

1 **Changes in zooplankton community, and seston and zooplankton fatty acid profiles at the**  
2 **freshwater/saltwater interface of the Chowan River, NC**

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## ABSTRACT

The variability in zooplankton fatty acid composition may be an indicator of larval fish habitat quality as fatty acids are linked to fish larval growth and survival. We sampled an anadromous fish nursery, the Chowan River, during spring of 2013 in order to determine how the seston fatty acid composition varied in comparison with the zooplankton community composition and fatty acid composition during the period of anadromous larval fish residency. The seston fatty acid profiles showed no distinct pattern in relation to sampling time or location. The mesozooplankton community composition varied spatially and the fatty acid profiles were typical of freshwater species in April. The Chowan River experienced a saltwater intrusion event during May, which resulted in brackish water species dominating the zooplankton community and the fatty acid profile showed an increase in polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The saltwater intrusion event was followed by an influx of freshwater due to high precipitation levels in June. The zooplankton community composition once again became dominated by freshwater species and the fatty acid profiles shifted to reflect this change; however, EPA levels remained high, particularly in the lower river. We found correlations between the seston, microzooplankton and mesozooplankton fatty acid compositions. These correlations show that seston composition was correlated to particular groups of fatty acids found in the zooplankton. These data suggest that anadromous fish nursery habitat likely experiences considerable spatial variability in fatty acid profiles of zooplankton prey and that are correlated to seston community composition and hydrodynamic changes. Our results also suggest that sufficient prey density as well as a diverse fatty acid composition is present in the Chowan River to support larval fish production.

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## Introduction

Estuaries are considered important nursery habitat for many ecologically and commercially important fish and invertebrates (Boesch and Turner 1984). 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). Hence, many fish have evolved life-history strategies whereby larvae and juvenile stages have residency periods in estuaries (McHugh 1967; Boehlert and

**Comment [T1]:** The introduction requires more recent references, most of the references are pre-2010. Look for new references in the Galloway, Kainz, Parrish, Richoux labs among others for more recent references on the subject at hand.

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**Comment [T2]:** More recent references

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62 Mundy 1988; Beck et al. 2001; Able 2005; Walsh et al. 2005). Higher growth rates of larval fish  
63 are possible because of zooplankton prey **that interact with fish** during their critical transition  
64 from yolk sac larvae to free-living, feeding larvae (Hjort 1914; Mullen et al. 1986; Rulifson et al.  
65 1993; Cooper et al. 1998; Martino and Houde 2010; Binon 2011). Many studies have related  
66 spatial and temporal overlap in zooplankton and fish populations to successful year class strength  
67 for fish (Hjort 1914; Townsend 1983; Newton 1996; Chick and Van Den Avyle 1999; Martino et  
68 al. 2010; Binon 2011). However, spatial and temporal overlap between predators and prey does  
69 not explain how fish nurseries function mechanistically. Therefore, the quality of prey can play a  
70 major role in determining the effectiveness of a nursery for early stages of fish.

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71 The quality (chemical composition) of zooplankton prey can influence fish growth,  
72 development, and survival (Fraser et al. 1989; Webster and Lovell 1990; Copeman et al. 2002;  
73 Rossi et al. 2006; Malzahn et al. 2007, Paulsen et al. 2014). Lipids are one class of compounds  
74 that are particularly important, having been shown to impact neural and vision development in  
75 fish (Gulati et al. 1997; Müller-Navarra et al. 2000; Kainz et al. 2004; Masclaux et al. 2012).  
76 Lipids and fatty acids may act as both dietary tracers in the food web and indicators of overall  
77 food quality. Fatty acids are chemically diverse, often incorporated into organisms unmodified,  
78 and different organisms have distinct profiles (Dalsgaard et al. 2003). The majority of aquatic  
79 organisms need specific dietary fatty acids for somatic development and fitness (Masclaux et al.  
80 2012). For example, 18:3n-3,  $\alpha$ -linolenic acid (ALA), and 18:2n-6, linoleic acid (LA) are labeled  
81 essential fatty acids because they cannot be directly synthesized by heterotrophic organisms and  
82 must come from the diet (Arts et al. 2009). Polyunsaturated fatty acids (i.e., 20:5n-3,  
83 eicosapentaenoic acid (EPA), 22:6n-3, docosahexaenoic acid (DHA), and 20:4n-6, arachidonic  
84 acid (ARA)) are required for all organisms and play a role in health and cell function (Dalsgaard

85 et al. 2003). In addition to these essential fatty acids, entire suites of fatty acids can be used to  
86 generate fatty acid profiles for comparison across trophic levels (Iverson et al. 2004). Thus, an  
87 organisms' fatty acid signature may indicate dietary consumption and nutritional quality of its  
88 prey (Goncalves et al. 2012).

89 Fatty acids are present in estuaries as a result of *de novo* synthesis by phytoplankton and  
90 the delivery of detrital material of plant origin (Dalsgaard et al. 2003). The free-floating portion  
91 of organic particulates is termed seston and the seston fatty acid composition is important because  
92 it forms the origin point for the propagation of fatty acids through the pelagic food web (ref).

93 Zooplankton assimilate fatty acids from the seston through direct consumption of  
94 phytoplankton cells, detritus and/or consumption of microzooplankton that graze phytoplankton  
95 or detritus (Wacker and von Elert 2001; Kainz, Arts, and Mazumder 2004; Vargas et al. 2006).

96 To date, studies of the relationship between seston and zooplankton fatty acid composition have  
97 shown variable patterns. Persson and Verde (2006) demonstrated in laboratory studies that

98 broad, zooplankton taxonomic groups (cladoceran *vs.* copepods) have different fatty acid profiles  
99 independent of the food source. The seston has been correlated to the fatty acid profile of

100 zooplankton *in situ* as well (Goulden and Place 1990; Brett et al 2006; Taipale et al. 2009;  
101 Gladyshev et al. 2010; Ravet, Brett, and Arhonditsis 2010). The mismatch of fatty acids in seston  
102 to zooplankton has also been shown in many studies (Desvillettes et al. 1997; Persson and Vrede  
103 2006; Rossi et al. 2006; Smyntek et al. 2008). There appears to be a difference in fatty acid  
104 transfer from seston to zooplankton when freshwater and marine ecosystems are compared. Most  
105 marine zooplankton species cannot convert precursor fatty acids and only obtain longer-chained  
106 fatty acids from their diet (Rossi et al. 2006; Persson and Verde 2006). However, it is clear that  
107 fatty acid composition of the seston changes as a result of local conditions, e.g., the salinity and

**Comment [T4]:** See Bergamino et al. 2015. Evidence of spatial and temporal changes in sources of organic matter in estuarine sediments: stable isotope and fatty acid analyses. *Hydrobiologia*  
Dalu et al. 2016. Effects of substrate on essential fatty acids produced by phytobenthos in an austral temperate river system. *Freshwater Science*

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108 temperature, nutrient concentration, and the degree of autotrophy or heterotrophy in the system  
109 (Farkas and Herodek 1964; Desvillettes et al. 1997; Wacker and von Elert 2001; Goncalves et al.  
110 2012). Therefore, combined knowledge of the changing nature of seston fatty acid composition,  
111 zooplankton community composition changes, and fatty acid profiles forms a useful base for  
112 assessing the quality of fish nursery habitat.

113 Zooplankton community composition in estuaries has been intensely studied and abiotic  
114 factors are thought to structure zooplankton communities (Ambler et al. 1985; Orsi 1986;  
115 Cervetto et al. 1999; Mouny and Dauvin 2002; Kimmel and Roman 2004; Lawrence et al. 2004;  
116 Islam et al. 2005). Zooplankton community composition in temperate estuaries is dominated by  
117 crustaceans in general and copepods and cladocerans in particular (Tackx et al. 2004, Marques et  
118 al. 2006; Winder and Jassby 2011; Chambord et al. 2016). The fatty acid (FA) profiles of  
119 crustacean zooplankton vary among species and are influenced by diet (Persson and Vrede 2006;  
120 Arts et al. 2009). Cladocerans are characterized by high levels of EPA and ARA and this is  
121 thought to be related to a life history strategy focused on high rates of somatic growth (Persson  
122 & Vrede 2006). In contrast, copepods have higher relative DHA levels because this fatty acid is  
123 critical for nervous system development (Arts et al. 2009). Copepods feature more developed  
124 nervous systems compared to cladocerans and this is a function of the active hunting of prey,  
125 mate location, and predator avoidance (Dalsgaard et al. 2003). Carnivorous crustacean  
126 zooplankton have shown to be richer in PUFAs and this is thought to be related to their food  
127 source (rotifers and smaller bodied cladocerans/copepods compared to phytoplankton) (Arts et  
128 al. 2009).

129 Here we assess the variability in fatty acid composition of seston and zooplankton at the  
130 freshwater/saltwater interface of an estuarine fish nursery, the Chowan River, North Carolina,

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131 USA. The Chowan River is considered a critical habitat for larval and juvenile blueback herring  
132 (*Alosa aestivalis*) and alewife (*A. pseudoharengus*), collectively known as river herring  
133 (NCDMF 2007). The river herring are of interest because they have been severely overfished and  
134 a moratorium on harvest is in place at various locations along the eastern United States,  
135 including North Carolina (ASMFC 2012). The Chowan also serves as a nursery habitat for  
136 American shad (*A. sapidissima*) and striped bass (*Morone saxatilis*); however, the status of  
137 the habitat for the latter species is unknown (Greene et al. 2009). The overall goal of our study  
138 was to determine if fatty acid profiles of the lower food web could be used to indicate habitat  
139 quality for an estuarine fish nursery. In order to achieve this goal, we determined the fatty acid  
140 composition of the seston, microzooplankton, and mesozooplankton during the period of larval  
141 fish residency in the Chowan River. We then compared the fatty acid profiles of these groups to  
142 determine the pathway of fatty acid transfer that was present in the Chowan River. We  
143 hypothesized that fatty acid profiles of micro- and mesozooplankton would relate to species  
144 composition and would reflect that of the seston. If supported, this would suggest that the  
145 “quality” of the larval fish forage, based on fatty acids, could be used to assess fish nursery  
146 quality.

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## 148 Materials and Methods

### 149 Study Site

150 The Chowan River is one of the largest tributaries that empty-drain into the Albemarle  
151 Sound (Fig. 1a and 1b) and is the 12<sup>th</sup> largest river basin in North  
152 Carolina (NCDENR 2006). and It's is mainly a freshwater estuary that experiences intermittent  
153 salinity intrusion, mainly in the winter months (Leech et al. 2009). The Chowan River was

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154 classified as “nutrient sensitive waters” in 1979 (NCDENR 2006) and has routinely experienced  
155 algal blooms and low dissolved oxygen levels ( $<3.0 \text{ mg L}^{-1}$ ). The entire river is classified as a  
156 Strategic Habitat Area for larval and juvenile river herring (NCDMF 2007). Sampling took place  
157 south of Holiday Island on a 34 km transect of Chowan River (Fig. 1c). Seven locations (4 km  
158 apart) were sampled between Holiday Island and the river mouth (Fig 1c). Sampling occurred on  
159 10-11 April, 31 May, and 25 June 2013, dates that span the residency for larvae of alewife,  
160 blueback herring, and striped bass. For our study, we divided the river into three sections: upper,  
161 middle, and lower. The main differences among these sections were (1) the distance from the  
162 Albemarle Sound and (2) the potential influence of salinity.

163 **Sample collection**  
164 **Water column properties and seston**

166 Vertical profiles of temperature ( $^{\circ}\text{C}$ ) and salinity were measured with a conductivity,  
167 temperature, and depth sensor (CTD, Yellow Springs Instruments, Castaway). Seston was  
168 collected a depth of 3 meters with a Niskin water sampler. The water samples were placed in  
169 brown bottles and brought back to the laboratory for processing.

171 **Zooplankton**

172 Zooplankton sampling occurred on 10 and 11 April, 31 May, and 25 June 2013 at seven  
173 sites on the Chowan River. Water depths ranged from 5.27 to 7.56 m- for zooplankton sampling.  
174 Two net tows were made with 0.5 m diameter nets of two different mesh sizes (60 and 200  $\mu\text{m}$ ).  
175 Two mesh sizes were used in order to generate an adequate representation of the zooplankton for  
176 the size range  $> 60 \mu\text{m}$ . The zooplankton samples between 60 and 200  $\mu\text{m}$  are designated

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Comment [T9]: is it seston or water samples for seston analysis. Seston sample processing was not properly mentioned in the manuscript

Comment [T10]: how many litres

Comment [T11]: this section should be separated into 2 sections for identification and FA samples. There is too much confusion here

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Comment [T12]: delete repetition, already mentioned above, study area

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177 microzooplankton and the > 200 µm zooplankton samples are designated mesozooplankton  
 178 throughout the remainder of the paper. The zooplankton net was towed obliquely through the  
 179 water for 1 minute (species composition) and 2 minutes (fatty acid composition) at an average  
 180 boat speed of 0.75 m s<sup>-1</sup>. The volume filtered was calculated using the volume of a cylinder  
 181 ( $V = \pi r^2 L$ ), where  $r$  was the radius of the plankton net (0.25 m) and  $L$  was determined using the  
 182 boat speed (m s<sup>-1</sup>) and the tow time (s). Each identification and count sample, depending on mesh  
 183 size, was filtered through a 200 or 60 µm filter, and zooplankton for composition were preserved  
 184 in 120 mL glass jar with 10 mL of 10% buffered formaldehyde, sucrose, and filtered  
 185 water. The addition of sucrose to the formalin helps to reduce ballooning of cladoceran bodies  
 186 and inflation of their carapace (Haney and Hall 1973). The 60 µm sample had a half a tablet of  
 187 Alka Seltzer added to keep rotifers from pulling in critical body parts (legs and arms) to ease  
 188 identification (Chick et al. 2010). The zooplankton samples for fatty acid analysis were collected  
 189 at seven sites on the Chowan River for April and June, and three sites in May. Due to limitations  
 190 related to sampling preparation, a subset of all the field site samples were analyzed for fatty acid  
 191 analyses in May. The zooplankton samples for fatty acids were filtered to remove excess water  
 192 and placed in a 1000 mL plastic container on ice, and processed in the laboratory.

**Comment [T14]:** how were these zooplankton size classes determined. That is not clear. The 200 um will collect >200 um whereas the 60 um mesh collects all zooplankton size classes. How did you determine the 60 and 200 um range

**Comment [T15]:** Repetition

**Comment [T16]:** am lost here, did you analyse all subsamples for april and june. What was really done

## 194 Laboratory Processing

### 195 Zooplankton Identification

196 Samples were filtered through a sieve (60 or 200 µm) to remove the sugar formalin  
 197 solution. A total of three subsamples (2 mL per subsample for microzooplankton and 5 mL  
 198 per subsample for mesozooplankton) were analyzed for the community composition using a  
 199 Hensen-Stempel pipette. Organisms were identified using a dissecting microscope and

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200 enumerated using Ward counting wheel. The zooplankton were identified to genus except for the  
201 freshwater copepods that were identified to order. Copepod nauplii were grouped together  
202 because identification can be difficult at this stage (Johnson and Allen 2012). If a species in a  
203 subsample comprised greater than 500 individuals, then that species was not counted for the  
204 other two subsamples. Species abundances ( $A$ ) were determined using the equation:  $A = A_s(V_t/V_s)$   
205 where  $A_s$  is the number of individuals in the subsample,  $V_t$  is the total volume filtered by the  
206 plankton net, and  $V_s$  is the volume of the subsample.

### 207 Lipid and fatty acid samples

208 The seston samples (300 ~~mL~~<sup>mL</sup>) were concentrated on a 47 mm GF/F filter (Whatman<sup>TM</sup>)  
209 and stored at -80°C until ready to process. The zooplankton samples were filtered through 200  
210 and 60 µm sieves. Each sample was visually analyzed to determine the dominant species with  
211 dissecting ~~micro~~<sup>micro</sup>scope, and detritus and phytoplankton were removed. The samples were  
212 concentrated on a GF/F filter (47 mm diameter) by mesh size (60, 200 µm), placed on paper  
213 towel to remove excess water, and stored at -80°C until ready to process.

214 Total lipids were extracted with chloroform-methanol (2:1, v/v) containing 0.01%  
215 butylated hydroxytoluene as an antioxidant (Folch et al. 1957). The organic solvent was  
216 evaporated under a stream of nitrogen and lipid concentration determined gravimetrically.  
217 Transmethylation of fatty acids was done according to the method described by Metcalfe and  
218 Schmitz (1969). A known amount of nonadecanoate acid (19:0) dissolved in hexane at a  
219 concentration of 8 mg ~~mL~~<sup>mL</sup><sup>-1</sup> (Nu Check Prep Inc.) was added as internal standard. The fatty  
220 acid methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas  
221 Chromatograph, Agilent Technologies, Inc.) using a 7693 mass spectrometer detector (Agilent  
222 Technologies, Inc.), a capillary column (Omegawax<sup>TM</sup> 250 fused silica capillary column, 30 mm

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223 ~~xx~~ 0.25 mm and 0.25 mm film thickness, Supleco®), and a 7890A autoinjector (Agilent  
224 Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.8 ~~ml~~mL min<sup>-1</sup> and the  
225 injection volume was 2 ~~ml~~mL. Initial temperature of the oven was 175 °C for 26 min, which was  
226 increased to 205 °C by increments of 2 °C min<sup>-1</sup>, then held at 205 °C for 24 min. The source and  
227 analyzer for the mass spectrometer was set at 230 °C. The individual fatty acid methyl esters  
228 were identified by comparing the retention times of authentic standard mixtures (FAME mix 37  
229 components, Supleco) and quantified by comparing their peak areas with that of the internal  
230 standard (Czesny and Dabrowski 1998). The results of individual fatty acid composition are  
231 expressed in percentage of total identified FAME.

232

### 233 *Statistical Analysis*

234 Multivariate statistics were conducted in PRIMER 6 (Clarke and Gorley 2006). Separate,  
235 Bray-Curtis similarity matrices were constructed for the following data sets: microzooplankton  
236 species composition (60 µm mesh), mesozooplankton species composition (200 µm mesh),  
237 seston fatty acid composition, microzooplankton fatty acid composition, and mesozooplankton  
238 fatty acid composition. A separate cluster analysis on each data set similarity matrix was  
239 performed. Each individual sample was associated with a location in the river (upper, middle,  
240 lower) and month (April, May, June) and these labels were used for visualization of samples in  
241 the cluster dendrogram. Groups identified from each cluster analysis were then compared using  
242 analysis of similarity (ANOSIM) to determine if difference between groups were significant at  
243 the  $\alpha$ -level = 0.05 level (Clarke and Gorley 2006). Finally, a similarity percentage analysis  
244 (SIMPER) test was used to compare similarities within groups (Clarke and Gorley 2006). The  
245 SIMPER test was set at 70% cumulative contribution.

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246 All fatty acid percent composition data for seston, microzooplankton, and  
247 mesozooplankton were combined into a single data set using the group designation from  
248 individual cluster analysis to determine correlations using Spearman rank correlation coefficient  
249 using RStudio (RStudio Team 2015). The correlations determine if similar fatty acid profiles are  
250 shown throughout the food web. The results allow us to determine the connections between the  
251 different trophic levels. We used correlations greater than or equal to 0.4, and only positive  
252 correlations.  
253

254 **Results**

255 *Salinity and Temperature*

256 The salinity was near zero (0.02 to 0.04) throughout the river in April. During May,  
257 salinities in the upper river remained low (0.07), but the water column became stratified in the  
258 middle and lower river, with salinities ranging from 1.05-1.66. The river freshened (0.04 to 0.08)  
259 again in June due to a tropical storm that brought heavy rains for a two-week period. North  
260 Carolina experienced the second wettest June since 1895 with rainfall that ranged from 15.2 to  
261 19.05 cm in the study area (Hiatt 2013). Water temperatures increased during the study period  
262 April ( $15.7 \pm 1.1$  °C), May ( $24.0 \pm 1.0$  °C) and June ( $26.2 \pm 0.3$  °C) but showed no differences  
263 between river sections.  
264

265 *Zooplankton Community Composition*

266 Microzooplankton could be separated into 2 distinct groups by cluster analysis at 65%  
267 similarity (Figure 2). The microzooplankton percent composition was statistically significant  
268 showing the two groups differed not by community composition but difference percent among

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Comment [T21]: statistics to prove this

Comment [T22]: where are the results for the zooplankton community composition for the identified taxa, present as a table. Instead of having 3 cluster figures, we all the results can be summed or summarised with 1 figure i.e. cluster analysis with each month showing the 3 river sections

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269 similar group members (ANOSIM,  $p$ -value = 0.005,  $R$  = 0.989). Group 1 consisted of the vast  
270 majority of the samples collected in 2013 across April, May, and June throughout the river  
271 (Figure 2). Group 2 consisted of two samples collected in the middle and lower river in June  
272 (Figure 2). Both groups were dominated by rotifers and copepod nauplii, but group two had a  
273 more even contribution of rotifers and copepod nauplii (Figure 2).

274 Three groups of mesozooplankton were differentiated at 50% similarity using cluster  
275 analysis (Figure 3). The mesozooplankton percent composition was statistically significant  
276 showing the three groups differed (ANOSIM,  $p$ -value = 0.001,  $R$  = 0.798). Group 3 consisted of  
277 samples from the May collection only, Group 4 consisted of a mixture of April and June samples  
278 in primarily the upper and middle river, and Group 5 consisted of one April upper site, May  
279 upper river section and June samples throughout the river (Figure 3). Mesozooplankton percent  
280 community composition was significantly different among the three groups (ANOSIM  $p$ -value =  
281 0.001). Group 3 mesozooplankton percent composition was dominated by *Acartia* spp. with a  
282 minor contribution by Bosminidae, Group 4 consisted primarily of equivalent percentages of  
283 Cyclopodia and Bosminidae, and Group 5 mesozooplankton percent community composition  
284 was characterized by higher percentages of *Leptodora* spp. and Calanoida (Figure 3).

## 286 Fatty Acid Composition

287 A total of 24 specific fatty acids were found in all samples (Tables 1A-3A). Fatty acids  
288 were first separated into broad categories: saturated fatty acids (SFA), monounsaturated fatty acids  
289 (MUFA), and polyunsaturated fatty acids (PUFA) (Figure 4a). Seston had a higher percent of  
290 SFA, and lower percent of MUFA and PUFA compared to micro- and mesozooplankton (Figure  
291 4a). Mesozooplankton and microzooplankton had a similar percent composition of SFA, MUFA,  
292 and PUFA (Figure 4a). There were eight dominant fatty acids found in all the samples, but the

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Comment [T23]: all sampled were collected in 2013, do you mean upper or all river sections excluding june mid and low

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Comment [T24]: this section should have subheadings for seston, zooplankton and larval fish. These results could be better presented using combined data for sites in an NMDS plot and a simple cluster plot. Why were similarities carried out at different levels i.e. one level will have sufficed. THIS SECTION ALSO NEEDS MAJOR IMPROVEMENTS. QUANTITATIVE RATHER THAN QUALITATIVE DATA WILL HAVE TOLD A BETTER STORY HERE

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percent composition varied (Fig 4b). The most common SFA was palmitic acid (16:0) (data not shown), the most common MUFAs were palmitoleic acid (16:1n-7) and oleic acid (18:1n-9), and the most common PUFAs were ALA (18:3n-3), 18:4n-3, EPA (20:5n-3), and DHA (22:6n-3) (Figure 4b; Tables A1-A3). A comparison of MUFAs and PUFAs among the seston and zooplankton showed that seston had the lowest overall percentages of MUFAs and PUFAs (Figure 4b). The microzooplankton fatty acid profile was characterized by a higher percentage of 18:1n-9 compared to the other MUFAs and PUFAs. In contrast, the mesozooplankton had the highest percent composition attributed to two PUFAs, EPA and DHA (Figure 4b).

Three groups were designated at 60% similarity using cluster analysis for the seston fatty acid composition (Figure 5a). There was a significance difference among these three groups for seston fatty acid composition using ANOSIM (Global R= 0.611, p-value= 0.001). The groups showed no distinct pattern in term of sampling time or location (Figure 5a). Seston fatty acid composition of Group A was characterized by 18:1n-9, 16:1n-7, ALA, EPA, Group B by 16:1n-7, 18:1n-9, 18:2n-6 and EPA, and Group C had similar percentage composition of MUFAs and PUFAs, except 18:2n-6 (Figure 5b and Table A4).

Three groups were designated at 70% similarity using cluster analysis for the microzooplankton fatty acid composition (Figure 6a). There was a significant difference among groups for microzooplankton fatty acid composition (ANOSIM, Global R= 0.997, p-value= 0.001). The groups segregated temporally, with Group D consisting of April samples only as did Group E, and Group F consisted of May and June samples only (Figure 6a). The Group D fatty acids were dominated by 18:1n-9 and to a lesser extent, 18:2n-6, Group E showed similar percent composition among the fatty acids, with higher percentages of 18:1n-9 and 20:5n-3

315 (EPA), and Group F also had similar percent composition of fatty acids; however, the PUFAs  
 316 20:5n-3 (EPA) and 22:6n-3 (DHA) had significant contribution (Figure 6b and Table A5).  
 317 Four groups were designated by cluster analysis at 77% similarity for the  
 318 mesozooplankton fatty acid composition (Figure 7a). There was a significance difference  
 319 between the four groups for mesozooplankton fatty acid composition using ANOSIM (Global  
 320  $R = 0.765$ ,  $p\text{-value} = 0.001$ ). Groups G and I consisted of April samples only whereas Group J  
 321 consisted of May and June samples only (Figure 7a). Group H showed spatial separation,  
 322 consisting on primarily upper river locations across all of the months (Figure 7a).  
 323 Mesozooplankton fatty acid composition for Group G was not significantly different compared  
 324 to Group H ( $p\text{-value} = 0.167$ , and  $0.143$ ) and all other groups were significantly different from  
 325 one another ( $p\text{-value} < 0.005$ ). The Group G and H fatty acids were dominated by 18:1n-9, Group  
 326 8 by 16:1n-7, 18:1n-9, and 20:5n-3 (EPA), Group I had similar percentages of fatty acids with  
 327 18:3n-3 (ALA), 20:5n-3 (DHA) and 22:6n-3 (EPA) having higher percentages, and Group J had  
 328 20:5n-3 (DHA) and 22:6n-3 (EPA) as dominant components of the fatty acids (Figure 7b and  
 329 Table A6).  
 330 The seston fatty acid composition (group A) in April from lower, middle, and upper  
 331 sections and June was correlated to microzooplankton fatty acid composition (Group D) in April  
 332 from lower, middle, and upper sections (Figure 8 and Table 1). The seston fatty acid  
 333 composition (Group B) in May from the lower and middle section and June from lower and  
 334 upper sections correlated to the microzooplankton fatty acid composition (Group F) in May from  
 335 middle and lower sections, and June from upper, middle and lower sections, and the  
 336 mesozooplankton fatty acid composition (Groups H, G, J) in April from lower section, May and  
 337 June from the middle and lower sections (Figure 8 and Table 1). The microzooplankton fatty

338 acid composition (Group F) correlated to the mesozooplankton fatty acid profiles (Groups H, I,  
339 J) in April, May and June for the upper, middle and lower sections (Figure 8 and Table 1). The  
340 seston fatty acid composition (Group C) in April from upper, middle and lower, and May from  
341 upper sections correlates to microzooplankton fatty acid composition (Group F) in May from  
342 middle and lower sections, and June from upper, middle and lower sections, and  
343 mesozooplankton fatty acid composition (Groups H, I, J) in April, May and June from all  
344 sections (Figure 8 and Table 1). The microzooplankton fatty acid composition (Group E) in April  
345 from lower and upper sections correlated to mesozooplankton fatty acid composition (Group J)  
346 in May and June from all sections (Figure 8 and Table 1). The microzooplankton fatty acid  
347 composition (Group F) in May from middle and lower sections, and June from upper, middle and  
348 lower sections correlated to mesozooplankton fatty acid composition (Group H, I, J) in April,  
349 May and June from all sections (Figure 8 and Table 1).

## 351 Discussion

352 The fatty acid (FA) dynamics observed in the Chowan River changed dramatically in  
353 association with physical variability in the water column, most notably salinity. Prior to the  
354 salinity intrusion, larval fish experienced a food web primarily based on a freshwater plankton  
355 assemblage that was proportionally higher in EPA relative to DHA (Figure 6, Group E; Figure 7,  
356 Group I). During the salinity intrusion, the proportion of DHA increased and remained elevated  
357 into June (Figure 6, Group F; Figure 7, Group J). The intrusion of saline water increased the  
358 overall proportion of omega-3 fatty acids in the River, presumably due to the increased fraction  
359 of micro- and mesozooplankton feeding on a more marine-like phytoplankton based food-web  
360 (Figure 4) and this signal propagated through the food web (Figure 8). Additionally, we observed

**Comment [T26]: DISCUSSION IS VERY LONG, IT NEEDS TO BE STREAMLINED AND ONLY FOCUS ON THE MAIN RESULTS**

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**Comment [T27]: CORRELATION OR REGRESSION SHOULD HAVE BEEN CARRIED OUT TO PROVE THESE FINDINGS**



361 that FA appeared to be incorporated relatively unchanged in micro and mesozooplankton in  
362 terms of relative composition; however, MUFA and PUFA percent compositions increased in  
363 zooplankton relative to seston. This suggested that MUFA and PUFA are bioaccumulated at  
364 higher trophic levels, as seen in other studies (Persson and Vrede 2006; Gladyshev et al. 2010;  
365 Ravett, Brett, Arhonditsis 2010; Burns, Brett, and Schallenberg 2011). Overall, the FA  
366 composition of the food web indicated that the Chowan River is likely to provide adequate  
367 nutrition in terms of FA composition for larval fish growth and development. This is based on  
368 the presence of significant proportions of PUFA present in the mesozooplankton throughout the  
369 nursery (Figure 6, 7).

**Comment [T28]:** WHY, YOU EXPECT THE MUFA AND PUFA TO BE HIGH IN SESTON THAN IN HIGH TROPHIC LEVELS

370 The seston fatty acid composition consisted mainly of saturated fatty acids (Figure 4a).  
371 Seston from freshwater and estuarine systems typically has a large percentage of SFA and this  
372 fraction has been attributed to detrital input, as opposed to originating from phytoplankton  
373 (Persson and Vrede 2006; Gladyshev et al. 2010; Ravett, Brett, Arhonditsis 2010; Burns, Brett,  
374 and Schallenberg 2011; Gonclaves et al 2012), Müller-Navarra et al. (2004) and Bec et al. (2010)  
375 analyzed seston and found phytoplankton only explained 27% of variance in FA composition  
376 and concluded that detritus and heterotrophic organisms also needed to be considered (Müller-  
377 Navarra et al. 2004; Bec et al. 2010). Bec et al (2010) therefore concluded that the seston can  
378 affect the fatty acid profiles of higher organisms, but may not relate individual groups of  
379 phytoplankton or microzooplankton. This agreed with our findings as seen in the reduced  
380 correlations between seston and the rest of the food web (Figure 8).

**Comment [T29]:** WHY

381 We did not examine the seston composition directly by counting phytoplankton cells or  
382 examining pigment concentrations, thus we were unable to attribute the origin of particular fatty  
383 acids to phytoplankton or other sources. However, we were able to use the available literature to

384 identify potential indicators of fatty acid origin. The top three fatty acids by percent composition  
385 varied by group, but 16:1n-7, 18:1n-9, 18:2n-6, and 18:3n-3 (ALA) were the most prevalent  
386 (Figure 5). Potential phytoplankton sources for these fatty acids may be diatoms, which have  
387 been shown to have increased 16:1n-7 and EPA in both freshwater and marine systems  
388 (Napolitano et al. 1997; Dalsgaard et al. 2003; Boschker et al. 2005; Arts et al. 2009; Bec et al.  
389 2010) and we observed this occurred in May and June (Figure 5, Group B, C), coincident with  
390 the salinity increase. Green algae have been shown to possess higher proportions of 18:2n-6, and  
391 ALA (Ahlgren et al. 1990; Dalsgaard et al. 2003; Boschker et al. 2005; Masclaux et al. 2012;  
392 Strandberg et al. 2015) and chlorophytes have fatty acid profiles higher in 18:2n-6, 18:3n-3, and  
393 18:4n-3 (Ahlgren et al. 1990; Dalsgaard et al. 2003; Boschker et al. 2005; Arts et al. 2009;  
394 Masclaux et al. 2012). Fatty acids corresponding to these phytoplankton groups were observed  
395 during April throughout the river and June in the middle and upper river. (Figure 5, Group A and  
396 C). One other sources of seston FA may be have been present, pine pollen, which is found in  
397 large quantities during spring. Pine pollen is transported to freshwater systems via aeolian  
398 deposition and floats at the surface (Masclaux et al. 2013). The fatty acid profile of pine pollen  
399 has a high percent composition of 18:1n-9 and 18:2n-6 (Masclaux et al. 2013), which can be  
400 observed in Figure 5, Group A. Obviously, seston FA are a mixture of multiple sources, thus the  
401 variability seen across the groups identified by the cluster analysis would be expected (Figure 5).

402       The fatty acid profiles were different throughout the sampling period with a change from  
403 decreased omega-3s to increased omega-3s in the system (Figure 6). This suggests a switch in  
404 microzooplankton diet had occurred over the sampling period and two pathways appear to be  
405 present during the study. The April microzooplankton fatty acid profiles for all river sections had  
406 a high percentage of 18:1n-9 and 18:2n-6 (Figure 6, Group D suggesting that the

407 microzooplankton could be consuming either terrestrial material or chlorophytes during this  
408 time. Two sites in April had an increase in omega-3s fatty acids (ALA, 18:4n-3, EPA, DHA)  
409 (Figure 6, Group E) and this would suggest a different dietary pathway that was reduced in SFA,  
410 perhaps consisting of either smaller microzooplankton such as ciliates or phytoplankton such as  
411 diatoms and/or dinoflagellates (Park and Marshall 2000; Gladyshev et al. 2010). The community  
412 was dominated by rotifers during this time and communities high in rotifer abundance have been  
413 shown to reflect the seston composition closely (Gladyshev et al. 2010). The microzooplankton  
414 fatty acid profiles in May and June at all river locations had an increase in 16:1n-7, and omega-  
415 3s (ALA, EPA, and DHA) (Figure 6, Group F). These changes can be directly related to the  
416 saltwater intrusion event in May and the likely increase in more marine algal sources for FAs.  
417 The changes in June could be the increased in copepod nauplii of Calanoid copepods (Figure 2,  
418 Group 2), and the presence of dinoflagellates and diatoms even when the system returned to  
419 freshwater. Our results are similar to systems where the phytoplankton composition was  
420 represented by diatoms and dinoflagellates by having increased 16:1n-7 and PUFAs (Müller-  
421 Navara et al. 2000; Dalsgaard et al. 2003; Gladyshev et al. 2010; Ravett, Brett, Arhonditsis  
422 2010).

423       The mesozooplankton fatty acid profiles throughout the river in April and in the upper  
424 river in May and June were defined by higher percentages of 16:1n-7, 18:1n-9, ALA, EPA, and  
425 DHA (Figure 7). These fatty acids profiles are similar to ones found in freshwater systems that  
426 have a mixed zooplankton composition (Persson and Verde 2006; Arts et al. 2009; Kainz et al.  
427 2009; Gladyshev et al 2010; Burns et al. 2011; Masclaux et al. 2012). This mirrored our species  
428 composition at these times and locations as the mesozooplankton community consisted of  
429 freshwater cladocerans and cyclopedia (Figure 3). Cladocerans have low or no DHA compared

430 to copepods and high EPA levels have been shown to correlate with the high somatic growth  
431 rates of cladocerans (Persson and Verde 2006). A saltwater intrusion changed the  
432 mesozooplankton species composition in May resulting in numerical dominance by *Acartia* spp.  
433 in the lower and middle sections of the river. *Acartia* spp. is the dominant copepod species in  
434 temperate, estuarine systems (Ambler et al. 1985; Orsi 1986; Cervetto et al. 1999; Mouny and  
435 Dauvin 2002; Kimmel and Roman 2004; Lawrence et al. 2004; Islam et al. 2005). The  
436 mesozooplankton fatty acid profiles in May were represented by 16:1n-7, EPA, and the highest  
437 observed percentages of DHA. This is clearly a reflection of the dominance of *Acartia* spp. in  
438 the system and a diet primarily consisting of marine algae higher in omega-3 FAs (Stottrup et al.  
439 1999; Persson and Verde 2006; Arts et al. 2009; Kainz et al. 2009; Gladyshev et al 2010;  
440 Masclaux et al. 2012). Mesozooplankton fatty acid percent composition in June at the lower and  
441 middle site remained similar to that observed in May, despite the species composition having  
442 returned to a mix of cladocerans and copepods (Figure 3). We saw a similar fatty acid profile in  
443 June as observed in May. This suggests that physical shifts in the system that result in seston  
444 changes may persist in the system despite shifts in zooplankton community composition.

445         The seston fatty acid profile correlated to the fatty acid profiles of the micro and  
446 mesozooplankton (Figure 8, Table 1). It was clear that seston Group A was only linked to  
447 microzooplankton Group D and this link was only present in April in the middle and upper  
448 portion of the river (Figure 8). This linkage was likely detrital in nature, as discussed above. The  
449 remaining correlation suggest links that follow two distinct paths that become clear at the  
450 mesozooplankton level. Mesozooplankton Group H showed strong correlations to seston groups  
451 B and C (Figure 8). Based on fatty acid composition (Figure 7), this group was higher in EPA  
452 versus DHA and this also agreed with the species composition data that showed a community

453 that consisted of Bosminidae cladocerans and freshwater calanoid copepods (Figure 3). The  
454 other pathway was correlated with the saltwater intrusion event and mesozooplankton Group J  
455 was correlated to microzooplankton Group F and seston Group B (Figure 8). The  
456 mesozooplankton community consisted primarily of the estuarine calanoid *Acartia* spp. (Figure  
457 3) and had a fatty acid signature with increased proportion of DHA relative to EPA (Figure 5).  
458 Our findings agree with laboratory and field studies across natural freshwater, brackish, and  
459 marine systems (Goulden and Place 1990; Brett et al. 2006; Rossi et al. 2006; Arts et al. 2009;  
460 Gladyshev et al 2010; Ravet, Brett, Arhonditis 2010). Gladyshev et al. (2010) found a similar  
461 correlation between the seston, especially the producer groups, and the consumers in a Siberian  
462 reservoir. Laboratory studies of mesozooplankton feeding on phytoplankton monocultures had  
463 similar trends for fatty acid profiles between the phytoplankton and consumers with an increase  
464 of PUFAs in the mesozooplankton (Brett et al. 2006; Arts et al. 2009).

465         The relevance of the food web fatty acid composition can be determined by examining  
466 the potential feeding behavior of larval fish within the Chowan River nursery. Alewife and  
467 blueback herring start feeding on smaller cladocerans and copepods at about 6 mm total length  
468 (Mullen et al. 1986). Binon (2011) reported that river herring at 6 mm notochord length had a  
469 maximum gape width of 400  $\mu$ m, and estimated maximum prey size of 200  $\mu$ m.  
470 Mesozooplankton were an important food resource for river herring <6 mm, and diets consisted  
471 of Bosminidae, rotifers, and copepod nauplii (Binon 2011). Alewife grew larger than blueback  
472 herring and over time began to feed selectively, whereas blueback herring continued to filter feed  
473 (Mullen et al. 1986). In the Connecticut River, the diet for blueback herring were dominated by  
474 rotifers for fish 5-12 mm, Bosminids for fish 12-16+ mm, and cyclopoid copepods for fish  
475 16+mm in total length (Crecco and Blake 1983). Based on these dietary studies, the larval and

476 juvenile river herring would be feeding across the size range of zooplankton prey that we  
477 sampled; however, fish would be consuming primarily microzooplankton early in the year  
478 (April) and mesozooplankton later in the season (May and June). In April, two pathways for FA  
479 propagation were present in the microzooplankton (Figure 6; Groups 4 and 5). Thus, fish feeding  
480 during this time may experience variability in the quality of the microzooplankton prey in terms  
481 of percentage of PUFAs (Figure 6). Larval fish need PUFAs (ALA, EPA and DHA) for growth,  
482 visual acuity, survival, and development of normal pigmentation (Bell et al. 1995; Bell and  
483 Sargent 1996; Rainuzzo et al. 1997; Sargent et al. 1999; Rossi et al. 2006). The shift to larger  
484 prey later in the year (May and June) resulted in a change in prey quality as the relative  
485 percentage of EPA and DHA increased. This was the result of a salinity intrusion into the middle  
486 and lower reaches of the estuary that was associated with dominance of the cladoceran *Acartia*  
487 spp. (Figure 3) and a significant increase in DHA and EPA (Figure 7; Group 10). This could  
488 allow larval and juvenile fish to consume prey with a higher proportion of PUFAs. It is unknown  
489 if river herring can elongate precursor FA into PUFAs. Even if fish can convert precursor FA,  
490 larval fish could not receive all nutritional need for those fatty acids (Agaba et al. 2005). The  
491 larval fish would not have to use energy for the conversion, and continue to put energy to growth  
492 (Wacker and von Elert 2001; Rossi et al 2006). This allows the larval fish survive and grow past  
493 the critical period.

494         The fish nursery present in the lower Chowan River may undergo significant changes  
495 during the critical time of larval fish growth. Our results demonstrate how changes in the seston  
496 community may propagate through the food-web. They also highlight that additional information  
497 concerning the fatty acid composition of the zooplankton prey base for larval fish can provide  
498 insight into habitat quality. Sheaves et al. (2004) pointed out the need to expand the nursery

499 habitat concept to include relevant ecosystem processes, particularly resource dynamics. This  
500 research begins to explore the mechanisms that allow nursery habitat to function. We plan  
501 further research to investigate the linkage between the seston community fatty acid composition,  
502 the zooplankton community fatty acid composition, and larval fish to determine how lower food  
503 web variability relates to larval fish condition and survival.

504

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744 **Tables**

745 **Table 1.** Spearman correlations for the seston (Groups A-C), microzooplankton (Groups D-F),  
 746 and mesozooplankton (Groups G-J). Italicized numbers = <0.05, and Bold numbers = <0.0001

	A	B	C	D	E	F	G	H	I
A	1								
B	0.64	1							
C	0.21	0.46	1						
D	0.42	-0.07	-0.04	1					
E	0.04	-0.07	0.36	<i>0.75</i>	1				
F	-0.11	0.59	0.55	-0.47	-0.14	1			
G	0.39	0.54	0.11	0.29	0.25	0.38	1		
H	0.5	0.82	<i>0.75</i>	0.21	0.36	<i>0.67</i>	0.54	1	
I	-0.53	-0.04	<i>0.68</i>	-0.25	0.36	<i>0.63</i>	0	0.36	1
J	-0.04	0.61	0.54	-0.43	0.75	<b>0.99</b>	0.43	<i>0.68</i>	<i>0.57</i>

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**Comment [T30]:** The black part should be white

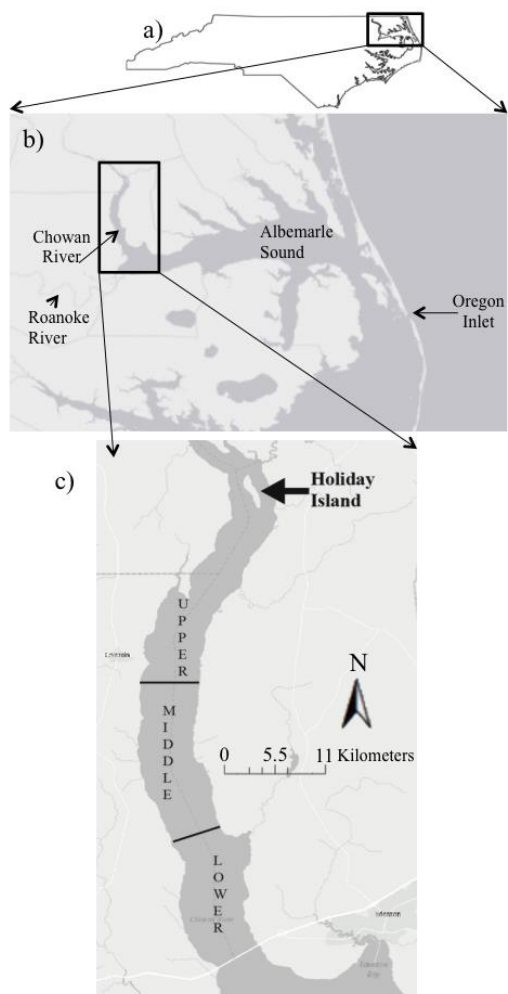
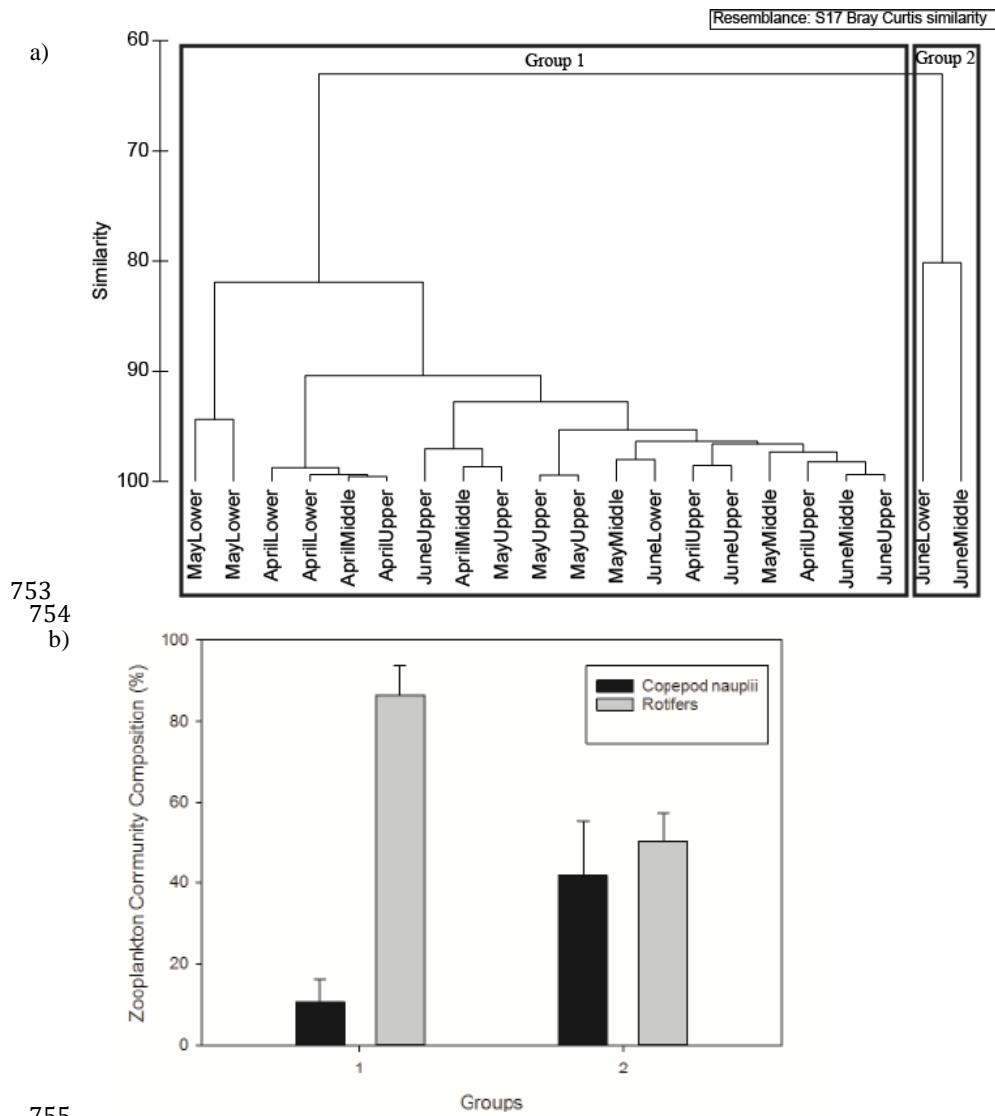


Figure 1. The overview of Albemarle Sound in North Carolina (a). The close up view of the location for two main tributaries (Chowan and Roanoke Rivers), and the Albemarle Sound in North Carolina (b). The three sections used to collect zooplankton on the Chowan River (c).



756 Figure 2. The two microzooplankton community composition groups from cluster analysis (a) at  
757 65% similarity. The mean microzooplankton community composition (%  $\pm$  S.D.) for the two  
758 groups from cluster analysis (b).

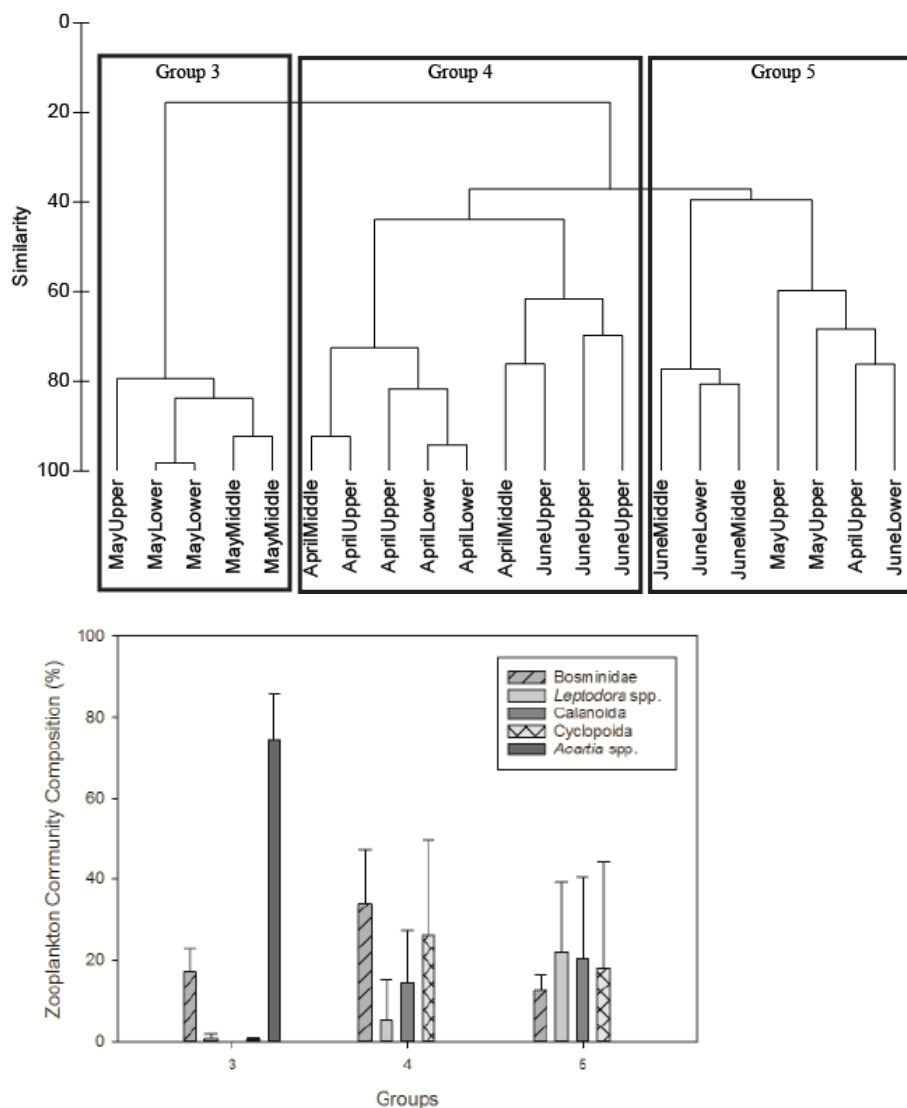


Figure 3. The three mesozooplankton community composition groups from cluster analysis (a) at 50% similarity. The mean mesozooplankton community composition (%  $\pm$  S.D.) for the three groups from cluster analysis (b).

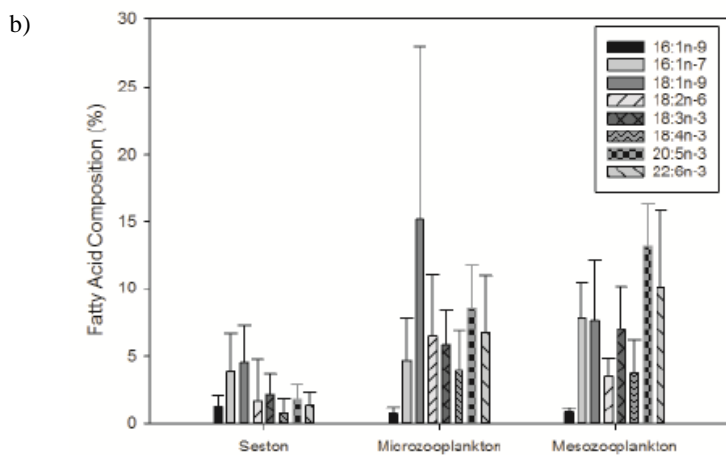
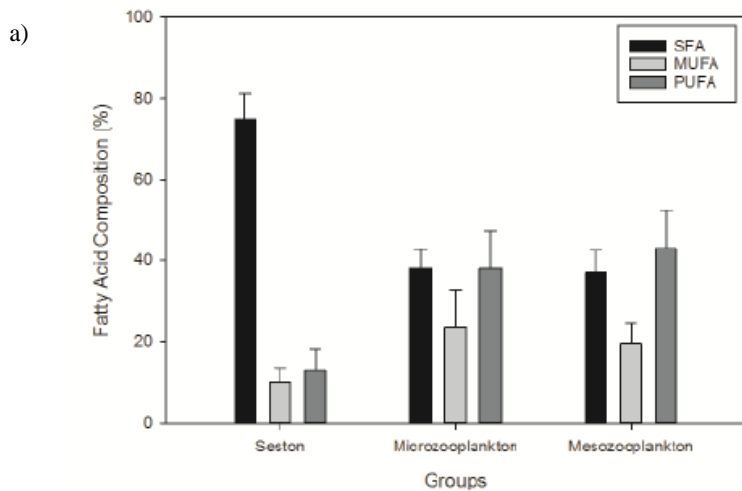


Figure 4. The mean ( $\pm$  S.D.) saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid composition (%) for the seston, microzooplankton, and mesozooplankton (a). The mean fatty acid composition ( $\pm$  S.D.) for the seston, microzooplankton, and mesozooplankton (b).

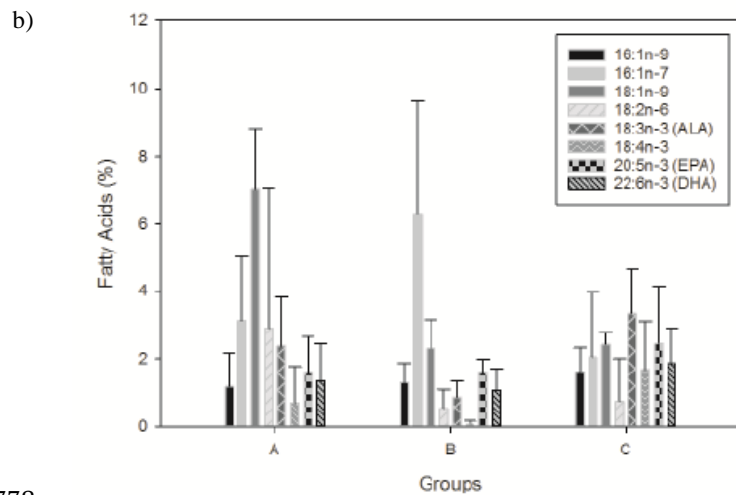
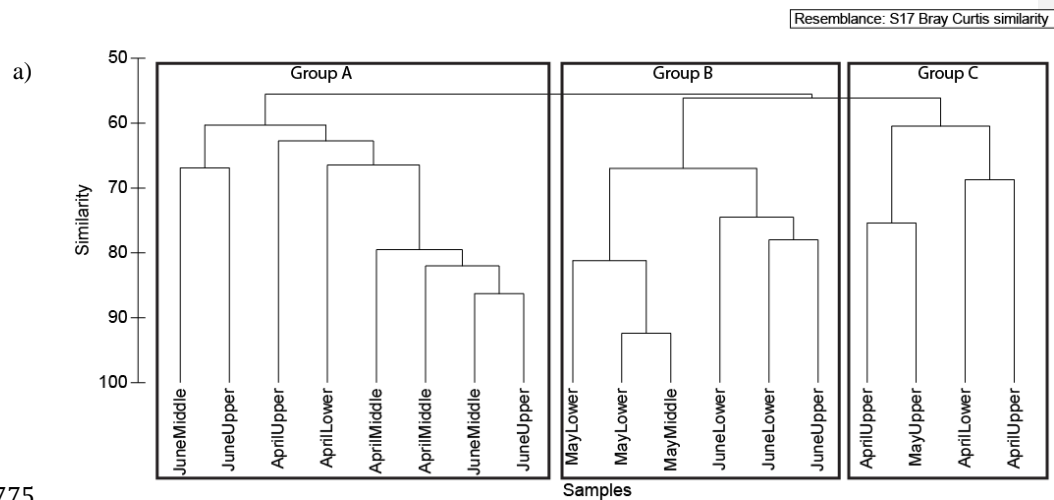
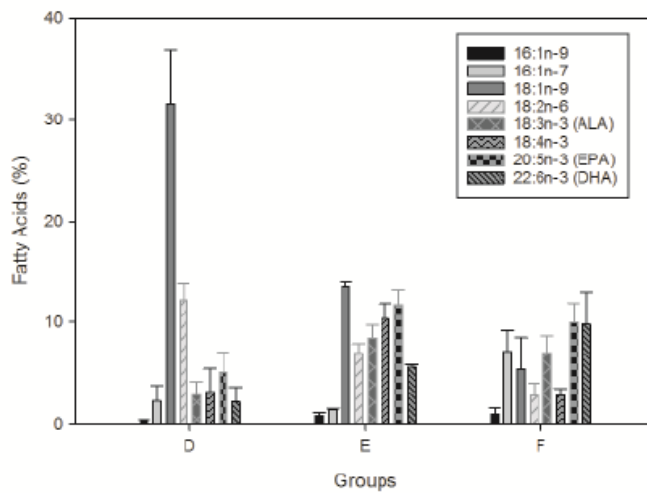
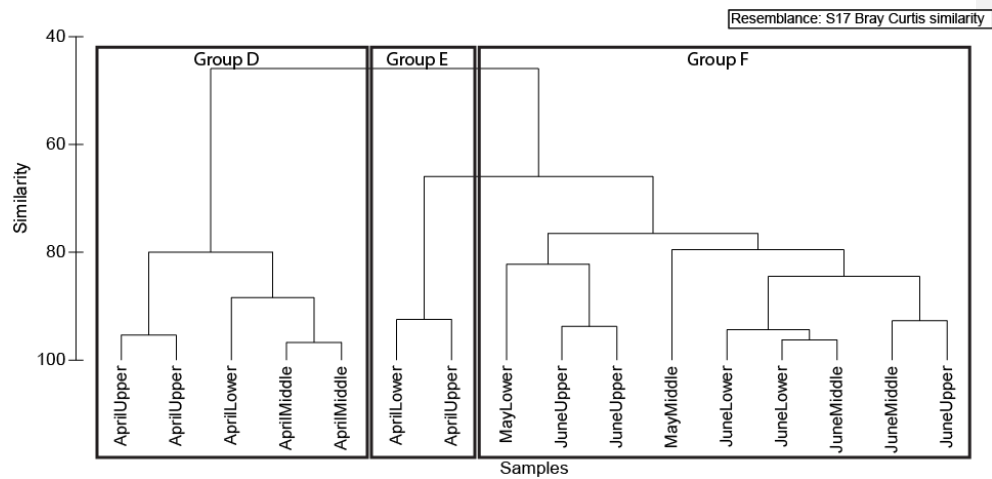


Figure 5. The three seston fatty acid composition groups from cluster analysis (a) at 60% similarity. The mean seston fatty acid composition ( $\pm$  S.D.) for the three groups from cluster analysis (b). The letters above the graphs represent significance differences using ANOSIM ( $p < 0.05$ ).



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Figure 6. The three microzooplankton fatty acid composition groups from cluster analysis (a) at 60% similarity. The mean microzooplankton fatty acid composition (% ,  $\pm$  S.D.) for the three groups from cluster analysis (b). The letters above the graphs represent significance differences using ANOSIM ( $p < 0.05$ ).



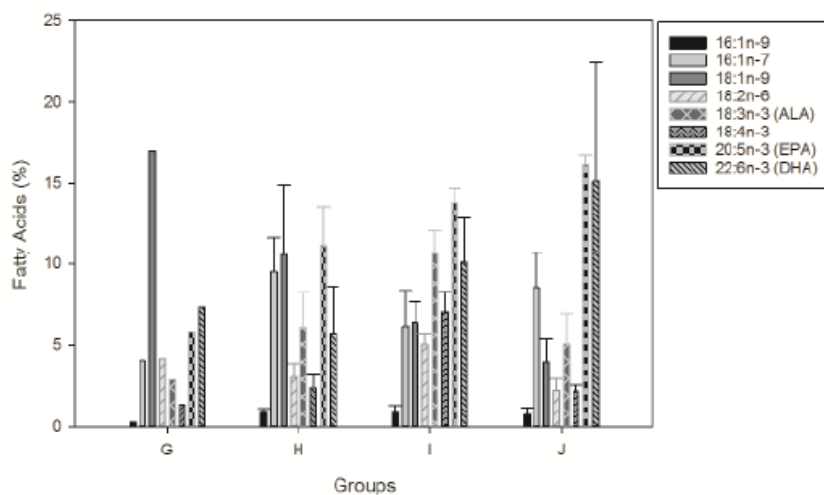
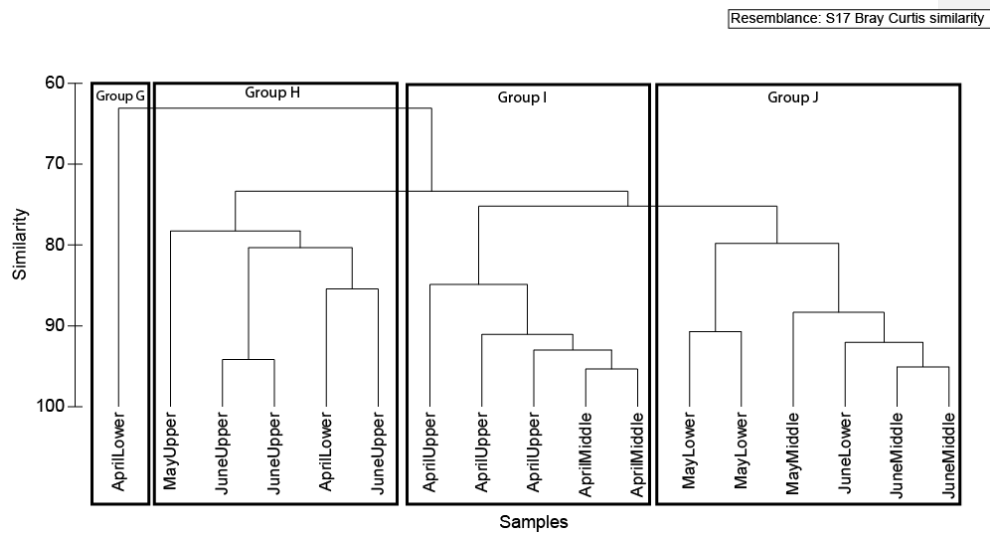


Figure 7. The four mesozooplankton fatty acid composition groups from cluster analysis (a) at 60% similarity. The mean mesozooplankton fatty acid composition (% ,  $\pm$  S.D.) for the four groups from cluster analysis (b). The letters above the graphs represent significance differences using ANOSIM ( $p < 0.05$ ).

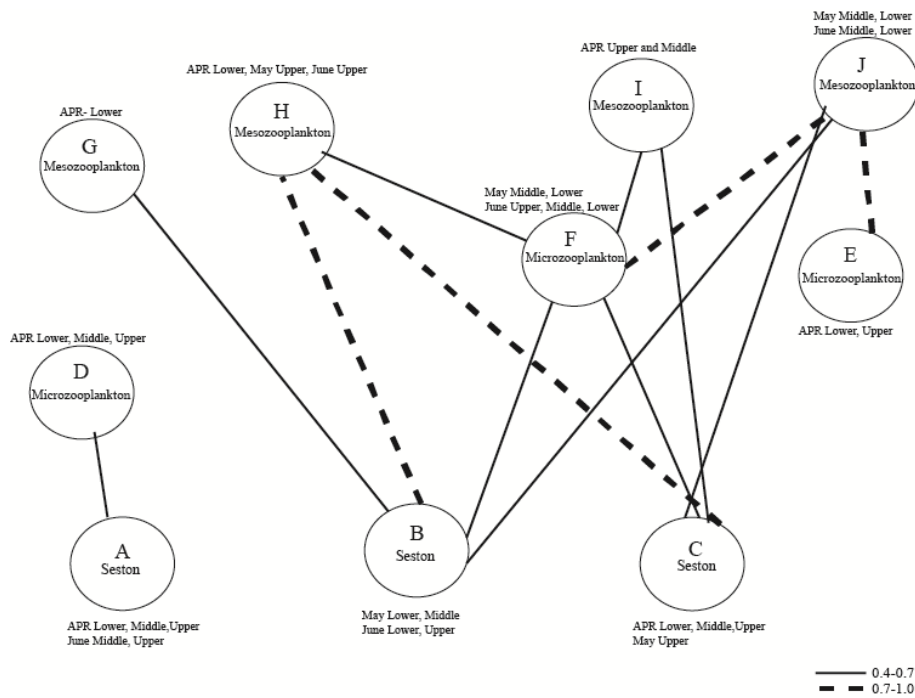


Figure 8. The seston, microzooplankton, and mesozooplankton groups correlated using Spearman correlation. The letters represent the group designation from individual cluster analysis. Straight line shows correlations between 0.4 and 0.7, and weighted dash line shows correlations between 0.7 and 1.0.

**Comment [T31]:** The line between C and J should be corrected and it should not cross others like that

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824 **Appendix**

825 Table A1: Mean fatty acid composition ( $\pm$  standard deviation) (percentage of total fatty acids  
826 detected) of seston from the Chowan River by group. SFA: saturated fatty acids, MUFA:  
827 monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	Seston Group		
	A (8)	B (5)	C (4)
14:0	6.6 $\pm$ 1.8	11.2 $\pm$ 5.1	7.6 $\pm$ 1.0
15:0	1.5 $\pm$ 1.5	1.3 $\pm$ 0.4	1.7 $\pm$ 0.3
16:0	47.7 $\pm$ 5.5	54.0 $\pm$ 5.1	50.8 $\pm$ 4.4
17:0	2.3 $\pm$ 0.4	2.7 $\pm$ 0.7	2.6 $\pm$ 0.2
18:0	14.2 $\pm$ 5.6	9.7 $\pm$ 1.2	11.4 $\pm$ 2.5
20:0	0.4 $\pm$ 0.3	0.3 $\pm$ 0.1	0.4 $\pm$ 0.2
<b><math>\Sigma</math>SFA</b>	<b>72.7</b>	<b>79.2</b>	<b>74.0</b>
16:1n-9	1.2 $\pm$ 1.0	1.3 $\pm$ 0.6	1.6 $\pm$ 0.7
16:1n-7	3.1 $\pm$ 1.9	6.3 $\pm$ 3.3	2.1 $\pm$ 1.9
18:1n-9	7.0 $\pm$ 1.8	2.3 $\pm$ 0.8	2.4 $\pm$ 0.3
18:1n-7	0.3 $\pm$ 0.2	0.1 $\pm$ 0.2	0.1 $\pm$ 0.1
20:1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.2 $\pm$ 0.1
<b><math>\Sigma</math>MUFA</b>	<b>11.7</b>	<b>10.1</b>	<b>6.4</b>
18:2n-6	2.9 $\pm$ 4.2	0.6 $\pm$ 0.6	0.8 $\pm$ 1.2
18:3n-3	2.4 $\pm$ 1.4	0.9 $\pm$ 0.5	3.3 $\pm$ 1.3
18:4n-3	0.7 $\pm$ 1.1	0.1 $\pm$ 0.1	1.7 $\pm$ 1.4
20:2n-6	0.5 $\pm$ 0.4	0.2 $\pm$ 0.2	0.7 $\pm$ 0.5
20:3n-6	0.4 $\pm$ 0.1	0.4 $\pm$ 0.3	0.3 $\pm$ 0.2
20:4n-6	0.9 $\pm$ 0.6	0.8 $\pm$ 0.4	0.8 $\pm$ 0.5
20:3n-3	0.6 $\pm$ 0.3	0.4 $\pm$ 0.3	1.1 $\pm$ 0.6
20:4n-3	0.6 $\pm$ 1.4	0.5 $\pm$ 0.3	2.3 $\pm$ 1.9
20:5n-3	1.6 $\pm$ 1.1	1.6 $\pm$ 0.4	2.4 $\pm$ 1.7
22:5n-6	0.7 $\pm$ 0.5	0.8 $\pm$ 0.8	1.2 $\pm$ 0.6
22:5n-3	0.8 $\pm$ 0.6	0.7 $\pm$ 0.6	1.2 $\pm$ 0.7
22:6n-3	1.4 $\pm$ 1.1	1.1 $\pm$ 0.6	1.9 $\pm$ 1.0
<b><math>\Sigma</math>PUFA</b>	<b>13.5</b>	<b>8.1</b>	<b>17.7</b>

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838 Table A2: Mean fatty acid composition (percentage of total fatty acids detected) of  
839 microzooplankton (<60µm) from the Chowan River by groups. SFA: saturated fatty acids,  
840 MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	Microzooplankton Groups		
	D (5)	E (2)	F (8)
14:0	2.6 ± 0.5	3.7 ± 0.9	5.9 ± 1.1
15:0	0.3 ± 0.1	0.5 ± 0.2	1.1 ± 0.3
16:0	27.4 ± 3.0	24.5 ± 0.3	23.5 ± 3.4
17:0	0.4 ± 0.1	0.6 ± 0.0	1.2 ± 0.3
18:0	3.4 ± 0.8	3.4 ± 0.0	7.1 ± 0.3
20:0	1.8 ± 0.4	0.8 ± 0.1	0.2 ± 0.1
ΣSFA	<b>35.9</b>	<b>33.5</b>	<b>39.0</b>
16:1n-9	0.3 ± 0.1	0.8 ± 0.3	0.9 ± 0.6
16:1n-7	2.2 ± 1.6	1.4 ± 0.1	7.1 ± 2.0
18:1n-9	31.6 ± 5.3	13.5 ± 0.5	5.3 ± 3.1
18:1n-7	0.7 ± 0.8	0.8 ± 0.2	1.9 ± 0.5
20:1	0.6 ± 0.2	0.8 ± 0.1	1.4 ± 0.6
ΣMUFA	<b>35.4</b>	<b>17.3</b>	<b>16.6</b>
18:2n-6	12.2 ± 1.6	6.9 ± 0.9	2.8 ± 1.2
18:3n-3	2.9 ± 1.3	8.4 ± 1.3	7.0 ± 1.7
18:4n-3	3.2 ± 2.3	10.3 ± 1.5	2.8 ± 0.7
20:2n-6	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
20:3n-6	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
20:4n-6	0.6 ± 0.7	0.4 ± 0.1	3.2 ± 0.5
20:3n-3	0.3 ± 0.1	1.0 ± 0.2	0.4 ± 0.1
20:4n-3	1.3 ± 0.4	2.8 ± 0.1	2.1 ± 0.3
20:5n-3	5.0 ± 2.0	11.7 ± 1.4	9.9 ± 2.0
22:5n-6	0.2 ± 0.2	1.0 ± 0.1	2.3 ± 0.9
22:5n-3	0.2 ± 0.2	0.2 ± 0.1	1.9 ± 1.1
22:6n-3	2.1 ± 1.5	5.6 ± 0.3	9.8 ± 3.2
ΣPUFA	<b>28.5</b>	<b>48.9</b>	<b>42.7</b>

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 855 Table A3: Mean fatty acid composition ( $\pm$  standard deviation) (percentage of total fatty acids  
 856 detected) of mesozooplankton from the Chowan River by group. SFA: saturated fatty acids,  
 857 MUFA: monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

	Mesozooplankton Groups			
	G (1)	H (5)	I (5)	J (5)
14:0	4.5	5.4 $\pm$ 0.9	5.2 $\pm$ 1.1	5.1 $\pm$ 1.1
15:0	1.1	1.1 $\pm$ 0.4	0.7 $\pm$ 0.0	0.9 $\pm$ 0.1
16:0	29.7	24.4 $\pm$ 2.6	19.5 $\pm$ 1.3	22.0 $\pm$ 1.5
17:0	1.3	1.4 $\pm$ 0.4	1.0 $\pm$ 0.1	1.4 $\pm$ 0.3
18:0	11.2	7.7 $\pm$ 1.2	5.1 $\pm$ 0.3	6.8 $\pm$ 0.7
20:0	0.4	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.1 $\pm$ 0.1
$\Sigma$ SFA	<b>48.2</b>	<b>40.2</b>	<b>31.7</b>	<b>36.3</b>
16:1n-9	0.3	0.9 $\pm$ 0.2	0.9 $\pm$ 0.4	0.8 $\pm$ 0.4
16:1n-7	4.1	9.5 $\pm$ 2.2	6.2 $\pm$ 2.2	8.6 $\pm$ 2.2
18:1n-9	17.0	10.6 $\pm$ 4.3	6.4 $\pm$ 1.2	4.0 $\pm$ 1.4
18:1n-7	2.1	3.9 $\pm$ 1.7	2.4 $\pm$ 0.4	3.0 $\pm$ 1.1
20:1	0.3	0.1 $\pm$ 0.1	0.3 $\pm$ 0.1	0.2 $\pm$ 0.1
$\Sigma$ MUFA	<b>23.8</b>	<b>25.0</b>	<b>16.2</b>	<b>16.6</b>
18:2n-6	4.2	3.0 $\pm$ 0.9	5.1 $\pm$ 0.6	2.2 $\pm$ 0.7
18:3n-3	2.9	6.1 $\pm$ 2.1	10.7 $\pm$ 1.5	5.1 $\pm$ 1.9
18:4n-3	1.4	2.4 $\pm$ 0.8	7.1 $\pm$ 1.2	2.2 $\pm$ 0.4
20:2n-6	0.2	0.2 $\pm$ 0.1	0.4 $\pm$ 0.1	0.2 $\pm$ 0.0
20:3n-6	0.1	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0
20:4n-6	3.7	4.2 $\pm$ 1.5	2.1 $\pm$ 0.6	2.5 $\pm$ 1.2
20:3n-3	0.2	0.2 $\pm$ 0.1	0.5 $\pm$ 0.3	0.2 $\pm$ 0.1
20:4n-3	0.8	0.6 $\pm$ 0.3	1.2 $\pm$ 0.6	0.8 $\pm$ 0.3
20:5n-3	5.9	11.2 $\pm$ 2.4	13.8 $\pm$ 0.9	16.2 $\pm$ 0.6
22:5n-6	0.4	0.4 $\pm$ 0.2	0.8 $\pm$ 0.2	1.8 $\pm$ 0.8
22:5n-3	0.4	0.3 $\pm$ 0.2	0.4 $\pm$ 0.2	0.6 $\pm$ 0.3
22:6n-3	7.3	5.7 $\pm$ 2.9	10.1 $\pm$ 2.8	15.1 $\pm$ 7.3
$\Sigma$ PUFA	<b>27.5</b>	<b>34.4</b>	<b>52.3</b>	<b>47.0</b>

869 Table A4: SIMPER similarity for seston fatty acid composition for the three groups. Sim/SD= Similarity standard deviation.  
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Group	Fatty Acids	Average Percent	Average Similarity	Sim/SD	Contribution %
<b>A</b>	18:1n-9	7.04	29.98	5.50	45.15
	16:1n-7	3.12	10.52	1.62	15.84
	18:3n-3	2.37	7.69	2.13	11.58
<b>B</b>	16:1n-7	6.53	32.44	2.88	44.86
	18:1n-9	2.27	12.82	4.84	17.72
	20:5n-3	1.55	9.67	3.31	13.37
<b>C</b>	18:3n-3	3.33	15.95	7.56	24.79
	18:1n-9	2.42	14.06	5.85	21.85
	20:5n-3	2.44	8.28	2.60	12.86
	22:6n-3	1.87	8.21	1.36	12.75

871 Table A5: SIMPER similarity for microzooplankton fatty acid composition for the three groups. Sim/SD= Similarity standard  
 872 deviation.

Group	Fatty Acids	Average Percent	Average Similarity	Sim/SD	Contribution%
<b>D</b>	18:1n-9	31.63	48.12	9.98	56.70
	18:2n-6	12.15	18.83	13.12	22.18
<b>E</b>	18:1n-9	13.46	22.41	N/A	24.23
	20:5n-3	11.72	18.32	N/A	19.81
	18:4n-3	10.29	15.74	N/A	17.02
	18:3n-3	8.43	12.87	N/A	13.92
<b>F</b>	20:5n-3	9.91	19.23	8.36	23.72
	22:6n-3	9.45	16.68	2.84	20.58
	16:1n-7	7.98	13.58	3.64	16.76
	18:3n-3	6.80	12.86	4.24	15.87

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904 Table A6: SIMPER similarity for mesozooplankton fatty acid composition for the four groups. Sim/SD= Similarity standard  
 905 deviation. Group G does not have a similarity breakdown because of n=1.  
 906

Groups	Fatty Acids	Average Percent	Average Similarity	Sim/SD	Contribution %
<b>G</b>	N/A	N/A	N/A	N/A	N/A
<b>H</b>	20:5n-3	11.24	20.41	17.74	25.07
	16:1n-7	9.53	16.68	4.37	20.49
	18:1n-9	10.58	15.72	3.84	19.31
	18:3n-3	6.14	10.08	4.61	12.38
<b>I</b>	20:5n-3	13.84	22.08	32.83	24.69
	18:3n-3	10.65	16.19	8.06	18.10
	22:6n-3	10.11	14.03	5.63	15.69
	18:4n-3	7.05	10.57	18.14	11.82
<b>J</b>	20:5n-3	16.04	28.86	28.57	33.99
	22:6n-3	16.58	22.04	4.19	25.96
	16:1n-7	8.51	13.29	7.64	15.65