River Herring Nursery Habitat in Albemarle Sound, North Carolina, Inferred from Otolith Microchemistry

by

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Abstract

River herring is a collective term used to describe two similar alosine species: alewife *Alosa pseudoharengus* and blueback herring *A. aestivalis*. Both of these anadromous species are native to the Atlantic coast of North America and spawn in North Carolina rivers. Consistent with populations along the east coast of North America, river herring populations in North Carolina have experienced drastic declines. Therefore, it is essential to identify nursery habitats used by these species. The goal of this study was to assess river herring nursery habitats in Albemarle Sound by examining growth of juvenile river herring and estimating survival to the adult stage using otolith microchemistry. Water samples were collected from the Alligator, Chowan, Perquimans, Roanoke, and Scuppernong rivers in the summer of 2010. Sr:Ca, Ba:Ca, and Mn:Ca ratios differed significantly between habitats. Magnesium (Mg) was detected consistently only in the Alligator River and was therefore excluded from most analyses. Juvenile river herring were collected from riverine and non-riverine Albemarle Sound habitats from June-October 2010. Concentrations of Mg, manganese (Mn), strontium (Sr), and barium (Ba) at the outer edge of otoliths were measured to determine habitat specific signatures that were used to classify river herring captured in non-riverine habitats to their river of origin. Total length, condition, and growth rates of juvenile river herring differed significantly between habitats. Concentrations of Mg, Mn, Sr, and Ba in otoliths differed significantly between rivers, allowing juvenile river herring to be classified to their river of capture with between 75-100% accuracy. Based on the growth metrics used, alewife nursery habitat was best in the Alligator, Chowan, Pasquotank, and Roanoke rivers along with non-riverine northwest and southwest Albemarle Sound habitats. Alewife nursery habitat was poor in the Little, North, Perquimans, Scuppernong and Yeopm rivers. Blueback herring nursery habitat was best in the non-riverine northwest and southwest Sound. Riverine habitats, particularly the Scuppernong and Perquimans rivers, provided poorer nursery habitat for blueback herring. However, juvenile alewife and blueback herring seemed to move out of the Chowan and Perquimans rivers into western Albemarle Sound habitats suggesting they may seek out nursery areas of higher quality than natal rivers can provide.

Adult blueback herring were captured in the Chowan, Perquimans and Scuppernong rivers. Using river specific elemental signatures obtained from juvenile river herring otoliths, adult blueback herring were classified to their river of origin. High percentages of adults returning to Albemarle Sound were predicted as originating from the Alligator, Chowan, and Roanoke rivers. Homing rates ranged from 0-60%, with highest rates of homing to the Chowan River, and lowest rates to the Perquimans and Scuppernong rivers. This analysis and the analysis of juveniles show that the Alligator, Chowan, and Roanoke rivers along with western Albemarle Sound habitats are high quality river herring habitats, which corresponds well with the strategic habitat areas (SHAs) designated by the state of North Carolina.

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Chapter 1: Introduction

River herring have experienced drastic declines in North Carolina, consistent with populations along the east coast (Schmidt et al. 2003). In 2007, North Carolina enacted a river herring harvest moratorium, but as of 2010 the time frame is not sufficient to determine if the moratorium is aiding in population recovery. Hightower et al. (1996) noted that river herring abundances increased following a period of no river herring fishing in Albemarle Sound, North Carolina, during the Civil War. However, this was long before significant anthropogenic changes to water quality and habitat. The human population in the coastal region of North Carolina has rapidly increased since 1980, consequences of which include degradation of water quality and aquatic habitat (Street et al. 2005). In addition, pollution from urban areas, agriculture and confined animal feeding operations (CAFOs) could lead to degraded water quality in Albemarle Sound (Spruill et al. 1998). Because of habitat degradation from pollution, and loss from shoreline development and impediments, it is plausible the harvest moratorium may not lead to population recovery. The consistent presence of river herring threshold levels over the years indicates that a percentage of river herring do spawn successfully, suggesting the existence of suitable river herring spawning and nursery habitats.

Nursery habitat is an area where juvenile fish are found at high densities, more successfully avoid predation, or have faster growth rates (Beck et al. 2001). Tributaries and western portions of Albemarle Sound, North Carolina, have been identified as river herring nursery habitat (Copeland et al. 1983). However, this distinction was made based on presence of juveniles; no distinction has been made as to which of these habitats might be better than others. In addition, knowledge of river herring nursery habitats were used in designating Strategic Habitat Areas (SHAs) in Albemarle Sound (Figure 1). The entire Chowan River, Roanoke River, most of the western Albemarle Sound shoreline, and large portions of the Alligator River are designated as SHAs (Deaton et al. 2010). One of the goals of these designations is to protect river herring spawning and nursery habitats (Deaton et al. 2010). Beck et al. (2001) proposed that the most important nursery habitats produce more adult recruits than other juvenile habitats based on a combination of four criteria: higher density, growth, survival of juveniles, and movement to adult habitats. If river herring management policies and protections are to be successful, it is essential to identify and protect the highest quality river herring nursery habitats.

The purpose of this study was to identify the most important river herring nursery habitats in Albemarle Sound, North Carolina. The study was broken into two parts: the first part focused on juvenile river herring in Albemarle Sound habitats, the second focused on adult river herring returning to Albemarle Sound during the spawning migration. The objectives of part one were: 1) to collect environmental data and water samples from Albemarle Sound tributaries for elemental analysis; 2) to collect juvenile river herring from tributaries and open Albemarle Sound habitats; 3) to examine growth of juvenile river herring; and 4) using elemental fingerprints in otoliths, to examine connectivity between habitats. The objectives of part two were: 1) to collect adult river herring from the Chowan, Perquimans, and Scuppernong rivers during the spawning run; 2) to examine differences in the elemental composition at the core of adult river herring otoliths; 3) to use river specific elemental signatures from the otoliths of juvenile river herring collected in 2010 (first part of study) to examine natal homing of adult river herring; and 4) to combine results from the two studies to identify habitats that function as important river herring nursery areas, and then compare these locations to existing SHA designations.

Site Description

The Albemarle Sound (Figure 1) encompasses 45,500 km^2 in northeastern North Carolina and extends approximately 90 km eastward from the mouth of the Roanoke River to Kitty Hawk Bay and Colington Island (Copeland et al. 1983). It is the drowned portion of the Roanoke and Chowan rivers and their floodplain (Copeland et al. 1983), covering portions of the piedmont and coastal plain of North Carolina (Riggs 1996). The Albemarle Sound is a shallow oligohaline system characterized by low salinity (0-5) (Copeand et al 1983), shallow water (< 9 m) (Giese et al. 1979), and high turbidity (Copeland et al. 1983). The sound has no direct connection to the ocean, but seawater intrusion does occur through Oregon Inlet, Croatan and Roanoke sounds (Giese et al. 1979; Copeland et al. 1983; Riggs 1996). The system is well mixed due to nearly constant wind action allowing only temporary stratification due to salinity and temperature (Giese et al. 1979; Copeland et al. 1983; Riggs 1996), although hypoxic conditions do occur and diurnal fluctuations in dissolved oxygen from photosynthetic activity can be significant (Bales et al. 1991). There are nine major tributaries including the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers (Figure 1).

Piedmont rocks are crystalline and include granite, slates, schists, and shales, while coastal plain rocks are sedimentary and composed of sand, clay, limestone and marl (Harned and Davenport 1990). The entire region is underlain by sediments and sedimentary rocks that thicken from west to east (Wilder et al. 1978; Copeland et al 1983). There are three aquifers: an upper aquifer consisting of sands and clays, a middle limestone aquifer (Castle Hayne), and a lower aquifer consisting of sand, silt, clay, shale, limestone and dolimite (Wilder et al. 1978).

Albemarle Sound lies within two geographic regions, the western Talbot Terrace and the eastern Pamlico Terrace separated by the Suffolk Scarp (Copeland et al. 1983; Riggs 1996). The western portion is geologically older and characterized by bluff shorelines and well drained sandy soils (Riggs 1996). The Chowan and Roanoke rivers, draining the western portion, carry a high volume of water and high sediment load (Riggs 1996). The eastern portion is characterized by poorly drained soils and pocosins composed of peat soils (Riggs 1996). Rivers originating in the eastern coastal plain drain swamps with low discharges of acidic black water and small sediment loads (Riggs 1996). The Albemarle Sound, Chowan River, and Roanoke River watersheds are primarily forested with some land use for agricultural purposes, including livestock in the Chowan and Roanoke river basins (Stanley 1989). Almost no land within these watersheds is considered urban (Stanley 1989).

Albemarle Sound is considered an important nursery habitat for anadromous species fish species including striped bass *Morone saxatilis*, American shad *Alosa sapidissima*, alewife *A. pseudoharengus*, and blueback herring *A. aestivalis* along with other shellfish and finfish species (Giese et al. 1979). Historically, much of commercial fisheries in the region have focused on these anadromous species, but there is also a large blue crab *Callinectes sapidus* fishery and smaller fisheries focusing on primarily freshwater species like catfish (Geise et al. 1979; Copeland et al. 1983; Epperly 1984).

Water samples were collected from the Alligator, Chowan, Perquimans, Roanoke and Scuppernong Rivers from June-October 2010 to obtain a representation of water chemistry in Albemarle Sound tributaries. Juvenile river herring were collected over the same time frame from all Albemarle Sound tributaries to capture spatial and temporal variation in growth characteristics and elemental concentrations in otoliths. Adult blueback herring were collected from the Chowan, and Perquimans rivers in April and May 2010 and from the Scuppernong River in 2009. No alewife were collected during this study.

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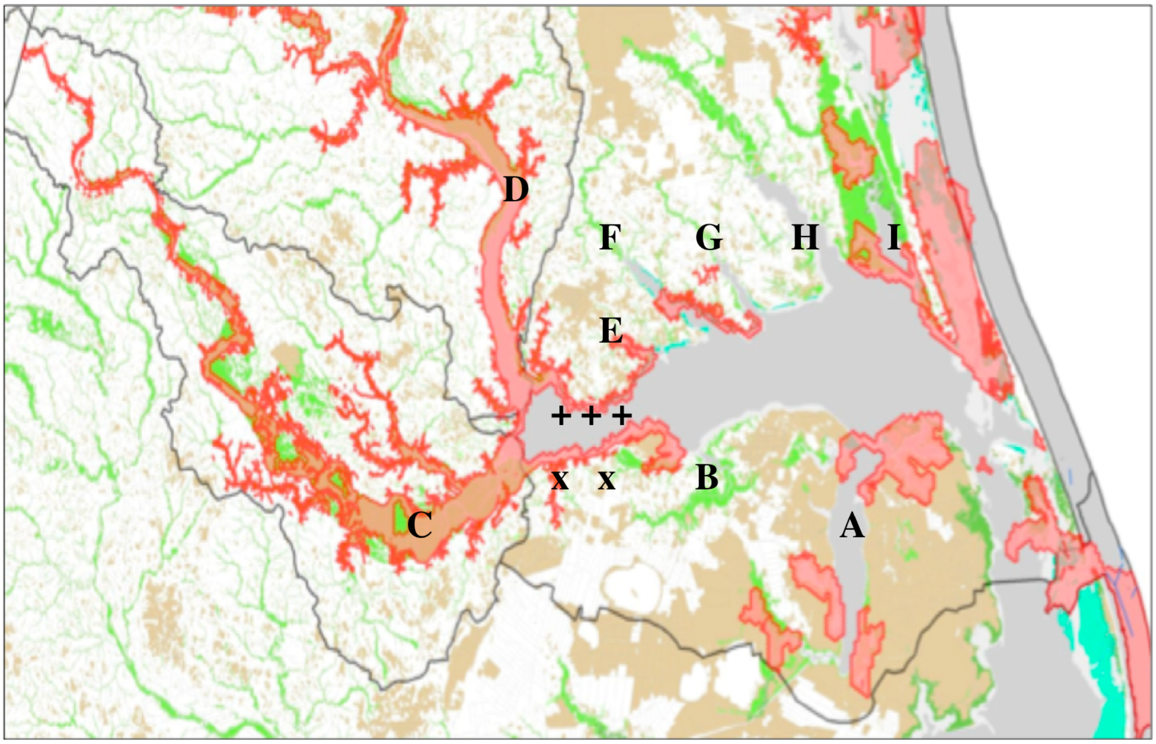

Figure 1. – Map of study site. A = Alligator, B = Scuppernong, C = Roanoke, D = Chowan, E =Yeopim, F = Perquimans, G = Little, H = Pasquotank and I = North. Locations marked with + are northwest Sound habitats and locations marked with X are southwest Sound habitats. Red areas mark Strategic Habitat Area (Sha) designations (from Deaton et al. 2010).

Chapter 2: Surface Water Chemistry of Tributaries to Albemarle Sound, North Carolina

Abstract

Concentrations of dissolved strontium (Sr), barium (Ba), manganese (Mn), and magnesium (Mg) in Albemarle Sound tributaries were investigated to examine the potential of using otolith microchemisty as a proxy for habitat use. Water samples and environmental data were collected monthly from the Alligator, Chowan Perquimans, Roanoke, and Scuppernong rivers from June-October 2010. Water samples were analyzed using an inductively-coupled plasma optical emission spectrometer (ICP-OES) for the elements Ca, Sr, Ba, Mn, and Mg. Elements were normalized to Ca to account for the role of Ca in otolith formation. Salinity differed between locations and water temperature; dissolved oxygen and pH differed between months but not locations. Sr:Ca, Ba:Ca, and Mn:Ca differed significantly between locations but only Sr:Ca and Mn:Ca differed significantly between months. Mg was detected consistently only in the Alligator River and therefore could not be used in statistical comparisons between watersheds. Using only Sr:Ca and Ba:Ca, water samples were classified to river of collection with a range of 46-90% accuracy. Comparison of results from this study and those of Mohan et al. (2012) show stability in dissolved elemental ratios between 2008 and 2010. The exception to this is the Sr:Ca ratio in Perquimans River, which was much higher in 2010 than it was in 2008. Stable differences in dissolved elemental ratios between Albemarle Sound watersheds should allow for the use of otolith microchemistry in reconstructing natal origins of anadromous fish from multiple year classes.

Introduction

Investigations of surface water chemistry provide information that can be useful in studies of otolith microchemistry (Dorval et al. 2005; Elsdon and Gillanders 2006). Concentrations of the elements strontium (Farrell and Campana 1996; Bath et al. 2000; Elsdon and Gillanders 2003a; Kraus and Secor 2004; Walther and Thorrold 2006; Mohan et al. 2012), barium (Bath et al. 2000; Elsdon and Gillanders 2003a; Elsdon and Gillanders 2004; Walther and Thorrold 2006; Miller 2009; Mohan et al. 2012), and manganese (Forrester 2005; Dorval et al. 2007; Mohan 2012) in the otoliths of fish have been shown to reflect concentrations in water. For other elements, like magnesium, which are more physiologically regulated (Campana 1999), the relationship between concentrations in the water and concentrations in the otolith are less clear (Wells et al. 2003; Dorval et al. 2007; Mohan et al. 2012).

Otolith microchemistry has been shown to be useful in discriminating natal habitats of fish (Thorrold et al. 1998a; Thorrold et al. 1998b; Walther et al. 2008). However, before this can be done it is important to examine differences in elemental concentrations between watersheds as a means of ground truthing what is found in otoliths. The goal of this study was to examine differences in Ba:Ca, Mg:Ca, Mn:Ca, and Sr:Ca ratios between Albemarle Sound watersheds. In addition, results from this study were compared to those obtained by Mohan et al. (2012), who conducted a similar study of Albemarle Sound watersheds in 2008.

Methods

Water Sample Collection and Preparation

Collection and preparation of water samples followed methods similar to those of Mohan et al. (2012). The Alligator, Chowan, Perquimans, Roanoke and Scuppernong rivers, all tributaries of Albemarle Sound, were chosen as water sample collection locations. Water samples and environmental data were collected once per month from June through October 2010 in order to capture temporal variation in the sample. Samples were collected at each site within a single day. Samples from all five locations were scheduled for collection within two or three consecutive days, but sampling in July, September, and October took longer when weather prevented collection of samples on consecutive days. Two replicate samples were collected from each river, one from a downstream location and one from an upstream location, to capture spatial variability within rivers. The exceptions to this were in June and September, when two water samples were taken at both upstream and downstream locations within each river, and in August when three samples were collected in the Chowan River. Environmental data -- water temperature (°C), dissolved oxygen (mg/L), salinity (ppt), and pH -- were collected with water samples. No environmental data were collected in the Roanoke and Scuppernong rivers in September due to equipment malfunctions.

Because the Albemarle Sound is well mixed (Copeland et al. 1983) and the objective of this study was to investigate surface water chemistry, water samples were collected at approximately 80-cm depth using a Masterflex peristaltic pump. Samples were pumped and filtered inline (Whatman glass microfiber filters: Grade GF/D = 1.5

µm; Grade GF/F = 0.7 µm) into new 125-mL, high-density fluorinated Nalgene bottles, rinsed with three sample volumes. Water samples were stored on ice during transport to the lab and acidified with trace-metal-grade nitric acid to pH less than 2.0. Samples were filtered using 0.2-µm syringe filters (Supor) to remove particulate fractions while retaining colloidal and dissolved fractions (Mohan et al. 2012). Acidification and filtration of samples usually occurred within eight hours of collection. Water samples were stored at 4°C until elemental analysis.

Water Sample Analysis

Water sample analysis followed methods described by Mohan et al. (2012). A Perkin Elmer inductively coupled plasma (ICP) optical emission spectrometer (Optima 2100 DV) was used to measure concentrations of Ca (ppm), Mg (ppm), Sr (ppb), Ba (ppb), and Mn (ppb). Samples were diluted with 10 parts of ultrapure water (18.5 Ω) to one part of sample. A stock standard solution (1,000 mg/L in 2% HNO_3) for each element was diluted to create an elemental specific calibration curve with five standards (lowest low, low, medium, high, highest high). The combined stock solution was analyzed before sample measurements, and quality control checks requiring greater than 90% recovery were issued after every 12 samples. Four water samples collected in the Roanoke River in September and two samples collected in the Scuppernong River in September were not analyzed due to instrument malfunctions. Concentrations of Sr, Ba, Mg, and Mn were normalized by dividing the concentration by the concentrations of Ca to account for the role of Ca in the uptake of elements in otoliths.

Statistical Analysis

Kruskal-Wallis tests were used to examine differences in water temperature, dissolved oxygen, salinity, pH, and ratios of Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca between sampling locations and months. Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca ratios were plotted against water temperature, dissolved oxygen, and salinity to examine how dissolved elemental ratios varied based on environmental factors. Quadratic discriminate function analysis was used to examine how elemental ratios could be used to classify water samples to river of collection.

Results

Environmental Variables

Salinity differed significantly between locations, and temperature, dissolved oxygen, and pH differed significantly between months (Table 1). Mean salinity was highest in the Alligator River (1.51 ppt) and lowest in the Chowan (0.08 ppt) and Roanoke (0.09 ppt) rivers. Mean temperature was 27.3°C in June, 30.6°C in July, and then decreased to 20.8°C in October (Figure 1). Mean dissolved oxygen was 5.8 mg/L in June, 7.8 mg/L in July, 9.1 mg/L in August, and 9.71 mg/L in September before declining to 6.3 mg/L in October (Figure 1). Differences in pH between months were significant, declining from 8.6 to 8.3 from June through October (Table 1). Significant differences in temperature between months were due to significant temperature differences in the Chowan and Roanoke rivers (Figure 1).

Elemental Variables

Sr and Ba were detected consistently at all locations during the entire sample season. Mg and Mn varied in detection between locations and months. Mg was detected in the Alligator River in all months, the Perquimans River in one September sample, and the Scuppernong River in August and September. No Mg was detected in the Chowan and Roanoke rivers. Mn was detected in the Chowan and Perquimans rivers in all months, in the Alligator River in September and October, the Roanoke River in August June and October (no September samples), and the Scuppernong River in June, July, August and October.

Mn:Ca, Sr:Ca, and Ba:Ca ratios differed significantly between locations, and Mn:Ca and Sr:Ca differed significantly between months (Table 1). The mean Sr:Ca ratio was highest in the Perquimans River (36.02) and lowest in the Roanoke River (11.12) (Figure 2). The mean Ba:Ca ratio was highest in the Perquimans River (10.85) and lowest in the Alligator River (2.41) (Figure 2). The mean Mn:Ca ratio was highest in the Perquimans (11.60) and Roanoke rivers (11.78) and lowest in the Alligator River (1.83) (Figure 2). Mean Mg:Ca was highest in the Alligator River (2.07), but was only detected consistently in the Alligator River (Figure 2). Sr:Ca peaked in August and September before declining in October, and Mn:Ca increased from June to July, was steady from July through September then increased from September to October (Figure 3).

Both the Sr:Ca and Ba:Ca ratios showed weak positive relationships with water temperature, while Mn:Ca showed no relationship (Figure 4). Sr:Ca showed a weak positive relationship with dissolved oxygen while Ba:Ca, and Mn:Ca showed no relationship (Figure 4). Both Ba:Ca and Mn:Ca showed weak negative relationships with salinity, while Sr:Ca showed no relationship (Figure 4).

Because Mg and Mn were detected infrequently at some locations, only Sr:Ca and Ba:Ca were used to classify water samples to collection locations. Multi-variate means differed significantly between locations (Pillai's trace statistic: $F = 10.14$, $df = 8, 116$, $P < 0.0001$) (Figure 5) allowing water samples to be classified to collection river with 46-90% accuracy (Table 2). Most misclassifications occurred to neighboring rivers; rivers that were more geographically isolated had the highest classification success (i.e., Alligator River).

Discussion

Environmental Variables

Salinity

Salinity was the only environmental variable that varied significantly between locations. Salinity appeared to follow a longitudinal gradient with highest mean salinity in the easternmost Alligator River and lowest mean salinity in the westernmost Chowan and Roanoke rivers. This is generally the pattern observed in Albemarle Sound with eastern locations close to the ocean having higher salinities than western portions further from the ocean (Copeland et al. 1983; Mohan et al. 2012). Although salt water can encroach into western Albemarle Sound, high freshwater inflow from the Chowan and Roanoke Rivers usually blocks saltwater intrusion (Giese et al. 1979). The Perquimans and Scuppernong rivers, originating in the central portion of Albemarle Sound, had similar mean salinities that were lower than those in the Alligator River and higher than those in the Chowan and Roanoke rivers. Mohan et al. (2012), found salinity was higher in the Alligator River compared to the Pasquotank River, Perquimans River and Batchelor Bay, results similar to those found here. Similar to this study, Mohan et al. (2012) found no differences in salinity between months.

Temperature and pH

Temperature and pH did not vary significantly between locations but did differ significantly between months. Although no differences were found in pH between months, Mohan et al. (2012) found differences in temperature between months. Temperature increased throughout the summer before declining in September and October, whereas pH declined throughout the summer. Declines in temperature and pH in October may be the result of heavy rains, which occurred at the beginning of October.

Dissolved Oxygen

No differences were found in dissolved oxygen between locations, but differences were found between months. These results agree with those found by Mohan et al. (2012) who found no differences in dissolved oxygen between locations but detected differences in dissolved oxygen between months. Dissolved oxygen was lowest in June and increased throughout the summer before decreasing in October. Mohan et al. (2012) found similar results, with dissolved oxygen increasing from July-October at most locations. The sharp decline in dissolved oxygen at most sites may have been the result of heavy rains that occurred at the beginning of October and caused major flooding in the area. Mohan et al. (2012) found that dissolved oxygen was closely related to water temperature, with highest dissolved oxygen occurring at the coldest water temperatures. This was not observed during this study. When water temperature dropped in October dissolved oxygen dropped as well. Again this may have been the result of flooding events.

Elemental Ratios

Strontium

Sr:Ca varied significantly between locations and months. Highest concentrations were found in the Perquimans River and lowest concentrations were found in the Roanoke River. Strontium has been shown to follow a salinity gradient with higher concentrations in saltwater than freshwater (Odom 1951; Rosenthal et al. 1970; Ingram and Sloan 1992) with some exceptions (Limburg and Siegel 2006). Strontium can vary over small spatial scales in systems like the Chesapeake Bay, along the east coast of North America (Dorval et al. 2005), and estuaries along the southern shore of Australia (Elsdon and Gillanders 2006). However, these studies have focused on investigating dissolved Sr along a salinity gradient. Fewer studies have demonstrated differences in Sr between locations in primarily low salinity or freshwater systems. Exceptions include Wells et al. (2003) investigating dissolved elemental concentrations in the Coeur d' Alene River, Idaho, Limburg and Siegel (2006) investigating dissolved elemental concentrations in the Hudson-Mohawk-Erie Canal system, and Humston et al. (2010) investigating dissolved elemental concentrations in James River and the Maury River.

Although salinity in Albemarle Sound is low and varies based on wind and rainfall, the eastern Albemarle Sound generally has higher salinity than the western sound (Copeland et al. 1983; Mohan et al. 2012; *this study*). In general, results from this study followed an expected pattern; the easternmost higher salinity Alligator River had high Sr:Ca whereas the western most Chowan and Roanoke Rivers had lower Sr:Ca. However, an anomaly occurred in the mid-salinity Perquimans River, which had extremely high Sr:Ca compared to the higher salinity Alligator River. This could be the result of groundwater discharges from the Castle Hayne aquifer (Harned and Davenport 1990) which contains water with Sr:Ca similar or higher to that found in seawater (Woods et al. 2000). It is also possible differences in bedrock, and sediment between Albemarle Sound watersheds (Harned and Davenport; Riggs 1996) could cause variation in dissolved Sr. Limburg and Siegel (2006) found high Sr:Ca in the freshwater Seneca River up to 500 km from the Atlantic Ocean, these values were similar to or higher than Sr:Ca values found in the tidal Hudson River. These unexpectedly high values were related to the weathering of rocks in the region (Limburg and Siegel 2006). The weak positive relationship between salinity and Sr:Ca, supports the hypothesis that high Sr in the Perquimans River is influenced by sources other than high salinity water.

Sr:Ca measurements from this study were similar to those observed by Mohan et al. (2012) with Sr:Ca being higher in the Perquimans River, than the Alligator River and Batchelor Bay (comparable to Chowan and Roanoke Rivers). However, Sr:Ca ratios from the Perquimans River in 2010 were much higher in my study than those observed by Mohan et al. (2012) in 2008 suggesting that the Perquimans River Sr:Ca ratio may fluctuate annually.

In addition, Mohan et al. (2012) found a strong positive relationship between salinity and Sr:Ca that was not observed in my study. This is probably because samples from my study were not collected over a strong salinity gradient, with mean salinity from my study being less than 1.0. However, Sr:Ca was somewhat high in the Alligator River where salinity was higher, suggesting salinity plays some role in predicting Sr:Ca in water.

Barium

Ba:Ca was significantly different between locations but not months, results that agree with Mohan et al. (2012). Ba was highest in the Perquimans River and lowest in the Alligator and Scuppernong rivers. Unlike Sr, Ba has been shown to have a negative relationship with salinity (Guay and Falkner 1998), although Coffey et al. (1997) found Ba maximums at mid-salinities of estuaries in the United States and Europe. Ba:Ca ratios have been shown to differ between locations in predominantly freshwater systems (Wells et al. 2003; Limburg and Siegel 2006; Humston et al. 2010). As expected, the higher salinity Alligator River had low Ba:Ca and the low salinity Chowan and Roanoke rivers had high Ba:Ca ratios. Again, an anomaly occurred in the Perquimans River where Ba:Ca was extremely high. It was expected that the Perquimans River would have Ba:Ca falling somewhere between values observed in the Alligator River and values observed in the Chowan and Roanoke rivers due to its location in a mid-salinity portion of the sound. This result is consistent with the mid-salinity Ba peak observed by Coffey et al. (1997). This study did find a somewhat weak negative relationship between salinity and Ba:Ca with some outlying points. A potential source of high Ba may be groundwater discharge, because salty groundwater can be enriched in Ba (Shaw et al. 1998)

Ba:Ca values in my study were similar to those found by Mohan et al. (2012) except for in the Chowan and Perquimans rivers, which had much higher Ba:Ca than any value reported by Mohan et al. (2012). My study recorded high Ba:Ca ratios in the Perquimans River, whereas Mohan et al. (2012) found low Ba:Ca values in the Perquimans River. My study did find low Ba:Ca values in the Alligator River, which compare well with the results of Mohan et al. (2012). My study showed a weak negative relationship between salinity and Ba:Ca, whereas Mohan et al. (2012) found a stronger negative relationship between salinity and Ba:Ca. Again, this may be the result of the low salinity gradient sampled in my study.

Manganese

Mn:Ca differed significantly between locations and months. Mn:Ca was highest in the Perquimans and Roanoke rivers and lowest in the Alligator River, results that agree with those of Mohan et al. (2012). Dissolved Mn has been related to reducing conditions in sediments during anoxic conditions (Brewer and Spencer 1971; Sundby et al. 1986; Laslett 1995). While no anoxic conditions were observed during this study it is possible fluctuations in dissolved oxygen between day and night could cause the release of Mn. Although the Albemarle Sound is well mixed (Copeland et al. 1983) dissolved oxygen can range from supersaturated conditions to hypoxia (Bales et al. 1993). In addition, diurnal fluctuations in dissolved oxygen can be large (Bales et al. 1993). Because we only sampled surface waters during the day, periods of low dissolved oxygen or hypoxia may not have been recorded during this study. However, dissolved Mn is water may provide a record of recent hypoxic events because it can remain dissolved for a number of days (Pakhomova et al. 2007). Despite the published relationship between hypoxia and Mn we found a weak negative relationship between dissolved oxygen and Mn:Ca. Mohan et al. (2012) observed highest Mn:Ca values at mid-salinities. While Mn:Ca measurements from mid-salinity areas were not common in this study, Mn:Ca was generally low in the higher salinity Alligator River.

Magnesium

Mg was detected consistently in the Alligator River, and infrequently in the Perquimans and Scuppernong rivers. This finding is somewhat troubling since Mohan et al. (2012) consistently measured Mg in the Alligator River, Pasquotank River, Perquimans River and Batchelor Bay. Since we used the same ICP-OES under the same parameters to quantify Mg, and Mg was consistently detected in samples from another study (Cathey et al. 2012 *in revision*) run simultaneously with our samples, it seems unlikely that hardware was an issue. The lack of Mg in our samples was probably due in part to not sampling a large salinity gradient. At most locations salinity rarely exceeded 0.1, with the exception of the Perquimans and Scuppernong rivers on a few occasions, and the Alligator River. These are all locations where salinity was greater than 0.1 on at least some occasions. Mohan et al. (2012) found a strong positive relationship between salinity and Mg:Ca indicating Mg may follow a similar pattern to Sr. This pattern was also observed by Dorval et al. (2005) in the Chesapeake Bay. In this study, the Alligator River consistently had salinities higher than 1.0 ppt and Mg was consistently detected suggesting a relationship between salinity and Mg. Other locations may have never had salinity high enough to have a quantifiable Mg:Ca ratio.

Multivariate Classification

Because Mg:Ca and Mn:Ca were not consistently detected, a multi-variate classification was difficult. Nevertheless, 46-90% of water samples were correctly classified to collection rivers using only Ba:Ca and Sr:Ca. Classification followed an expected pattern with rivers in similar geographic proximity having similar multi-variate means, and geographically isolated rivers having more distinct means. Using Mg:Ca, Mn:Ca, Ba:Ca, and Sr:Ca Mohan et al. (2012) classified water samples to collection locations with 76-81% accuracy. This suggests that using more variables may increase classification accuracy in my study. In addition, the classification of water samples somewhat followed the expected longitudinal gradient. The Chowan and Roanoke rivers classified primarily based on Ba:Ca, the Perquimans River classified based on Sr:Ca and the Alligator and Scuppernong rivers fell in the middle. The expected pattern would have seen the Alligator and Perquimans rivers switch places to more consistently follow geographic locations.

Conclusions

Sr:Ca, Ba:Ca, and Mn:Ca ratios differed significantly between rivers. Mg was detected consistently in the Alligator River only and was excluded from most analyses. Despite using only two variables (Ba:Ca and Sr:Ca) water samples were classified to collection rivers with 46-90% accuracy. In addition, results from this study were comparable to results reported by Mohan et al. (2012). While values in my study were not always comparable on a one-to-one basis, rankings of sample sites for individual elements were often similar. For example both my study and Mohan et al. (2012) found the Perquimans River to have the highest Sr:Ca values. This finding suggests elemental ratios may be stable from year to year. A large salinity gradient was not sampled in this study and most sampling locations were freshwater locations. While dissolved elemental concentrations in rivers may fluctuate between seasons, they are thought to remain stable from year to year (Wells et al. 2003; Bickford and Hannigan 2005). This information, combined with our comparison to Mohan et al. (2012), suggest differences in elemental concentrations between rivers may be useful in classifying fish to natal Albemarle Sound tributaries using otolith microchemistry.

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Table 1. – Results of Kruskal-Wallis tests examining differences in water temperature (C), dissolved oxygen (mg/L), salinity (ppt), and pH, and element:Ca ratios between locations and months. For each one-way comparison α was set at 0.05.

Variable	Effect	chi-squared	df	p
Temperature	Location	1.56	4	0.8161
	Month	46.10	4	<0.0001*
Dissolved Oxygen	Location	1.42	4	0.8399
	Month	18.61	4	0.0009*
Salinity	Location	41.63	4	<0.0001*
	Month	2.52	4	0.641
pH	Location	2.79	4	0.5933
	Month	9.69	4	0.046*
Mg/Ca	Location			
	Month	6.71	4	0.1522
Mn/Ca	Location	9.1013	4	<0.0001*
	Month	15.5	4	0.0038*
Sr/Ca	Location	28.15	4	<0.0001*
	Month	17.44	4	0.0016*
Ba/Ca	Location	34.59	4	<0.0001*
	Month	0.92	4	0.9212

Table 2. – Results of quadratic discriminant function analysis used to classify water samples to collection river.

Location	N	ALLI	CHOW	PERQ	ROAN	SCUPP	% Correct
ALLI	14	12	0	0	0	1	85.7
CHOW	15	2	7	0	6	0	46.7
PERQ	13	0	1	9	1	2	69.2
ROAN	10	0	0	0	9	1	90.0
SCUPP	12	2	0	0	4	6	50.0

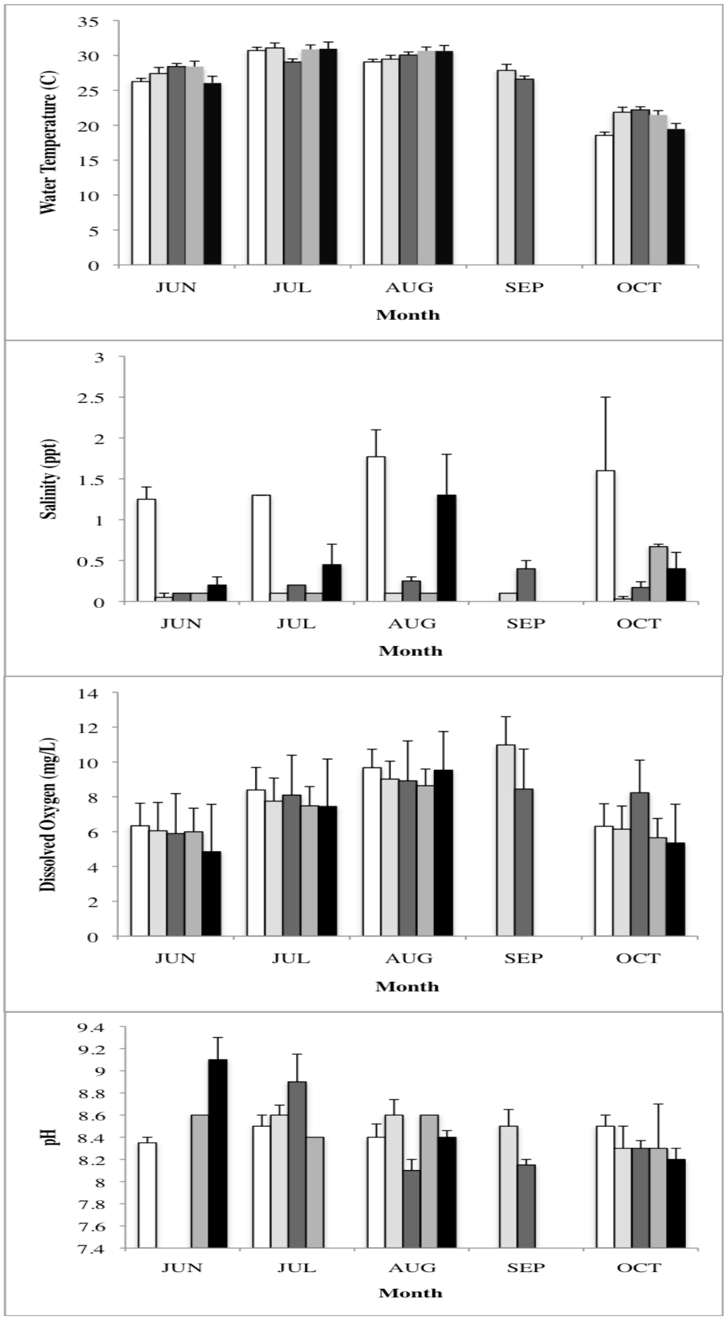

Figure 1. – Spatial and temporal (June-October 2008) variation (± SE) of water temperature (C), dissolved oxygen (mg/L), salinity (ppt), and pH. White bars = ALLI, light gray = CHOW, light black = PERQ, dark gray = ROAN, black = SCUPP.

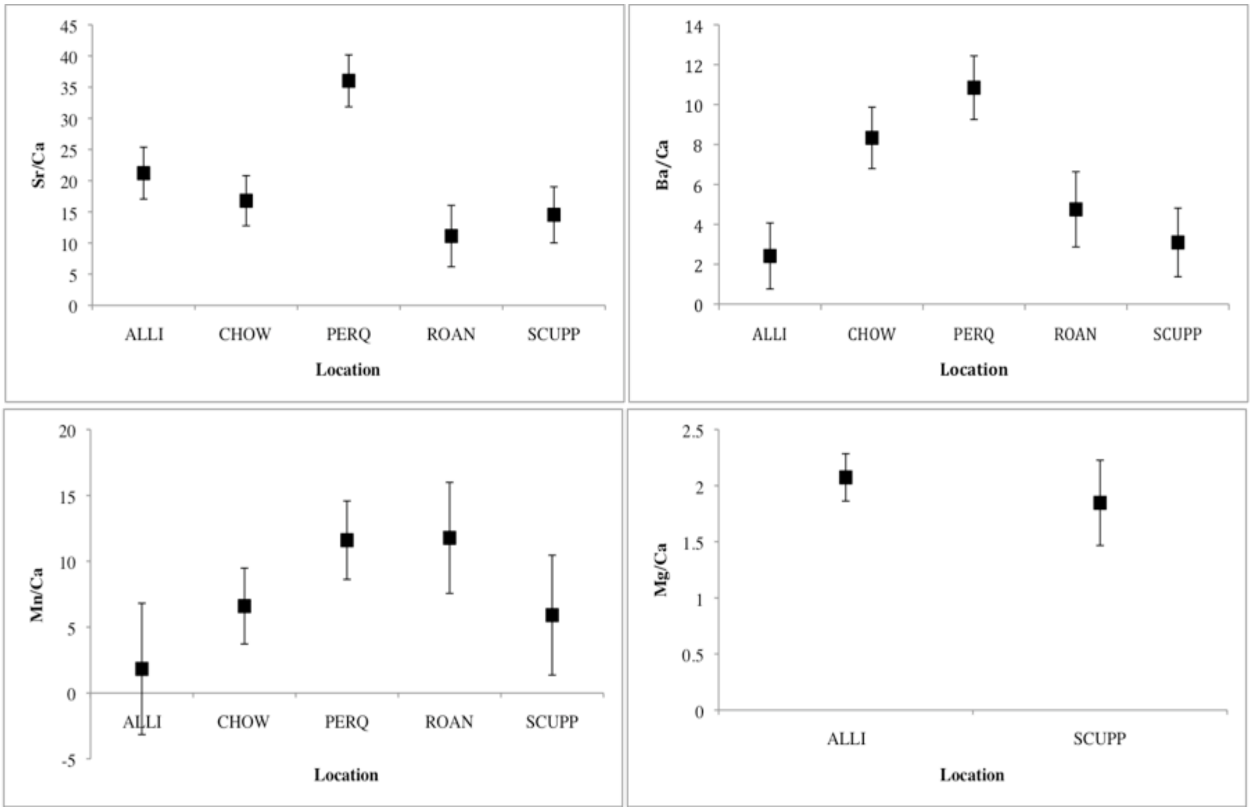

Figure 2. – Mean (± SE) Sr:Ca, Ba:Ca, Mn:Ca, and Mg:Ca in water samples collected in the Alligator (ALLI), Chowan (CHOW), Perquimans (PERQ), Roanoke (ROAN), and Scuppernong (SCUPP) rivers from June-October 2010.

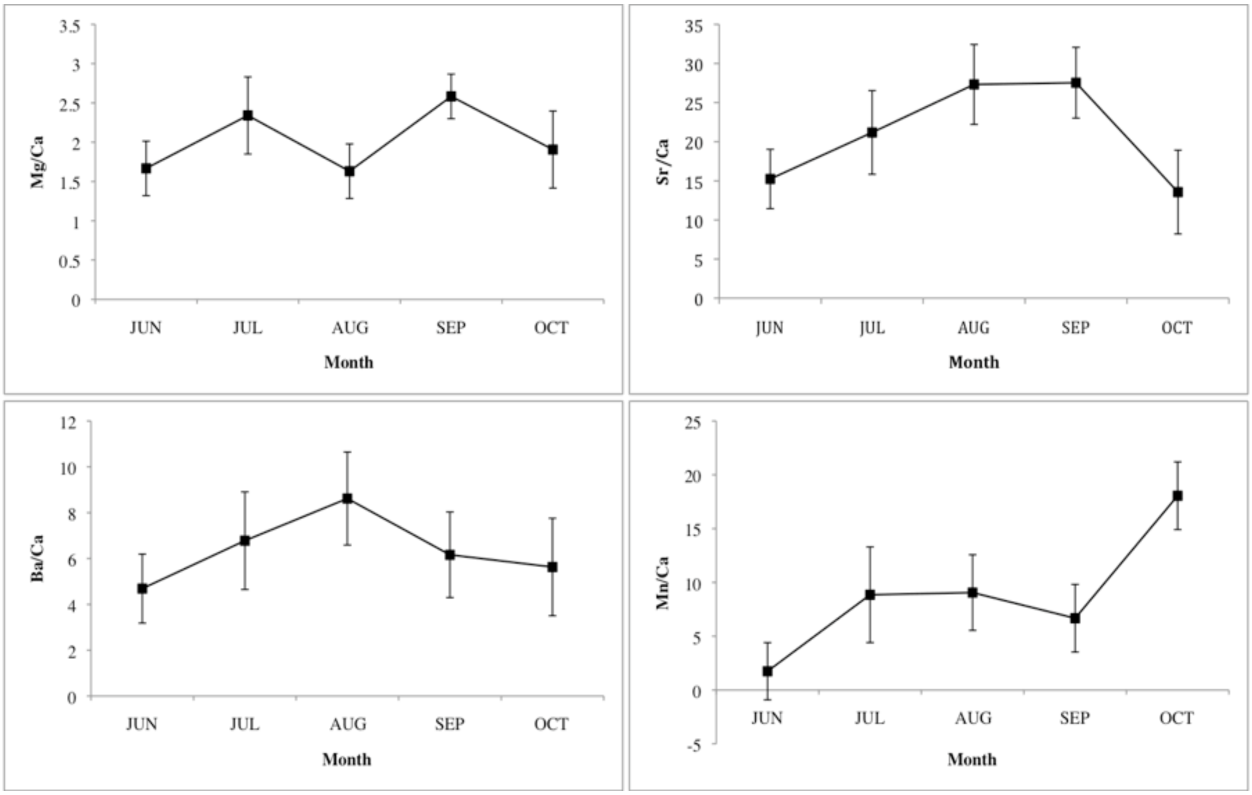

Figure 3. – Mean (± SE) Sr:Ca, Ba:Ca, Mn:Ca, and Mg:Ca by month (June-October 2010) in water samples collected from tributaries of Albemarle Sound.

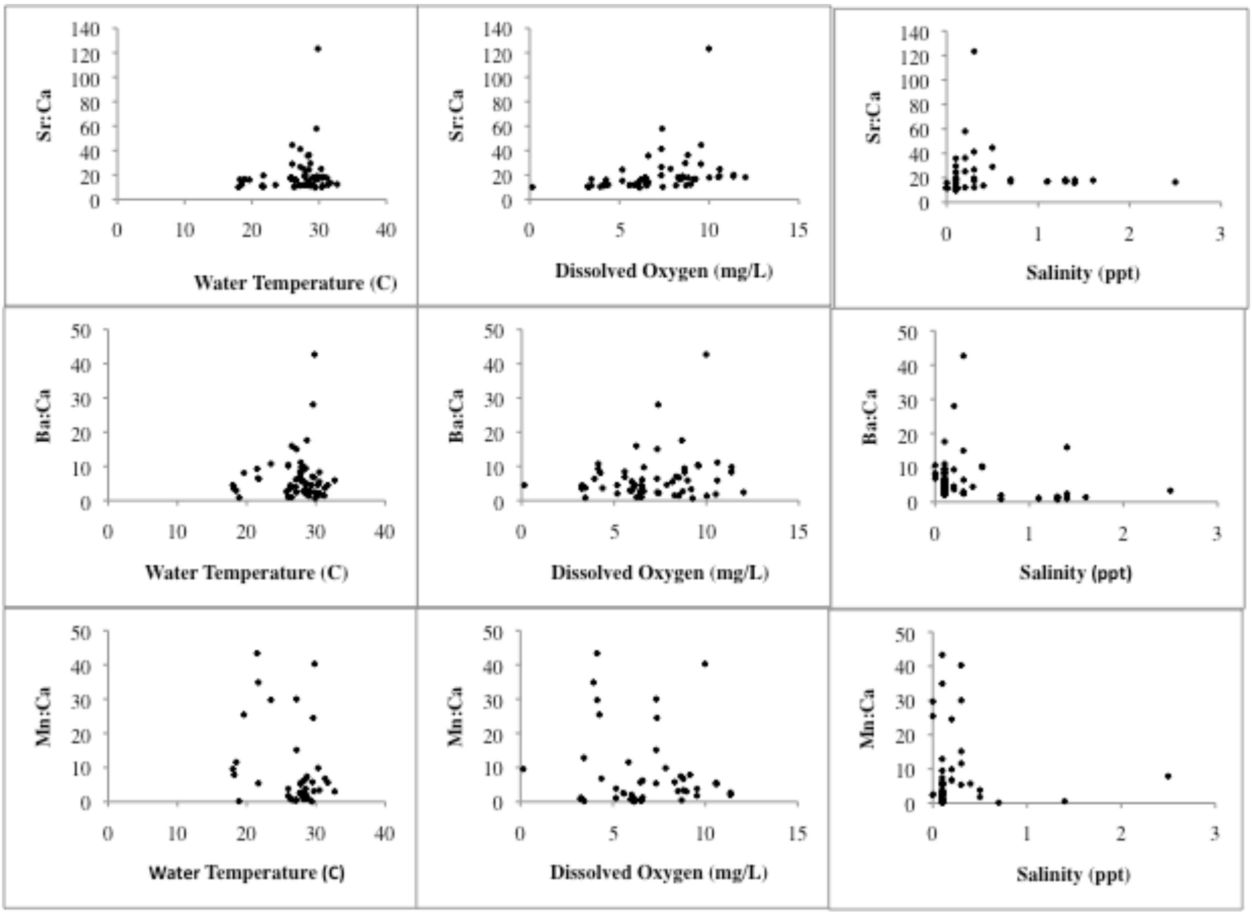

Figure 4. – Plots of relationships between water temperature (C), dissolved oxygen (mg/L) and salinity (ppt) and element:Ca. Water samples and environmental data collected in the Alligator, Chowan, Perquimans, Roanoke, and Scuppernong rivers from June-October 2010.

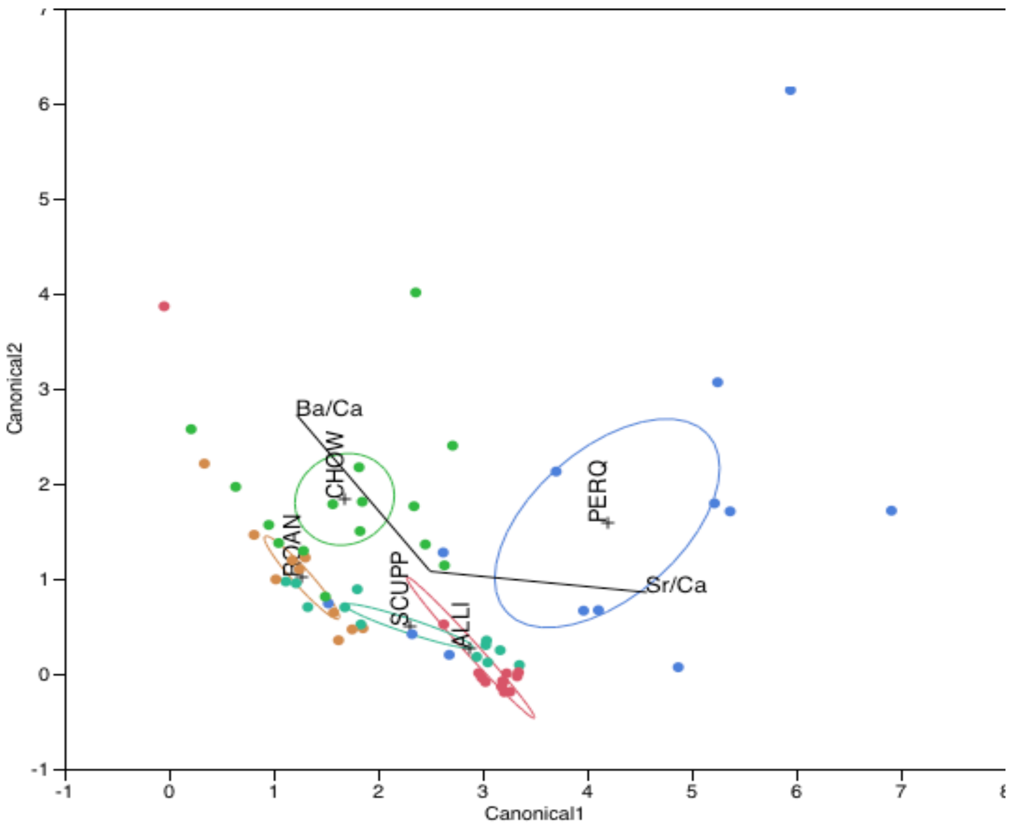

Figure 5. - Plot of first two canonical variates obtained using quadratic discriminant function analysis to classify water samples to collection rivers using Sr:Ca and Ba:Ca. Alligator (ALLI) = red, Chowan (CHOW) = green, Perquimans (PERQ) = blue, Roanoke (ROAN) = orange, Scuppernong (SCUPP) = aqua. Group centroids are marked with (+), ellipses represent 95% confidence ellipse for each location.

Chapter 3: Tracking Nursery Habitat Use of Juvenile River Herring in a Large Lagoonal Estuary

Abstract

River herring is a collective term used to describe two similar alosine species: alewife *Alosa pseudoharengus* and blueback herring *A. aestivalis*. Both of these anadromous species are native to the Atlantic coast of North America and spawn in North Carolina rivers. Consistent with populations along the east coast of North America, river herring populations in North Carolina have experienced drastic declines. Therefore, it is essential to identify nursery habitats used by these species. Juvenile river herring were collected from riverine and non-riverine Albemarle Sound habitats from June-October 2010. Total length, condition, and growth rates were measured to examine growth of fish from different habitats. Concentrations of Mg, Mn, Sr and Ba at the outer edge of otoliths were measured to determine habitat specific signatures that were used to classify river herring captured in non-riverine habitats to their river of origin. Total length, condition, and growth rates of alewife and blueback herring differed significantly between habitats. Concentrations of Mg, Mn, Sr, and Ba in otoliths differed significantly between rivers, allowing river herring to be classified to their river of capture with between 75-100% accuracy. Based on the growth metrics used, alewife nursery habitat was best in the Alligator, Chowan, Pasquotank, and Roanoke rivers along with non-riverine northwest and southwest sound habitats. Blueback herring nursery habitat was best in the non-riverine northwest and southwest sound. Riverine habitats provided poorer nursery habitat for blueback herring and the Scuppernong and Perquimans rivers provided poorer nursery habitat for alewife. However, alewife and blueback herring

seemed to move out of the Chowan and Perquimans rivers into western Albemarle Sound habitats suggesting they may seek out higher quality nursery areas. High quality river herring nursery habitats identified in this study correlate well with Strategic Habitat Areas (SHAs) designated by the state of North Carolina.

Introduction

Otolith microchemistry is a method that has been employed to investigate questions related to fisheries and ecology. Otoliths are paired calcareous structures formed from calcium carbonate, primarily in the form of aragonite, used by fish for balance and hearing. Despite being composed predominantly of calcium carbonate and protein matrix, approximately 31 trace elements have been detected in otoliths (Campana 1999). Experimental evidence suggests that strontium (Farrell and Campana 1996; Bath et al. 2000; Elsdon and Gillanders 2003a; Kraus and Secor 2004; Walther and Thorrold 2006; Mohan et al. 2012), barium (Bath et al. 2000; Elsdon and Gillanders 2003a; Elsdon and Gillanders 2004; Walther and Thorrold 2006; Miller 2009; Mohan et al. 2012), and manganese (Forrester 2005; Dorval et al. 2007; Mohan 2012) are incorporated into otoliths in ratios similar to concentrations in water, thus allowing the chemical composition of otoliths to be used as natural tags (Elsdon and Gillanders 2003b). Since otoliths are metabolically inert, the elements incorporated into the otolith reflect the environmental history of the fish from its time of hatch to time of death (Elsdon and Gillanders 2003b). For example, elemental concentrations at the outer edge of an otolith correspond to environmental conditions experienced by the fish just prior to capture, whereas elemental concentrations at the core of otoliths correspond to conditions experienced by the fish when it was hatched (Elsdon and Gillanders 2003b). This finding has led to the use of otolith microchemisty in reconstructing habitat use throughout the life of a fish. Analysis of elemental concentrations in otoliths has been used to infer early life habitat use of marine fish including Atlantic cod *Gadus morhua* (Campana et al. 1994; Campana et al. 2000), blue groper *Achoerodus viridis* (Gillanders and Kingsford 1996), weakfish *Cynoscion regalis* (Thorrold et al. 1998a; Thorrold et al. 2001) and freshwater fish including smallmouth bass *Micropterus dolomieu* (Humston et al. 2010) and yellow perch *Perca flavescens* (Brazner et al. 2004a; Brazner et al. 2004b).

The use of otolith elemental concentrations to discriminate between natal rivers of anadromous fish species is of particular interest because it provides information about early life habitat use and the stock to which a fish belongs (Thorrold et al. 1998a). Otolith microchemistry has been used to discriminate natal origins and early life habitat use of anadromous species including Atlantic salmon *Salmo salar* (Veinott and Porter 2005), chinook salmon *Oncorhynchus tshawytscha* (Barnett-Johnson et al. 2008), chum salmon *Oncorhynchus keta* (Sohn et al. 2005), striped bass (Morris et al. 2003) and American shad *A. sapidissima* (Thorrold et al. 1998a; Walther et al. 2008; Walther and Thorrold 2008). Using elemental concentrations in American shad otoliths, Thorrold et al. (1998a) were able to correctly classify juveniles to the Connecticut, Hudson, and Delaware rivers with nearly 90% accuracy, and suggested that natal rivers of adult fish could be determined by analyzing the juvenile portion of adult otoliths. Expanding on the work of Thorrold et al. (1998a), Walther et al. (2008) and Walther and Thorrold (2008) were able to classify juvenile American shad to natal rivers across the species natural range using a combination of elemental and isotopic ratios, and were then able to predict whether adult American shad returning to the York River, Virginia originated in the Mattaponi or Pamunkey River, both of which are tributaries of the York River (Walther et al. 2008).

River herring is a collective term used to classify two anadromous alosine species: alewife *A. pseudoharengus* and, blueback herring *A. aestivalis*. Both species are native to the east coast of North America, with blueback herring ranging from Nova Scotia to Florida, and alewife from Nova Scotia to South Carolina (Munroe 2002; Greene et al. 2009).

Despite similarities in life history and range, there is spatial and temporal differences in spawning behavior, with alewife spawning earlier along shore eddies or deep pools, and blueback herring spawning later in the main stem of rivers (Loesch and Lund 1977; Messieh 1977), and in rice paddies (Thomas et al. 1992) and impoundments in South Carolina (Meador et al. 1984). In North Carolina, river herring spawn in coastal rivers and Lake Mattamuskeet (Rulifson and Wall 2006) from approximately March through May in lotic and lentic habitats (Walsh et al. 2005), and from mid-April to mid-May in the Roanoke River (Harris and Hightower 2010). Further to the south in Lake Mattamuskeet, North Carolina, alewife spawn in April (Tyus 1974). Larval blueback herring utilize lotic and lentic habitats whereas alewife larvae remain in backwater areas (Walsh et al. 2005). Otolith microchemistry seems an appropriate method for investigations of river herring habitat use for many of the same reasons as American shad. River herring remain in natal rivers for extended periods before migrating to the ocean (Walton 1983; Limburg 1998; Kosa and Mather 2001), and there is a pressing need to understand stock structure and habitat use due to drastic declines in population throughout the species range (Schmidt et al. 2003).

Beck et al. (2001) suggested that a habitat is a nursery if its production of individuals that recruit to the adult population is greater than from other habitats in which juveniles occur. Nursery habitats must promote greater contribution to the adult population based on a combination of four criteria: higher density, growth, survival of juveniles, and movement to adult habitats (Beck et al. 2001). Western portions of Albemarle Sound, N.C., and its tributaries have been identified as nursery habitat for river herring (Copeland et al 1983). However, these designations are based on presence of juvenile alewife or blueback herring. There has been little work done regarding which habitats may provide better nursery habitat based on growth, or connectivity between nursery habitats. The purpose of my study was to examine Albemarle Sound habitats that may function as important nursery areas for river herring by 1) collecting juvenile river herring from tributaries and open sound habitats, 2) examining growth, and 3) using elemental fingerprints in otoliths to examine connectivity between habitats.

Methods

Site Description

The Albemarle Sound, in northeastern North Carolina, is the drowned portion of the Roanoke River and its floodplain, extending approximately 90 km eastward from the mouth of the Roanoke River to Kitty Hawk Bay and Colington Island (Copeland et al. 1983). The Albemarle Sound is a shallow oligohaline system with salinities ranging from 0-5 ppt (Copeland et al. 1983). The sound has no direct connection to the ocean but seawater intrusion does occur through Oregon Inlet, Croatan and Roanoke sounds (Copeland et al. 1983; Riggs 1996). The system is well mixed due to nearly constant wind action allowing only temporary stratification due to salinity and temperature (Riggs 1996). There are eight major tributaries including the Chowan, Perquimans, Little, Pasquotank, North, Scuppernong, Yeopim, and Alligator rivers (Chapter 1, Figure 1).

Fish Collection and Otolith Removal

The goal was to collect five juvenile alewife and five juvenile blueback herring per month from June-October 2010 in the Alligator, Chowan, Little, North, Pasquotank Perquimans, Scuppernong, and Yeopim rivers in order to capture any monthly variation in elemental signatures. These fish were provided by the North Carolina Division of Marine Fisheries (NCDMF) bottom trawl and beach seine surveys. River herring from the Roanoke River were captured by independent seine collections in July and August 2010. In addition to river herring from tributaries, the NCDMF also provided fish from five locations (three along the northwest shore and two along the southwest shore) in western Albemarle Sound (Chapter 1, Figure 1). Fish from the three northwest shore locations were pooled, and fish from the two southwest shore locations were pooled. All fish were frozen until they could be processed.

Fish were identified to species (alewife or blueback), measured for total length (mm) and weighed (g), and saggital otoliths were removed. Fulton's condition factor (K) was calculated using the formula:

$$K = (\text{weight}/\text{total length}^3) * 100{,}000.$$

Upon removal, otoliths were placed on a glass slide in a drop of distilled water to remove tissue and allowed to dry for ~24 hours in a fume hood. Otolith pairs were then transferred to 1.5-ml microcentrifuge polypropylene vials for storage until elemental analysis could be performed. The left otolith was used for microchemical analysis and the right was used for ageing.

Otoliths used for ageing were mounted with super glue (Gorilla^{TM}) on a glass slide, sulcus down. Otoliths were then ground to the midplane using a series of 9-µm, 3-µm, and 0.3-µm alumina lapping films (Pace Technologies). Age estimation followed similar methods to those used by Walsh et al. (2005). Age was estimated by performing at least two increment counts. If the first two counts differed by less than five increments an average was taken to calculate a final age. If the first two counts differed by more than five increments then a third count was made; if this count was less than five increments from one of the first two counts, those counts were used to calculate age. Sismour (1994) (as reviewed by Walsh et al. 2005) determined that increment formation begins two days post hatch, therefore two days were added to each count to calculate a final age. Growth rate was then calculated using the formula:

Growth rate (mm/day) = Total Length (mm)/Age (days).

Otolith Preparation and Analysis

Microchemical analysis was performed using laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS) as described by Halden and Friedrich (2008) and Mohan et al. (2012). Otoliths were embedded in epoxy resin (Buehler Epoxicure ®) and ground to the core in a dorso-ventral transverse section using 320, 400, and 600- grit wet sandpaper and ultrasonically cleaned for 2 minutes. Scratches on the surface of the otolith were removed by polishing with Buehler diamond polishing suspensions (9-µm and 0.05-µm) on a polishing wheel to create a completely smooth surface for laser ablation. Polished, mounted otoliths were then cleaned again with ultrapure water and digitally photographed.

Elements were quantified using a Thermo-Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Operating parameters for LA-ICP-MS included: 15-µm beam size; 2 μms^{-1} scan speed; repetition rate 20 Hz; and 75% power, using low resolution (R = 300) mode. The isotopes counted were ^{44}Ca, ^{25}Mg, ^{88}Sr, ^{138}Ba, ^{55}Mn, ^{63}Cu, ^{66}Zn, and ^{208}Pb. Calcium (as 56 wt. % CaO) was used as the internal standard. NIST 610 glass was used for external calibration and to monitor any instrument drift. Laser scans were across the entire width of the otolith. Isotope counts were converted to ppm and plotted versus laser distance.

^{25}Mg, ^{88}Sr, ^{138}Ba, and ^{55}Mn were the only elements consistently found above limits of detection and thus were the only elements used in analyses. Because juvenile river herring can move between Albemarle Sound locations, elemental concentrations at the outer edge of the otolith were used to develop river specific elemental signatures. The outer edge of the otolith represents recent growth, which was assumed to have occurred in the river the fish was captured. Elemental concentrations in the outer 35-µm of one side of the otolith transect were averaged to obtain mean values for each fish. Based upon otolith width and number of daily increments a 35-µm section of otolith typically represented approximately 10 days of the fishes life A 35-µm section of otolith located just beyond the core was used to predict river of origin of river herring captured in non-riverine Albemarle Sound habitats. This was done because preliminary investigations of line scan data revealed Mg spikes at the core of the otolith inconsistent with Mg values at the outer edge, and in water samples (Zapf 2012, Chapter 2). It was hypothesized that these spikes may be due to maternal input of Mg, thus decoupling the value of Mg in the otolith core from that in the water.

Statistical Analysis

Physical Characteristics of Fish

Kruskal-Wallis tests were used to examine differences in total length, weight, and Fulton's condition factor between capture locations. One-way ANOVA was used to examine differences in growth rate between capture locations. When significant differences were detected Tukey's HSD was used to examine which locations differed significantly.

Elemental Data

Elemental concentration data were split into two groups: fish caught in riverine habitats and fish caught in non-riverine habitats. In rivers and months in which both blueback herring and alewife were collected, Welch's t-tests were used to compare elemental concentrations at the outer edge of otoliths to examine if elements are incorporated into otoliths of the two species in similar proportions.

One-way ANOVA was used to test for differences in Mg, Mn, Sr, and Ba in the otoliths of fish caught in the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers. Tukey's HSD was used to examine which locations differed significantly. Quadratic discriminant function analysis (QDFA) was used to assess how elemental concentrations can be used to classify fish to river of capture and Pillai's trace statistic was used to assess differences in multivariate means between rivers (JMP ® 2007). Multivariate means were used classify fish of unknown origin, captured in non-riverine Albemarle Sound habitats, using group centroids and Mahalanobis distance, which is the distance from a point to the multivariate mean (JMP ® 2007). Based on Mahalanobis distance, probabilities of belonging to each group (river) were calculated, and the fish was classified to the group (river) with the highest probability (JMP ® 2007).

Results

Elemental Analysis

Sufficient numbers of alewife and blueback herring needed to perform statistical analysis comparing elemental uptake between the two species were captured in the Chowan River in July and August, the Perquimans River in June, and the Yeopim River in July. No significant differences ($\alpha = 0.05$) were detected between elemental concentrations in alewife and blueback herring otoliths on these capture occasions (Figure 1). Because no statistically significant differences were detected and any differences were not thought to significantly alter the multivariate mean, alewife and blueback herring were pooled for elemental analyses.

Concentrations of Mg ($F_{(8.113)} = 8.96$, $p < 0.0001$), Mn ($F_{(8.113)} = 9.61$, $p < 0.0001$), Sr ($F_{(8,113)} = 24.36$, $p < 0.0001$), and Ba ($F_{(8,113)} = 20.28$, $p < 0.0001$) differed significantly in otoliths collected from the nine rivers included in analysis. Mg concentrations were highest in otoliths from the Little and Scuppernong rivers and lowest in Alligator River otoliths (Figure 2). Mn concentrations were highest in otoliths from the Scuppernong River, and lowest in otoliths from the Alligator River (Figure 2). Sr concentrations were highest in otoliths from the Yeopim River, and lowest in otoliths from the Chowan River (Figure 2). Ba concentrations were highest in otoliths from the Yeopim River, and lowest in otoliths from the North and Pasquotank rivers (Figure 2).

Using QDFA, 86% of the 122 fish were correctly classified to their rivers of capture (Table 1). Multivariate means differed significantly between locations (Pillai's trace statistic: $F = 13.70$, $df = 32, 452$, $P < 0.0001$) although there was overlap of 95% confidence intervals between some rivers (Figure 3). Fish were classified with 100% accuracy to the Little, North, Pasquotank, Roanoke, Scuppernong and Yeopim rivers (Table 1). Overall classification accuracy was decreased due to lower classification success to the Alligator, Chowan, and Perquimans rivers. Misclassifications were most common to locations with similar group centroids (Figure 3). One fish captured in the Alligator River was classified to the Chowan River and one was classified to the Little River (Table 1). Seven fish captured in the Chowan River were classified to the neighboring Roanoke River, and one to the Perquimans River (Table 1). Five fish from the Perquimans River were classified to the Alligator (2), Little (1), North (1), Pasquotank (1), and Yeopim (2) rivers (Table 6). Ba was the most important element in classifying fish to the Chowan and Roanoke rivers (Figure 3). Sr was important in classifying fish to the Alligator, Little, North and Pasquotank rivers (Figure 3). Sr and Mg were important in classifying fish to the Perquimans and Yeopim rivers, and Ba and Mn were important in classifying fish to the Scuppernong River (Figure 3).

Alewife

Collection

A total of 535 alewife were used for this study (Table 2). Alewife were captured in nine riverine and two non-riverine locations including the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers and the northwest and southwest sound. The greatest numbers of alewife were captured in the southwest sound (n = 158), Perquimans River (n = 91), and the Chowan River (n = 86). The fewest alewife were captured in the North River (n = 9), Pasquotank River (n = 6) and Roanoke River (n = 10) (Table 2). Alewife were captured in four consecutive months (June-September) in the Chowan River, northwest sound, Perquimans River, and southwest sound. At the seven other locations alewife were captured less frequently. Alewife were only captured in July in the Roanoke and Yeopim rivers. The majority of alewife from the Scuppernong River was captured in June, with only one fish being captured in July and August (Table 3).

Physical Characteristics

Total length of alewife increased throughout the summer at every location (Table 3). Results of Kruskal-Wallis tests revealed significant differences between locations and months (Table 4). Alligator River alewife had the greatest mean total length, Scuppernong River alewife had the lowest mean total length (Figure 4), but the majority (42 of 44) of the Scuppernong River fish were caught in June potentially biasing the sample. Examining monthly variation in total length, alewife caught in the Perquimans

River were consistently smaller than alewife caught in other locations during the same period (Table 3).

In general, the condition factors of alewife in the various watersheds increased throughout the summer (Table 3), with the exception of alewife from the Alligator River, northwest sound, and Yeopim River (Table 3). Mean condition factor of alewife was highest in the Yeopim River and lowest in the North and Scuppernong Rivers (Figure 5).

Mean growth rate of alewife was highest (0.63 mm/day) in the Pasquotank and Yeopim rivers, and lowest (0.51 mm/day) in the Perquimans River (Table 5). Significant differences in growth rate of alewife was observed between locations ($F_{(9.104)} = 3.92$, p 0.0003). Tukey's HSD test showed that mean growth rates of alewife in the Alligator, Chowan, southwest sound, and Yeopim were significantly higher than growth rates of alewife from the Perquimans River (Figure 6).

Classification of Alewife from Non-riverine Habitats

Discriminant scores obtained from classifying juvenile river herring to capture locations were used to predict the river of origin of alewife captured in northwest and southwest sound habitats. In the northwest sound alewife originated from five watersheds: the Chowan, Little, North, Pasquotank and Perquimans rivers (Table 6). All of these watersheds are located along the north shore of the Albemarle Sound (Chapter1, Figure 1). Alewife captured in the southwest sound in June were predicted to have originated from the Perquimans and Roanoke rivers (Table 6). Alewife captured in the southwest sound in July were predicted to have originated from the Chowan, North, Pasquotank, and Perquimans rivers (Table 6). Alewife captured in the southwest sound in

August were predicted to originate from the Alligator, Chowan, Pasquotank, Perquimans, and Scuppernong rivers (Table 6); this was the only month in which alewife from south shore watersheds were captured in western sound habitats.

Blueback herring

Collection

A total of 509 blueback herring were collected from seven watersheds and two regions: the Alligator, Chowan, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers, and the northwest and southwest sound (Table 2). The greatest numbers of blueback herring were captured in the Chowan River, northwest sound, and southwest sound but large numbers were also captured in the Perquimans and Yeopim rivers (Table 2). The Perquimans River was the only river were blueback herring were captured during the entire study (June-October) (Table 7). Single blueback herring were captured in the Alligator River during June and August, and a single fish was captured in the Scuppernong River during August. Of the three blueback herring captured in the Roanoke River two were collected in July and one in August (Table 7).

Physical Characteristics

Total length of blueback herring increased throughout the summer at every location (Table 7). Results of Kruskal-Wallis tests revealed significant differences in total length between locations and months for blueback herring (Table 4). Mean total length was longest in the southwest sound, northwest sound and Roanoke River, and shortest in the Yeopim River (Figure 4).

Monthly variation in blueback herring total length was difficult to assess because they were not caught consistently from month to month. However, July caught blueback herring were smallest in the Yeopim River and longest in the Roanoke River. August caught fish were smallest in the Pasquotank River and longest in the southwest sound (Table 7).

In general, condition factor decreased throughout the summer (Table 7). Only in the Pasquotank River fish was there an increase in condition factor between summer and fall caught fish (Table 7). Highest condition blueback herring were captured in the Yeopim River and lowest condition blueback herring were captured in the northwest sound, Pasquotank River, and Perquimans River (Figure 5).

Overall, mean growth rate of blueback herring was faster than alewife, growing about 0.97 mm/day throughout the region. Growth was highest in the northwest and southwest sound, and lowest in the Perquimans River (Table 5). These differences were small but significantly different ($F_{(5.34)} = 10.81$, $p < 0.0001$) (Figure 6).

Classification of Blueback Herring from Non-riverine Habitats

Discriminant scores obtained from classifying juvenile river herring to capture locations were used to predict the river of origin of blueback herring captured in northwest and southwest sound habitats. In August, fish were predicted to originate primarily from the Chowan (45%), and Perquimans (27%) rivers, with minor contributions from the Roanoke (18%) and Pasquotank (9%) rivers (Table 6). In October 80% of blueback herring captured in the northwest sound were from the Chowan River, with the Perquimans River (10%) and Roanoke River (10%) providing minor contributions (Table 6). In the southwest sound, fish originated primarily from the Chowan (80%) and Roanoke rivers (20%). By September southwest sound fish were still primarily from the Chowan (70%) but fish from the Pasquotank, Perquimans, and Scuppernong rivers were also present (Table 6).

Discussion

Elemental Concentrations and Classification Accuracy

There were no statistically significant differences between the concentrations of Mg, Mn, Sr, and Ba at the outer edge of otoliths from alewife and blueback herring captured in the Chowan River in July, the Chowan River in August, the Perquimans River in June and the Yeopim River in July. These are the only months and locations where alewife and blueback herring were captured together and therefore were the only comparisons possible. Gahagan (2010) found significant differences in Sr:Ca, but not Ba:Ca between otoliths of alewife and blueback herring captured in Connecticut rivers. However, Gahagan (2010) conducted whole otolith analysis of Sr:Ca rather than the most recent growth, so differences in Sr:Ca may have occurred due to differences in lifetime habitat use. In general, there was no clear pattern of differences in elemental concentrations between alewife and blueback herring, and concentrations of most elements were similar, so it seemed reasonable to pool alewife and blueback herring otoliths when analyzing elemental concentrations between locations.

Statistically significant differences in the concentrations of Mg, Mn, Sr and Ba at the outer edge of otoliths were found between rivers. Generally, rivers with similar geographic locations had similar elemental concentrations. Magnesium was similar across rivers with the exception of the Scuppernong and Little rivers, which had elevated Mg concentrations.

Mn varied between rivers with highest concentrations of Mn found in otoliths of river herring from the Chowan, Little, North and Scuppernong rivers. The Alligator and Pasquotank had decreased Mn concentrations. Dissolved Mn has been related to reducing conditions in sediments during anoxic conditions (Brewer and Spencer 1971; Sundby et al. 1986; Laslett 1995) and Mn concentrations in otoliths have been used to infer anoxic conditions in the Neuse River (Thorrold and Shuttleworth 2000); the Baltic Sea (Limburg et al. 2011), and Albemarle Sound (Mohan et al 2012). It is possible that increased Mn in the otoliths of river herring from Chowan, Little, North and Scuppernong rivers is the result of anoxic conditions in these rivers.

Sr has been shown to follow a salinity gradient with higher concentrations in saltwater than freshwater (Odom 1951; Rosenthal et al. 1970; Ingram and Sloan 1992), although there are exceptions (Limburg and Siegel 2006; Brown and Severin 2009). Salinity in Albemarle Sound is generally very low and can vary based on wind patterns and rainfall. The eastern Albemarle Sound generally has higher salinity than the western sound due to proximity to the Atlantic Ocean (Copeland et al. 1983; Mohan et al. 2012). Based on salinity, Sr in river herring otoliths had a somewhat unexpected pattern. While river herring captured in the easternmost Alligator River had high Sr concentrations, river herring from the other eastern rivers -- the North and Pasquotank rivers -- had Sr concentrations that were not significantly different than western rivers. This is probably because despite being closer to the ocean these rivers are primarily freshwater with little saltwater intrusion. The exceptions are the Perquimans and Yeopim rivers, which had extremely high Sr concentrations far exceeding that of any other river. This is interesting because the Perquimans and Yeopim rivers are located more towards the western portion of the sound and would be thought to be less influenced by seawater. This suggests there may be some other source of Sr in these rivers. While this finding is unexpected, studies of elemental concentrations in the otoliths of yellow perch from Lake Superior show Sr can vary between locations in an entirely freshwater habitat (Brazner et al. 2004a; Brazner et al. 2004b). In addition, studies of elemental concentrations in the otoliths of smallmouth bass show Sr can differ between the main stem and tributaries of freshwater portions of the James River, Virginia (Humston et al. 2010).

Unlike Sr, Ba has been shown to have a negative relationship with salinity (Coffey et al. 1997; Guay and Falkner 1998). Concentrations of Ba in otoliths somewhat followed the expected pattern based on salinity, with fish from western most rivers -- the Chowan, Roanoke and Scuppernong rivers -- having slightly elevated otolith Ba concentrations compared to those from eastern rivers. However, concentrations of Ba from river herring captured in the Yeopim and Perquimans rivers were extremely high compared to other rivers. High Ba concentrations in these rivers was not unexpected because these rivers are located in the western portion of the sound, but when comparing these values to other western rivers the concentrations seem high. It should be noted that the Yeopim and Perquimans rivers also had very high Sr concentrations. De Vries et al. (2005) conducted laboratory studies on black bream *Acanthopagrus butcheri* and found that Sr facilitated the uptake of otolith Ba in black bream raised in brackish water;

perhaps this mechanism is responsible for the high Sr and Ba concentrations in the otoliths of river herring captured in the Yeopim and Perquimans rivers.

Results obtained in this study were similar to those of Mohan et al. (2012), who examined concentrations of Mg, Mn, Sr and Ba at the outer edge of otoliths from striped bass reared in cages in Batchelor Bay, the Perquimans River, the Pasquotank River, and the Alligator River. No significant differences were found in striped bass otolith Mg concentrations between these locations (Mohan et al. 2012), results similar to that of this study. This study also found decreased otolith Mn in Alligator River and Pasquotank River fish compared to fish from the Perquimans River. Mohan et al. (2012) found decreased otolith Mn in Alligator, Pasquotank and Batchelor Bay striped bass compared to Perquimans River striped bass. Batchelor Bay is located at the mouth of the Chowan and Roanoke rivers, so while they are in similar geographic locations they are not necessarily interchangeable. This study also found similar results with Sr and Ba as Mohan et al. (2012).

Differences in otolith elemental concentrations allowed for relatively high classification of juvenile river herring to their rivers of capture. Interestingly, positions in canonical space seemed to be influenced by both longitudinal and latitudinal location of rivers. The western most rivers -- the Chowan and Roanoke -- are located adjacent to each other and had similar multi-variate means. The Scuppernong River, located on the south shore toward the central part of the sound, does not closely neighbor other rivers and there was very little classification overlap with other rivers. The Yeopim and Perquimans rivers are located furthest west of the rivers located on the north shore of the sound. These two rivers had similar multi-elemental means with some classification overlap between the rivers. Classification to these rivers was influenced by the high Sr and Ba concentrations in these rivers. The Little River, on the north shore, is located between the Perquimans and Pasquotank rivers. However, the multi-elemental mean from the Little River was distinct from both the Perquimans and Pasquotank rivers. The Pasquotank and North rivers, located the furthest east on the north shore, had similar multi-elemental means, and both had similar means to the Alligator River, which is located the furthest east on the south shore.

High classification was not unexpected, as other studies have found differences in elemental concentrations over small geographic areas, and have had high classification success using elemental concentrations. Thorrold et al. (1998a) found significant differences in the concentrations of Mg, Mn, Sr and Ba in the otoliths of weakfish caught at different locations within Doboy Sound, Pamlico Sound, Chesapeake Bay, Delaware Bay, and Peconic Bay. Thorrold et al. (1998b) found significant differences in the concentrations of Mg, K, Mn, Sr, and Ba in the otoliths of American shad caught at different locations within the Connecticut, Hudson and Delaware rivers. Brazner et al. (2004) were able to classify yellow perch to western Lake Superior wetlands with an average of 76% accuracy using multi-elemental signatures. Humston et al. (2010) were able to classify smallmouth bass to the James River, and a tributary (the Maury River) with approximately 87% accuracy using a Sr:Ca, Rb:Ca, Mg:Ca, and Ba:Ca ratios. In addition, Mohan et al. (2012) were able to classify cage-reared striped bass to Albemarle Sound habitats with 59-63% accuracy using concentrations of Mg, Mn, Sr, and Ba at the edge of otoliths. Using only Sr:Ca and Ba:Ca ratios, Gahagan (2010) was able to classify juvenile alewife to tributaries of the Connecticut River with 50-100% accuracy and blueback herring with 20-57% accuracy. Differences in Albemarle Sound elemental concentrations could arise from differences in sediment composition (Riggs 1996), and geological characteristics (Copeland et al. 1983) between watersheds and differing anthropogenic uses of watershed areas (Copeland et al. 1983).

Alewife Nursery Habitat

While the goal of this study was not to quantify abundance or catch per unit effort, the sample does represent a fairly complete record of NCDMF summer river herring sampling. Therefore, variation in the number of river herring captured at each location provides some insight as to when river herring were present in each habitat. Alewife were captured at all 11 habitats considered in this study. However, low numbers of alewife were collected from North, Pasquotank, and Roanoke rivers. This does not necessarily mean that river herring were not abundant in these locations, just that they were not captured as frequently.

Significant differences were found in total length, weight, condition, and growth rate of alewife between habitats. Examining growth rates of larval alewife in the Roanoke River Walsh et al. (2005) calculated growth rates of 0.65 mm/day in 1996 and 0.41 mm/day in 1997, values not considerably different from growth rates calculated in this study. In addition, growth rates from this study were not considerably different from results obtained by Iafrate and Oliveira (2008) studying alewife in the Herring River, Massachusetts. Total length of alewife from this study was not considerably different from results obtained by Grabe (1996) studying alewife in the Hudson River, Yako et al. (2002) studying alewife from streams in Massachusetts or Iafrate and Oliveira (2008).

However, total length of alewife from this study were somewhat greater on average than results found by Gahagan et al. (2010) studying alewife in Bride Lake, Connecticut. Condition of alewife from this study was considerably higher on average than results obtained by Iafrate and Oliveira (2008).

Using the growth metrics of total length, growth rate, and condition to assess nursery habitat for alewife in Albemarle Sound, the Alligator, Chowan, Pasquotank, and Roanoke rivers along with northwest and southwest sound habitats should be considered high quality nursery habitat. Alewife utilizing these habitats had longer total lengths, better condition, and better growth rates suggesting faster or increased growth.

Based on the predicted origins of alewife captured in the north and southwest sounds, no single source appeared to contribute higher percentages of fish. Low percentages of alewife captured in the northwest and southwest sound locations originated from rivers that promote higher growth. One possible explanation for this is that alewife in these rivers may not leave if the habitat is suitable. Steady catches of alewife throughout the summer in the Chowan River suggests that this may be a possibility. Yako et al. (2002) related emigration of juvenile alewife to declines in *Bosmina* spp. Density suggesting that alewife may leave a location if food is insufficient. Leech et al. (2009) concluded that zooplankton biomass in the Chowan River is sufficient to support river herring forage providing support for the Chowan River being a quality nursery habitat for alewife. However, Leech et al. (2009) also concluded that degraded water quality, including low dissolved oxygen, may pose a threat to the Chowan River as alewife nursery habitat. A number of researchers have linked emigration of alewife to environmental factors including water temperature, flow, and precipitation events

(Richkus 1975; Yako et al. 2002; Iafrate and Oliveira 2008; Gahagan et al. 2010). Therefore, changes in any of these parameters may cause alewife to move from their current habitat. If there are no drastic changes in environmental conditions alewife may remain in one location if conditions are favorable. Alewife from the Alligator and Pasquotank rivers may be under-represented in the sample because alewife leaving these rivers may never migrate into western portions of Albemarle Sound.

Alewife captured in the Perquimans and Scuppernong rivers had smaller total lengths, and lower condition and growth rates indicating that some aspect of these habitats does not promote growth of juvenile alewife. However, these metrics can be somewhat misleading. Juvenile alewife captured in the Perquimans River had what seemed to be low growth potential while alewife captured in northwest and southwest sound habitats had higher total length, condition and growth rates. However, examining the predicted origins of these alewife reveals that 20% of the alewife (n = 2) captured in the northwest sound in August originated from the Perquimans River. In the southwest sound 80% (n = 4) of alewife captured in June, 30% (n = 3) of alewife captured in July, and 20% (n = 1) of alewife captured in August originated from the Perquimans River. This indicates that while alewife utilizing the Perquimans River may have lower growth, at least a portion of juvenile Perquimans River alewife moved to non-riverine habitats that may offer a growth advantage, as alewife in the northwest and southwest sound had higher growth rates than alewife from the Perquimans River. Rulifson et al. (2009a) noted the presence of many confined animal feeding operations (CAFOs) within the Perquimans River watershed, and suggested CAFOs may contribute to degraded water quality within the Perquimans River. In addition, striped bass reared in cages within the

Perquimans River had slower growth rates compared to striped bass reared in cages within the Alligator River, Pasquotank River, and Batchelor Bay (Rulifson et al. 2009a). The same habitat effects that cause slow growth in juvenile Perquimans River striped bass may be acting on juvenile alewife in the river as well.

Information on natal origins of juvenile alewife indicates that they may move long distances in search of suitable habitat, as a large portion of the alewife caught in the southwest sound are predicted to originate from the Perquimans River, even though the Perquimans River is on the north shore of the sound. A similar pattern holds true for alewife from the North River. Although alewife captured in this location had what seemed to be lower growth, a portion of alewife captured in the north and southwest sound were predicted to have originated from the North River (30% from northwest sound in August, 20% from southwest sound in August). This not only suggests that alewife from the North River may move to find more suitable habitat, but they may move long distances, as the North River is located in the easternmost portions of Albemarle Sound. Long distance upstream movement by alewife is not entirely surprising as Burbridge et al. (1974) noted blueback herring in the James River, Virginia moving upstream, and suggested these fish may move upstream due to higher zooplankton abundances upstream than downstream. In addition, Massmann (1963) suggested American shad may move upstream in the Mattaponi and Pamunkey rivers, Virginia, potentially in search of better habitats.

A total of 44 alewife were captured in the Scuppernong River from June-August, with 42 of those alewife being captured in June. These fish had low total lengths, and low growth rates. Similar to the Perquimans River, this suggests that alewife utilizing the

Scuppernong River have lower growth than fish from other habitats. However, unlike alewife from the Perquimans, alewife from the Scuppernong River do not seem to move to habitats that offer higher growth potential. Only 20% (n = 1) of alewife captured in August, and 10% (n = 1) of alewife captured in September in the southwest sound were classified as originating from the Scuppernong River. No alewife captured in the northwest sound were predicted to have originated from the Scuppernong River.

There are three hypotheses as to why juvenile alewife from the Scuppernong River are not moving into habitats that may promote higher growth: 1) alewife are not spawning in the Scuppernong River, 2) juvenile alewife may not survive to move into non-riverine Albemarle Sound habitats, and 3) juvenile alewife leaving the Scuppernong River move east, not west. Hypothesis 1 seems unlikely due to the small size of the alewife captured in the Scuppernong River in June, suggesting they may not have had sufficient time to move from their river of origin, and spawning condition adult alewife are captured in the Scuppernong River. Hypothesis 2 seems reasonable since only two alewife were captured in the Scuppernong River after June suggesting large numbers of alewife were not present in this habitat after June, and they do not appear to have moved to other habitats. Hypothesis 3 is certainly possible, since all southwestern sample locations were west of the mouth of the Scuppernong River and juvenile alewife would have to swim east to get to the ocean. However, juvenile river herring do not always migrate directly to the ocean and juvenile blueback herring have been shown to migrate upstream (Burbidge 1974). Juvenile alewife captured in central and eastern sound habitats were not analyzed for this study, but this is partly because high numbers of alewife were not captured in these areas, suggesting that very few alewife regardless of origin were using these habitats other than to move to the ocean. This suggests that juvenile alewife are not transitioning from the Scuppernong River to non-riverine Albemarle Sound habitats, which is a necessary transition prior to ocean emigration. Data from this study suggest that juvenile alewife utilizing the Scuppernong River have reduced growth compared to other Albemarle Sound habitats, and may have reduced survival implying the Scuppernong River offers poor quality alewife nursery habitat.

Similar to the Perquimans River, Rulifson et al. (2009a) noted the presence of CAFOs within the Scuppernong River watershed. CAFOs may contribute to poor water quality and poor growth of alewife within the Scuppernong River. In addition, juvenile alewife in the Scuppernong River had significantly higher Mn in their otoliths compared to otoliths of fish from other rivers. Otolith Mn has been used to track hypoxic conditions in the Neuse River (Thorrold and Shuttleworth 2000); the Baltic Sea (Limburg et al. 2011), and Albemarle Sound (Mohan et al. 2012). It is possible that high Mn concentrations in the otoliths of juvenile alewife is the result of hypoxic events within the river. If this is the case hypoxia may contribute to low growth and apparent poor survival of fish in the Scuppernong River. In addition, Rulifson et al. (2009b) noted an historical river herring spawning area, Lake Phelps, within the Scuppernong River watershed may be inaccessible due to water level fluctuations and impediments. This could at least partially explain low catches of juvenile alewife in the Scuppernong River.

Bluback Herring Nursery Habitat

Walsh et al. (2005) calculated growth rates for larval blueback herring of 0.60 mm/day in 1996 and 0.42 mm/day in 1997. These results are considerably lower than growth rates found in my study. Walsh et al. (2005) sampled larval blueback herring while we sampled juvenile blueback herring, indicating analysis of different life stages may cause growth rate calculations to vary. Growth rates of blueback herring from this study are considerably higher than growth rates reported by Iafrate and Oliveira (2008), suggesting possible growth differences between northern and southern locations. Total lengths of blueback herring measured in this study were not considerably different than those reported by Grabe (1996) or Iafrate and Oliveira (2008) but are slightly lower than those reported by O'Leary and Kynard (1986) and Yako et al. (2002). In addition, condition of blueback herring from this study was slightly higher than condition reported by Iafrate and Oliveira (2008), again suggesting possible differences in growth between northern and southern locations.

Non-riverine Albemarle Sound habitats seem to offer some growth advantage for blueback herring compared to riverine Albemarle Sound habitats. While blueback herring captured in the Alligator and Roanoke rivers did have high mean total lengths these locations had low sample sizes. Although blueback herring captured in the north and southwest sounds have somewhat low mean condition compared to other locations, it should be noted that at no location was condition particularly high and was not considered strongly in designation of high quality nursery areas. Growth rates and total lengths of blueback herring were highest in the north and southwest sounds suggesting these areas offer high quality nursery habitat for blueback herring. Growth rates of blueback herring in the Perquimans River were significantly lower than other locations. Similar to alewife, blueback herring captured in the Perquimans River seem to have decreased growth compared to blueback herring captured at other locations. The pattern of alewife moving from the Perquimans River to non-riverine habitats holds true for blueback herring as well.

Unlike alewife, there is a fairly clear distinction between growth of blueback herring captured in non-riverine habitats compared to riverine habitats. Large numbers of blueback herring seem to move out of riverine habitats that promote low growth, and into non-riverine habitats that promote higher growth. Studying spawning and nursery habitat of blueback herring in the Rappahannock River, Virginia, O'Connell and Angermeier (1997) concluded that headwaters provide spawning and nursery habitat for river herring but also noted that small streams and headwaters are more likely to be affected by land use, and impediments (O'Connell and Angermeier 1997). Examining water quality and hatching success of blueback herring eggs in the Chowan River, Waters and Hightower (1997) found increased hatching success in main-stem sites compared to tributary locations, and found tributaries had lower dissolved oxygen and higher nutrient concentrations than main-stem sites. Declines in dissolved oxygen in tributaries of Albemarle Sound may lead to poor growth in these habitats, which in turn may cause blueback herring to seek better conditions in non-riverine habitats. Dissolved oxygen in tributaries of Albemarle Sound declined from Septermber to October (Zapf 2012, Chapter 2) potentially leading to blueback herring leaving these habitats. In addition, pH in tributaries of Albemarle Sound generally declined from June-October (Zapf 2012, Chapter 2), and mortality of blueback herring has been shown to increase with declining pH (pH 5.0-7.8) (Klauda et al. 1987). Though pH during this study never fell below what is considered the lethal limit for blueback herring, decreasing pH could lead to blueback herring leaving habitats.

A large portion of blueback herring captured in non-riverine western Albemarle Sound habitats originated from the Chowan River, suggesting the Chowan River also functions as essential nursery habitat, although blueback herring may not remain in the Chowan River long. Of the 509 blueback herring examined for this study, 111 were from the Chowan River, which is by far the most from any riverine location. However, all of these fish were captured in July (n = 70), and August (n = 41). Dissolved oxygen and pH in the Chowan River declined significantly from August through October, indicating the absence of juvenile blueback herring in the Chowan after August may be the result of decreasing environmental factors.

Conclusions

Concentrations of Mg, Mn, Sr and Ba in the otoliths of river herring varied significantly between rivers allowing for high classification of alewife and blueback herring to their river of capture. This allowed river herring captured in non-riverine western Albemarle Sound habitats to be classified to their river of origin.

Quality nursery habitat for alewife is found in the Alligator, Chowan, Pasquotank, and Roanoke rivers along with non-riverine northwest and southwest Albemarle Sound based on total length, condition, and growth rate of alewife captured in these habitats. Degraded alewife nursery habitat is found in the Scuppernong and Perquimans rivers. However, many alewife captured in non-riverine northwest and southwest sound habitats are predicted to have originated from the Perquimans River, suggesting juvenile alewife from the Perquimans River may seek out more favorable habitat.

Non-riverine habitats offer higher quality blueback herring nursery habitat than riverine habitats. The Perquimans River seems to be offer degraded nursery habitat for juvenile river herring. However, a portion of blueback herring captured in the non-riverine western Albemarle Sound habitats are predicted to originate from the Perquimans River suggesting these fish were seeking more favorable habitats. A large portion of the blueback herring captured in non-riverine habitats were predicted to originate from the Chowan River, suggesting blueback herring leave the Chowan River in search of better habitats.

The association between land use and river herring spawning and nursery habitat has been well established (O'Connell and Angermeier 1997; Waters and Hightower 1997). In particular small headwater and tributary streams may be affected by agricultural land use (O'Connell and Angermeier 1997; Waters and Hightower 1997). Large portions of the Albemarle Sound watershed are used for agricultural purposes (Spruill et al. 1998). Large numbers of CAFOs are present in the Perquimans and Scuppernong river watersheds potentially causing poor water quality and decreased growth of fish (Rulifson et al. 2009a). In addition, water quality in the Scuppernong River may be degraded due to high dissolved nitrate, dissolved phosphorous, dissolved ammonia, and pesticides (Spruill et al. 1998).

In general, the Chowan, Roanoke and Alligator rivers, and non-riverine western Albemarle Sound habitats are high quality river herring nursery areas. The state of North Carolina has designated Strategic habitat Areas (SHAs) in Albemarle Sound, one goal of which is to protect spawning and nursery habitat for river herring (Deaton et al. 2010). The entire Chowan and Roanoke rivers, along with most of the shoreline of the western

Albemarle Sound and large portions of the Alligator River are designated as strategic habitats (Deaton et al. 2010). SHAs are less dense in central sound locations like the Perquimans and Scuppernong rivers (Deaton et al. 2010). Findings from my study support existing strategic habitat designations, in terms of nursery habitat for river herring.

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Table 1. – Reslts of quadratic discriminant function analysis used to classify juvenile river herring to river of capture. Alligator = ALI, Chowan = CHOW, Little = LITT, North = NORT, Pasquotank = PASQ, Perquimans = PERQ, Roanoke = ROAN, Scuppernong = SCUPP, Yeopim = YEOP.

		Predicted River									
Capture Location	n	ALLI	CHOW	LITT	NORT	PASQ	PERQ	ROAN	SCUPP	YEOP	% Correct
ALLI	15	13	1	1	0	0	0	0	0	0	86.7
CHOW	38	0	30	0	0	0	1	7	0	0	79
LITT	5	0	0	5	0	0	0	0	0	0	100
NORT	5	0	0	0	5	0	0	0	0	0	100
PASQ	8	0	0	0	0	8	0	0	0	0	100
PERQ	28	2	0	1	1	1	21	0	0	2	75
ROAN	10	0	0	0	0	0	0	10	0	0	100
SCUPP	5	0	0	0	0	0	0	0	5	0	100
YEOP	8	0	0	0	0	0	0	0	0	8	100

Table 2. - Number of alewife and blueback herring caught in the Alligator (ALLI), Chowan (CHOW), Little (LITTLE), North (NORTH), Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim (YEOPIM) Rivers. Fish from the NW SOUND were collected at three locations along the northwest shore of Albemarle Sound and fish from the SW SOUND were collected at two locations along the southwest shore of Albemarle Sound. Fish were collected from June-October 2010.

Location	Alewife	Blueback herring
ALLI	22	2
CHOW	86	111
LITTLE	39	0
NORTH	9	0
NW SOUND	34	129
PASQ	6	17
PERQ	91	43
ROAN	10	3
SCUPP	44	1
SW SOUND	158	167
YEOPIM	36	36
Total	535	509

Table 3. – TL, weight, condition (K) of alewife caught in the Alligator (ALLI), Chowan (CHOW), Little (LITTLE), North (NORTH), Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim (YEOPIM) Rivers by month. Fish from the NW SOUND were collected at three locations along the northwest shore of Albemarle Sound and fish from the SW SOUND were collected at two locations along the southwest shore of Albemarle Sound. Fish were collected from June-October 2010.

Location	Month	n	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
ALLI	JUL	15	72.05 ± 0.86	3.51 ± 0.10	0.94 ± 0.02
	AUG	3	72.87 ± 1.58	3.59 ± 0.27	0.92 ± 0.01
	SEP	4	97.86 ± 2.97	8.57 ± 0.66	0.91 ± 0.02
CHOW	JUN	29	53.02 ± 0.98	1.36 ± 0.08	0.88 ± 0.01
	JUL	34	70.34 ± 1.45	3.47 ± 0.23	0.95 ± 0.02
	AUG	9	71.47 ± 0.87	3.40 ± 0.11	0.93 ± 0.01
	SEP	14	77.41 ± 0.98	4.49 ± 0.17	0.96 ± 0.01
LITTLE	JUN	30	46.23 ± 0.80	0.86 ± 0.05	0.84 ± 0.02
	JUL	9	74.07 ± 2.35	4.35 ± 0.42	1.05 ± 0.02
NORTH	JUN	8	49.47 ± 2.34	1.09 ± 0.15	0.84 ± 0.03
	SEP	1	105.17	10.84	0.93
NW SOUND	JUN	2	61.53 ± 0.10	2.66 ± 0.26	1.14 ± 0.10
	JUL	6	65.94 ± 1.28	2.71 ± 0.22	0.94 ± 0.03
	AUG	25	71.34 1.04	3.47 ± 0.18	0.94 ± 0.01
	SEP	1	97.85	8.05	0.86
PASQ	JUN	3	51.71 ± 2.71	1.20 ± 0.21	0.85 ± 0.02
	JUL	3	80.52 ± 1.25	5.5 ± 0.20	1.05 ± 0.02
PERQ	JUN	21	37.84 ± 0.65	0.48 ± 0.03	0.86 ± 0.01
	JUL	27	47.94 ± 1.51	1.12 ± 0.11	0.95 ± 0.03
	AUG	34	62.93 ± 0.81	2.54 ± 0.08	1.10 ± 0.01
	SEP	9	72.34 ± 1.45	3.17 ± 0.13	0.83 ± 0.02
ROAN	JUL	10	68.41 ± 0.94	3.00 ± 0.12	0.93 ± 0.02
SCUPP	JUN	42	41.1 ± 0.58	0.60 ± 0.02	0.86 ± 0.02
	JUL	1	68.71	3.5	1.08
	AUG	1	71.84	2.93	0.79
SW SOUND	JUN	101	65.7 ± 0.49	2.81 ± 0.06	0.98 ± 0.01
	JUL	43	66.64 ± 0.64	2.93 ± 0.09	0.98 ± 0.01
	AUG	13	69.67 ± 0.75	3.44 ± 0.15	1.01 ± 0.03
	SEP	1	83.29	5.26	0.91
YEOPIM	JUL	31	64.9 ± 0.52	2.77 ± 0.07	1.01 ± 0.01
	AUG	5	75.92 ± 3.46	3.94 ± 0.50	0.89 ± 0.06

Table 4. – Results of Kruskal-Wallis tests examining spatial and temporal differences in total length, weight and Fulton's condition factor (K) of alewife and blueback herring captured in the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim Rivers from June-October 2010. Alewife and blueback herring were also captured in Northwest and Southwest Albemarle Sound Locations. No blueback herring were captured in the Little or North Rivers. One blueback herring was captured in the Scuppernong River and was excluded from analysis. No alewife were captured in October.

Variable	Species	Effect	chi-squared	df	p-value
TL	ALE	Location	215.25	10	<0.0001
		Month	192.65	3	<0.0001
	BB	Location	143.96	7	<0.0001
		Month	303.73	4	<0.0001
Weight	ALE	Location	207.18	10	<0.0001
		Month	179.8	3	<0.0001
	BB	Location	115.68	7	<0.0001
		Month	240.05	4	<0.0001
K	ALE	Location	113.63	10	<0.0001
		Month	49.93	3	<0.0001
	BB	Location	118.81	7	<0.0001
		Month	172.79	4	<0.0001

Table 5. – Mean growth rate (mm/day) of alewife and blueback herring captured in the Alligator (ALLI), Chowan (CHOW), Little (LITTLE), North (NORTH), Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim (YEOPIM) Rivers. Alewife and blueback herring were aslo captured at northwest and southwest Albemarle Sound habitats. No blueback herring were captured in the Little, North, and Scuppernong Rivers.

Location	Species	n	Growth Rate (mm/day) ±S.E.
ALLI	ALE	19	0.61 ± 0.01
	BB	1	0.97
CHOW	ALE	25	0.57 ± 0.01
	BB	13	0.96 ± 0.001
LITTLE	ALE	4	0.58 ± 0.01
NORTH	ALE	1	0.52
NW Sound	ALE	11	0.55 ± 0.02
	BB	10	0.97 ± 0.001
PASQ	ALE	3	0.63 ± 0.04
	BB	4	0.96 ± 0.001
PERQ	ALE	18	0.51 ± 0.01
	BB	3	0.95 ± 0.001
ROAN	ALE	7	0.59 ± 0.02
	BB	1	0.97
SCUPP	ALE	3	0.59 ± 0.08
SW Sound	ALE	19	0.60 ± 0.01
	BB	7	0.97 ± 0.001
YEOPIM	ALE	5	0.63 ± 0.06
	BB	3	0.96 ± 0.002

Table 6. – Predicted river of origin of juvenile river herring caught at Northwest and Southwest Albemarle Sound habitats by month based on multi-variate means established using otoliths of fish caught in each river. ALLI = Alligator, CHOW = Chowan, LITTLE = Little, NORTH = North, PASQ = Pasquotank, PERQ = Perquimans, ROAN = Roanoke, SCUPP = Scuppernong.

Location	Month	Species	n	Predicted Origin	Percent
NW Sound	AUG	ALE	10	CHOW	10
				LITTLE	20
				NORTH	30
				PASQ	20
				PERQ	20
		BB	11	CHOW	45
				PASQ	9
				PERQ	27
				ROAN	18
	OCT	BB	10	CHOW	80
				PERQ	10
				ROAN	10
SW Sound	JUN	ALE	5	PERQ	80
				ROAN	20
	JUL	ALE	10	CHOW	20
				NORTH	20
				PASQ	30
				PERQ	30
	AUG	ALE	5	ALLI	20
				CHOW	20
				PASQ	20
				PERQ	20
				SCUPP	20
		BB	5	CHOW	80
				ROAN	20
	SEP	BB	10	CHOW	70
				PASQ	10
				PERQ	10
				SCUPP	10

Table 7. – Mean total length (TL), weight (w) and Fulton's condition factor (K) of blueback herring caught in the Alligator (ALLI), Chowan (CHOW), Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim (YEOPIM) Rivers by month. Fish from the NW SOUND were collected at three locations along the northwest shore of Albemarle Sound and fish from the SW SOUND were collected at two locations along the southwest shore of Albemarle Sound. Fish were collected from June-October 2010.

Location	Month	n	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
ALLI	JUN	1	47.86	0.94	0.86
	AUG	1	59.35	1.54	0.74
CHOW	JUL	70	48.93 ± 0.37	0.94 ± 0.02	0.80 ± 0.01
	AUG	41	54.24 ± 0.76	1.32 ± 0.06	0.81 ± 0.01
NW SOUND	AUG	70	52.56 ± 0.32	1.13 ± 0.02	0.77 ± 0.01
	SEP	5	58.51 ± 1.45	1.50 ± 0.13	0.74 ± 0.02
	OCT	54	61.08 ± 0.58	1.63 ± 0.07	0.70 ± 0.01
PASQ	AUG	15	51.17 ± 0.62	1.00 ± 0.04	0.74 ± 0.02
	OCT	2	54.8 ± 3.53	1.30 ± 0.06	0.80 ± 0.12
PERQ	JUN	11	42.95 ± 1.03	0.68 ± 0.03	0.86 ± 0.03
	AUG	1	49.95	0.89	0.71
	SEP	1	54.15	1.17	0.74
	OCT	30	57.26 ± 0.38	1.31 ± 0.03	0.70 ± 0.01
ROAN	JUL	2	51.55 ± 1.57	1.07 ± 0.23	0.77 ± 0.10
	AUG	1	69.75	2.67	0.79
SCUPP	AUG	1	47.88	0.88	0.8
SW SOUND	JUL	15	49.75 ± 0.73	1.00 ± 0.05	0.80 ± 0.01
	AUG	31	55.31 ± 0.59	1.35 ± 0.04	0.79 ± 0.01
	SEP	121	58.03 ± 0.40	1.46 ± 0.03	0.74 ± 0.01
YEOPIM	JUL	29	46.61 ± 0.47	0.90 ± 0.02	0.88 ± 0.01
	AUG	6	51.73 ± 0.80	1.14 ± 0.04	0.82 ± 0.02
	SEP	1	58.29	1.42	0.72

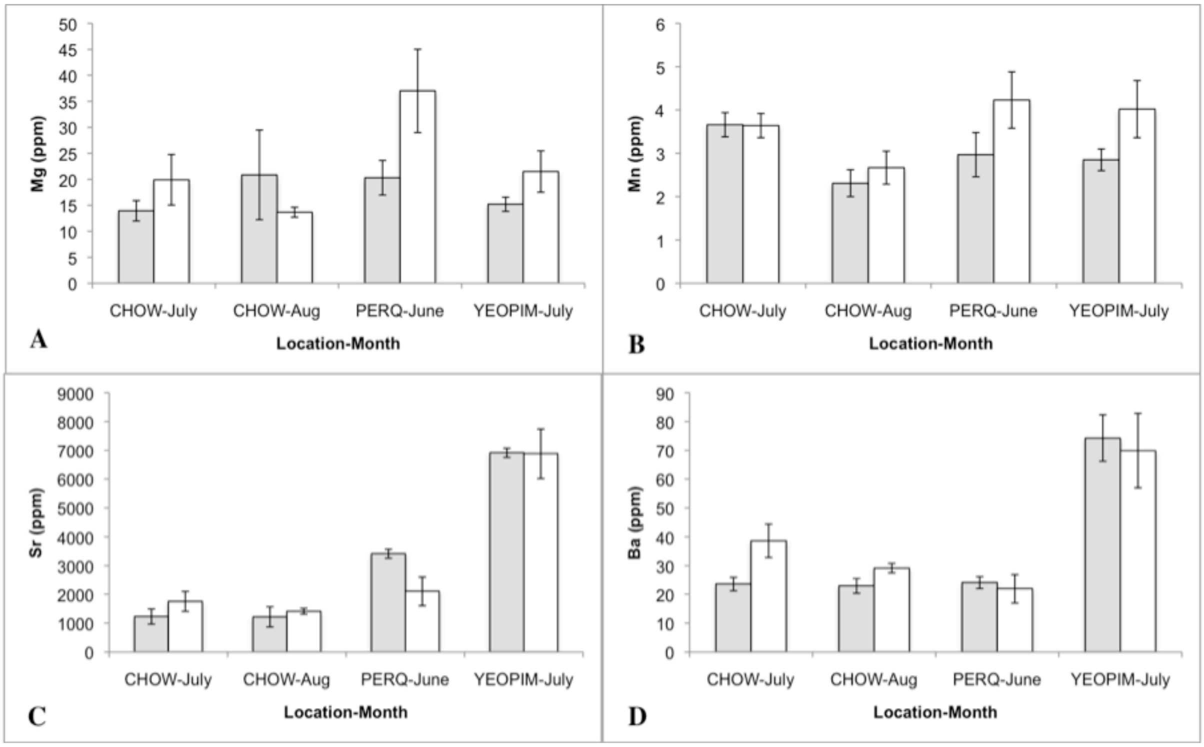

Figure 1. – Mean (± S.E.) Mg (A), Mn (B), Sr (C), and Ba (D) at the outer edge of of alewife (white bars) and blueback herring (grey bars) captured in the Chowan River (CHOW) in July and August, the Perquimans River (PERQ) in June and the Yeopim River in July.

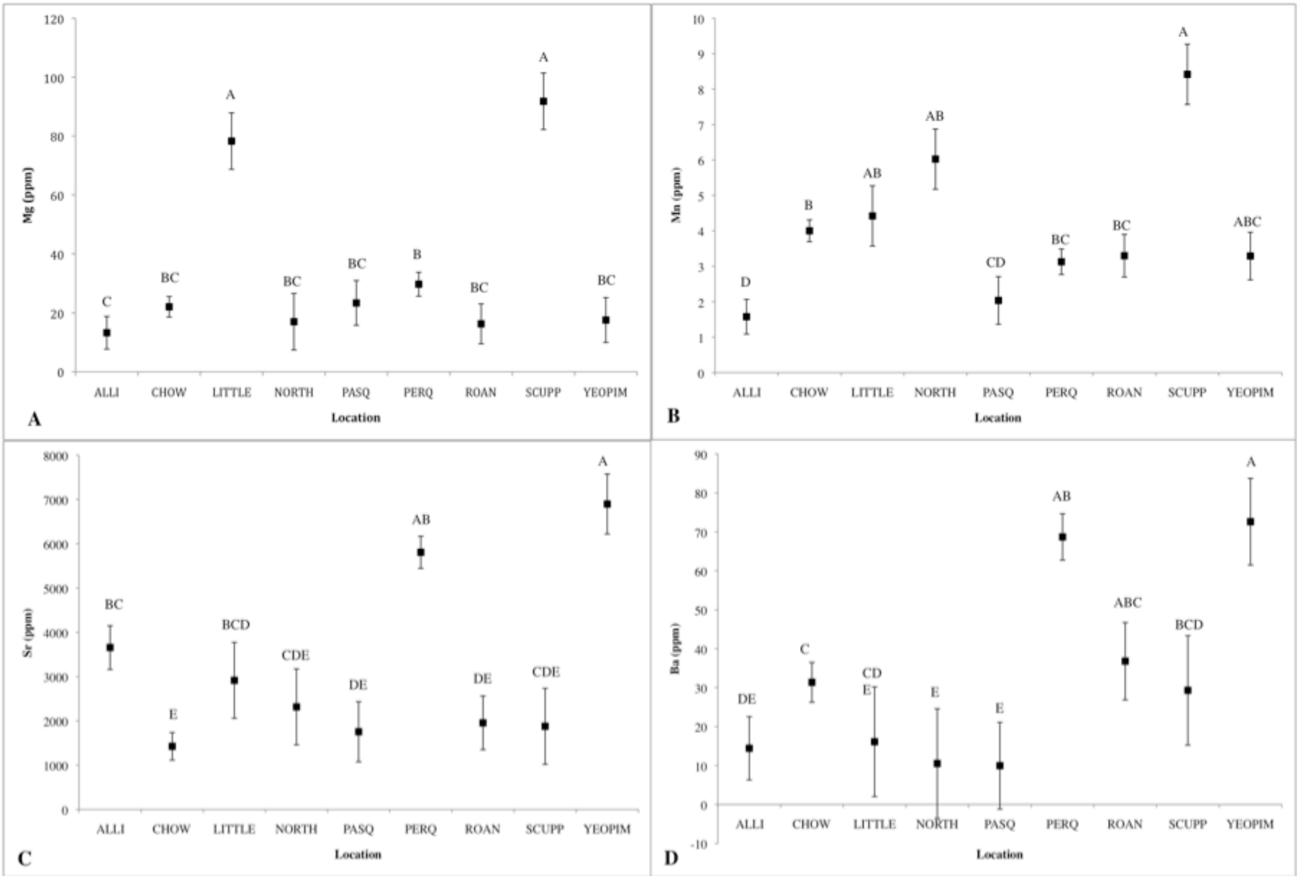

Figure 2. – Mean (± S.E.) Mg (A), Mn (B), Sr (C), and Ba (D) (ppm) at the outer edge of the otoliths of river herring (alewife and blueback herring combined) captured in the Alligator (ALLI), Chowan (CHOW), Little, North, Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim Rivers from June-October 2010. Locations not connected by the same letter are significantly different.

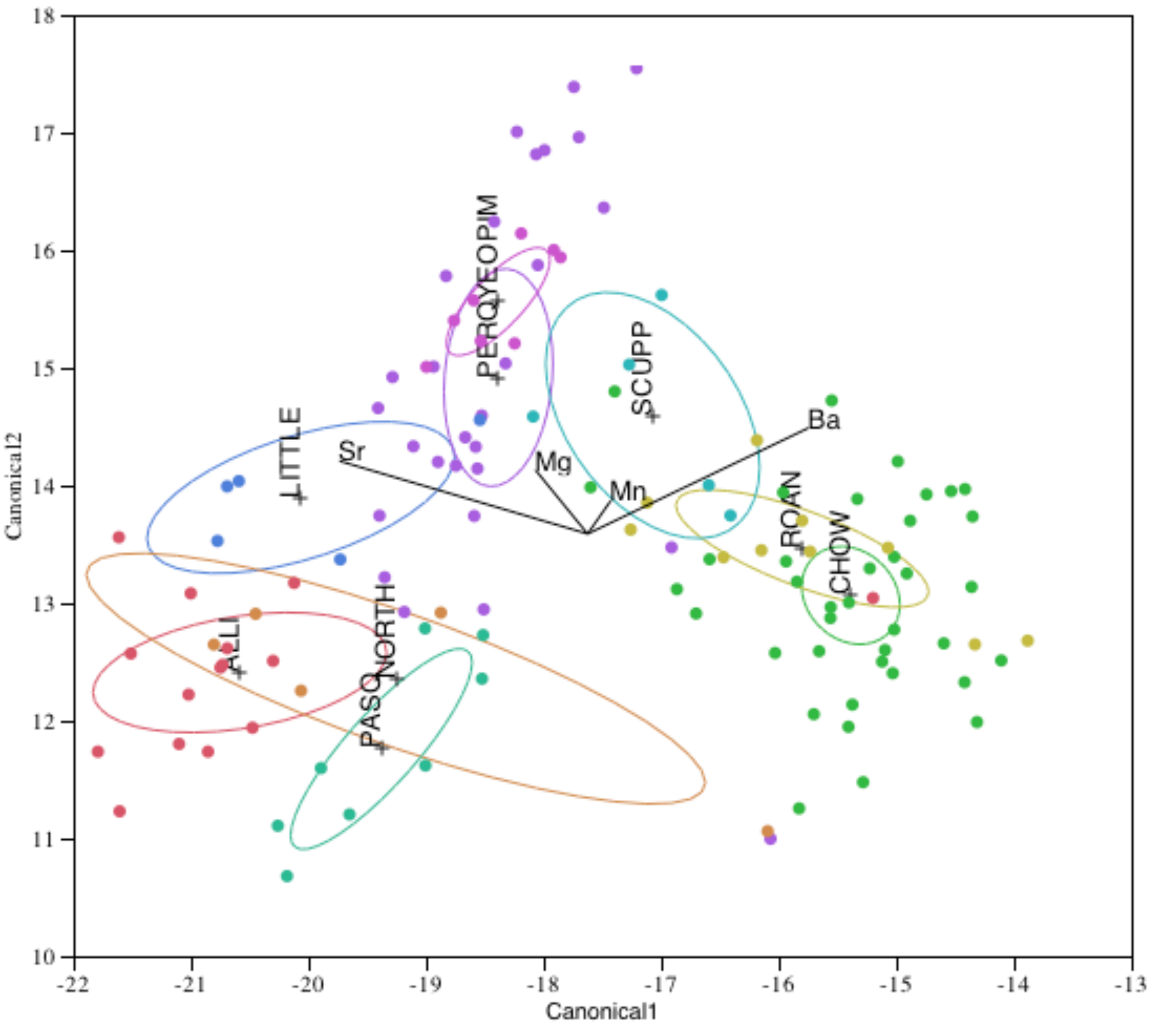

Figure 3. - Plot of first two canonical variates obtained using quadradic discriminant function analysis to classify juvenile river herring to their river of capture. Alligator (ALLI) = red, Chowan (CHOW) = green, Little = blue, North = orange, Pasquotank (PASQ) = blue green, Perquimans (PERQ) = purple, Roanoke (ROAN) = yellow, Scuppernong (SCUPP) = aqua. Group centroids are marked with (+), ellipses represent 95% confidence ellipse for each location.

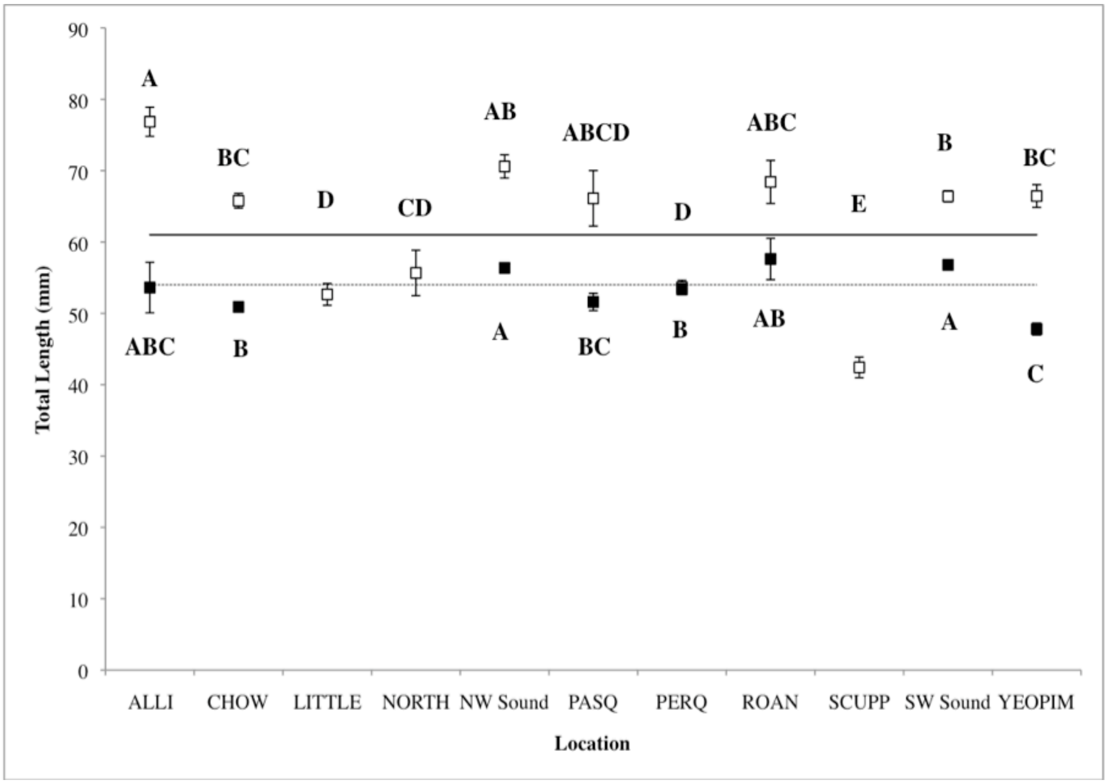

Figure 4. – Mean total length (± S.E.) of alewife (white squares), and blueback herring (black squares) captured in Albemarle Sound tributaries. Top line represents mean total length of alewife, bottom line represents mean total length of blueback herring. Top letters indicate significant differences in total length of alewife, and bottom letters indicate significant differences in total length of blueback herring.

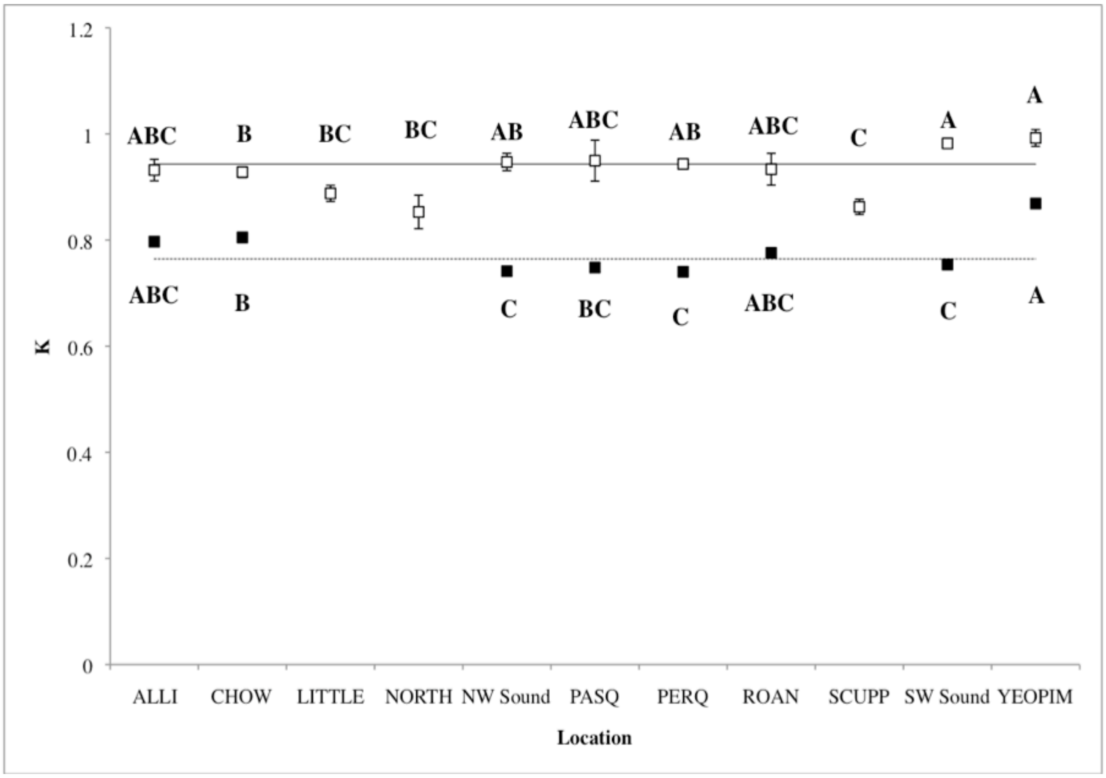

Figure 5. – Mean condition (± S.E.) of alewife (white squares), and blueback herring (black squares) captured in Albemarle Sound tributaries. Top line represents mean condition of alewife, bottom line represents mean condition of blueback herring. Top letters indicate significant differences in condition of alewife, and bottom letters indicate significant differences in condition of blueback herring.

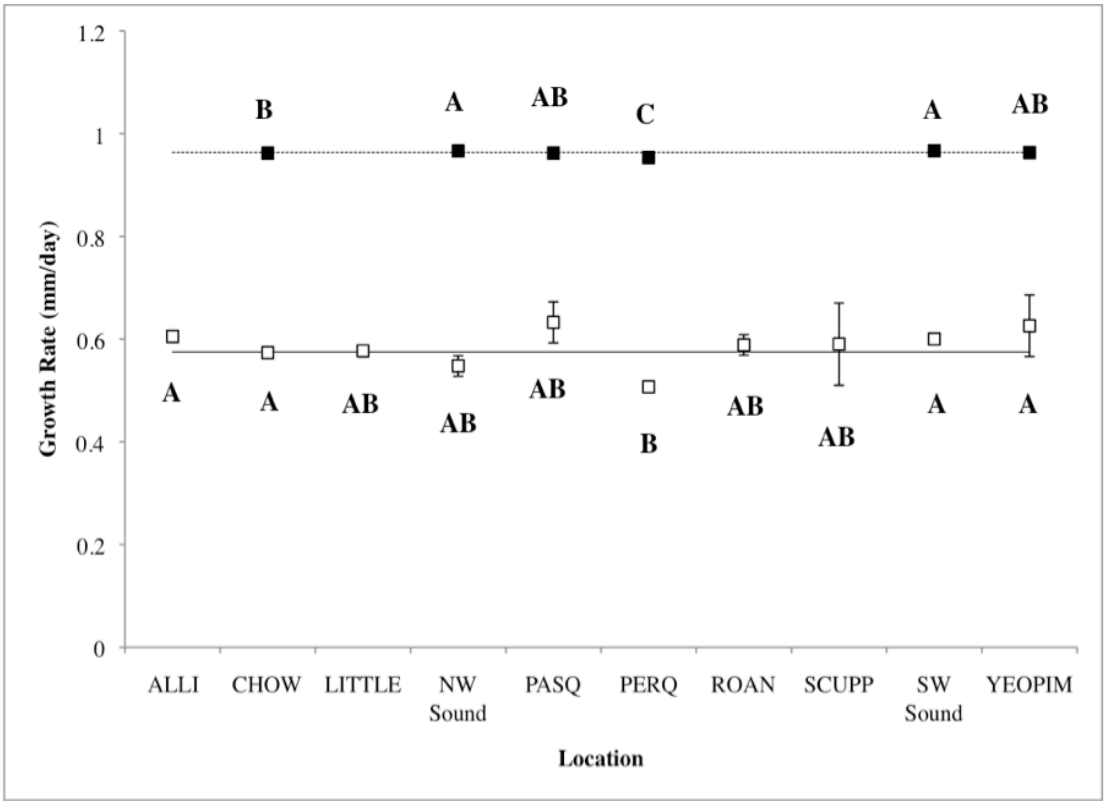

Figure 6. – Mean growth rate (± S.E.) of alewife (white squares), and blueback herring (black squares) captured in Albemarle Sound tributaries. Top line represents mean growth rate of blueback herring, bottom line represents mean growth rate of alewife. Top letters indicate significant differences in growth rate of blueback herring, and bottom letters indicate significant differences in growth rate of alewife.

Chapter 4: Estimating Stock Structure and Natal Homing of Adult River Herring Returning to Tributaries of Albemarle Sound, North Carolina

Abstract

River herring is a collective term used to describe two similar alosine species: alewife *Alosa pseudoharengus* and blueback herring *A. aestivalis*. Adult river herring were collected in the Chowan and Perquimans rivers in April and May 2010, and the Scuppernong River in 2009. Two methods using multi-elemental signatures of Mg, Mn, Sr, and Ba in river herring otoliths were used to estimate stock structure and natal homing. The first method used only elemental signatures in the otoliths of adult fish captured in each river to classify fish to their river of capture. No adult alewife were collected for the study, likely a result of sampling after spawning had occurred. Adult blueback herring classified to their river of capture with between 60 and 100% accuracy depending on the number of year classes considered in analysis. The second method utilized ground truthed elemental signatures in the otoliths of juvenile river herring collected in nine tributaries of the Albemarle Sound in the summer of 2010 (Zapf 2012, Chapter 3). This method estimated varying percentages of adult blueback herring homing to natal tributaries and the spawning run in all rivers was made up of fish from a number of sources. Homing rates of adult blueback herring were between 0-64% depending on the river. Low percentages of adult blueback herring were predicted to originate from the Perquimans and Scuppernong rivers suggesting these locations may offer poor river herring nursery habitat.

Introduction

The implementation of proper regulations for improved fisheries management relies on accurate information concerning life history characteristics including stock structure (Begg and Waldman 1999). A stock is a reproductively isolated population with minimal mixing from outside sources, which can be treated as a single unit for management purposes (Hubert and Fabrizio 2007). Stock identification is important because it provides information on how fishing effort and mortality are distributed (Begg and Waldman 1999). If the degree to which stocks are separated or mixed is known, then regulations can be geared toward fisheries where multiple stocks are differentially exploited (Ricker 1981).

Stock discrimination can be accomplished using otolith microchemistry (Begg and Waldman 1999). Otoliths are metabolically inert; therefore, elements incorporated into the otolith reflect the environmental history of the fish from its time of hatch to time of death (Elsdon and Gillanders 2003a). Experimental evidence suggests that strontium (Farrell and Campana 1996; Bath et al. 2000; Elsdon and Gillanders 2003a; Kraus and Secor 2004; Walther and Thorrold 2006; Mohan et al. 2012), barium (Bath et al. 2000; Elsdon and Gillanders 2003a; Elsdon and Gillanders 2004; Walther and Thorrold 2006; Miller 2009; Mohan et al. 2012), and manganese (Forrester 2005; Dorval et al. 2007; Mohan et al. 2012) are incorporated into otoliths in ratios similar to concentrations in water, thus allowing the chemical composition of otoliths to be used as natural tags (Elsdon and Gillanders 2003b).

Generally, two analytical techniques have been used in stock discrimination studies utilizing otolith microchemistry (Thresher 1999; Miller et al. 2005). The first is analyzing the elemental composition of otoliths from adult fish caught at the same or separate locations, and then discriminating stocks based on multi-elemental signatures. Fish with similar otolith elemental concentrations are assumed to have experienced similar natal environments and therefore belong to the same stock. This method is used in a manner similar to stock structure studies utilizing meristic and morphometric analysis. Meristic counts and morphometric analysis have been used to discriminate alewife *Alosa pseudoharengus* stocks (Messieh 1977) and American shad *Alosa sapidissima* stocks (Carscadden and Leggett 1975; Melvin et al. 1992). Analyzing the elemental composition of adult otoliths has been used to infer stock structure of orange roughy *Hoplostethus atlanticus* off the coast of southern Australia (Edmonds et al. 1991), yellow-eye mullet *Aldrichetta forsteri* in estuaries along the coast of Australia (Edmonds et al. 1992), Atlantic cod *Gadus morhua* collected at spawning grounds in the northwest Atlantic (Campana et al. 1994), and striped bass *Morone saxatilis* from rivers along the Atlantic coast of North America (Morris et al. 2003). However, this method is somewhat limited, only providing information on similarity of otolith elemental composition from fish caught in the same location; it does not provide specific information on where fish originated.

The second method is analyzing the elemental composition of otoliths from juvenile fish captured in natal habitats to obtain location-specific elemental signatures. This has been done for a number of species including weakfish *Cynoscion regalis* in estuaries along the Atlantic coast of North America (Thorrold et al 1998a), yellow perch *Perca flavescens* in Lake Superior wetlands (Brazner et al. 2004), Atlantic salmon *Salmo salar* parr from streams in Newfoundland, Canada (Veinott and Porter 2005), American shad in rivers along the Atlantic coast of North America (Thorrold et al. 1998b; Walther et al. 2008; Walther and Thorrold 2008), and striped bass in tributaries of Albemarle Sound, North Carolina (Mohan et al. 2012). This method identifies river specific elemental signatures, which can then be used to identify natal origins, and thus to which stock an adult fish belongs. Location specific elemental signatures have been used to investigate questions regarding stock mixing of Atlantic cod in the northeast Atlantic (Campana et al. 2000), spawning site fidelity of weakfish (Thorrold et al. 2001), natal homing of anadromous American shad (Walther et al. 2008), nursery habitat of delta smelt *Hypomesus transpacificus* in the San Francisco Bay estuary (Hobbs et al. (2007), and the dispersal of young-of-year smallmouth bass *Micropterus dolomieu* between tributaries and the main stem of the James River, Virginia (Humston et al. 2010). In addition, this method holds promise for investigating the nursery role hypothesis proposed by Beck et al. (2001), in which identifying the natal origins of adult fish is essential for testing the hypothesis. Studies utilizing ground truthed elemental signatures in otoliths to infer stock structure of adult fish are, at this point, rare due to issues that arise when elemental signatures are not temporally stable (Patterson et al. 1999; Campana et al. 2000; Gillanders 2002; Elsdon and Gillanders 2006; Walther et al. 2008). If this is the case studies must span from the time signatures are identified, in juveniles, to a point of interest in the life of the fish. In many cases this can be a number of years, entailing multiple collections and sets of analyses, which may not be feasible.

River herring is a collective term for two similar alosine species: alewife and blueback herring *A. aestivalis*. Both are native to the east coast of North America, with blueback herring ranging from Nova Scotia to Florida, and alewife from Nova Scotia to

South Carolina (Munroe 2002; Greene et al. 2009). River herring are anadromous, meaning they are born in freshwater; migrate to the ocean after 3-9 months (Kosa and Mather 2001), then return to freshwater rivers after 3-5 years to spawn (Loesch and Lund 1977; Davis and Schultz 2009). Evidence from meristic counts (Messieh 1977), mark-recapture data (Jessop 1994), and genetic analyses (Bentzen and Peterson 2005; Willis 2006) suggests river herring return to natal tributaries to spawn. In addition, experimental evidence suggests river herring can use olfaction to discriminate natal waters (Thunberg 1971). Landlocked populations of alewife and blueback herring do exist (Schmidt et al. 2003), and non-anadromous populations of blueback herring are thought to exist in the Hudson and Mohawk Rivers (Limburg et al. 2001),

Despite similarities in range and life history there are spatial and temporal differences in the spawning behavior of alewife and blueback herring, with alewife spawning earlier along shore eddies or deep pools and blueback herring spawning later in the mainstem of rivers (Loesch and Lund 1977; Messieh 1977), and in rice paddies (Thomas et al. 1992) and impoundments in South Carolina (Meador et al 1984). In North Carolina, river herring spawn in coastal rivers and Lake Mattamuskeet (Rulifson and Wall 2006) from approximately March through May in lotic and lentic habitats (Walsh et al. 2005), and from mid-April to mid-May in the Roanoke River (Harris and Hightower 2010). Further to the south in Lake Mattamuskeet, North Carolina alewife spawn in April (Tyus 1974). Historically, spawning runs have been dominated by older repeat spawners (Davis and Schultz 2009). Examining scales, Creed (1985) found 85% of blueback herring captured in the Chowan River, North Carolina were repeat spawners, with some having spawned as many as six times. However, in a more recent study, Rulifson et al. (2009) concluded that 18.8% of male and 27.8% of female blueback herring returning to the Scuppernong River, North Carolina in 2007 were repeat spawners. Current spawning runs are typically comprised of 4-5 year old first time spawners (Moser and Patrick 2000; Davis and Schultz 2009). Although river herring are not classified as semelparous (Greene et al. 2009) post spawn mortality can be high (Durbin et al. 1979).

The human population in the coastal region of North Carolina has rapidly increased since 1980, consequences of which include degradation of water quality and aquatic habitat (Street et al. 2005). Despite a relatively small geographic area, the tributaries of Albemarle Sound, North Carolina, utilized by river herring for spawning, have variable watershed characteristics based on geographic location, geology, and anthropogenic use of the watershed (Copeland et al. 1983; Riggs et al. 1996). River herring have experienced drastic declines in North Carolina, consistent with populations along the east coast of North America (Schmidt et al. 2003). If river herring exhibit tributary fidelity, then some populations may be increasingly susceptible to habitat degradation and overfishing. Identifying river specific populations allows management efforts to be directed toward individual rivers (Edmonds et al. 1991). The ability to examine natal origins and straying rates of river herring between tributaries of Albemarle Sound provide information that may guide managers in implementing fishing regulations, restoring habitat and initiating stocking programs. However, at this point an extensive database of elemental signatures for Albemarle Sound tributaries has not been developed making investigations of natal homing and straying rates difficult. Also, we do not know whether watershed specific signatures are temporally stable, although limited evidence suggests it may be (Zapf 2012, Chapter 2).

The objectives of this study were: 1) to collect river herring from the Chowan, Perquimans, and Scuppernong rivers during the spawning run; 2) to examine differences in the elemental composition at the core of adult river herring otoliths; 3) to use ground truthed elemental signatures from the otoliths of juvenile river herring collected in 2010 (Zapf 2012, Chapter 2) to examine natal homing and rates of straying of adult river herring; and 4) to compare results of adult otolith analysis (objective 2) and river specific signatures (objective 3) to examine the effectiveness of each method for inferring stock structure and natal homing of Albemarle Sound river herring.

Methods

Site Description

The Albemarle Sound in northeastern North Carolina extends approximately 90 km eastward from the mouth of the Roanoke River to Kitty Hawk Bay and Colington Island. It is the drowned portion of the Roanoke River and its floodplain (Copeland et al. 1983). The Albemarle Sound is a shallow oligohaline system with salinities ranging from 0-5 ppt (Copeland et al. 1983). The sound has no direct connection to the ocean but seawater intrusion does occur through Oregon Inlet, Croatan and Roanoke sounds (Copeland et al. 1983; Riggs 1996). The system is well mixed due to nearly constant wind action allowing only temporary stratification due to salinity and temperature (Riggs 1996). There are nine major tributaries including the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers.

Fish Collection and Otolith Removal

Adult river herring were collected from the Chowan and Perquimans rivers in April and May 2010 and from the Scuppernong River in 2009. Fish from the Scuppernong River and a portion of the fish from the Chowan River were collected from commercial pound nets. The North Carolina Division of Marine Fisheries (NCDMF) bridge net survey provided additional fish from the Chowan River and all of the fish from the Perquimans River. The goal of the NCDMF bridge net survey is to assess tributaries utilized by river herring for spawning. Gill nets are hung from bridges across the length of a tributary stream, although the net does not always stretch across the whole tributary. The nets are set on Monday, checked every day and pulled on Friday. If river herring are caught in the net, they are pulled and moved upstream to the next bridge or overpass. The Chowan River watershed is sampled each year and the other sampling site changes each year.

Fish were identified to species (alewife or blueback herring), sexed, measured for total length (TL) and fork length (FL) (mm), and weighed (g). Gonads were removed and weighed, and otoliths were removed using plastic forceps. Upon removal otoliths were rinsed with distilled water and gently scrubbed to remove tissue. Left and right otoliths were stored separately in 1.5-ml microcentrifuge polypropylene vials and allowed to air dry for 24 hours. Each otolith was photographed using Image-Pro® Plus version 6.2 on an Olympus SZX16 scope in order to obtain a permanent record of otoliths used for analysis.

Although scales commonly have been used in age determination of alosines (Walton 1983; Jessop 1990), otoliths have been shown to accurately record age

(Kornegay 1978; Davis and Schultz 2009). Otoliths were aged whole by three independent readers using a compound microscope until there was agreement between at least two readers (Kornegay 1978; Libby 1985; LaBay and Lauer 2006). Each fish was then assigned to a year class by subtracting age from year of capture (2009 for Scuppernong fish, 2010 for Chowan and Perquimans fish). Eighty otoliths were chosen randomly (15 Scuppernong fish, 21 Perquimans fish, and 44 Chowan fish) and sent to the University of Manitoba for elemental analysis.

Otolith Preparation and Analysis

Microchemical analysis was performed using laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS) as described by Halden and Friedrich (2008) and Mohan et al. (2012). Otoliths were embedded in epoxy resin (Buehler Epoxicure ®) and a 2-mm thick dorso-ventral transverse section was cut using a diamond blade Isomet saw (Buehler model 646). Cut sections were then embedded in 25-mm diameter plexiglass ringmounts. Sections were ground down using 320, 400, and 600 grit wet sandpaper to expose the core and ultrasonically cleaned for 2 minutes. Scratches on the surface of the otolith were removed by polishing with Buehler diamond polishing suspensions (9-µm and 0.05-µm) on a polishing wheel to create a smooth surface. Polished, mounted otoliths were cleaned with ultrapure water and digitally photographed.

Elements were quantified using a Thermo-Finnigan Element 2 ICP-MS coupled to a Merchantek LUV 213 Nd-YAG laser. Operating parameters for LA-ICP-MS included: 30-µm beam size; 2-μms^{-1} scan speed; repetition rate 20 Hz; 75% power, low resolution (R = 300) mode. Isotopes counted included ^{44}Ca, ^{25}Mg, ^{88}Sr, ^{138}Ba, ^{55}Mn, ^{63}Cu, ^{66}Zn,

^{208}Pb. Calcium (as 56 wt. % CaO) was used as the internal standard and NIST 610 glass was used for external calibration and to monitor instrument drift. Laser scans were across the entire width of the otolith. Isotope counts were converted to ppm and plotted versus laser distance.

Elemental concentrations in an approximately 48-μm section of otolith located slightly beyond the core were averaged to obtain an elemental signature of the natal river for each fish. The 48-μm was thought to represent approximately 10-20 days of the fishes life based on previous results (Zapf 2012, Chapter 3). A section beyond the otolith core was used because preliminary investigations of line scan data revealed Mg spikes at the core of the otolith inconsistent with Mg values throughout the otolith, and in water samples from Albemarle Sound tributaries (Zapf 2012, Chapter 2). We hypothesize that these spikes may be due to maternal input of Mg, thus decoupling the value of Mg in the otolith core from that in the water.

Statistical Analysis

Kruskal-Wallis tests were used to examine differences in $\log_{10}$-transformed concentrations of Mg, Mn, Sr, and Ba in the otoliths of river herring captured in each river. If significant differences were detected, then Tukey's HSD test was used to identify which rivers differed significantly.

Quadratic discriminant function analysis (QDFA) was used to assess how multi-elemental signatures utilizing $\log_{10}$-transformed concentrations of Mg, Mn, Sr, and Ba could be used to classify fish to their river of capture. This was done for all year classes combined (2002-2007), and then for the 2005 and 2006-year classes separately. Analysis of the 2005-year class included fish from all three rivers, while analysis of the 2006-year class included only fish from the Chowan and Perquimans rivers. Differences in multi-elemental signatures between sites was assessed using Pillai's trace statistic (JMP ® 2007).

River specific multi-elemental signatures obtained from a previous study of elemental concentrations in the otoliths of juvenile river herring captured in Albemarle Sound tributaries (Zapf 2012, Chapter 3) were used to predict natal rivers of adult river herring. Adult fish were classified using group centroids created by the juvenile data set and Mahalanobis distance, which is the distance from a point to the multivariate mean (JMP ® 2007). Based on Mahalanobis distance, probabilities of belonging to each group (river) were calculated, and the fish was classified to the group (river) with the highest probability (JMP ® 2007). The results of this analysis were then compared to results obtained analyzing adult otoliths to assess the effectiveness of these methods in stock discrimination and identifying natal homing.

Results

Catch Data

Ninety-four blueback herring from the Chowan River, 20 from the Perquimans River, and 32 from the Scuppernong River were analyzed for this study (Table 1). No alewife were collected during this study, probably due to late initiation of collection. Alewife generally spawn earlier than blueback herring (Loesch and Lund 1977; Messieh 1977) and since collection for this study was initiated in April, the alewife spawning run had probably already ended. More male blueback herring than female blueback herring were captured in both the Chowan (66 of 94) and Scuppernong (23 of 32) rivers. In the Perquimans River more females than males were captured (12 of 20) (Table 1). No blueback herring from the Scuppernong River were weighed or measured because of damage to fish during the freezing and storage process.

Female blueback herring captured in the Chowan and Perquimans rivers had greater mean total length (259 mm) and weight (147 g) than males (248 mm, 125 g) captured in those rivers. Female (272 mm, 184 g) and male (265 mm, 154 g) blueback herring captured in the Perquimans River had greater mean total lengths and weights than female (259 mm, 147 g) and male (248 mm, 125 g) blueback herring captured in the Chowan River (Table 1). Mean age did not differ greatly between any of the rivers, with mean age ranging from 4.48 to 4.75 years (Table 1).

Elemental Analysis

Kruskal-Wallis tests examining differences in the concentrations of Mg, Mn, Sr, and Ba in the otoliths of blueback herring from the Chowan, Perquimans, and Scuppernong rivers revealed significant differences in the concentrations of Sr and Ba between rivers ($\alpha = 0.05$) (Table 2). Tukey's HSD showed fish captured in the Perquimans River to have significantly higher otolith Sr than those captured in the Chowan and Scuppernong rivers, and blueback herring captured in the Chowan River to have significantly higher otolith Ba than fish captured in the Perquimans and Scuppernong rivers (Figure 1).

Classification

Using QDFA, adult blueback herring from the 2002-2007 year classes were classified to the Chowan River with 84.4% accuracy, the Perquimans River with 76.2% accuracy, and the Scuppernong River with 60% accuracy (Table 3). Multi-elemental signatures were significantly different between rivers (Pillai's trace statistic: $F = 4.89$, $df = 8, 152$, $P < 0.0001$), and there was little overlap of 95% confidence intervals (Figure 2). Fish were classified to the Chowan River using Ba, to the Perquimans River using Sr, and to the Scuppernong River using Mg (Figure 2).

Blueback herring from the 2005-year class were classified to the Chowan River with 65.2% accuracy, the Perquimans River with 100% accuracy, and the Scuppernong River with 100% accuracy (Table 3). Multi-elemental signatures were significantly different between rivers (Pillai's trace statistic: $F = 3.01$, $df = 8, 62$, $P = 0.0064$); however, 95% confidence intervals of the Chowan and Scuppernong Rivers overlapped significantly (Figure 3). Fish were classified to the Chowan River using Ba, the Perquimans River using Sr and Mg, and the Scuppernong River using Mn (Figure 3).

Blueback herring from the 2006-year class were classified to the Chowan River with 94.4% accuracy, and the Perquimans River with 87.5% accuracy (Table 3). Multi-elemental signatures of the two rivers were significantly different (Pillai's trace statistic: $F = 8.78$, $df = 4, 21$, $P = 0.0002$), and the 95% confidence intervals did not overlap (Figure 4). Fish were classified to the Chowan River primarily using Ba, and to the Perquimans River using Sr (Figure 3).

River specific multi-elemental signatures from juvenile river herring caught in Albemarle Sound tributaries (Zapf 2012, Chapter 2) were used to predict the natal river of adult river herring returning to the Chowan, Perquimans, and Scuppernong rivers. Of the blueback herring returning to the Chowan River, 64.4% were predicted to originate from the Chowan River and 17.8% were predicted to originate from the Roanoke River (Table 4). Smaller percentages of blueback herring captured in the Chowan River were predicted as originating from the Alligator, Pasquotank, and Perquimans rivers (Table 4). Of the river herring captured in the Chowan River that were classified as originating from the Chowan River, the mean probability of being classified to the Chowan River was 0.95 (Table 5). The group with the next highest probability was fish classified to the Roanoke River with a probability of 0.80 (Table 5). Of the Chowan River fish from the 2005-year class, 52.2% were classified as originating from the Chowan River, 17.4% were from the Alligator and 17.4% were from the Roanoke. Lower numbers were predicted as originating from the Pasquotank and Perquimans rivers (Table 6). Of the Chowan River fish from the 2006-year class, 83.3% were predicted to originate from the Chowan River while lower numbers were predicted to originate from the Alligator and Roanoke rivers (Table 6).

Of the blueback herring returning to the Perquimans River, 28.6% were predicted to originate from the Perquimans River, 23.8% from the Alligator River, and 14.3% from the Chowan River (Table 4). Smaller percentages of blueback herring captured in the Perquimans River were predicted to originate in the Little, North, Pasquotank, and Scuppernong rivers (Table 4). River herring captured in the Perquimans River that were predicted as originating from the Alligator, Chowan, and Perquimans rivers all had high mean probabilities of originating from these rivers (Table 5). Of the Perquimans River fish, 25% from the 2005-year class and 37.5% from the 2006-year class were predicted as originating from the Perquimans River (Table 6). Lesser numbers were predicted as originating from the Alligator River to the southeast, Chowan River to the west, Little, North, and Pasquotank rivers to the east, and the Scuppernong River to the south (Table 6).

Of the blueback herring returning to the Scuppernong River, 40% were predicted as originating from the Chowan River (Table 4). Smaller percentages were predicted to have originated in the Alligator, North, Pasquotank, Perquimans and Roanoke rivers (Table 4). Of the river herring captured in the Scuppernong River, those that were predicted to originate from the Chowan River had a high mean probability, 0.86, of originating from that river (Table 5). Of the Scuppernong River fish from the 2005-year class 40% were predicted to originate from the Alligator River, 40% from the Chowan River and 20% from the Roanoke River (Table 6).

In total 46.91% of the blueback herring analyzed in this study were predicted as originating from the Chowan River (Table 7). Lesser percentages of blueback herring were predicted as originating from the Alligator, Little, North, Pasquotank, Perquimans, Roanoke and Scuppernong rivers (Table 7).

Discussion

Catch

Of 148 blueback herring collected, 64% were captured in the Chowan River, 14% in the Perquimans River and 22% in the Scuppernong River. Female blueback herring were larger than male blueback herring, consistent with other studies (Loesch and Lund 1977; Durbin et al. 1979; McBride et al. 2010), and the mean age of spawning fish was between 4 and 5 consistent with what has been found in other studies (Loesh and Lund 1977; Moser and Patrick 2000; Rulifson et al. 2009).

Elemental Concentrations

Significant differences in the concentrations of Sr and Ba were found in the otoliths of blueback herring captured in the Chowan, Perquimans, and Scuppernong rivers. Zapf (2012, Chapter 3) found significant differences in the concentrations of Mg, Mn, Sr and Ba in the otoliths of juvenile river herring caught in eight Albemarle Sound tributaries, and Mohan et al. (2012) found significant differences in Mn, Sr, and Ba concentrations in the otoliths of juvenile striped bass held in cages of four Albemarle Sound habitats: Batchelor Bay, the Perquimans, Pasquotank and Alligator rivers. In general, elemental concentrations from adult fish otoliths, matched what was found in the otoliths of juveniles with a few exceptions.

Juvenile river herring captured in the Scuppernong River had increased Mg compared to juvenile river herring collected in the Chowan and Perquimans rivers (Zapf 2012, Chapter 3). However, this was not reflected in otoliths of adults captured in the Scuppernong River because the 2009 Scuppernong adults were predicted to originate from a number of sources, but not from the Scuppernong River itself.

Juvenile river herring from the Scuppernong River were found to have increased otolith Mn, compared to Chowan and Perquimans river juveniles (Zapf 2012, Chapter 3*),* while no significant differences in Mn were found in the otoliths of adults captured in the Chowan, Perquimans and Scuppernong rivers. Again this is probably because small percentages of adult fish were predicted as originating from the Scuppernong River.

Adult blueback herring captured in the Chowan and Scuppernong rivers had similar otolith Sr, while juveniles captured in the Scuppernong River had higher otolith Sr than juveniles from the Chowan River (Zapf 2012, Chapter 3). This is likely because the majority of the blueback herring returning to the Scuppernong River were predicted to originate from the Chowan and Roanoke rivers, both of which had juveniles with low otolith Sr (Zapf 2012, Chapter 3).

Chowan River adult blueback herring had significantly higher otolith Ba than fish from the Perquimans and Scuppernong rivers. Juvenile river herring from the Perquimans River had higher otolith Ba than fish from the Chowan and Scuppernong rivers (Zapf 2012, Chapter 3). High percentages of adult fish from other sources (particularly the Alligator River) being captured in the Perquimans River may have altered mean Ba concentrations for these fish, causing Chowan River adult fish to have significantly higher mean Ba concentrations.

Classification

Using QDFA, adult blueback herring from all year classes (2002-2009) classified to the Chowan River with 84.44% accuracy, the Perquimans River with 76.19% accuracy, and the Scuppernong River with 60% accuracy. This suggests fish caught within the same river have similar otolith elemental concentrations allowing them to be classified to that river. It is also suggests elemental signatures remain somewhat stable over time, as the sample represents the 2002-2007 year classes and fish were still classified to rivers of capture with high accuracy. This result is not entirely surprising because while dissolved elemental concentrations in rivers may fluctuate between seasons, they are thought to remain stable from year to year (Wells et al. 2003; Bickford and Hannigan 2005). Comparing dissolved elemental concentrations measured in water samples collected in 2010 to the results of a similar study conducted in 2008 (Mohan et al. 2012), Zapf (2012, Chapter 2) concluded there may be evidence that some elemental concentrations in some Albemarle Sound tributaries may be stable on an annual basis.

Classifying adult fish using river specific elemental signatures results in classification discrepancies between this method and the adult otolith classification method. For example, when using ground truthed elemental signatures to classify adult fish to the Chowan River, 64.44% of blueback herring captured in the Chowan River were predicted to originate from the Chowan River with high probability. This is far lower than the 84.44% of blueback herring classified to the Chowan River using only elemental concentrations in adult otoliths. However, 17.78% of adult blueback herring from the Chowan River were predicted to have originated from the Roanoke River; in total, 82.22% of adult blueback herring captured in the Chowan River were predicted to originate in either the Chowan or Roanoke river. This number is much closer to what was found using only adult otolith signatures. Elemental signatures in the otoliths of juvenile river herring captured in the Chowan and Roanoke Rivers were similar, and otoliths from both rivers had elevated Ba and low Sr concentrations, which clearly separated them from other rivers (Zapf 2012, Chapter 3*)*. Because no adult river herring from the Roanoke River were analyzed, adult fish from the Chowan River could not be misclassified as Roanoke River fish. Therefore, Roanoke River fish were classified with Chowan River fish because signatures from the Roanoke and Chowan rivers were similar. Using only adult otoliths to classify fish may result in misclassifications, particularly if some watersheds are not represented in the sample. Similar trends were observed when analyzing adult otoliths by year class.

Classification using only adult otoliths initially revealed high rates of natal homing to the Chowan, Perquimans, and Scuppernong rivers. However, when compared to results obtained by classification based on river specific signatures, results seemed to be less precise. Because adult fish were not collected from every tributary it was not possible to classify adult fish to every tributary using adult otoliths, because fish originating outside the Chowan, Perquimans, and Scuppernong rivers had to be classified to one of the three rivers. Overall, classification using only adult otoliths may be useful in identifying natal homing and straying rates of fish over a large geographic area (Edmonds et al. 1991; Edmonds et al. 1992; Patterson et al. 1999; Miller et al. 2005), but may be less useful when attempting to discriminate natal origins of fish over smaller scales, due to similarity in elemental signatures of neighboring watersheds (Zapf 2012, Chapter 2 and 3). However, this information may be useful in support of other more precise methods.

Natal Homing

This study did not directly assess percentages of river herring originating outside of Albemarle Sound watersheds. Evidence from studies employing a number of methods suggests that river herring return to natal rivers to spawn (Messieh 1977; Jessop 1994; Bentzen and Patterson 2005; Willis 2006), and the majority of the fish in this study classified to Albemarle Sound tributaries with relatively high probabilities. Therefore, it seems reasonable to assume the majority of river herring returning to Albemarle Sound tributaries to spawn originated from the Albemarle Sound. American shad, an anadromous alosine, is thought to return to natal rivers to spawn (Melvin et al. 1986), and some evidence suggests spawning American shad return to natal tributaries (Carscadden and Leggett 1975). However, using otolith microchemistry techniques Walther et al. (2008) estimated 6% of American shad returning to the York River, Virginia originated from other Atlantic Coast watersheds, suggesting that while the majority of American shad home to natal rivers to spawn there is some straying. Walther et al. (2008) concluded based on their study, and a previous study by Olney et al. (2006) that while American shad largely home to natal watersheds lower percentages home to natal tributaries of the York River.

Differing rates of natal homing for blueback herring were predicted for the Chowan, Perquimans, and Scuppernong rivers. 64.4% of the blueback herring captured in the Chowan River were predicted as originating from the Chowan River, 28.6% of the blueback herring captured in the Perquimans River were predicted as originating in the Perquimans River, and no blueback herring captured in the Scuppernong River were predicted as originating from the Scuppernong River. Examining straying by year class indicates straying rates do vary slightly between year classes, with a slight trend toward older fish straying more. However, low sample sizes prevent the identification of any strong trend.

Straying was somewhat common between neighboring rivers, particularly the Chowan and Roanoke rivers. Mixing of Chowan River and Roanoke River blueback herring seems reasonable particularly if olfaction is used by river herring to discriminate natal watersheds (Thunberg 1971) due to the close proximity of these rivers (Chapter 1,

Figure 1). Despite the predominance of small scale straying, there does seem to be straying over longer distances. Many fish that were predicted to originate from the Alligator River (easternmost tributary) were captured in the Chowan (westernmost), Perquimans (north central) and Scuppernong rivers (south central).

Employing mark recapture of spawning adult river herring in the Saint John River, New Brunswick, Jessop (1994) estimated 63-97% of river herring show fidelity to specific sites within the river. Examining meristic characteristics of alewife returning to the St. John River, New Brunswick Messieh (1977) concluded 20-82% of alewife show site fidelity to natal tributaries and are not as specific in homing to natal tributaries to spawn, compared to American shad and Atlantic salmon. Bentzen and Patterson (2005) found genetic differences in alewife from the St. Croix River, Maine and the Gaspreau and LaHave Rivers in Nova Scotia, suggesting spawning alewife home to natal watesheds. In addition, Bentzen and Patterson (2005) found small but significant genetic differences in alewife collected in two tributaries of the St. Croix River, Dennis and Milltown streams. This finding led to the conclusion that to some degree alewife home to natal tributaries (Bentzen and Patterson 2005; Willis 2006). Gahagan (2010), concluded 85% of alewife and 81% of blueback herring returning to tributaries of the Connecticut River were homing to natal streams, however Gahagan (2010) cautioned that these results should be interpreted cautiously due to the analytical methods used and the geographic and temporal scales investigated.

Ocean tagging of river herring in the inner Bay of Fundy, Canada, indicated that river herring are able to migrate long distances along the eastern seaboard of North America (Rulifson et al. 1987). While the majority of alewife (known as “gaspereau” in

Canada) were recaptured in streams in Nova Scotia and New Brunswick, at least two blueback herring tagged in the inner Bay were recovered from the Roanoke River the following spring (Rulifson et al. 1987).

While results of my study of homing rates (0-64%) fall within the range of reported homing rates for alewife and blueback herring, they are on the low end and it is unclear whether reports of alewife homing rates can be used as a proxy for blueback herring. Low rates of homing from my study are partially due to low rates of homing in the Perquimans and Scuppernong rivers. Two potential explanations for this are 1) poor survival of individuals from these rivers, and 2) fish from these locations are spawning in other locations. Zapf (2012, Chapter 3) concluded the Perquimans and Scuppernong rivers provide poor nursery habitat for juvenile river herring based on growth of juveniles caught in these locations. Poor growth of juveniles in these locations could cause poor survival to the adult stage. Overall, low percentages of adults predicted as originating from the Perquimans and Scuppernong rivers suggests poor survival may be possible. In addition, Zapf (2012, Chapter 3) found juvenile river herring move from the Perquimans River to better nursery habitats in western Albemarle Sound. This movement may cause juvenile river herring to not imprint on a ‘true’ natal river. Western Albemarle Sound habitats utilized by juvenile river herring are located at the mouth of the Chowan and Roanoke rivers. If river herring use olfaction to distinguish between natal waters, as suggested by Thunberg (1971), they may home to the Chowan and Roanoke rivers. The small percentage (4.4%) of adult blueback herring captured in the Chowan River that are predicted as originating from the Perquimans suggests this is a possibility.

Conclusions

Two methods were used to examine stock structure, and natal homing of blueback herring returning to Chowan, Perquimans and Scuppernong rivers to spawn. Based on these analysis natal homing rates ranging from 0-64% were calculated, results which fall on the low end of results reported for alewife and blueback herring in other watersheds. Blueback herring returning to the Chowan River had the highest homing rates (64%), Perquimans River blueback herring had homing rates of 28%, and no blueback herring returning to the Scuppernong River were predicted as originating from the Scuppernong River. Low homing rates to the Perquimans and Scuppernong rivers is likely the result of these locations being poor river herring nursery habitats (Zapf 2012, Chapter 3).

Beck et al. (2001) proposed that the most important nursery habitats are those that produce the largest number of adults that recruit to the adult population. Based on the low numbers of adult river herring predicted to originate from the Perquimans and Scuppernong rivers it appears the findings of Zapf (2012, Chapter 3) may be valid. There does appear to be some relationship between low growth of juveniles in the Perquimans and Scuppernong rivers and recruitment to the adult population, and these locations are probably poor river herring nursery habitats.

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Table 1. – Mean total length, weight, and age (± S.E.) of female and male river herring captured in the Chowan (CHOW), Perquimans (PERQ), and Scuppernong (SCUPP) rivers.

River	Sex	n	Total Length (mm)	Weight (g)	Age
CHOW	F	28	259.71 ± 2.45	147.85 ± 4.92	4.57 ± 0.12
	M	66	248.47 ± 2.05	125.32 ± 2.23	4.48 ± 0.08
PERQ	F	12	272.33 ± 2.98	184.69 ± 8.02	4.75 ± 0.22
	M	8	265.38 ± 3.63	154.36 ± 4.14	4.5 ± 0.33
SCUPP	F	9	N/A	N/A	4.56 ± 0.34
	M	23	N/A	N/A	4.48 ± 0.18

Table 2. – Results of Kruskal-Wallis tests examining differences in the mean concentrations of Mg, Mn, Sr, and Ba in the otoliths of river herring captured in the Chowan, Perquimans, and Scuppernong rivers. Differences were considered significant if p-value was < 0.05.

Variable	chi-square	df	p-value
Mg	0.7699	2	0.6805
Mn	2.1936	2	0.3339
Sr	16.5323	2	0.0003
Ba	21.9184	2	<0.0001

Table 3. – Results of quadratic discriminant function analysis used to classify adult river herring to river of capture. Chowan = CHOW, Perquimans = PERQ, and Scuppernong = SCUPP.

			Predicted River of Origin			
Year Classes	River of Capture	n	CHOW	PERQ	SCUPP	% Correct
2002-2006	CHOW	45	38	6	1	84.44
	PERQ	21	0	16	5	76.19
	SCUPP	15	3	3	9	60
2005	CHOW	23	15	5	3	65.22
	PERQ	8	0	8	0	100
	SCUPP	5	0	0	5	100
2006	CHOW	18	17	1		94.44
	PERQ	8	1	7		87.5

Table 4. – Predicted river of origin of adult blueback herring caught in the Chowan (CHOW), Perquimans (PERQ), and Scuppernong (SCUPP) rivers based on multi-variate signatures established using otoliths of juvenile alewife and blueback herring captured in the Alligator (ALLI), Chowan (CHOW), Little, North, Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim rivers (Zapf 2012, Chapter 2).

		Predicted River of Origin (%)							
Capture Location	n	ALLI	CHOW	LITTLE	NORTH	PASQ	PERQ	ROAN	SCUPP
CHOW	45	11.11	64.44	0	0	2.22	4.44	17.78	0
PERQ	21	23.81	14.29	9.52	4.76	9.52	28.57	4.76	4.76
SCUPP	15	20	40	0	6.67	6.67	6.67	20	0

Table 5. – Mean probability (± S.E.) of blueback herring collected in the Chowan (CHOW), Perquimans (PERQ), and Scuppernong (SCUPP) rivers being classified to predicted river. Alligator = ALLI, Chowan = CHOW, Little = LITTLE, North = NORTH, Pasquotank = PASQ, Perquimans = PERQ, Roanoke = ROAN, and Scuppernong = SCUPP.

River of Capture	Predicted River	n	Mean Probability (± S.E.)
CHOW	ALLI	5	0.64 ± 0.08
	CHOW	29	0.95 ± 0.02
	PASQ	1	0.99
	PERQ	2	0.67 ± 0.13
	ROAN	8	0.8 ± 0.05
PERQ	ALLI	5	0.84 ± 0.08
	CHOW	3	0.89 ± 0.08
	LITTLE	2	0.74 ± 0.02
	NORTH	1	0.68
	PASQ	2	0.73 ± 0.27
	PERQ	6	0.84 ± 0.08
	ROAN	1	0.96
	SCUPP	1	0.83
SCUPP	ALLI	3	0.68 ± 0.18
	CHOW	6	0.86 ± 0.06
	NORTH	1	1
	PASQ	1	0.53
	PERQ	1	0.92
	ROAN	3	0.75 ± 0.09

Table 6. - Predicted river of origin of adult river herring captured in the Chowan (CHOW), Perquimans (PERQ), and Scuppernong (SCUPP) rivers by year class. Alligator = ALLI, Pasquotank = PASQ, Roanoke = ROAN.

			Percent in Year Class					
Capture Location	Predicted River	n	2002	2003	2004	2005	2006	2007
CHOW	ALLI	5	0	0	0	80	20	0
	CHOW	29	0	0	0	41.4	51.7	6.9
	PASQ	1	0	0	0	100	0	0
	PERQ	2	0	0	0	100	0	0
	ROAN	8	0	0	0	50	25	25
PERQ	ALLI	5	20	0	0	60	20	0
	CHOW	3	0	0	0	0	66.7	33.3
	LITTLE	2	0	0	0	50	50	0
	NORTH	1	0	0	0	0	100	0
	PASQ	2	0	0	50	50	0	0
	PERQ	6	0	0	16.7	33.3	50	0
	ROAN	1	0	0	100	0	0	0
	SCUPP	1	0	0	0	100	0	0
SCUPP	ALLI	3	0	0	33.3	66.7	0	0
	CHOW	6	0	50	16.7	33.3	0	0
	NORTH	1	0	0	0	0	100	0
	PASQ	1	0	100	0	0	0	0
	PERQ	1	0	0	100	0	0	0
	ROAN	3	0	33.3	33.3	33.3	0	0

Table 7. – Percentage of river herring caught in the Chowan (CHOW), Perquimans (PERQ), and Scuppernong (SCUPP) rivers, predicted to originate from the Alligator (ALLI), Chowan (CHOW), Little, North, Pasquotank (PASQ), Perquimans (PERQ), Roanoke (ROAN), Scuppernong (SCUPP), and Yeopim rivers based on multi-variate signatures established using otoliths of juvenile river herring captured in these tributaries (Zapf 2012, Chapter 2).

Predicted River	Percent of Sample
ALLI	16.05
CHOW	46.91
LITTLE	2.47
North	2.47
PASQ	4.94
PERQ	11.11
ROAN	14.81
SCUPP	1.23
YEOPIM	0

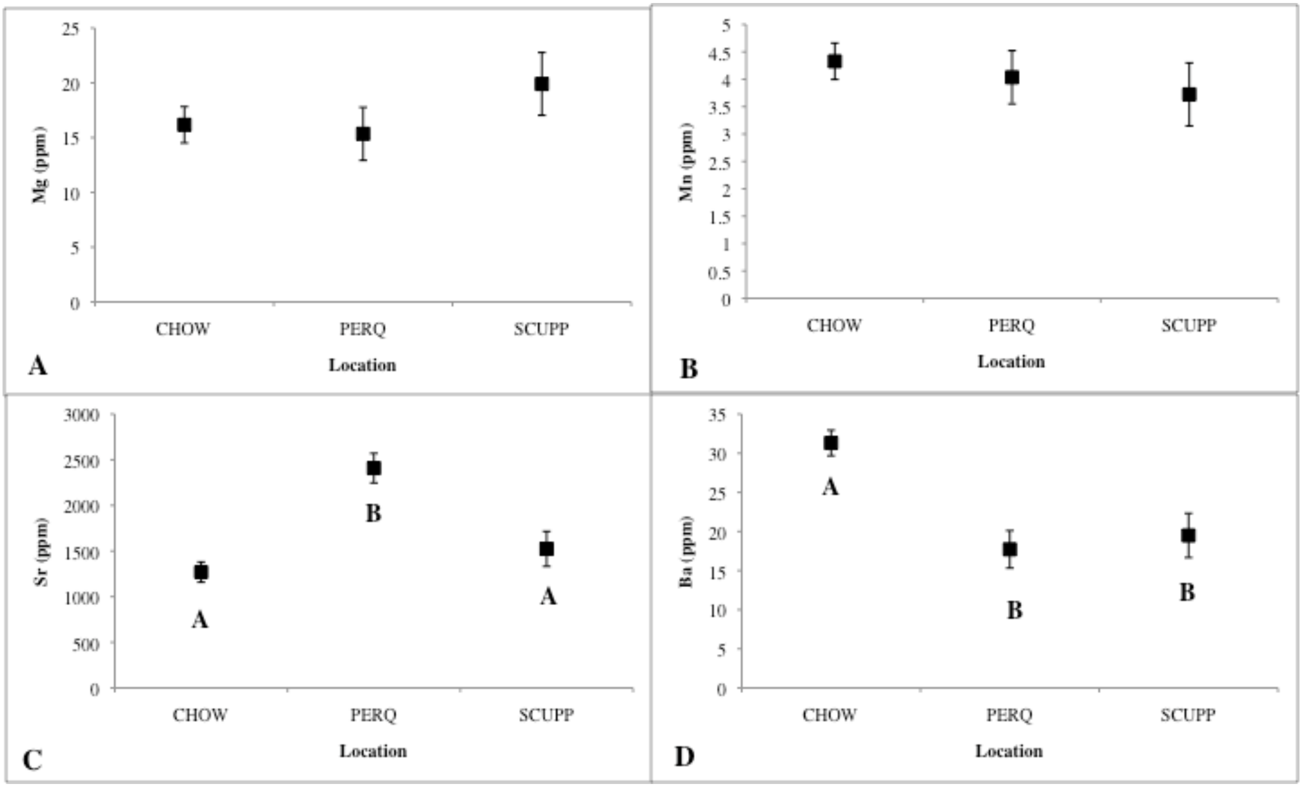

Figure 1. – Mean (± S.E.) Mg (A), Mn (B), Sr (C), and Ba (D) (ppm) in the otoliths of river herring captured in the Chowan (CHOW), Perquimans (PERQ), and Scuppernong (SCUPP) Rivers. In panels C and D locations not connected by the same letter were found to be significantly different based on Tukey's HSD test.

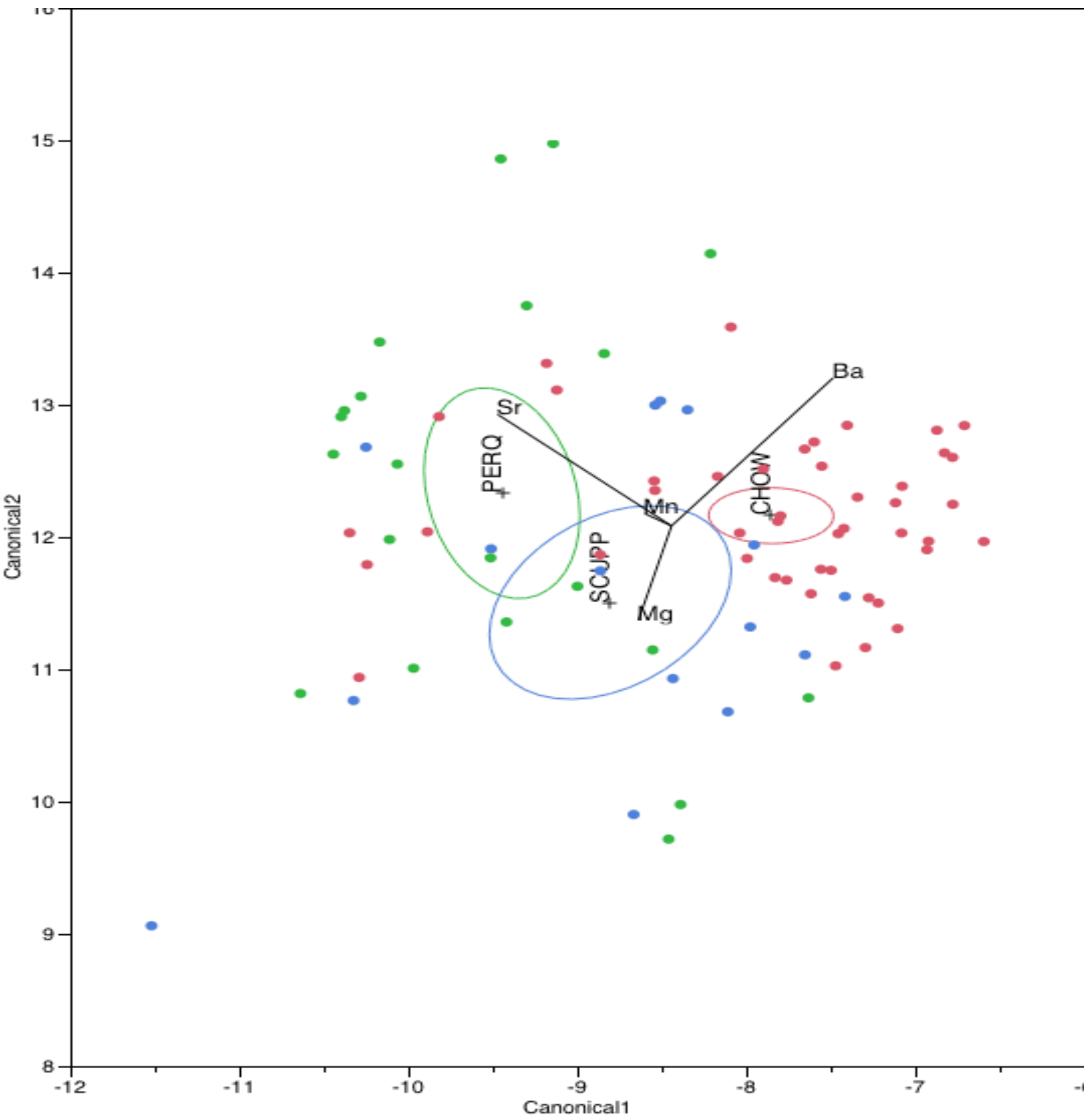

Figure 2. – Plot of first two canonical variates obtained using quadratic discriminant function analysis to classify adult river herring from the 2002-2007 year classes to their river of capture. Chowan (CHOW) = Red, Perquimans (PERQ) = Green, and Scuppernong (SCUPP) = Blue. Group centroids are marked with (+), ellipses represent 95% confidence ellipse for each location.

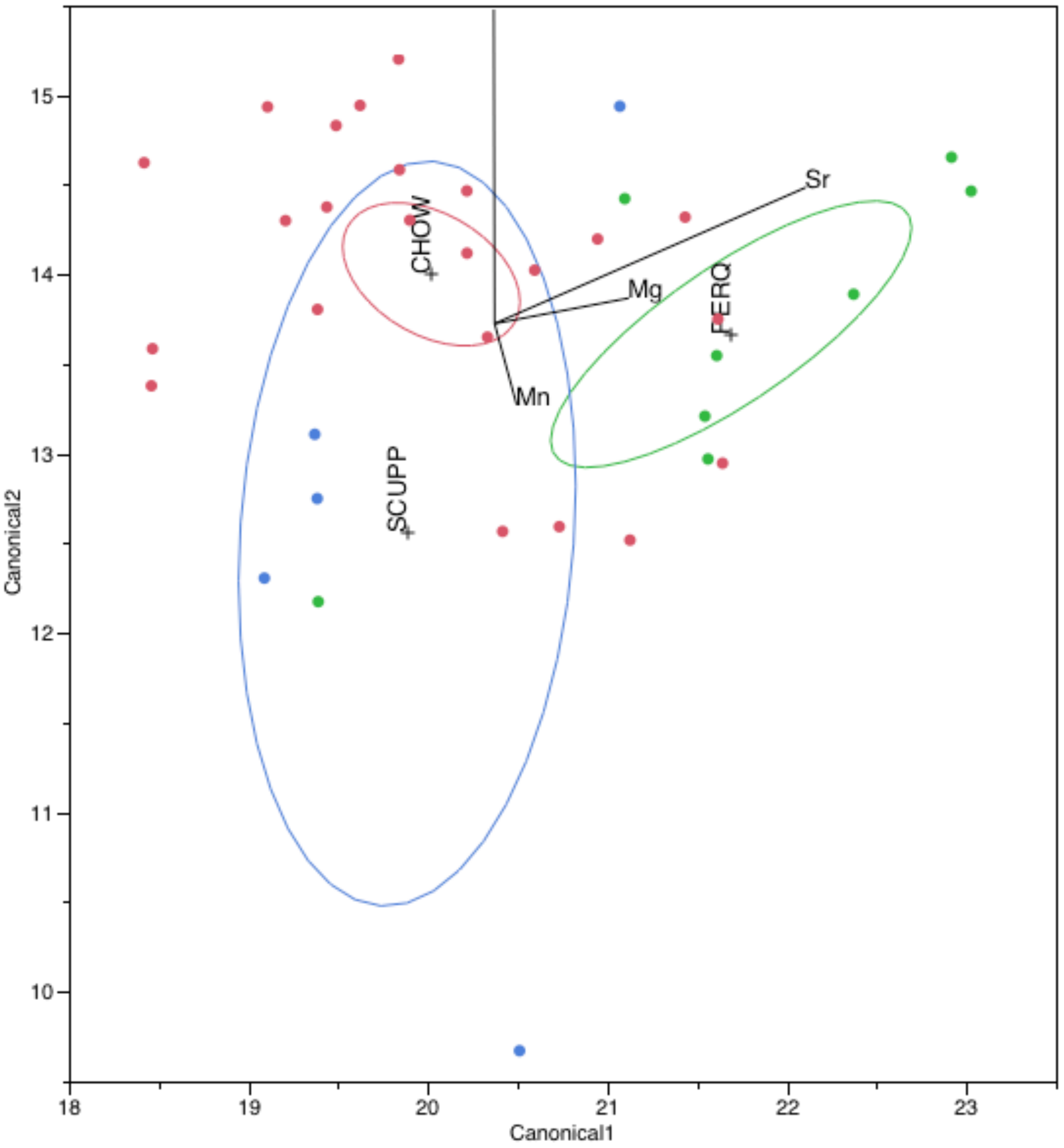

Figure 3. – Plot of first two canonical variates obtained using quadratic discriminant function analysis to classify adult river herring from the 2005 year class to their river of capture. Chowan (CHOW) = Red, Perquimans (PERQ) = Green, and Scuppernong (SCUPP) = Blue. Group centroids are marked with (+), ellipses represent 95% confidence ellipse for each location.

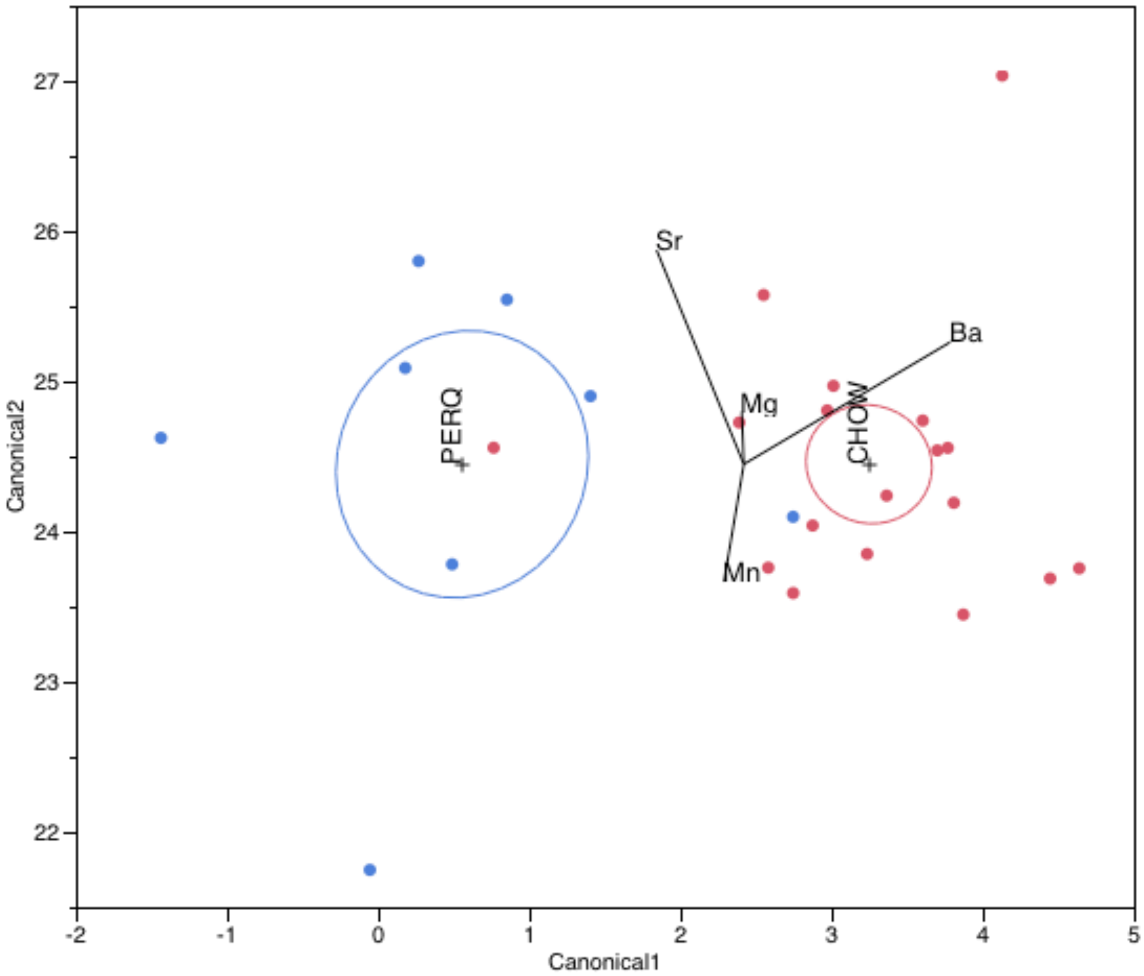

Figure 4. – Plot of first two canonical variates obtained using quadratic discriminant function analysis to classify adult river herring from the 2006 year class to their river of capture. Chowan (CHOW) = Red, Perquimans (PERQ) = Blue. Group centroids are marked with (+), ellipses represent 95% confidence ellipse for each location.

Chapter 5: River Herring Nursery Habitat

Introduction

Historically, tributaries and western portions of Albemarle Sound, North Carolina, have been designated as river herring nursery habitat (Copeland et al. 1983). However, this designation was made based on presence of river herring eggs, larvae and juveniles. Beck et al. (2001) suggests the most important nursery habitats produce more adult recruits than other juvenile habitats based on a combination of four criteria: higher density, growth, survival of juveniles, and movement to adult habitats. The overarching goal of this study was to identify important river herring nursery habitats in Albemarle Sound using criteria proposed by Beck et al. (2001).

Methods

A two-fold approach was used to evaluate river herring nursery habitat. First, juvenile river herring were collected from tributaries and western portions of Albemarle Sound during the summer of 2010. Total length, condition and growth rate of these fish were measured. In addition, origins of river herring collected in western Albemarle Sound habitats were predicted using elemental signatures in otoliths. Habitats in which river herring had high total lengths, condition, and growth rates were considered to be higher quality nursery areas. Second, adult river herring were collected from the Chowan, Perquimans and Scuppernong Rivers during the 2010-spawning run. Natal origins of these fish were predicted using elemental signatures in otoliths. Elemental signatures were compared to dissolved elemental ratios from water samples collected in a number of Albemarle Sound tributaries to ground truth what was found in otoliths. Rivers that produced high percentages of adult fish were considered to be higher quality nursery habitat. Results from the two approaches were combined to designate high quality river herring nursery habitat in Albemarle Sound.

Results/Discussion

Water chemistry

Water samples were collected monthly from the Alligator, Chowan, Perquimans, Roanoke, and Scuppernong rivers from June-October 2010. These sampling locations and dates were chosen to coincide with the collection of juvenile river herring.

Sr:Ca was high in water samples from the Perquimans River, and had mid-level concentrations in samples from the Alligator River. Sr:Ca was low in water samples from the Chowan, Roanoke and Scuppernong Rivers. These results compared favorably to Sr concentrations in the otoliths of juvenile river herring collected from these locations. Sr was high in the otoliths of fish from the Perquimans River, had mid-level concentrations in the otoliths of fish from the Alligator River, and low in the otoliths of fish from the Chowan, Roanoke, and Scuppernong rivers.

Ba:Ca was high in water samples from the Perquimans River, had mid-level concentrations in water samples from the Chowan, Roanoke and Scuppernong rivers, and low in water samples from the Alligator River. These results compared favorably to Ba concentrations in the otoliths of juvenile river herring from these locations. Ba was high in the otoliths of fish from the Perquimans River, had mid-level concentrations in the otoliths of fish from the Chowan, Roanoke and Scuppernong rivers, and low in the otoliths of fish from the Alligator River.

Manganese did not follow the patterns of Sr and Ba. Mn:Ca was high in water samples from the Perquimans and Roanoke rivers, somewhat high in water samples from the Chowan and Scuppernong rivers and low in water samples from the Alligator River. These results did not compare well with what was found in the otoliths of juvenile river herring from these locations, although the finding of low Mn:Ca in Alligator River water samples did match with low Mn concentrations in the otoliths of river herring from the Alligator River. Mn:Ca in water samples from the Chowan, Perquimans, and Roanoke rivers compared favorably to what was found in otoliths from these locations. Differences in Mn:Ca in water samples from these locations were not large and neither were the differences in Mn between otoliths from these locations. However, elevated Mn in the otoliths of river herring from the Scuppernong River did not match the lower Mn:Ca ratios found in water samples from the Scuppernong River. One explanation is that the only river herring otoliths obtained from Scuppernong River fish were collected at the beginning of June, a date which may not be reflected in the water sampling schedule.

Mg:Ca was only detected in water of the Alligator, Perquimans, and Scuppernong rivers, and was only detected consistently in the Alligator River. Because of this pattern it was difficult to make comparisons with what was found in otoltihs. However, otoliths of river herring from the Alligator River had very low Mg concentrations compared to what was found in water samples. In addition, Scuppernong River fish had very high Mg in their otoliths and the Scuppernong River was one location were Mg:Ca was measured.

Mg concentrations in the otoliths of river herring from the Perquimans River was slightly elevated compared to other rivers and the Perquimans River was one location where Mg:Ca was measured. Overall, Mg:Ca in water samples appeared to be a poor predictor of Mg in otoliths. This is not surprising since Mg is physiologically regulated by fish (Campana 1999), and the relationship between Mg concentrations in water and Mg concentrations in otoliths is poorly understood (Wells et al. 2003; Dorval et al. 2007; Mohan et al. 2012).

Classification of water samples to river of collection followed a similar pattern to classification of juvenile river herring to river of capture, despite using only Sr:Ca and Ba:Ca values to classify water samples. Water samples and fish were both classified to the Chowan and Roanoke rivers based primarily on Ba concentrations, and water samples and fish were classified to the Perquimans River based on Sr concentrations. When classifying water samples, the Alligator River and Scuppernong River had similar multi-variate means. When classifying fish, the Alligator and Scuppernong rivers had very different multi-variate means. This suggests that using more variables in classification would increase differences between the two rivers. Overall, otolith chemistry appeared to reflect water chemistry, allowing elemental signatures in otoliths to be used in classification of river herring to natal watersheds.

Juveniles

Significant differences in multi-elemental signatures were found between rivers, which allowed juvenile river herring to be classified to their river of capture with between 75 and 100% accuracy using quadratic discriminant function analysis. These multi-elemental signatures were used to predict the river of origin of juvenile river herring captured in northwestern and southwestern Albemarle Sound habitats. Predicting the origins of these fish is important because it provides examples of habitat connectivity and information on survival of individuals from different habitats. This is particularly important when investigating potentially degraded habitats and whether fish from these habitats survive and utilize other habitats.

Growth was used as an indicator of quality nursery habitat. Three growth metrics were used: total length, condition and growth rate. Habitats in which juvenile river herring had greater total length, condition, and growth rates were considered to be better nursery habitats. Based on these growth metrics the Alligator, Chowan, Pasquotank, and Roanoke rivers along with northwest and southwest Albemarle Sound habitats were considered high quality alewife nursery habitat, while the Perquimans and Scuppernong rivers were considered to be lower quality habitats. Based on the growth metrics used in this study northwest and southwest Albemarle Sound habitats were considered to be high quality blueback herring nursery habitat, while riverine habitats, particularly the Perquimans River, were considered lesser habitats.

In general, northwest and southwest Albemarle Sound habitats were considered to be high quality nursery habitat for both alewife and blueback herring. Large numbers of alewife and blueback herring caught in these habitats were predicted as originating in the Chowan and Perquimans rivers. A portion of juvenile alewife captured in the northwest and southwest sound was predicted as originating from the Perquimans River. This finding suggests that while the Perquimans River may not function as high quality alewife nursery habitat, alewife originating from the Perquimans River may seek out higher quality habitats. Lesser numbers of alewife appeared to have migrated from other rivers implying that other rivers may offer sufficient nursery habitat for alewife. Large numbers of blueback herring captured in northwest and southwest sound habitats were predicted as originating from the Chowan, Roanoke and Perquimans rivers. Based on growth metrics it was found that non-riverine Albemarle Sound habitats were better nursery areas for blueback herring than riverine habitats. This could explain the movement from riverine to non-riverine habitats by juvenile blueback herring.

Adults

Adult blueback herring were collected from the Chowan and Perquimans rivers in April and May 2010 and from the Scuppernong River in 2009. No alewife were collected during this portion of the study. Significant differences were found in the concentrations of Sr and Ba in otoliths. Differences in elemental concentrations allowed fish from all year classes to be classified to their river of capture with between 60 and 84.44% accuracy. Fish from the 2005-year class classified to their rivers of capture with between 65.22 and 100% accuracy, and fish from the 2006-year class classified to their rivers of capture with between 87.5 and 94.44% accuracy. These findings show that fish caught within rivers have similar elemental concentrations in their otoliths allowing them to be classified together. These results show that groups of fish caught in the same rivers have elemental concentrations in their otoliths that are similar enough for them to be grouped together. However, this finding implies some degree of natal homing in blueback herring.

Elemental signatures from juvenile river herring otoliths were used to predict the origins of adult river herring. Using this method 64.44% of the blueback herring returning to the Chowan River were predicted as originating from the Chowan River, 28.57% of the blueback herring returning to the Perquimans River were predicted as originating from the Perquimans River, and no blueback herring returning to the Scuppernong River were predicted as originating from the Scuppernong River. These findings suggest low rates of natal homing to the Perquimans and Scuppernong rivers. While the numbers may be somewhat biased because fish were only collected from the Chowan, Perquimans and Scuppernong rivers almost half (46.91%) of blueback herring were predicted as originating from the Chowan River. Smaller percentages were predicted as originating from the Alligator (16.05%), Roanoke (14.81%) and Perquimans (11.11%) rivers. Very low percentages of blueback herring were also predicted as originating from the Little, North, Pasquotank, and Scuppernong rivers. No blueback herring were predicted as originating from the Yeopim River.

Nursery Habitat

Beck et al. (2001) proposed that important nursery habitats are those areas that produce more adult recruits than other juvenile habitats based on a combination of four criteria: higher density, growth, survival of juveniles, and movement to adult habitats. Applying that definition to this study, it can be concluded that the Alligator, Roanoke and Chowan rivers along with northwestern and southwestern Albemarle Sound habitats are important river herring nursery habitat. These areas supported high growth in juvenile alewife and blueback herring and contributed large percentages of adults to the spawning population. Though no adult river herring were captured in the Alligator or Roanoke Rivers they still made up a high percentage of the adult river herring returning to the Chowan, Perquimans and Scuppernong rivers. This suggests that these rivers provide high quality river herring nursery habitat.

Juvenile river herring captured in the Perquimans River had low growth potential. However, many of the juvenile river herring captured in western Albemarle Sound habitats were predicted as originating from the Perquimans River. Therefore, despite the Perquimans River itself appearing to be degraded nursery habitat, river herring from the Perquimans River do move to higher quality habitats. In addition, 11.11% of the adult blueback herring returning to Albemarle Sound tributaries were predicted as originating from the Perquimans River. While the Perquimans River does not seem to be the best river herring nursery habitat in Albemarle Sound, it certainly is not the worst. A portion of the juvenile river herring spawned in the Perquimans River survive to utilize higher quality habitats, and a percentage of these fish survive to spawn as adults. The connection between the Perquimans River and western Albemarle Sound habitats, along with the connection between the Chowan River and western Albemarle Sound habitats, demonstrates the importance of connectivity between habitats in the survival of juvenile fish.

Based on findings from this study the Scuppernong River is probably a poor nursery habitat for river herring. Juvenile river herring captured in the Scuppernong River had low growth and did not appear to leave the river to utilize other higher quality habitats. The lack of juvenile Scuppernong River river herring in non-riverine Albemarle Sound habitats possibly indicates poor survival. In addition, only 1.23% of adult river herring returning to Albemarle Sound tributaries were predicted as originating from the Scuppernong River, and none of these fish were captured in the Scuppernong River. Blueback herring in spawning condition were captured in the Scuppernong; however, all of these fish were predicted as originating in locations other than the Scuppernong River. While fish do seem to be spawning in the Scuppernong River, they do not appear to survive to adulthood and return to Albemarle Sound to spawn. This implies the Scuppernong River may be acting as a population sink, where the spawning population of blueback herring in the Scuppernong River is maintained by strays from other rivers and contributes no recruits to the spawning population (Pulliam 1988). Low percentages of blueback herring were predicted as originating from the Little, North, and Pasquotank rivers and no fish were predicted as originating from the Yeopim River. However, because no adult river herring were collected from these rivers, it is difficult to say whether these locations are poor river herring nursery habitat. Growth of river herring in the Pasquotank and Yeopim rivers was somewhat high suggesting they might be important river herring nursery habitat. However, without collecting adult river herring from these rivers it is difficult to assess survival to the adult stage.

Conclusions and Recommendations

Of the 11 Albemarle Sound habitats examined in this study, the Alligator, Chowan, and Roanoke rivers along with non-riverine northwest and southwest Albemarle Sound habitats seem to offer high quality nursery habitat for alewife and blueback herring. This conclusion is based on growth of juveniles in these habitats and survival of fish from these habitats to the adult stage. Although no adult river herring were collected in the Alligator or Roanoke rivers, adult fish predicted as originating from these rivers were not uncommon in the sample, implying the quality of these rivers as nursery habitat. It is more difficult to assess the quality of the North, Little, Pasquotank, and Yeopim rivers as river herring nursery habitat. While inferences can be made based on growth of juveniles in these habitats, no adults were collected in these rivers and few adults were predicted as originating from these rivers.

Of the habitats examined in this study the Perquimans and Scuppernong rivers appear to be poor river herring nursery habitat. Poor quality of these habitats could be due to decreased water quality, shoreline development, or impediments restricting access to spawning locations. Rulifson et al. (2009a) reported large numbers of confined animal feeding operations (CAFOs) in areas around the Perquimans and Scuppernong Rivers. Mohan et al. (2012) found elevated Mn in the otoliths of cage reared striped bass in the Perquimans River and suggested this could be the result of dissolved Mn being released from sediments during anoxic conditions (Rulifson et al. 2009a). In addition striped bass utilizing the Perquimans River had slower growth rates than fish in other rivers. Geffen et al. (2003) found elevated Mn in the otoliths of plaice *Pleuronectes platessa* captured near sewage sludge dumping grounds near the mouth of the Mersey River, so it is possible Mn in otoliths could be an indicator of degraded habitat. While high concentrations of Mn were not found in the otoliths of river herring captured in the Perquimans River, high Mn concentrations were found in the otoliths of river herring captured in the Scuppernong River. The large number of CAFOs in the Scuppernong River region (Rulifson et al. 2009a) along with high Mn in river herring otoliths, and poor growth of Scuppernong River river herring suggests the habitat may be degraded. In addition, water quality in the Scuppernong River may be degraded due to high dissolved nitrate, dissolved phosphorous, dissolved ammonia, and pesticides (Spruill et al. 1998). River herring ascending the Scuppernong River may not be able to access historical spawning grounds in Phelps Lake due to obstructions and low water levels (Rulifson et al. 2009b).

While this study highlights the need to restore degraded nursery habitats, like the Perquimans and Scuppernong rivers, it also demonstrates the importance of habitats like the Alligator, Chowan, and Roanoke rivers, as well as western portions of Albemarle Sound. These habitats are all considered to be quality river herring nursery habitat but they are very different watersheds. The Alligator River has minimal human development and is surrounded by the Alligator River National Wildlife Refuge. Minimum human development probably allows for ample river herring habitat in this area and could offer a significant buffer to runoff from agricultural activity and CAFOs in the region. The Chowan and Roanoke rivers are similar watersheds in that they both originate in Virginia and flow into the western Albemarle Sound. While there are many CAFOS located near the upper Chowan River there are no major urban areas in the region and, at this time, little shoreline development. It is likely that this has allowed for sufficient nursery habitat in the Chowan River. While the town of Plymouth, NC, does lie within the Roanoke River watershed it still appears to be an important river herring nursery habitat. The western Albemarle Sound is also an important river herring nursery area, likely for many of the same reasons it is an important nursery area for striped bass *Morone saxatilis* (Copeland et al. 1983).

Fish from the Alligator, Chowan, and Roanoke rivers appear to make up large portion of the spawning population and strongly supplement spawning runs in the Perquimans and Scuppernong rivers. Straying rates may be beneficial in that blueback herring may not continuously home to degraded habitats where spawning success is low (Hill et al. 2002). In addition, it is possible that with habitat improvement in degraded watersheds spawning populations could be reestablished. Conversely, degradation to quality habitats could drastically decrease Albemarle Sound river herring populations.

The state of North Carolina has designated Strategic Habitat Areas (SHAs) in Albemarle Sound, one goal of which is to protect spawning and nursery habitat for river herring (Deaton et al. 2010). The entire Chowan River, Roanoke River, most of the western Albemarle Sound shoreline, and large portions of the Alligator River are designated as Strategic Habitat Areas (Deaton et al. 2010). Findings from this study support the placement of existing strategic habitats, in terms of nursery habitat for river herring.

Limitations and Future Research

High concentrations of Mg at the core of otoliths, inconsistent with what was found in water samples and throughout the otolith, prevented analysis of the core of the otolith. Instead, a portion of the otolith just beyond the core was used as a proxy for natal origins. While this portion of the otolith is within the first few days of life it may not be reflective of the exact natal origin. Therefore, when natal origins are referred to in this study it would probably be more accurate to say natal nursery, or early life habitat. While not being able to analyze otolith cores is not ideal, it was not a limitation in this study.

This study was limited by the small number of juvenile and adult river herring captured in some watersheds. In addition, no juvenile river herring from eastern Albemarle Sound locations were analyzed during this study. Examining multiple year classes of juvenile river herring would elucidate whether trends in growth are consistent from year to year. In addition, it is necessary to examine the natal origins of river herring from multiple spawning runs over many years to determine true homing rates.

Based on findings from this and other studies it is clear that alewife and blueback herring should be analyzed separately (Schmidt et al. 2003). While this study found no significant differences in elemental concentrations in the otoliths of alewife and blueback herring captured simultaneously, differences in life history and habitat use suggest the two species may not be interchangeable. Therefore, conclusions made about blueback herring nursery habitat in this study may not be applicable to alewife and vice versa. Because no adult alewife were collected in this study it is necessary to collect alewife for a more thorough examination of alewife habitat. In addition, combining genetics with otolith analysis may yield stronger discrimination between river herring populations (Miller et al. 2005). Genetic analyses have been used successfully in investigating alewife population structure over small distances similar to those present in the Albemarle Sound (Bentzen and Patterson 2005; Willis 2006; Palkovacs et al. 2008).

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