Abstract

Characterizing Environmental and Physicochemical Conditions in Nursery Areas of River Herring in Chowan River, North Carolina

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Anadromous fishes such as blueback herring *Alosa aestivalis* and alewife *A. pseudoharengus*, utilize sounds, rivers, and tributaries during spawning. Collectively known as river herring, these two ecologically important species spawn in Chowan River, North Carolina. River herring have been important commercially since the early 1900s. Over time, overfishing, declining water quality, and degradation of habitat likely caused population declines resulting in a moratorium in the state of North Carolina in 2007. This study examined how water quality and water chemistry of Chowan River influenced larval river herring abundance, health, and nutritional condition. I hypothesized that variability in abiotic conditions could structure nursery habitat into groups based on river herring abundances. Ichthyoplankton samples were collected weekly from March 18 through May 26, 2011 at nine stations in Chowan River basin. Biologically higher abundances (number/100m^3 ± SD) of river herring were found in two creeks, Catherine's Creek (1583 ± 2698) and Wiccacon Creek (1316 ± 3027), when compared to the remaining sites (mean = 182 ± 384). Nutrient values (mean concentration ± SD), chlorophyll α (6.17 ± 6.23), phosphate (0.022 ± 0.019), and organic nitrogen (0.21 ± 0.012) were related to

habitat with higher larval fish abundance. Larval nutritional condition does not seem to be significantly correlated with these physicochemical factors; however, standard lengths of larvae are significantly longer in high abundance sites. Results from this study will aid state agencies in completing surveys of larval river herring abundance in Chowan River and will also aid management agencies in sustaining and rebuilding nursery habitat for river herring and many other species in Chowan River, NC and other bodies of water.

CHARACTERIZING ENVIRONMENTAL AND PHYSICOCHEMICAL CONDITIONS IN NURSERY AREAS OF RIVER HERRING IN CHOWAN RIVER, NORTH CAROLINA

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Introduction

The success of a fish developing from egg to adulthood is a delicate balance involving spatial, temporal, chemical, and environmental influences. There are many factors including pollution, habitat loss and degradation, decreased access to spawning habitat, overfishing, and increased predation that contribute to reproductive failure in different fish species (Bozeman and Van Den Avyle 1989; Sabo et al. 1991; Spruill 1998; O'Connell and Angermeier 1999; Bilkovic 2000; Kocovsky et al. 2008; NMFS 2009; Harris and Hightower 2010). Pollution, dam construction, and deforestation are a few factors that can contribute to changing the chemical properties and equilibrium of a river system. Many affected riverine ecosystems provide spawning and nursery habitat for a number of fish species. Slight changes in geography and water chemistry effect anadromous fish spawning (Dahlberg 1970; Crecco and Savoy 1984; Chambers 1991; Limburg 1994; Beasley and Hightower 2000; Limburg 2001; Burdick and Hightower 2006; Greene et al. 2009).

Some anadromous fish populations are declining, making them of interest to fisheries managers and lending themselves to scrutiny and research (McDowall 1992; Musick et al. 2000). Knowing the abundance of a species can aid managers in determining the fate of a fish population. Blueback herring, *Alosa aestivalis,* and Alewife, *A. pseudoharengus*, collectively known and managed as "river herring" because of similar appearance and commercial value, are two species important to fisheries management and research (Figure 2). Failed egg production, acute contamination, daily mortality, or predation cause variability in the abundance of eggs and larvae during development (Houde 1989). Strong links between population recruits, health, and survival make the larval stage an advantageous proxy for stock analysis (Bergenius et al. 2002).

The river herring fishery is one of the oldest fisheries in North America, dating to 350 years old in some portions of the country (NMFS 2009). Declining catches began in 1960 along

the entire population range. (Figure 2) (Schmidt et al. 2003a). In response to the decline, river herring have been classified as a Species of Concern (SOC) by the National Oceanic and Atmospheric Administration (NOAA) (NMFS 2009). The SOC classification by NOAA in conjunction with state Fisheries Management Plans (FMP) prompted enactment of river herring moratoriums. There are currently four states with an active moratorium: Connecticut, Massachusetts, Rhode Island, and North Carolina.

Research involving stock assessment, population density, spawning location, larval abundance, and restoration efforts of river herring have been conducted in many river systems (Rulifson et al. 1981; Rulifson et al. 1982; Sabo et al. 1991; Rulifson 1994; Schmidt et al. 2003b; Overton and Rulifson 2007; Kocovsky et al. 2008; Overton et al. 2012). However, the FMP for river herring in North Carolina gives first priority for spawning and nursery area surveys in Chowan River. Watershed surveys conducted to determine areas of spawning runs and areas for high brood stock yield are important as well to help prevent overharvest. Lastly, water quality and water chemistry surveys should be completed to evaluate the impact on river herring (NCDMF 2007).

North Carolina has many rivers and streams that serve as important fishing and spawning habitats for river herring (Rohde et al. 2009). River herring have faced reproductive failure, in addition to overharvest, because of pollution and habitat loss throughout their range including North Carolina (Schmidt et al. 2003a). Sounds and tributaries within North Carolina once supported large commercial and recreational fishing catches. Post 1970, Albemarle Sound was the primary location of almost all of the fish landed in North Carolina (NMFS 2009). Within Albemarle Sound, Chowan River was once a large spawning site for river herring (O'Rear 1983; Hightower et al. 1996). A pound-net fishery was also centered in Chowan River (NCDMF

2007). Adults targeted and harvested by the commercial fishery before spawning reduce the spawning potential of the population. In conjunction with pressure from fisheries, habitat degradation and alteration caused a decline in river herring spawning (Hightower et al. 1996). The continued decline in the river herring population warranted the need for the current moratorium and an effort at conservation, sustainability, and population rebuilding efforts.

Houde (1989) discussed the need to study larval stages of fish to adequately gauge a population. Thus, identifying and studying suitable spawning and nursery habitat is an important link to conserving and rebuilding the river herring population (Kocovsky et al. 2008). Both species of river herring have similar life histories; however, their spawning behavior is somewhat different. Alewives prefer to spawn in lentic waters, while blueback herring spawn in lentic and lotic waters (Walsh et al. 2005; Harris and Hightower 2010). Blueback herring and alewife eggs are difficult to speciate, and thus are grouped together as river herring eggs, which range in size from 0.80-1.11 mm (Harris and Hightower 2010). These eggs are initially slightly adhesive and negatively buoyant, sinking to the bottom and attaching to substrate. River herring eggs have an incubation time range of 2.1 days to 15 days depending on water temperature (Klauda et al. 1991). Yolk-sac larvae range from 2-5 mm total length (TL) at hatching, lose the yolk-sac at 3-5 days post hatch, and are 6 mm long at about 10 days old (Loesch 1987; Klauda et al. 1991). Overton et al. (2012) showed that yolk-sac larvae and post-larvae of river herring are planktonic and can be dispersed by passive transport mechanisms.

Water quality and water chemistry have significant effects on larval fish and their spawning and nursery areas (Bozeman and Van Den Avyle 1989; Houde 1989; Sabo et al. 1991; O'Connell and Angermeier 1997; Spruill 1998; Walsh et al. 2005; Waters and Hightower 2007; Kocovsky et al. 2008). All larval fish require specific conditions during the early developmental

stages (Sabo et al. 1991; NCDMF 2007). Sabo et al. (1991) studied floodplain ponds along the west bank of Mississippi River and differentiated between ponds that were regarded as high and low-quality nursery habitats by larval abundance in those ponds. They found that larval fish abundances were affected by dissolved oxygen or dissolved ion concentrations (alkalinity, nitrate, phosphorous, organic carbon, and chlorophyll a). Higher dissolved oxygen and ion concentrations were related to higher larval abundance. Subtle factors such as chronic water contaminant-induced mortality can cause significant losses to recruitment (Houde 1989). Dissolved oxygen, conductivity, and pH are also important parameters that lower the hatch rates of river herring eggs, which will reduce the abundance of larval individuals (Waters and Hightower 2007).

Nutritional condition of fish larvae can be a useful tool to determine the health of a year class or fish population. Dry weights have a strong relationship to muscle energy density (Hartman and Brandt 1995; Wuenschel et al. 2006). Fish with low dry weight or high energy density are in good condition (Hartman and Brandt 1995). A high energy density demonstrates that the larval fish is dedicating more energy to growth than to storage and is thus reducing predation through faster growth (Wuenschel et al. 2006). Measurement of morphological characters and comparison to dry weights is often used as an indicator of larval fish condition (McGurk 1985).

The River Herring Plan Development Team (PDT) and Advisory Council have recently recommended updating the river herring spawning and nursery area surveys and a baseline stock assessment for river herring was completed in 2011. The PDT hoped to develop protocol that would aid in programs to restore the stock of river herring. A comprehensive report was not available at the time of this thesis. O'Rear (1983) performed surveys of larval river herring in

1981. He concluded that larval river herring were abundant in minimum flow tributaries of the Chowan River system. My project explored many of these same nursery habitats to compare current conditions to the results found in O'Rear (1983).

Research Objectives

The goal of my thesis was to address the functionality and suitability of Chowan River as a spawning and nursery habitat of river herring. This research project examined the factors that affect larval rearing habitat and what influence these have on river herring. My initial hypothesis was that variability in abiotic (physicochemical) conditions could structure nursery habitat and affect larval abundance. My first objective was to complete an abundance assessment of larval river herring in Chowan River and its tributaries by surveying the larval river herring production in Chowan River. I collected river herring larvae using pushnets and driftnets to estimate overall abundance. A second objective addressed during my research was characterizing the physicochemical condition of Chowan River and its tributaries. Temperature, dissolved oxygen, salinity, pH, velocity, alkalinity, ammonia, chloride, total and dissolved nitrogen, phosphates, calcium, magnesium, sodium, and potassium were all characterized. Describing the physicochemical parameters for the sampling sites and comparing them to larval abundance permitted creation of an index of experienced habitat for high and low abundance areas.

The third objective of my thesis was a thorough evaluation of the nutritional health of larval river herring. Using three methods for describing nutritional condition, I related nutritional condition to physicochemical parameters and abundance estimates. Fulton's condition index (K) uses dry weight related to standard length to estimate a relative health index among individuals. The thin-plate spline (TPS) suite of geometric morphometric programs allowed for a thorough examination of size and shape differences between fish found at high and

low abundance sites. Finally, using a qualitative histological scoring method, I was able to characterize the health of vital tissues that contribute to the survival of larval river herring. Analysis was performed on three levels: between sites, between developmental stages (pre- and post-flexion), and also between high and low abundance sites. Numerous studies address nutritional condition through lab-reared larvae, but fewer studies calculate this for wild-caught individuals(Koslow et al. 1985; McGurk 1985; Margulies 1993; Ferron and Leggett 1994; Clemmesen and Doan 1996; Suthers 1998; Høie et al. 2000; Swaim and Boeing 2008).

Methods

Site Description

Nine stations were sampled weekly within Chowan River Basin (Tributaries , Sarem Creek, Wiccacon Creek, Bennett's Creek, Catherine's Creek, and Rockyhock Creek, and three main stem sites) and the Meherrin River, (Figure 3; Table 1). The northern most stations (North Chowan and Meherrin River) were accessed using the North Carolina Wildlife Resource Commission (NCWRC) boat ramp, Shoup's Landing. The second two sites, Sarem Creek and Wiccacon Creek, were accessed via the Gatesville NCWRC boat ramp. The three mid-river sampling sites, Bennett's Creek, Catherine's Creek, and Holiday Island, were accessed using the Cannons Ferry NCWRC boat ramp. The final two sampling locations, Rockyhock Creek and South Chowan, were sampled by launching from an unnamed boat ramp on Cowpen Neck Rd., Edenton, NC located at 36°06'33.82"N, 76°41'07.03"W after being given permission from the land owner.

Field Sampling

Water quality and ichthyoplankton samples were collected weekly from March 18 through May 26 of 2011. This time represents the spawning and nursery rearing period in Chowan River and permitted the collection of fish at various stages between hatching and metamorphosis (Rulifson and Overton 2005). Sampling was conducted during the day (0700-1400) because it is safer and there is no difference in catch abundance between day and night sampling (Overton and Rulifson 2007). To address any diel effects of larval dispersal, sampling times were varied at each site weekly by starting with the northern most sites on the first week of sampling, starting with the southernmost sites on next week and continuing to alternate the starting site each week.

A bow-mounted, surface-paired pushnet supported from an aluminum frame mounted on a 5.8-m boat was used to collect larval fishes. Each net was constructed of 500 µm nitex mesh with a Dacron® collar sewn at the mouth, had a 0.5-m square opening, and a mouth-to-tail ratio of 1:5. Bow-mounted pushnets were selected because they are more effective than obliquely towed plankton nets in the collection of river herring larvae (Overton and Rulifson 2007). The net mesh size was chosen to prevent excessive clogging and to allow comparison with long-term ichthyoplankton sampling programs (Zincone and Rulifson 1991; Overton and Rulifson 2007; Binion 2011; Riley 2012). Each net was equipped with a calibrated mechanical flowmeter (Model MF315, SeaGear Corp., Melbourne, Florida) mounted inside the mouth of the net. The nets were pushed upstream at an average speed of 1.4 m/s for 2.0 minutes, filtering between 37 and 57 m^3 of water. Pushnet samples were condensed and preserved immediately after collection. The contents from the portside were preserved in buffered 10% formalin solution and the right side preserved in 95% ethanol. This technique was used so that color and body structure of the larvae can be preserved by formalin to aid in identification and aging the larvae with otoliths can be accomplished from fish preserved in alcohol. Samples preserved in alcohol were split using a Folsom plankton splitter once back in the lab. Half of this sample was fixed in Bouin's solution for histological preparations. The remaining half was again preserved in 95% ethanol. The larvae preserved in formalin solution were stored for an average of 50 days at 20° C to allow morphometric dimensions to stabilize (McGurk 1985).

Water temperature (°C), dissolved oxygen (mg/L), pH, conductivity, and salinity (ppt) profiles were completed for every site on each sampling day for surface and bottom water using a multi-parameter water quality meter (YSI Professional Plus, YSI, Inc., Yellow Springs, OH). Air temperature (°C) and wind speed (m/s) were measured using a portable digital anemometer

(Skymate Model SM-18, Campbell Scientific, Inc., Logan, UT). Water flow velocity was measured at each site from an anchored position. Current velocity (m/s) and direction were measured 1 m below the surface using a portable water velocity meter (Model 201D, Marsh-McBirney, Inc., Frederick, MD). Readings were averaged over ten seconds to determine velocity. Surface water samples (1 L) were collected for analysis of alkalinity, chlorophyll α, nitrate, nitrite, phosphate, chloride, magnesium, sodium, potassium, and calcium. All water samples were preserved in rinsed bottles and placed on ice until filtered (muffled Whatman 934-AH filters, 0.47 mm diameter, 1.5 micron retention).

Daily water discharge estimated for Chowan River was based on five USGS gauging stations located within the watershed, including Potecasie Creek (02053200), Ahoskie Creek (02053500), Nottoway River (020470000), Meherrin River (02052000), and the Blackwater River (02049500) (EPA 2012). Discharge at all five of these sites was summed to determine a total, daily, average stream-flow. These values were divided by the watershed area upstream (7992.7 km^2) and then multiplied by the total area of the watershed (12,664.04 km^2). This approach was taken because there was no gauging station located near the mouth of the river. Groundwater inputs and wind effects could cause error in these calculations. These sources of input do introduce possible sources of error that could not be avoided.

Laboratory Processing of Samples

Larval Fish Speciation

Alosine species were separated from the other groups and further separated for river herring. Larval fish abundances were standardized as the number of fish sampled per 100 m^3. For samples containing more than 100 alosine larvae, sub-sampling was done. When a sample contained less than 100 alosine larvae, all of the fish present were identified. If a sample contained greater than 100 larvae, a subsample (1 liter aliquot) was taken. My goal was to

sample a minimum of 10% of the whole sample, with an upper limit near 200 larvae. However, I never split the original sample more than five times (minimum 1/32 aliquot of the original sample). Abundance estimates within each site were calculated by averaging the catch at each station. Finally, all non-river herring alosine larvae were identified to species.

Protocol for speciation was to examine larvae from each sampling event and identify each larva. Non-clupeid larvae were sorted out and preserved, and clupeid larvae were sorted to the lowest taxonomic level possible. Throughout the speciation process, several identification keys were used (Chambers et al. 1976a; Sismour 1994a; Walsh et al. 2005). After samples were identified to the lowest taxonomic level, a blind study was performed to validate identification of blueback herring and alewife because of the similarities present between the species. During this blind study of identification keys, considerable variability was found in descriptions and counts of morphological characteristics used for taxonomic classification.

To ensure proper identification of and differentiation between the larvae, a thorough literature review of keys used to speciate blueback herring and alewife was completed. There were six larval identification manuals and upwards of twenty peer-reviewed papers that were analyzed (See Appendix 1). After the literature review, I found considerable overlap between blueback herring and alewife in critical differentiating characteristics, such as myomere counts and morphometric ratios (Cianci 1965; Mansueti and Hardy 1967; Lippson et al. 1974; Chambers et al. 1976a; Lam 1977; Jones et al. 1978; Auer 1982; Bulak 1985; Wallus 1990; Sismour 1994a; Fahay 2007). Personal communication with Dr. Tom Shultz at Duke Marine Laboratory, who is performing genetic analysis of river herring on Chowan River, has compounded the ambiguity in classification of river herring larvae. Dr. Shultz has recently found that blueback herring and alewife are hybridizing in Chowan River. This information,

paired with the difficulty in accurately differentiating the river herring larvae in our samples, led me to group blueback herring and alewife together as "river herring" for data analysis. While I initially attempted to use current identification keys to differentiate between blueback herring and alewife, it should be noted that this study closely mimics O'Rear (1983). In his study, O'Rear groups blueback herring and alewife together as "river herring".

Larval Nutritional Condition

The nutritional condition of the larvae in Chowan River was examined to determine if there were any nutritional differences among sites. Geometric morphometric analyses, dry weight comparisons (Fulton's Condition Factor, K), and histological preparations were used to assess larval condition. All larvae with bodies straight enough for histological examination were pooled and photographed. Pooling the larvae for histological analysis allowed for an unbiased approach when examining condition. After the analysis was completed, the location of capture was used to determine differences in condition among sampling stations. The larvae were photographed on their left sides in the sagittal plane using a dissecting microscope at 40-x magnification. The microscope was fitted with a high-resolution video camera and still images were recorded. Specimens for condition comparisons were randomly selected to ensure that observations of size, body condition and recent feeding history were well represented.

Studies on fish have shown that a decrease in health causes a collapse in morphological measurements (McGurk 1985; Margulies 1993; Ruehl et al. 2011). Geometric morphometric analyses were used to examine this relationship This method allowed me to analyze shape variation differences in the larvae (Rohlf and Marcus 1993). Because distinct changes in morphology occur around 9 mm (SL) (Chambers et al. 1976a), larvae used for morphometric analysis were separated into pre-flexion and post-flexion for the highest three abundance sites

and the lowest three abundance sites. Fish were photographed digitally at a resolution of 6 pixels mm^{-1} and digitized using tpsDig software to digitize homologous landmarks for each developmental stage (30 landmarks for 100 pre-flexion individuals and 13 landmarks for 150 post-flexion larvae) (Rohlf 2006) (Figure 4).

For Fulton's Condition factor, (K), a subsample of larvae (n=350) was measured (SL) and dried at 60° C for 24 hours to a constant weight. These samples were then placed in a dessicator for transfer and storage during weight measurements. A Cahn microbalance was used to weigh each sample individually. Fulton's condition index was used to quantify the overall condition of the larvae. The index is the condition factor, K:

$$K = (W/L^3) \times 100,$$

where *W* is dry weight (µg), L is standard length (mm), and 100 is a scaling constant. Fulton's condition index was compared qualitatively with the other larval condition methods (geometric morphometrics and histological preparations). Because distinct changes in morphology occur around 9 mm (SL) (Chambers et al. 1976a), Fulton's K values were separated into pre-flexion and post-flexion for main stem and tributary sampling sites.

The final method used to determine larval condition involved histological staining of larval sagittal sections. The same subsamples used for the geometric morphometric analysis were used for this process (N=300). The larvae were fixed in Bouin's solution for 24 hours after capture and then transferred to 85% ethanol until analysis. All larvae were placed in histological cassettes and transferred into paraffin wax. Each larva was mounted in a paraffin block and serially sectioned in 5 or 10 µm sections (Generally, pre-flexion larvae were sectioned at 5 µm and post-flexion at 10 µm). All sections were stained with Periodic Acid-Schiff's reagent to examine relative amounts of glycogen storage in liver hepatocytes. A hemotoxylin counter stain

was used to help orient anatomical features in the fish. A qualitative method of determining the health (brightness of the stain in the liver) was used to compile an index of larval condition. This histological grading system was based on the cellular condition of three body tissues: liver, gut lining, and musculature (Margulies 1993). Each tissue was assigned a grade from 3 (healthy) to 1 (degraded) (Figure 5).

Analysis of variance (ANOVA) was used to compare larval condition determined by Fulton's condition index (K). Geometric morphometric methods were used to analyze shape variation. A generalized Procrustes superimposition using TPSRELW (Rohlf 2005) adjusted for position, orientation, and scale. A principal components analysis was used to calculate the relative warps (principal components of shape) from the aligned landmark constellations that placed components of shape into decreasing order (Ruehl et al. 2011). The last four components were null because of the Procrustes procedure (two for translation, one for orientation, and one for scaling) leaving components serving as shape variables for analysis (56 for pre-flexion and 22 for post-flexion larvae). Centroid size, the square root of the sum of squared distances between the landmarks and the centroid of the landmarks, was used as a covariate to characterize and statistically adjust for general and species-specific allometries (Bookstein 1997; Zelditch 2004). Centroid size is highly correlated with standard length but is less correlated with shape (Ruehl et al. 2011).

To examine variation in size, I tested for differences in centroid size among sites with analysis of variance. For an explanation of the major factors contributing to shape differences, the first two principal components of shape were plotted. Procrustes distance was used to estimate the amount of shared shape variation and unique shape variation between sites.

Histological analysis of body tissues was used to assess the nutritional condition of river herring larvae. The percentage occurrence of each nutritional condition (1-3) was estimated for all samples. An analysis of variance was performed to determine any differences among the percent occurrence among sites.

Water Sample Analysis

Chemical analysis was performed for all water samples taken during peak spawning weeks while field sampling (middle 7 weeks) to perform a physicochemical characterization of each sampling site. On occasion, sampling duplicates were collected from the same site to allow for comparison over a brief (less than 5 minutes) period. One-liter samples were filtered using muffled Whatman 934-AH, 0.47 mm diameter, 1.5 micrometer retention filters. Filters were frozen immediately at -80° C to prevent degradation. A 0.5 L sample of filtered water was also frozen at -80° C to test for dissolved anions and nutrients. The second 0.5 L sample of filtered water was refrigerated for analysis of four major cations (Magnesium, Potassium, Calcium, and Sodium). Surface and bottom water samples were also taken to analyze for alkalinityto determine if an influence from the Castle Hayne Aquifer was present. The frozen filters and water samples were tested for concentrations of chlorophyll α, ammonia, nitrate, nitrite, dissolved Kjieldhal nitrogen, chloride, and phosphate. These analyses were performed using standard operating procedures in the environmental testing lab at East Carolina University using a SmartChem™ Discrete Analyzer (Westco Scientific).

For cations (calcium, magnesium, potassium, and sodium), samples were filtered a second time to 0.45 μm and compared to the standards of 1/10, 3/30, 5/50, 7/70, 10/100, 30/300, and 50/500; the first number of each standard indicates the concentration (ppm) of Ca^{2+}, Mg^{2+}, and K^{+}, and the second number is the concentrations of Na^{+}. Concentrations were measured with

a Perkin-Elmer Inductively Coupled Argon Plasma – Optical Emission Spectrometer (ICP-OES – model Optima 2100DV). Cation data collected from the ICP-OES were processed using WinLab32 for ICP. The standards used simulated the proportions and encompassed the ranges expected in the ambient environment. Analytical duplicates always yielded results within 10% of one another.

Physicochemical parameters including chlorophyll a, inorganic and organic dissolved nitrogen, phosphorous, chlorides, magnesium, sodium, potassium, and calcium were assessed using ANOVA. Data were evaluated for normality using Q-Q plots to ensure that assumptions of ANOVA were satisfied. When necessary, data were logarithmically transformed before statistical analysis to normalize observations and stabilize the variance. Spatial and temporal differences between environmental parameters were evaluated using a one-way analysis of variance (ANOVA). If the ANOVA was significant ($P \leq 0.05$), differences in environmental factors among sites were further examined using the tukey's post-hoc test. Unless otherwise noted, all statistical analyses on environmental variables were performed using SPSS statistical software and all visualization techniques were performed using SigmaPlot graphical software.

Statistical analyses were conducted to determine whether site or environmental parameters accounted for a significant amount of variability in the spatiotemporal distribution of river herring. Catch Per Unit Effort (CPUE) was analyzed with environmental and chemical parameters that included water temperature, dissolved oxygen, salinity, and pH including chlorophyll a, inorganic and organic dissolved nitrogen, phosphorous, chlorides, sodium, magnesium, calcium, and potassium. Linear regressions of each parameter were plotted against CPUE to determine relationships. A Pearson's correlation matrix was also computed to determine relationships among the physicochemical measures and larval abundance.

Physicochemical variables with correlations greater than 0.40 were considered biologically important.

A cluster analysis was performed to visualize similarities among sites. Groups, or clusters, were formed based on the degree of dissimilarity among the sites. Clusters formed at 90% similarity were kept for other analyses. Principal Components Analysis (PCA) was used to reduce dimensions in the data. This allowed the conversion of mutually dependent variables into significant and independent variables (Brosse et al. 2001). Component axes retained for interpretation were those that explained >75% of the cumulative variance and those with an eigenvalue greater than 1.0. Based on loading relationships between the first and second principle components, combinations of physicochemical factors were made. To determine if there were any linear relationships between the newly formed reduced variables, multiple regression analysis was conducted. This allowed for the visualization of effects from the newly reduced variables.

Results

Habitat and physicochemical factors

During my sampling season (March 18-May 26 2011) conditions on Chowan River exhibited weather patterns typical of southeastern United States. Seasonal air temperatures were 5.40 C – 34.30 C and precipitation measured 16.9 cm. Mean daily river flow (±SD) from Chowan River Basin varied from 23 to 192 cm/s during sampling with a mean flow of 72 ± 38 cm/s. Mean Flows were 80 ± 59 cm/s slower than 2009 and 75 ± 113 cm/s slower than 2010. Discharge rates for Chowan River during larval collection peaked 1-2 weeks prior to larval abundance peaks and generally decreased throughout collection (Figure 6).

Temporal variation of dissolved oxygen, pH, and conductivity was observed. Water temperature varied temporally, but was consistent with patterns from previous years (Figure 7). Water temperatures (mean ± SD) were lowest in March (13.0 ± 2.1 °C) and increased through April (18.6 ± 2.7 °C) into May (22.7 ± 2.2 °C) with temperatures during the peak capture period (April 29 – May 5, 2011) at 21 ± 0.7 °C. Dissolved oxygen (mean ± SD) levels decreased throughout the sampling season with values for March, April, and May of 7.32 ± 1.67 mg/L, 5.95 ± 1.99 mg/L, and 5.21 ± 1.98 mg/L, respectively. Dissolved oxygen levels were not above 70% saturation throughout the sampling period, but hypoxia (< 3.0 mg/L) occurred infrequently, accounting for only 8% of readings. Anoxic conditions (< 0.5 mg/L) were not observed during sampling. Salinity only reached the detection limit of the YSI meter (> 0.10 ppt) 17% of the time with samples greater than 0.16 ppt occurring only 3% of the time. The highest salinity reading (0.57 ppt) was on the last day of sampling after the peak abundance of river herring larvae had already occurred. Water at all of the sampling sites flowed predominantly downstream and surface currents were similar among sites, ranging from 0.01 – 0.12 m/s, with a mean velocity (±SD) of 0.05 ± 0.03 m/s.

All physicochemical parameters varied spatially as well (Figures 8-9). A correlation matrix was constructed of all *r* values between the physicochemical parameters (Table 2). Catherine's Creek (site 6), which had the highest abundance (1583 alosines/100m^3) of larval river herring, had the highest concentrations (±SD) of chlorophyll α (6.17 ± 6.23 μg/L), phosphate (0.023 ± 0.020 mg/L), and potassium (6.66 ± 4.63 mg/L). However, Catherine's Creek did not have the lowest concentrations of any variables. Rockyhock Creek (site 8), which had one of the lowest abundances (94 alosines/100m^3), had the highest concentrations (±SD) of ammonia (0.09 ± 0.08 mg/L), nitrate and nitrite (0.27 ± 0.11 mg/L), and calcium (17.8 ± 5.7 mg/L). South Chowan (site 9), which had the lowest abundance (53 alosines/100m^3), had the highest concentrations (±SD) of sodium (42.9 ± 23.1 mg/L), chloride (66.3 ± 35.7 mg/L) and magnesium (6.46 ± 2.83 mg/L). South Chowan also had the lowest concentrations (±SD) of ammonia (0.04 ± 0.02 mg/L) and dissolved kjeldhal nitrogen (0.41 ± 0.15 mg/L).

Larval abundance

A total of 46,612 fish (all fish combined) were collected from 99 pushnet samples. Larvae were caught at all sites on all dates with the exception of Catherine's Creek (Site 6) on March 31, 2011 and Bennett's Creek (site 5) on May 26, 2011. The mean catch between the port and starboard nets was not significantly different ($p = 0.946$). The highest abundances were in Catherine's Creek (38%), and Wiccacon Creek (30%). The majority of the fishes (94%) were captured in tributaries (Sarem, Wiccacon, Bennett's, Catherine's, and Rockyhock Creeks).

Larval alosines were identified to species for hickory shad *A. mediocris* (3.7%), American shad, *A. sapidissima* (0.3%), and to a combined river herring for blueback herring and alewife, *Alosa spp.* (95.1%), from the rest of the fishes in the samples. The frequency of occurrence for shads and river herring did not differ with site (Table 3). Alosines (N = 44,755)

comprised 96% of the total catch. Alosines were collected during 9 of 11 weeks of sampling. Other clupeids including gizzard shad *Dorosoma cepedium*, were also present in samples.

Spatial variation in the abundance of larval river herring was observed among sites. Abundances at site 4 (Wiccacon Creek) and site 6 (Catherine's Creek) were numerically higher (1316 ± 3027; 1583 ± 2698 respectively) than the rest of the sites (mean = 182 ± 384). However, there was not a statistically significant difference between sites ($F = 0.923$; $P = 0.501$). Peaks in river herring abundance were observed over a three week period during sampling between April 20 and May 5, 2011. Before and after this peak, there were steep increases and declines in abundance, respectively.

Relationship between fish abundance and physicochemical factors

A Pearson's correlation matrix showed which of the physicochemical factors were important to alosine abundance (CPUE alosines/100m^3). Ammonia ($r = 0.261$, $p = 0.039$), Organic nitrogen ($r = 0.392$, $p = 0.001$), and Phosphorous ($r = 0.361$, $p = 0.004$) all had significant correlations with CPUE. Significant linear regressions were found with Ammonia ($p = 0.039$, $r^2 = 0.068$), Organic nitrogen ($p = 0.001$, $r^2 = 0.154$), and Phosphorous ($p = 0.004$, $r^2 = 0.130$) (Table 4).

A dendrogram from cluster analysis shows four clusters at 90% similarity (Figure 10). Site 9 (South Chowan) comprises cluster 1, site 8 (Rockyhock Creek) forms cluster 2, sites 1 (North Chowan) and 7 (Holiday Island) occupy cluster 3, and sites 2 (Meherrin River), 3 (Sarem Creek), 4 (Wiccacon Creek), 5 (Bennett's Creek), and 6 (Catherine's Creek) form cluster 4. When directly comparing CPUE with the dendrogram from the cluster analysis, cluster 4 holds sampling sites with the highest abundance (Figure 11).

Principal components analysis was used to corroborate the results of the correlation and cluster analyses, summarize the variation observed between sites, and reduce the dimensions in

the data. The scree plot from the PCA identified three factorial axes that explained 87% of the total variability (Figure 12; Table 5). All physicochemical variables were biologically important on at least one of the principal components. Principal Component I accounted for most of the variation (42%) and was characterized by a positive correlation with chlorophyll α, inorganic nitrogen, chloride, phosphorous, and all cations. There was a negative correlation with organic nitrogen. Principal Component II explained 31% of the variability and was associated with a positive correlation with chlorophyll α, organic and inorganic nitrogen, phosphorous, calcium, potassium, and magnesium. There was a negative correlation with sodium and chloride. Principal Component III explained 15% of the variability and was associated with a negative correlation with ammonia, inorganic nitrogen, and calcium. All other factors were positively correlated with Component III (Figure 13).

After close examination of the correlation, regression, and Principal Components Analysis, and the loadings associated with the first two components, some decisions were made on reducing the dimensionality of the data. Ammonia, nitrate, and nitrite were combined to account for all organic nitrogen together. Sodium and chloride concentrations were combined because of a highly significant correlation ($r = 0.652$, $p = <0.0001$) and similar loadings in components 1 and 2 (0.756 and 0.829 respectively for component 1; -0.606 and -0.455 respectively for component 2). Calcium, potassium, and magnesium were also grouped together as the remaining cations because of highly significant correlations and similar loadings in the first component. A multiple regression analysis of the reduced variables returned an r^2 value of 0.943 and significance value (p) of 0.161.

Larval nutritional condition

The standard length and dry weight of pre-flexion river herring ranged from 3.2 to 9 mm and 11 to 233 µg with a mean length (±SD) of 6.3 ± 1.2 mm and weight (±SD) of 45.0 ± 34.6 µg. Post-flexion larvae ranged from 9.1 to 21 mm with a mean length (±SD) of 12.1 ± 2.5 mm and 49 to 5822 µg with a mean weight (±SD) of 788.3 ± 913 µg. Fulton's condition index (K) for pre-flexion larvae ranged from 4.5 to 64.3 with a mean (±SD) of 16.2 ± 6.7. Post-flexion larvae ranged from 4.4 to 64.6 with a mean (±SD) of 32.1 ± 12.7. Condition indices between developmental stage and sites indicated that site 8 (Rockyhock Creek) had a significantly higher condition index than site 1 (North Chowan) for pre-flexion larvae (there was a difference of 7.7, $p = 0.019$). There were no other significant differences in Fulton's Condition for pre- or post-flexion larvae. Visual comparisons between pre- and post-flexion larvae with the cluster analysis performed on all physicochemical factors also show that there are no trends in condition among the clusters (Figures 14-15).

There was a biological gradient between centroid sizes for high and low abundances in both pre- and post-flexion developmental stages, however there was no significant difference ($p > 0.05$). For pre-flexion individuals, centroid size (mean ± SD) (a multivariate measure of size) was significantly smaller ($F = 6.179$, $p = 0.015$) for high abundance sites (827 ± 122) compared to low abundance sites (926 ± 208). For post-flexion individuals, centroid size was significantly larger ($F = 26.615$, $p = 0.000$) for high abundance (541 ± 144) than low abundance (402 ± 35) (Figure 16). After accounting for size with the Procrustes superimposition, plots of the first two principal components of shape revealed that there was no separation along the first component and the second component between high and low abundances for either developmental stage (Figure 17).

A total of 124 larval river herring were examined for qualitative histological nutritional condition. Examination of the liver hepatocytes, midgut epithelium and midgut musculature produced a good composite index of nutritional condition for river herring larvae. The condition of all three tissues varied across the gradient range (1-3). The mean (mean ± SD) condition of the liver hepatocytes was 1.94 (0.54). Hepatocyte cytoplasm ranged from slightly granular, with abundant intracellular vacuoles, to completely dark. Vacuolized hepatocytes stained darkly with PAS stain, indicating that the vacuoles were areas of glycogen storage.

The alimentary tract of all larvae was coiled, with evidence of feeding found in some individuals. The condition of the epithelial cells in the midgut ranged from having compact, prominent cells (healthy), to a darkened contracted condition (degraded). Mean values for midgut cells was 1.96 (0.44). Healthy (Grade 3) midgut epithelial tissue was significantly correlated and occurred in association with healthy liver hepatocytes (Table 6). The trunk musculature showed compact muscle fibers in healthy (Grade 3) individuals and separated muscle fibers in degraded larvae. The mean value for trunk musculature nutritional condition was 1.68 (0.58). The composite nutritional score (the sum of the three tissue grades) of each larva was classified as either healthy (total score of 7 to 9), average (total score of 6), or starving (total score of 3 to 5). The mean for composite nutritional condition was 5.50 (0.52).

Discussion

Abundances of river herring collected in this study are higher than larval densities reported in other North Carolina systems (O'Rear 1983; Hightower et al. 1996; Overton and Rulifson 2007; Binion 2011; Overton et al. 2012; Riley 2012). Few studies have examined larval river herring abundance on Chowan River since O'Rear (1983), which led to the sites sampled during my study being chosen based on his historical sites. O'Rear (1983) reported for two years of sampling 0.3 larva/m. My mean abundance was 1.3 larva/m, capturing over four times as many larvae. On Roanoke River, a similar study to mine was conducted in 2009 and 2010 (Riley 2012). Abundances (number/100m^3) in my study (464 ± 1499) were fifteen times higher than 2009 (30.8 ± 149.8) and one hundred thirteen times higher than 2010 (4.1 ± 20.9).

The high numbers of larval river herring in Chowan River may exist for several reasons. First, the Atlantic States Marine Fisheries Council instituted a moratorium on landing adult river herring in 2007. The four years without fishing pressure on adults may have allowed for partial recovery of the stock. Second, the foraging base of Chowan River Basin appears sufficient to support river herring recovery (Leech et al. 2008). Crustacean zooplankton abundance (preferred food of river herring) is in greater abundance than in previous years.

Spatial differences in larval river herring abundance were observed among sites, with the highest abundances in tributaries. Abundances at site 4 (Wiccacon Creek) and site 6 (Catherine's Creek) were numerically higher than the rest of the sites. Statistical analysis returned non-significant differences among sites, but a closer examination of the data shows high variability in abundance numbers from week to week. Out of the eleven weeks that were sampled, 95% of the larvae captured were collected over a four week period (April 20 – May 13, 2011). The low abundance numbers from the other eight weeks may have reduced the significance of the data. Future analysis should be explored on only the four weeks

encompassing the larval capture peak. This will reduce the variation in the data and may strengthen the statistical analyses.

The highest abundances of larval river herring were found at sampling sites with the highest concentrations of chlorophyll α, phosphate, and potassium. Values of calcium, nitrate, and nitrite were also in the top three in Catherine's Creek, which had the highest catch per unit effort (CPUE). Larval fish densities seem to be most likely affected and positively correlated with dissolved oxygen or dissolved ion concentrations (Sabo et al. 1991; ÖHman et al. 2006). Sabo et al. (1991) found significantly higher abundances of fish in ponds with higher values of phosphorous and chlorophyll α. Concentrations of chlorophyll α indicate primary production concentrations that could support a food base for larval river herring. Chlorophyll α levels during this study ranged from 0 – 21.9 µg/L, which is well below the state's water quality maximum of 40 µg/L. Although these levels were low, abundances of river herring were still very high. My findings are consistent with Leech et al. (2008) who found lower than historical concentrations of chlorophyll α throughout Chowan River basin during a multiyear study. One explanation for low chlorophyll α concentrations is that all of the phytoplankton is being consumed by organisms in the system (Leech et al. 2008). Further increases in chlorophyll α could continue to boost larval abundances.

Changes in phytoplankton biomass (chlorophyll α) over the past several decades are partially due to changes in nutrient inputs to Chowan River basin (NCDENR 2006). The group effect of physicochemical factors led to the multivariate statistical approach taken in this study. Cluster analysis and Principal Components Analysis (PCA) on physicochemical factors showed that there may be a collective effect on abundance. As suites of nutrients increase, chlorophyll α will increase, and larval river herring abundance will increase. A positive outlook from this

research is that although levels of important physicochemical factors were low (all measurements lower or within state standards), the abundance of river herring was still high. Improving these levels will increase food sources and the number of larvae that can be supported in Chowan River.

The water quality in Chowan River may still be an impediment. Physiological stress, alterations in behavior, and reduced recruitment in fish and other aquatic organisms increase as dissolved oxygen decreases below 5 mg/L (Breitburg 1992; Breitburg 2002; Ludsin et al. 2009). This alteration in behavior may have been witnessed during sampling when I was able to see larval herring swimming at the surface while in the boat at Catherine's Creek during one of the peak capture weeks. During those four weeks of peak abundance, DO at the two highest abundance sites ranged from 2.08 – 5.42 with a mean (± SD) of 3.70 ± 1.41 mg/L. Prolonged exposures to DO levels < 3 mg/L may cause death. Mean DO levels during this study were below 5 mg/L. Data from the 1970s – 1990s to the present show that low dissolved oxygen has been a persistent problem in Chowan River Basin (Leech et al. 2008). If levels of DO rise, abundances of river herring could increase even further.

Distinct migratory and spawning cues were not suspected given low flows (23 – 192 cm/s) seen during this study. The flows (± SD) experienced were on average 80 ± 59 cm/s slower than 2009 and 75 ± 113 cm/s slower than 2010. Environmental cues (water temperature) are recognized by spawning adults via river flow. Low flows would not seem to be beneficial in this transport. However, larval abundances during this study were 15 – 113 times higher than those in similar studies in the neighboring Roanoke River (Overton and Rulifson 2007; Binion 2011; Riley 2012). Mean flow in Chowan River (23 – 192 cm/s) were much lower than those seen in Roanoke River (300 – 600 cm/s). High advection rates of larvae were suspected in

Roanoke River (Riley 2012). Low flows in Chowan River may be beneficial in retaining larvae within creeks used as nursery habitats for increased abundances. This could also mean that there are more larvae being retained in systems with favorable conditions leading to higher abundances.

Historical spawning populations in Chowan River have been higher than any other river in the North Carolina system (NCDMF 2007; NMFS 2009). Habitat degradation because of landscape alterations throughout the water shed has limited the functionality of the spawning habitat. The North Carolina Division of Natural and Environmental Resources has taken steps, including a complete GIS assessment of river herring habitat in Chowan River, to reverse the deleterious effects of habitat alterations (McNaught et al. 2010). The states diligence in regulating fishing pressure, increasing water quality, and reversing habitat degradation are more reasons that abundances during this study were higher than O'Rear (1983).

Larval fish do not choose the nursery habitat in which they will be reared. Instead, they are confined to the environmental conditions experienced while being transported downstream from their natal origin. I hypothesized that characterizing the habitat experienced by larvae would help explain variations in abundance and nutritional condition. Results discussed above explain how increased levels of nutrients allow for higher abundances of larvae. Larval condition is another important factor affecting larval abundance (Suthers 1998). Larvae in better condition should contribute more to standing stock biomass than those in poor condition.

Three measures of condition were explored during this study: geometric morphometrics, dry weights (Fulton's condition index), and a histological comparison of internal tissues. Using these three methods, I was able to completely analyze any differences in larval condition among sampling sites. Geometric morphometrics allowed me to explore response to spatial variation in

relation to physicochemical factors (Ruehl et al. 2011). I tested for habitat-associated morphology among pre- and post-flexion larvae and found no shape differences among sites. I did, however, find differences in standard lengths between high and low abundance sites for both developmental stages. For pre-flexion individuals, there was a larger centroid size (standard length) in low abundance sites. This shows that these low abundance pre-flexion larvae have shallower body depths in relation to length. Shallower body depths could be an indication of poor nutritional condition (Ruehl et al. 2011). Conversely, post-flexion larvae had larger centroid size in high abundance sites. This shows that fish in higher abundances are larger than low abundance individuals. Larger individuals could also indicate fish in better nutritional condition.

Geometric morphometrics alone does not completely describe nutritional condition. To further assess the morphometric condition of the larvae, I used Fulton's condition index to quantify the overall condition of the larvae (Bolger and Connolly 1989). Fulton's condition index was not independently used because it has proven most useful when coupled with other growth and condition indices for larval fishes (Lochmann et al. 1997; Suthers 1998). In a study conducted on river herring in Roanoke River, Fulton's condition index ranged from 6.3 to 87.6 with a mean (± SD) of 19.76 (± 7.6) (Riley 2012). Fulton's condition index ranged from 4.4 to 64.6 in my study with a mean (± SD) of 24.15 (± 9.7). This comparison shows that larval river herring in Chowan River exhibit more robust nutritional condition than those caught in Roanoke River. Analysis completed within the study sites of this project only showed one significant difference between site 1 (North Chowan) and site 8 (Rockyhock Creek) for pre-flexion larvae ($p = 0.019$). In agreement with the geometric morphometric analysis, Fulton's condition index shows that none of the physicochemical factors are driving condition.

The final component of the nutritional condition assessment was histological qualification. Histological analysis is a sensitive indicator of nutritional condition (Margulies 1993). Glycogen is stored in the liver and is the first energy source depleted at the onset of malnourishment in fish larvae (O'Connell and Paloma 1981). In my study as well as another study involving histological analysis, depletion of glycogen reserves is associated with moderate to severe deterioration in other body tissues, namely mid-gut epithelium and trunk musculature (Margulies 1993). For Chowan River, this seems to corroborate the results from the habitat characterization analysis. I showed that key nutrient levels and potential food sources (indicated by chlorophyll α) are at historically low levels in this system. Poor nutritional condition of larval river herring is related to the range of parameters (physicochemical) observed during my study.

I initially hypothesized that larval nutritional condition would be significantly impacted by habitat. Strong links between population recruits, health, and survival make the larval stage an advantageous proxy for stock analysis (Bergenius et al. 2002). During this study however, there were no significant differences in condition between high abundance and low abundance sites. While there are subtle differences between sites, overall nutritional health determined by histological examination of larval river herring in Chowan River is average at best. The composite nutritional score (the sum of the three tissue grades) of each larva was classified as either healthy (total score of 7 to 9), average (total score of 6), or starving (total score of 3 to 5). The mean for composite nutritional condition was 5.5 (0.52). Future efforts to improve water quality in Chowan River should be taken to allow a more conducive environment for larval rearing. Differences in physicochemical parameters seem to be biologically important in determining abundances of larval river herring, but do not seem to have an effect on larval nutritional condition when they are below optimum levels.

Management plans are an essential part of sustaining and increasing populations of fish. Evidence was given for estuarine-dependent fish stocks declining because of poor recruitment (Rulifson and Manooch III 1990; Overton et al. 2012). Low recruitment is a function of habitat quality encountered during spawning, and thus early life stages (Houde 1989). Continued research into habitat characterization as it relates to larval abundance in Chowan River could contribute to further increases in larval production, and translate to larger stocks. A complete characterization of all habitat experienced by larval fish along the transport from egg to metamorphosis would better describe nutritional condition and needs for water quality improvement. This study shows that Chowan River Basin, even with access to spawning sites and high abundances, has water quality unsuitable for optimum development of larval river herring. If management agencies are able to continue improvements of water quality in Chowan River and its tributaries, river herring stocks may return to historical levels.

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Table 1. Latitudinal and Longitudinal coordinates of sampling sites on Chowan River, North Carolina between March 18 and May 26, 2011.

Site Number	Site Name	Latitude	Longitude
1	North Chowan	36°26’11.46”N	76°56’41.67”W
2	Meherrin River	36°26’11.64”N	76°57’20.59”W
3	Sarem Creek	36°22’02.40”N	76°46’17.48”W
4	Wiccacon Creek	36°20’48.19”N	76°45’55.11”W
5	Bennett's Creek	36°19’08.31”N	76°42’26.06”W
6	Catherine's Creek	36°18’17.37”N	76°40’35.54”W
7	Holiday Island	36°16’33.11”N	76°40’56.18”W
8	Rockyhock Creek	36°06’40.53”N	76°41’10.67”W
9	South Chowan	36°06’15.11”N	76 °42’15.66”W

Table 2. Pearson Correlations conducted on physicochemical parameters from Chowan River, North Carolina. * indicates significant correlations (P < 0.05). ** indicates highly significant correlations (P < .001). Note that correlations > 0.40 are considered biologically relevant.

	Chlorophyll α	Ammonia	Nitrate/Nitrite	Organic Nitrogen	Cloride	Phosphorous	Calcium	Potassium	Magnesium	Sodium
Chlorophyll α	1	0.263*	0.202	0.194	-0.086	-0.054	0.051	-0.01	-0.038	-0.15
Ammonia	0.263*	1	0.073	0.665**	-0.088	0.083	0.123	-0.089	-0.07	-0.178
Nitrate/Nitrite	0.202	0.073	1	-0.208	0.218	-0.16	0.398**	0.074	0.215	0.05
Organic Nitrogen	0.194	0.665**	-0.208	1	-.291*	0.191	0.174	0.163	0.019	-0.175
Cloride	-0.086	-0.088	0.218	-.291*	1	-0.016	0.173	0.136	0.533**	0.852**
Phosphorous	-0.054	0.083	-0.16	0.191	-0.016	1	0.182	0.252*	0.225	0.088
Calcium	0.051	0.123	0.398**	0.174	0.173	0.182	1	0.865**	0.815**	0.429**
Potassium	-0.01	-0.089	0.074	0.163	0.136	0.252*	0.865**	1	0.882**	0.495**
Magnesium	-0.038	-0.07	0.215	0.019	0.533**	0.225	0.815**	0.882**	1	0.768**
Sodium	-0.15	-0.178	0.05	-0.175	0.852**	0.088	0.429**	0.495**	0.768**	1

Table 3. Number and percent frequency of occurrence in samples of larval alosines identified from ichthyoplankton samples collected in Chowan River, North Carolina during spring 2011.

	Site 1 (North Chowan)		Site 2 (Meherrin River)		Site 3 (Sarem Creek)		Site 4 (Wiccacon Creek)		Site 5 (Bennett's Creek)		Site 6 (Catherine's Creek)		Site 7 (Holiday Island)		Site 8 (Rockyhock Creek)		Site 9 (South Chowan)	
Species	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
River Herring	998	100	1,407	100	5,918	100	13,087	100	2,637	91	16,046	91	758	100	987	100	571	100
Hickory shad	21	18	5	18	55	36	672	27	15	27	871	36	1	9	9	9	6	18
American shad	2	18	8	18	17	18	49	27	8	27	49	36	1	9	2	18	1	9

Table 4. Descriptive statistics and estimated parameters for physicochemical parameters (explanatory variable) and log transformed river herring abundances (fish/100m3) (dependent variable). Slope (B1) and intercept (B0) estimates were generated using linear regression techniques.

Parameter	B_0	B_1	r^2	P	95% Confidence Interval
Chl α	1.79	0.03	0.022	0.255	-0.025 - 0.087
NH_4	1.41	7.92	0.145	0.002*	2.95 - 12.9
NO_3NO_2	2.18	-2.05	0.030	0.181	-5.08 - 0.98
DKN	0.20	3.03	0.296	0.000*	1.83 - 4.24
Log_{10} Cl	3.01	-0.88	0.059	0.058	-1.793 - 0.031
PO_4	1.46	30.86	0.182	0.001*	14.0 - 47.7
Log_{10} Ca	1.31	0.62	0.020	0.271	-0.50 - 1.75
Log_{10} K	1.53	0.66	0.024	0.23	-0.43 - 1.74
Log_{10} Mg	1.79	0.22	0.004	0.629	-0.693 - 1.137
Log_{10} Na	2.20	-0.28	0.008	0.477	-1.07 - 0.51

Table 5. Component matrix from Principal Components Analysis on all physicochemical factors from sampling in Chowan River, North Carolina.

Physicochemical Parameter	Component 1	2	3
Chlorophyll α	0.156	0.799	0.334
Ammonia	-0.057	0.683	-0.648
Nitrate/Nitrite	0.760	0.316	-0.539
Organic Nitrogen	-0.580	0.689	0.116
Chloride	0.829	-0.455	0.025
Phosphorous	0.164	0.489	0.607
Calcium	0.737	0.524	-0.314
Potassium	0.689	0.549	0.360
Magnesium	0.961	0.068	0.179
Sodium	0.756	-0.606	0.106

Table 6. Pearson correlation conducted on qualitative measures of nutritional condtion from histological preparations of larval river herring from Chowan River, North Carolina between March 18 and May 26, 2011.

	Liver	Midgut	Muscle
Liver	1	0.480**	0.544**
Midgut	0.480**	1	0.369**
Muscle	0.544**	0.369**	1

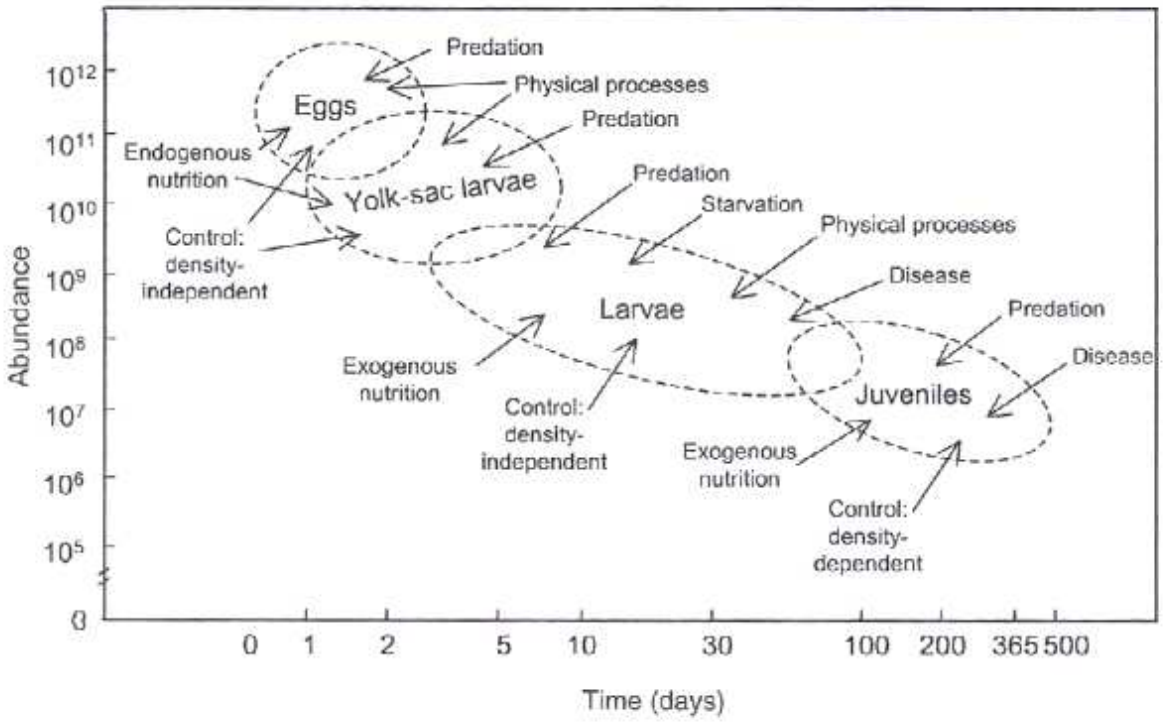


Figure 1. A conceptualization of the recruitment process in fishes including factors that affect mortality and growth. Log_{10} scales are used on both axes. Reproduced from Houde (1987).

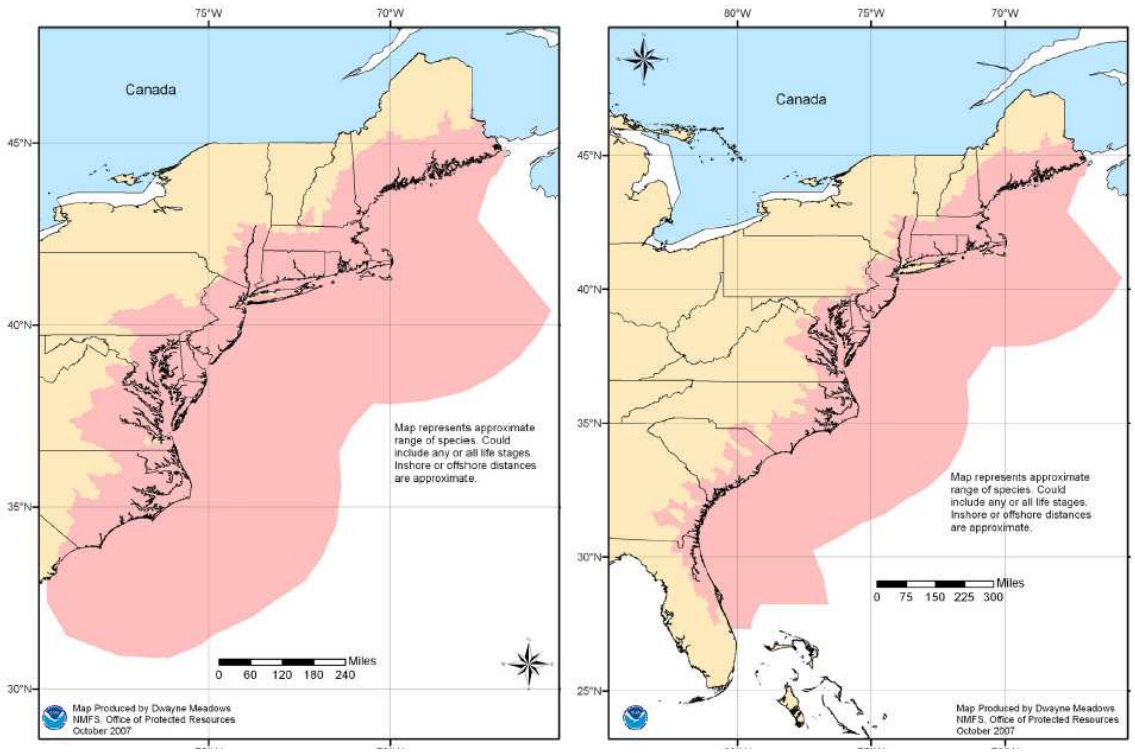


Figure 2. Maps of eastern United States showing the distribution of river herring (NMFS 2009). Left map shows alewife distributions, right map shows blueback herring distributions. Maps represent approximate range of species. River herring could appear at any or all life stages in shaded areas.

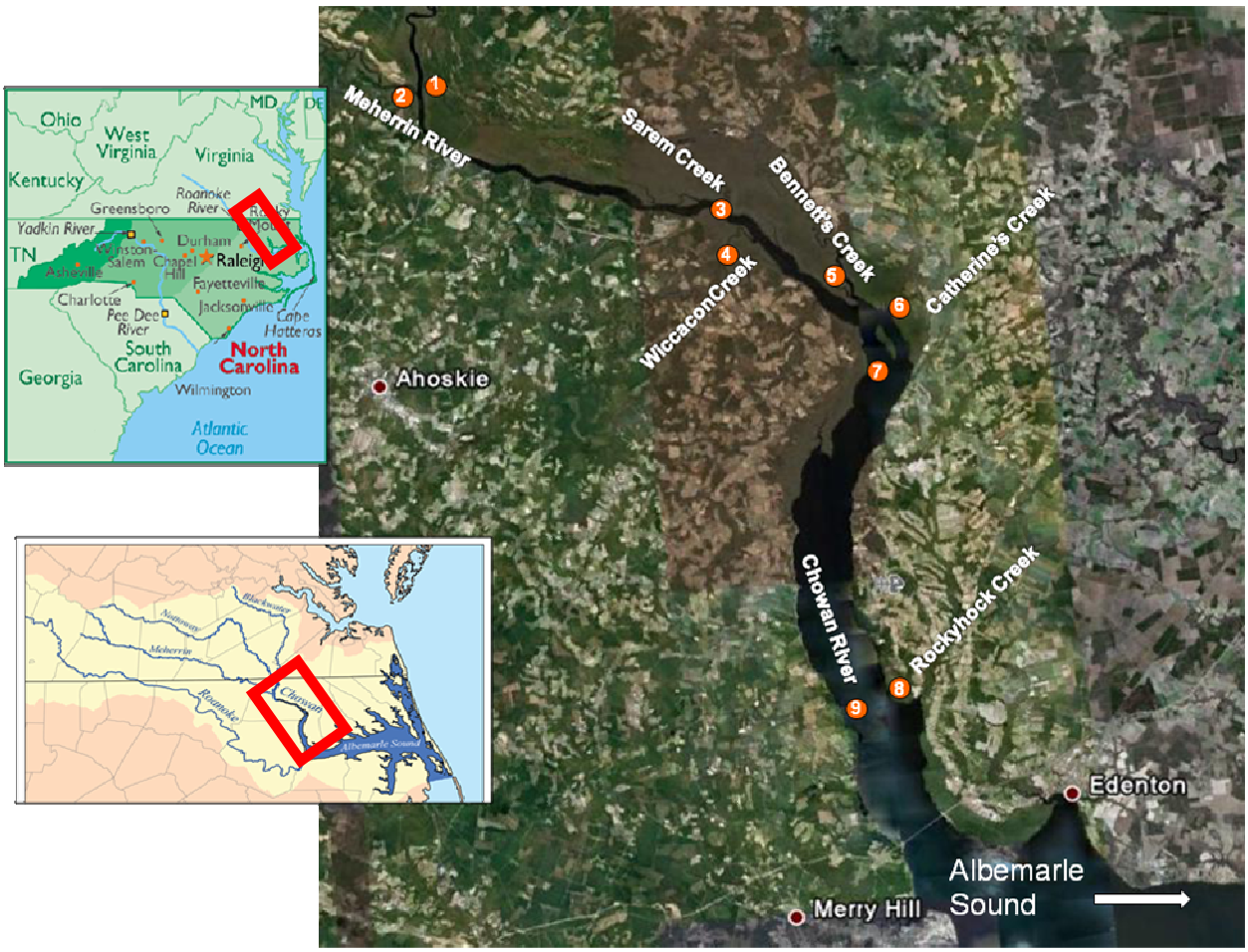


Figure 3. Map of Chowan River, North Carolina. Orange circles represent nine sampling locations including Meherrin River, Bennett's, Catherine's, Rockyhock, Sarem, and Wiccacon Creeks. Additional sampling sites located in the main stem of Chowan River North of the Meherrin River outlet (1), at Holiday Island (7), and south of the Rockyhock Creek outlet (9).

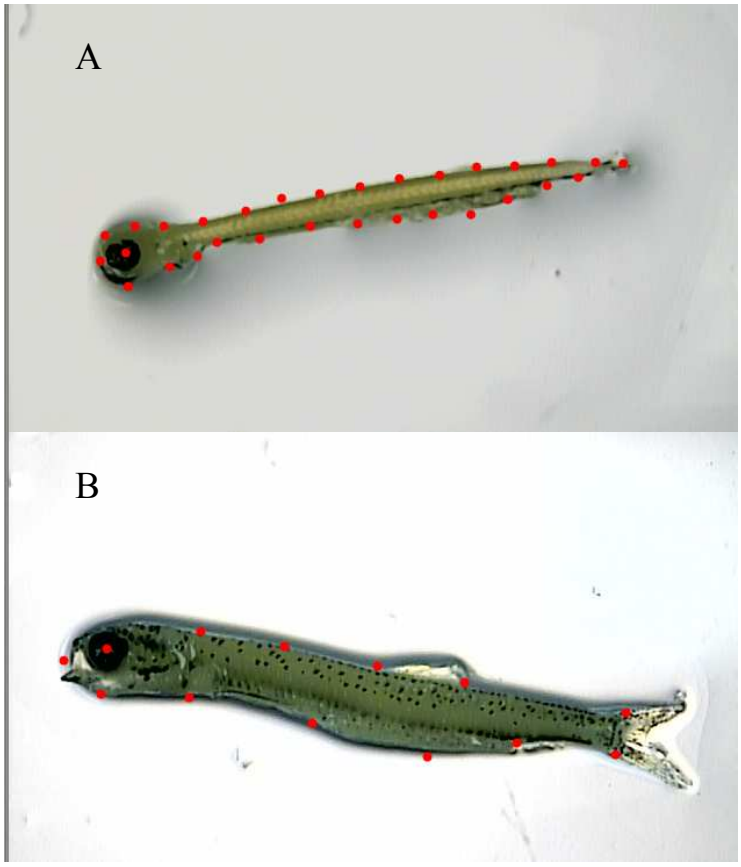


Figure 4. A – TPSDig landmarks for geometric morphometric analysis of pre-flexion larvae from Chowan River, North Carolina between March 18 and May 26, 2011. B – TPSDig landmarks for geometric morphometric analysis of post-flexion larvae from Chowan River, North Carolina between March 18 and May 26, 2011.

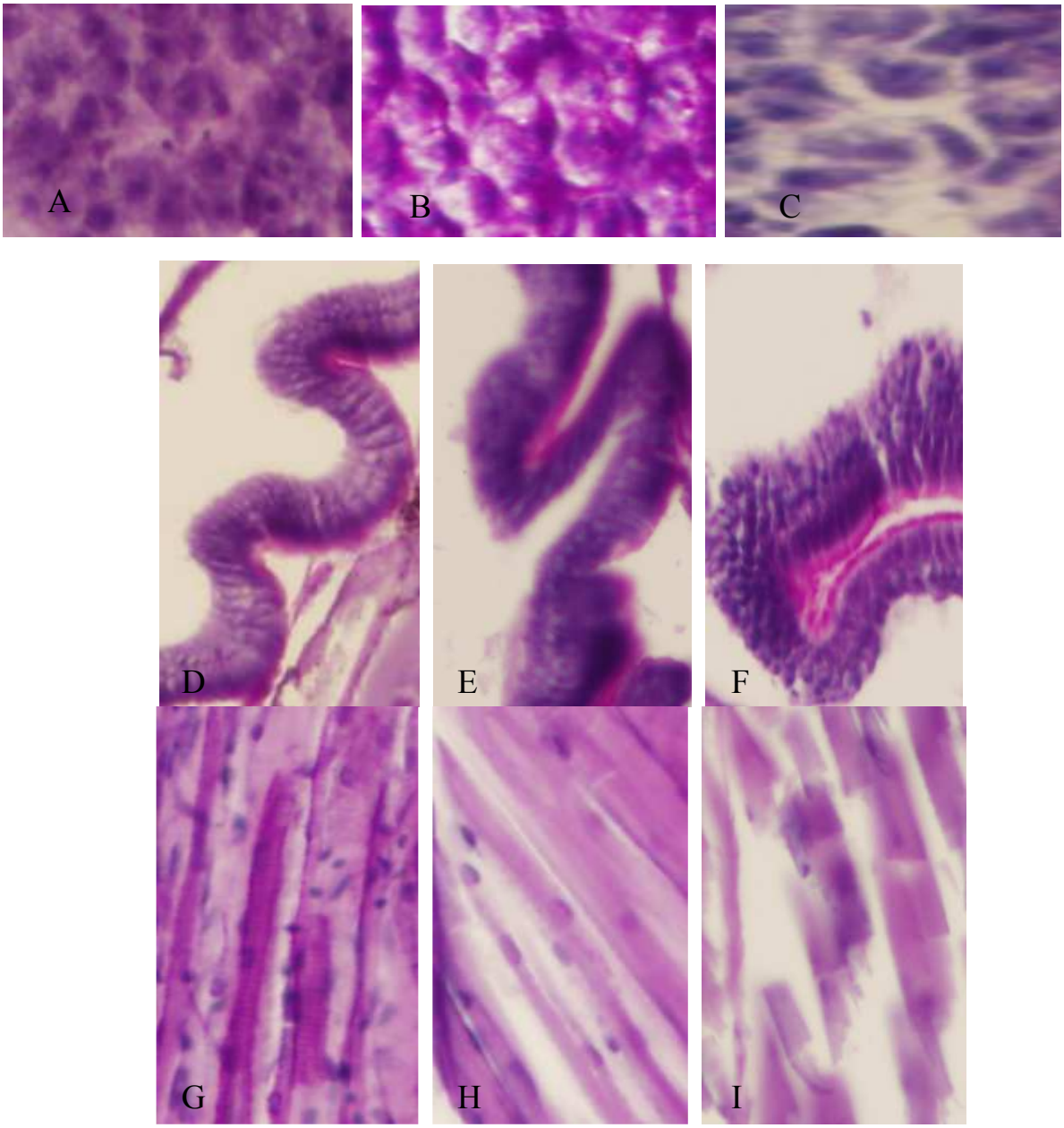


Figure 5. Histological grading system based on the cellular condition of three body tissues: liver, gut lining, and musculature. A – Healthy liver (grade 3). B – Midgrade liver (grade 2). C – Degraded liver (grade 1). D – Healthy gut lining (grade 3). E – Midgrade gut lining (grade 2). F – Degraded gut lining (grade 1). G – Healthy musculature (grade 3). H – Midgrade musculature (grade 2). I – Degraded musculature (grade 1). All samples collected from Chowan River, North Carolina between March 18 and May 26, 2011.

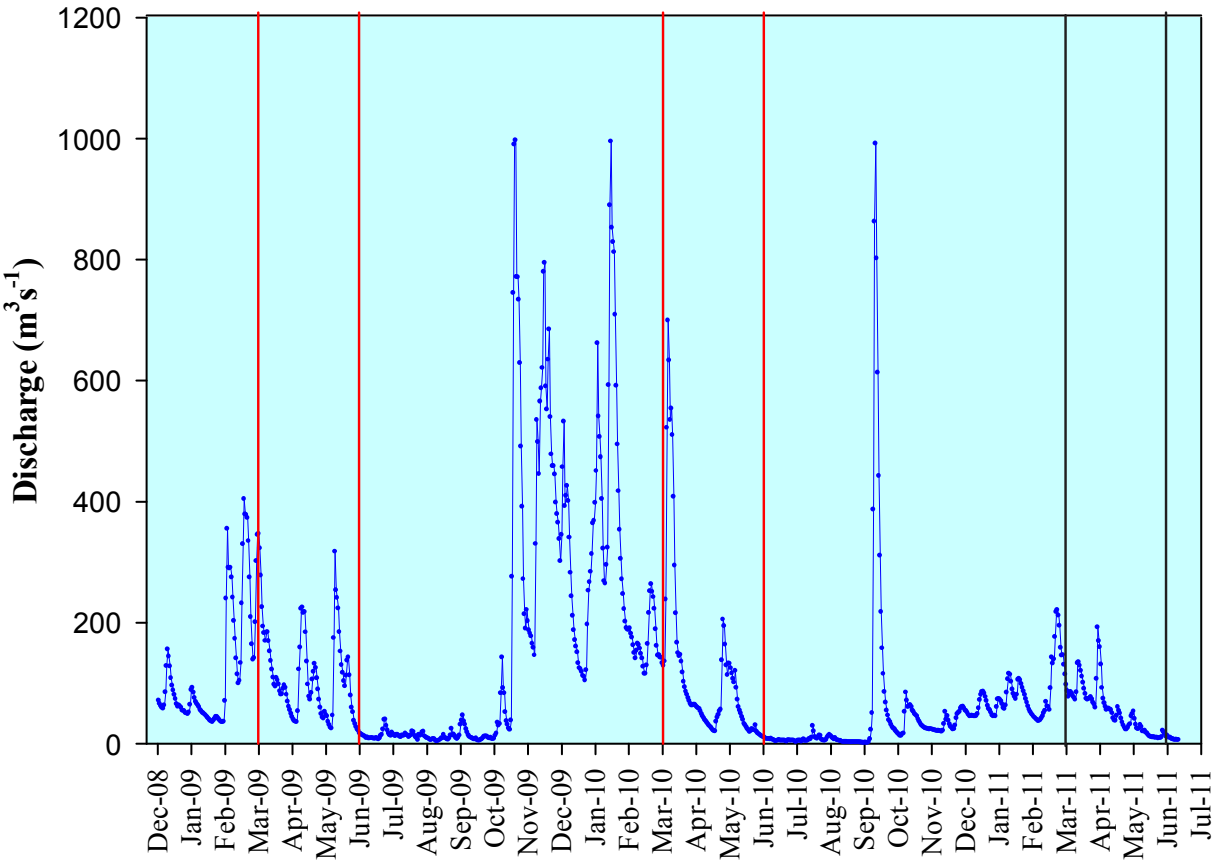


Figure 6. Estimated discharge for the Chowan River based on five USGS gauging stations within the watershed, including Potecasi Creek (02053200), Ahoskie Creek (02053500), Nottoway River (020470000), Meherrin River (02052000), and the Blackwater River (02049500). Discharge at all five of these sites was summed to determine a total daily average stream flow. These values were divided by the total gauge area (7992.7 km^2) and then multiplied by the total area of the watershed (12, 665.04 km^2). Red lines indicate known peak spawning times of river herring. Black lines indicate sampling season (March 18 to May 26, 2011) during this study.

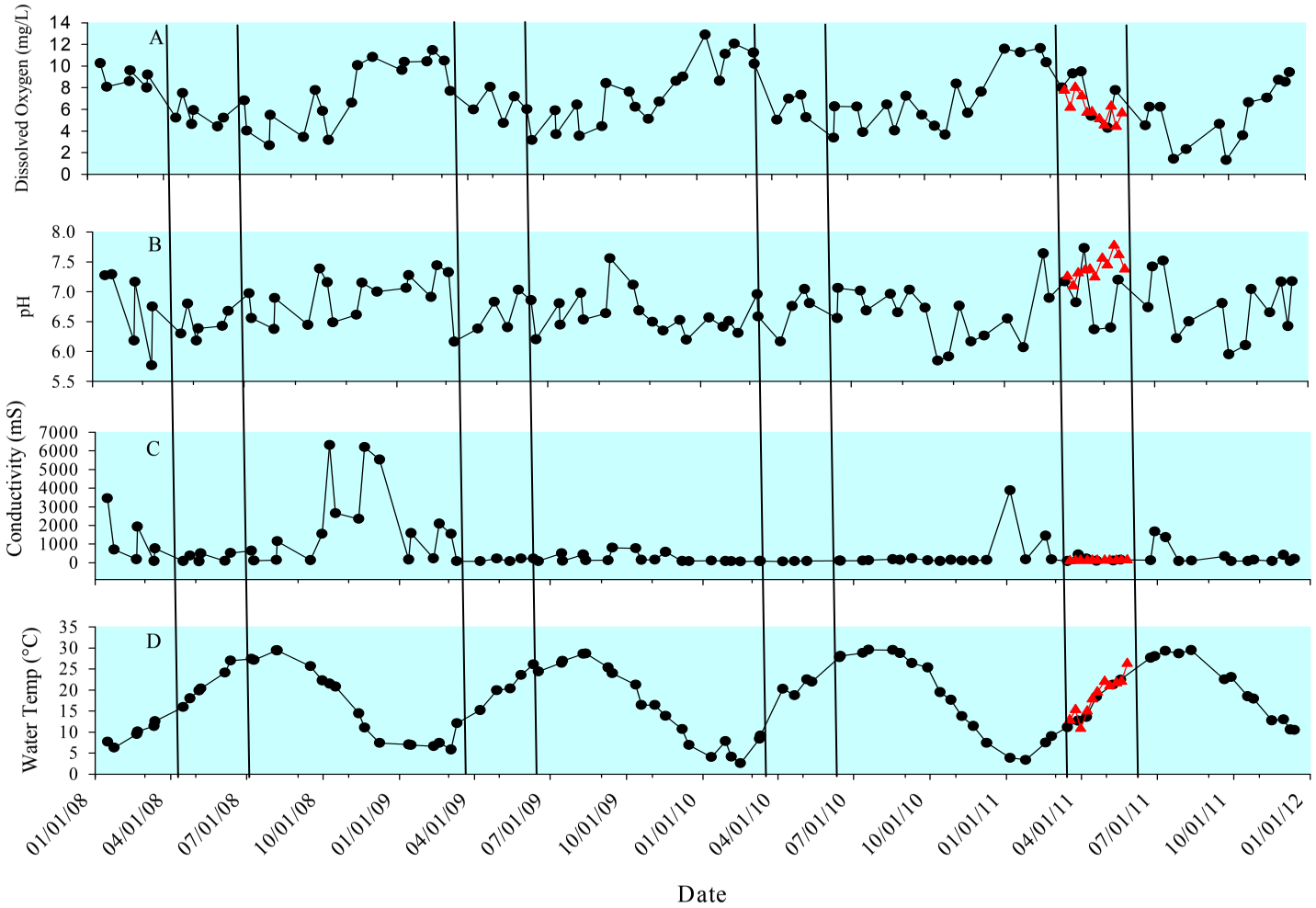


Figure 7. Black line - Estimated values for four environmental parameters in Chowan River based on five USGS gauging stations within the watershed, including Potecasi Creek (02053200), Ahoskie Creek (02053500), Nottoway River (020470000), Meherrin River (02052000), and the Blackwater River (02049500). Environmental parameters at all five of these sites were summed to determine a total daily average. Red line – Mean values of environmental parameters taken during sampling (March 18 – May 26, 2011) at nine sampling sites (North Chowan, Meherrin River, Sarem Creek, Wiccacon Creek, Bennett's Creek, Catherine's Creek, Holiday Island, Rockyhock Creek, and South Chowan). Environmental parameters at all nine of these sites were summed to determine a total daily average.

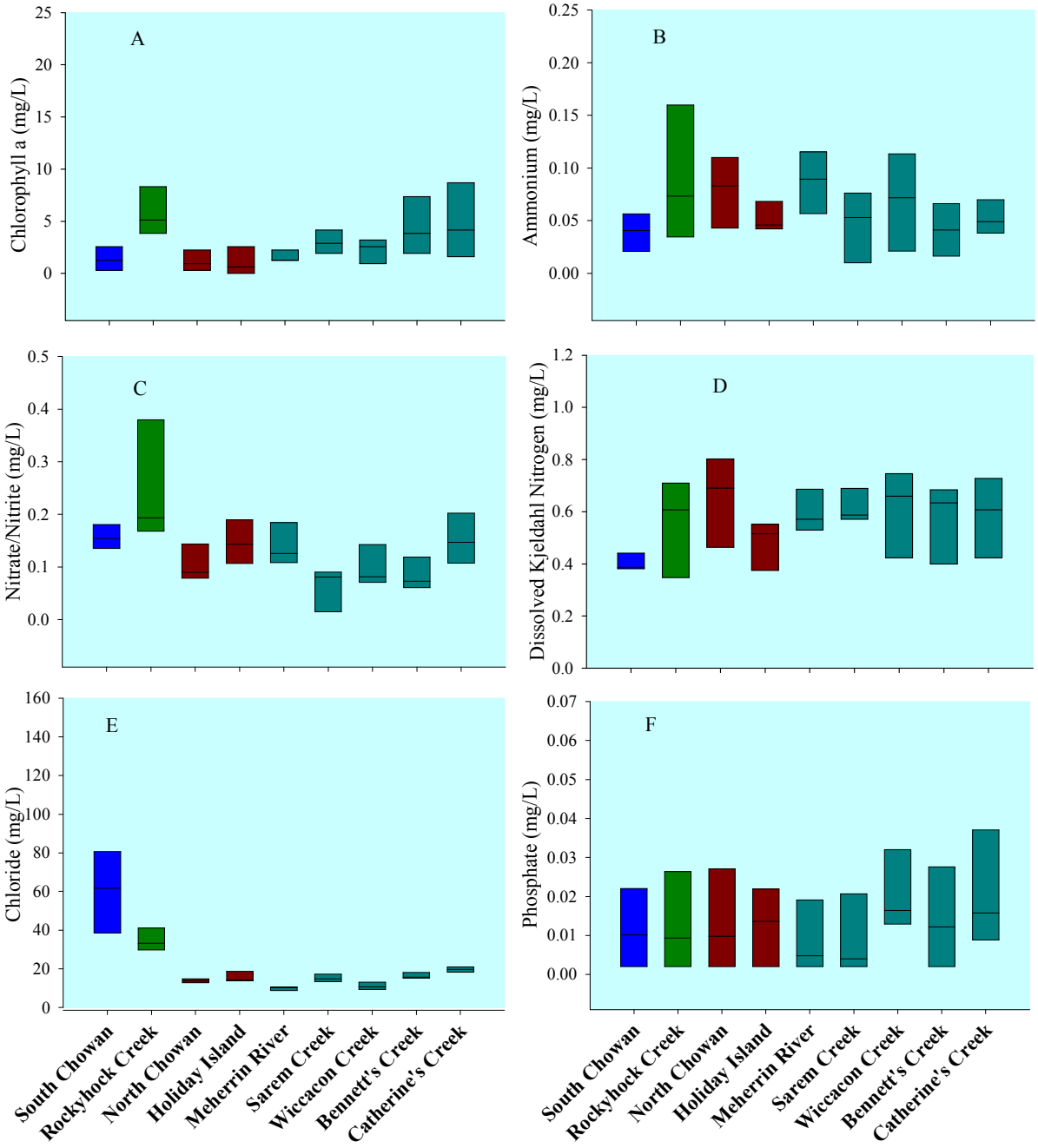


Figure 8. Boxplots of the median and upper and lower quartiles of six physicochemical parameters (A – Chlorophyll α, B – Ammonia, C – Nitrate/Nitrite, D – Dissolved Kjeldhal Nitrogen, E – Chloride, F – Phosphate) from all sampling sites along Chowan River, North Carolina from March 18 to May 26, 2011. Colors of boxes indicate groupings from Cluster Analysis.

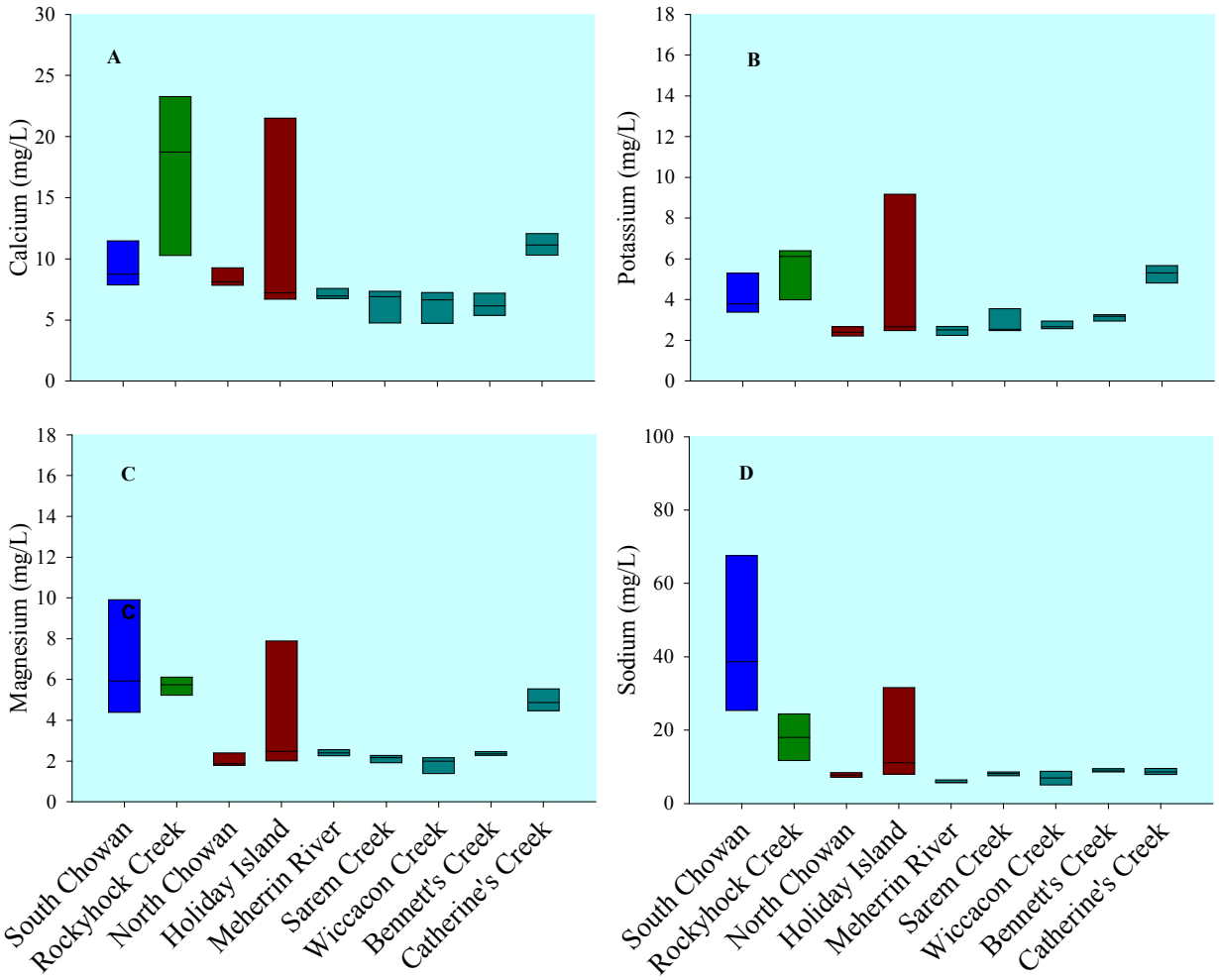


Figure 9. Boxplots of the median and upper and lower quartiles of four cation parameters (A – Calcium, B – Potassium, C – Magnesium, D – Sodium) from all sampling sites along Chowan River, North Carolina between March 18 and May 26, 2011. Colors of bars indicate grouping from Cluster Analysis.

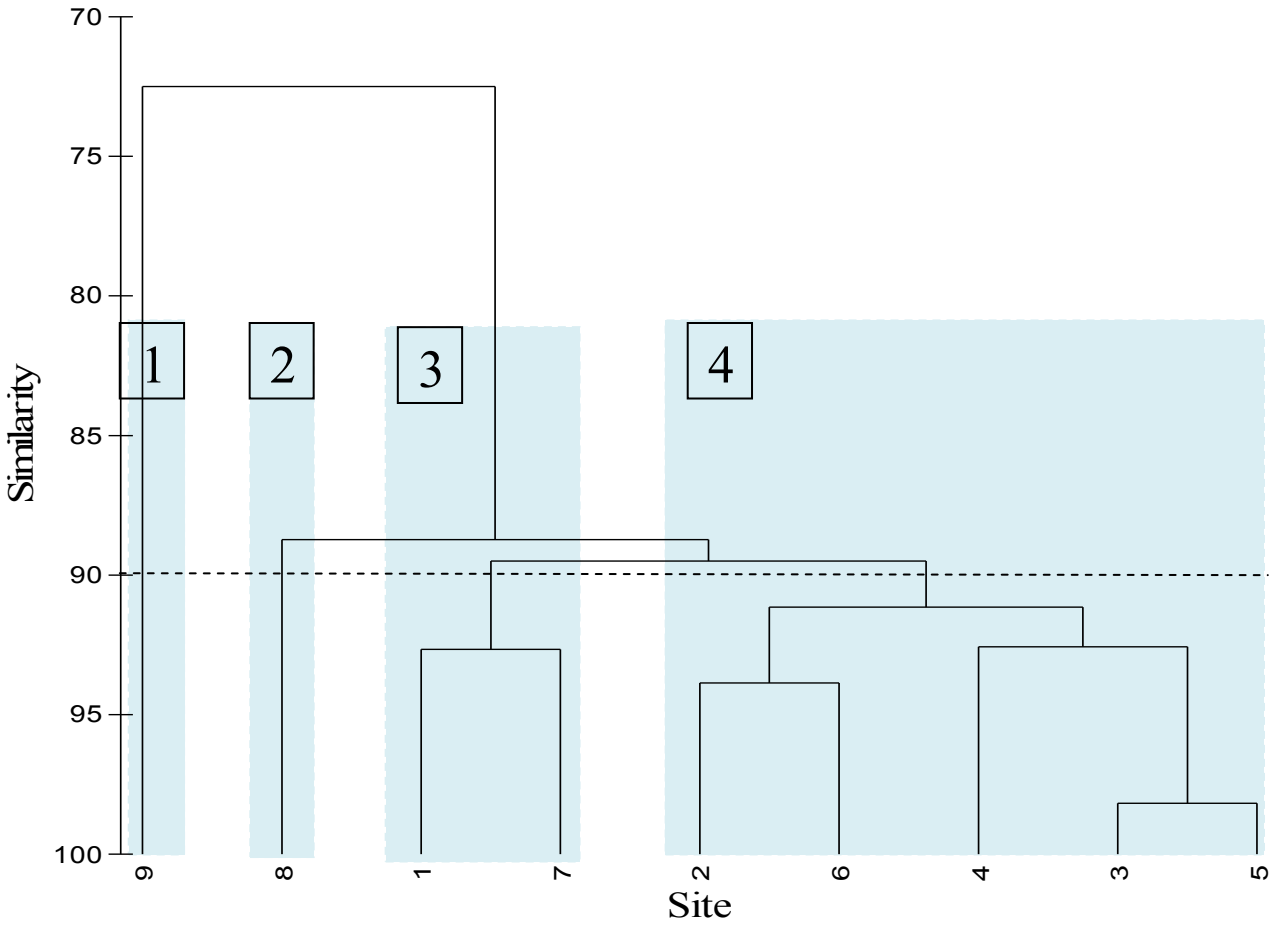


Figure 10. Cluster analysis using physicochemical factors to group similar sites from samples collected on Chowan River, North Carolina (March 18 – May 26, 2011). Dotted line indicates 90% similarity. Numbered boxes indicate cluster number.

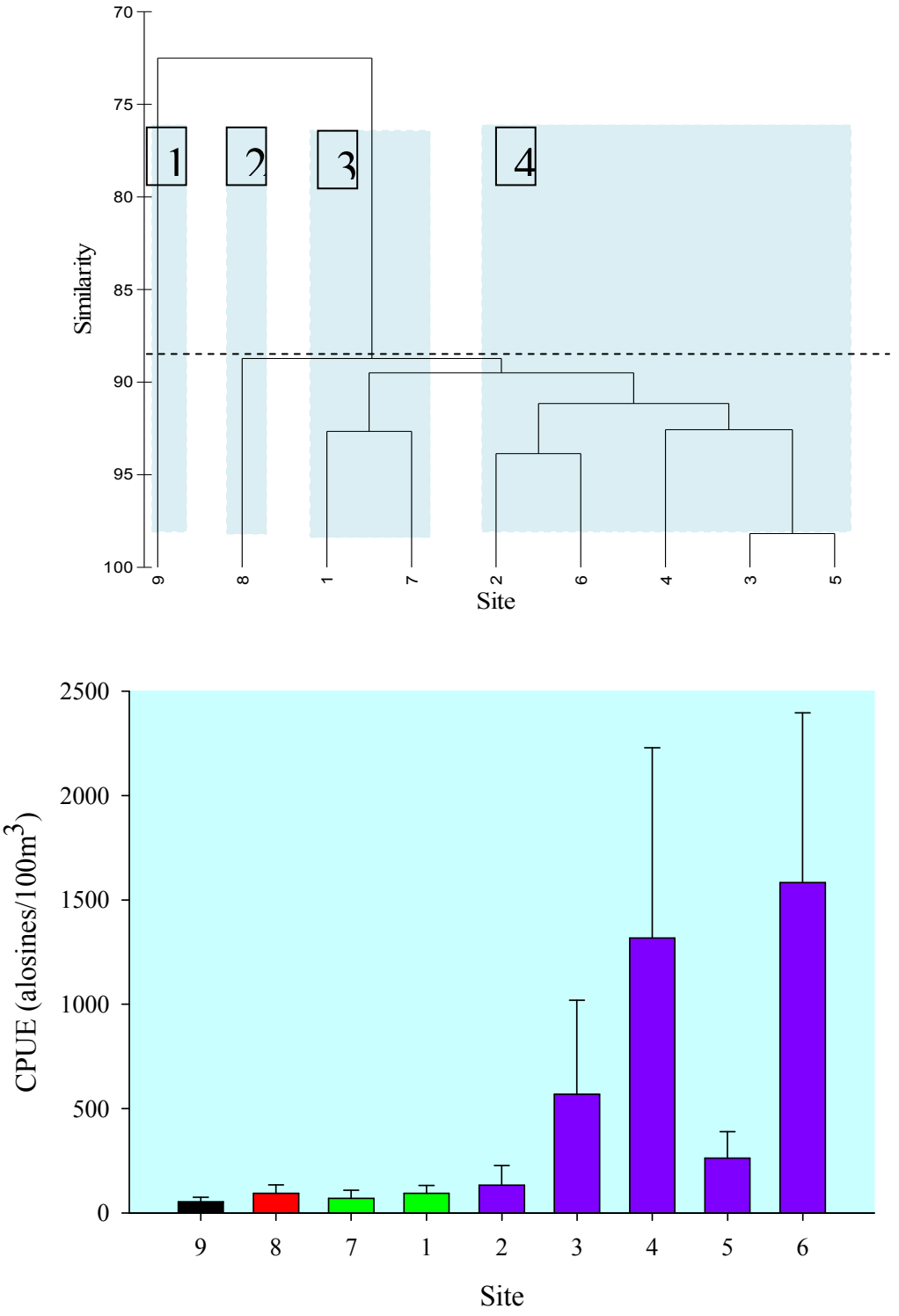


Figure 11. A - Cluster analysis by site showing four clusters at 90% similarity from samples collected on Chowan River, North Carolina between March 18 and May 26, 2011. B - Bar graph of CPUE arranged in order by cluster. Note that all of the highest abundance sites fall within cluster 4.

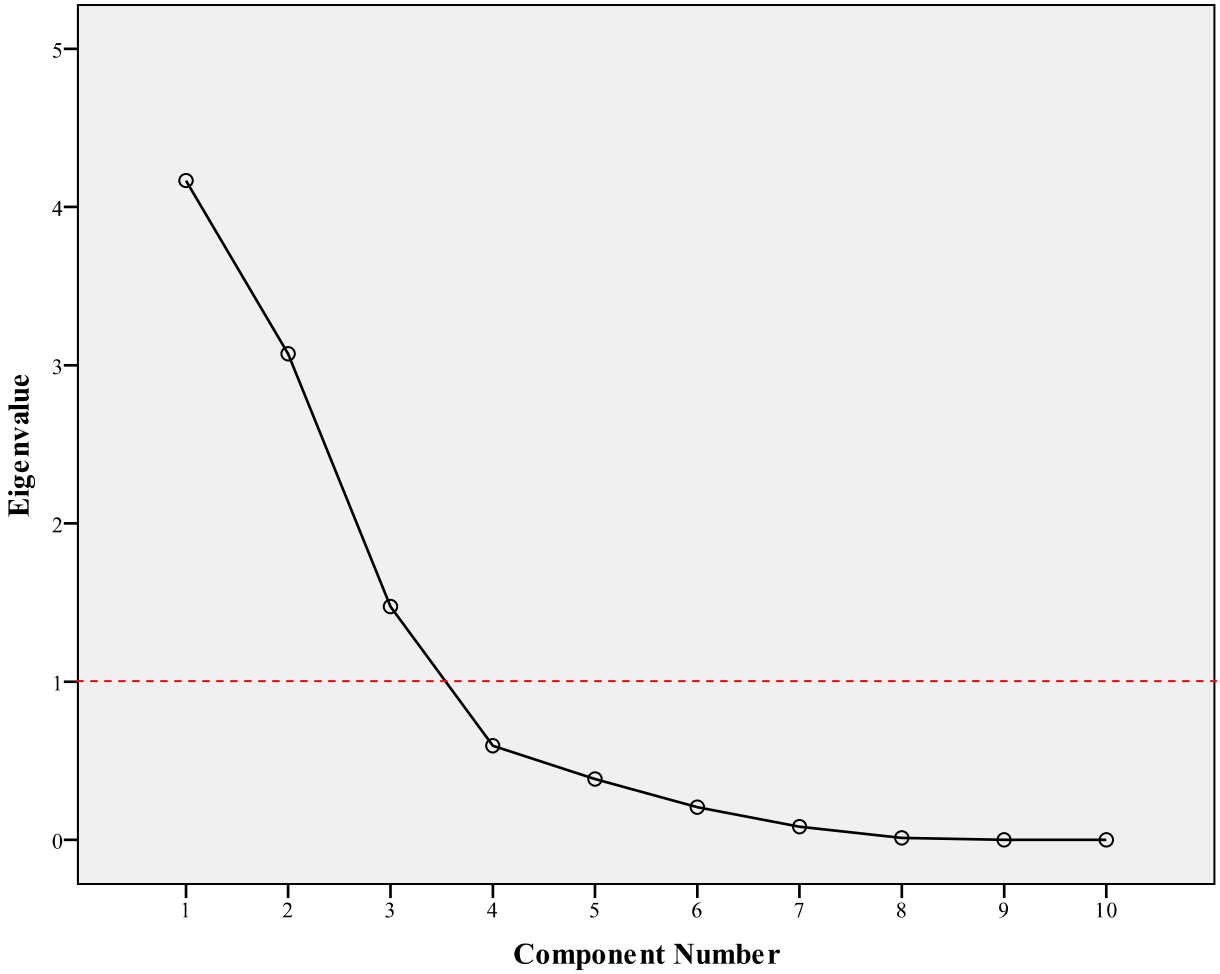


Figure 12. Scree plot from Principal Components Analysis on all physicochemical factors from sampling in Chowan River, North Carolina between March 18 and May 26, 2011. Components with eigenvalues greater than 1 were kept for analysis (3 components).

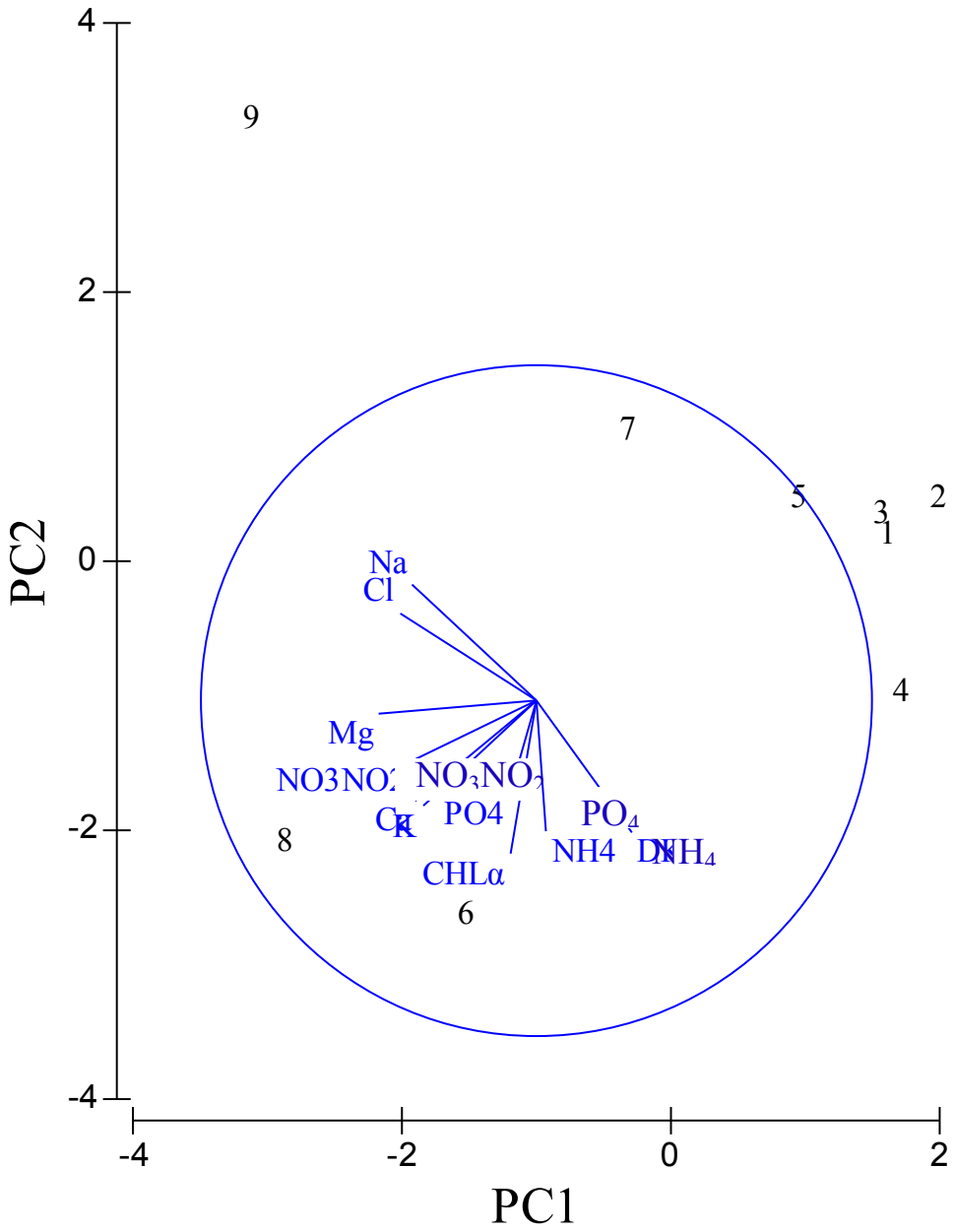


Figure 13. The correlation circle associated with Principal Components (PC) 1 and 2 from physicochemical samples taken from Chowan River, North Carolina between March 18 and May 26, 2011. Correlation circle shows a projection of the initial variables (physicochemical parameters) in the PC space. When two parameters are on the same side of the centroid and close to each other, they are significantly positively correlated. If they are orthogonal, they are not correlated. If they are on the opposite side of the centroid, they are significantly negatively correlated. As physicochemcial parameters reach the blue circle they have stronger effects on sample site. Numbers correspond to sample sites.

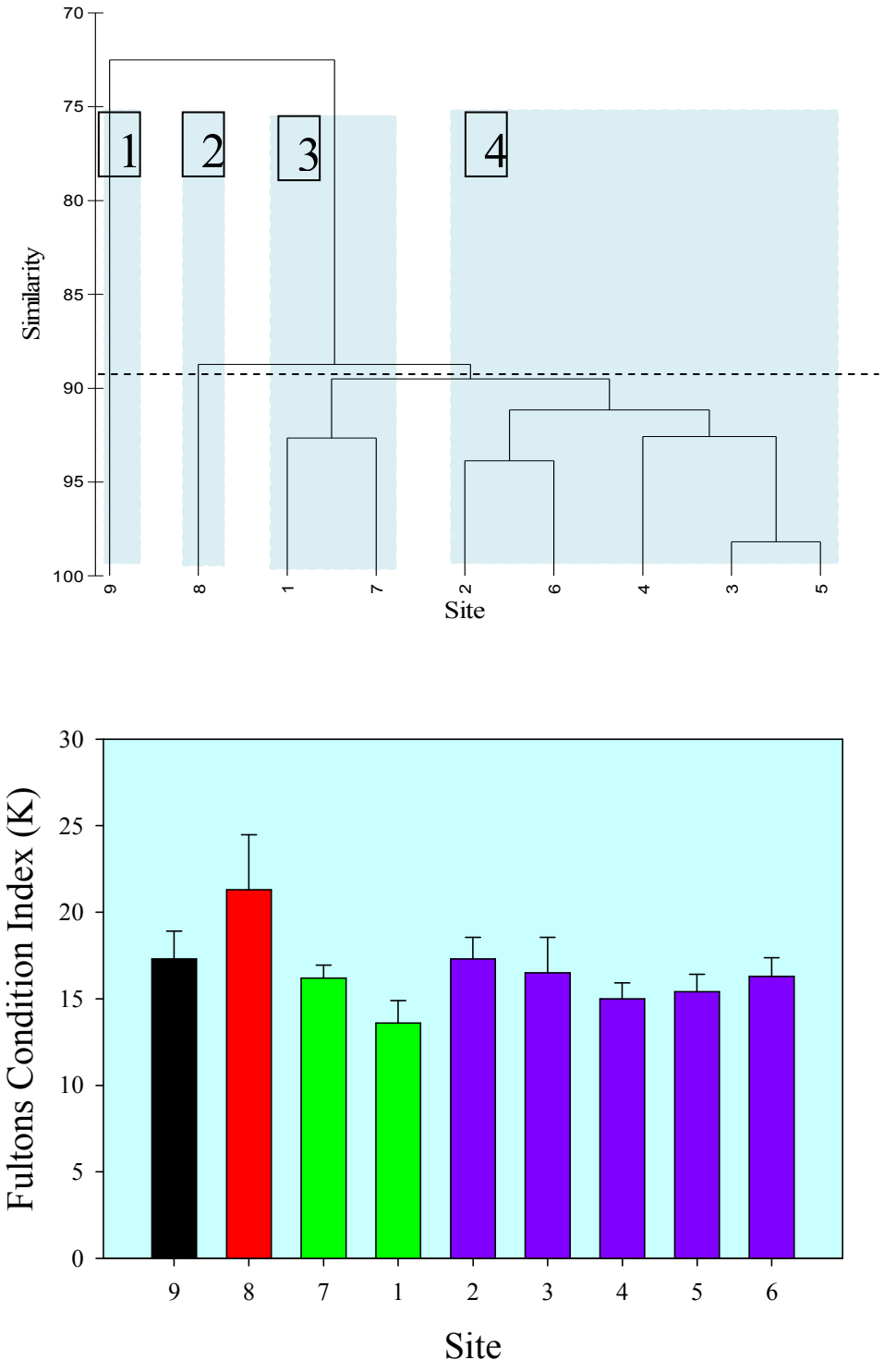


Figure 14. A - Cluster analysis by site showing four clusters at 90% similarity. B - bar graph of Fulton's Condition Index for pre-flexion (< 9 mm) alosine larvae captured on Chowan River, North Carolina between March 18 and May 26, 2011. Bars are arranged in order by cluster.

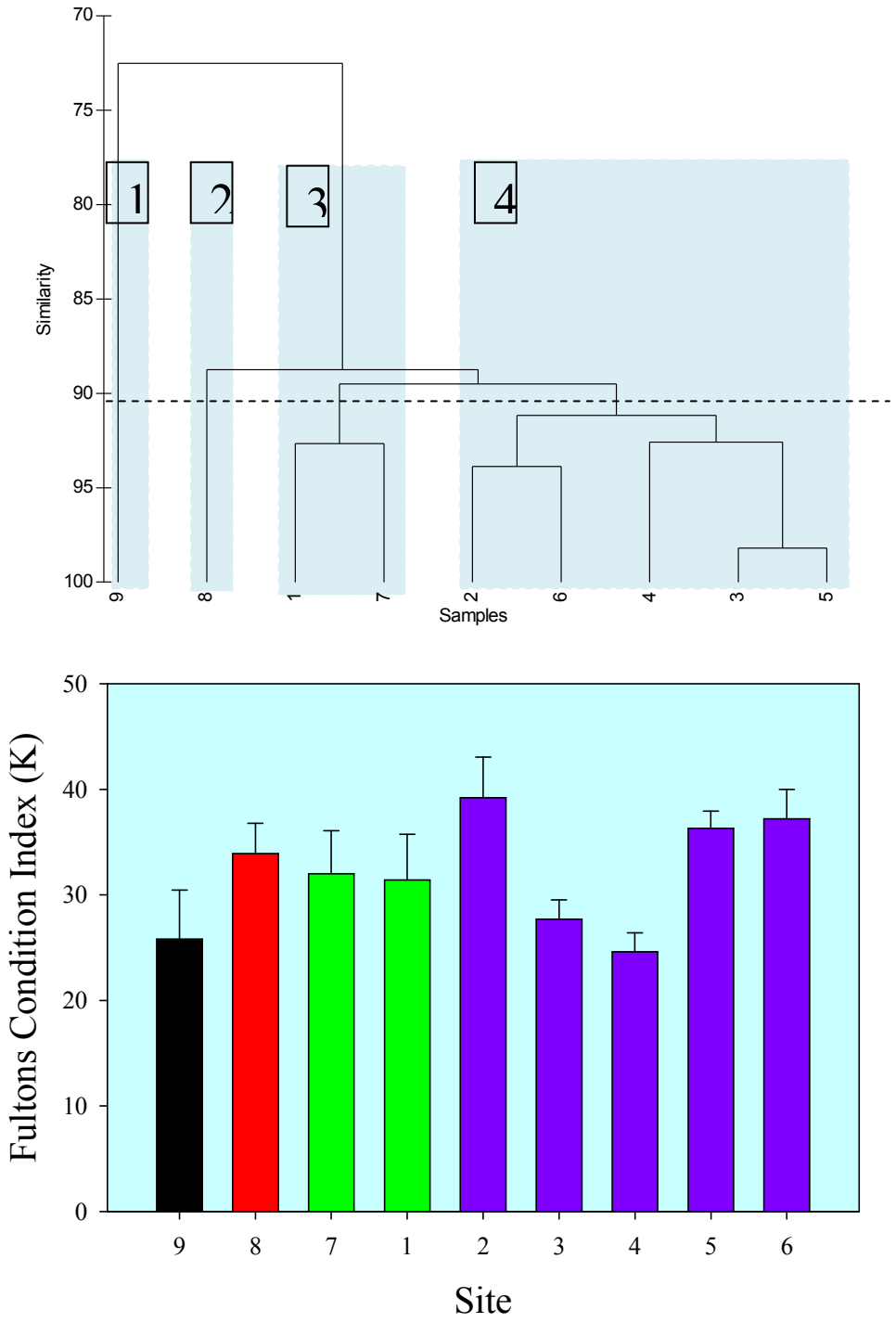


Figure 15. A - Cluster analysis by site showing four clusters at 90% similarity. B - bar graph of Fulton's Condition Index for post-flexion (> 9 mm) alosine larvae captured on Chowan River, North Carolina between March 18 and May 26, 2011. Bars are arranged in order by cluster.

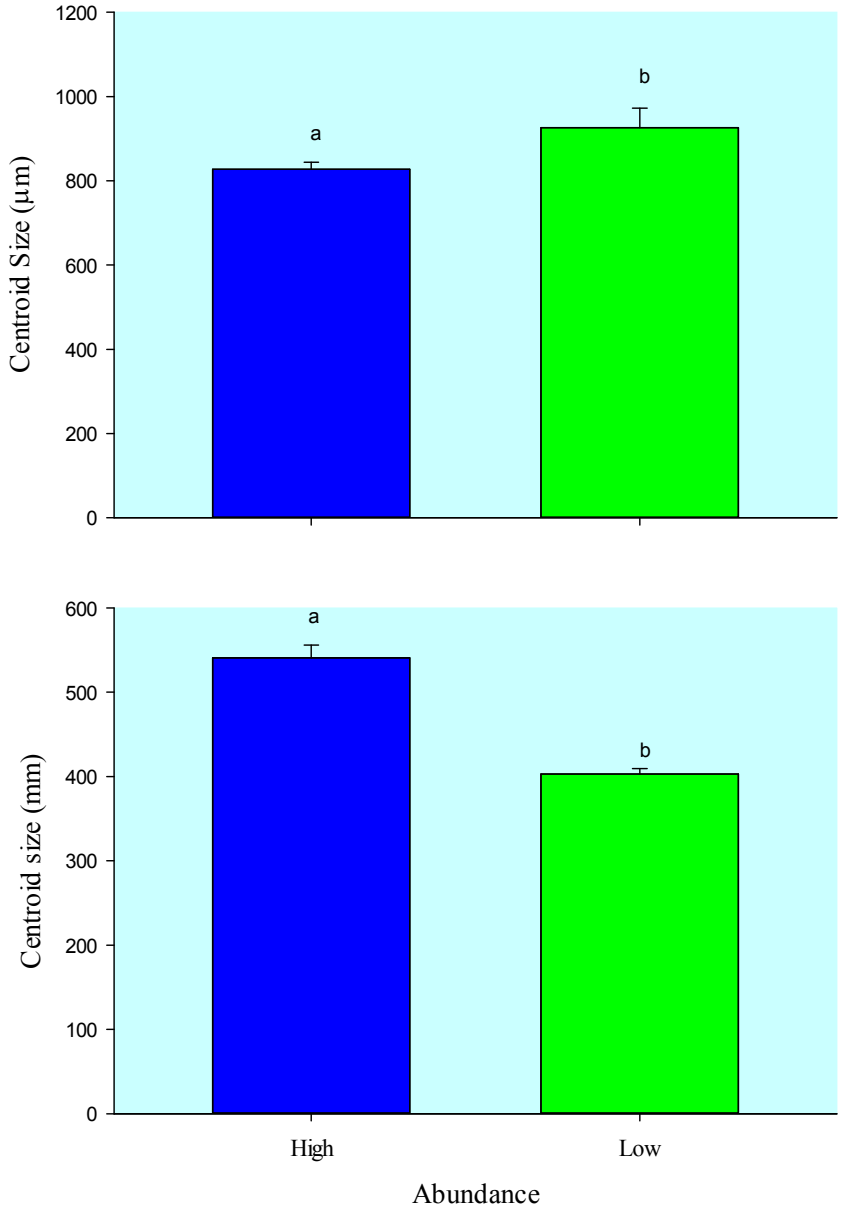


Figure 16. Size variation (mean ± SE) between high and low abundance sites for pre-flexion (top) and post-flexion (bottom) from Chowan River, North Carolina between March 18 and May 26, 2011. Centroid size is a multivariate measure of size analogous to standard length used as a covariate in shape analysis. Bars with the same letters are not different from each other ($p > 0.05$).

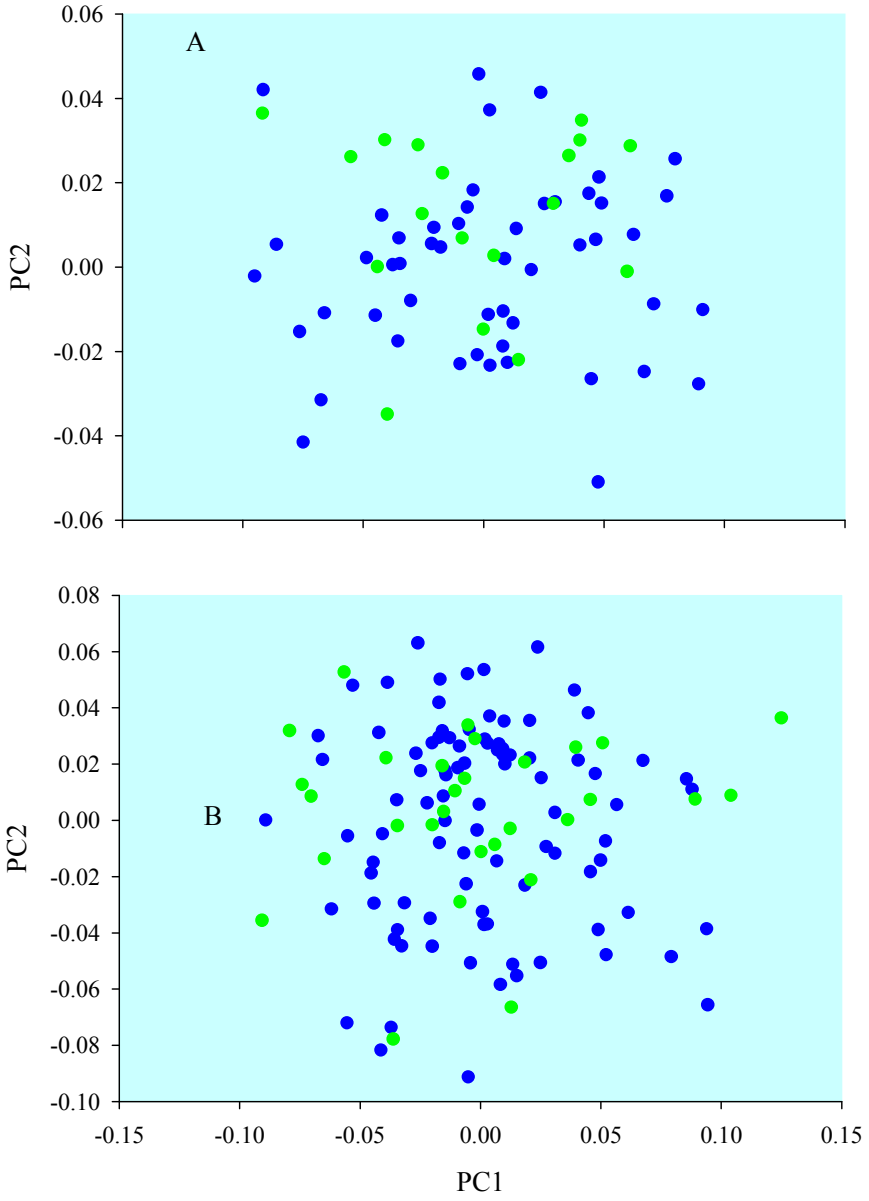
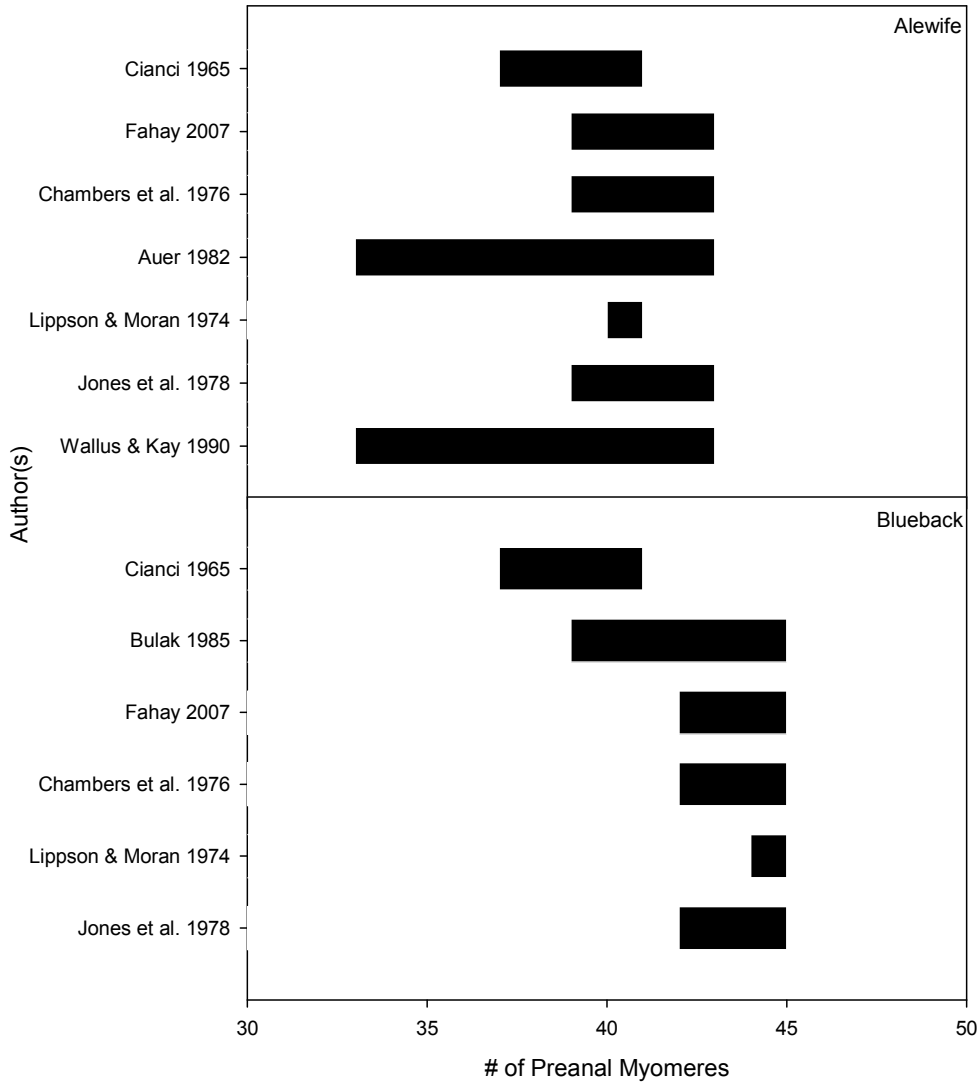
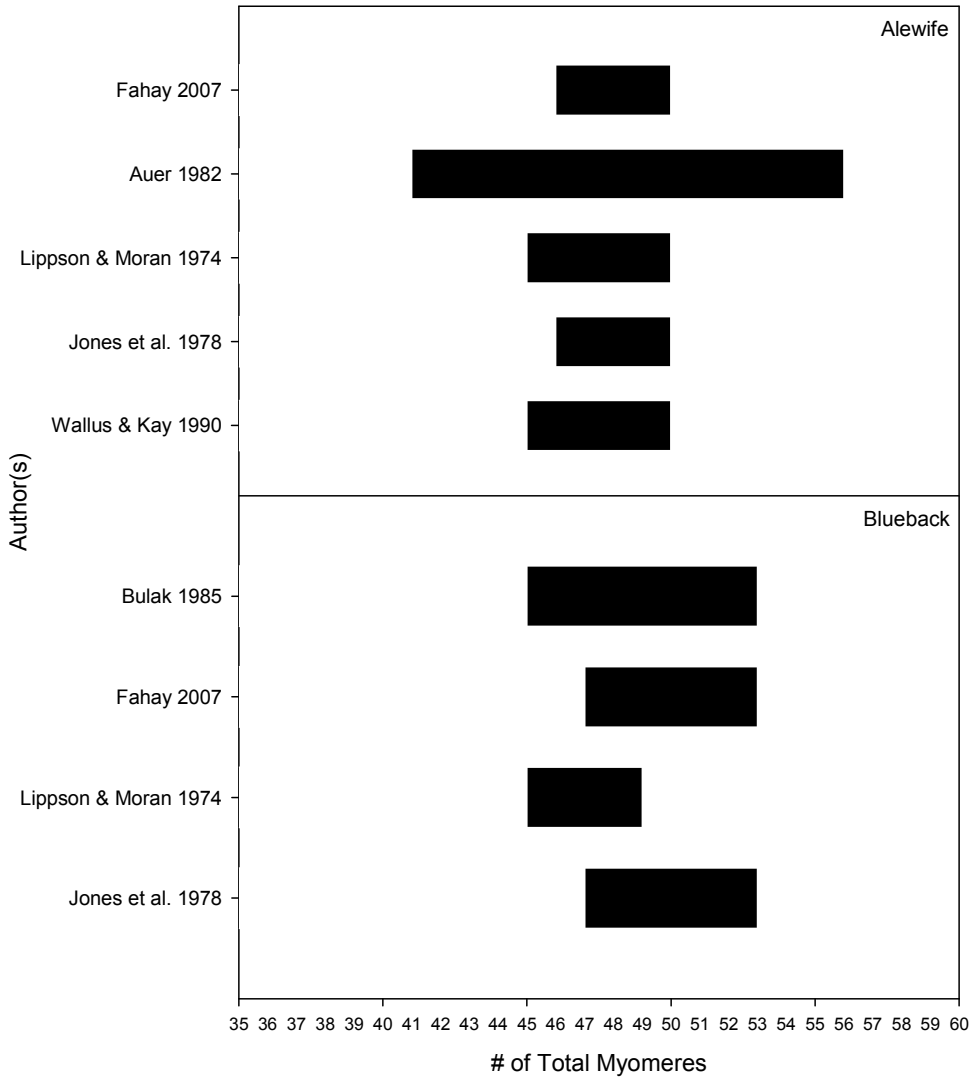


Figure 17. Principal component (PC) plots from two principal components analysis, pre-flexion (A) and pos-flexion (B), from high and low abundance sites on Chowan River, North Carolina between March 18 and May 26, 2011. A – PC1 explained 33% of the variance, PC2 explained 20%. B – PC1 explained 35% of the variance, PC2 explained 28%.

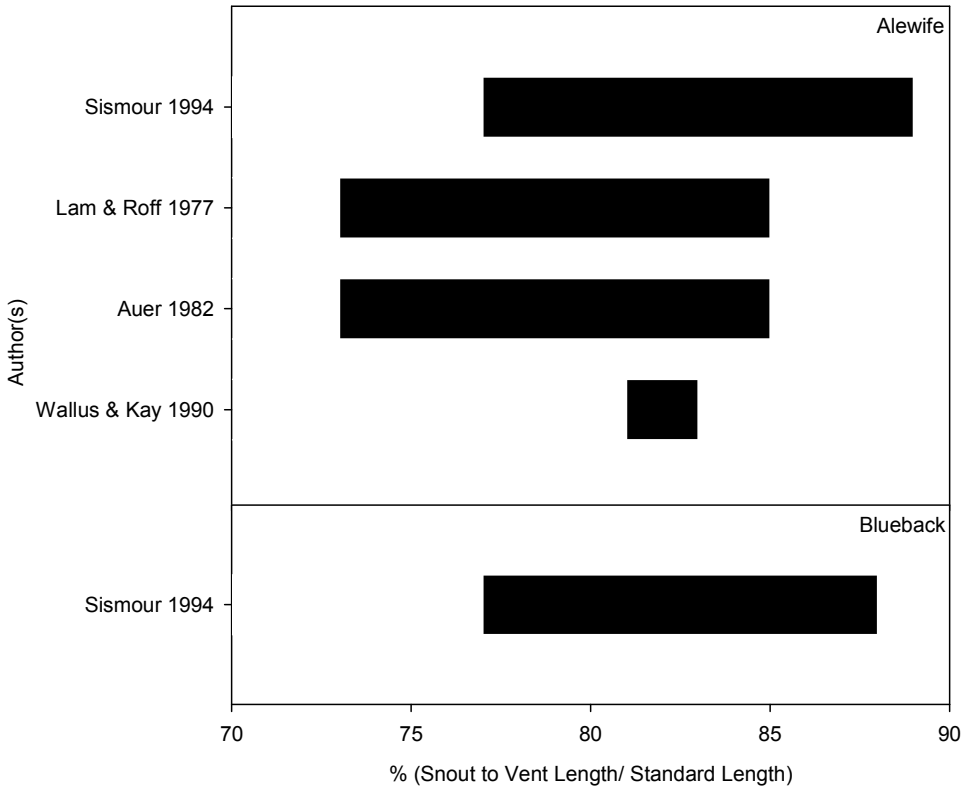
APPENDIX 1: RIVER HERRING IDENTIFICATION PAPER REVIEW



A comparison of preanal myomere counts for larval river herring as reported by various references (Cianci 1965; Lippson et al. 1974; Chambers et al. 1976b; Jones et al. 1978; Auer 1982; Bulak 1985; Wallus 1990; Fahay 2007).



A comparison of total myomere counts for larval river herring as reported by various references (Lippson et al. 1974; Jones et al. 1978; Auer 1982; Bulak 1985; Wallus 1990; Fahay 2007).



A comparison of snout to vent length (SVL) percent of standard length (SL) values for larval river herring as reported by various references (Lam 1977; Auer 1982; Wallus 1990; Sismour 1994b).

APPENDIX 2: ANIMAL USE PROTOCOL APPROVAL

East Carolina University

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

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

March 1, 2011

Anthony Overton, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Overton:

Your Animal Use Protocol entitled, "Can Spawning Habitat be Characterized and Prioritized Based on the Presence of Early Life Stages of River Herring?" (AUP #D254) was reviewed by this institution's Animal Care and Use Committee on 3/1/11. The following action was taken by the Committee:

"Approved as submitted"

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

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure