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

Deborah A. Lichti¹, Jacques Rinchard² and David G. Kimmel¹,³

¹ Department of Biology, East Carolina University, Greenville, NC, United States of America
² Department of Environmental Science and Biology, State University of New York College at Brockport, Brockport, NY, United States of America
³ Alaska Fisheries Science Center, National Oceanic and Atmospheric Administration, Seattle, WA, United States of America

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. 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.

Subjects Aquaculture, Fisheries and Fish Science, Biochemistry, Zoology, Freshwater Biology
Keywords Zooplankton, Fatty acid, Estuarine systems, Freshwater, Seston, Community

How to cite this article Lichti et al. (2017), Changes in zooplankton community, and seston and zooplankton fatty acid profiles at the freshwater/saltwater interface of the Chowan River, North Carolina. PeerJ 5:e3667; DOI 10.7717/peerj.3667
INTRODUCTION

Estuaries are considered important nursery habitat for many ecologically and commercially important fish and invertebrates (Beck et al., 2001; Boesch & Turner, 1984; Sheaves et al., 2015; Sheaves, 2016). Estuaries function as fish nurseries because they are highly productive, support large planktonic populations across multiple size ranges, and fish within estuaries generally have higher growth rates compared to other habitats (Beck et al., 2001). Hence, many fish have evolved life-history strategies whereby larval and juvenile stages have residency periods in estuaries (McHugh, 1967; Boehlert & Mundy, 1988; Beck et al., 2001; Able, 2005; Walsh, Settle & Peters, 2005). Beck et al. (2001) defined nursery habitat as an area that contributes to higher production of individuals that move to juvenile habitat compared to other areas. Higher growth rates of larval fish are possible because of zooplankton prey that are present during their critical transition from yolk sac to free-living, feeding larvae (Hjort, 1914; Mullen, Fay & Moring, 1986; Rulifson et al., 1993; Cooper et al., 1998; Martino & Houde, 2010; Binion, 2011). However, spatial and temporal overlap between predators and prey does not completely explain how fish nurseries function mechanistically. The quality of prey can play a major role in determining the effectiveness of a nursery for early stages of fish.

The quality (chemical composition) of zooplankton prey can influence fish growth, development, and survival (Fraser et al., 1989; Webster & Lovell, 1990; Copeman et al., 2002; Rossi et al., 2006; Malzahn et al., 2007; Paulsen et al., 2014). Fatty acids are chemically diverse, often incorporated into organisms unmodified, and different organisms have distinct profiles (Dalsgaard et al., 2003). Fatty acids are one class of compounds found in lipids that are particularly important, impacting neural and vision development in fish (Gulati & Demott, 1997; Müller-Navarra et al., 2000; Kainz, Arts & Mazumder, 2004; Masclaux et al., 2012). Fatty acids may act as both dietary tracers in the food web and indicators of overall food quality (Iverson et al., 2004). The majority of organisms need specific dietary fatty acids for somatic development and fitness (Masclaux et al., 2012). These fatty acids, 18:3ω-3, α-linolenic acid (ALA), and 18:2ω-6, linoleic acid (LA), are labeled essential fatty acids because they cannot be directly synthesized by heterotrophic organisms and must come from the diet (Arts, Brett & Kainz, 2009). Polyunsaturated fatty acids (i.e., 20:5ω-3, eicosapentaenoic acid (EPA), 22:6ω-3, docosahexaenoic acid (DHA), and 20:4ω-6, arachidonic acid (ARA)) are required for all organisms and play a role in health and cell function (Dalsgaard et al., 2003). Thus, an organisms’ fatty acid signature may indicate dietary consumption and nutritional quality of its prey (Goncalves et al., 2012).

Fatty acids are present in estuaries as a result of de novo synthesis by phytoplankton and the delivery of detrital material of plant origin (Dalsgaard et al., 2003). The free-floating portion of organic and inorganic particles is termed seston (Postel, Fock & Hagen, 2000). The organic portion of seston is important because it forms the origin point for the propagation of fatty acids through the pelagic food web. Zooplankton assimilate fatty acids from the seston through direct consumption of phytoplankton cells, detritus and/or consumption of microzooplankton that graze phytoplankton or detritus (Wacker & Von Elert, 2001; Kainz, Arts & Mazumder, 2004; Vargas, Escribano & Poulet, 2006). To date,
studies of the relationship between seston and zooplankton fatty acid composition have shown variable patterns. Persson & Vrede (2006) demonstrated in laboratory studies that broad zooplankton taxonomic groups (cladoceran vs. copepods) have different fatty acid profiles independent of the food source. The seston has been correlated to the fatty acid profile of zooplankton in situ as well (Goulden & Place, 1990; Brett et al., 2006; Taipale et al., 2009; Gladyshev et al., 2010; Ravet, Brett & Arhonditis, 2010). The mismatch of fatty acids in seston to zooplankton has also been shown in many studies (Desvillettes et al., 1997; Persson & Vrede, 2006; Rossi et al., 2006; Smyntek et al., 2008). Most marine zooplankton species cannot convert precursor fatty acids and only obtain longer-chained fatty acids from their diet, compared to freshwater zooplankton species that can convert precursor fatty acids (Rossi et al., 2006; Persson & Vrede, 2006). However, it is clear that the fatty acid composition of the seston changes as a result of local conditions, e.g., the salinity and temperature, nutrient concentration, and the degree of autotrophy or heterotrophy in the system (Farkas & Herodek, 1964; Desvillettes et al., 1997; Wacker & Von Elert, 2001; Goncalves et al., 2012). Therefore, combined knowledge of the changing nature of seston fatty acid composition, zooplankton community composition changes, and fatty acid profiles forms a useful base for assessing the quality of fish nursery habitat.

Zooplankton community composition in estuaries has been intensely studied and abiotic factors are thought to structure zooplankton communities (Ambler, Cloern & Hutchinson, 1985; Orsi & Mecum, 1986; Cervetto, Gaudy & Pagano, 1999; Mouny & Dauvin, 2002; Kimmel & Roman, 2004; Lawrence, Valiela & Tomasky, 2004; Islam, Ueda & Tanaka, 2005). Zooplankton community composition in temperate estuaries is dominated by crustaceans in general, and copepods and cladocerans in particular (Tackx et al., 2004; Marques et al., 2006; Winder & Jassby, 2011; Chambord et al., 2016). Cladocerans are characterized by high levels of EPA and ARA and this is thought to be related to a life history strategy focused on high rates of somatic growth (Persson & Vrede, 2006). In contrast, copepods have higher relative DHA levels because this fatty acid is critical for nervous system development (Arts, Brett & Kainz, 2009). Copepods feature more developed nervous systems compared to cladocerans and this is a function of active hunting of prey, mate location, and predator avoidance (Dalsgaard et al., 2003). Carnivorous crustacean zooplankton have shown to be richer in PUFAs and this is thought to be related to their food source (rotifers and smaller bodied cladoceran/copepods compared to phytoplankton) (Arts, Brett & Kainz, 2009).

Here we explore the species composition and variability in fatty acid composition of the lower food web at the freshwater/saltwater interface of an estuarine fish nursery, the Chowan River, North Carolina, USA. The Chowan River is considered a critical habitat for larval and juvenile blueback herring (Alosa aestivalis), alewife (A. pseudoharengus), collectively known as river herring (NCDMF, 2007). The river herring are of interest because they have been severely overfished and a moratorium on harvest is in place at various locations along the eastern United States, including North Carolina (ASMFC, 2012). The Chowan River also serves as a nursery habitat for American shad (A. sapidissima) and striped bass (Morone saxatilis); however, the status of the habitat for the latter species is unknown (Greene et al., 2009).
The overall goal of our study was to determine if species and fatty acid composition of the lower food web could be used to indicate habitat quality of an estuarine fish nursery. In order to achieve this goal, we examined the spatial and temporal variability in species composition of microzooplankton and mesozooplankton as well as the fatty acid composition of the seston, microzooplankton, and mesozooplankton during the period of larval fish residency in the Chowan River. Our specific objectives were to determine: (1) if differences in the species composition and fatty acid composition were present in the system; (2) if so, were there patterns in species composition and fatty acid composition in time and space; (3) if particular species were related to the species composition patterns and if particular fatty acids were related to the fatty acid composition patterns; (4) if changes in species and fatty acid composition were related to changes in salinity dynamics; (5) if patterns in fatty acid composition correlated across trophic levels. We hypothesized that species composition would be related to salinity and that fatty acid profiles of micro- and mesozooplankton would relate to species composition and would reflect that of the seston. If supported, this would suggest that the quality of the larval fish forage, based on fatty acids, could be used to assess fish nursery quality.

MATERIALS AND METHODS

Study site
The Chowan River is one of the largest tributaries that drains into the Albemarle Sound (Figs. 1A and 1B) and is the 12th largest river basin in North Carolina (NCDENR, 2006). It is mainly a freshwater estuary that experiences intermittent salinity intrusion, mainly in the winter months (Leech, Ensign & Piehler, 2009). The Chowan River was classified as “nutrient sensitive waters” in 1979 (NCDENR, 2006) and has routinely experienced algal blooms and low dissolved oxygen levels (<3.0 mg L⁻¹). The entire river is classified as a Strategic Habitat Area for larval and juvenile river herring (NCDMF, 2007). Sampling took place south of Holiday Island on a 34 km transect of Chowan River (Fig. 1C). Seven locations (4 km apart) were sampled between Holiday Island and the river mouth (Fig. 1C). Sampling occurred on 10–11 April, 31 May, and 25 June 2013, dates that span the residency for larvae of alewife, blueback herring, and striped bass. For our study, we divided the river into three sections: upper, middle, and lower. The main differences among these sections were (1) the distance from the Albemarle Sound and (2) the potential influence of salinity.

Sample collection
Water column properties. Vertical profiles of temperature (°C) and salinity were measured with a conductivity, temperature, and depth sensor (CTD, Yellow Springs Instruments, Castaway). Water samples were collected at a depth of 3 m with a Niskin water sampler.

Zooplankton. Water depths ranged from 5.27 to 7.56 m during zooplankton sampling. Two horizontal net tows were made with 0.5 m diameter nets of two different mesh sizes (60 and 200 µm). Two mesh sizes were used in order to generate an adequate representation of the zooplankton for the size range >60 µm. The zooplankton samples between 60 and
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). Map data: ESRI, HERE, DeLorme, MapmyIndia, OpenStreetMap contributors, GIS user community.
200 μm are designated microzooplankton and the >200 μm zooplankton samples are designated mesozooplankton throughout the remainder of the paper. The zooplankton net was towed obliquely through the water for 1 min (species composition) and 2 min (fatty acid composition) at an average boat speed of 0.75 m s$^{-1}$. The volume filtered was calculated using the volume of a cylinder ($V = \pi r^2 L$), where $r$ was the radius of the plankton net (0.25 m) and $L$ was determined using the boat speed (m s$^{-1}$) and the tow time (s). Each identification and count sample, depending on mesh size, was filtered through a 60 or 200 μm filter, and zooplankton for composition were preserved in a 120 mL glass jar with 10 ml of 10% buffered formaldehyde, sucrose, and filtered water. The addition of sucrose to the formalin helps to reduce ballooning of cladoceran bodies and inflation of their carapace (Haney & Hall, 1973). The 60 μm sample had a half tablet of Alka Seltzer added to keep rotifers from pulling in critical body parts (legs and arms) to ease identification (Chick et al., 2010). The zooplankton samples for fatty acid analysis were collected at seven sites on the Chowan River for April and June, and three sites in May. Due to limitations related to sampling preparation in May, a subset of the field sites was sampled for fatty acid analyses. The zooplankton samples for fatty acids were placed in a 1,000 mL plastic container on ice, and processed in the laboratory.

**Laboratory processing**

**Zooplankton identification.** Samples were filtered through a sieve (60 or 200 μm) to remove the sugar formalin solution, and then added to a beaker with a known volume of water. A total of three subsamples (2 mL per subsample for microzooplankton and 5 mL per subsample for mesozooplankton) were analyzed for community composition using a Hensen-Stempel pipette. Organisms were identified using a dissecting microscope and enumerated using a Ward counting wheel. The zooplankton were identified to genus except for the freshwater copepods that were identified to order. Copepod nauplii were grouped together because identification can be difficult at this stage (Johnson & Allen, 2012). If a species in a subsample comprised greater than 500 individuals, then that species was not counted for the other two subsamples. Species abundances ($A$) were determined using the equation: $A = A_s(V_t/V_s)$ where $A_s$ is the number of individuals in the subsample, $V_t$ is the total volume of water in the beaker, and $V_s$ is the volume of the subsample.

**Lipid and fatty acid samples.** The water samples (300 mL) were concentrated on a 0.7 μm Whatman™ GF/F filter (47 mm diameter) and stored at −80 °C until ready to process, which constituted the seston material. The zooplankton samples were filtered through 60 and 200 μm sieves stacked to collect species based on size. Each sample was visually analyzed to determine the dominant species with a dissecting microscope, and detritus and phytoplankton were removed. The samples were concentrated on a GF/F filter (47 mm diameter) by mesh size (60, 200 μm), and stored at −80 °C until ready to process.

Total lipids were extracted with chloroform-methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene as an antioxidant (Folch, Lees & Sloane Stanley, 1957). The organic solvent was evaporated under a stream of nitrogen and lipid concentration determined gravimetrically. Transmethylation of fatty acids was done according to the
The method described by Metcalfe & Schmitz (1961). A known amount of nonadecanoate acid (19:0) dissolved in hexane at a concentration of 8 mg ml\(^{-1}\) (Nu Check Prep Inc.) was added as internal standard. The fatty acid methyl esters (FAME) were separated by gas chromatography (Agilent 7890A Gas Chromatograph; Agilent Technologies, Inc., Santa Clara, CA, USA) using a 7693 mass spectrometer detector (Agilent Technologies, Inc., Santa Clara, CA, USA), a capillary column (Omegawax\(^\text{TM}\) 250 fused silica capillary column, 30 mm × 0.25 mm and 0.25 mm film thickness, Supleco\(^\circledR\)), and a 7890A autoinjector (Agilent Technologies, Inc.). Helium was used as the carrier gas at a flow of 1.3 ml min\(^{-1}\) and the injection volume was 2 mL. Initial temperature of the oven was 175 °C for 26 min, which was increased to 205 °C by increments of 2 °C min\(^{-1}\), then held at 205 °C for 24 min. The source and analyzer for the mass spectrometer was set at 230 °C. The individual fatty acid methyl esters were identified by comparing the retention times of authentic standard mixtures (FAME mix 37 components, Supleco) and quantified by comparing their peak areas with that of the internal standard (Czesny & Dabrowski, 1998). The results of individual fatty acid composition are expressed in percentage of total identified FAME.

**Statistical analysis.** We performed a series of multivariate analyses to address our specific objectives. We used PERMANOVA a part of the PRIMER 6 statistical software package (Clarke & Gorley, 2006), to test for overall differences between the microzooplankton and mesozooplankton community composition, and fatty acid profiles of seston, microzooplankton, and mesozooplankton. PERMANOVA is a non-parametric technique related to ANOVA, but uses permutations and fewer assumptions compared to the traditional ANOVA approach (Anderson, 2001). As such, it is particularly well suited to multivariate data sets that violate the traditional assumptions of ANOVA and also have low sample sizes, as was our case (Anderson, 2001).

If PERMANOVA detected differences, we then generated separate, Bray-Curtis similarity matrices for microzooplankton species composition (60 µm mesh), mesozooplankton species composition (200 µm mesh), seston fatty acid composition, microzooplankton fatty acid composition, and mesozooplankton fatty acid composition. A separate cluster analysis was performed using PRIMER 6 in order to reveal patterns over time and space for each similarity matrix. Each individual sample was associated with a location in the river (upper, middle, lower) and month (April, May, June) and these labels were used for visualization of samples in the cluster dendrogram. Next, a similarity percentage analysis (SIMPER) test was used to compare similarities within groups and determine the species or fatty acids that contributed to each grouping from the cluster analysis (Clarke & Gorley, 2006). The SIMPER test was set at 70% cumulative contribution.

We then wanted to determine if salinity and temperature were related to the observed patterns and we used redundancy analysis for this purpose (Legendre & Legendre, 1998). The redundancy analysis was carried out in the R environment (R v3.2.3, R Core Development Team, 2015) using rda function in the vegan package (Oksanen et al., 2017). Finally, we used a Mantel matrix comparison to correlate fatty acid profiles between the three groups (seston, microzooplankton, and mesozooplankton). The mantel.rtest function in the ade4
Table 1 Results of SIMPER analysis for each group; all the zooplankton species that contributed community composition, and their corresponding values in % are given. Dash marks represent species that were not included in the contribution of 70%.

| Species          | Overall similarity | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|------------------|--------------------|---------|---------|---------|---------|---------|
| Microzooplankton | 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   | –       | –       | –       |         |

| Mesozooplankton  | Percent composition|         |         |         |         |         |

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

RESULTS

Salinity and temperature

Salinity was near zero (0.02–0.04) throughout the river in April. During May, salinities in the upper river remained low (0.07), but the water column became stratified in the middle and lower river, with salinities ranging from 0.28–1.66. The river returned to freshwater (0.04–0.08) again in June due to a tropical storm that brought heavy rains for a two-week period. North Carolina experienced the second wettest June since 1895 with rainfall that ranged from 15.2 to 19.05 cm in the study area (Hiatt, 2013). Water temperatures increased during the study period April (15.7 ± 1.1 °C), May (24.0 ± 1.0 °C) and June (26.2 ± 0.3 °C).

Zooplankton community composition

There were significant differences between the overall microzooplankton and mesozooplankton community composition (PERMANOVA, p = 0.001). Microzooplankton could be separated into two distinct groups by cluster analysis at 65% similarity (Fig. 2A). Group 1 consisted of the vast majority of the samples collected across April, May, and June throughout the river (Fig. 2A). Group 2 consisted of two samples collected in the middle and lower river in June (Fig. 2A). Both groups were dominated by rotifers and copepod nauplii, but group 2 had a higher contribution of copepod nauplii (Fig. 2B and Table 1).

Three groups of mesozooplankton were differentiated at 50% similarity using cluster analysis (Fig. 3A). Group 3 consisted of samples from the May collection only, Group 4 consisted of a mixture of April and June samples in primarily the upper and middle river, and Group 5 consisted of one April upper site, May upper river section and June samples throughout the river (Fig. 3A). Group 3 mesozooplankton percent composition was dominated by Acartia spp. with a minor contribution by Bosminidae, Group 4 consisted primarily of equivalent percentages of Cyclopoida and Bosminidae, and
Figure 2  The two microzooplankton community composition groups from cluster analysis (A) at 65% similarity. The mean microzooplankton community composition (% ± S.D.) for the two 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 (%, ±S.D.) for the three groups from cluster analysis (B).
Group 5 mesozooplankton percent community composition was characterized by higher percentages of *Leptodora* spp. and Calanoida (Fig. 3B and Table 1).

**Fatty acid composition**

A total of 24 specific fatty acids were found in all samples (Tables A1–A3). Fatty acids were first separated into broad categories: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Fig. 4A). Seston had a higher percent of SFA, and lower percent of MUFA and PUFA compared to micro- and mesozooplankton (Fig. 4A). Mesozooplankton and microzooplankton had a similar percent composition of SFA, MUFA, and PUFA (Fig. 4A). There were eight dominant fatty acids found in all the samples, but the percent composition varied (Fig. 4B). The most common SFA was palmitic acid (16:0), the most common MUFAs were palmitoleic acid (16:1ω-7) and oleic acid (18:1ω-9), and the most common PUFAs were ALA, 18:4ω-3, EPA, and DHA (Fig. 4B; Tables A1–A5). A comparison of MUFAs and PUFAs among the seston and zooplankton showed that seston had the lowest overall percentages of MUFAs and PUFAs (PERMANOVA, $p = 0.001$, Fig. 4B). There was a difference between the microzooplankton fatty acid profile and the mesozooplankton fatty acid profile (PERMANOVA, $p = 0.025$). The microzooplankton fatty acid profile was characterized by a higher percentage of 18:1ω-9 compared to the other MUFAs and PUFAs. In contrast, the mesozooplankton had the highest percent composition attributed to two PUFAs, EPA and DHA (Fig. 4B).

**Seston**

Three groups were designated at 60% similarity using cluster analysis for the seston fatty acid composition (Fig. 5A). The groups showed no distinct pattern in terms of sampling time or location (Fig. 5A). Seston fatty acid composition of Group A was characterized by 18:1ω-9, 16:1ω-7, ALA, and EPA, Group B by 16:1ω-7, 18:1ω-9, 18:2ω-6 and EPA, and Group C had similar percentage composition of MUFAs and PUFAs, except 18:2ω-6 (Fig. 5B and Table 2).

| Seston | Microzooplankton | Mesozooplankton |
|--------|-------------------|-----------------|
| 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 | 3.42 | 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 |

**Table 2** Results of SIMPER analysis for each group; the fatty acids that contributed to the differences in fatty acid profile, and their corresponding values in % are given. Dash marks represent fatty acids that were not included in the contribution of 70%. Group G had a sample size <2.
Figure 4  The mean (±S.D.) saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acid composition (%) for the seston, microzooplankton, and mesozooplankton (A). The mean fatty acid composition (% ± S.D.) for the seston, microzooplankton, and mesozooplankton (B).
Three groups were designated at 70% similarity using cluster analysis for the microzooplankton fatty acid composition (Fig. 6A). The groups segregated temporally, with Group D and E consisting of April samples only, and Group F consisted of May and June samples only (Fig. 6A). The Group D fatty acids were dominated by 18:1ω-9 and to a lesser extent, 18:2ω-6, Group E showed similar percent composition among the fatty acids, with higher percentages of 18:1ω-9 and EPA, and Group F also had similar percent
composition of fatty acids; however, the PUFAs EPA and DHA had significant contribution (Fig. 6B and Table 2).

**Mesozooplankton**

Four groups were designated by cluster analysis at 77% similarity for the mesozooplankton fatty acid composition (Fig. 7A). Groups G and I consisted of April samples only whereas Group J consisted of May and June samples only (Fig. 7A). Group H showed spatial...
Figure 7  The four mesozooplankton fatty acid composition groups from cluster analysis (A) at 60% similarity. The mean mesozooplankton fatty acid composition (% ± S.D.) for the four groups from cluster analysis (B).

separation, consisting of primarily upper river locations across all of the months (Fig. 7A). The Group G and H fatty acids were dominated by 18:1ω-9, Group 8 by 16:1 ω-7, 18:1ω-9, and EPA, Group I had similar percentages of fatty acids with ALA, DHA and EPA having higher percentages, and Group J had DHA and EPA as dominant components of the fatty acids (Fig. 7B and Table 2).

Redundancy analysis and Mantel matrix correlations
Salinity was the main factor found to be correlated to observed patterns in the species composition of both microzooplankton and mesozooplankton (Redundancy, p = 0.004).
However, salinity was not correlated to changes in the seston (Redundancy, \( p = 0.490 \)) or microzooplankton (Redundancy, \( p = 0.27 \)) fatty acid profiles. Salinity was associated with the changes in the mesozooplankton fatty acid profiles (Redundancy, \( p = 0.034 \)). Seston fatty acid profiles were correlated to the microzooplankton fatty acid profiles (\( p = 0.013 \)) based on the Mantel matrix comparison, but not correlated to the mesozooplankton fatty acid profiles (\( p = 0.340 \)). The microzooplankton fatty acid profiles were correlated to the mesozooplankton fatty acid profiles (Mantel, \( p = 0.059 \)).

**DISCUSSION**

We found temporal and spatial differences in the species and fatty acid composition of the lower food web that were mainly related to a salinity intrusion that occurred during the study period during May. Prior to the salinity intrusion, larval fish would have encountered a freshwater plankton assemblage that was dominated by rotifers, Bosminidae, and cyclopoid copepods throughout the river. This assemblage was proportionally higher in EPA relative to DHA. During the salinity intrusion, the microzooplankton remained dominated by rotifers; however, the mesozooplankton community became dominated by the copepod *Acartia* spp. Concurrently, the proportion of DHA increased and remained elevated into June, particularly in the middle and lower river. The intrusion of saline water increased the overall proportion of omega-3 fatty acids in the river, presumably due to the increased fraction of micro- and mesozooplankton feeding on a more marine-like phytoplankton based food web and this signal propagated through the food web. Additionally, we observed that FA appeared to be incorporated relatively unchanged in micro and mesozooplankton in terms of relative composition; however, MUFA and PUFA percent compositions increased in zooplankton relative to seston. This suggested that MUFA and PUFA are bioaccumulated at higher trophic levels, as seen in other studies (*Persson & Vrede, 2006*; *Gladyshev et al., 2010*; *Ravet, Brett & Arhonditsis, 2010*; *Burns, Brett & Schallenberg, 2011*). Overall, the FA composition of the food web indicated that the Chowan River is likely to provide nutrition in terms of FA composition for larval fish growth and development. This is based on the presence of higher chain (>20 carbons) PUFAs present in the mesozooplankton throughout the nursery.

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

We did not examine the seston composition directly by counting phytoplankton cells or examining pigment concentrations, thus we were unable to attribute the origin of
particular fatty acids to phytoplankton or other sources. However, we were able to use the available literature to identify potential indicators of fatty acid origin. The top four fatty acids by percent composition varied by group, but 16:1\(\omega-7\), 18:1\(\omega-9\), 18:2\(\omega-6\), and ALA were the most prevalent. Potential phytoplankton sources for these fatty acids may be diatoms, which have been shown to have increased 16:1\(\omega-7\) and EPA in both freshwater and marine systems (Napolitano et al., 1997; Dalsgaard et al., 2003; Boscher, Krombamp & Middelburg, 2005; Arts, Brett & Kainz, 2009; Bec et al., 2010) and we observed this occurred in May and June coincident with the salinity increase. Green algae (Chlorophytes) have been shown to possess higher proportions of 18:2\(\omega-6\), and ALA, and 18:4\(\omega-3\) (Ahlgren et al., 1990; Dalsgaard et al., 2003; Boschker, Krombamp & Middelburg, 2005; Masclaux et al., 2012; Strandberg et al., 2015). Fatty acids corresponding to these phytoplankton groups were observed during April throughout the river and June in the middle and upper river. One other source of seston FA may have been present, pine pollen, which is found in large quantities during spring. Pine pollen is transported to freshwater systems via aerial deposition and floats at the surface, and the pine pollen fatty acid profile has a high percent composition of 18:1\(\omega-9\) and 18:2\(\omega-6\) (Masclaux et al., 2013), which can be observed in our samples. Obviously, seston FA are a mixture of multiple sources, thus the variability seen across the groups identified by the cluster analysis would be expected.

The microzooplankton fatty acid profiles were different throughout the sampling period with a change from decreased omega-3s to increased omega-3s in the system. This suggests a switch in microzooplankton diet had occurred over the sampling period and two pathways appear to be present during the study. The April microzooplankton fatty acid profiles for all river sections had a high percentage of 18:1\(\omega-9\) and 18:2\(\omega-6\) suggesting that the microzooplankton could be consuming either terrestrial material or chlorophytes during this time. Two sites in April had an increase in omega-3 fatty acids (ALA, 18:4\(\omega-3\), EPA, DHA), and this would suggest a different dietary pathway that was reduced in SFA, perhaps consisting of either smaller microzooplankton such as ciliates or phytoplankton such as diatoms and/or dinoflagellates (Park & Marshall, 2000; Gladyshev et al., 2010). The community was dominated by rotifers during this time and communities high in rotifer abundance have been shown to closely reflect the seston composition (Gladyshev et al., 2010). The microzooplankton fatty acid profiles in May and June at all river locations had an increase in 16:1\(\omega-7\), and omega-3s (ALA, EPA, and DHA). These changes can be correlated to the likely increase in diatoms and dinoflagellates from the saltwater intrusion event. The changes in June could be the increase in copepod nauplii of Calanoid copepods, and the presence of dinoflagellates and diatoms even when the system returned to freshwater. Our results are similar to systems where the phytoplankton composition was represented by diatoms and dinoflagellates by having increased 16:1\(\omega-7\) and PUFAs (Müller-Navarra et al., 2000; Dalsgaard et al., 2003; Gladyshev et al., 2010; Ravet, Brett & Arhonditsis, 2010).

The mesozooplankton fatty acid profiles throughout the river in April and in the upper river in May and June were defined by higher percentages of 16:1\(\omega-7\), 18:1\(\omega-9\), ALA, EPA, and DHA, and mixed mesozooplankton community consisting of cladoceran and copepods. These fatty acids profiles are similar to those found in freshwater systems that have a mixed zooplankton composition (Persson & Vrede, 2006; Arts, Brett & Kainz, 2009;
Cladocerans have low or no DHA compared to copepods and high EPA levels have been shown to correlate with the high somatic growth rates of cladoceran (Persson & Vrede, 2006). A saltwater intrusion changed the mesozooplankton species composition in May resulting in numerical dominance by *Acartia* spp. in the lower and middle sections of the river. *Acartia* spp. is the dominant copepod species in temperate, estuarine systems (Ambler, Cloern & Hutchinson, 1985; Orsi & Mecum, 1986; Cervetto, Gaudy & Pagano, 1999; Mouny & Dauvin, 2002; Kimmel & Roman, 2004; Lawrence, Valiela & Tomasky, 2004; Islam, Ueda & Tanaka, 2005). The mesozooplankton fatty acid profiles in May were represented by 16:1ω-7, EPA, and the highest observed percentages of DHA. This is clearly a reflection of the dominance of *Acartia* spp. in the system and a diet primarily consisting of marine algae higher in omega-3 FAs (Stottrup, Bell & Sargent, 1999; Persson & Vrede, 2006; Arts, Brett & Kainz, 2009; Kainz et al., 2009; Gladyshev et al., 2010; Masclaux et al., 2012). Mesozooplankton fatty acid percent composition in June at the lower and middle site remained similar to that observed in May, despite the species composition having returned to a mix of cladocerans and copepods. This suggests that physical shifts in the system that result in seston changes may persist in the system despite shifts in zooplankton community composition.

The relevance of the food web fatty acid composition can be determined by examining the potential feeding behavior of larval fish within the Chowan River nursery. Alewife and blueback herring start feeding on smaller cladocerans and copepods at about 6 mm total length (Mullen, Fay & Moring, 1986). Binion (2011) reported that river herring at 6 mm notochord length had a maximum gape width of 400 µm, and estimated maximum prey size of 200 µm, which would result in mesozooplankton being an important food resource. In the Connecticut River, the diet for blueback herring were dominated by rotifers for fish 5–12 mm, Bosminidae for fish 12–16 mm, and cyclopoid copepods for fish >16 mm in total length (Crecco & Blake, 1983). Based on these dietary studies, the larval and juvenile river herring would be feeding across the size range of zooplankton prey that we sampled; however, fish would be consuming primarily microzooplankton early in the year (April) and mesozooplankton later in the season (May and June). In April, two pathways for FA propagation were present in the microzooplankton. Thus, fish feeding during this time may experience variability in the quality of the microzooplankton prey in terms of percentage of PUFAs. Larval fish need PUFAs (ALA, EPA and DHA) for growth, visual acuity, survival, and development of normal pigmentation (Bell et al., 1995; Bell & Sargent, 1996; Rainuzzo, Reitan & Olsen, 1997; Sargent et al., 1999; Rossi et al., 2006). The shift to larger prey later in the year (May and June) resulted in a change in prey quality as the relative percentage of EPA and DHA increased. This was the result of a salinity intrusion into the middle and lower reaches of the estuary that was associated with dominance of the cladoceran *Acartia* spp. and a significant increase in DHA and EPA. This could allow larval and juvenile fish to consume prey with a higher proportion of PUFAs. It is unknown if river herring can elongate precursor FA into PUFAs. Even if fish can convert precursor FA, larval fish could not receive all nutritional need for those fatty acids (Agaba et al., 2005). The larval fish would not have to use energy for the conversion, and could continue to put energy into...
growth (Wacker & Von Elert, 2001; Rossi et al., 2006). This allows the larval fish to survive and grow past the critical period.

The fish nursery present in the lower Chowan River may undergo significant changes during the critical time of larval fish growth and our results demonstrate how changes in the seston community may propagate through the food web. The results also highlight that additional information concerning the fatty acid composition of the zooplankton prey base for larval fish can provide insight into habitat quality, our stated goal. Sheaves et al. (2015) pointed out the need to expand the nursery habitat concept to include relevant ecosystem processes, particularly resource dynamics. This research begins to explore the mechanisms that allow nursery habitat to function. We plan further research to investigate the linkage between the seston community fatty acid composition, the zooplankton community fatty acid composition, and larval fish to determine how lower food web variability relates to larval fish condition and survival.

ACKNOWLEDGEMENTS

I would like to thank S Lichti, C Krahforst, J Osborne, A Powell, M Baker, and E Diaddorio for help in the field collection. I would like to thank L Stratton, C Kolb, and R Pattridge for help in Dr. Jacques Rinchard’s lab in processing my fatty acid samples. I would like to thank Dr. Ariane Peralta for help with the improved statistical analysis, and two anonymous reviewers for comments, which helped to improve the manuscript.

APPENDIX

Table A.1 Mean fatty acid composition (±standard deviation) (percentage of total fatty acids detected) of seston from the Chowan River by group.

| 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 |

(continued on next page)
### Table A.1 (continued)

| Seston group | A (8) | B (5) | C (4) |
|--------------|-------|-------|-------|
| 18:2o-6      | 2.9 ± 4.2 | 0.6 ± 0.6 | 0.8 ± 1.2 |
| 18:4o-3      | 2.4 ± 1.4 | 0.9 ± 0.5 | 3.3 ± 1.3 |
| 20:2o-6      | 0.7 ± 1.1 | 0.1 ± 0.1 | 1.7 ± 1.4 |
| 20:3o-6      | 0.5 ± 0.4 | 0.2 ± 0.2 | 0.7 ± 0.5 |
| 20:4o-6      | 0.4 ± 0.1 | 0.4 ± 0.3 | 0.3 ± 0.2 |
| 20:3o-3      | 0.9 ± 0.6 | 0.8 ± 0.4 | 0.8 ± 0.5 |
| 20:4o-3      | 0.6 ± 0.3 | 0.4 ± 0.3 | 1.1 ± 0.6 |
| 22:5o-6      | 0.6 ± 0.3 | 0.7 ± 0.6 | 1.2 ± 0.7 |
| 22:6o-3      | 0.8 ± 0.6 | 1.1 ± 0.6 | 1.9 ± 1.0 |
| ∑PUFA        | 13.5  | 8.1   | 17.7  |

**Notes.**
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

### Table A.2  Mean fatty acid composition (percentage of total fatty acids detected) of microzooplankton (<60 µm) from the Chowan River by groups.

| 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  |
| 16:1o-9                 | 0.3 ± 0.1 | 0.8 ± 0.3 | 0.9 ± 0.6 |
| 16:1o-7                 | 2.2 ± 1.6 | 1.4 ± 0.1 | 7.1 ± 2.0 |
| 18:1o-9                 | 31.6 ± 5.3 | 13.5 ± 0.5 | 5.3 ± 3.1 |
| 18:1o-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  |
| 18:2o-6                 | 12.2 ± 1.6 | 6.9 ± 0.9 | 2.8 ± 1.2 |
| 18:3o-3                 | 2.9 ± 1.3 | 8.4 ± 1.3 | 7.0 ± 1.7 |
| 18:4o-3                 | 3.2 ± 2.3 | 10.3 ± 1.5 | 2.8 ± 0.7 |
| 20:2o-6                 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| 20:3o-6                 | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.2 ± 0.1 |
| 20:4o-6                 | 0.6 ± 0.7 | 0.4 ± 0.1 | 3.2 ± 0.5 |

(continued on next page)
### Table A.2  (continued)

| Microzooplankton Groups | D (5) | E (2) | F (8) |
|-------------------------|-------|-------|-------|
| 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 |

Notes.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

### Table A.3  Mean fatty acid composition (± standard deviation) (percentage of total fatty acids detected) of mesozooplankton from the Chowan River by group.

| 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 |

Notes.
SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
ADDITIONAL INFORMATION AND DECLARATIONS

Funding
This work was funded by NSF Grant DEB-0951414 to DG Kimmel. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
NSF: DEB-0951414.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Deborah A. Lichti conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables.
• Jacques Rinchard conceived and designed the experiments, performed the experiments, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
• David G. Kimmel conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, reviewed drafts of the paper.

Data Availability
The following information was supplied regarding data availability:
The raw data has been supplied as a Supplementary File.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.3667#supplemental-information.

REFERENCES

Able KW. 2005. A re-examination of fish estuarine dependence: evidence for connectivity between estuarine and ocean habitats. *Estuarine, Coastal, and Shelf Science* 64:5–17 DOI 10.1016/j.ecss.2005.02.002.

Agaba MK, Tocher DR, Zheng XZ, Dickson CA, Dick JR, Teale DR. 2005. Cloning and functional characterization of polyunsaturated fatty acid elongates of marine and freshwater teleost fish. *Journal of Comparative Physiology B* 142:342–352.

Ahlgren G, Lundstedt L, Brett M, Forsberg C. 1990. Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. *Journal of Plankton Research* 12:809–818 DOI 10.1093/plankt/12.4.809.

Ambler JW, Cloern JE, Hutchinson A. 1985. Seasonal cycles of zooplankton from San Francisco Bay. *Hydrobiologia* 129:177–197 DOI 10.1007/BF00048694.
Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26:32–46 DOI 10.1111/j.1442-9993.2001.01070.pp.x.

Arts MT, Brett MT, Kainz MJ. 2009. *Lipids in aquatic ecosystems*. New York: Springer.

ASMFC (Atlantic States Marine Fisheries Commission). 2012. River herring benchmark stock assessment volume 1. Stock Assesment Report No. 12-02, May 2012..

Bec A, Perga ME, Desvilettes C, Bourdier G. 2010. How well can the fatty acid content of lake seston be predicted from its taxonomic composition?. *Freshwater Biology* 55:1985–1972.

Beck MW, Heck Jr KL, Able KW, Childers DL, Eggleston DB, Gillanders BM, Halpern B, Hays CG, Hoshino K, Minello TJ, Orth RJ, Sheridan PF, Weinstein MP. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *BioScience* 51:633–641 DOI 10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2.

Bell MV, Batty RS, Dick JR, Fretwell K, Navarro JC, Sargent JR. 1995. Dietary deficiency of docosahexanenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids* 30(5):443–449 DOI 10.1007/BF02536303.

Bell MV, Sargent JR. 1996. Lipid nutrition and fish recruitment. *Marine Ecology Progress Series* 134:315–316 DOI 10.3354/meps134315.

Binion S. 2011. Evaluating spatial and temporal overlap between larval alosines and potential zooplankton prey in lower Roanoke River and Albemarle Sound, North Carolina. Thesis, East Carolina University, Greenville, NC.

Boehlert GW, Mundy BC. 1988. Role of behavioral and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. *American Fisheries Society Symposium* 3:51–67.

Boesch DF, Turner RF. 1984. Dependence of fisheries species on salt marshes: the role of food and refuge. *Estuaries and Coasts* 7:460–468 DOI 10.2307/1351627.

Boschker HT, Krombamp JC, Middelburg JJ. 2005. *Limnology and Oceanography* 50:70–80 DOI 10.4319/lo.2005.50.1.0070.

Brett MT, Müller-Navarra DC, Ballantyne AP, Ravet JL, Goldman CR. 2006. *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography* 51:2428–2437 DOI 10.4319/lo.2006.51.5.2428.

Burns C, Brett MT, Schallenberg M. 2011. A comparison of the trophic transfer of fatty acids in freshwater plankton by cladocerans and calanoid copepods. *Freshwater Biology* 56:889–903 DOI 10.1111/j.1365-2427.2010.02534.x.

Cervetto G, Gaudy R, Pagano M. 1999. Influence of salinity on the distribution of Acartia tonsa (Copepoda, Calanoida). *Journal of Experimental Marine Biology and Ecology* 239:33–45 DOI 10.1016/S0022-0981(99)00023-4.

Chambord S, Maris T, Colas F. van Engeland, T, Sossu AC, Azemar F, Le Coz M, Cox T, Buisson L, Souissi S, Meire P, Tackx M. 2016. Mesozooplankton affinities in a recovering freshwater estuary. *Estuarine, Coastal, and Shelf Science* 177:47–59 DOI 10.1016/j.ecss.2016.04.016.
Chick JH, Levchuk AP, Medley KA, Havel JH. 2010. Underestimation of rotifer abundance a much greater problem than previously appreciated. Limnology and Oceanography: Methods 8:79–87 DOI 10.4319/lom.2010.8.0079.

Clarke KR, Gorley RN. 2006. Primer V6: user manual/tutorial. Plymouth: Primer-E Ltd.

Cooper JE, Rulifson RA, Isely JJ, Winslow SE. 1998. Food habits and growth of juvenile striped bass, Morone saxatilis, in Albemarle Sound, North Carolina. Estuaries 21:307–317 DOI 10.2307/1352477.

Copeman LA, Parrish CC, Brown JA, Harel M. 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (Limanda ferruginea): a live food enrichment experiment. Aquaculture 210:285–304 DOI 10.1016/S0044-8486(01)00849-3.

Crecco VA, Blake MM. 1983. Feeding ecology of coexisting larvae of American shad and blueback herring in the Connecticut River. Transactions of the American Fisheries Society 112:498–507.

Czesny S, Dabrowski K. 1998. The effect of fatty acids concentration in wild and domesticated walleye (Stizostedion vitreum) eggs on embryos’ viability. Aquatic Living Resources 11:371–378 DOI 10.1016/S0990-7440(99)80002-3.

Dalsgaard J, St. John M, Kattner G, Müller-Navarra D, Hagen W. 2003. Fatty acids trophic markers in the pelagic marine environment. Advances in Marine Biology 46:225–340 DOI 10.1016/S0065-2881(03)46005-7.

Desvilettes CH, Bourdier G, Amblard CH, Barth B. 1997. Use of fatty acids for the assessment of zooplankton grazing on bacteria, protozoans and microalgae. Freshwater Biology 38:629–637 DOI 10.1046/j.1365-2427.1997.00241.x.

Farkas T, Herodek S. 1964. The effect of environmental temperature on the fatty acid composition of crustacean plankton. Journal of Lipid Research 5:369–373.

Folch J, Lees M, Sloane Stanley GH. 1957. A simple method for isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226:497–509.

Fraser AJ, Sargent JR, Gamble JC, Seaton DD. 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton, and herring (Clupea harengus L.) larvae. Marine Chemistry 27:1–18 DOI 10.1016/0304-4203(89)90024-8.

Gladyshev MI, Sushchik NN, Makhutova ON, Dubovskaya OP, Kravchuk ES, Khromechek EB. 2010. Correlations between fatty acid composition of seston and zooplankton and effects of environmental parameters in a eutrophic Siberian reservoir. Limnologica 40:343–357 DOI 10.1016/j.limno.2009.12.004.

Goncalves AMM, Azeiteiro UM, Pardal MA, De Troch M. 2012. Fatty acid profiling reveals seasonal and spatial shifts in zooplankton diet in a temperate estuary. Estuarine, Coastal and Shelf Science 109:70–80 DOI 10.1016/j.ecss.2012.05.020.

Goulden CE, Place AR. 1990. Fatty acid synthesis and accumulation rates in daphniids. Journal of Experimental Zoology 256:168–178 DOI 10.1002/jez.1402560207.

Greene KE, Zimmerman JL, Laney RW, Thomas-Blate JC. 2009. Atlantic coast diadromous fish habitat: a review of utilization, threats, recommendations for conservation,
and research needs. Atlantic States Marine Fisheries Commission Habitat Management Series, no. 9. Washington, D.C: Atlantic State Marine Fisheries Commission.

Gulati RD, Demott WR. 1997. The role of food quality for zooplankton: remarks on the state-of-the-art, perspectives and priorities. Freshwater Biology 38:753–768 DOI 10.1046/j.1365-2427.1997.00275.x.

Haney JF, Hall DJ. 1973. Sugar-coated Daphnia: a preservation technique for Cladocera. Limnology and Oceanography 18:331–333 DOI 10.4319/lo.1973.18.2.0331.

Hiatt A. 2013. Climate Summary: June 2013. State Climate Office of North Carolina. Available at http://nc-climate.ncsu.edu/climateblog?id=28 (accessed on 15 September 2013).

Hjort J. 1914. Fluctuations in the great fisheries of northern Europe viewed in the light of biological research. Rapports et Procès-Verbaux des Réunions 29:1–228.

Islam MS, Ueda H, Tanaka M. 2005. Spatial distribution and trophic ecology of dominant copepods associated with turbidity maximum along the salinity gradient in a highly embayed estuarine system in Ariake Sea, Japan. Journal of Experimental Marine Biology and Ecology 316:101–115 DOI 10.1016/j.jembe.2004.11.001.

Iverson SJ, Field C, Bowen WD, Blanchard W. 2004. Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecological Monographs 74:211–235 DOI 10.1890/02-4105.

Johnson WS, Allen DM. 2012. Zooplankton of the Atlantic and Gulf coasts: a guide to their identification and ecology. 2nd edition. Baltimore: John Hopkins University Press.

Kainz M, Arts MT, Mazumder A. 2004. Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnology and Oceanography 49:1748–1793 DOI 10.4319/lo.2004.49.5.1784.

Kainz MJ, Perga MT, Arts, Mazumder A. 2009. Essential fatty acid concentration of different seston sizes and zooplankton: a field study of monomictic coastal lakes. Journal of Plankton Research 31:635–645 DOI 10.1093/plankt/fbp015.

Kimmel DG, Roman MR. 2004. Long-term trends in mesozooplankton abundance in Chesapeake Bay USA: influence of freshwater input. Marine Ecology Progress Series 267:71–83 DOI 10.3354/meps267071.

Lawrence D, Valiela I, Tomasky G. 2004. Estuarine calanoid copepod abundance in relation to season, salinity, and land-derived nitrogen loading, Waquoit Bay, MA. Estuarine, Coastal and Shelf Science 61:547–557 DOI 10.1016/j.ecss.2004.06.018.

Leech D, Ensign S, Piehler M. 2009. Zooplankton Assessment Project (ZAP): reassessing prey availability for river herring in the Chowan River Basin- Year 2. North Carolina Sea Grant Report. FRG 08-EP-06/ 09-EP-03.

Legendre P, Legendre L. 1998. Numerical ecology. 2nd edition. Amsterdam: Elsevier.

Malzahn AM, Aberle N, Clemmensen C, Boersma M. 2007. Nutrient limitation of primary producers affects planktivorous fish condition. Limnology and Oceanography 52:2062–2071 DOI 10.4319/lo.2007.52.5.2062.

Marques SC, Azeiteiro UM, Marques JC, Neto JM, Pardal UM. 2006. Zooplankton and ichthyoplankton communities in a temperate estuary: spatial and temporal patterns. Journal of Plankton Research 28:297–312.
Martino EJ, Houde ED. 2010. Recruitment of striped bass in Chesapeake Bay: spatial and temporal environmental variability and availability of zooplankton prey. *Marine Ecology Progress Series* **409**:213–228 DOI 10.3354/meps08586.

Masclaux H, Bec A, Kainz MJ, Perriere F, Desvilettes C, Bourdier G. 2012. Accumulation of polyunsaturated fatty acids by cladocerans: effects of taxonomy, temperature and food. *Freshwater Biology* **57**:696–703 DOI 10.1111/j.1365-2427.2012.02735.x.

Masclaux H, Perga ME, Kagami M, Desvilettes C, Bourdier G, Bec A. 2013. How pollen organic matter enters freshwater food web. *Limnology and Oceanography* **58**:1185–1195 DOI 10.4319/lo.2013.58.4.1185.

McHugh JL. 1967. Estuarine nekton. *American Association for the Advancement of Science Publications* **83**:581–620.

Metcalfe LD, Schmitz AA. 1961. The rapid preparation of fatty acid esters for gas chromatographic analysis. *Analytical Chemistry* **33**:363–364 DOI 10.1021/ac60171a016.

Mouny P, Dauvin JC. 2002. Environmental control of mesozooplankton community structure in the Seine estuary (English Channel). *Oceanologica Acta* **25**:13–22 DOI 10.1016/S0399-1784(01)01177-X.

Mullen DM, Fay CW, Moring JR. 1986. Species profiles: life history and environmental requirements of coastal fishes and invertebrates (North Atlantic) alewife/blueback herring. Biological report 82. USFWS and Army Corps of Engineers.

Müller-Navarra DC, Brett MT, Liston AM, Goldman CR. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* **403**:74–77 DOI 10.1038/47469.

Müller-Navarra DC, Brett MT, Park S, Chandra S, Ballantyne AP, Zorita E, Goldman CR. 2004. Unsaturated fatty acid content in seston and tropho-dynamic coupling in lakes. *Nature* **427**:69–72 DOI 10.1038/nature02210.

Napolitano GE, Pollero RJ, Gayoso AM, MacDonald BA, Thompson RJ. 1997. Fatty acid as trophic markers of phytoplankton blooms in the Bahia Blanca Estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). *Biochemical Systematics and Ecology* **25**:739–755 DOI 10.1016/S0305-1978(97)00053-7.

NCDENR (North Carolina Department of Environment and Natural Resources). 2006. *Basinwide assessment report Chowan River Basin*. Raleigh: NCDENR.

NCDMF (North Carolina Division of Marine Fisheries). 2007. *North Carolina fisheries management plan amendment 1 river herring*. Morehead City: NCDMF.

Oksanen J, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O’Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H. 2017. Vegan: community ecology package. R package version 2.4-3. Available at *https://CRAN.R-project.org/package=vegan*.

Orsi JJ, Mecum WL. 1986. Zooplankton distribution and abundance in the Sacramento-San Joaquin delta in relation to environmental factors. *Estuaries* **9**:326–339 DOI 10.2307/1351412.

Park GS, Marshall HG. 2000. The trophic contributions of rotifers in tidal freshwater and estuarine habitats. *Estuarine, Coastal, and Shelf Science* **51**:729–742 DOI 10.1006/ecss.2000.0723.
Paulsen M, Hammer C, Malzahn AM, Polte P, Von Dorrien C, Clemmesen C. 2014. Nutritional situation for larval Atlantic herring (Clupea harengus L.) in two nursery areas in the western Baltic Sea. ICES Journal of Marine Science 71:991–1000 DOI 10.1093/icesjms/fst168.

Persson J, Vrede T. 2006. Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. Freshwater Biology 51:887–900 DOI 10.1111/j.1365-2427.2006.01540.x.

Postel L, Fock H, Hagen W. 2000. Chapter 4 Biomass and abundance. In: ICES Zooplankton Methodology Manual. Academic Press.

Rainuzzo JR, Reitan KI, Olsen Y. 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155:103–115 DOI 10.1016/S0044-8486(97)00121-X.

Ravet JL, Brett MT, Arhonditsis GB. 2010. The effects of seston lipids on zooplankton fatty acid composition in Lake Washington, Washington, USA. Ecology 9:180–190.

Rossi S, Sabates A, Latasa M, Reyes E. 2006. Lipid biomarkers and trophic linkages between phytoplankton, zooplankton and anchovy (Engraulis encrasicolus) larvae in the NW Mediterranean. Journal of Plankton Research 28:551–562 DOI 10.1093/plankt/fbi140.

R Core Development Team. 2015. RStudio: integrated development for R. Boston, MA: RStudio, Inc. Available at http://www.rstudio.com/.

Rulifson RA, Cooper JE, Stanley DW, Shepherd ME, Wood SF, Daniel DA. 1993. Food and feeding of young finfish species in lower Roanoke River, Batchelor Bay, and western Albemarle Sound, North Carolina, 1982-1988. Albemarle-Pamlico Estuarine Study APES 90-16:21-27 to US Environmental Protection Agency.

Sargent J, McEvoy L, Estevez A, Bell G, Bell M, Henderson J, Tocher D. 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179:217–229.

Sheaves M. 2016. Simple processes drive unpredictable differences in estuarine fish assemblages: baselines for understanding site specific ecological and anthropogenic impacts. Estuarine, Coastal and Shelf Science 170:61–69 DOI 10.1016/j.ecss.2015.12.025.

Sheaves M, Baker R, Nagelkerken I, Connolly RM. 2015. True value of estuarine and coastal nurseries for fish: incorporating complexity and dynamics. Estuaries Coas 38:401–414 DOI 10.1007/s12237-014-9846-x.

Smyntek PM, Teece MA, Schulz KL, Storch AJ. 2008. Taxonomic differences in the essential fatty acid composition of groups of freshwater zooplankton relate to reproductive demands and generation time. Freshwater Biology 53:1768–1782 DOI 10.1111/j.1365-2427.2008.02001.x.

Strandberg U, Hiltunen M, Jelkanen E, Taipale SJ, Kainz MJ, Brett MT, Kankaala P. 2015. Selective transfer of polyunsaturated fatty acids from phytoplankton to planktivorous fish in large boreal lakes. Science of the Total Environment 536:858–865.

Stottrup JG, Bell JG, Sargent JR. 1999. The fate of lipids during development and cold storage of eggs in the laboratory-reared calanoid copepod, Acartia tonsa Dana, and in response to different algal diets. Aquaculture 176:257–269 DOI 10.1016/S0044-8486(99)00062-9.
Tackx ML, De Pauw N, Van Mieghem R, Azemar F, Hannouti A, Van Damme S, Fiers F, Daro N, Meire P. 2004. Zooplankton in the Schelde estuary, Belgium and the Netherlands. Spatial and temporal patterns. *Journal of Plankton Research* **26**:133–141 DOI 10.1093/plankt/fbh016.

Taipale S, Kankaala P, Hamalainen K, Jones RI. 2009. Seasonal shifts in the diet of the lake zooplankton revealed by phospholipid fatty acid analysis. *Freshwater Biology* **54**:90–104.

Vargas CA, Escribano R, Poulet S. 2006. Phytoplankton food quality determines time windows for successful zooplankton reproductive pulses. *Ecology* **87**:2992–2999 DOI 10.1890/0012-9658(2006)87[2992:PFQDTW]2.0.CO;2.

Wacker A, Von Elert E. 2001. Polyunsaturated fatty acids: evidence for non-substitutable biochemical resources in *Daphnia galeata*. *Ecology* **82**:2507–2520 DOI 10.1890/0012-9658(2001)082[2507:PFQDTW]2.0.CO;2.

Walsh HJ, Settle LR, Peters DS. 2005. Early life history of blueback herring and alewife in the lower Roanoke River, North Carolina. *Transactions of the American Fisheries Society* **134**:910–926 DOI 10.1577/T04-060.1.

Webster CD, Lovell RT. 1990. Response of striped bass larvae fed brine shrimp from different sources containing different fatty acid compositions. *Aquaculture* **90**:49–61 DOI 10.1016/0044-8486(90)90282-R.

Winder M, Jassby AD. 2011. Shifts in zooplankton community structure: implications for food web processes in the upper San Francisco Estuary. *Estuaries and Coasts* **34**:675–690 DOI 10.1007/s12237-010-9342-x.