophic food web dynamics and structure of nursery areas for anadromous fish in the Tar/Pamlico River and three tributaries, North Carolina, U.S.A.

Introduction

Estuaries are considered important nursery habitat for many ecologically and commercially important fish and invertebrates (Beck et al. 2001, Boesch and Turner 1984, Sheaves et al. 2015, Sheaves 2016). Estuaries function as fish nurseries because they are highly productive, support large planktonic populations across multiple size ranges, and fish within estuaries generally have higher growth rates compared to other habitats (Beck et al. 2001). The nursery habitat concept leaves out multiple factors (e.g., abiotic and biotic factors) that affect fish, and does not include larval fish life stage (Sheaves et al. 2015). Three major aspects should be considered when investigating the nursery habitat concept: connectivity/population dynamics, ecological/eco-physiological factors, and resource dynamics (Sheaves et al. 2015). I incorporated two overall components (ecological/eco-physiological factors and resource dynamics) by investigating food webs, resource availability, and eco-physiological factors in this study.

An important component of a functional nursery habitat is the composition of the food source for fish, primarily zooplankton, which can be affected by abiotic conditions in the system (Sheaves et al. 2015). Since estuaries are important nursery habitats, nutrient dynamics in estuarine ecosystems that alter the phytoplankton composition could result in changes in the quality of food for zooplankton (Müller-Navarra et al. 2000, Paerl et al. 2003). High growth rates of larval fish are possible if zooplankton prey that are present during their critical transition from yolk sac to free-living, feeding larvae (Hjort 1914; Mullen et al. 1986; Rulifson et al. 1993; Cooper et al. 1998; Martino and Houde 2010; Binon 2011). However, this spatial and temporal

overlap between predators and prey does not completely explain how fish nurseries function mechanistically. The quality of prey also plays a major role in determining the effectiveness of a nursery for early stages of fish development (Fraser et al. 1989; Webster and Lovell 1990; Copeman et al. 2002; Rossi et al. 2006; Malzahn et al 2007, Paulsen et al. 2014). Investigating how estuaries, dynamic systems, affect the food web, and especially larval fish, can give us a better understanding of nursery habitat.

An organism's chemical composition (e.g., lipids) can be used to study how abiotic factors affect the different food sources for zooplankton and larval fishes (Fraser et al. 1989; Webster and Lovell 1990; Copeman et al. 2002; Rossi et al. 2006; Malzahn et al 2007, Paulsen et al. 2014). Fatty acids are chemically diverse, often incorporated into organisms unmodified, and different organisms have distinct profiles (Dalsgaard et al. 2003). Fatty acids are one class of compounds found in lipids that are particularly important, impacting neural and vision development in fish (Gulati et al. 1997; Müller-Navarra et al. 2000; Kainz et al. 2004; Masclaux et al. 2012). Fatty acids may act as both dietary tracers in the food web and indicators of overall food quality (Iverson et al. 2004). The majority of organisms need specific dietary fatty acids for somatic development and fitness (Masclaux et al. 2012). These fatty acids, 18:3n-3, α-linolenic acid (ALA), and 18:2n-6, linoleic acid (LIN), are labeled essential fatty acids because they cannot be directly synthesized by heterotrophic organisms and must come from the diet (Arts et al. 2009). Polyunsaturated fatty acids (i.e., 20:5n-3, eicosapentaenoic acid (EPA), 22:6n-3, docosahexaenoic acid (DHA)) are required for all organisms and play a role in health and cell function (Dalsgaard et al. 2003). Thus, an organisms' community composition can change the fatty acid signature that would indicate dietary consumption and nutritional quality of its prey (Goncalves et al. 2012).

Phytoplankton biomass in estuarine ecosystems are affected by nutrients, light availability, water residence time, and grazing (Hutchinson 1961, Fisher et al. 1988, Cole et al. 1992, Paerl et al. 2004, Sommer & Sommer 2006, Valdes-Weaver et al. 2006, Paerl 2009). Along an estuarine continuum, phytoplankton biomass is often lower in the freshwater reaches and increases as salinity increases toward an area of highest biomass, called the chlorophyll maximum (Fisher et al. 1988, Paerl et al. 2003, Paerl et al. 2004, Paerl 2009). This is because P-limitation becomes ameliorated as increasing salinity concentrations cause phosphate to become bioavailable (Fisher et al. 1988, Paerl et al. 2003, Paerl et al. 2004, Paerl 2009). Nutrients have increased throughout estuaries from agricultural production, increased population in coastal areas, and from runoff during larger rain events and flooding, which results in an increase of phytoplankton biomass (Cloern 2001, Paerl et al. 2003, Flemer and Champ 2006, Walters et al. 2009). In turbid and well-mixed systems, light can be a limiting factor for phytoplankton biomass (Cole et al. 1992, Irigoien & Castel 1997). Suspended particulate matter in the system can result in decreased primary production, and occur in areas even when nutrients are not limiting and have low phytoplankton biomass (Cole et al. 1992, Irigoien & Castel 1997). Phytoplankton biomass is related to water retention time, and higher retention time allows phytoplankton to grow and reproduce (Fisher et al. 1988, Paerl et al. 2004, Valdes-Weaver et al. 2006, Paerl 2009). During wet years, increased rain and flooding flushes nutrients and sediment down river, and into the sound (Fisher et al. 1988, Paerl et al. 2004, Valdes-Weaver et al. 2006, Paerl 2009). Both of these can result in the reduction of phytoplankton biomass (Fisher et al. 1988, Paerl et al. 2004, Valdes-Weaver et al. 2006, Paerl 2009). Finally, grazing by zooplankton can reduce the phytoplankton biomass, but have been shown to not regulate primary production (Hutchinson 1961, McManus & Ederington-Cantrell 1992, Sommer & Sommer 2006). All these

factors need to be considered when evaluating the phytoplankton biomass in the system, which result in changes to the phytoplankton community composition as well.

Nutrients can impact overall chlorophyll *a* levels; however, less attention is paid to the phytoplankton community composition. For example, different phytoplankton groups uptake nutrients at different rates. Diatoms use nitrate and are found in increased biomass when there is increased water clarity in estuaries (Paerl et al. 2003, Domingues et al. 2011). However, dinoflagellates respond to environmental perturbations differently, and can consume dissolved organic matter by osmomixotrophy (Paerl et al. 2003, Wacker and Weithoff 2009). During the spring when temperatures increase in freshwater areas of estuaries, chlorophytes and cryptophytes outcompete other phytoplankton by having efficient growth rates and enhanced nutrient uptake rates for ammonium and nitrate (Pinckey et al. 1999, Paerl et al. 2003, Valdes-Weaver et al. 2006, Domingues et al. 2011). These changes in phytoplankton composition and biomass can, therefore, result in differences in phytoplankton.

The origin of fatty acids in most aquatic food webs comes from phytoplankton production (Farkas and Herodek 1964; Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). However, in estuaries, fatty acids may enter the system via detrital material (Farkas and Herodek 1964; Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). The two combined sources are often reflected in the composition of the seston, the inorganic and organic material in the pelagic environment (Postel et al. 2000). The fatty acid composition of the seston changes as a result of local conditions, such as temperature, nutrient concentration, and the degree of autotrophy or heterotrophy in the system (Farkas and Herodek 1964; Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). The detrital material of the seston would result in increased amounts of saturated fatty acids (SFA)

(Persson and Vrede 2006, Gladyshev et al. 2010, Ravett, Brett, Arhonditsis 2010, Burns, Brett, and Schallenberg 2011, Gonclaves et al 2012).

The phytoplankton component of seston can be evaluated using pigments, which indicate phytoplankton functional groups because specific accessory pigments are found in particular groups (Paerl et al. 2003). Diatoms have the primary indicator pigment fucoxanthin, and have increased percent composition of 16:1n-7, and omega-3 fatty acids especially EPA (Napolitano et al. 1997; Dalsgaard et al. 2003; Paerl et al. 2003, Boschker et al. 2005; Arts et al. 2009; Bec et al. 2010) (Table 10). Photosynthetic dinoflagellates have the primary indicator pigment peridinin, and have increased DHA, an important omega-3 fatty acid in the fatty acid profile (Paerl et al. 2003, Art et al. 2009, Strandberg et al. 2015) (Table 10). Cryptophytes are a group of algae that have the primary indicator pigment alloxanthin, and had increased percent composition of omega-3, ALA and 18:4n-3 (Paerl et al. 2003, Strandburg et al. 2015) (Table 10). Zeaxanthin can be found in prochlorophytes, cyanobacteria, green algae, and chrysophytes, which could result in differences found the in the fatty acid composition (Paerl et al. 2003, Strandburg et al. 2015) (Table 10). The common fatty acid found throughout those species is ALA, a lower chain omega-3, and essential fatty acid throughout the food web (Strandberg et al. 2015). Chlorophyll *b* is a primary indicator pigment in green algae or chlorophytes (Paerl et al. 2003). Chlorophytes have increased percent composition of 18:1n-9, omega-6 (18:2n-6), and omega-3 (ALA) (Ahlgren et al. 1990; Dalsgaard et al. 2003; Boschker et al. 2005; Masclaux et al. 2012; Strandberg et al. 2015) (Table 10). These changes in the seston fatty acids would affect the grazers, especially the zooplankton.

Zooplankton fatty acid profiles are linked to species composition and the food sources in the system. Zooplankton community composition in estuaries has been intensely studied and

abiotic factors, especially salinity are thought to structure zooplankton communities (Ambler et al. 1985; Orsi 1986; Cervetto et al. 1999; Mouny and Dauvin 2002; Kimmel and Roman 2004; Lawrence et al. 2004; Islam et al. 2005). Cladocerans, copepods, and rotifers are the dominant groups of zooplankton found in the freshwater areas of estuaries; however copepods are dominant in brackish water (Tackx et al. 2004, Marques et al. 2006, Winder and Jassby 2011, Chambord et al. 2016). For the smaller bodied zooplankton, rotifers are usually dominant in freshwater, and copepod nauplii in brackish water (Park and Marshall 2000a & 2000b). Cladocerans are characterized by high levels of EPA and this is thought to be related to a life history strategy focused on high rates of somatic growth (Persson & Vrede 2006). In contrast, copepods have higher relative DHA levels because this fatty acid is critical for nervous system development (Arts et al. 2009). Copepods feature more developed nervous systems compared to cladocerans; this is a function of active hunting of prey, mate location, and predator avoidance (Dalsgaard et al. 2003). Rotifer fatty acid profiles are closely related to their food source, which is usually composed of lower chain fatty acids (Gladyshev et al. 2010). Copepod nauplii have fatty acid profiles similar to adult copepods with increased DHA (Arts et al. 2009). These changes in zooplankton community composition result in larval fish consuming very different diets depending on where they are residing.

The species composition and variability in fatty acid composition of the lower food web for an estuarine fish nursery was explored in the Tar/Pamlico River, North Carolina, USA. The Tar/Pamlico River is considered nursery habitat for larval and juvenile blueback herring (*Alosa aestivalis*), alewife (*A. pseudoharengus*), collectively known as river herring (NCDMF 2015). The river herring are of interest because they have been severely overfished and a moratorium on harvest is in place at various locations along the eastern United States, including North Carolina

(ASMFC 2012). Historically, river herring have been found throughout tributaries of the Tar/Pamlico River, but little is known about the population because of decreased monitoring and management (NCDMF 2015). Strategic habitat areas (SHAs) are defined as areas that contribute most to the integrity of the system and for fish as "locations of individual fish habitats or systems of habitats that have been identified to provide exceptional habitat functions or that are particularly at risk due to eminent threats, vulnerability or rarity" (Deaton et al. 2006). My study sites are considered SHAs, but river herring were not considered in the decision for Tar/Pamlico River (NCDMF 2015). This study will help to better understand the nursery habitat in these SHAs, and possible in the future be included in any changes to SHAs.

The overall goal of my study was to investigate the lower food web dynamics within fish nursery habitat in Tar/Pamlico River. I determined if species and fatty acid composition of the lower food web varied in relation to abiotic factors of the sampling site that are used to indicate habitat quality of an estuarine fish nursery. In order to achieve this goal, I examined the spatial and temporal variability of abiotic factors, phytoplankton pigments, zooplankton species composition, as well as the fatty acid composition of the seston and zooplankton during larval fish residency in the three sites (Tranters and Blounts Creek, and lower Tar River), and to brackish water sites (Blounts Bay and Pantego Creek). I hypothesized that phytoplankton pigments would be related to nutrient dynamics, and zooplankton species composition would change from cladoceran and copepods to *Acartia* spp. with increased salinity. I related abiotic factors (temperature, dissolved oxygen, pH, and nutrients) to patterns in phytoplankton pigments and zooplankton species composition. I hypothesized that the seston fatty acid would relate to the phytoplankton pigment, and increased chlorophyll *a* would result in increased omega-3s. I predicted that fatty acid profiles of zooplankton would have increased EPA and DHA for *Acartia*

spp. compared to the freshwater sites, and in freshwater, the copepods would have increased DHA compared to cladocerans, which would have EPA and rotifers with ALA. I related the zooplankton fatty acid profiles to the zooplankton community composition for brackish water sites, and freshwater sites. I hypothesized that zooplankton fatty acid profile would reflect the seston fatty acids in freshwater sites, but differ for brackish water sites. I compared the fatty acid profiles between the seston fatty acids and zooplankton fatty acid profiles. If supported, this would suggest that the quality of the larval fish forage, based on fatty acids, and lower trophic food web could be used to assess fish nursery quality.

Materials and Methods

Study Site

The Tar/Pamlico River begins in the Piedmont region and is the 4th largest river basin in North Carolina (NCDWQ 2010). The Tar/Pamlico River is a major freshwater source to the Pamlico Sound (NCDWQ 2010) (Fig. 28a & b). The entire basin is classified as nutrient sensitive waters, and nutrient enrichment is the main water quality issue, which has also led to nuisance algal blooms (Stanley 1992 & NCDWQ 2010). The Tar/Pamlico River flows into the Pamlico Sound and has a salinity gradient throughout the year in parts of the river. Historically, river herring have been found throughout the freshwater tributaries (NCDMF 2015).

Sampling occurred at three tributaries (Tranters, Blounts and Pantego Creeks) and two sites (Blounts Bay and Lower Tar River) on the Tar/Pamlico River (Fig. 28c). The tributaries were chosen for their historic larval river herring presence. The two Tar/Pamlico River sites were selected comparison to open water near two of the tributaries. Sampling occurred weekly from

March through May, weather permitting. I had a total of 9 sampling trips in 2016, and 11 sampling trips in 2017. Water depths ranged from 1.83 to 6.55 m during all sampling trips.

Sample Collection

Water column properties

I measured vertical profiles of temperature (°C), salinity, dissolved oxygen (mg L^{-1}), and pH using a YSI Pro handheld multi-sensor reader (Yellow Springs Instruments) and a conductivity, temperature, and depth sensor (CTD, Yellow Springs Instrument, Castaway). Water samples were collected at a depth of 1 meter with a Niskin water sampler.

Zooplankton

Two horizontal net tows were done using 0.5 m diameter nets of two different mesh sizes (60 and 200 µm). Two mesh sizes were used in order to generate an adequate representation of the zooplankton for the size range > 60 µm. The zooplankton samples between 60 and 200 µm are designated microzooplankton and the > 200 µm zooplankton samples are designated mesozooplankton throughout this study. The zooplankton net was towed obliquely through the water for 2 minutes at an average boat speed of 1.06 m s^{-1}. Each zooplankton composition sample, depending on mesh size, was filtered through a 60 or 200 µm filter, and the zooplankton were preserved in a 120 mL glass jar with 10 ml of 10% buffered formaldehyde, sucrose, and filtered 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 60 µm sample had a half tablet of Alka Seltzer added to keep rotifers from pulling in critical body parts (legs and arms) to ease identification (Chick et al. 2010). The fatty acid zooplankton samples by mesh size were placed in individual 1000 mL brown plastic containers on ice, and processed in the laboratory.

Laboratory Processing

Zooplankton Identification

Samples were filtered through a sieve (60 or 200 μm) to remove the sugar formalin solution, and then added to a beaker with a known volume of water. A total of three subsamples (2 mL per subsample for microzooplankton and 5 mL per subsample for mesozooplankton) were analyzed for community composition using a Hensen-Stempel pipette. If 1000 individuals of single species were counted in one subsample, then that species was not counted in the other two subsamples. Organisms were identified using a dissecting microscope and enumerated using a Ward counting wheel. 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 developmental stage (Johnson and Allen 2012).

Phytoplankton Pigment Samples

The water samples (150 to 500 mL) were concentrated on a 0.07 μm Whatman™ GF/F filter (47 mm diameter), and stored at -80°C until further processing. Each filter was placed into 100% acetone, and sonicated (Qsonica®) for 30 seconds. The filters were left for 24 hours in acetone in a -20°C freezer to extract the phytoplankton pigments, and then filtered into small brown vials. The samples were run on the Shimadzu high pressure liquid chromatograph (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) to separate the pigments over 52 minutes using the methods of Mantoura and Llewellyn (1983) and Van Heukelem and Thomas (2001). The samples were analyzed for six pigments (peridinin, fucoxanthin, alloxanthin, zeaxanthin, chlorophyll *a*, and chlorophyll *b*), and the areas under the peak were calculated. The areas of the peak were converted into μg L^{-1} per each pigment.

Nutrient Samples

Water that was filtered through a 0.07 µm Whatman GF/F filter (47 mm diameter) was placed in a 200 mL plastic bottle and frozen at -20°C for nutrient analysis. Ammonium (NH_4^+), nitrate and nitrite ($NO_3^- + NO_2^-$), and orthophosphate (PO_4^{3-}) were analyzed using an automated system on the *SmartChem200* discrete analyzer (Unity Scientific, Milford, MA, U.S.A.) in 2016, and a Seal Autoanalyzer 3 (SEAL Analytical, Mequon, WI, U.S.A.) in 2017. The methods used were EPA method 350.1 for ammonium EPA, method 353.2 for nitrate+nitrite, and EPA method 365.1 for orthophosphate (O'Dell 1993). Dissolved organic carbon was measured on a Shimadzu TOC‐V total carbon analyzer with a TNM‐1 nitrogen module (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.) in 2016, and a Teledyne Tekmar Torch analyzer with a total N module (Teledyne Tekmar Instruments, Mason, OH, U.S.A.) in 2017. The total dissolved phosphorus was analyzed using the manual persulfate method, and concentrations read on the spectrometer (APHA 1999).

Lipid and Fatty Acid Samples

The water samples (100 to 500 mL) were concentrated on three 0.7 µm Whatman™ GF/F filters (47 mm diameter), which constituted the seston material, and were stored at -80°C until lipid analysis. The zooplankton samples were filtered through stacked 60 and 200 µm sieves to collect species based on size. Each sample was visually analyzed to determine the dominant species using a dissecting microscope, and detritus and phytoplankton were removed. The samples were concentrated on a GF/F filter (47 mm diameter) by mesh size (60, 200 µm), and stored at -80°C until lipid analysis. Zooplankton from 60 and 200 µm mesh containing low biomass were combined for total lipids and fatty acid extraction.

Total lipids were extracted with chloroform-methanol (2:1, *v*/*v*) containing 0.01% butylated hydroxytoluene as an antioxidant (Folch et al. 1957). The organic solvent was evaporated under a stream of nitrogen and lipid concentration determined gravimetrically. Transmethylation of fatty acids was done according to the method described by Metcalfe and Schmitz (1969). A known amount of nonadecanoate acid (19:0) dissolved in hexane at a concentration of 8 mg mL^{-1} (Nu Check Prep Inc.) was added as an internal standard. The fatty acid methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas Chromatograph, Agilent Technologies, Inc.) using a 7693 mass spectrometer detector (Agilent Technologies, Inc.), a capillary column (Omegawax^{TM} 250 fused silica capillary column, 30 mm x 0.25 mm and 0.25 mm film thickness, Supleco®), and a 7890A autoinjector (Agilent Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.3 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^{-1}, 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) 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. I calculated the sestons' omega-3 to omega-6 ratio to determine the dominant source of fatty acids.

Statistical Analyses

I performed a series of multivariate analyses to address my specific objectives using the R environment (R v3.4.3, R Core Development Team 2017). I determined if abiotic and nutrient factors were related to the observed patterns in phytoplankton pigments for combined, freshwater

and brackish water sites, and zooplankton community composition, and if the fatty acid profiles were related to the observed patterns in the phytoplankton pigment, and freshwater and brackish water zooplankton community composition using redundancy analysis (Legendre and Legendre 1998). The redundancy analysis was carried using the *rda* function (Oksanen et al. 2018) in the vegan package (Oksanen et al. 2017).

I used permutational analysis of variance (PERMANOVA) using the *adonis* function in the vegan package (Oksanen et al. 2018), to examine how sampling site and year influenced the phytoplankton pigment composition and zooplankton community composition. I also used the PERMANOVA to determine differences between sampling site and year for seston and zooplankton fatty acid profiles. PERMANOVA is a non-parametric technique related to ANOVA, but uses permutations and fewer assumptions compared to the traditional ANOVA approach (Anderson 2001). As such, it is particularly well suited to multivariate data sets of low sample size that also violate the traditional assumptions of ANOVA, as was in my case (Anderson 2001).

I generated an ordination plot based on Principle Coordinates Analysis (PCoA) to visually show the results for site and year for the phytoplankton pigment composition, zooplankton community composition, and the interaction between seston and zooplankton fatty acid profiles. Finally, I used a Mantel matrix comparison to correlate fatty acid profiles between the seston, and zooplankton using *mantel.rtest* function in the ade4 package (Oksanen et al. 2017).

Results

Abiotic Conditions

Salinity ranges varied throughout the Tar/Pamlico River and three tributaries (Fig. 29 & Table 11). Pantego Creek, and Blounts Bay had brackish water in both years, Blounts Creek salinity was characterized by freshwater, except for a few times in 2017 when salinity was around 1 (Fig. 29 & Table 11). Tranters Creek and Lower Tar River salinity were characterized by freshwater in both years (Fig. 29 & Table 11). Overall, temperature increased from the start of sampling in March (10-14°C) to the end in May (22-25°C) (Table 11). The pH levels were similar throughout the sites and sampling periods with a range of 6.2 to 8.5 in 2016, and an increased range in 2017 of 6.6 to 8.5 (Table 11).

Dissolved organic carbon (DOC) had higher concentrations in 2016 and 2017 for Pantego Creek than at other sites (Fig. 30 & Table 12). Blounts Creek, Blounts Bay, and lower Tar River had decreased DOC concentrations in 2017 compared to 2016 (Fig. 30 & Table 12). Tranters Creek had similar DOC concentrations to the other sites with an increase in May of 2016, and similar concentrations in 2017 to the other sites (Fig. 30 & Table 12).

Ammonium (NH_4^+) concentrations were similar throughout the sites in 2016 except for an increase during early May in Pantego Creek (Fig. 31a & Table 12). Blounts Creek in 2017 had the lowest ammonium concentrations compared to the other sites (Fig. 31a & Table 12). Tranters Creek and lower Tar River had similar concentrations with an increase in mid- to end of April in 2017 (Fig. 31a & Table 12). Ammonium concentrations increased at the end of April and May in Blounts Bay in 2017, and NH_4^+ increased in Pantego Creek at the end of March and April in 2017 (Fig. 31a & Table 12).

Nitrate and nitrite (NO_x^-) concentrations were similar for Blounts Creek, lower Tar River, and Tranters Creek in 2016 and 2017 (Fig. 31b and Table 12). The NO_x^- concentrations in

Blounts Bay were higher than these other sites in March of 2016 and May of 2017 (Fig. 31b & Table 12). Pantego Creek had higher NO_x^- concentrations in March, a spike in May of 2016, and spikes in March and end of April in 2017 (Fig. 31b & Table 12).

Orthophosphate (PO_4^{3-}) concentrations were higher in Blounts Creek for 2016 (Fig. 31c & Table 12). The PO_4^{3-} concentrations were similar in the lower Tar River and Tranters Creek for both years (Fig. 31c & Table 12). Lower Tar River had similar PO_4^{3-} concentrations in 2016 and 2017 (Fig. 4c & Table 12). Pantego Creek had higher PO_4^{3-} concentrations in 2016 and reduced concentrations except for a spike at the end of April in 2017 (Fig. 31c & Table 12). Blounts Bay had high PO_4^{3-} concentrations in March of 2016, but in 2017 there were two spikes at the end of April and middle of May (Fig. 31c & Table 12).

Phytoplankton Pigments

Phytoplankton pigment composition differed with the interaction of year and site (PERMANOVA, $R^2= 0.10$, $p<0.001$) (Fig. 32). Blounts Bay in 2017 and Pantego Creek in both years had a similar phytoplankton pigment composition (Fig. 32). Tranters and Blounts Creeks had a similar phytoplankton pigment composition in 2016 (Fig. 32). Phytoplankton pigment composition was similar for Tranters Creek and lower Tar River in 2017 (Fig. 32). Tranters Creek, Blounts Creek, and lower Tar River site in 2017 appeared to be different along the PCoA2 axis compared to 2016 (Fig. 32). Chlorophyll *a* concentrations were low for both years, and similar throughout the sampling period on Tranters Creek and lower Tar River (Fig. 33). Blounts Creek had similar chlorophyll *a* concentrations for both years except there was a spike in May of 2017 (Fig. 33). Blounts Bay had higher chlorophyll *a* concentrations in 2017 compared to 2016 (Fig. 33). Chlorophyll *a* concentrations in Pantego Creek were consistent throughout the

sampling period in 2017, but in 2016 there were two spikes at the end of March and middle of April (Fig. 33).

Individual phytoplankton pigments varied over the sampling period and across sites during 2016 and 2017. There were low concentrations of accessory phytoplankton pigments in Tranters Creek and lower Tar River for both years (Fig. 34). Fucoxanthin, alloxanthin, and chlorophyll *b* were the main pigments, albeit at low concentrations, in Tranters Creek and lower Tar River (Fig. 34). Blounts Creek had low concentrations of accessory phytoplankton pigments over both years (Fig. 34). Peridinin was present in 2016, and in 2017 fucoxanthin, alloxanthin, and chlorophyll *b* were present Blounts Creeks (Fig. 34). Blounts Bay had elevated concentrations in peridinin and fucoxanthin for both years, but higher concentrations were observed in 2017 overall. An increase in alloxanthin, zeaxanthin, and chlorophyll *b* occurred at Blounts Bay in 2017 (Fig. 34). Fucoxanthin, peridinin, and alloxanthin had increased concentrations in Pantego Creek for 2016, with a spike at the beginning of May (Fig. 34). Pantego Creek in 2017 had similar concentrations of peridinin, fucoxanthin, and zeaxanthin, but also had alloxanthin and chlorophyll *b* present (Fig. 34).

Abiotic factors and nutrients were correlated to the phytoplankton pigment composition when freshwater and brackish water sites were combined (RDA, R^2=0.33, p= 0.001). The first two axes with all pigments account for 92.2% of variance in the phytoplankton pigments (Fig. 35a). The RDA1 axis was correlated with salinity (63%), TDP (-62%), PO_4^{3-} (-62%), and pH (54%), while the RDA2 axis correlated with DOC (50%) (Fig. 35a). Peridinin was correlated with salinity and DOC (Fig. 35a). Fucoxanthin, alloxanthian and chlorophyll *b* were correlated with pH (Fig. 8a). Chlorophyll *a* was negatively correlated with PO_4^{3-} and TDP (Fig. 35a).

Abiotic factors and nutrients were correlated to the phytoplankton pigment composition at the freshwater sites (RDA, R^2=0.41, *p*= 0.001), but not at the brackish water sites (RDA, R^2=0.33, *p*= 0.416). The first two axes with all pigments accounted for 93% of variance in the phytoplankton pigments for freshwater sites (Fig. 35b). RDA1 was correlated with pH (-67%), and PO_4^{3-} (33%), while RDA2 correlated with DOC (-69%) and NOx (63%) (Fig. 35b). Peridinin was positively correlated with DOC (Fig. 35b). Fucoxanthin, alloxanthian and chlorophyll *b* were correlated with pH (Fig. 35b). Chlorophyll *a* was negatively correlated with PO_4^{3-} and NOx^- (Fig. 35b).

Zooplankton Community Composition

Microzooplankton community composition differed between sites (PERMANOVA, R^2= 0.18, p<0.001), but not year (Fig. 36). Pantego Creek, Blounts Bay, and Tranters Creek were all similar across the years, but Blounts Creek and lower Tar River differed across years for the microzooplankton composition (Fig. 36). Copepod nauplii were the dominant species throughout both years at all sites except Blounts Creek in 2016 (Fig. 37). Blounts Creek in 2016 had 50/50 composition of copepod nauplii and rotifers (Fig. 37). In 2016, rotifers were present in higher percent composition for Blounts Bay, lower Tar River, and Tranters Creek compared to 2017 (Fig. 37). In 2017, there was a spike of rotifers in Pantego Creek during April, and in Tranters Creek during May (Fig. 37). Salinity, temperature and pH were the major drivers for the microzooplankton community composition (RDA, R^2=0.27, p=0.013).

Mesozooplankton community composition differed by site (PERMANOVA, R^2= 0.15, p<0.001) and year (PERMANOVA, R^2= 0.40, p<0.001) (Fig. 38). *Acartia* spp. was dominant in Pantego Creek and Blounts Bay both years (Fig. 39). Calanoida were present in Blounts Bay at the end of March and April in 2016 (Fig. 39). Blounts and Tranters Creek, and lower Tar River

had similar mesozooplankton composition of *Bosmina* spp. Chydoridae, Calanoida, and Cyclopodia during both years (Fig. 39). Salinity and temperature were the main drivers for changes in the mesozooplankton community composition (RDA, R^2=0.47, p=0.001).

Fatty Acid Composition

A total of 22 specific fatty acids were measured in all samples (Tables 13-14). Fatty acids were first separated into broad categories: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Table 10-11). Seston had the highest percent SFA at all sites, and the lowest percent of MUFAs and PUFAs (Fig.40). The zooplankton samples had increased PUFAs and at all sites compared to seston (Fig.40). The most common SFA was palmitic acid (16:0), the most common MUFAs were palmitoleic acid (PA, 16:1n-7) and oleic acid (OA, 18:1n-9), and the most common PUFAs were LIN (18:2n-6), ALA, SA (18:4n-3), EPA and DHA (Table 10-11).

Seston

Seston had increased mean percent of PA (16:1n-7) for both years in Blounts Bay and Pantego Creek and in 2017 for Blount Creeks (Fig. 41). Blounts and Tranters Creek, and lower Tar River had a mean percent increase of LIN (18:2n-6) in 2017 for seston (Fig. 41). Seston had a mean increase in PA (16:1n-7) and ALA (18:3n-3) in 2016 for Blounts Creek (Fig. 41). Seston in Tranters Creek and lower Tar River had a mean percent increase in PA (16:1n-7) and OA (18:1n-9) in 2016 (Fig. 41). Omega-3 to omega-6 ratio in seston determines if the system has increased phytoplankton compared to detrital material. Seston fatty acid profiles varied in the omega-3 to omega-6 ratio with the highest ratio at the end of April in 2017 for Pantego Creek

(Fig. 42). The ratio was greater in 2016 for Blounts and Pantego Creeks, and in 2017 for Blounts Bay (Fig. 42). The lowest ratios were in lower Tar River, and Tranters Creek (Fig. 42).

Seston fatty acids were correlated to the phytoplankton pigment composition at the freshwater sites (RDA, r^2=0.39, p= 0.002). The first two axes with all pigments account for 94% of variance in the phytoplankton pigment. RDA1 correlated with OA (18:1n-9) (-80%), PA (16:1n-7) (-51%), LIN (18:2n-6) (-38%), and EPA (-24%) (Fig. 43a). RDA2 correlated with ALA (68%), SA (33%), SFA (-30%), and DHA (-30%) (Fig. 43a). Peridinin was correlated with LIN (18:2n-6), ALA, and PA (16:1n-7) (Fig. 43a). Fucoxanthin, alloxanthin, and chlorophyll *b* were correlated with SA (18:4n-3) (Fig. 43a). Chlorophyll *a* was correlated with SFA and DHA (Fig. 43a).

Seston fatty acids were correlated to the phytoplankton pigment composition at the brackish water sites (RDA, r^2=0.60, p= 0.001). The first two axes with all pigments account for 88% of variance in the phytoplankton pigment. RDA1 correlated with PA (16:1n-7) (-51%), LIN (18:2n-6) (-50%), EPA (-32%), SA (18:4n-3) (22%), SFA (15%), and ALA (-11%) (Fig. 43b). RDA2 correlated with DHA (-60%) and OA (18:1n-9) (-42%) (Fig. 43b). Peridinin was correlated with EPA, ALA, and PA (16:1n-7) (Fig. 43b). Chlorophyll *b* and zeaxanthin were correlated with SA (18:4n-3) and SFA (Fig. 43b). Alloxanthin was correlated with LIN (18:2n-6), and ALA (Fig. 43b). Chlorophyll *a* was positively correlated with OA (18:1n-9) well fucoxanthin was negatively correlated with OA (Fig. 43b).

Zooplankton

Zooplankton fatty acid profiles were similar throughout the sampling period during the two years. Zooplankton had increased mean percent of DHA and EPA in Blounts Bay and

Pantego Creek for both years (Fig. 44). Zooplankton had a similar percent composition of EPA and DHA at Tranters and Blounts Creek, and lower Tar River both years (Fig. 44). There was an increase in mean percent of ALA at Tranters and Blounts Creek for both years and in the lower Tar River in 2017 (Fig. 44).

Zooplankton fatty acids were correlated to the freshwater zooplankton community composition (Redundancy, R^2=0.35, p= 0.001). The first two axes accounted for 81% of variance in the zooplankton community composition. RDA1 was positively correlated with LIN (18:2n-6) (46%) and negatively correlated with DHA (83%), and EPA (78%) (Fig. 45a). RDA2 was negatively correlated with PA (16:1n-7) (43%) and positively correlated with OA (18:1n-9) (41%) and ALA (29%) (Fig.45a). *Bosmina* spp. and rotifers were correlated with EPA and OA (18:1n-9) (Fig. 45a). Calanoida, copepod nauplii and Cyclopodia were correlated with DHA and SA (Fig. 45a). Chydoridae was correlated with PA (16:1n-7), and Daphniidae was correlated with LIN (18:2n-6), ALA and OA (18:1n-9) (Fig. 45a).

Zooplankton fatty acids were correlated to the brackish water zooplankton community composition (Redundancy, R^2=0.46, p= 0.001). The first two axes accounted for 99% of variance in the zooplankton community composition. RDA1 was positively correlated with DHA (65%), and negatively correlated with LIN (61%) (Fig. 45b). RDA2 was negatively correlated with PA (16:1n-7) (44%), SA (18:4n-3) (43%), EPA (24%), and ALA (19%), and positively correlated with OA (18:1n-9) (55%) (Fig.45b). *Acartia* spp. were correlated with DHA and SA (18:4n-3) (Fig. 45b). Copepod nauplii were correlated with LIN (18:2n-6) and OA (18:1n-9). Rotifers were correlated with PA (16:1n-7), ALA and EPA (Fig. 45b).

Relationships between trophic levels

Zooplankton and seston differed in fatty acid composition (PERMANOVA, R^2=0.35, p<0.001) (Fig. 46). Zooplankton from freshwater had similar fatty acid profiles to the seston along PCoA axis 1, and similar fatty acid profiles to zooplankton from brackish water along PCA axis 2 (Fig. 46). Seston fatty acid profiles from Pantego Creek were different from the other seston samples along PCoA axis 2 (Fig. 46). Seston fatty acid profiles were correlated to the zooplankton fatty acid profiles (Mantel, r=0.26, p= 0.002).

Discussion

Overall, the fatty acid composition of the food web indicated that the Tar/Pamlico River is likely to provide nutrition in terms of fatty acid composition for zooplankton and larval fish growth and development. Phytoplankton pigments were related to patterns in salinity, nutrient concentrations, and the fatty acid composition of seston. Chlorophyll *a* concentrations were highest at the brackish water sites, and lowest at the freshwater sites for both years, likely due to increased light and nutrient availability. There was an increase in omega-3 to omega-6 ratio in 2016 compared to 2017. The increase in salinity resulted in the zooplankton composition being dominated by *Acartia* spp. at brackish water sites. Zooplankton from brackish water had increased mean percent of DHA and EPA compared to zooplankton from freshwater. However, the freshwater assemblages had similar mean percent EPA to DHA throughout both years. Additionally, I observed that fatty acids appeared to be incorporated relatively unchanged in zooplankton in terms of relative composition to seston; however MUFA and PUFA percent composition increased in zooplankton relative to seston. This suggests that MUFAs and PUFAs are bioaccumulated at higher trophic levels, as seen in other studies (Persson and Verde 2006; Gladyshev et al. 2010; Ravett, Brett, Arhonditsis 2010; Burns, Brett, and Schallenberg 2011).

This is based on the presence of omega-6 and omega-3 PUFAs present in the seston, and zooplankton throughout the nursery.

Abiotic factors were correlated to phytoplankton pigments when the all sites were considered. Phytoplankton pigments and abiotic factors were not correlated at the brackish water sites when examined alone, but were correlated at freshwater sites. Chlorophyll *a* concentrations were highest when salinity increased and nutrients decreased presumably due to uptake in the brackish water sites. Estuaries have chlorophyll maximum areas that are usually found in areas of increased salinity where retention time for nutrients increases and light limitation from particulate matter is alleviated (Fisher et al. 1988, Paerl et al. 2003, Paerl et al. 2004). Freshwater reaches of the Tar/Pamlico River had the lowest chlorophyll *a* concentrations despite increased nutrient concentrations, particularly phosphorus. Even though I did not measure light levels or flow, it is likely that light was limiting in these areas, as has been observed in other studies. For example, increased flow rates and light limitation resulted in reduction of chlorophyll *a* in the Neuse River, Chesapeake Bay, and Delaware River in the freshwater reaches (Fisher et al. 1988, Paerl et al. 2003, Paerl et al. 2004). Primary production in freshwater is typically phosphorus limited, though nitrogen limitation may also occur (Fisher et al. 1988, Paerl et al. 2003, Paerl et al. 2004). Another time that nutrients increased, but chlorophyll *a* decreased at all sites was during a rain event that resulted in substantial flooding of the Tar/Pamlico River in April 2017 (Fig. 47). There was a spike of nutrients (Fig 4) and all sites were characterized by freshwater (Fig. 29), but chlorophyll *a* levels dropped (Fig 33). During storm events, the Neuse River has a similar reaction with increased nutrients being flushed out to Pamlico Sound (Paerl et al. 2003, Paerl et al. 2004, Valdes-Weaver et al. 2006).

Peridinin was found to be in higher concentrations in March when DOC had lower concentrations. Peridinin is indicative of dinoflagellates, which have been shown to consume DOC through osmomixotrophy (Paerl et al. 2003, Wacker and Weithoff 2009). Historically, dinoflagellates have been present in the brackish portion of the Pamlico River from January to March because of the increase in nitrate and the longer water retention time after increased rainfall in winter (Mallin 1994, Valdes-Weaver et al. 2006). Fucoxanthin was the most dominant pigment for both years in the brackish water sites. Fucoxanthin is indicative of diatoms in the system, which have more efficient nitrate uptake rates compared to other phytoplankton in the system (Paerl et al. 2003, Valdes-Weaver et al. 2006). In estuaries, diatoms are present in mid-spring during periods of elevated river flow and salinity (Mallin et al. 1991, Peierls et al. 2003, Domingues et al. 2005, Paerl 2006). Alloxanthin and chlorophyll *b* indicate cryptophytes and green algae respectively and were found at the two brackish water sites. Alloxanthin and chlorophyll *b* were present at freshwater sites and when salinity levels were lower (4-7) compared to the more brackish water sites (Ahel et al. 1996). In the Tagus and Neuse estuaries, green algae and cryptophytes were found in lower saline areas (0-10) later in the spring and summer compared to higher salinity sites (Gameiro et al. 2004, Valdes-Weaver et al. 2006). Overall, phytoplankton composition was a mix of the five pigments, which is similar to Neuse River phytoplankton composition during spring and early summer throughout fresh and brackish waters (Valdes-Weaver et al. 2006). These changes seen in nutrients and phytoplankton composition can change the fatty acid composition.

The seston fatty acid composition consisted of mainly SFAs. Seston from estuarine systems typically has a larger percentage of SFA and this fraction has been attributed to detrital input, as opposed to originating from phytoplankton (Persson and Vrede 2006, Gladyshev et al.

2010, Ravett, Brett, Arhonditsis 2010, Burns, Brett, and Schallenberg 2011, Gonclaves et al 2012). The phytoplankton pigments did not correlate with indicator fatty acids in both fresh and brackish water because low chlorophyll *a* values were low here and the seston likely was comprised of heterotrophic organisms and detritus. Phytoplankton has been found to only explain 26% of variance in fatty acid composition in seston of lakes, and the rest is detritus and heterotrophic organisms (Müller-Navarra et al. 2004). In estuaries, the phytoplankton composition should explain more variability in the fatty acid composition compared to large lakes, resulting in a higher correlation with phytoplankton seston fatty acids (Farkas and Herodek 1964, Desvilettes et al. 1997, Wacker and von Elert 2001, Gladyshev et al. 2010, Ravet et al. 2010, Goncalves et al. 2012). One observed difference was the increase in omega-3 to omega-6 ratio in the brackish water sites, which likely resulted from a change in the relative contribution of source phytoplankton and detrital material. Omega-3s mostly originate in aquatic systems, and would be seen in greater amounts with the presence of increased chlorophyll *a* (Dalsgaad et al. 2003, Arts et al. 2009, Twining et al. 2016). One concern with fatty acids is the same fatty acid can be used for multiple biomarkers (Dalsgaad et al. 2003, Arts et al. 2009). Seston from freshwater sites had increased PA (16:1n-7), OA (18:1n-9), LIN (18:2n-6), and ALA (18:3n-3), but these fatty acids can also be related to bacteria, and increased organic material made up of terrestrial debris. For example, fatty acid PA (16:1n-7) has been related to bacteria composition while omega-6 fatty acids, especially 18:2n-6, have been related to terrestrial plant material (Dalsgaad et al. 2003, Arts et al. 2009, Kelly & Scheibling 2012, Twining et al. 2016). The increase in diatoms resulted in fatty acid profiles that had increased PA (16:1n-7) and EPA, which are considered diatom fatty acid markers (Dalsgaad et al. 2003, Arts et al. 2009, Strandberg et al. 2015). When dinoflagellates had a higher concentration at Blounts Bay, there

was an increase in DHA in the seston fatty acid profile. Dinoflagellates have increased DHA as a fatty acid marker (Dalsgaad et al. 2003, Arts et al. 2009, Strandberg et al. 2015). Overall, the increase in phytoplankton pigment resulted in a correlation to seston fatty acids in the brackish water sites, but caution needs to be taken when phytoplankton biomass is low because there could be correlations to detrital material and heterotrophic organisms.

Zooplankton composition was correlated to changes in salinity levels throughout the sampling sites. Microzooplankton were dominated by copepod nauplii both years and at all sites; however, there was an increase in rotifers in the freshwater sites during 2016. Copepod nauplii are the dominant smaller bodied zooplankton in estuaries, but rotifers can be a dominant species in the freshwater rivers (Park and Marshall 2000a & 2000b). In this study, *Acartia* spp. was the dominant mesozooplankton species at the brackish water sites during both years. *Acartia* spp. is the dominant copepod species in temperate, estuarine systems (Ambler et al. 1985; Orsi 1986; Cervetto et al. 1999; Mouny and Dauvin 2002; Kimmel and Roman 2004; Lawrence et al. 2004; Islam et al. 2005). The freshwater assemblage was a mix of cladocerans (*Bosmina* spp. Chydoridae, and Daphniidae), and copepods (Calanoida and Cyclopoida) in the present study. This species composition is found throughout riverine and temperate estuaries (Leech et al. 2009, Lichti et al. 2017).

Zooplankton fatty acid composition was related to the zooplankton composition. Different copepod life stages had a presence at all sites in both years, which resulted in the higher mean percentage of DHA observed. Copepods from the brackish water sites had higher percent composition of DHA compared to freshwater sites. This is clearly a reflection of the dominance of *Acartia* spp. and copepod nauplii in the system and a diet primarily consisting of marine algae higher in omega-3 FAs (Stottrup et al. 1999; Persson and Verde 2006; Arts et al.

2009; Kainz et al. 2009; Gladyshev et al 2010; Masclaux et al. 2012). The one exception was copepod nauplii found in the brackish water sites that had increased LIN (18:2n-6), and OA (18:1n-9). The presence of these two fatty acids could relate to a food sources with a possible terrestrial signal (Dalsgaad et al. 2003, Twining et al. 2016). Rotifers had similar fatty acid composition in brackish and freshwater with an increase in EPA. Rotifer fatty acid profiles represent more of their diet as compared to copepod nauplii (Kennari et al. 2008, Wacker and Weithoff 2009). Cladocerans had increased EPA, ALA, and LIN (18:2n-6) throughout the freshwater sites and years. Cladocerans including *Bosmina* spp. and Chydoridae have increased EPA, and little to no DHA, even when prey were high in DHA levels (Persson and Verde 2006). The increase in EPA for cladocerans is related to life history strategies of increased reproduction (Persson and Verde 2006). The presence of ALA and LIN (18:2n-6) are related to the food source for cladoceran, which could be phytoplankton, detritus or heterotrophic microplankton (Goulden and Place 1990, Desvilettes et al. 1997, Brett et al 2006, Rossi et al. 2006, Smyntek et al. 2008, Taipale et al. 2009, Gladyshev et al. 2010, Ravet, Brett, and Arhoditsis 2010). Zooplankton fatty acid profiles have been correlated to seston fatty acid profiles in some studies (Goulden and Place 1990, Desvilettes et al. 1997, Brett et al 2006, Rossi et al. 2006, Smyntek et al. 2008, Taipale et al. 2009, Gladyshev et al. 2010, Ravet, Brett, and Arhoditsis 2010). The differences in the zooplankton fatty acid composition seen at the same site, or when transitioning from fresh to brackish water habitat result in changes for larval fish prey base.

The nursery habitats in Tar/Pamlico River have the critical fatty acids EPA and DHA present and therefore may support larval river herring growth and survival is not likely limited by lack of these fatty acids. However, further investigations are still required to prove this conclusively. This study demonstrated that investigating the lower trophic food web using fatty

acid analysis can be used to assess the quality of nursery habitat and provided baseline data on the fatty acid composition of the planktonic food web that river herring prey upon. Salinity was associated with changes in the nutrient dynamics that resulted in changes to phytoplankton biomass and pigment composition. This suggests that future changes in climate forcing and nutrient conditions would result in shifts in the fatty acid composition of the plankton food web. Fatty acid composition can be used to assess the quality of the prey base available to larval fish and to assess the differences in nursery habitat. The zooplankton in both fresh and brackish water contained DHA and EPA, as well as other essential fatty acids. Larval fish affected by changes in food sources could result in strong or weak year class strength at the beginning of life by altering larval fish growth and development (Takeuchi et al. 1997, Perga et al. 2009, Paulsen et al. 2014, Taipale et al. 2018). This information can be used to define the baseline for lower trophic food web (phytoplankton and zooplankton) in the Tar/Pamlico River, and start informing what fatty acid are available for zooplankton and larval fish through their diet. These data can help determine if the nursery habitat quality is undergoing change by comparing it to the baseline fatty acid information present in this dissertation.

SHAs in North Carolina were designated based on past information to determine the areas that should be managed. These designations used mostly land cover, improved water quality, and fish data (Deaton et al. 2006). The SHAs in the Tar/Pamlico River were chosen without considering river herring spawning or nursery habitat (NCDMF 2015). The addition of river herring spawning and nursery habitat to the SHA classification would allow for a better understanding of a population that may be returning after a collapse and manage for nursery habitat that have been affected by nutrient additions. The addition of fatty acid information from the lower trophic levels into the nursery habitat provides another layer to the definition of the

SHAs by allowing pelagic habitat to be assessed. These data could help to designate important nursery habitat under the SHAs for river herring. The next logical step is determining how food quality, (e.g. the ratio of EPA and DHA required for optimum growth) affect larval river herring growth. Despite these gaps, it is clear that fish nurseries in the Tar/Pamlico River can be impacted by abiotic factors that change the fatty acid composition of the prey base available for larval fish. Incorporating the lower trophic level of fatty acid analysis in determining fish nursery and SHAs may serve as an important monitoring tool to determine if the larval habitat is contributing to a lack of river herring recovery.

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Figures:

Fig. 28: The overview of North Carolina (a). The close-up view of the location for the Tar/Pamlico River(b). The five sites for sampling on the Tar/Pamlico River (c).

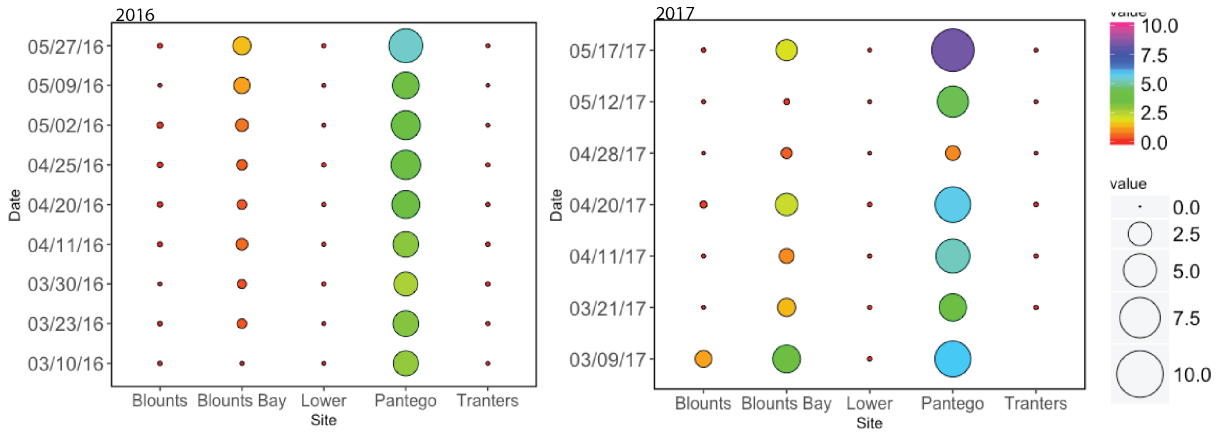


Fig. 29: Salinity over the sampling dates comparing the 5 sites for 2016 and 2017.

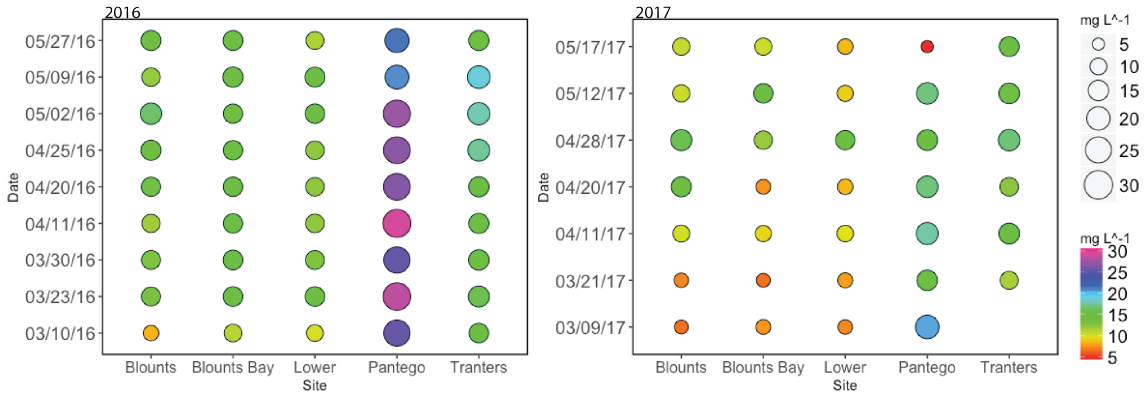


Fig. 30: Dissolved organic carbon (mg L^{-1}) over the sampling dates comparing the five sites for 2016 and 2017.

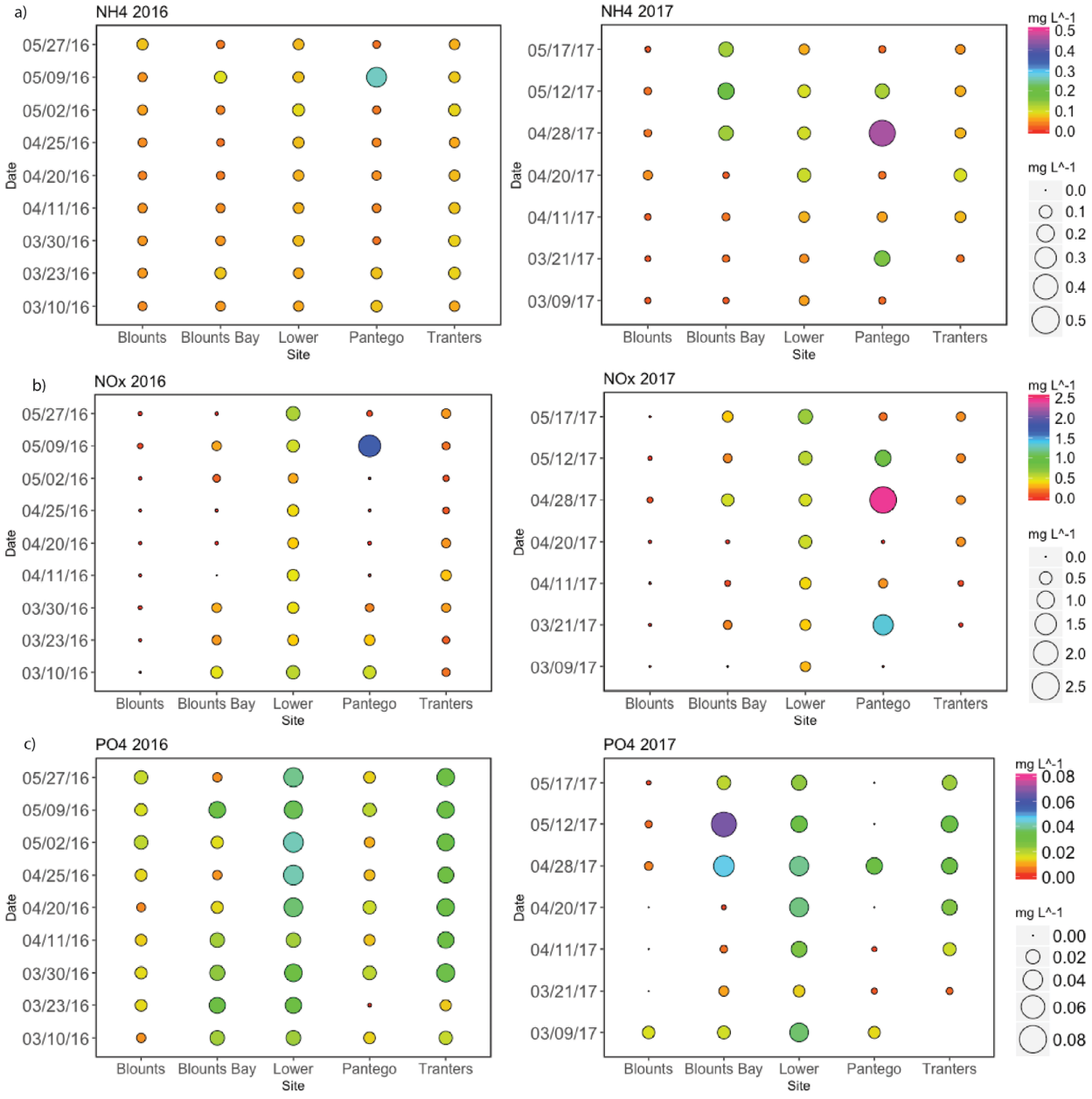


Fig. 31: Nutrient data from a) ammonium (NH_4, mg L^{-1}), b) nitrate and nitrite (NOx, mg L^{-1}), and c) orthophosphate (PO_4, mg L^{-1}) for the five sites over the sampling dates for 2016 and 2017.

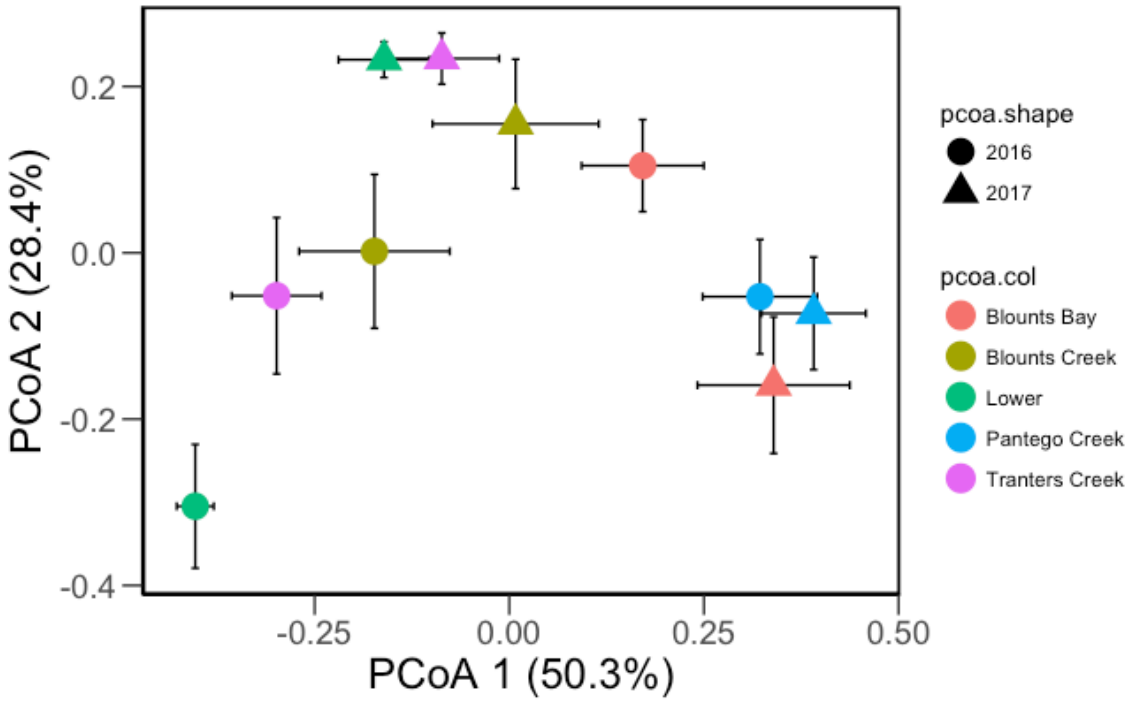


Fig. 32: Ordination from Principal Coordinates Analysis depicting the accessory phytoplankton pigment composition. Symbols are colored according to sampling sites on the Chowan River.

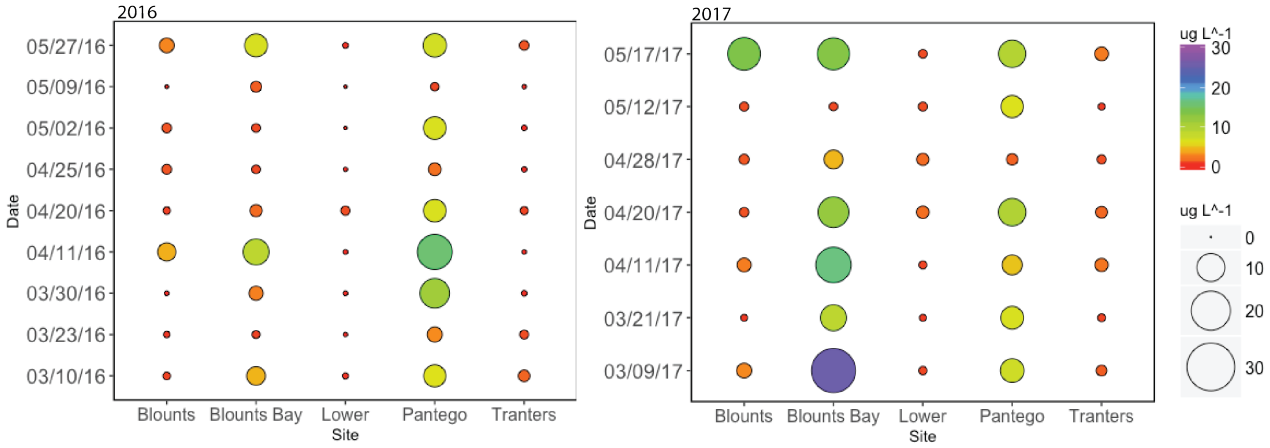


Fig. 33: Chlorophyll *a* concentrations (µg L^{-1}) for each sampling location over 2016 and 2017.

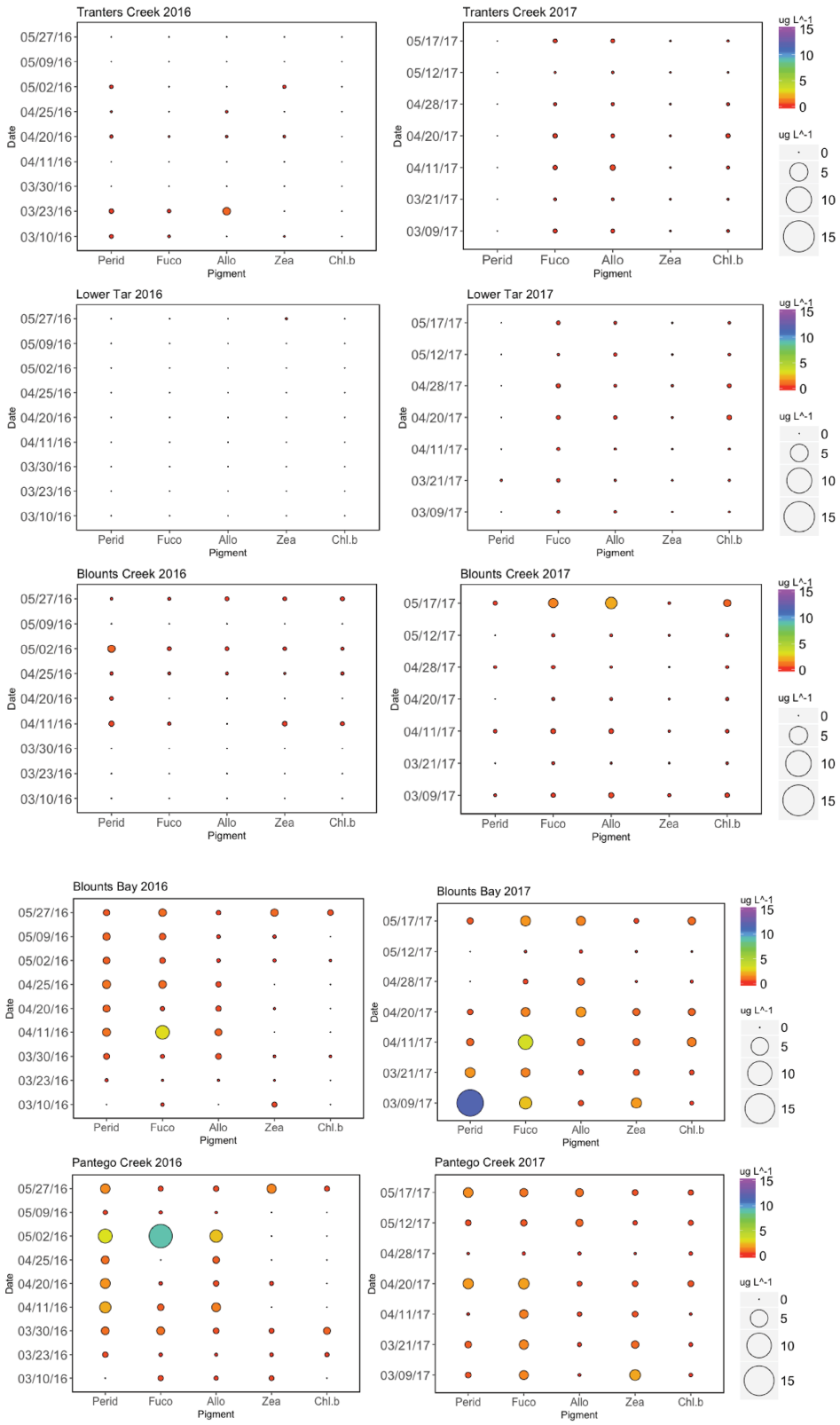
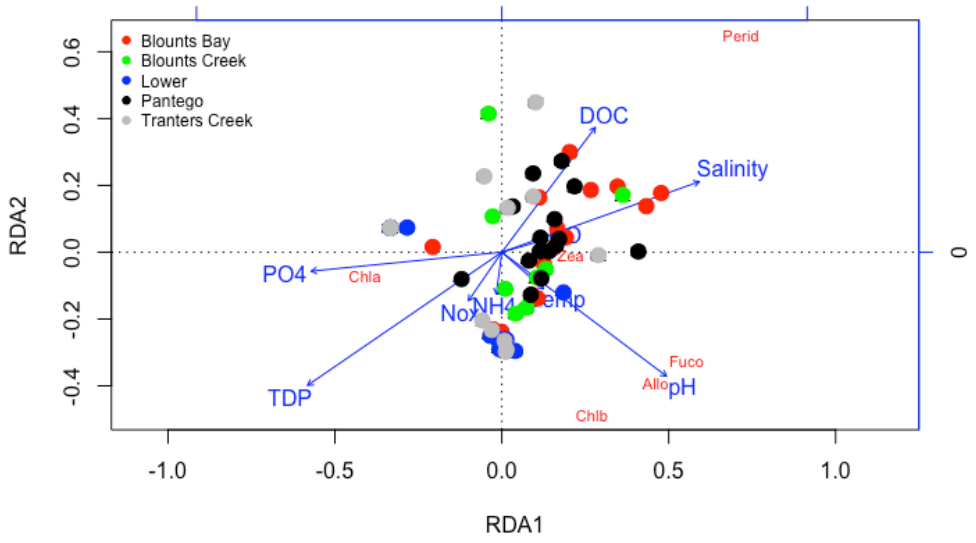


Fig. 34: Phytoplankton pigment concentration (ug L^{-1}) in 2016 and 2017 for Tranters Creek, lower Tar River, Blounts Creek, Blounts Bay, and Pantego Creek.

a)



b)

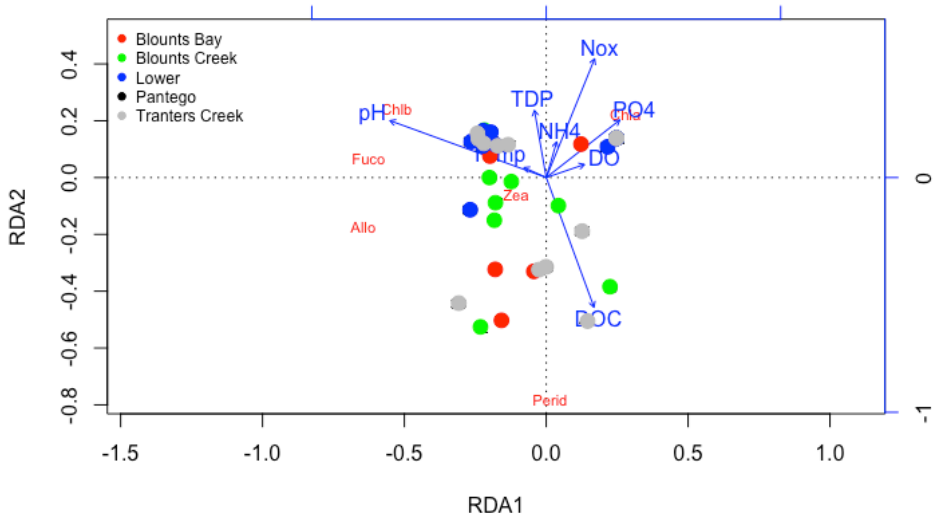


Fig. 35: Correlation plots of the redundancy analysis (RDA) for the phytoplankton pigment and environmental variables for combined a) fresh and brackish water sites, and b) freshwater sites. Brackish water sites RDA was not included because of no patterns found. Pigment Abbreviations: Perd= Peridinin, Fuco=Fucoxanthin, Allo= Alloxanthin, Chl a = Chlorophyll *a*; Chl.b = Chlorophyll *b*, Zea=Zeaxanthin. Abiotic Abbreviations: Temp = Temperature, DO= Dissolved Oxygen, DOC= Dissolved organic carbon, TDP= Total dissolved phosphorus, NH4= Ammonium, Nox= Nitrate+Nitrite, PO4= Orthophosphate

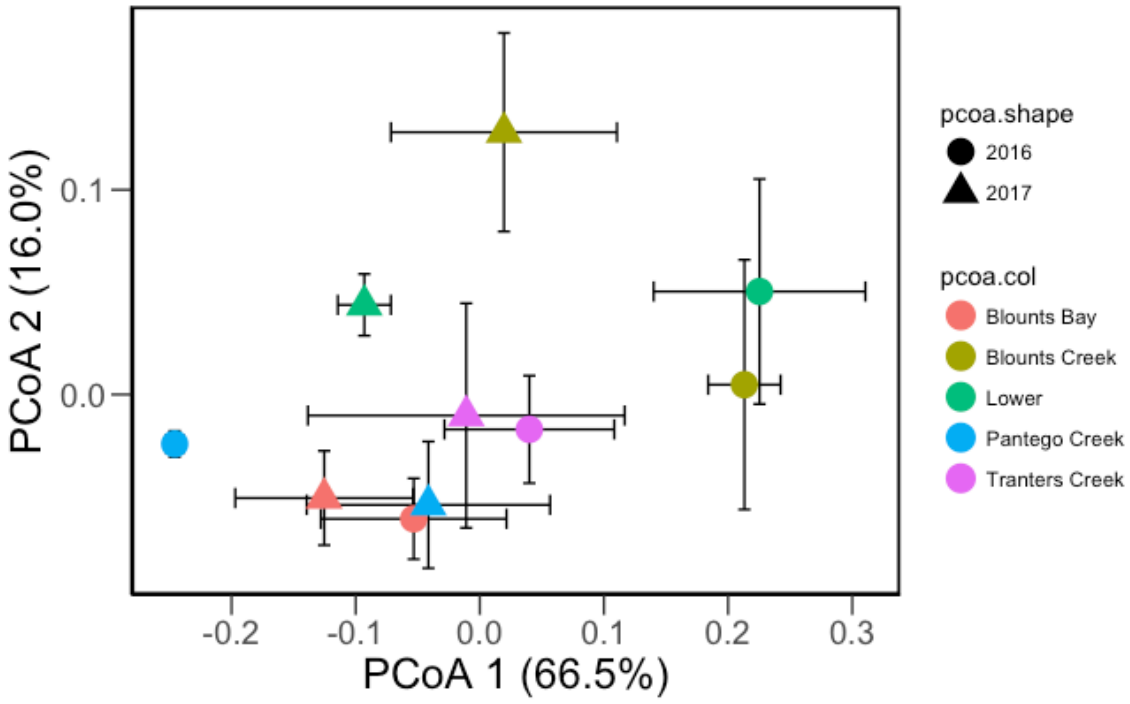


Fig. 36: Ordination from Principal Coordinates Analysis depicting the microzooplankton community composition for 2016 and 2017. Symbols are different to represent the year. Symbols are colored according to sampling sites on the Chowan River.

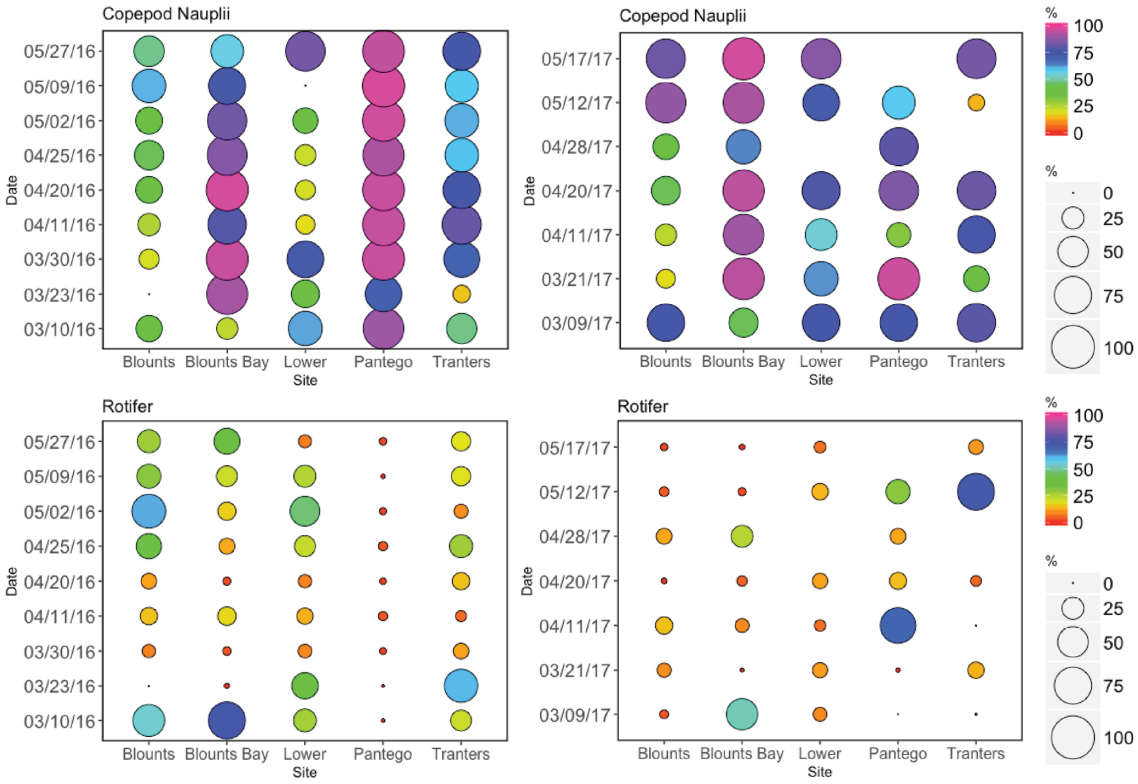


Fig. 37: Microzooplankton percent composition for rotifers and copepod nauplii by site for sampling period during 2016 and 2017.

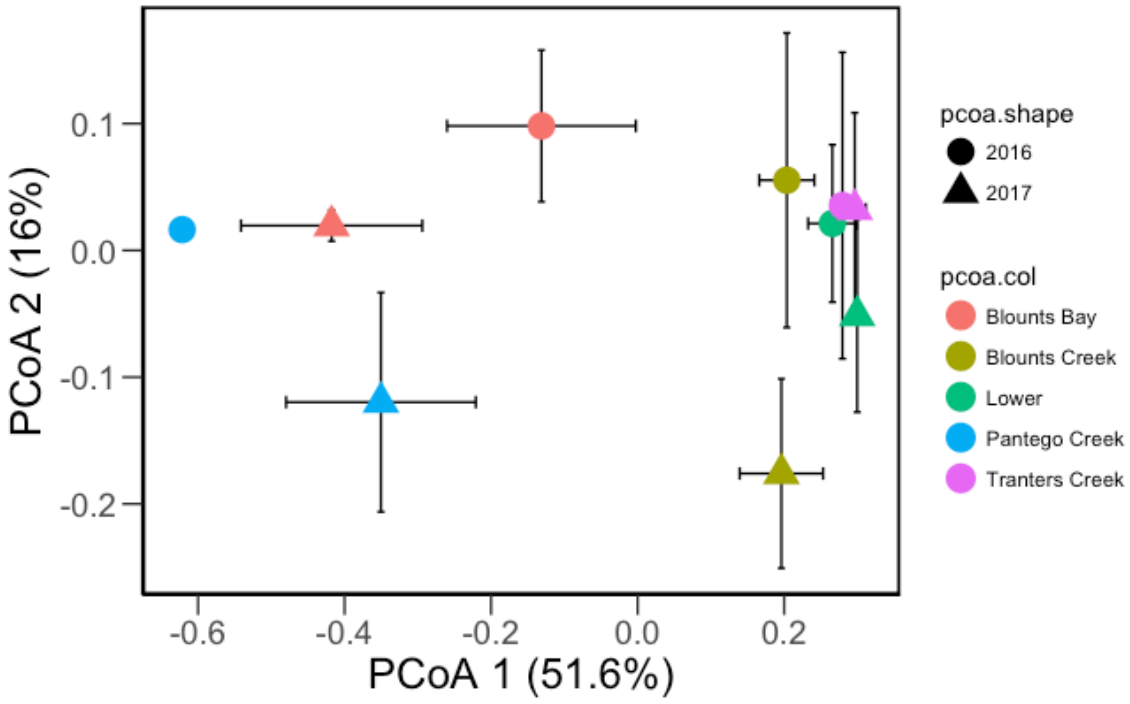


Fig. 38: Ordination from Principal Coordinates Analysis depicting the mesozooplankton community composition for 2016 and 2017. Symbols are different to represent the year. Symbols are colored according to sampling sites on the Chowan River.

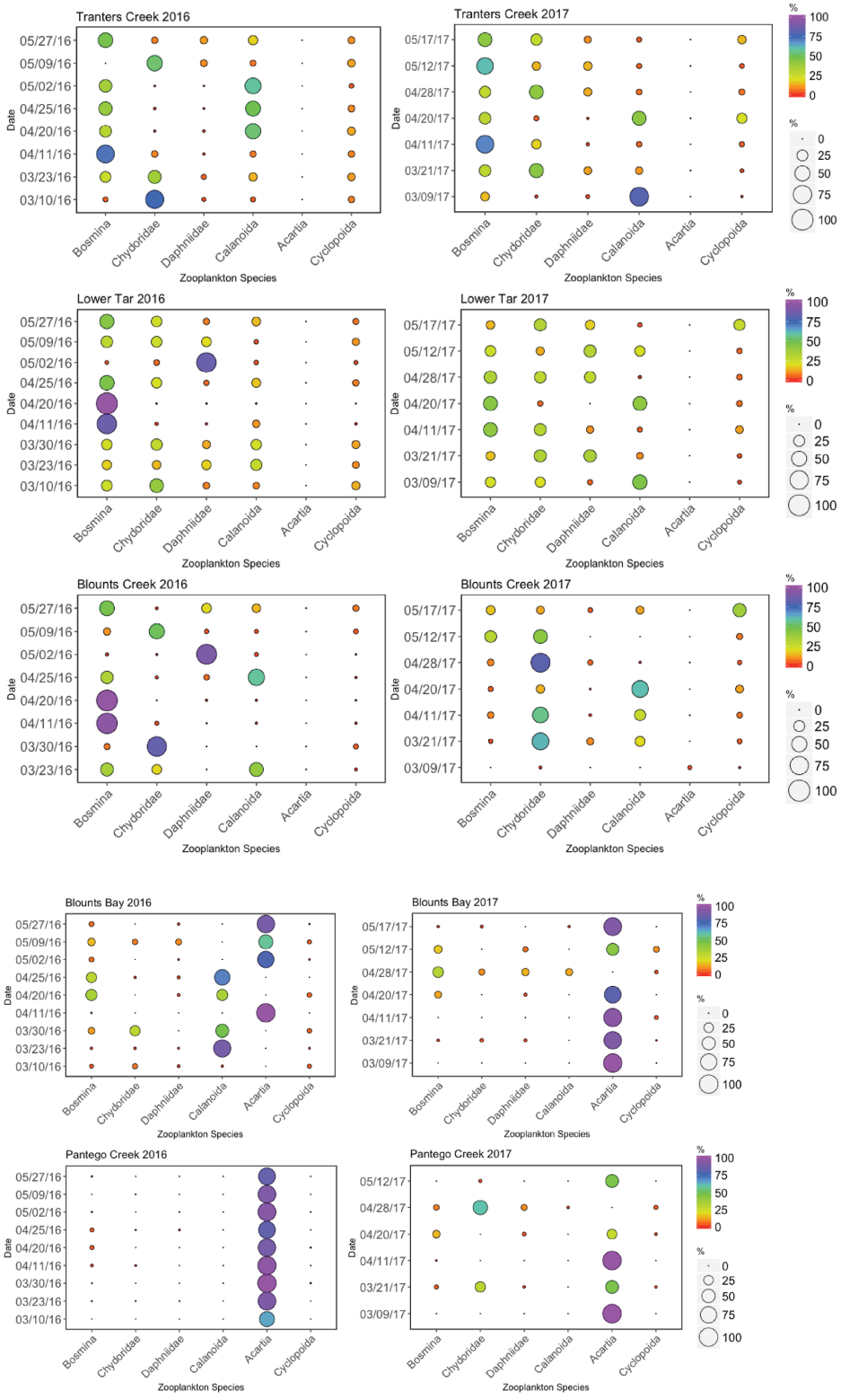


Fig. 39: Mesozooplankton percent composition for the six dominant species by site for sampling period during 2016 and 2017.

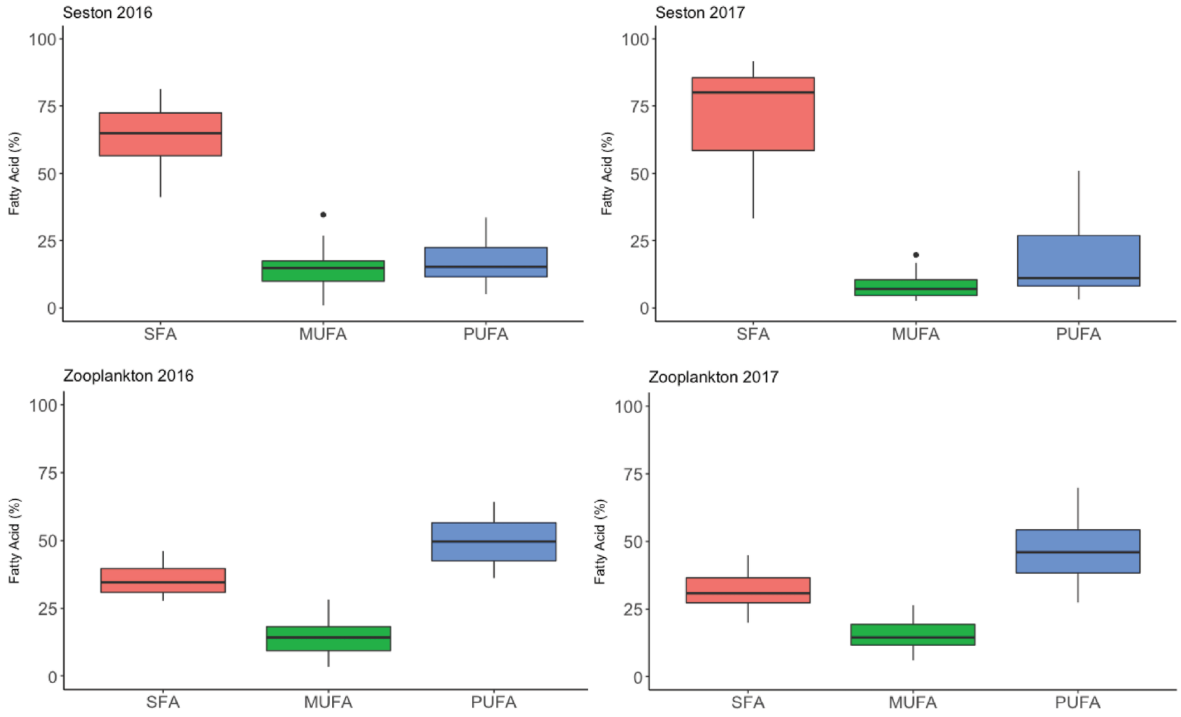


Fig. 40: Box plot for saturated fatty acid (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) for seston and zooplankton for 2016 and 2017.

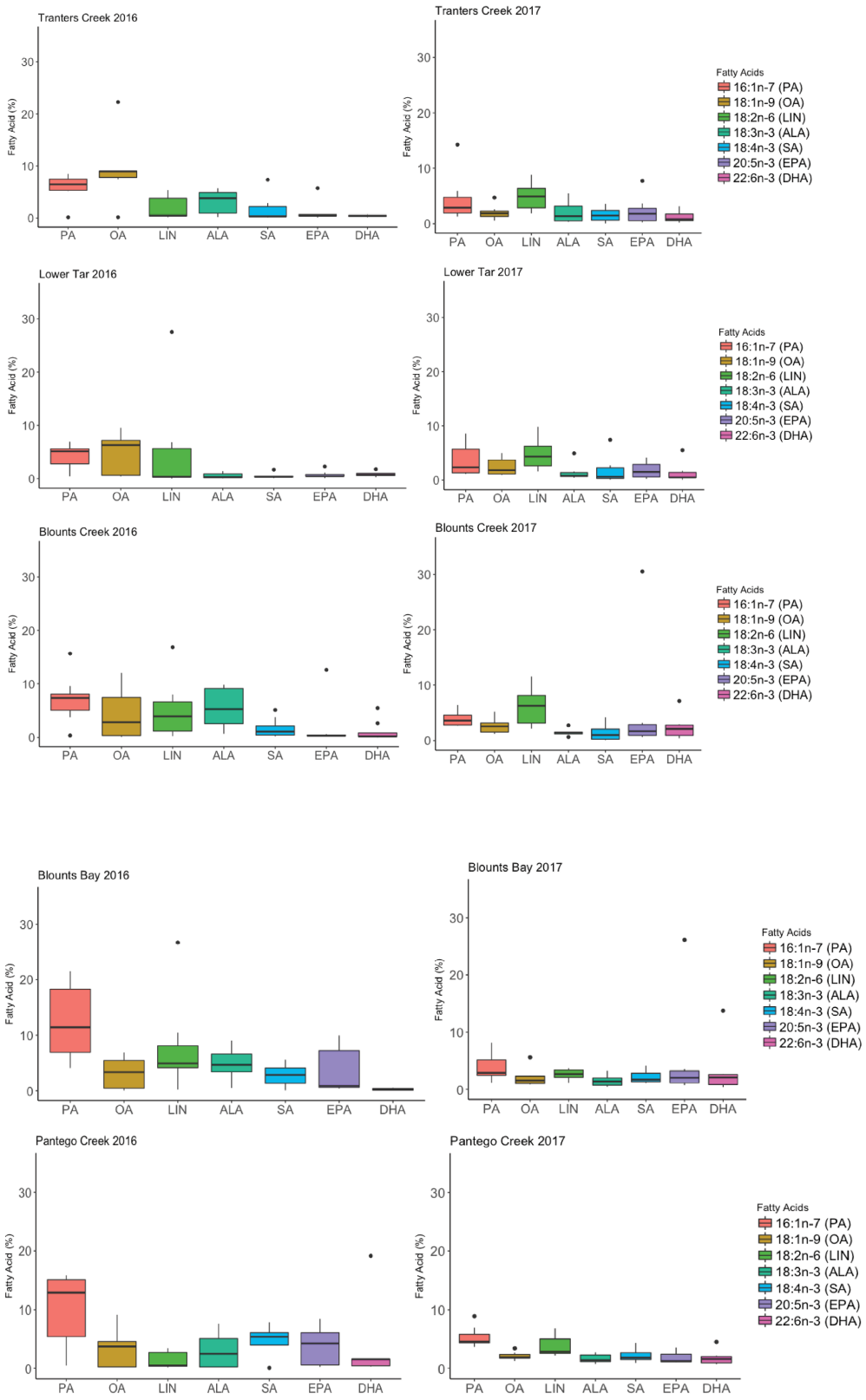


Fig. 41: Boxplot of seston fatty acid percent composition for five sites for 2016 and 2017.

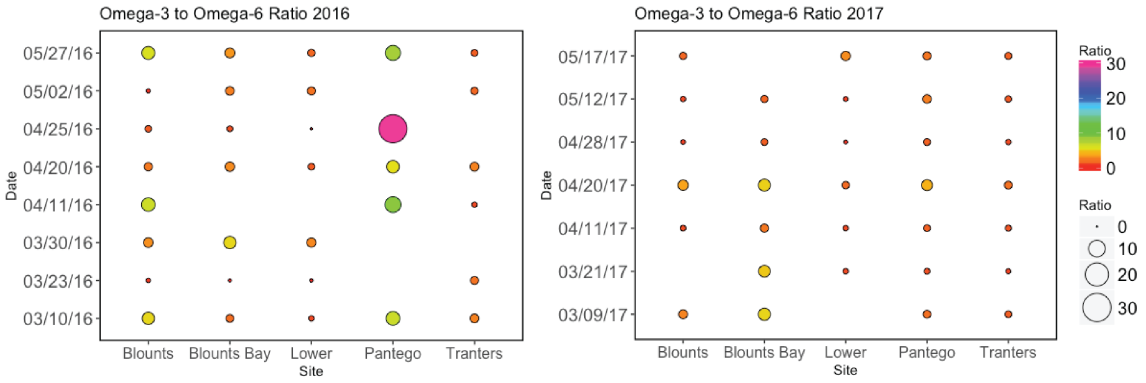
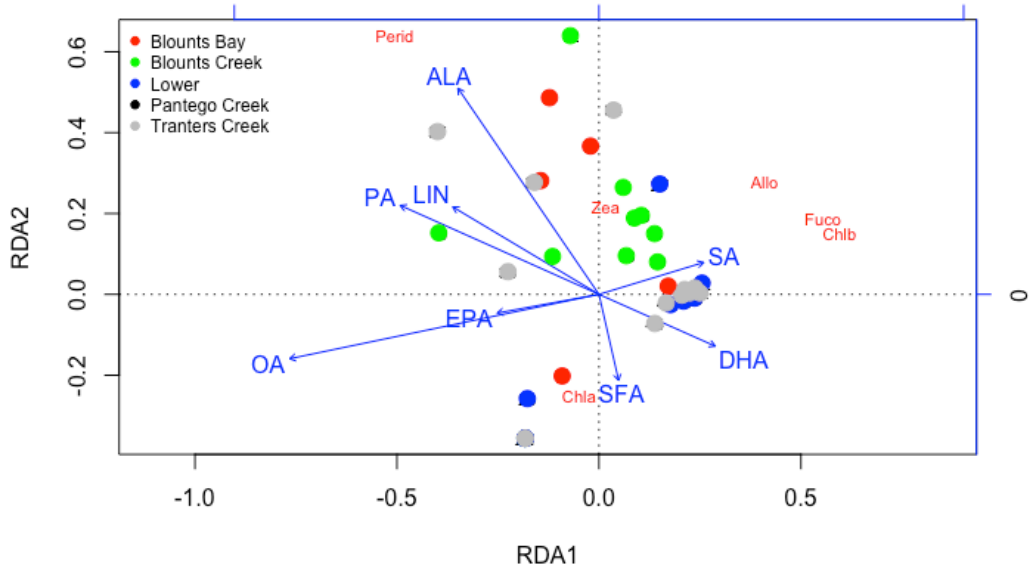


Fig. 42: Seston ratio of omega-3 to omega-6 for by site during the sampling period for 2016 and 2017.

a)



b)

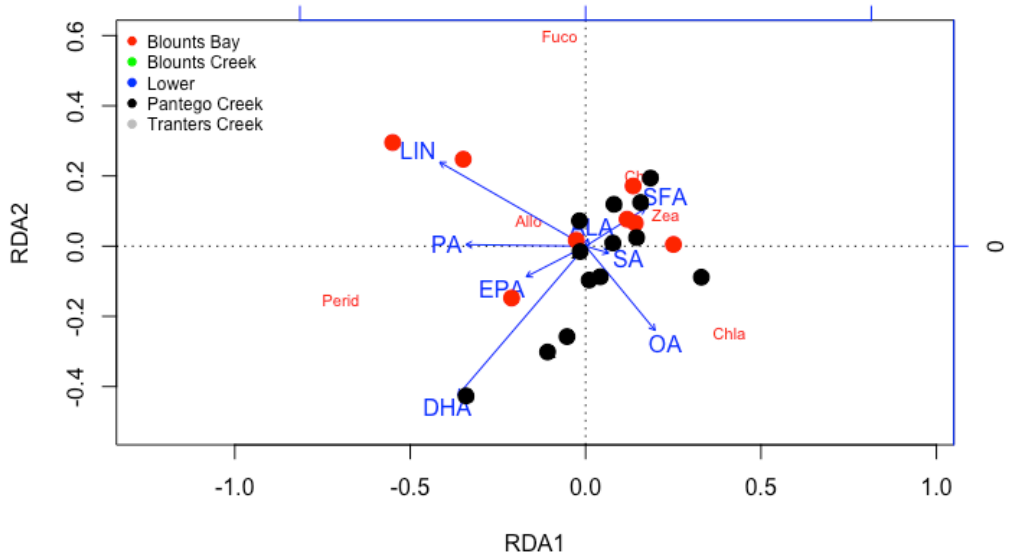


Fig. 43: Seston composition correlation plots of the redundancy analysis (RDA) to the fatty acid composition for seston at a) freshwater sites (Tranters Creek, lower Tar River, and Blounts Creek, some dates from Blounts Bay) and b) brackish water sites (Pantego Creek and Blounts Bay). Pigment Abbreviations: Perd= Peridinin, Fuco=Fucoxanthin, Allo= Alloxanthin, Chl a = Chlorophyll *a*; Chl.b = Chlorophyll *b*, Zea=Zeaxanthin. Fatty Acids: SFA=Saturated Fatty Acids, PA=16:1n-7, OA=18:1n-9, LIN=18:2n-6; ALA=18:3n-3, SA=18:4n-3, EPA=20:5n-3, DHA=22:6n-3

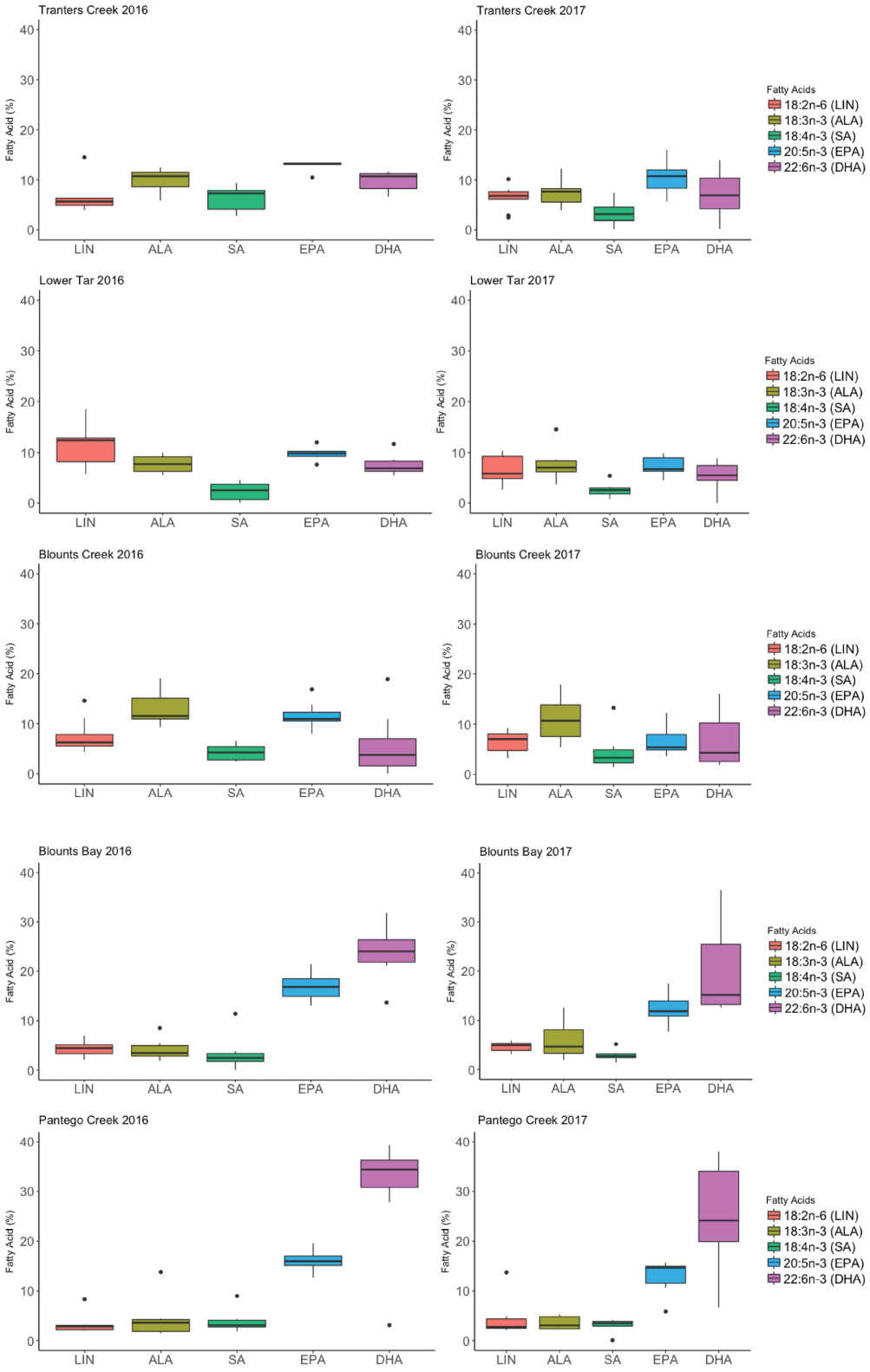
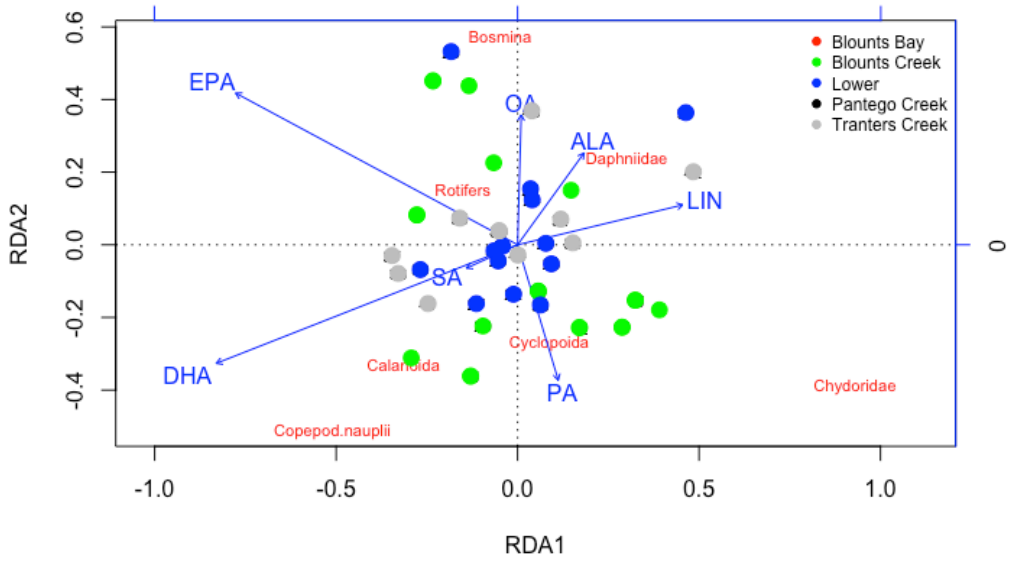


Fig. 44: Boxplot of zooplankton fatty acid percent composition for five sites in 2016 and 2017.

a)



b)

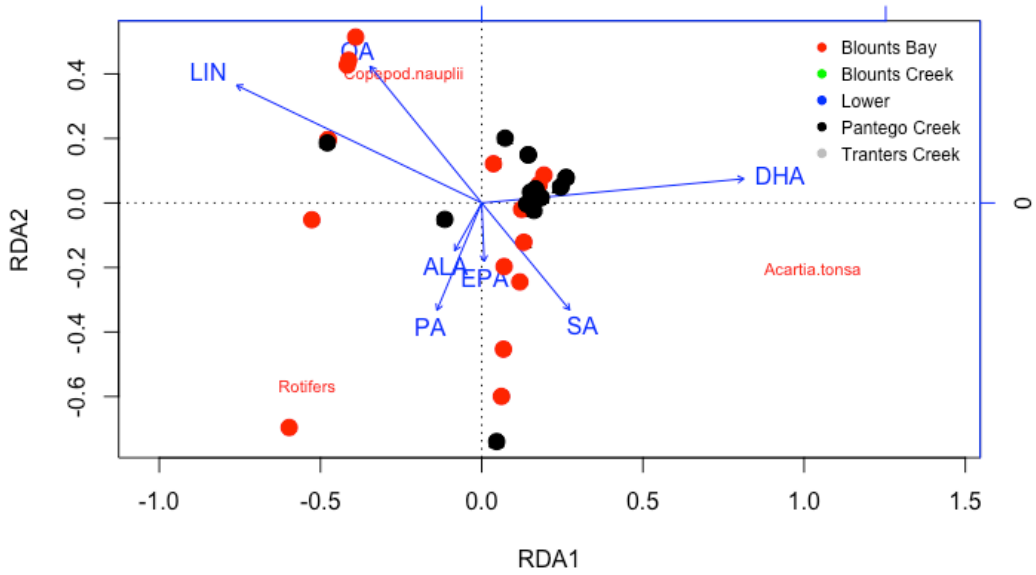


Fig. 45: Zooplankton community composition correlation plots of the redundancy analysis (RDA) to the fatty acid composition with zooplankton for a) freshwater sites (Tranters Creek, lower Tar River, and Blounts Creek) and b) brackish water sites (Pantego Creek and Blounts Bay). Fatty Acids: SFA=Saturated Fatty Acids, PA=16:1n-7, OA=18:1n-9, LIN=18:2n-6; ALA=18:3n-3, SA=18:4n-3, EPA=20:5n-3, DHA=22:6n-3

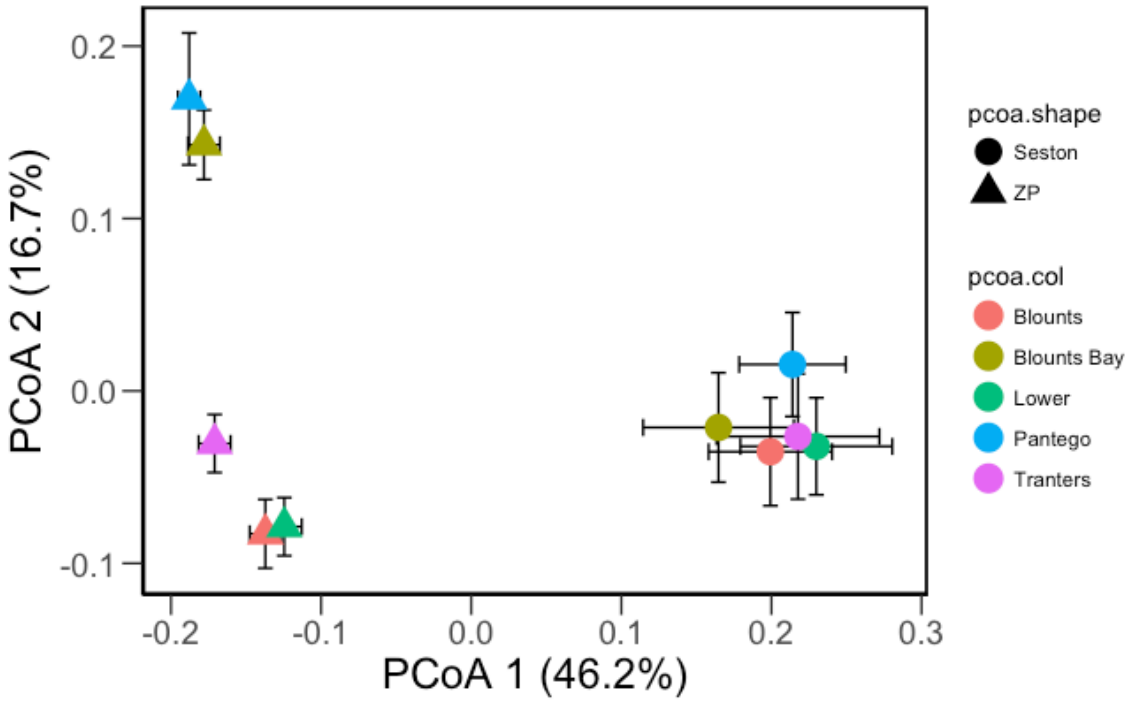
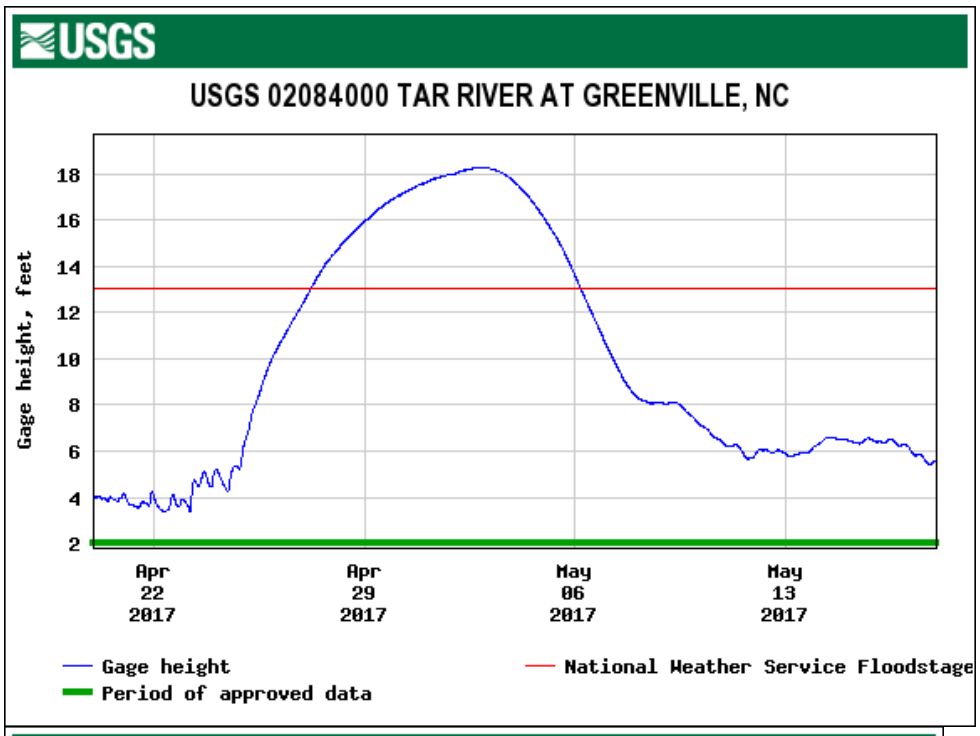


Fig. 46: Ordination from Principal Coordinates Analysis depicting the fatty acid composition for seston and zooplankton in 2016 and 2017. Symbols are different to represent the type of sample. Symbols are colored according to sampling sites on the Chowan River.



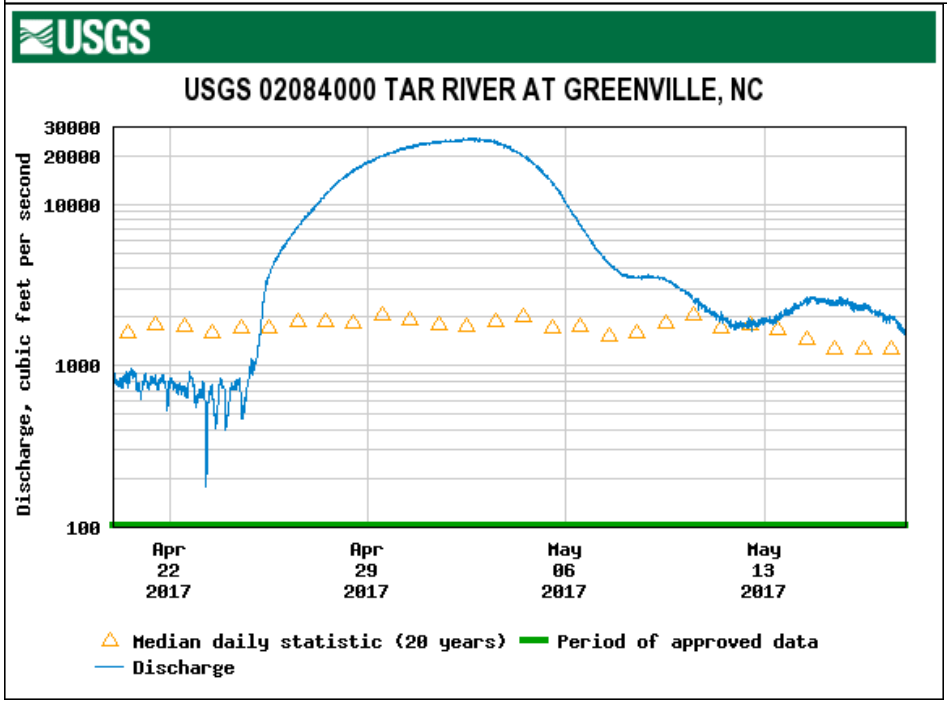


Fig. 47: Discharge and gage height from the USGS for the Tar River at Greenville, North Carolina from April 20 to May 17, 2017. This sit is above all sampling sites, and shows the flooding that occurred. (https://nwis.waterdata.usgs.gov/usa/nwis/uv/?cb_00045=on&cb_00060=on&cb_00065=on&cb_72255=on&format=gif_default&site_no=02084000&period=&begin_date=2017-04-20&end_date=2017-05-17)

Tables:

Table 10: Phytoplankton functional groups with indicator pigments and fatty acids.

Phytoplankton Functional Group	Phytoplankton Pigment	Fatty Acids
Dinoflagellates	Peridinin	DHA
Diatoms	Fucoxanthin	16:1n-7, EPA
Crytomonad	Alloxanthin	ALA, 18:4n-3, EPA
Green Algae	Chlorophyll b	ALA, EPA, 18:2n-6
Cyanobacteria	Zeaxanthin	ALA, 18:4n-3

Table 8: Mean abiotic factor (±S.D.) by site and year in the Tar/Pamlico River system. Range is below the mean.

	n	Temperature (°C)	Salinity	Dissolved Oxygen (%)	Dissolved Oxygen (mg/L)	Specific Conductivity (µS/cm)	Conductivity (µS/cm)	pH
				2016				
Tranters Creek	9	18.3±3.0	0.0±0.0	64.1±15.8	6.1±1.8	209.2±319.8	181.1±275.1	6.8±0.4
		12.6-22.0	0.0-0.1	39.5-83.5	3.5-8.9	76.7-1061.0	69.4-914.0	6.4-7.6
Lower Tar River	9	17.8±2.9	0.0±0.0	75.4±13.7	7.2±1.5	199.7±299.3	171.6±255.5	7.0±0.2
		13.0-21.9	0.0-0.1	45.0-90.6	4.3-9.5	82.2-997.0	73.1-852.0	6.8-7.3
Blounts Creek	9	17.6±3.7	0.1±0.0	53.0±13.7	5.2±1.7	249.2±234.7	212.5±193.6	6.5±0.2
		11.1-22.4	0.0-0.1	35.5-77.1	3.2-8.5	80.2-855.0	74.5-706.0	6.2-6.8
Blounts Bay	9	18.1±4.0	0.6±0.5	96.6±12.8	9.1±1.0	1370.4±871.3	1238.9±872.2	7.6±0.5
		13.5-24.7	0.1-1.5	82.7-126.9	7.5-10.6	107.6-2864.0	84.1-2857.0	7.1-8.5
Pantego Creek	8	18.8±45.0	3.6±0.8	96.0±15.3	8.8±1.5	6589.5±1426.4	5885.1±1777.7	7.5±0.4
		13.8-25.2	2.9-5.4	66.4-116.9	5.8-10.4	5082-9579	4244-9618	6.8-7.9
				2017				
Tranters Creek	5	18.7±5.0	0.0±0.0	61.7±15.4	5.9±2.2	89.1±21.0	78.1±20.0	7.3±0.5
		10.2-22.3	0.0-0.1	51.3-88.0	4.6-9.9	66.6-118.9	61.4-112.9	6.9-8.1
Lower Tar River	6	18.2±4.7	0.0±0.0	76.3±14.5	7.3±2.0	101.9±20.4	88.3±19.4	7.9±0.6
		10.4-22.5	0.0-0.1	52.1-92.1	4.7-10.1	69.3-126.3	63.2-120.3	7.3-8.5
Blounts Creek	6	18.3±4.5	0.3±0.5	50.9±20.3	4.9±2.3	518.6±904.3	422.7±707.5	7.8±0.6
		11.5-22.8	0.0-1.2	22.4-83.1	2.1-9.1	66.3-2351.0	62.0-1852.0	7.0-8.5
Blounts Bay	6	18.4±5.2	1.6±1.2	91.4±12.4	8.6±1.8	3051.1±2223.7	2600.2±1850.8	7.7±0.5
		10.2-22.3	0.1-3.5	71.9-101.3	6.3-11.0	261.4-6308.0	239.0-4975.0	7.0-8.2
Pantego Creek	6	18.8±5.2	4.8±2.5	88.7±9.7	8.1±1.4	8462.7±4245.8	7527.5±4228.8	7.3±0.4
		10.5-23.9	0.9-8.3	70.9-99.0	6.1-10.0	1845-14359	1749-14064	6.6-7.6

Table 9: Mean nutrient factors (±S.D.) by site and year in the Tar/Pamlico River system. Range is below the mean. NH4 = ammonium, NOx = nitrate and nitrite, PO4 = orthophosphate, TDP= Total dissolved phosphorus, and DOC= dissolved organic carbon

		2016				
	n	NH_4 (mg/L)	NOx (mg/L)	PO_4 (mg/L)	TDP (mg/L)	DOC (mg/L)
Tranters Creek	9	0.08±0.01	0.21±0.08	0.03±0.01	1.97±0.49	15.84±1.79
		0.06-0.09	0.11-0.34	0.01-0.03	1.14-2.54	13.69-18.85
Lower Tar River	9	0.07±0.01	0.44±0.10	0.03±0.01	1.89±0.69	12.59±1.30
		0.06-0.09	0.30-0.61	0.02-0.04	0.84-2.65	10.08-14.29
Blounts Creek	9	0.05±0.01	0.03±0.02	0.01±0.00	1.01±0.42	13.03±2.24
		0.04-0.08	0.01-0.09	0.01-0.02	0.60-1.73	8.43-16.51
Blounts Bay	9	0.05±0.02	0.17±0.16	0.02±0.01	1.12±0.58	13.55±1.00
		0.03-0.09	0.00-0.45	0.01-0.03	0.41-1.91	11.19-14.80
Pantego Creek	9	0.07±0.07	0.32±0.54	0.01±0.01	0.87±1.12	25.59±2.87
		0.03-0.26	0.01-1.67	0.00-0.02	0.21-3.83	20.78-29.05
		2017				
Tranters Creek	6	0.07±0.02	0.18±0.10	0.02±0.01	1.79±0.56	14.29±1.96
		0.03-0.10	0.04-0.25	0.00-0.03	1.08-2.48	11.56-16.72
Lower Tar River	7	0.08±0.02	0.48±0.12	0.03±0.01	2.41±0.53	9.21±1.96
		0.05-0.11	0.32-0.65	0.01-0.04	1.67-3.14	7.34-13.32
Blounts Creek	7	0.03±0.01	0.03±0.04	0.00±0.01	0.81±0.23	10.83±3.48
		0.02-0.05	0.00-0.10	0.00-0.02	0.57-1.08	6.62-16.07
Blounts Bay	7	0.08±0.07	0.21±0.18	0.02±0.02	1.88±1.40	9.62±2.52
		0.02-0.17	0.00-0.51	0.02-0.07	0.66-4.56	6.63-13.44
Pantego Creek	7	0.13±0.15	0.72±0.89	0.01±0.01	0.84±0.45	15.32±4.82
		0.03-0.46	0.00-2.41	0.00-0.03	0.55-1.84	5.10-20.36

Table 10: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of seston by site and year from the Tar/Pamlico River system by month. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	2016					2017				
	Tranters n=4	**Lower Tar n=6**	**Blounts Creek n=6**	**Blounts Bay n=7**	**Pantego n=4**	**Tranters n=7**	**Lower Tar n=6**	**Blounts Creek n=6**	**Blounts Bay n=6**	**Pantego n=7**
14:0	5.4±0.7	5.6±2.7	6.9±1.4	9.4±3.3	11.8±2.7	2.6±1.8	14.9±28.7	2.9±1.3	2.9±2.0	3.2±1.0
15:0	3.3±3.7	0.7±0.6	3.9±6.5	0.9±0.5	1.1±0.4	1.5±0.4	1.6±1.0	1.7±1.1	1.6±1.8	1.5±0.7
16:0	36.4±5.5	46.8±9.9	42.2±9.2	34.3±5.6	39.5±5.7	60.5±25.5	47.2±33.6	51.6±24.4	60.3±28.5	57.5±18.3
17:0	2.0±1.2	4.4±1.7	3.6±1.9	1.6±0.9	1.7±0.2	0.3±0.4	0.9±0.9	1.1±1.1	0.2±0.4	0.6±0.6
18:0	14.6±5.0	13.5±6.7	8.9±7.1	11.4±4.3	9.0±2.3	6.0±4.0	7.1±3.7	7.7±4.8	4.4±2.7	6.9±3.5
20:0	0.2±0.3	0.5±0.4	0.3±0.2	0.9±2.0	0.2±0.1	2.9±3.4	3.0±4.2	3.2±4.2	3.7±5.5	3.4±4.7
∑SFA	61.9±14.5	71.4±8.5	65.8±6.3	58.5±7.7	63.3±6.9	73.9±18.8	74.7±19.1	68.2±19.0	73.1±21.9	73.2±12.0
16:1n-9	1.6±1.9	1.5±1.7	2.7±3.0	1.2±1.5	0.6±0.5	0.3±0.3	0.3±0.2	0.3±0.3	0.1±0.1	0.2±0.2
16:1n-7	6.4±1.2	4.0±2.8	6.7±5.1	12.5±7.0	11.1±7.2	4.5±4.6	3.7±3.1	4.0±1.5	3.9±2.6	5.4±1.9
18:1n-9	12.3±6.6	5.2±3.9	4.2±4.8	3.2±2.9	2.2±2.3	2.0±1.4	2.5±1.7	2.7±1.5	2.2±1.8	2.1±0.7
18:1n-7	1.0±1.6	0.6±0.5	0.3±0.2	0.6±0.6	0.1±0.1	1.5±1.5	1.2±1.0	1.3±0.6	0.9±0.7	1.4±0.5
∑MUFA	21.3±8.9	11.3±5.1	13.9±5.3	17.4±5.8	13.9±8.8	8.4±6.6	7.7±6.0	8.2±3.4	7.2±4.9	9.1±2.6
18:2n-6	2.7±2.8	5.9±10.9	6.0±6.1	8.1±8.8	1.1±1.6	4.9±2.5	4.9±3.1	6.2±3.7	2.7±1.0	3.9±1.8
18:3n-3	4.0±2.4	0.5±0.5	4.2±3.0	4.9±2.9	2.0±2.3	2.1±2.1	1.5±1.7	1.5±0.7	1.6±1.1	1.7±0.8
18:4n-3	2.7±3.4	0.6±0.6	1.6±1.8	2.8±2.1	3.9±2.7	1.6±1.3	2.0±2.9	1.4±1.6	2.2±1.2	2.2±1.2
20:2n-6	0.4±0.1	0.5±0.4	0.4±0.4	0.3±0.2	0.3±0.1	0.3±0.4	0.5±0.9	0.1±0.1	0.2±0.3	0.2±0.2
20:3n-6	0.8±0.8	0.4±0.3	0.3±0.1	0.2±0.2	0.2±0.1	0.1±0.1	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1
20:4n-6	0.6±0.8	0.8±0.5	0.3±0.3	0.3±0.2	0.2±0.2	1.2±1.5	1.6±2.1	1.1±1.1	0.9±1.4	0.8±1.0
20:3n-3	0.4±0.1	1.0±1.8	0.4±0.3	0.4±0.3	3.7±6.7	0.5±0.4	1.3±1.3	0.9±1.1	1.1±1.8	0.6±0.6
20:4n-3	0.3±0.1	0.6±0.3	0.5±0.4	0.4±0.2	0.2±0.2	1.4±2.0	0.4±0.9	0.9±1.6	0.3±0.4	1.8±3.0
20:5n-3	1.8±2.7	0.8±0.8	0.4±0.1	3.8±4.1	3.4±3.8	2.4±2.6	1.8±1.6	6.4±11.9	5.9±10.0	1.8±1.0
22:5n-6	0.4±0.3	1.0±0.9	0.5±0.5	0.4±0.3	0.2±0.1	0.2±0.2	0.1±0.1	0.2±0.2	0.1±0.1	0.3±0.5
22:5n-3	0.2±0.1	0.6±0.4	0.6±0.3	0.3±0.2	0.6±0.9	0.3±0.4	0.1±0.1	0.1±0.1	0.1±0.1	0.3±0.4
22:6n-3	0.4±0.2	0.7±0.3	1.5±2.2	0.3±0.2	5.7±9.0	1.3±1.1	1.5±2.1	2.6±2.5	3.6±5.1	1.8±1.3
∑PUFA	14.6±8.2	13.4±9.4	16.7±5.9	22.2±5.9	21.4±8.8	16.1±11.9	15.8±13.2	21.7±17.0	18.7±16.9	15.5±9.2
Omega-6	4.9±2.0	8.6±10.3	7.5±5.7	9.3±8.6	2.1±1.6	6.6±4.4	7.1±5.9	7.8±4.4	4.0±2.3	5.3±3.1
Omega-3	9.7±6.3	4.7±2.8	9.2±3.6	12.9±4.0	19.3±8.2	9.5±7.6	8.6±8.8	13.9±13.9	14.7±14.8	10.2±6.5

Table 11: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of zooplankton by site and year from the Tar/Pamlico River system by month. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	2016					2017				
	Tranters n=6	**Lower Tar n=6**	**Blounts Creek n=8**	**Blounts Bay n=8**	**Pantego n=7**	**Tranters n=9**	**Lower Tar n=7**	**Blounts Creek n=12**	**Blounts Bay n=7**	**Pantego n=6**
14:0	4.8±1.0	4.6±1.2	4.3±0.9	5.0±1.5	4.4±1.3	6.3±1.4	7.1±2.7	5.7±3.7	4.8±2.4	6.1±1.9
15:0	2.1±2.0	1.8±1.3	2.2±2.0	0.7±0.3	0.9±0.6	1.8±1.4	1.8±0.6	1.5±0.7	0.9±0.6	1.2±0.7
16:0	20.7±3.9	24.7±3.0	22.0±4.6	22.1±2.8	19.9±3.1	17.6±4.0	18.4±5.1	20.3±3.5	18.3±3.7	17.3±2.2
17:0	1.3±0.3	1.4±0.4	1.4±0.2	1.1±0.2	1.1±0.3	0.9±0.4	0.9±0.5	0.6±0.4	0.7±0.4	0.7±0.4
18:0	5.5±0.6	6.5±0.9	6.3±0.9	6.2±1.2	5.6±0.7	4.9±1.6	5.3±2.2	4.5±1.7	5.0±1.6	4.6±0.9
20:0	0.0±0.0	0.5±0.7	0.1±0.2	0.2±0.4	0.0±0.0	0.5±0.4	0.8±0.6	0.4±0.2	0.3±0.3	0.2±0.1
∑SFA	34.5±4.2	39.5±4.5	36.3±4.5	35.3±4.1	31.9±4.9	32.0±7.2	34.4±9.0	32.9±4.1	30.2±7.7	30.0±4.3
16:1n-9	1.0±0.8	0.9±0.5	1.7±1.3	0.2±0.1	0.2±0.3	1.4±0.7	1.7±0.4	3.1±1.3	0.7±0.4	0.5±0.3
16:1n-7	4.4±3.9	2.7±3.9	2.9±4.6	3.7±4.3	3.7±2.5	5.7±1.5	6.4±1.8	9.2±4.2	3.0±0.9	5.5±2.8
18:1n-9	6.2±3.3	12.2±3.9	9.0±2.9	3.7±2.5	3.2±1.8	4.7±1.9	5.2±2.2	5.4±1.7	4.0±1.8	3.4±1.6
18:1n-7	4.0±1.0	4.3±1.2	4.8±2.3	2.2±0.5	2.0±1.2	3.1±0.9	2.9±1.0	3.0±0.5	1.8±0.9	1.9±1.1
∑MUFA	15.6±4.6	20.1±4.3	18.4±6.5	9.5±4.3	9.1±4.5	14.8±3.7	16.2±3.8	20.7±4.4	9.4±3.0	11.3±3.0
18:2n-6	6.8±3.9	11.5±4.6	7.5±3.5	4.3±1.6	3.4±2.2	6.4±2.4	6.7±3.0	6.5±2.0	4.6±1.1	4.7±4.5
18:3n-3	9.9±2.5	7.7±1.8	13.0±3.3	4.1±2.1	4.4±4.3	7.2±2.5	7.8±3.4	11.0±3.9	5.9±4.0	3.5±1.4
18:4n-3	6.3±2.7	2.3±1.9	4.3±1.6	3.3±3.5	3.9±2.4	3.6±2.3	2.6±1.5	4.1±3.2	2.9±1.2	2.9±1.5
20:2n-6	0.6±0.3	0.2±0.3	0.2±0.2	0.4±0.2	0.2±0.2	0.3±0.4	0.5±0.5	0.3±0.2	0.2±0.2	0.2±0.2
20:3n-6	0.1±0.1	0.1±0.0	0.1±0.1	0.0±0.0	0.0±0.0	1.2±3.1	0.8±1.4	0.1±0.2	3.5±9.3	0.0±0.0
20:4n-6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	7.2±7.0	5.9±4.9	5.1±3.5	4.2±6.2	3.5±6.0
20:3n-3	0.2±0.3	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.8±1.0	0.8±1.7	0.5±0.6	0.7±0.7	0.9±0.7
20:4n-3	0.7±0.6	0.0±0.0	0.4±0.4	0.6±1.3	0.5±0.5	0.5±0.5	1.3±2.6	0.6±0.5	0.7±0.6	0.7±0.7
20:5n-3	12.8±1.1	9.8±1.4	11.7±2.6	16.9±2.8	16.1±2.2	10.4±3.2	7.4±1.9	6.8±2.9	12.4±3.1	12.7±3.8
22:5n-6	2.3±1.9	0.0±0.0	1.8±1.9	1.1±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22:5n-3	0.1±0.2	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.3±0.4	0.2±0.3	0.3±0.4	0.3±0.3	0.5±0.4
22:6n-3	9.8±2.1	7.6±2.3	5.7±6.4	23.8±5.4	30.1±12.5	7.2±4.5	5.4±2.9	6.5±4.9	20.2±9.2	24.8±11.7
∑PUFA	49.5±6.3	39.3±2.1	44.7±7.1	54.8±4.2	58.7±5.9	45.1±9.4	39.3±8.8	41.7±5.9	55.7±11.3	54.6±8.3
Omega-6	9.8±3.5	11.8±4.8	9.7±3.2	5.8±2.5	3.6±2.2	15.1±10.2	14.0±6.9	12.0±4.8	12.6±11.4	8.5±10.3
Omega-3	39.7±7.1	27.5±3.0	35.1±7.5	49.0±4.7	55.1±7.7	30.0±10.9	25.4±6.0	29.7±7.6	43.1±11.2	46.0±15.8

Chapter 5: Incorporating the nursery habitat concept into the North Carolina's Strategic Habitat Areas

My dissertation examined the nursery habitat concept by assessing the lower trophic food web using fatty acid analysis as a tool. All of my research sites (Chowan and Tar/Pamlico River) are considered strategic habitat areas in North Carolina, and this research was meant to add to the criteria for defining important fish habitats that are not listed as primary nursery habitat. I was able to answer the question "Are all fish nursery areas equal?", and the overall answer is no based on differences in fatty acid composition observed across trophic levels. However, it is important to note that future research needs to be done to determine the exact concentrations of fatty acids required to support larval river herring growth and survival. My work indicates that including more factors than physical habitat alone would improve the ability to predict possible future effects on important nursery habitat.

Nursery Habitat Concept

The nursery habitat definition has been extant for many years to describe areas used by important commercial species during the mobile juvenile stage in estuaries. Beck et al. (2001) redefined and clarified the nursery habitat concept and developed a number of hypotheses with testable predictions. Nurseries were defined as areas where juvenile fish or invertebrates had higher densities, were able to avoid predation, and experienced faster growth rates compared to other habitats (Beck et al. 2001). Furthermore, a nursery functions "for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur" (Beck et al. 2001). Most nursery habitat research focuses on salt marshes, sea grass meadows, coral reefs, and mangroves. My research looked at the nursery habitat concept, but focused on

the larval stage of an anadromous fish, a life-history stage that is not currently included in the concept (Beck et al. 2001, Sheaves et al. 2015). Also, my research focused on an anadromous species that is pelagic, a habitat that has harder to define features, but not less important to study. Pelagic systems are dynamic and monitoring them is hard because nutrients, phytoplankton, and zooplankton are subject to short-term fluctuations with greater frequency as compared to seagrass beds that are more static. I extended the study one step further by also including the adult anadromous fish characteristics to determine if they are returning to the spawning ground able to spawn and produce offspring that have resources available through the yolk sac until first feeding. Therefore, my research expanded the nursery concept to include other components that rarely are considered when defining nursery habitat.

Researchers have pointed out that the nursery habitat concept/hypothesis does not take into consideration the complex ecosystem dynamics occurring in nursery areas (Sheaves et al. 2015). Sheaves et al. (2015) discussed that management agencies need to reconsider nursery habitat quality not only by what emerges at the end in terms of fish biomass, but also to focus on the complex mechanisms that determine how these habitats function. Coastal and estuarine areas are heavily impacted by anthropogenic effects including eutrophication, climate change, and fishing pressure (Flemer and Champ 2006, Barbier et al. 2011). These external stressors and complex food web interactions occurring within the nursery habitat are not incorporated into the current definition (Sheaves et al. 2015). My research encompasses two areas (ecological/ecophysiological factors and resource dynamics) by focusing on food webs, resource availability, and eco-physiological factors that impact larval and juvenile fish in nursery habitats (Sheaves et al. 2015, Chapter 1, Fig. 1). These factors are interrelated, but I will go through how my research touches on each of these factors.

First, ecophysiological factors are the physical conditions and how they affect the fish and the location of the nursery habitat (Sheaves et al. 2015). These physical conditions can include dissolved oxygen levels, salinity ranges, temperature, and nutrients. For example, if the site was hypoxic, then fish could not reside in the area even if the habitat might otherwise be considered a nursery habitat. Larval fish have tolerance ranges for temperature and salinity and any changes beyond these tolerance ranges could result in changes in nursery habitat suitability (Sheaves et al. 2015). For example, Blounts Bay in the Tar/Pamlico River is a salinity transient zone. In 2016 it was freshwater early in the sampling period, had lower salinity levels later in the year, but salinity was higher throughout the whole sampling period in 2017. Larval fish can have changes in growth because of amount of energy required for ionic and osmotic regulation (Winger & Lasier 1994). Also, spawning populations could move to another location if conditions are not good for offspring, resulting in this nursery habitat being unoccupied by larval and juvenile fish during higher salinity events. Overall, the major abiotic factor that differed between the two rivers was salinity, which was more variable in the Tar/Pamlico River than the Chowan River. I also sampled other abiotic factors, (e.g. nutrient concentrations) because even if nutrients do not directly affect the larval fish, nutrients can impact other trophic levels. I measured inorganic nitrogen and phosphorus because they have an impact on phytoplankton biomass and composition. Even though they do not have direct effects on the larval fish these changes could affect the food web, as will be described below.

Second, food webs in nursery habitat research often consider the food web from "top-down" perspective, i.e. how do predators affect the juvenile fish population (Sheaves et al. 2015)? In contrast, my research focused on the food web from the "bottom-up" perspective by examining how nutrient dynamics affected phytoplankton composition, the primary source of

fatty acids in aquatic food webs. Fatty acids can act as both dietary tracers in the food web and indicators of overall food quality (Iverson et al. 2004). I used fatty acids as tracers across three trophic levels, from phytoplankton to larval fish in the Chowan River and from phytoplankton to zooplankton in the Tar/Pamlico River. Overall, I saw that the fatty acid profiles did move up the food web resulting in bioaccumulation of particular fatty acids ultimately resulting in an increased percentage of omega-3s in each trophic level in both river systems. The omega-3 and omega-6 fatty acids are indicators of food quality for fish because of their role in somatic growth, fitness, membrane development, and cell function (see introductions of chapters 3, 4) and both a significant fraction of both fatty acid types were found in fish. Nutrient regimes and climate changes may alter food webs in the future and my work shows how these impacts would result in differences in the fatty acid composition that might occur in the nursery habitat. For example, nutrient changes can result in altered phytoplankton composition and possibly phytoplankton that is not edible or lower in quality (Müller-Navarra et al. 2000, Müller-Navarra et al. 2004). Temperature changes from warmer winters could result in a mismatch of the prey abundance to when larval fish are present in the system or prey composition could be affected by salinity changes from increased rain events or drought (Litzow, et al. 2006, Valdes-Weaver et al. 2006). All of these factors result in changes to fatty acid resource availability, which could become limiting for larval fish in the future.

Finally, resource availability is determined by the food quantity and quality throughout the nursery habitat for larval fish (Sheaves et al. 2015). I took this one step further and investigated the food availability for the prey of larval fish. When I compare and contrast the two rivers in my study, there are differences seen between the rivers. Phytoplankton biomass was higher in the tributaries of the Chowan River compared to the open water sites, and had

increased concentrations in 2017 and Tar/Pamlico River had the highest phytoplankton biomass in the brackish water sites compared to freshwater. The phytoplankton biomass at the freshwater sites of the Tar/Pamlico River was low, which could indicate that the system was net heterotrophic. A net heterotrophic system would have reduced quantities and percentages of omega-3 and omega-6 fatty acids. Overall, I observed higher quantities of zooplankton in Chowan River sites compared to Tar/Pamlico River at the freshwater sites, but I cannot compare between the two years due to equipment malfunction on the boat (i.e., no hose to wash down nets in 2016 for both rivers). Washing zooplankton nets resulted in possible loss of biomass since I could not wash the net the same for both years. Larval river herring (blueback herring, (*Alosa aestivalis*), and alewife (*A. pseudoharengus)*) were more abundance in the Chowan River and tributaries compared to Tar/Pamlico River.

However, the prey quantities are only half the story, I used species composition, and fatty acid analysis to determine the "quality" of prey for zooplankton and larval fish. Phytoplankton pigments were similar between the two systems with peridinin and fucoxanthin being more abundant compared to the other pigments. In the Chowan River, alloxanthin and chlorophyll *b* had increased abundances compared to Tar/Pamlico River. Peridinin and fucoxanthin represent dinoflagellates and diatoms, which have increased EPA and DHA fatty acids compared to other phytoplankton. Alloxanthin and chlorophyll *b* are indicators for cryptophytes and green algae, which have ALA and EPA present in the fatty acid profile. Zooplankton community composition had increased percentage of rotifers and cladocerans in Chowan River and tributaries, reflecting the greater influence of freshwater. This led to an increased percentage of ALA and EPA compared to DHA. In the Tar/Pamlico River, zooplankton community composition had increased copepod nauplii and a mix of cladocerans/copepods in freshwater and

Acartia spp. in brackish water. The percent of EPA and DHA were relatively equal in the freshwater sites, and there was increased percent of DHA in the brackish water sites. The decrease in EPA to DHA amounts or ratios in the system could result in decreases in larval fish growth rate from having to use energy for bioconversion of precursor fatty acids.

Nursery habitat areas are dynamic systems and the changes to the lower trophic food web could result in effects moving up the food web that otherwise would not be detected. For example, a change in phytoplankton from diatoms to cyanobacteria could happen in a short time frame and disappear quickly due to a storm-event. It would be possible to determine a lack of sufficient fatty acids by examining larval tissue for some time after this event using the fatty acid approach. These changes are likely to be especially important during the first-feeding period and early during the larval stage when a fish's feeding and swimming capacity are under development. Juveniles and even late-stage larvae may be better able to weather such changes due to increased swimming capacity allowing them to better select habitats or due to the ability to eat a larger variety of food sources as they grow. Furthermore, juveniles and late-stage larvae may have more energy stores allowing them to better weather periods of low food quality.

Strategic Habitat Areas

Turning to management, many state agencies have used the nursery habitat concept to designate areas of important nursery habitat, but have not considered the dynamic nature of the system (Beck et al. 2001, Sheaves et al. 2015). Also, many of the species considered in defining the nursery habitat concept only become commercially important once they reach the ocean (Beck et al. 2001). In contrast, I studied river herring, which were harvested commercially when returning to the spawning grounds. Two other species are similar to river herring in this respect:

salmon on the west coast are an important commercial species when returning to the spawning grounds as well as species of sturgeon, many which are threatened or endangered. Both species could benefit from a better understanding of nursery habitat function that could be applied through management.

In North Carolina, strategic habitat areas (SHAs) are defined as areas that contribute most to the integrity of the system and for fishes as "locations of individual fish habitats or systems of habitats that have been identified to provide exceptional habitat functions or that are particularly at risk due to eminent threats, vulnerability or rarity" (Deaton et al. 2006). The model used to determine SHAs incorporated land use, water quality, and fish data when available, but did not take into account any of the other food web dynamics (Deaton et al. 2006). In the Chowan River, the whole river and its tributaries were designated SHA because it was already designated as anadromous fish spawning areas (AFSAs) (NCDMF 2015). The SHAs designated incorporated both spawning habitat and the migration route (NCDMF 2015). The Tar/Pamlico River is not fully designated as SHAs, but all the sites I sampled are considered SHAs. That being said, it is important to note that river herring were not considered in the SHAs designation for the Tar/Pamlico River, but other fish species especially striped bass, were considered (NCDMF 2015).

As estuaries continue to experience anthropogenic effects, there is a greater need to understand how the lower trophic food web in SHAs respond to human activity because it is this lower trophic level food web that supports fish survival and recruitment. Any change to the lower food web would result in a possible change to the habitat for different fish species. I can take the SHAs model a step further by showing how the abiotic conditions impact the lower trophic food web. Here, I will step through each level of my research and demonstrate how it

helps improve the model for SHA designation. First, if I only collected data on nutrients and abiotic conditions (temperature, DO, salinity, pH), then I may be missing important information that may help define SHAs. Nutrients drive phytoplankton dynamics in estuaries as has been demonstrated from long-term studies on eutrophication in estuarine systems (Paerl et al. 2003). Excessive nutrients have been linked to harmful algae blooms and hypoxia in estuaries, which have been studied extensively in North Carolina (Paerl et al. 2003). However, as my work demonstrated, changing nutrient conditions and phytoplankton community composition changes are directly linked to the fatty acid composition in the planktonic food web. Therefore, by linking nutrients to fatty acid changes, one can begin to assess food "quality" changes that may be impacting larval and juvenile fish in the nursery area. This cannot be done when examining nutrients alone.

Second, if I only collected samples for chlorophyll *a*, then I would know when the phytoplankton biomass is high, but not which functional group is causing those changes. Changes in the functional group are directly linked to changes in the fatty acid composition of the plankton. For example, a mixed phytoplankton assemblage of dinoflagellates, diatoms, cryptophytes, and green algae would have the presence of ALA, EPA and DHA fatty acids. However, when diatoms dominated the system, there was an increase in EPA compared to when dinoflagellates were present when an increase in DHA was observed. The increase of cryptophytes and green algae in the Chowan River resulted in increased in ALA, SA, and EPA. The presence of omega-3s are important for growth and reproduction of the zooplankton, which could lead to a better prey base for larval river herring compared to if I found cyanobacteria as the dominant phytoplankton, which is higher in ALA but has very low levels of EPA or DHA (Fig. 48). This could result in reduced growth and reproduction of zooplankton and a possible

reduction in the prey base (Fig. 48). Both rivers have been classified as nutrient sensitive since the 1970s from excess nutrients. Over the last few years, there has been a return of cyanobacteria blooms during the summer. These blooms could be potentially toxic to organisms, but also could harm the system in terms of low nutritive quality for prey.

Third, knowledge of zooplankton composition and fatty acid profiles provided a better understanding of the prey base for larval fish. Zooplankton composition changes varied considerably, being dominated at different times by smaller cladocerans, larger cladocerans, and copepods. Therefore, size limitation may also play a role in larval and juvenile river herring feeding. For copepods, Cyclopoida are usually smaller than Calanoida, and may be able to be consumed earlier in the larval fish diet compared to Calanoida due to larval fish mouth gape width. Chowan River sites had higher percent of cladoceran especially *Bosmina* spp. and *Daphnia* spp. compared to Cyclopoida and Calanoida especially in 2017. There was an increase in Cyclopoida in 2016. Rockyhock Creek on the Chowan River would be a river to more closely investigate during the time of larval river herring residency, as the zooplankton population was mostly rotifers, which usually have a fatty acid higher in ALA compared to EPA and DHA. In the freshwater reaches of the Tar/Pamlico River, the zooplankton were a mix of cladoceran (*Bosmina* spp., Daphniidae, and Chydoridae), and copepods (Calanoida and Cyclopoida). The brackish water sites in the Tar/Pamlico River had *Acartia* spp. as the dominant species. Cladoceran species were correlated to higher EPA levels, and copepod species were correlated to increased DHA levels throughout both systems, which could change the fatty acids being consumed from the diet (see Chapters 3 and 4). Abundances tell us only part of the story because the species composition could switch from smaller to larger cladoceran, and depending on the phytoplankton composition the fatty acids could be lower in the needed fatty acid composition.

Managers could say that the abundance is adequate for larval fish as was reported by Leech et al. (2009), but without knowing the quality of this prey base, river herring may still be experiencing poor growth and survival in the presence of high zooplankton abundances. In the Chowan, the Catherine and Lower Chowan River had similar abundances over the two years but very different fatty acid profiles with the Lower Chowan River site having increased DHA compared to Catherine Creek.

Finally, the larval fish were studied to determine abundance and fatty acid composition. Just knowing if the larval river herring are present is not enough to define nursery habitat. If the prey base is not supportive of growth and survival of the larval fish present then these fish will not survive to adulthood. River herring had similar fatty acid profiles and percent composition for all the sites on the Chowan River. This would demonstrate that overall the larval river herring may be consuming different diets, but are bioaccumulating omega-3s from these diets in the Chowan River sites. This suggests that river herring are able to either bioconvert from precursor fatty acids or selectively feed on the zooplankton community to attain the fatty acids necessary for growth and survival. Obviously, more studies are needed to investigate this important finding. Finally, I wanted to determine if female river herring were returning to the spawning grounds with stored lipids and ovaries with a fatty acid profile consisting of DHA and EPA. If the females were of "poor quality" when they returned to spawning grounds (low DHA and EPA levels in the ovary), then nursery habitat would not matter if they are unable to spawn or if their spawned eggs did not contain sufficient yolk for larval survival to first feeding. I determined that female river herring had storage lipids to allow spawning, and the ovaries had high percent of DHA and EPA (two important fatty acids for development and growth of larval fish). Larval river herring have a yolk sac that should be able to allow them to survive until first feeding.

Overall, I found that the Chowan River and Tar/Pamlico River had different lower trophic food webs during the time larval river herring are present in the system. Also, having sampled the brackish water in the Tar/Pamlico River allowed us to see a picture of what is in the system when larval river herring move to brackish water. If only physical habitat was explored, these two river systems would have been found to be similar since river herring return to similar habitat conditions for spawning, but investigating the lower trophic level allowed us to find differences and start asking questions as to how these differences may affect the larval fish. For example, if a cyanobacteria bloom occurred during the larval river herring residency in some areas and not others, then these methods would be able to help determine what may happen to zooplankton fatty acid profile which could result in changes in the larval river herring fatty acid profiles, growth or survival or if hypoxic conditions reduces the available habitat. River herring continue to experience a lack of recovery and a long-term data set would have allowed managers to investigate if there are differences in the lower trophic food web between when populations were abundant and now. Another important point is that having a long-term data set would have allowed the study of how changes in the system affect larval fish if river herring continue to experience a lack of recovery. This would only be possible if continued monitoring of changes in the lower trophic level food web are ongoing to assess how larval river herring are doing at the beginning of life. These data would give managers and researchers information to manage nursery habitats and assess ongoing changes.

Overall, I concluded from the data collected that the female river herring, and lower trophic food web from community composition and fatty acid profiles does not seem to be affecting the recovery of the river herring in North Carolina. There were higher chain omega-3 present in the system throughout the food web, and larval river herring had increased amounts of

DHA and EPA from other trophic levels either through selective feeding or the ability of convert lower chain fatty acid to higher chain fatty acids or bioaccumulation of specific fatty acids. One area that was not resolved in this research was the concentration or ratios of fatty acids that is needed by the river herring for growth and survival and there is currently no literature available that addresses this. This indicates that a laboratory study to determine larval river herring fatty acid requirements would be a logical next stop. Such information could be incorporated into the SHA model and help managers determine appropriate nursery habitat for river herring. In conclusion, this research is the first step into incorporating more data into the basic nursery habitat concept, and the first step in helping managers by determining a baseline data set on the similarities and differences within and between to SHAs (the Chowan and Tar/Pamlico River systems) using a fatty acid approach.

Future Research

I was able to determine that incorporating the lower trophic food web into the nursery habitat concept and SHAs would allow for a better understanding of how changes affect larval river herring, but there are still gaps in my research. First, there needs to be more information about the larval river herring, in particular studies on diet and growth. The next logical step is determining how food quality, (e.g. the ratio of EPA and DHA required for optimum growth) affects larval river herring growth. Furthermore, the realized diets of larval river herring from stomach analysis would determine if I was measuring non-preferred prey using net sampling. Larval fish growth could be correlated to food items each week if otoliths were removed from the fish, and growth each day was back calculated (Stevenson and Campana 1992). Second, measuring zooplankton length to determine the size structure of the prey available and measuring the size of the prey consumed by larval river herring at different sizes would help identify the

size ranges of prey consumed and this could then be related to fatty acid composition of these species.. Finally, targeted sequencing analysis of the seston filters using genomic techniques would determine the phytoplankton community composition, especially when chlorophyll *a* concentrations are low. This would help better determine the microplankton composition since I only used five accessory pigments. I used the five main accessory pigments for fresh and brackish water, but I could have missed more minor species that are also important. In the Chowan River especially at the lower site and Rockyhock Creek, I collected a type of blue-greenish material in May 2016 and April 2017 that did not appear to relate to any of the pigments I analyzed. I want to determine if it is cyanobacteria because even though it did not appear to be a bloom, this may show how early cyanobacteria blooms starts to show up in the system. Future research in these systems will help to improve the model used for designating SHAs by allowing the management agency to incorporate more data.

In conclusion, my dissertation expanded the nursery habitat concept to include the larval fish stage, incorporated the pelagic environment, and included the lower trophic food web. This research could be applied to other species besides river herring to improve understanding how changes in the lower trophic food web would affect species differently and allow multispecies management to occur. My dissertation results are beneficial to incorporate into the model for SHAs areas because it helps to strengthen the data set used to determine important habitat that needs management. Also, my research shows that nursery habitat could be considered in the SHAs throughout both regions to allow for improved management for river herring populations in the future.

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Figure:

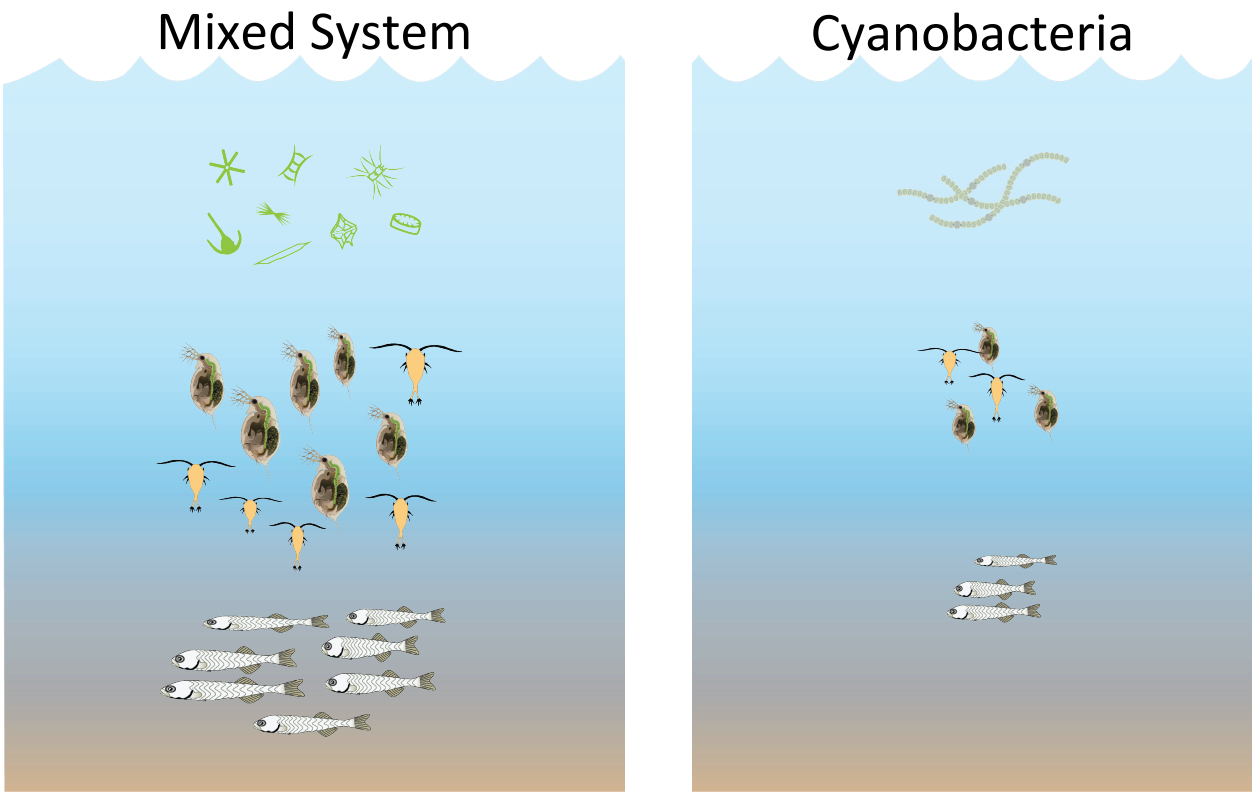

Figure 48: Comparison of the trophic levels (phytoplankton, zooplankton, and larval fish), and the predictions from a mixed system versus a system dominated by cyanobacteria.

APPENDIX A: ANIMAL CARE PROTOCOL

East Carolina University

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

January 12, 2016

David Kimmel, Ph.D.
Department of ICSP/Biology
Howell Science
East Carolina University

Dear Dr. Kimmel:

Your Animal Use Protocol entitled, "Linking Water Quality, Food Quality, and Larval Fish Condition to Determine Strategic Habitat Area Quality" (AUP #D333) was reviewed by this institution's Animal Care and Use Committee on January 11, 2016. The following action was taken by the Committee:

"Approved as submitted"

Note: A minor administrative change was made adding 70,000 unknown bycatch species to the table. This was a clear, thorough, and well-written protocol!

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP and are familiar with its contents.**

Sincerely yours,

Eddie Johnson/jd

Eddie Johnson, MS
Interim Chair, Animal Care and Use Committee

EJ/jd

enclosure

East Carolina University

Animal Care and
Use Commitee
212 Ed Warren Life
Sciences Building
East Carolina University
Greenville, NC 27834

252-744-2436 office
252-744-2355 fax

December 20, 2016

Ariane Peralta, Ph.D.
Department of Biology
Howell Science Building
East Carolina University

Dear Dr. Peralta:

The Amendment to your Animal Use Protocol entitled, "Linking Water Quality, Food Quality, and Larval Fish Condition to Determine Strategic Habitat Area Quality" (AUP #D333) was reviewed by this institution's Animal Care and Use Committee on December 19, 2016. The following action was taken by the Committee:

"Approved as submitted"

Please contact Aaron Hinkle at 744-2997 prior to hazard use

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies. **Please ensure that all personnel associated with this protocol have access to this approved copy of the AUP and are familiar with its contents.**

Sincerely yours,

S McRae

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

Enclosure

APPENDIX B: Scientific Collection Permits

Scientific Fish Collecting License

AUTHORITY
STATUTES
113-261, 113-262, & 113-272.4

North Carolina Wildlife Resources Commission
Division of Inland Fisheries
MSC 1721
Raleigh, NC 27699-1721

Phone: (919) 707-0220
Fax: (919) 707-0028

PERMITTEE/LICENSEE

DEBORAH A LICHTI
103 SUNSHINE LN UNIT D
WINTERVILLE, NC 28590

PERMIT NUMBER
16-SFC00186

EFFECTIVE	EXPIRES
04/27/2016	**12/31/2016**

Conditions and Authorizations:

Print Date: **04/28/2016**

The above licensee is authorized to collect the species/organisms by the sampling methods as listed below without restriction to season, size, or creel limits unless otherwise specified under Special Conditions. These species/organisms may be collected in the River Basins/Counties listed below. Sampling in Collection-Sensitive Waters is not authorized under this license unless specified under Special Conditions or allowed through a Commission-issued Endangered Species Permit. For a current list of Collection-Sensitive Waters, see www.ncwildlife.org/Licensing/fishcollection.aspx. The take or possession of federally or state listed endangered, threatened, or special concern species is not authorized under this license except in conjunction with a valid Endangered Species Permit. The licensee is required to comply with all requirements and laws specified by the U.S. Fish and Wildlife Service and National Marine Fisheries Service as they pertain to federally-listed species. For a current list of federally or state listed endangered, threatened, or special concern species, see www.ncwildlife.org/Licensing/fishcollection.aspx.

SPECIES/ORGANISMS:

Alosa aestivalis; Alosa pseudoharengus; Clupeidae

RIVER BASINS/COUNTIES:

CHOWAN - Chowan, Gates, Hertford
TAR-PAMLICO - Beaufort, Pitt

SAMPLING METHOD:

Net, Other

ASSISTANTS:

SPECIAL CONDITIONS:

This permit/license is non-transferable and expires at midnight on the above specified expiration date.

ISSUED BY: Christian T Waters

TITLE: **Chief**

16-SFC00186

Scientific Fish Collecting License

AUTHORITY

STATUTES

113-261, 113-262, & 113-272.4

North Carolina Wildlife Resources Commission
Division of Inland Fisheries
MSC 1721
Raleigh, NC 27699-1721

Phone: (919) 707-0220
Fax: (919) 707-0028

PERMITTEE/LICENSEE

DEBORAH A LICHTI
103 SUNSHINE LN UNIT D
WINTERVILLE, NC 28590

PERMIT NUMBER

17-SFC00186

EFFECTIVE	EXPIRES
01/01/2017	**12/31/2017**

Conditions and Authorizations:

Print Date: **12/02/2016**

The above licensee is authorized to collect the species/organisms by the sampling methods as listed below without restriction to season, size, or creel limits unless otherwise specified under Special Conditions. These species/organisms may be collected in the River Basins/Counties listed below. Sampling in Collection-Sensitive Waters is not authorized under this license unless specified under Special Conditions or allowed through a Commission-issued Endangered Species Permit. For a current list of Collection-Sensitive Waters, see www.ncwildlife.org/Licensing/fishcollection.aspx. The take or possession of federally or state listed endangered, threatened, or special concern species is not authorized under this license except in conjunction with a valid Endangered Species Permit. The licensee is required to comply with all requirements and laws specified by the U.S. Fish and Wildlife Service and National Marine Fisheries Service as they pertain to federally-listed species. For a current list of federally or state listed endangered, threatened, or special concern species, see www.ncwildlife.org/Licensing/fishcollection.aspx.

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TAR-PAMLICO - Beaufort, Pitt

SAMPLING METHOD:

Net, Other

ASSISTANTS:

SPECIAL CONDITIONS:

This permit/license is non-transferable and expires at midnight on the above specified expiration date.

ISSUED BY: Christian T Waters

TITLE: **Chief**