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

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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. Salinity was the main factor correlated to the observed pattern in species composition, and fatty acid changes in the mesozooplankton. 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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2 **freshwater/saltwater interface of the Chowan River, NC**

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31 **Abstract**

32   The variability in zooplankton fatty acid composition may be an indicator of larval fish

33 habitat quality as fatty acids are linked to fish larval growth and survival. We sampled an

34 anadromous fish nursery, the Chowan River, during spring of 2013 in order to determine how the

35 seston fatty acid composition varied in comparison with the zooplankton community

36 composition and fatty acid composition during the period of anadromous larval fish residency.

37 The seston fatty acid profiles showed no distinct pattern in relation to sampling time or location.

38 The mesozooplankton community composition varied spatially and the fatty acid profiles were

39 typical of freshwater species in April. The Chowan River experienced a saltwater intrusion event

40 during May, which resulted in brackish water species dominating the zooplankton community

41 and the fatty acid profile showed an increase in polyunsaturated fatty acids (PUFA), in particular

42 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The saltwater intrusion event

43 was followed by an influx of freshwater due to high precipitation levels in June. The zooplankton

44 community composition once again became dominated by freshwater species and the fatty acid

45 profiles shifted to reflect this change; however, EPA levels remained high, particularly in the

46 lower river. We found correlations between the seston, microzooplankton and mesozooplankton

47 fatty acid compositions. Salinity was the main factor correlated to the observed pattern in species

48 composition, and fatty acid changes in the mesozooplankton. These data suggest that

49 anadromous fish nursery habitat likely experiences considerable spatial variability in fatty acid

50 profiles of zooplankton prey and that are correlated to seston community composition and

51 hydrodynamic changes. Our results also suggest that sufficient prey density as well as a diverse

52 fatty acid composition is present in the Chowan River to support larval fish production.

53

54 **Introduction**

55   Estuaries are considered important nursery habitat for many ecologically and

56 commercially important fish and invertebrates (Beck et al. 2001, Boesch and Turner 1984,

57 Sheaves et al. 2015, Sheaves 2016). Estuaries function as fish nurseries because they are highly

58 productive, support large planktonic populations across multiple size ranges, and fish within

59 estuaries generally have higher growth rates compared to other habitats (Beck et al. 2001).

60 Hence, many fish have evolved life-history strategies whereby larvae and juvenile stages have

61 residency periods in estuaries (McHugh 1967; Boehlert and Mundy 1988; Beck et al. 2001; Able

62 2005; Walsh et al. 2005). Beck et al. (2001) defined nursery habitat as an area that contributes to

63 higher production of individuals that move to juvenile habitat compared to other areas. Higher

64 growth rates of larval fish are possible because of zooplankton prey that are present during their

65 critical transition from yolk sac to free-living, feeding larvae (Hjort 1914; Mullen et al. 1986;

66 Rulifson et al. 1993; Cooper et al. 1998; Martino and Houde 2010; Binon 2011). However,

67 spatial and temporal overlap between predators and prey does not completely explain how fish

68 nurseries function mechanistically. The quality of prey can play a major role in determining the

69 effectiveness of a nursery for early stages of fish.

70   The quality (chemical composition) of zooplankton prey can influence fish growth,

71 development, and survival (Fraser et al. 1989; Webster and Lovell 1990; Copeman et al. 2002;

72 Rossi et al. 2006; Malzahn et al 2007, Paulsen et al. 2014). Fatty acids are chemically diverse,

73 often incorporated into organisms unmodified, and different organisms have distinct profiles

74 (Dalsgaard et al. 2003). Fatty acids are one class of compounds found in lipids that are

75 particularly important, impacting neural and vision development in fish (Gulati et al. 1997;

76 Müller-Navarra et al. 2000; Kainz et al. 2004; Masclaux et al. 2012). Fatty acids may act as both

77   dietary tracers in the food web and indicators of overall food quality (Iverson et al. 2004). The

78   majority of organisms need specific dietary fatty acids for somatic development and fitness

79   (Masclaux et al. 2012). These fatty acids, 18:3ω-3, α-linolenic acid (ALA), and 18:2ω-6, linoleic

80   acid (LA), are labeled essential fatty acids because they cannot be directly synthesized by

81   heterotrophic organisms and must come from the diet (Arts et al. 2009). Polyunsaturated fatty

82   acids (i.e., 20:5ω-3, eicosapentaenoic acid (EPA), 22:6ω-3, docosahexaenoic acid (DHA), and

83   20:4ω-6, arachidonic acid (ARA)) are required for all organisms and play a role in health and

84   cell function (Dalsgaard et al. 2003). Thus, an organisms' fatty acid signature may indicate

85   dietary consumption and nutritional quality of its prey (Goncalves et al. 2012).

86   Fatty acids are present in estuaries as a result of *de novo* synthesis by phytoplankton and

87   the delivery of detrital material of plant origin (Dalsgaard et al. 2003). The free-floating portion

88   of organic and inorganic particles is termed seston (Postel et al. 2000). The organic portion of

89   seston is important because it forms the origin point for the propagation of fatty acids through the

90   pelagic food web. Zooplankton assimilate fatty acids from the seston through direct consumption

91   of phytoplankton cells, detritus and/or consumption of microzooplankton that graze

92   phytoplankton or detritus (Wacker and von Elert 2001; Kainz, Arts, and Mazumder 2004; Vargas

93   et al. 2006). To date, studies of the relationship between seston and zooplankton fatty acid

94   composition have shown variable patterns. Persson and Verde (2006) demonstrated in laboratory

95   studies that broad zooplankton taxonomic groups (cladoceran vs. copepods) have different fatty

96   acid profiles independent of the food source. The seston has been correlated to the fatty acid

97   profile of zooplankton *in situ* as well (Goulden and Place 1990; Brett et al 2006; Taipale et al.

98   2009; Gladyshev et al. 2010; Ravet, Brett, and Arhoditsis 2010). The mismatch of fatty acids in

99   seston to zooplankton has also been shown in many studies (Desvilettes et al. 1997; Persson and

100   Vrede 2006; Rossi et al. 2006; Smyntek et al. 2008). Most marine zooplankton species cannot

101   convert precursor fatty acids and only obtain longer-chained fatty acids from their diet, compared

102   to freshwater zooplankton species that can convert precursor fatty acids (Rossi et al. 2006;

103   Persson and Verde 2006). However, it is clear that the fatty acid composition of the seston

104   changes as a result of local conditions, e.g., the salinity and temperature, nutrient concentration,

105   and the degree of autotrophy or heterotrophy in the system (Farkas and Herodek 1964;

106   Desvilettes et al. 1997; Wacker and von Elert 2001; Goncalves et al. 2012). Therefore,

107   combined knowledge of the changing nature of seston fatty acid composition, zooplankton

108   community composition changes, and fatty acid profiles forms a useful base for assessing the

109   quality of fish nursery habitat.

110   Zooplankton community composition in estuaries has been intensely studied and abiotic

111   factors are thought to structure zooplankton communities (Ambler et al. 1985; Orsi 1986;

112   Cervetto et al. 1999; Mouny and Dauvin 2002; Kimmel and Roman 2004; Lawrence et al. 2004;

113   Islam et al. 2005). Zooplankton community composition in temperate estuaries is dominated by

114   crustaceans in general, and copepods and cladocerans in particular (Tackx et al. 2004, Marques

115   et al. 2006; Winder and Jassby 2011; Chambord et al. 2016). Cladocerans are characterized by

116   high levels of EPA and ARA and this is thought to be related to a life history strategy focused on

117   high rates of somatic growth (Persson & Vrede 2006). In contrast, copepods have higher relative

118   DHA levels because this fatty acid is critical for nervous system development (Arts et al. 2009).

119   Copepods feature more developed nervous systems compared to cladocerans and this is a

120   function of active hunting of prey, mate location, and predator avoidance (Dalsgaard et al. 2003).

121   Carnivorous crustacean zooplankton have shown to be richer in PUFAs and this is thought to be

122   related to their food source (rotifers and smaller bodied cladoceran/copepods compared to

123   phytoplankton) (Arts et al. 2009).

124   Here we explore the species composition and variability in fatty acid composition of the

125   lower food web at the freshwater/saltwater interface of an estuarine fish nursery, the Chowan

126   River, North Carolina, USA. The Chowan River is considered a critical habitat for larval and

127   juvenile blueback herring (*Alosa aestivalis*), alewife (*A. pseudoharengus*), collectively known as

128   river herring (NCDMF 2007). The river herring are of interest because they have been severely

129   overfished and a moratorium on harvest is in place at various locations along the eastern United

130   States, including North Carolina (ASMFC 2012). The Chowan River also serves as a nursery

131   habitat for American shad (*A. sapidissima*) and striped bass (*Morone saxatilis*); however, the

132   status of the habitat for the latter species is unknown (Greene et al. 2009).

133   The overall goal of our study was to determine if species and fatty acid composition of

134   the lower food web could be used to indicate habitat quality of an estuarine fish nursery. In order

135   to achieve this goal, we examined the spatial and temporal variability in species composition of

136   microzooplankton and mesozooplankton as well as the fatty acid composition of the seston,

137   microzooplankton, and mesozooplankton during the period of larval fish residency in the

138   Chowan River. Our specific objectives were to determine: 1) if differences in the species

139   composition and fatty acid composition were present in the system; 2) if so, were there patterns

140   in species composition and fatty acid composition in time and space; 3) if particular species were

141   related to the species composition patterns and if particular fatty acids were related to the fatty

142   acid composition patterns; 4) if changes in species and fatty acid composition were related to

143   changes in salinity dynamics; 5) if patterns in fatty acid composition correlated across trophic

144   levels. We hypothesized that species composition would be related to salinity and that fatty acid

145   profiles of micro- and mesozooplankton would relate to species composition and would reflect

146   that of the seston. If supported, this would suggest that the quality of the larval fish forage, based

147   on fatty acids, could be used to assess fish nursery quality.

148   **Materials and Methods**

149   **Study Site**

150   The Chowan River is one of the largest tributaries that drains into the Albemarle Sound

151   (Fig. 1a and 1b) and is the 12th largest river basin in North Carolina (NCDENR 2006). It is

152   mainly a freshwater estuary that experiences intermittent salinity intrusion, mainly in the winter

153   months (Leech et al. 2009). The Chowan River was classified as "nutrient sensitive waters" in

154   1979 (NCDENR 2006) and has routinely experienced algal blooms and low dissolved oxygen

155   levels (< 3.0 mg L^{-1}). The entire river is classified as a Strategic Habitat Area for larval and

156   juvenile river herring (NCDMF 2007). Sampling took place south of Holiday Island on a 34 km

157   transect of Chowan River (Fig. 1c). Seven locations (4 km apart) were sampled between Holiday

158   Island and the river mouth (Fig 1c). Sampling occurred on 10-11 April, 31 May, and 25 June

159   2013, dates that span the residency for larvae of alewife, blueback herring, and striped bass. For

160   our study, we divided the river into three sections: upper, middle, and lower. The main

161   differences among these sections were (1) the distance from the Albemarle Sound and (2) the

162   potential influence of salinity.

163   *Sample collection*

164   *Water column properties*

165   Vertical profiles of temperature (°C) and salinity were measured with a conductivity,

166   temperature, and depth sensor (CTD, Yellow Springs Instruments, Castaway). Water samples

167   were collected at a depth of 3 meters with a Niskin water sampler.

168   *Zooplankton*

169   Water depths ranged from 5.27 to 7.56 m during zooplankton sampling. Two horizontal

170   net tows were made with 0.5 m diameter nets of two different mesh sizes (60 and 200 μm). Two

171   mesh sizes were used in order to generate an adequate representation of the zooplankton for the

172   size range > 60 μm. The zooplankton samples between 60 and 200 μm are designated

173   microzooplankton and the > 200 μm zooplankton samples are designated mesozooplankton

174   throughout the remainder of the paper. The zooplankton net was towed obliquely through the

175   water for 1 minute (species composition) and 2 minutes (fatty acid composition) at an average

176   boat speed of 0.75 m s^{-1}. The volume filtered was calculated using the volume of a cylinder (

177   $V = \pi r^2 L$), where r was the radius of the plankton net (0.25 m) and L was determined using the

178   boat speed (m s^{-1}) and the tow time (s). Each identification and count sample, depending on mesh

179   size, was filtered through a 60 or 200 μm filter, and zooplankton for composition were preserved

180   in a 120 mL glass jar with 10 ml of 10% buffered formaldehyde, sucrose, and filtered water. The

181   addition of sucrose to the formalin helps to reduce ballooning of cladoceran bodies and inflation

182   of their carapace (Haney and Hall 1973). The 60 μm sample had a half tablet of Alka Seltzer

183   added to keep rotifers from pulling in critical body parts (legs and arms) to ease identification

184   (Chick et al. 2010). The zooplankton samples for fatty acid analysis were collected at seven sites

185   on the Chowan River for April and June, and three sites in May. Due to limitations related to

186   sampling preparation in May, a subset of the field sites was sampled for fatty acid analyses. The

187   zooplankton samples for fatty acids were placed in a 1000 mL plastic container on ice, and

188   processed in the laboratory.

189 *Laboratory Processing*

190 *Zooplankton Identification*

191   Samples were filtered through a sieve (60 or 200 µm) to remove the sugar formalin

192 solution, and then added to a beaker with a known volume of water. A total of three subsamples

193 (2 mL per subsample for microzooplankton and 5 mL per subsample for mesozooplankton) were

194 analyzed for community composition using a Hensen-Stempel pipette. Organisms were

195 identified using a dissecting microscope and enumerated using a Ward counting wheel. The

196 zooplankton were identified to genus except for the freshwater copepods that were identified to

197 order. Copepod nauplii were grouped together because identification can be difficult at this stage

198 (Johnson and Allen 2012). If a species in a subsample comprised greater than 500 individuals,

199 then that species was not counted for the other two subsamples. Species abundances (*A*) were

200 determined using the equation: $A = A_s(V_t V_s^{-1})$ where A_s is the number of individuals in the

201 subsample, V_t is the total volume of water in the beaker, and V_s is the volume of the subsample.

202 *Lipid and fatty acid samples*

203   The water samples (300 mL) were concentrated on a 0.7 µm Whatman™ GF/F filter (47

204 mm diameter) and stored at -80°C until ready to process, which constituted the seston material.

205 The zooplankton samples were filtered through 60 and 200 µm sieves stacked to collect species

206 based on size. Each sample was visually analyzed to determine the dominant species with a

207 dissecting microscope, and detritus and phytoplankton were removed. The samples were

208 concentrated on a GF/F filter (47 mm diameter) by mesh size (60, 200 µm), and stored at -80°C

209 until ready to process.

210   Total lipids were extracted with chloroform-methanol (2:1, *v/v*) containing 0.01%

211 butylated hydroxytoluene as an antioxidant (Folch et al. 1957). The organic solvent was

212 evaporated under a stream of nitrogen and lipid concentration determined gravimetrically.

213 Transmethylation of fatty acids was done according to the method described by Metcalfe and

214 Schmitz (1969). A known amount of nonadecanoate acid (19:0) dissolved in hexane at a

215 concentration of 8 mg ml^{-1} (Nu Check Prep Inc.) was added as internal standard. The fatty acid

216 methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas

217 Chromatograph, Agilent Technologies, Inc.) using a 7693 mass spectrometer detector (Agilent

218 Technologies, Inc.), a capillary column (Omegawax^{TM} 250 fused silica capillary column, 30 mm

219 x 0.25 mm and 0.25 mm film thickness, Supleco®), and a 7890A autoinjector (Agilent

220 Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.3 ml min^{-1} and the

221 injection volume was 2 mL. Initial temperature of the oven was 175°C for 26 min, which was

222 increased to 205°C by increments of 2°C min^{-1}, then held at 205°C for 24 min. The source and

223 analyzer for the mass spectrometer was set at 230°C. The individual fatty acid methyl esters were

224 identified by comparing the retention times of authentic standard mixtures (FAME mix 37

225 components, Supleco) and quantified by comparing their peak areas with that of the internal

226 standard (Czesny and Dabrowski 1998). The results of individual fatty acid composition are

227 expressed in percentage of total identified FAME.

228 *Statistical Analysis*

229 We performed a series of multivariate analyses to address our specific objectives. We

230 used PERMANOVA a part of the PRIMER 6 statistical software package (Clarke and Gorley

231 2006), to test for overall differences between the microzooplankton and mesozooplankton

232 community composition, and fatty acid profiles of seston, microzooplankton, and

233 mesozooplankton. PERMANOVA is a non-parametric technique related to ANOVA, but uses

234 permutations and fewer assumptions compared to the traditional ANOVA approach (Anderson

235   2001). As such, it is particularly well suited to multivariate data sets that violate the traditional

236   assumptions of ANOVA and also have low sample sizes, as was our case (Anderson 2001).

237   If PERMANOVA detected differences, we then generated separate, Bray-Curtis

238   similarity matrices for microzooplankton species composition (60 µm mesh), mesozooplankton

239   species composition (200 µm mesh), seston fatty acid composition, microzooplankton fatty acid

240   composition, and mesozooplankton fatty acid composition. A separate cluster analysis was

241   performed using PRIMER 6 in order to reveal patterns over time and space for each similarity

242   matrix. Each individual sample was associated with a location in the river (upper, middle, lower)

243   and month (April, May, June) and these labels were used for visualization of samples in the

244   cluster dendrogram. Next, a similarity percentage analysis (SIMPER) test was used to compare

245   similarities within groups and determine the species or fatty acids that contributed to each

246   grouping from the cluster analysis (Clarke and Gorley 2006). The SIMPER test was set at 70%

247   cumulative contribution.

248   We then wanted to determine if salinity and temperature were related to the observed

249   patterns and we used redundancy analysis for this purpose (Legendre and Legendre 1998). The

250   redundancy analysis was carried out in the R environment (R v3.2.3, R Core Development Team

251   2015) using rda function (Oksanen et al. 2013) in the vegan package (Oksanen et al. 2017).

252   Finally, we used a Mantel matrix comparison to correlate fatty acid profiles between the three

253   groups (seston, microzooplankton, and mesozooplankton). The mantel.rtest function in the ade4

254   package (Oksanen et al. 2017) in the R environment (R v3.2.3, R Core Development Team 2015)

255   was used.

256   **Results**

257   *Salinity and Temperature*

258   Salinity was near zero (0.02 to 0.04) throughout the river in April. During May, salinities

259   in the upper river remained low (0.07), but the water column became stratified in the middle and

260   lower river, with salinities ranging from 0.28-1.66. The river returned to freshwater (0.04 to

261   0.08) again in June due to a tropical storm that brought heavy rains for a two-week period. North

262   Carolina experienced the second wettest June since 1895 with rainfall that ranged from 15.2 to

263   19.05 cm in the study area (Hiatt 2013). Water temperatures increased during the study period

264   April (15.7 ± 1.1°C), May (24.0 ± 1.0°C) and June (26.2 ± 0.3°C).

265   *Zooplankton Community Composition*

266   There were significant differences between the overall microzooplankton and

267   mesozooplankton community composition (PERMANOVA, $p = 0.001$). Microzooplankton could

268   be separated into two distinct groups by cluster analysis at 65% similarity (Figure 2a). Group 1

269   consisted of the vast majority of the samples collected across April, May, and June throughout

270   the river (Figure 2a). Group 2 consisted of two samples collected in the middle and lower river in

271   June (Figure 2a). Both groups were dominated by rotifers and copepod nauplii, but group 2 had a

272   higher contribution of copepod nauplii (Figure 2b and Table 1).

273   Three groups of mesozooplankton were differentiated at 50% similarity using cluster

274   analysis (Figure 3a). Group 3 consisted of samples from the May collection only, Group 4

275   consisted of a mixture of April and June samples in primarily the upper and middle river, and

276   Group 5 consisted of one April upper site, May upper river section and June samples throughout

277   the river (Figure 3a). Group 3 mesozooplankton percent composition was dominated by *Acartia*

278   spp. with a minor contribution by Bosminidae, Group 4 consisted primarily of equivalent

279   percentages of Cyclopoida and Bosminidae, and Group 5 mesozooplankton percent community

280   composition was characterized by higher percentages of *Leptodora* spp. and Calanoida (Figure

281   3b and Table 1).

282   *Fatty Acid Composition*

283   A total of 24 specific fatty acids were found in all samples (Tables A1-A3). Fatty acids

284   were first separated into broad categories: saturated fatty acids (SFA), monounsaturated fatty

285   acids (MUFA), and polyunsaturated fatty acids (PUFA) (Figure 4a). Seston had a higher percent

286   of SFA, and lower percent of MUFA and PUFA compared to micro- and mesozooplankton

287   (Figure 4a). Mesozooplankton and microzooplankton had a similar percent composition of SFA,

288   MUFA, and PUFA (Figure 4a). There were eight dominant fatty acids found in all the samples,

289   but the percent composition varied (Figure 4b). The most common SFA was palmitic acid (16:0),

290   the most common MUFAs were palmitoleic acid (16:1ω-7) and oleic acid (18:1ω-9), and the

291   most common PUFAs were ALA, 18:4ω-3, EPA, and DHA (Figure 4b; Tables A1-A3). A

292   comparison of MUFAs and PUFAs among the seston and zooplankton showed that seston had

293   the lowest overall percentages of MUFAs and PUFAs (PERMANOVA, $p = 0.001$, Figure 4b).

294   There was a difference between the microzooplankton fatty acid profile and the

295   mesozooplankton fatty acid profile (PERMANOVA, $p = 0.025$). The microzooplankton fatty

296   acid profile was characterized by a higher percentage of 18:1ω-9 compared to the other MUFAs

297   and PUFAs. In contrast, the mesozooplankton had the highest percent composition attributed to

298   two PUFAs, EPA and DHA (Figure 4b).

299   **Seston**

300   Three groups were designated at 60% similarity using cluster analysis for the seston fatty

301   acid composition (Figure 5a). The groups showed no distinct pattern in terms of sampling time or

302   location (Figure 5a). Seston fatty acid composition of Group A was characterized by 18:1ω-9,

303 16:1ω-7, ALA, and EPA, Group B by 16:1ω-7, 18:1ω-9, 18:2ω-6 and EPA, and Group C had

304 similar percentage composition of MUFAs and PUFAs, except 18:2ω-6 (Figure 5b and Table 2).

Microzooplankton

306 Three groups were designated at 70% similarity using cluster analysis for the

307 microzooplankton fatty acid composition (Figure 6a). The groups segregated temporally, with

308 Group D and E consisting of April samples only, and Group F consisted of May and June

309 samples only (Figure 6a). The Group D fatty acids were dominated by 18:1ω-9 and to a lesser

310 extent, 18:2ω-6, Group E showed similar percent composition among the fatty acids, with higher

311 percentages of 18:1ω-9 and EPA, and Group F also had similar percent composition of fatty

312 acids; however, the PUFAs EPA and DHA had significant contribution (Figure 6b and Table 2).

Mesozooplankton

314 Four groups were designated by cluster analysis at 77% similarity for the

315 mesozooplankton fatty acid composition (Figure 7a). Groups G and I consisted of April samples

316 only whereas Group J consisted of May and June samples only (Figure 7a). Group H showed

317 spatial separation, consisting of primarily upper river locations across all of the months (Figure

318 7a). The Group G and H fatty acids were dominated by 18:1ω-9, Group 8 by 16:1ω-7, 18:1ω-9,

319 and EPA, Group I had similar percentages of fatty acids with ALA, DHA and EPA having higher

320 percentages, and Group J had DHA and EPA as dominant components of the fatty acids (Figure

321 7b and Table 2).

Redundancy analysis and Mantel matrix correlations

323 Salinity was the main factor found to be correlated to observed patterns in the species

324 composition of both microzooplankton and mesozooplankton (Redundancy, $p = 0.004$).

325 However, salinity was not correlated to changes in the seston (Redundancy, $p = 0.490$) or

326   microzooplankton (Redundancy, $p = 0.27$) fatty acid profiles. Salinity was associated with the

327   changes in the mesozooplankton fatty acid profiles (Redundancy, $p = 0.034$). Seston fatty acid

328   profiles were correlated to the microzooplankton fatty acid profiles ($p = 0.013$) based on the

329   Mantel matrix comparison, but not correlated to the mesozooplankton fatty acid profiles ($p =$

330   0.340). The microzooplankton fatty acid profiles were correlated to the mesozooplankton fatty

331   acid profiles (Mantel, $p = 0.059$).

332   **Discussion**

333   We found temporal and spatial differences in the species and fatty acid composition of

334   the lower food web that were mainly related to a salinity intrusion that occurred during the study

335   period during May. Prior to the salinity intrusion, larval fish would have encountered a

336   freshwater plankton assemblage that was dominated by rotifers, Bosminidae, and cylopoid

337   copepods throughout the river. This assemblage was proportionally higher in EPA relative to

338   DHA. During the salinity intrusion, the microzooplankton remained dominated by rotifers;

339   however, the mesozooplankton community became dominated by the copepod *Acartia* spp..

340   Concurrently, the proportion of DHA increased and remained elevated into June, particularly in

341   the middle and lower river. The intrusion of saline water increased the overall proportion of

342   omega-3 fatty acids in the River, presumably due to the increased fraction of micro- and

343   mesozooplankton feeding on a more marine-like phytoplankton based food web and this signal

344   propagated through the food web. Additionally, we observed that FA appeared to be

345   incorporated relatively unchanged in micro and mesozooplankton in terms of relative

346   composition; however, MUFA and PUFA percent compositions increased in zooplankton

347   relative to seston. This suggested that MUFA and PUFA are bioaccumulated at higher trophic

348   levels, as seen in other studies (Persson and Verde 2006; Gladyshev et al. 2010; Ravett, Brett,

349   Arhonditsis 2010; Burns, Brett, and Schallenberg 2011). Overall, the FA composition of the food

350   web indicated that the Chowan River is likely to provide nutrition in terms of FA composition

351   for larval fish growth and development. This is based on the presence of higher chain (>20

352   carbons) PUFAs present in the mesozooplankton throughout the nursery.

353   The seston fatty acid composition consisted mainly of saturated fatty acids. Seston from

354   freshwater and estuarine systems typically has a large percentage of SFA and this fraction has

355   been attributed to detrital input, as opposed to originating from phytoplankton (Persson and

356   Vrede 2006; Gladyshev et al. 2010; Ravett, Brett, Arhonditsis 2010; Burns, Brett, and

357   Schallenberg 2011; Gonclaves et al 2012). Müller-Navarra et al. (2004) and Bec et al. (2010)

358   analyzed seston and found phytoplankton only explained 27% of variance in FA composition

359   and concluded that detritus and heterotrophic organisms also needed to be considered (Müller-

360   Navarra et al. 2004; Bec et al. 2010). Bec et al (2010) therefore concluded that the seston can

361   affect the fatty acid profiles of higher organisms, but may not relate individual groups of

362   phytoplankton or microzooplankton. This agreed with our findings as seen in the reduced

363   correlations between seston and the mesozooplankton.

364   We did not examine the seston composition directly by counting phytoplankton cells or

365   examining pigment concentrations, thus we were unable to attribute the origin of particular fatty

366   acids to phytoplankton or other sources. However, we were able to use the available literature to

367   identify potential indicators of fatty acid origin. The top four fatty acids by percent composition

368   varied by group, but 16:1ω-7, 18:1ω-9, 18:2ω-6, and ALA were the most prevalent. Potential

369   phytoplankton sources for these fatty acids may be diatoms, which have been shown to have

370   increased 16:1ω-7 and EPA in both freshwater and marine systems (Napolitano et al. 1997;

371   Dalsgaard et al. 2003; Boschker et al. 2005; Arts et al. 2009; Bec et al. 2010) and we observed

372   this occurred in May and June coincident with the salinity increase. Green algae (Chlorophytes)

373   have been shown to possess higher proportions of 18:2ω-6, and ALA, and 18:4ω-3 (Ahlgren et

374   al. 1990; Dalsgaard et al. 2003; Boschker et al. 2005; Masclaux et al. 2012; Strandberg et al.

375   2015). Fatty acids corresponding to these phytoplankton groups were observed during April

376   throughout the river and June in the middle and upper river. One other source of seston FA may

377   have been present, pine pollen, which is found in large quantities during spring. Pine pollen is

378   transported to freshwater systems via aerial deposition and floats at the surface, and the pine

379   pollen fatty acid profile has a high percent composition of 18:1ω-9 and 18:2ω-6 (Masclaux et al.

380   2013), which can be observed in our samples. Obviously, seston FA are a mixture of multiple

381   sources, thus the variability seen across the groups identified by the cluster analysis would be

382   expected.

383   The microzooplankton fatty acid profiles were different throughout the sampling period

384   with a change from decreased omega-3s to increased omega-3s in the system. This suggests a

385   switch in microzooplankton diet had occurred over the sampling period and two pathways appear

386   to be present during the study. The April microzooplankton fatty acid profiles for all river

387   sections had a high percentage of 18:1ω-9 and 18:2ω-6 suggesting that the microzooplankton

388   could be consuming either terrestrial material or chlorophytes during this time. Two sites in

389   April had an increase in omega-3 fatty acids (ALA, 18:4ω-3, EPA, DHA), and this would

390   suggest a different dietary pathway that was reduced in SFA, perhaps consisting of either smaller

391   microzooplankton such as ciliates or phytoplankton such as diatoms and/or dinoflagellates (Park

392   and Marshall 2000; Gladyshev et al. 2010). The community was dominated by rotifers during

393   this time and communities high in rotifer abundance have been shown to closely reflect the

394   seston composition (Gladyshev et al. 2010). The microzooplankton fatty acid profiles in May

395 and June at all river locations had an increase in 16:1ω-7, and omega-3s (ALA, EPA, and DHA).

396 These changes can be correlated to the likely increase in diatoms and dinoflagellates from the

397 saltwater intrusion event. The changes in June could be the increase in copepod nauplii of

398 Calanoid copepods, and the presence of dinoflagellates and diatoms even when the system

399 returned to freshwater. Our results are similar to systems where the phytoplankton composition

400 was represented by diatoms and dinoflagellates by having increased 16:1ω-7 and PUFAs

401 (Müller-Navara et al. 2000; Dalsgaard et al. 2003; Gladyshev et al. 2010; Ravett, Brett,

402 Arhonditsis 2010).

403   The mesozooplankton fatty acid profiles throughout the river in April and in the upper

404 river in May and June were defined by higher percentages of 16:1ω-7, 18:1ω-9, ALA, EPA, and

405 DHA, and mixed mesozooplankton community consisting of cladoceran and copepods. These

406 fatty acids profiles are similar to those found in freshwater systems that have a mixed

407 zooplankton composition (Persson and Verde 2006; Arts et al. 2009; Kainz et al. 2009;

408 Gladyshev et al 2010; Burns et al. 2011; Masclaux et al. 2012). Cladocerans have low or no

409 DHA compared to copepods and high EPA levels have been shown to correlate with the high

410 somatic growth rates of cladoceran (Persson and Verde 2006). A saltwater intrusion changed the

411 mesozooplankton species composition in May resulting in numerical dominance by *Acartia* spp.

412 in the lower and middle sections of the river. *Acartia* spp. is the dominant copepod species in

413 temperate, estuarine systems (Ambler et al. 1985; Orsi 1986; Cervetto et al. 1999; Mouny and

414 Dauvin 2002; Kimmel and Roman 2004; Lawrence et al. 2004; Islam et al. 2005). The

415 mesozooplankton fatty acid profiles in May were represented by 16:1ω-7, EPA, and the highest

416 observed percentages of DHA. This is clearly a reflection of the dominance of *Acartia* spp. in

417 the system and a diet primarily consisting of marine algae higher in omega-3 FAs (Stottrup et al.

418   1999; Persson and Verde 2006; Arts et al. 2009; Kainz et al. 2009; Gladyshev et al 2010;

419   Masclaux et al. 2012). Mesozooplankton fatty acid percent composition in June at the lower and

420   middle site remained similar to that observed in May, despite the species composition having

421   returned to a mix of cladocerans and copepods. This suggests that physical shifts in the system

422   that result in seston changes may persist in the system despite shifts in zooplankton community

423   composition.

424   The relevance of the food web fatty acid composition can be determined by examining

425   the potential feeding behavior of larval fish within the Chowan River nursery. Alewife and

426   blueback herring start feeding on smaller cladocerans and copepods at about 6 mm total length

427   (Mullen et al. 1986). Binon (2011) reported that river herring at 6 mm notochord length had a

428   maximum gape width of 400 μm, and estimated maximum prey size of 200 μm, which would

429   result in mesozooplankton being an important food resource. In the Connecticut River, the diet

430   for blueback herring were dominated by rotifers for fish 5-12 mm, Bosminidae for fish 12-16

431   mm, and cyclopoid copepods for fish >16mm in total length (Crecco and Blake 1983). Based on

432   these dietary studies, the larval and juvenile river herring would be feeding across the size range

433   of zooplankton prey that we sampled; however, fish would be consuming primarily

434   microzooplankton early in the year (April) and mesozooplankton later in the season (May and

435   June). In April, two pathways for FA propagation were present in the microzooplankton. Thus,

436   fish feeding during this time may experience variability in the quality of the microzooplankton

437   prey in terms of percentage of PUFAs. Larval fish need PUFAs (ALA, EPA and DHA) for

438   growth, visual acuity, survival, and development of normal pigmentation (Bell et al. 1995; Bell

439   and Sargent 1996; Rainuzzo et al. 1997; Sargent et al. 1999; Rossi et al. 2006). The shift to larger

440   prey later in the year (May and June) resulted in a change in prey quality as the relative

441   percentage of EPA and DHA increased. This was the result of a salinity intrusion into the middle

442   and lower reaches of the estuary that was associated with dominance of the cladoceran *Acartia*

443   spp. and a significant increase in DHA and EPA. This could allow larval and juvenile fish to

444   consume prey with a higher proportion of PUFAs. It is unknown if river herring can elongate

445   precursor FA into PUFAs. Even if fish can convert precursor FA, larval fish could not receive all

446   nutritional need for those fatty acids (Agaba et al. 2005). The larval fish would not have to use

447   energy for the conversion, and could continue to put energy into growth (Wacker and von Elert

448   2001; Rossi et al 2006). This allows the larval fish to survive and grow past the critical period.

449   The fish nursery present in the lower Chowan River may undergo significant changes

450   during the critical time of larval fish growth and our results demonstrate how changes in the

451   seston community may propagate through the food web. The results also highlight that additional

452   information concerning the fatty acid composition of the zooplankton prey base for larval fish

453   can provide insight into habitat quality, our stated goal. Sheaves et al. (2004) pointed out the

454   need to expand the nursery habitat concept to include relevant ecosystem processes, particularly

455   resource dynamics. This research begins to explore the mechanisms that allow nursery habitat to

456   function. We plan further research to investigate the linkage between the seston community fatty

457   acid composition, the zooplankton community fatty acid composition, and larval fish to

458   determine how lower food web variability relates to larval fish condition and survival.

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692   Tables
693   Table 1: Results of SIMPER analysis for each group; all the zooplankton species that contributed
694   community composition, and their corresponding values in % are given. Dash marks represent
695   species that were not included in the contribution of 70%

| | Microzooplankton | | Mesozooplankton | | |
	Group 1	Group 2	Group 3	Group 4	Group 5
Overall Similarity	**91.18**	**80.18**	**84.39**	**57.16**	**52.52**
Species	**Percent Composition**		**Percent Composition**		
Bosminidae	-	-	17.26	34.25	12.20
Leptodora spp.	-	-	-	-	21.80
Calanoida	-	-	-	33.32	26.09
Cyclopoida	-	-	-	10.24	9.07
Acartia spp.	-	-	74.66	-	-
Copepod nauplii	10.60	41.61	-	-	-
Rotifer	86.47	50.46	-	-	-

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726 Table 2: Results of SIMPER analysis for each group; the fatty acids that contributed to the
727 differences in fatty acid profile, and their corresponding values in % are given. Dash marks
728 represent fatty acids that were not included in the contribution of 70%.
729 Group G had a sample size < 2.

	Seston			Microzooplankton			Mesozooplankton			
	Group A	Group B	Group C	Group D	Group E	Group F	Group G	Group H	Group I	Group J
Overall Similarity	66.40	72.32	64.34	84.88	92.49	81.05	N/A	81.42	89.42	84.89
Fatty Acids	Percent Composition			Percent Composition			Percent Composition			
16:1ω-7	3.12	6.53	-	-	-	7.98	-	9.53	-	8.51
18:1ω-9	7.04	2.27	2.42	31.63	13.46	-	17.01	10.58	-	-
18:2ω-6	-	-	-	12.15	-	-	-	-	-	-
18:3ω-3 (ALA)	2.37	-	3.33	-	8.43	6.80	-	6.14	10.65	-
18:4ω-3	-	-	-	-	10.29	-	-	-	7.05	-
20:5ω-3 (EPA)	-	1.55	2.44	-	11.72	9.91	-	11.24	13.84	16.40
22:6ω-3 (DHA)	-	-	1.87	-	-	9.45	-	-	10.11	16.58

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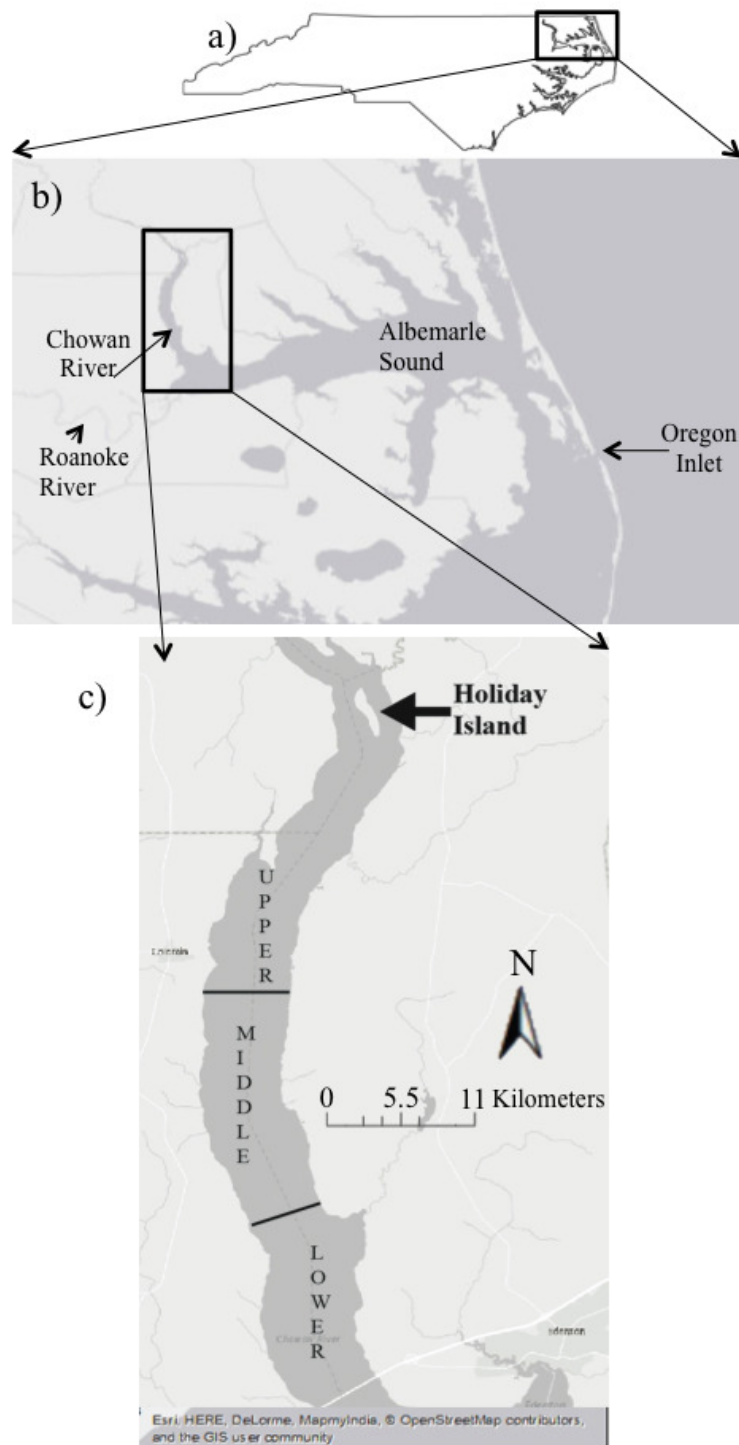
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757 Figure 1. The overview of Albemarle Sound in North Carolina (a). The close up view of the
758 location for two main tributaries (Chowan and Roanoke Rivers), and the Albemarle Sound in
759 North Carolina (b). The three sections used to collect zooplankton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

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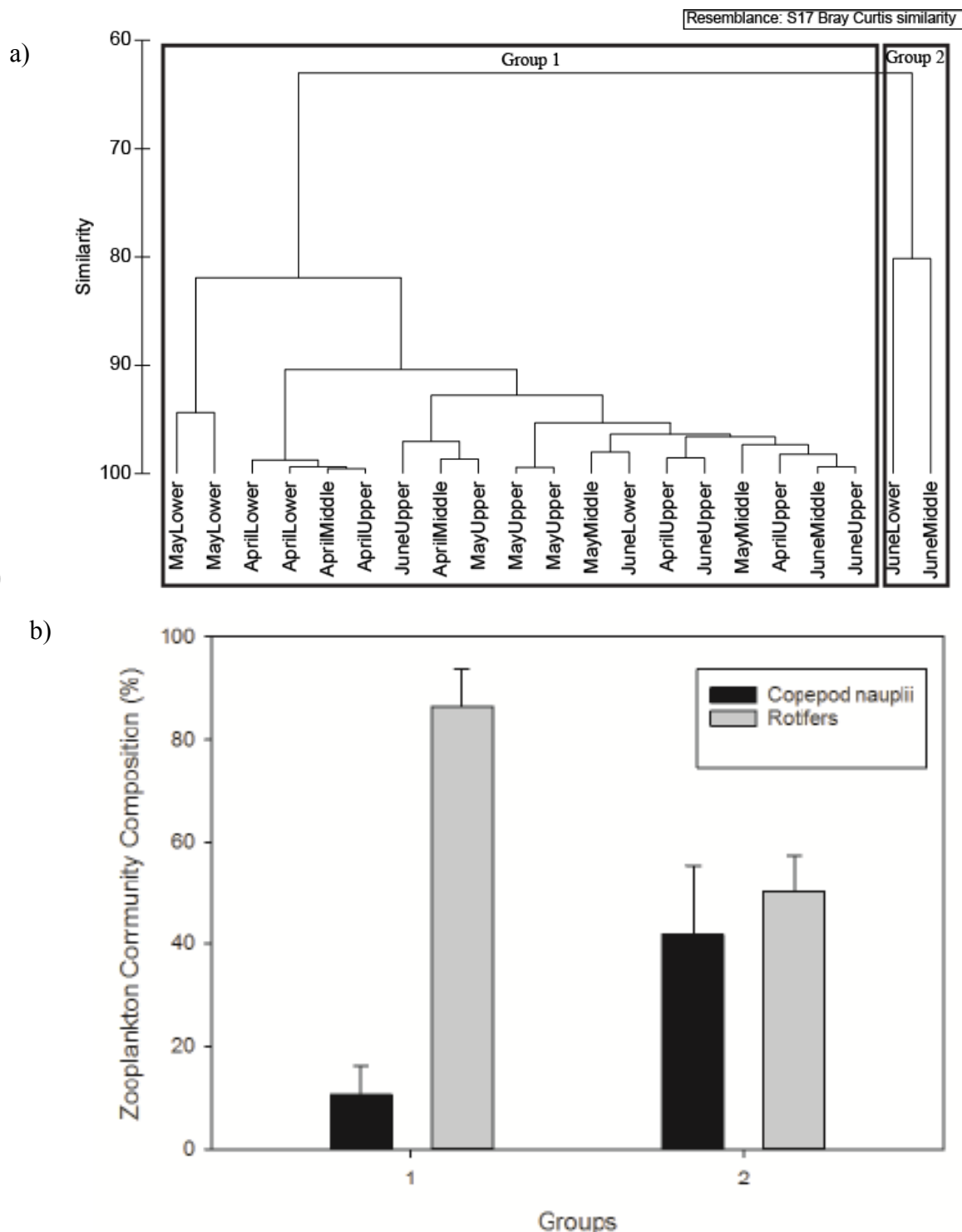
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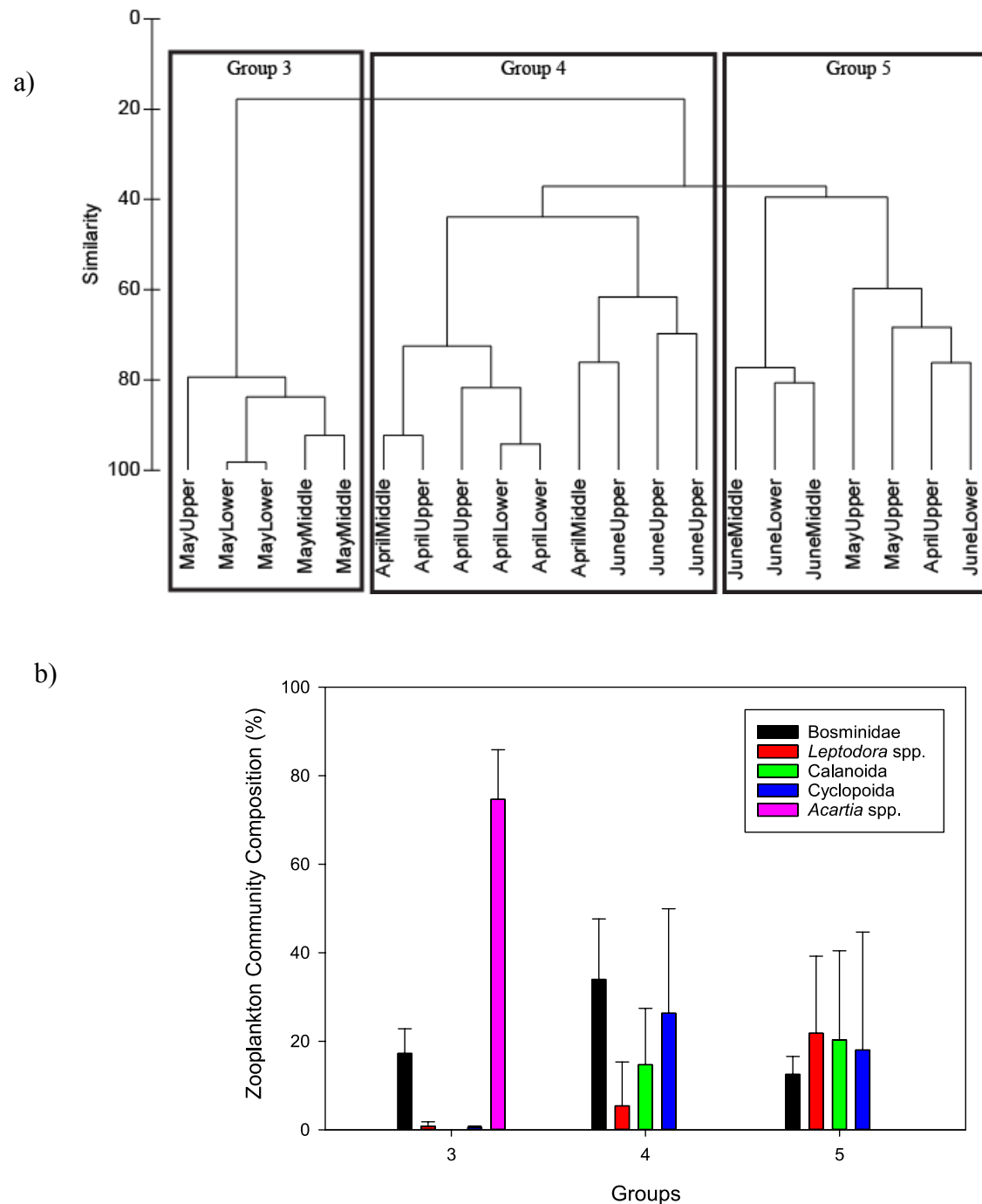
763   Figure 2. The two microzooplankton community composition groups from cluster analysis (a) at
764   65% similarity. The mean microzooplankton community composition (%, ±S.D.) for the two
765   groups from cluster analysis (b).

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770   Figure 3. The three mesozooplankton community composition groups from cluster analysis (a) at
771   50% similarity. The mean mesozooplankton community composition (%, ±S.D.) for the three
772   groups from cluster analysis (b).

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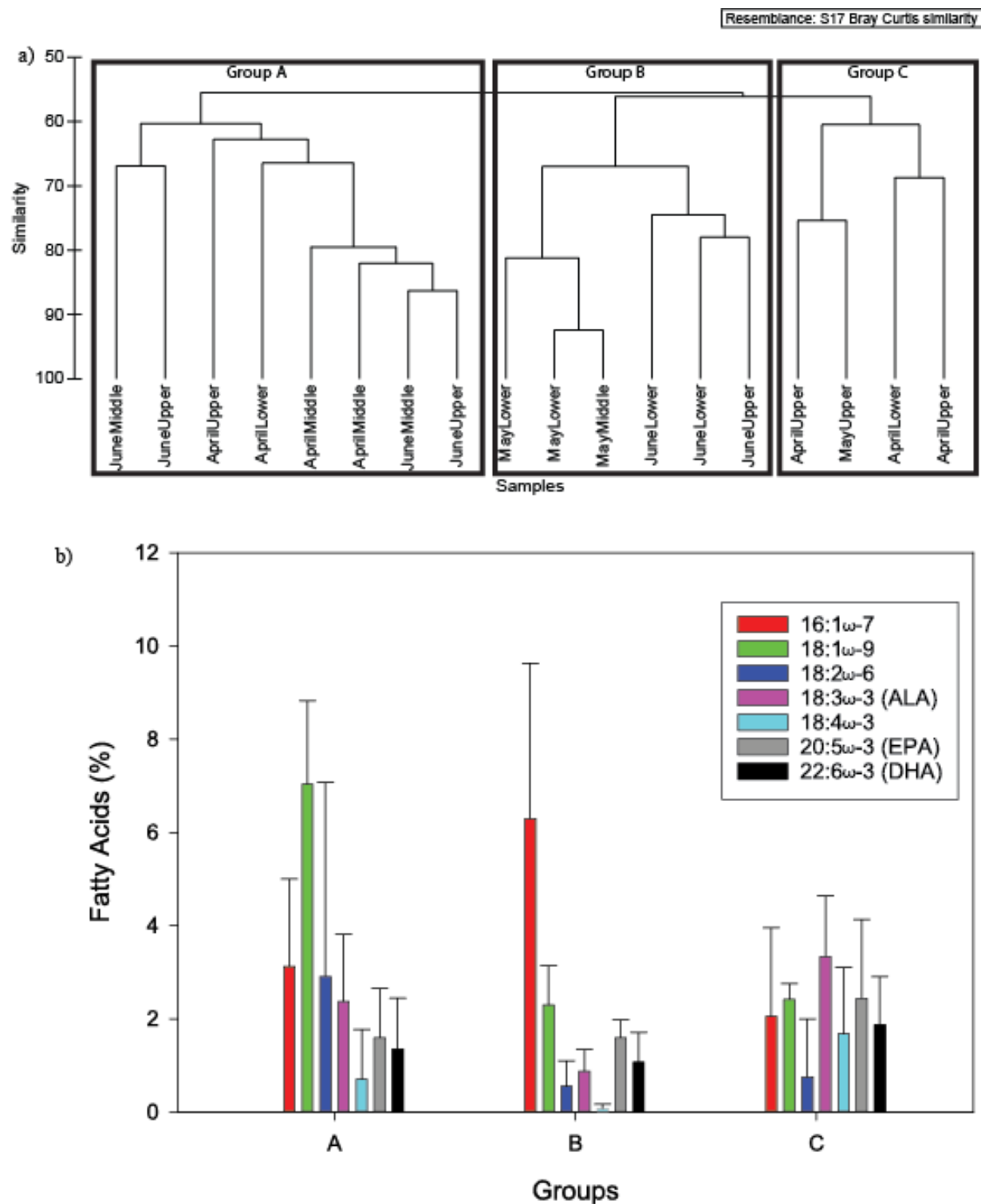
776 Figure 4. The mean (± S.D.) saturated (SFA), monounsaturated (MUFA), and polyunsaturated
777 (PUFA) fatty acid composition (%) for the seston, microzooplankton, and mesozooplankton (a).
778 The mean fatty acid composition (%, ± S.D.) for the seston, microzooplankton, and
779 mesozooplankton (b).

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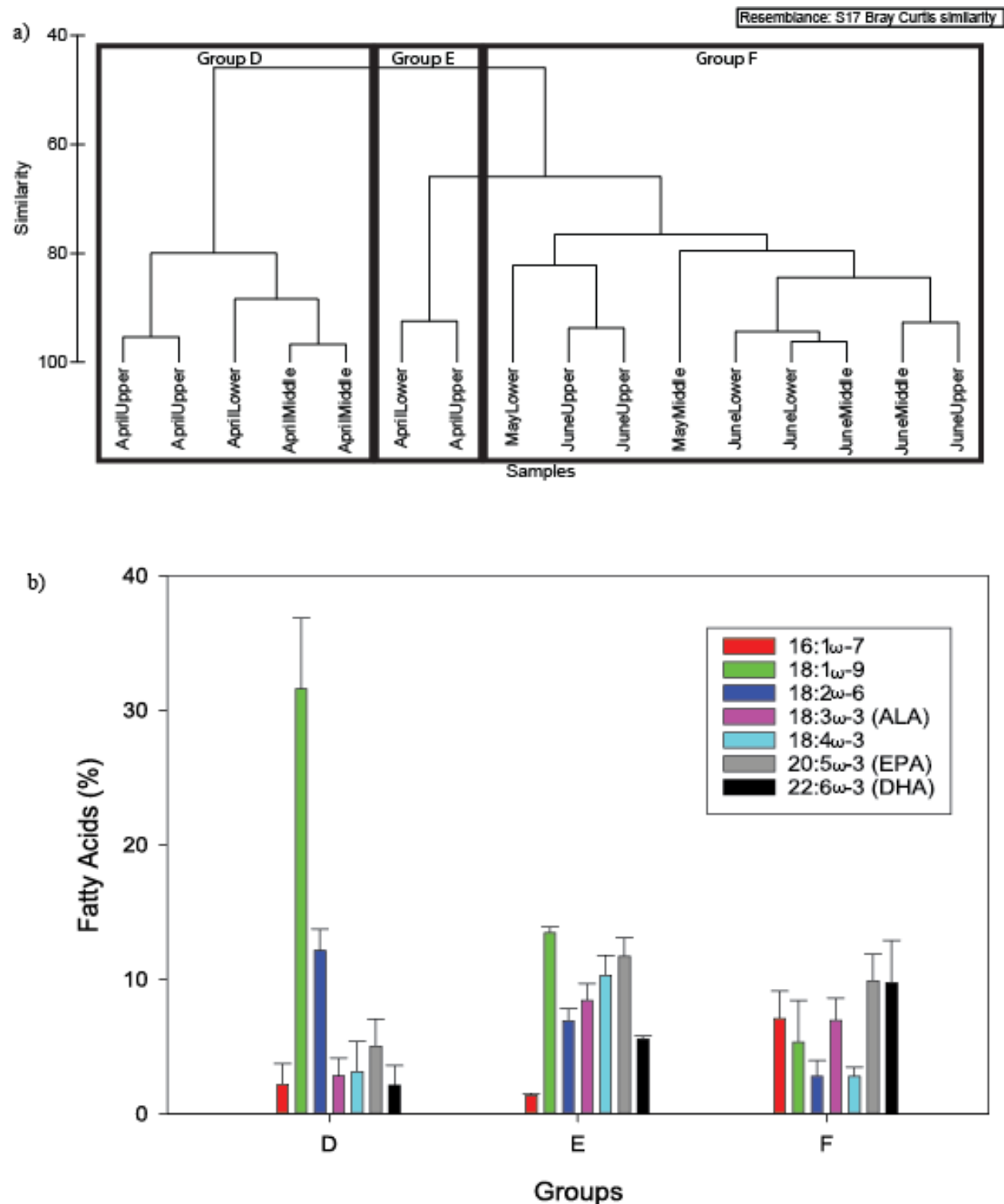
782 Figure 5. The three seston fatty acid composition groups from cluster analysis (a) at 60%
783 similarity. The mean seston fatty acid composition (%, ± S.D.) for the three groups from cluster
784 analysis (b).

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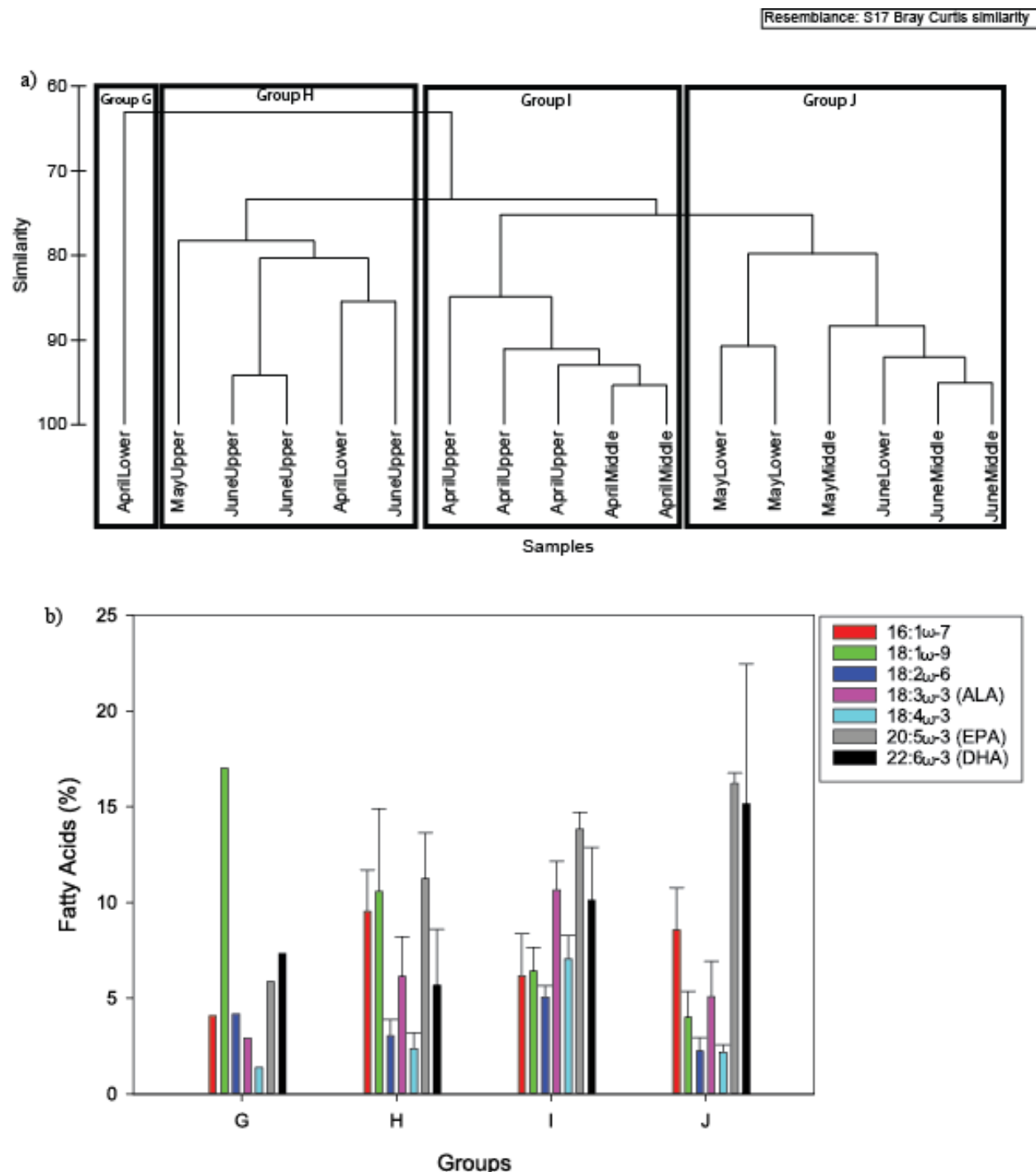
786   Figure 6. The three microzooplankton fatty acid composition groups from cluster analysis (a) at
787   60% similarity. The mean microzooplankton fatty acid composition (%, ± S.D.) for the three
788   groups from cluster analysis (b).

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790 Figure 7. The four mesozooplankton fatty acid composition groups from cluster analysis (a) at
791 60% similarity. The mean mesozooplankton fatty acid composition (%, ± S.D.) for the four
792 groups from cluster analysis (b).
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799 **Appendix**

800 Table A1: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids
801 detected) of seston from the Chowan River by group. SFA: saturated fatty acids, MUFA:
802 monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

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

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814   Table A2: Mean fatty acid composition (percentage of total fatty acids detected) of
815   microzooplankton (<60μm) from the Chowan River by groups. SFA: saturated fatty acids,
816   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
∑SFA	**35.9**	**33.5**	**39.0**
16:1ω-9	0.3 ± 0.1	0.8 ± 0.3	0.9 ± 0.6
16:1ω-7	2.2 ± 1.6	1.4 ± 0.1	7.1 ± 2.0
18:1ω-9	31.6 ± 5.3	13.5 ± 0.5	5.3 ± 3.1
18:1ω-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
∑MUFA	**35.4**	**17.3**	**16.6**
18:2ω-6	12.2 ± 1.6	6.9 ± 0.9	2.8 ± 1.2
18:3ω-3	2.9 ± 1.3	8.4 ± 1.3	7.0 ± 1.7
18:4ω-3	3.2 ± 2.3	10.3 ± 1.5	2.8 ± 0.7
20:2ω-6	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
20:3ω-6	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
20:4ω-6	0.6 ± 0.7	0.4 ± 0.1	3.2 ± 0.5
20:3ω-3	0.3 ± 0.1	1.0 ± 0.2	0.4 ± 0.1
20:4ω-3	1.3 ± 0.4	2.8 ± 0.1	2.1 ± 0.3
20:5ω-3	5.0 ± 2.0	11.7 ± 1.4	9.9 ± 2.0
22:5ω-6	0.2 ± 0.2	1.0 ± 0.1	2.3 ± 0.9
22:5ω-3	0.2 ± 0.2	0.2 ± 0.1	1.9 ± 1.1
22:6ω-3	2.1 ± 1.5	5.6 ± 0.3	9.8 ± 3.2
∑PUFA	**28.5**	**48.9**	**42.7**

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830   a: Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of
831   mesozooplankton from the Chowan River by group. SFA: saturated fatty acids, MUFA:
832   monounsaturated fatty acids, and PUFA: polyunsaturated fatty acids.

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

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