Population Structure of River Herring in Albemarle Sound, North Carolina, Inferred from Geometric Morphometrics and Otolith Shape Analysis

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Abstract

Alewife Alosa pseudoharengus and Blueback Herring Alosa aestivalis, collectively known as River herring, use tributaries of the Albemarle Sound, North Carolina as spawning and nursery habitats. Stocks of these anadromous fish have experienced dramatic declines in North Carolina, and show no sign of recovery. Although the state has designated considerable resources to the management of river herring, we still do not fully understand river herring utilization of North Carolina's estuaries, and know little about the structure and composition of populations. Determining the population, or "stock" structure of species is crucial for the proper distribution of management efforts. We utilized two robust stock identification methods to identify distinct groups of River Herring. Using geometric morphometric analysis, we found that groups of juvenile Alewife and Blueback herring from different tributaries of the Albemarle Sound had significantly different overall body shapes, despite apparent mixing between groups. Overall body shape of adult Blueback Herring was not significantly different at the tributary level, but did differ significantly at the state level between North Carolina and New Jersey. Elliptical Fourier analysis of otolith shape revealed the same

pattern as geometric morphometric analysis on adult Blueback Herring, with significant differences in otolith shape at the state level but not the tributary level. Our results suggest that a portion of spawning adult River Herring returning to the Albemarle Sound may return to non-natal tributaries to spawn.

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CHAPTER 1: GENERAL INTRODUCTION

'River Herring' is a collective term applied to two similar alosine species: Alewife *Alosa pseudoharengus* and Blueback Herring *Alosa aestivalis*. These anadromous species spend their adult lives at sea and return to estuarine tributaries to spawn. River Herring 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 at al. 2009). Alewife and Blueback Herring, despite similarities in geographical range and life history, display differences in spawning behavior. Alewife spawn earlier and prefer lentic habitats such as deep pools and along-shore eddies, while Blueback Herring spawn later and prefer lotic systems such as main-stems of rivers (Loesch and Lund 1977; Messieh 1977). In North Carolina, River Herring spawn in coastal rivers and lakes from approximately March through June and return to the ocean shortly thereafter (Walsh et al. 2005). Juveniles migrate to the ocean after 3 to 9 months of life and return to natal tributaries after 3 to 5 years to spawn (Loesch and Lund 1977; Messieh 1977; Jessop 1994). River Herring are an important ecological, economic, and cultural component of coastal marine, estuarine, and riverine ecosystems. The two species provide an important energy-flow link between marine and freshwater food webs (West et al. 2010), and are an important prey resource for coastal birds and fishes (Walter and Austin 2003).

The majority of River Herring in North Carolina are found in the Albemarle Sound and its tributaries. This large oligohaline estuary (~45,500 km^2) is located in northeastern North Carolina and extends approximately 90 km from east to west, averaging approximately 12 km in width and 4-6 m in depth (Copeland et al. 1983; ASMFC 2012). Nine major tributaries drain into the sound including: the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers (Figure 1). The Albemarle Sound joins with Currituck Sound to the northeast and Roanoke and Croatan Sounds to the southeast, and connects to the Atlantic Ocean via Oregon Inlet.

River Herring populations have been declining since the 18th century (Limburg and Waldman 2009; Hall et al. 2012), and landings have decreased by 93% since 1970 (ASMFC 2012). Formerly robust commercial and recreational fisheries in North Carolina's sounds and rivers have completely collapsed (Schmidt et al. 2003) (Figure 2). Historically, River Herring have been caught for personal consumption and bait in every major North Carolina river system (NCDMF 2013). The Albemarle Sound and its tributaries once supported the most productive fisheries for River Herring on the entire U.S. east coast. These fisheries have virtually disappeared (Hightower et al.1996; Greene et al. 2009). Blueback Herring recruitment in the Chowan River averaged 28.9 million age-3 fish per year between 1972 and 1985. That average dropped to 3.6 million fish between 1986 and 2007 and fell to 522,000 fish from 2007-2012 (ASMFC 2012) (Figure 3). Alewife recruitment in the Chowan River averaged 7.5 million age-3 fish per year between 1972 and 1986. From 1987 to 2007 that average dropped to 587,000 fish and fell further to 317,000 fish between 2007 and 2012 (Figure 4). These declines in recruitment have contributed to drastic reductions in Spawning Stock Biomass (SSB) of Alewife and Blueback Herring (ASMFC 2012)

The 2005 North Carolina River Herring Stock Assessment stated that River Herring stocks were overfished and that overfishing was still occurring (ASMFC 2012). In 2007, North Carolina adopted the NC River Herring Fishery Management Plan that mandated a no-harvest provision for commercial and recreational River Herring fisheries in the state. Despite years of strict regulations and the 2007 provision, populations of River Herring have not shown signs of recovery (NCDMF 2013). Currently, River Herring are listed as *depleted* in the Albemarle Sound Area by the North Carolina Division of Marine Fisheries (NCDMF 2013).

The human population in North Carolina's coastal region has increased rapidly since 1980, leading to degradation of water quality and the destruction of aquatic habitats. Pollution from urban, agricultural, and industrial inputs has been attributed as the cause of declines in water quality in the Albemarle Sound (Spruill et al. 1998). Rulifson (1994) suggested that chemical pollution, turbidity, and low dissolved oxygen could contribute to the decline in River Herring stocks. Physical obstructions such as dams and road bridges with culverts may also affect migration and spawning. Offshore mid-water trawl fisheries such as the Atlantic Herring fishery can unintentionally harvest River Herring as bycatch in the open ocean. (NCDMF 2007). These negative anthropogenic impacts on important spawning tributaries and oceanic habitats could be one reason why harvest restrictions and moratoriums have not lead to River Herring population recovery in North Carolina.

North Carolina's River Herring are managed in coastal waters by NCDMF and in inland waters by the North Carolina Wildlife Resources Commission (NCWRC). The 2000 Albemarle Sound River Herring Fishery Management Plan designated two management areas in NC: The Albemarle Sound River Herring Management Area (ASRHMA) and the Chowan River Herring Management Area (CRHMA). The ASRHMA is delineated as follows: "Albemarle Sound and all its Coastal, Joint and Inland water tributaries; Currituck Sound; Roanoke and Croatan sounds and all their Coastal, Joint and Inland water tributaries, including Oregon Inlet, north of a line from Roanoke Marshes Point 35° 48.3693' N -75° 43.7232' W across to the north point of Eagles Nest Bay 35° 44.1710' N - 75°31.0520' W." The CRHMA is delineated as follows: "Northwest of a line from Black Walnut Point 35° 59.9267' N - 76° 41.0313' W to Reedy Point 36°02.2140' N - 76° 39.3240' W, to the North Carolina/Virginia state line; including the Meherrin River (ASMFC 2012)."

Tributaries and western portions of the Albemarle Sound have been identified as River Herring nursery habitats (Copeland et al. 1983). NCDMF, in accordance with the North Carolina Coastal Habitat Protection Plan (CHPP), has designated a large portion of the Albemarle Sound and its tributaries as Strategic Habitat Areas (SHAs). Through these designations, state agencies seek to protect important River Herring spawning and nursery habitats (Deaton et al. 2010).

Although the state has designated considerable resources to the management of River Herring, we still do not fully understand River Herring use of North Carolina's estuaries, and are still learning about the population structure of spawning adults and resulting young-of-year (Zapf 2012). Currently there is a pressing need to identify the population structure of River Herring and determine which habitats may provide the best nursery habitats in order to formulate appropriate management and protection efforts. NCDMF has drafted a set of research priorities for River Herring restoration. These priorities include a mandate to "focus research on within-species variation in genetic, otolith microchemistry, reproductive, meristic, morphological, and ecological characteristics found in River Herring from various NC river systems." (NCDMF 2013).

Population, or 'stock' identification and delineation are fundamentally important in fisheries management. To manage a fishery successfully, it is important to understand the population structure of a species, as different groups of fish can be exploited in different ways and be exposed to different environmental conditions (Begg and Waldman 1999). Populations are separated using a number of methods to quantify variations in characteristics between groups of fish. These variations can be summarized in two categories: genetic variation and phenotypic variation. Analysis of genetic variation between groups of fish can directly elucidate population structure. Molecular genetic techniques have been used to identify reproductive isolation between populations, allowing delineation of management units and conservation efforts from an evolutionary standpoint (Altukhov 1981; Begg et al. 1999; Palkovacs et al. 2013). Genetic similarity, however, does not necessarily imply stock homogeneity within a management context. Genetically similar stocks can display variable phenotypic traits that can affect their responses to various stressors such as harvest and environmental degradation (Begg et al. 1999). Phenotypic variation between groups of fish can be analyzed to delineate population structure using somatic measurements of meristic counts, body shape, and otolith shape. Both meristic and morphometric divergence between groups of fish can be the result of differences in environments and/or genetic differentiation. The extent to which these two factors influence morphometric variation is often difficult to determine (Ihssen et al. 1992; Begg et al. 1999). Although it does not directly imply genetic differentiation between populations of fish in all cases, morphometric variation can indicate limited mixing and life history differences between populations and therefore identify distinct units of fish (Armstrong and Cadrin 2001; Jorgensen et al. 2008).

Traditional and geometric morphometric analyses are among the traditional mainstays of stock identification, and have been used for decades to identify variation between populations of various clupeids including: American Shad *Alosa sapiddissima* (Carscadden and Legget 1975; Melvin et al. 1992), Atlantic Herring *Clupea harengus* (Armstrong and Cadrin 2001; King 1985), Pacific Herring *Clupea palasii* (Meng and Stocker 1984; Kartavtsev et al. 2008), and River Herring (Cronin-Fine et al. 2013). With the development of image processing software, morphometric analyses have become increasingly efficient and powerful in delineating populations of fish (Cadrin and Friedland 1999). Geometric morphometric analysis involves the digitization of discrete landmarks on images of fish using software such as tps-Dig2. Unlike traditional truss-based morphometrics, which involves measuring linear distances between landmarks, geometric morphometric analysis creates Cartesian (*X,Y*) coordinates for each landmark. Raw Cartesian coordinates for all samples are then superimposed upon each other and rotated upon a common centroid. This process, called "Procrustes superimposition," removes variation between samples associated with size, location, and orientation within the image. Procrustes superimposition creates a new set of coordinates (Procrustes coordinates) from the modified raw Cartesian coordinates (Rohlf 1999). Principle Component Analysis (PCA) can be used to explore shape variation between samples and reduce data dimensionality. Discriminant Analysis (DA) can be used to test for significant differences in shape between samples and to assign individuals to a priori designated groups (Webster and Sheets 2010). These methods are relatively inexpensive compared to other stock identification methods and can feasibly be accomplished without killing specimens.

Otolith shape analysis has become more prevalent in recent decades as imaging and computing technologies have flourished. Otoliths grow throughout the lives of fish and remain “metabolically inert;” not strongly affected the drastic changes in body condition that can confound analyses of body morphometrics. Unlike structures such as scales and bones, otolith material is unlikely to change once deposited (Campana and Nielson 1985). Otolith shape is species-specific and can vary geographically within a species range (L’Abee-Lund, 1988; Lombarte and Lleonart 1993). Shape can also vary among ages and sexes within a given stock (Casselman et al. 1981; Bird et al.1986; Castonguay et al. 1991). Like morphometric analysis, otolith shape analysis provides a phenotypic-based assessment of population structure. Differences in otolith shape can be attributed to partial geographic isolation between groups of fish (Casselman et al. 1981; Ihssen et al. 1992), and therefore can elucidate population differences (Begg and Brown 2000; Galley et al. 2006; Treinen-Crespo et al. 2012). Many studies have utilized otolith shape analysis to discriminate stocks of Atlantic Mackerel *Scomber scombrus* (Castonguay et al. 1991), Atlantic Cod *Gadus morhua* (Campana and Casselman 1993; (Galley et al. 2006), King Mackerel *Scomberomorus cavalla* (DeVries et al. 2002), Atlantic Herring *Clupea harengus* (Burke et al. 2008), Horse Mackerel *Trachurus trachurus* (Stransky et al. 2008), Black Scabbardfish *Aphanopus carbo* (Farias et al. 2009), Anglerfish *Lophius piscatorius* (Cañás et al. 2012), and White Grunt *Haemulon plumierii* (Treinen-Crespo et al. 2012). Elliptical Fourier Analysis (EFA) is an efficient method to create and analyze outlines of otoliths from two-dimensional images (Kuhl and Giardina 1982). EFA is considered to be the most powerful shape analysis for identifying large and small-scale differences between otolith outline shapes (Campana and Casselman 1993). Image processing software such as SHAPE (Iwata and Ukai 2002) creates a set of Elliptical Fourier Descriptors (EFDs), or harmonics, from chain-coded contours of each otolith. SHAPE software can normalize these EFDs to eliminate effects of size and orientation of the otoliths in sample images. These EFDs can be incorporated into multivariate analyses to identify the effects of variables on otolith shape and classify fish to putative stocks.

Begg and Waldman (1999) suggest that a 'holistic' approach be used in stock identification, using various techniques to separate distinct units of fish. Combining more than one technique allows for the strongest inferences on stock structure because it involves multiple aspects of the biology of a species. The modern concept of 'stock' describes "the characteristics of the units assumed homogeneous for particular management purposes (Begg and Waldman 1999)." In this way, stock determinations can be relatively plastic, and may not solely rely on genetic differentiation. Employing various stock identification methods into a single study allows comparisons between different analyses. Various studies have identified discrepancies between phenotypic and genetic analyses (Kinsey et al.; 1994; Leslie and Grant 1990; Safford and Booke 1992; Pepin and Carr 1993). These studies found morphometric variation in groups of fish within genetically homogeneous stocks, suggesting that morphometric variation was environmentally induced. These findings agree with the assertion that phenotypic data can be more useful than genetic data in detecting small-scale, environmentally induced variation in fish (Grant and Utter 1984; Campana and Casselman 1993).\

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CHAPTER 2: POPULATION STRUCTURE OF JUVENILE AND ADULT RIVER HERRING

1. Introduction

Alewife (*Alosa pseudoharengus*) and Blueback Herring (*Alosa aestivalis*), collectively known as River Herring, are native to the east coast of North America. Blueback Herring range from Nova Scotia to Florida and Alewife range from Nova Scotia to South Carolina (Munroe 2002; Greene at al 2009). These anadromous species spend ta portion of their juvenile and adult lives at sea and return to coastal rivers to spawn. River Herring are an important ecological, economic, and cultural component of coastal marine, estuarine, and riverine ecosystems. The two species provide an important energy-flow link between marine and freshwater food webs (West et al. 2010), and are an important prey resource for coastal birds and fishes (Walter and Austin 2003.) North Carolina's River Herring fishery was historically among the largest freshwater fisheries in the world (ASMFC 2012). Stocks of these fish have drastically declined since the mid- 1970's, causing the collapse of many North Carolina fisheries (Hightower et al. 1996; Schmidt et al. 2003; Limburg and Waldman 2009). The 2005 North Carolina River Herring Stock Assessment stated that River Herring stocks were overfished and that overfishing was still occurring (ASMFC 2012). In 2007, North Carolina adopted the NC River Herring Fishery Management Plan that mandated a no-harvest provision for commercial and recreational River Herring fisheries in the state. Despite years of strict regulations and the 2007 no-harvest provision, populations of River Herring have not shown signs of recovery (NCDMF 2013).

Population, or ‘stock’ identification and delineation are fundamentally important in fisheries management. To manage a fishery successfully, it is important to understand the population structure of a species, as different groups of fish can be exploited in different ways and be exposed to different environmental conditions (Begg and Waldman 1999; Campana and Casselman 1993). Populations are separated using a number of methods to quantify variations in genetic and phenotypic characteristics between groups of fish (Ihssen et al. 1981). Traditional and geometric morphometric analyses are among the mainstays of stock identification, and have been used for decades to identify phenotypic variation between populations of various clupeids including: American Shad *Alosa sapiddissima* (Carscadden and Legget 1975; Melvin et al. 1992), Atlantic Herring *Clupea harengus* (Armstrong and Cadrin 2001; King 1985), Pacific Herring *Clupea palsii* (Meng and Stocker 1984; Kartavtsev et al. 2008), and River Herring (Cronin-Fine et al. 2013). With the development of image processing software, morphometric analyses have become increasingly efficient and powerful in delineating populations of fish (Cadrin and Friedland 1999).

Otolith shape analysis has become has emerged as a stock identification tool in recent decades as imaging and computing technologies have flourished. Otoliths grow throughout the lives of fish and remain “metabolically inert;” not strongly affected the drastic changes in body condition that can confound analyses of body morphometrics. Unlike structures such as scales and bones, otolith material is unlikely to change once deposited (Campana and Nielson 1995; Casselman 1987). Otolith shape is species-specific and can vary geographically within a species (L’Abee-Lund, 1988; Lombarte and Lleonart 1993). Shape can also vary among ages and sexes within a given stock

(Casselman et al. 1981; Bird et al.1986; Castonguay et al. 1991). Like morphometric analysis, otolith shape analysis provides a phenotypic-based assessment of population structure. Differences in otolith shape can be attributed to partial geographic isolation between groups of fish (Ihssen et al. 1981; Casselman et al. 1981), and therefore can elucidate population differences (Begg and Brown 2000; Galley et al. 2006; Treinen-Crespo et al. 2012). Many studies have utilized otolith shape analysis to discriminate stocks of Atlantic Mackerel *Scomber scombrus* (Castonguay et al. 1991), Atlantic Cod *Gadus morhua* (Campana and Casselman 1993; Galley et al. 2006), King Mackerel *Scomberomorus cavalla* (DeVries et al. 2002), Atlantic Herring *Clupea harengus* (Burke et al. 2008), Horse Mackerel *Trachurus trachurus* (Stransky et al. 2008), Black Scabbardfish *Aphanopus carbo* (Farias et al. 2009), Anglerfish *Lophius piscatorius* (Cañás et al. 2012), and White Grunt *Haemulon plumierii* (Treinen-Crespo et al. 2012

The objective of this study was to examine the population structure of juvenile and adult River Herring in North Carolina's Albemarle Sound using two robust stock identification tools. We also sought to compare the results of geometric morphometric analysis and otolith shape analysis to determine if these two tools produce similar population designations.

2. Methods

2.1 Study Site

The majority of River Herring in North Carolina are found in the Albemarle Sound and its tributaries (Figure 1). This large oligohaline estuary (~45,500 km^2) is located in northeastern North Carolina and extends approximately 90 km from east to west, averaging approximately 12 km in width and 4-6 m in depth (Copeland et al. 1983; ASMFC 2012). Nine major tributaries drain into the sound including: the Alligator, Chowan, Little, North, Pasquotank, Perquimans, Roanoke, Scuppernong, and Yeopim rivers. The Albemarle Sound joins with Currituck Sound to the northeast and Roanoke and Croatan Sounds to the southeast, and connects to the Atlantic Ocean via Oregon Inlet.

2.2 Sample Collection

Alewife and Blueback Herring samples were collected from tributaries and portions of the main Albemarle Sound during the spring and fall months of 2010, 2013, and 2014 in collaboration with the North Carolina Division of Marine Fisheries (NCDMF). A small sample of adults was collected in 2014 from the Metedeconk River in New Jersey to allow for comparisons at the state level. Juveniles were collected from the NCDMF Program 100: Anadromous Juvenile Survey and adults from the Program 150: Adult Anadromous Spawning Area Survey. Juvenile fish were sampled by NCDMF technicians via trawl and beach seine and adults were sampled via gill net.

Frozen samples were thawed, sexed, measured (mm total length and fork length), weighed (0.1 g), and identified by species according to peritoneal coloration (Bigelow and Schroeder 1953). Fulton's condition factor (K) was calculated using the formula: $K = (\text{weight}/\text{total length}^3) \times 100{,}000$ (Murphy and Willis 1996). After a standard image was taken for morphometric analysis, fish were dissected. Sagittal otoliths were removed and stored to dry in plastic centrifuge vials. Otoliths were aged whole by three independent readers under 5x magnification using an Olympus SZX16 Research

Stereomicroscope until there was agreement between at least two readers. (LaBay and Lauer 2006; Libby 1985).

2.3 Geometric Morphometrics

Landmark-based geometric morphometric analysis was conducted on juvenile Alewife and Blueback Herring caught in 2013, and juvenile and adult Blueback Herring caught in 2014. Frozen samples were thawed and placed in a dissection tray. Morphometric images (.tiff) were captured using a Nikon D5100 digital camera mounted on an adjustable frame. Ten discrete landmarks were digitized upon sample images using tps-Dig2 software (http://life.bio.sunysb.edu/morph/) (Figure 5, Table 1). This software created Cartesian coordinates (*X*-*Y*) for each landmark digitized upon each specimen. Morpho-J software was then used to perform a Procrustes superimposition of raw landmark data for each sample. This process rotates landmark configurations for each sample around a common centroid and removes the effect of size differences between samples (Rohlf 1999). Procrustes superimposition created a new set of *X*,*Y* (Proctrustes) coordinates upon which we ran Principle Component Analysis (PCA) to visualize shape differences between groups and Linear Discriminant Analysis (LDA) to classify samples according to age, sex, and capture location. Group centroids and Mahalanobis distances between groups were calculated, and classification success rate was determined based on the percentage of individuals correctly assigned to their original sample.

2.4 Otolith Shape Analysis

We examined the otolith shapes of adult Blueback Herring caught in 2010 and 2014. Otoliths were placed sulcus-down on a dark background and bitmap (.bmp) images were captured using an Olympus DP71 camera integrated into the Olympus SZX16 Research Stereomicroscope system. Closed form Fourier analysis was applied to two-dimensional images of otoliths using the SHAPE version 1.2 suite of programs (Iwata and Ukai 2002). These programs extracted the contours of otoliths and derive Elliptical Fourier Descriptors (EFDS), or harmonics, for each sample. This process effectively captured otolith outline information in a quantifiable manner (Tracey et al. 2006). The ChainCoder program was used to extract the contour of each otolith and record data as chain-codes. We then used the CHC2NEF to derive normalized EFDs from chain-coded contours through Fourier transformation (Farias et al. 2007). This transformation eliminated the effects of size and orientation of otoliths in sample images (Kuhl and Giardina 1982) (Figure 6). The PrinComp program created Principle Component Analysis (PCA) of EFD's to visualize shape differences between samples. We then used LDA to classify individuals to ad-hoc designations (location of capture, sex, age.) Multivariate analyses were conducted using JMP vers. 11.2 software. SAS Institute Inc., Cary, NC, 2007.

3. Results

3.1 Geometric Morphometrics

A total of 464 juvenile and adult River Herring were analyzed using geometric morphometric analysis (Table 2). In order to minimize temporal effects on morphometric characters, we only analyzed fish caught within a 2 month range. Juvenile samples were separated by location of capture and subjected to multivariate analysis (PCA and LDA). Data was resampled using the Bootstrap function (100 replicates) in JMP to test LDA models. Juvenile Alewife (n=67) showed significant differences in body shape between capture locations in Albemarle Sound (Pillai's trace: P<0.0001; Figures 7 and 8). Classification percentages from LDA ranged from 30% to 100% correct (Table 3). Juvenile Blueback Herring from 2013 (n=176) and 2014 (n=109) also showed significant differences in body shape between capture locations (Pillai's trace: P<0.0001; Figures 9 and 10). Classification scores ranged from 53-96.3% for 2013 juvenile Blueback Herring and from 83.3-100% for 2014 juvenile Blueback Herring (Table 3; Figures 11 and 12).

Multivariate analysis of adult Blueback Herring (n=112) revealed that morphometric characteristics differed significantly between males and females; therefore we separated the sexes during further analyses (Figure 13: Table 4). Males differed significantly at the state (Pillai's trace: P<0.0001) level but not at the tributary level (Pillai's trace: P>0.05) levels (Figure 14). This could be a result of inadequate sample size for rivers other than the Chowan (Table 2). Classification scores for males ranged from 50-85.7% correct to tributary of capture (Table 5), and 82.8 and 92.9% at the state level for North Carolina and New Jersey (Table 6). No adult Alewife were collected in either 2013 or 2014.

Age had a significant effect on body shape of adult Blueback Herring. We chose to examine age-3 male fish because the majority of samples were determined to be that age. These fish differed significantly between states (Pillai's trace: P<0.0051) but not tributaries (Pillai's trace: P>0.05), again likely due to inadequate sample size.

Classification scores ranged from 75-90.2% correct to tributary of capture, and 82.2 and 90.9% at the state level for North Carolina and New Jersey

3.2 Otolith Shape Analysis

We derived and analyzed the outlines of 155 adult Blueback Herring otoliths from specimens captured in 2010 (n=80) and 2014 (n=75) (Table 7). The 2014 otoliths were excised from the same fish that were used in the previous geometric morphometric analysis, allowing for direct comparisons between the two stock identification methods. We used the SHAPE suite of programs to analyze 20 Elliptical Fourier Descriptors, or harmonics, that describe the mean shape of the otolith samples. From these EFD's, we derived principle components for each sample.

We found no significant effect of age or sex on otolith shape (P>0.05) and therefore pooled samples for spatial analysis (Table 8). There were no significant differences in otolith shape between the three tributaries for Blueback herring caught in 2010. Classification success ranged from 45.7-66.7% (Table 9). Blueback herring caught in 2014 did not display significant differences the two between tributaries of capture despite high classification success (98.2 and 100% for the Chowan and Yeopim rivers, respectively) (Table 10). Significant differences (P=0.0499) were found between fish caught in New Jersey (n=22) and North Carolina in 2014, with classification rates of 79.9 and 75%, respectively (Table 10, Figure 15).

4. Discussion

The modern concept of 'stock' describes "the characteristics of the units assumed homogeneous for particular management purposes (Begg and Waldman 1999)." In this way, stock determinations can be relatively plastic, and may not solely rely on one stock identification method. Begg and Waldman (1999) suggest that a ‘holistic’ approach be used in stock identification, using various techniques to separate distinct units of fish. Combining more than one technique allows for the strongest inferences on stock structure because it involves multiple aspects of the biology of a species. The application of these different methods allows for comparative studies based upon the same samples or datasets. These practices allow regulatory bodies to develop stronger management strategies based on comprehensive stock determinations (Begg et al. 1999).

4.1 Geometric Morphometrics

Geometric morphometric analysis revealed significant body shape differences at the tributary level for juvenile River Herring. Alewife collected in 2013 displayed significant differences between tributaries but highly variable classification percentages from LDA (30-100%). Juvenile Alewife caught in the Currituck Sound were completely unique from those caught in tributaries of the Albemarle Sound proper (100% classification). This could be a result of differences in environmental characteristics between the Currituck Sound and the other, more riverine capture locations that may have different physical conditions. Fish shape has been shown to differ between fish occupying areas with different flow regimes (Meyers and Belk 2014). Further study should be conducted with both juvenile and adult River Herring to determine if

occupying the Currituck Sound are truly unique.

Analysis of juvenile Blueback Herring from 2013 showed 100% classification success for Pasquotank River specimens. Classification was considerably less for Chowan River (59.6%) and Western Albemarle fish (69.6%), which seemed to intermix.

This pattern seems intuitive, as the Chowan River and Western Albemarle are adjacent to one another, and are separated from the Pasquotank River three other rivers. Cronin-Fine et al. 2013 reported that morphometric characters tend to be more similar between groups of fish from neighboring water bodies than those of distant water bodies. Interestingly, juvenile Blueback Herring specimens collected in 2014 displayed a different pattern, with 100 % classification of Chowan River fish. Juvenile Blueback Herring specimens caught in Western Albemarle, according to LDA, contained a mixture of Yeopim River and Chowan River fish along with those classified as Western Albemarle fish. Specimens caught in the Yeopim contained Western Albemarle fish, but no Chowan fish. The population structure displayed by the juvenile 2014 Blueback Herring seems to align with the current NCDMF management strategy that isolates the Chowan River as a distinct management area and encompasses the remaining areas of the Albemarle Sound area as another management unit (ASMFC 2012).

It is important to consider the potential effects of allometric growth when examining the body shape of juvenile fishes. Changes in body shape during ontogeny of young fish may confound morphometric analyses. Fish caught at different times may display shape differences that are driven my somatic development and not different population characteristics (Cadrin et al. 2005). We attempted to minimize the effects of allometric growth by limiting the maximum temporal difference between times of capture to two months. Even with this temporal limitation, there still may be some confounding of analysis due to allometric growth. Our adult specimens are not subject to this problem.

Along with allometric growth, fish condition could potentially have an effect on body shape. Healthy fish could have different body shapes than unhealthy or emaciated fish. We calculated Fulton's condition factor (K) for each specimen and examined whether this factor was different between specimens caught in different locations and during different times. We found that both juvenile and adult Alewife and Blueback Herring differed in K factor between locations and months of capture.

Adult Blueback Herring differed significantly in body shape between North Carolina and New Jersey, but not between the two rivers within the Albemarle Sound. This result was consistent when ages and sexes were isolated. Cronin-Fine et al. (2013) found similar results in comparing Alewife caught in Maine versus Massachusetts and subsequently comparing Alewife caught in different rivers and lakes within Maine. Morphometric differences were much greater between Maine and Massachusetts fish than the differences between fish caught in different areas within Maine. One of the drawbacks of our study was the inability to collect adult Blueback Herring from any tributaries except for the Chowan and Yeopim rivers, and any adult Alewife, for geomorphometric analysis. Even so, the resulting data do show a lack of differentiation between samples from tributaries within the Albemarle Sound, which may be due to the fact that a portion of adult Alewives and Blueback Herring do not return to natal tributaries to spawn. Messieh (1977) suggested that Alewife may wander from natal tributaries and spawn in adjacent areas during spawning migrations. This would result in mixing between different local populations of spawning adults that display different morphometric characteristics. Results of our geometric morphometric analysis Sound support the suggestion of Messieh (1977), as the Yeopim and Chowan rivers are adjacent watersheds.

4.2 Otolith Shape Analysis

Fourier analysis of adult otoliths produced results similar to those from geometric morphometric analysis. We found significant differences in otolith shape at the state level but not the tributary level. Adult Blueback Herring collected in 2010 from the Chowan, Perquimans, and Scuppernong rivers had low classification rates and seemed to intermix. Otoliths analyzed from 2014 were taken from the same specimens used in geometric morphometric analyses, which allows for direct comparisons between the two stock identification methods. Geometric morphometrics yielded slightly higher classification percentages than those generated by otolith shape analysis. These results agree with those found in a study by Vergara-Solana et al. (2013) who compared body and otolith shape of Pacific Sardine *Sardinops sagax*. This study found body shape to be more discriminatory between groups but considered population structure interpretations afforded by both methods to be relatively similar.

Our results suggesting the apparent mixing between populations of spawning adults in the Albemarle Sound agree with those found by previous studies involving River Herring in the Albemarle Sound. Zapf (2012) used otolith microchemistry to determine that adult Blueback Herring stray from natal tributaries and spawn in non-natal tributaries. Palkovacs et al. (2013) examined 15 polymorphic microsatellite loci to map genetic differentiation among River Herring populations occupying 20 rivers along the U.S. species range (ME to NC). Populations from the Chowan, Roanoke, and Alligator rivers did not show significant genetic differentiation.

River Herring in North Carolina are managed in coastal waters by NCDMF and in inland waters by the North Carolina Wildlife Resources Commission (NCWRC). The

2000 Albemarle Sound River Herring Fishery Management Plan designated two management areas in NC: The Albemarle Sound River Herring Management Area (ASRHMA) and the Chowan River Herring Management Area (CRHMA). The ASRHMA includes the Sound proper and all of its tributaries as well as the Currituck, Roanoke, and Croatan Sounds. The CRHMA isolates the Chowan River as a distinct management area (ASMFC 2012). Results from our study suggest that the current River Herring management regime in North Carolina is appropriate, if not conservative, according to population structure. Future research should examine otolith and body shape of juvenile and adult River Herring over a number of years so determine the consistency of these patterns in population structure. Ideally, samples from all tributaries of the Albemarle Sound, including the Currituck Sound, should be collected to comprehensively study how these imperiled species utilize North Carolina's habitats. As the state continues restoration efforts to restore historical spawning runs, it will become important to determine if those fish that make up the newly restored runs become distinct population units. Geometric morphometric and otolith shape analyses are quick, cost-effective ways to answer these questions.

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Zapf, D. H. 2012. River Herring Nursery Habitat in Albemarle Sound, North Carolina, Inferred from Otolith Microchemistry. MS Thesis, East Carolina University, Greenville, North Carolina.

Figures and Tables

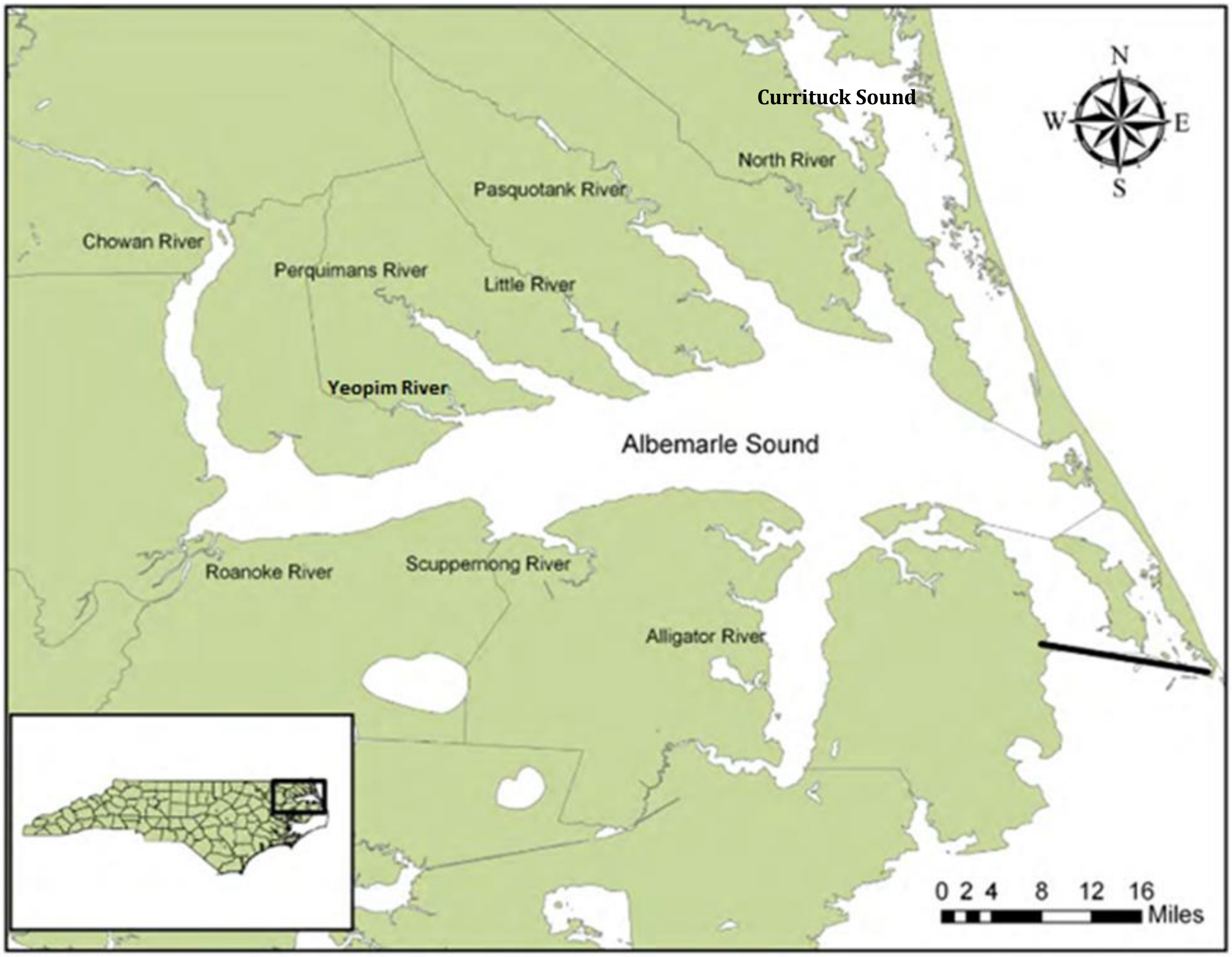

Figure 1. The Albemarle Sound and its major tributaries. The Currituck Sound, although not a tributary of the Albemarle, is included because samples were collected from the water body .

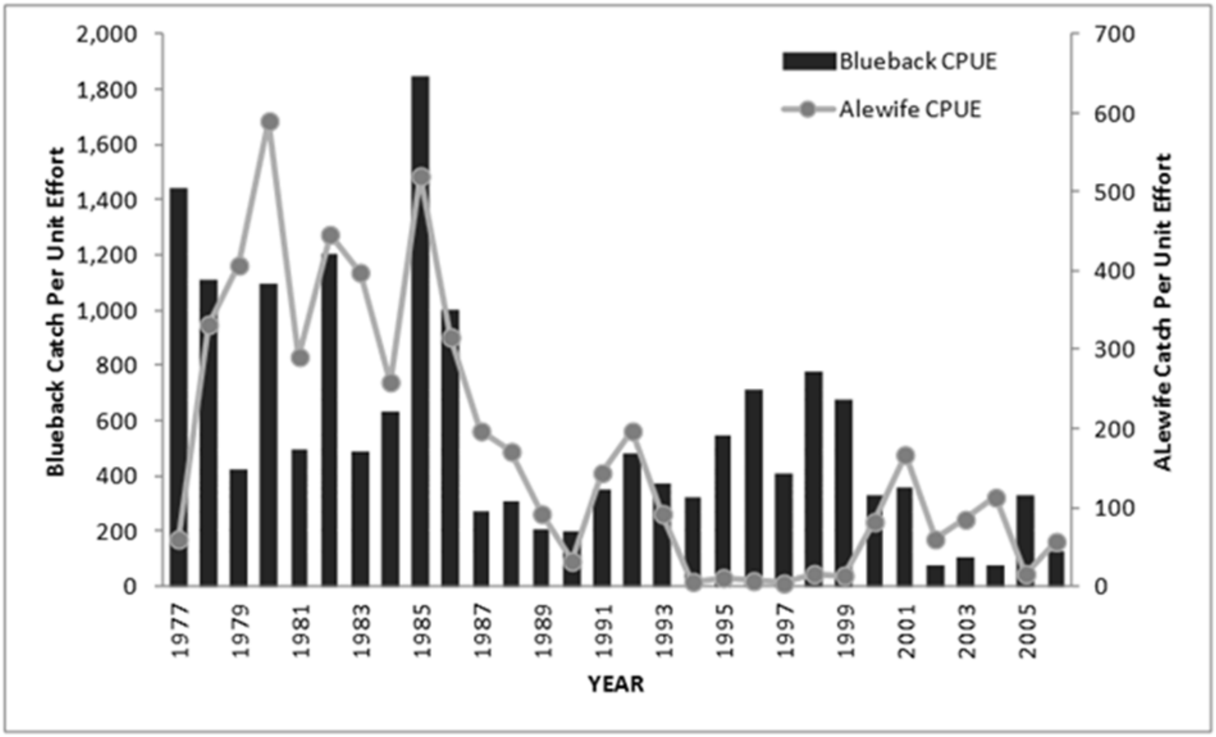

Figure 2. Catch per unit effort (CPUE) for Blueback Herring and Alewife from the Chowan River commercial pound net fishery (ASMFC 2012).

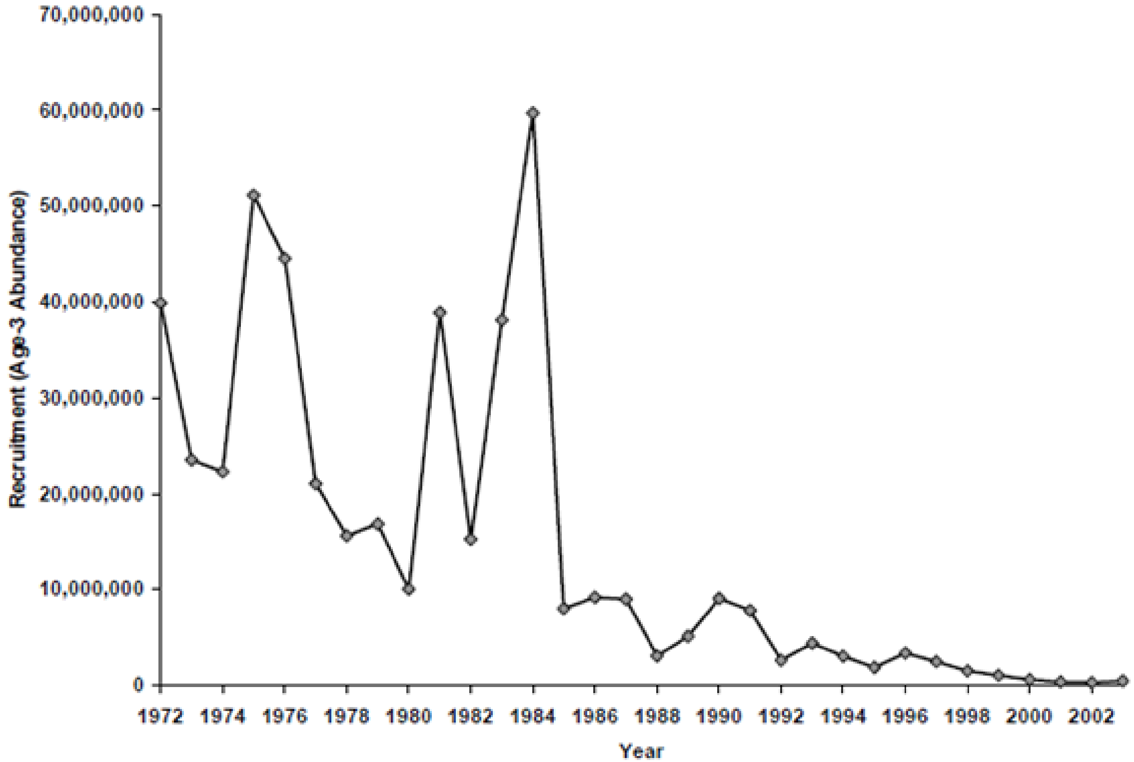

Figure 3. Annual recruitment estimates for Blueback Herring in the Chowan River (ASMFC 2012).

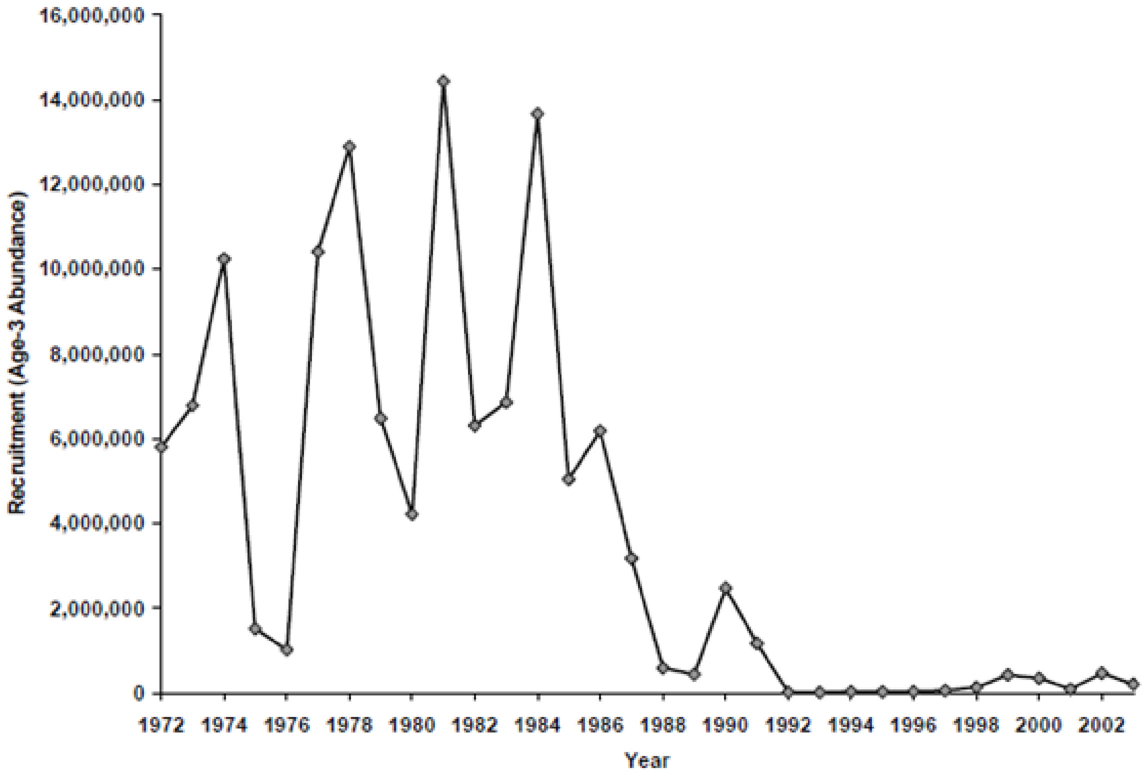

Figure 4. Annual recruitment estimates for Alewife in the Chowan River (ASMFC 2012).

Figure 5. A representative digital image used to digitize the 10 morphological landmarks used in geometric morphometric analysis.

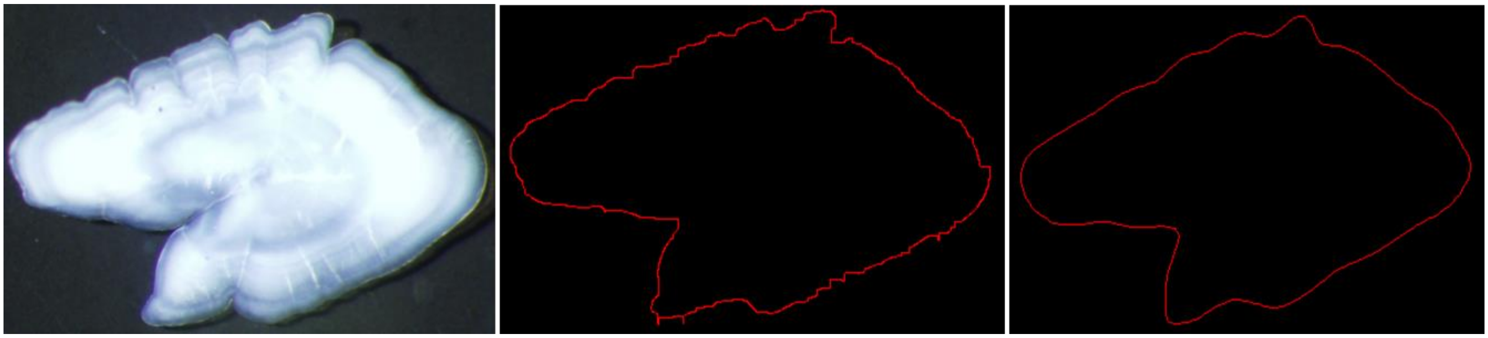

Figure 6. Photograph of an age-3 male Blueback Herring caught in the Scuppernong River (*left*). The chain coded contour of the sample otolith derived from ChainCoder software (*middle*). Depiction of otolith shape as described by the normalized Elliptical Fourier Descriptors (EFDs) that were calculated from the chain-coded contours via Fourier transformation (*right*).

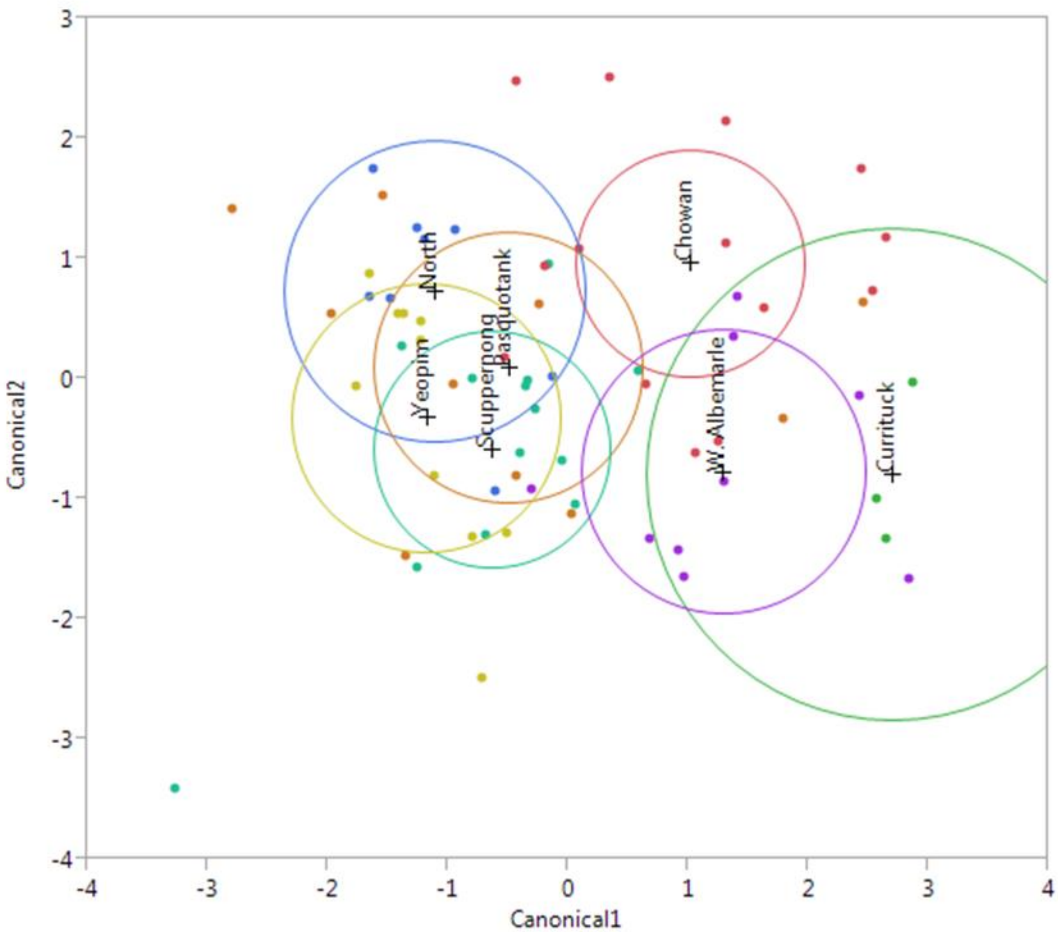

Figure 7. Plot of the first two canonical variates obtained from linear discriminant analysis used to classify juvenile Alewife caught in 2013 to their location of capture. Group centroids are indicated by (+) marks, and ellipses represent the 95% confidence region for each location.

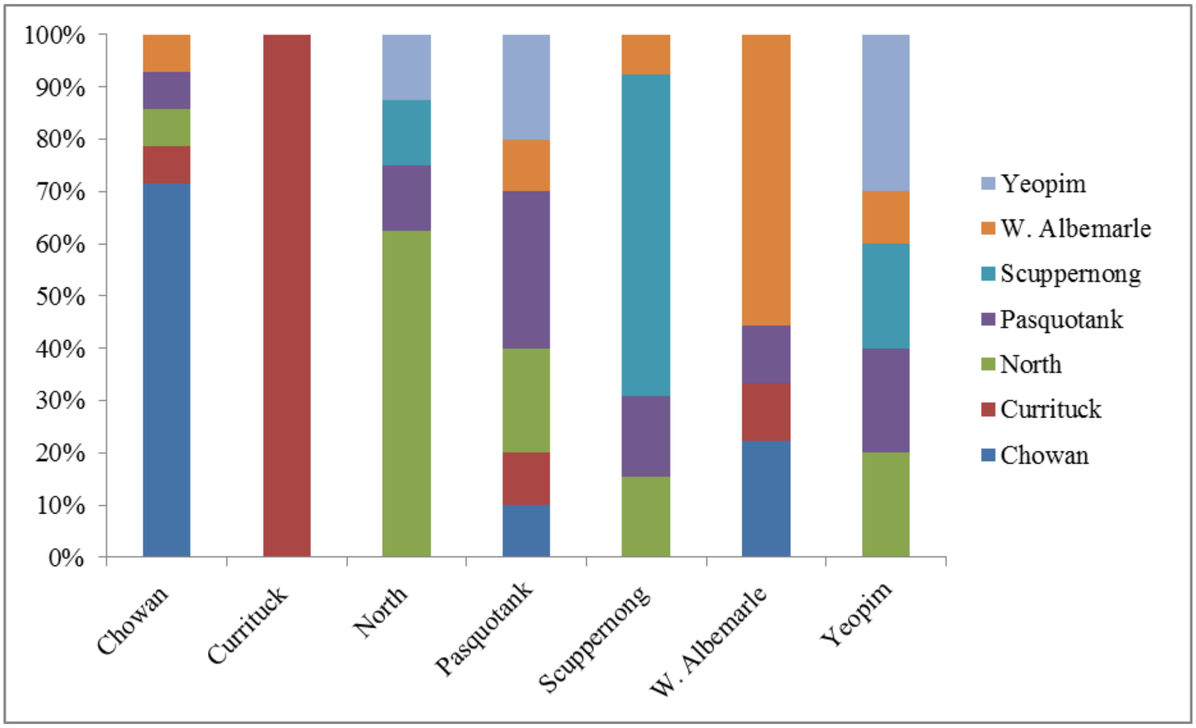

Figure 8. Bar graph depicting results from linear discriminant analysis of Procrustes coordinates derived from juvenile Alewife caught in 2013. Colors of bars correspond with fish that display shape characteristics of a given capture location. Colored areas of each bar represent the percentage of fish from each location that make up the total sample.

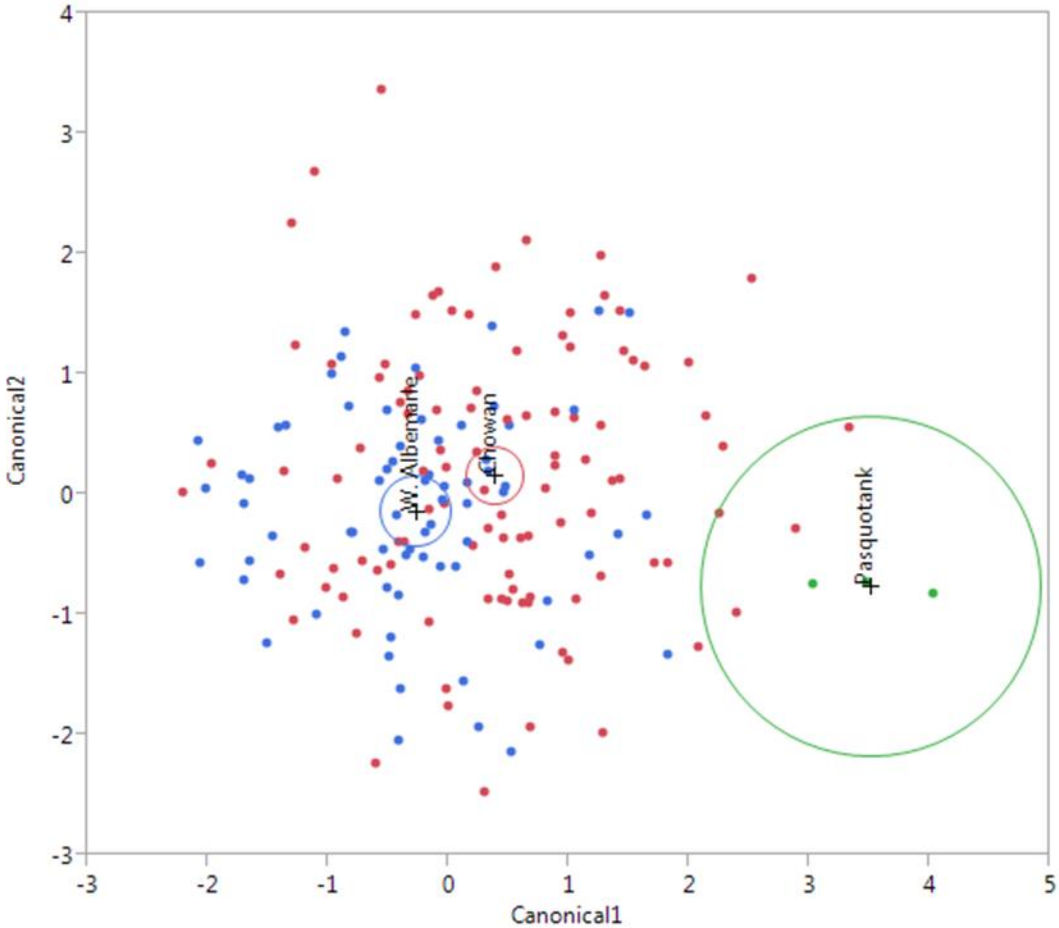

Figure 9. Plot of the first two canonical variates obtained from linear discriminant analysis used to classify juvenile Blueback Herring caught in 2013 to their location of capture. Group centroids are indicated by (+) marks and ellipses represent the 95% confidence region for each location.

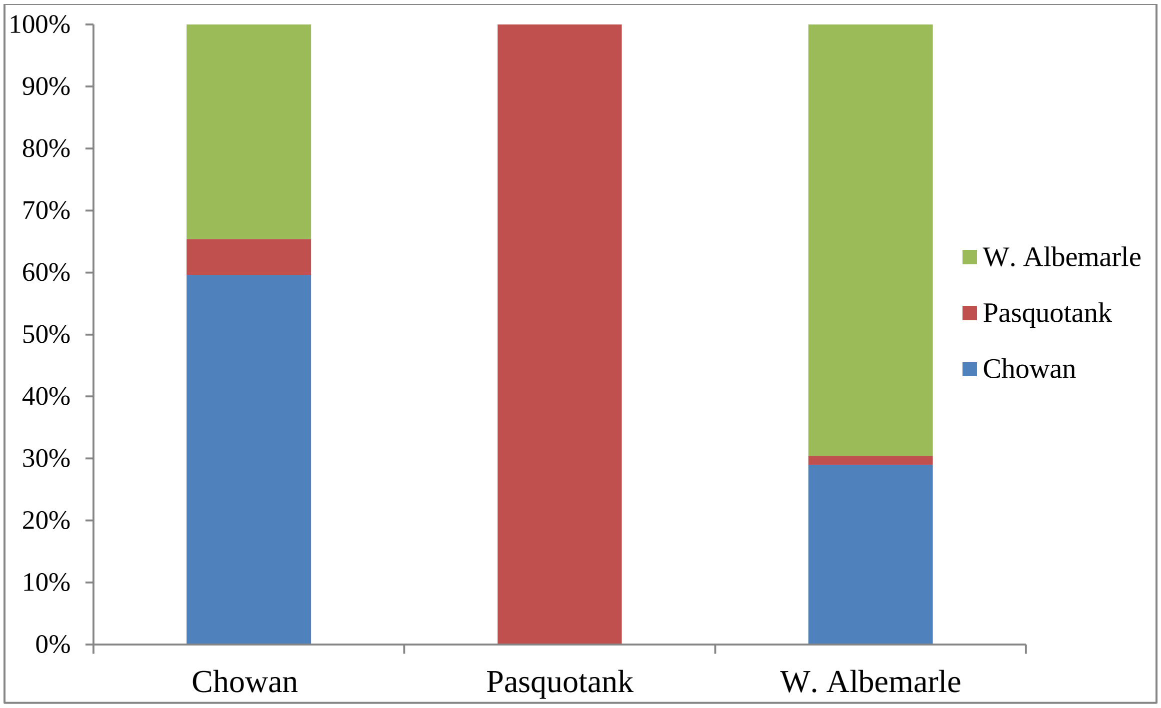

Figure 10. Bar graph depicting results from linear discriminant analysis of Procrustes coordinates derived from juvenile Blueback Herring caught in 2013. Colors of bars correspond with fish that display shape characteristics of a given capture location. Colored areas of each bar represent the percentage of fish from each location that make up the total sample.

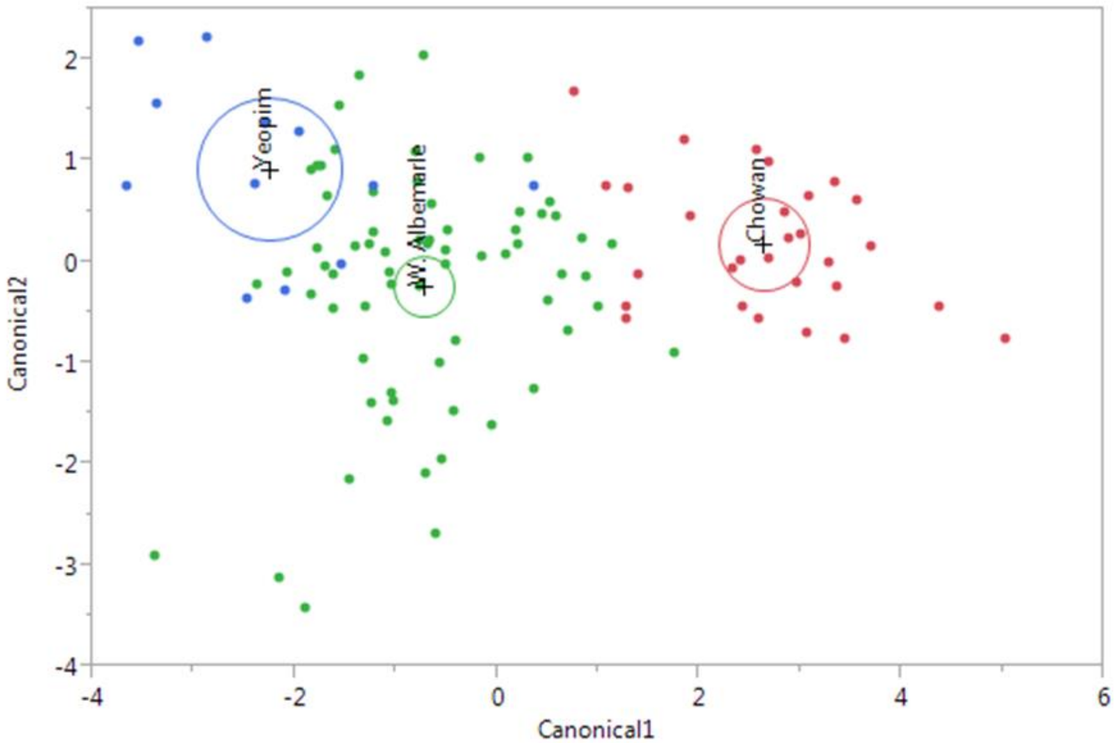

Figure 11. Plot of the first two canonical variates obtained from linear discriminant analysis used to classify juvenile Blueback Herring caught in 2014 to their location of capture. Group centroids are indicated by (+) marks and ellipses represent the 95% confidence region for each location.

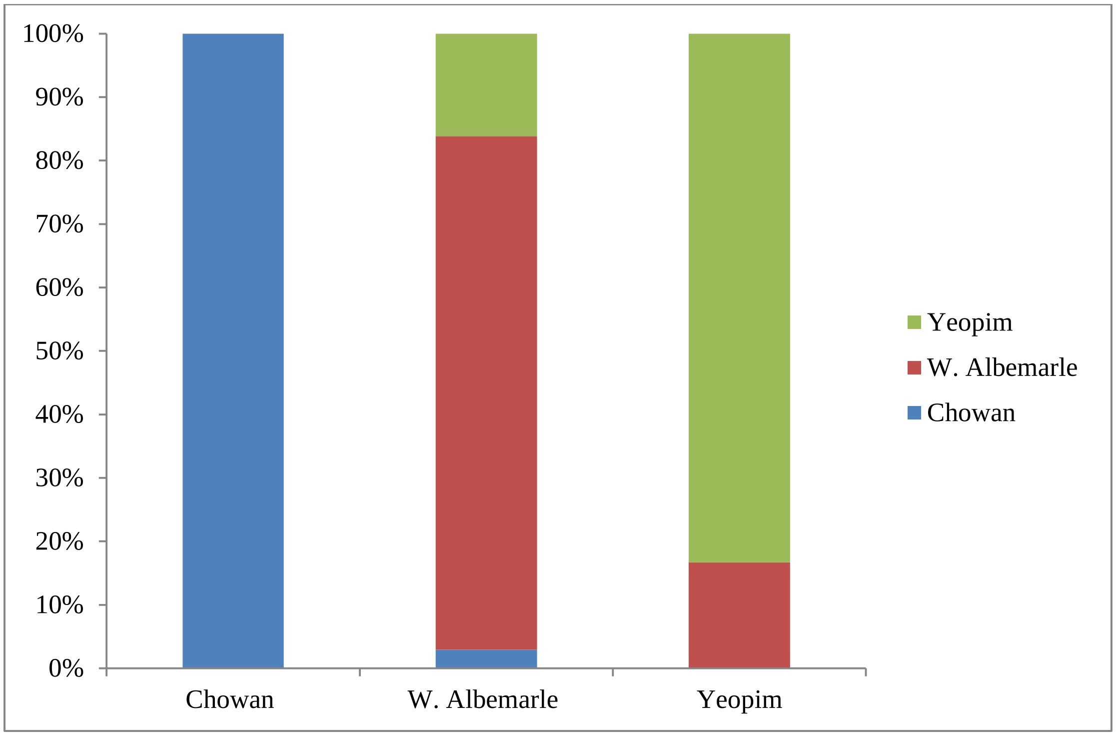

Figure 12. Bar graph depicting results from linear discriminant analysis of Procrustes coordinates derived from juvenile Blueback Herring caught in 2014. Colors of bars correspond with fish that display shape characteristics of a given capture location. Colored areas of each bar represent the percentage of fish from each location that make up the total sample. ‘

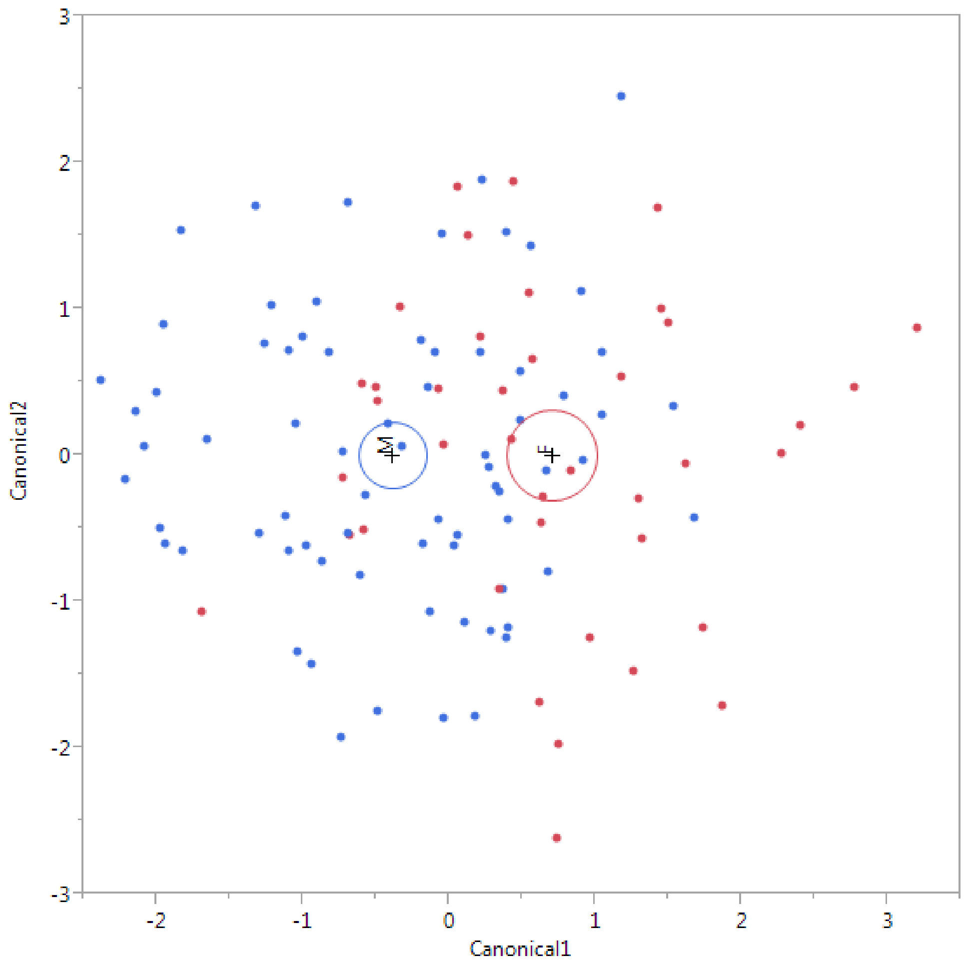

Figure 13. Plot of the first two canonical variates obtained from linear discriminant analysis of Procrustes coordinates used to classify adult male (M) and female (F) Blueback Herring caught in 2014. Group centroids are indicated by (+) marks and ellipses represent the 95% confidence region for each location.

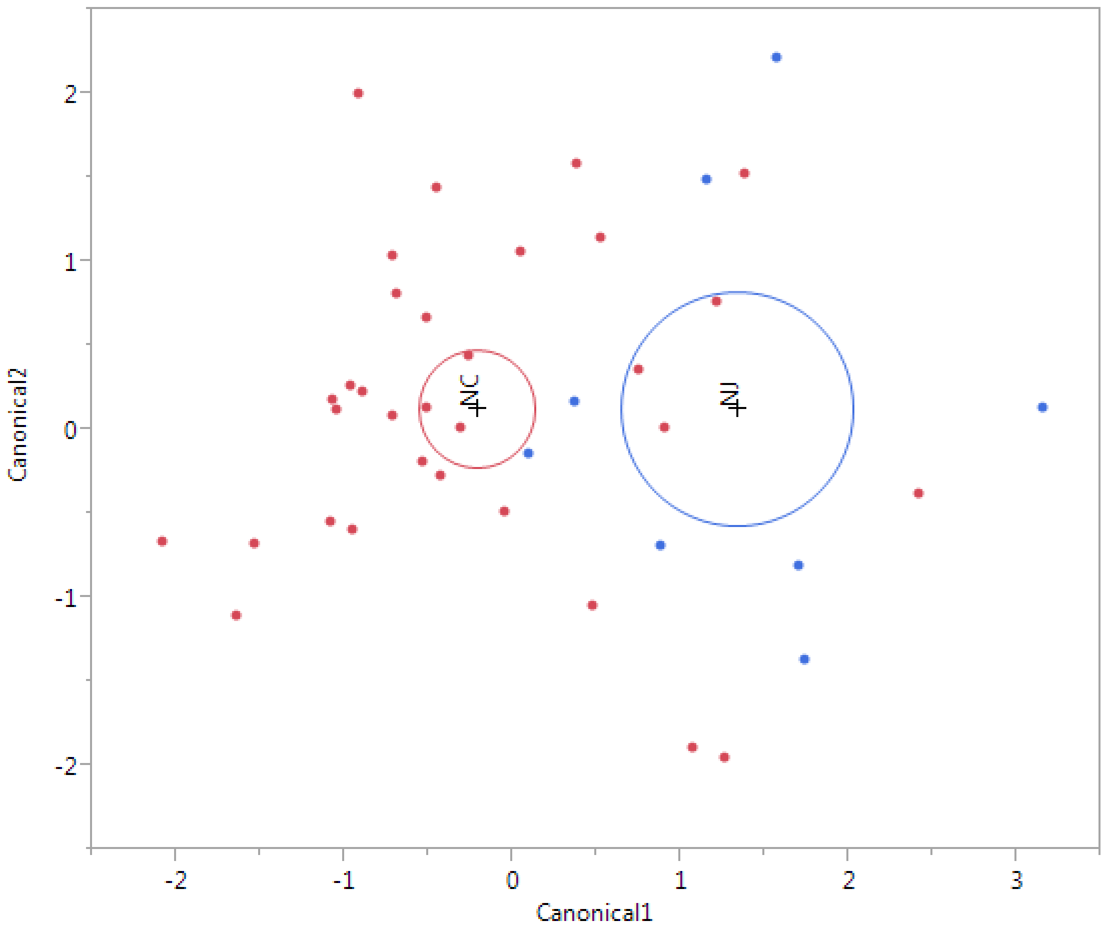

Figure 14. Plot of the first two canonical variates obtained from linear discriminant analysis used to classify adult female Blueback Herring caught in 2014 to their state of capture. Group centroids are indicated by (+) marks and ellipses represent the 95% confidence region for each location.

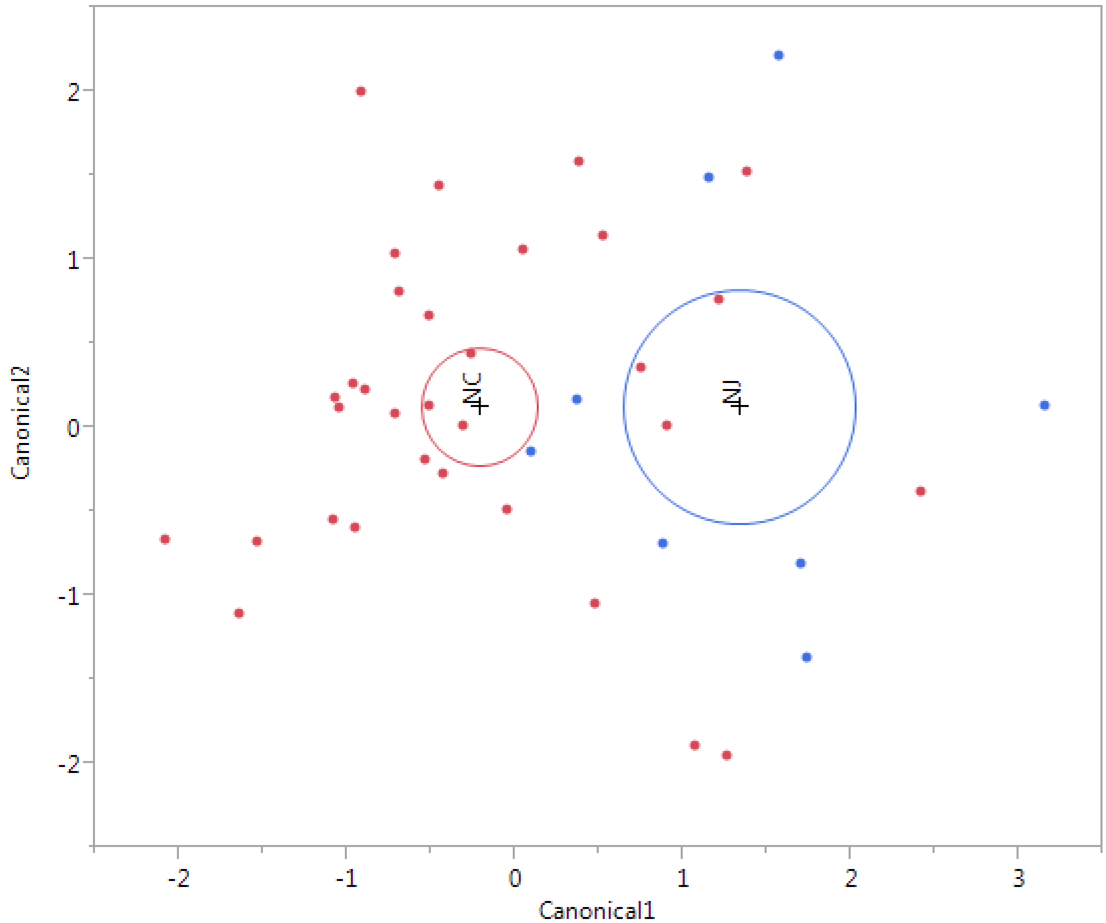

Figure 15. Plot of the first two canonical variates obtained from linear discriminant analysis used to classify adult female Blueback Herring caught in 2014 to their state of capture. Group centroids are indicated by (+) marks and ellipses represent the 95% confidence region for each location.

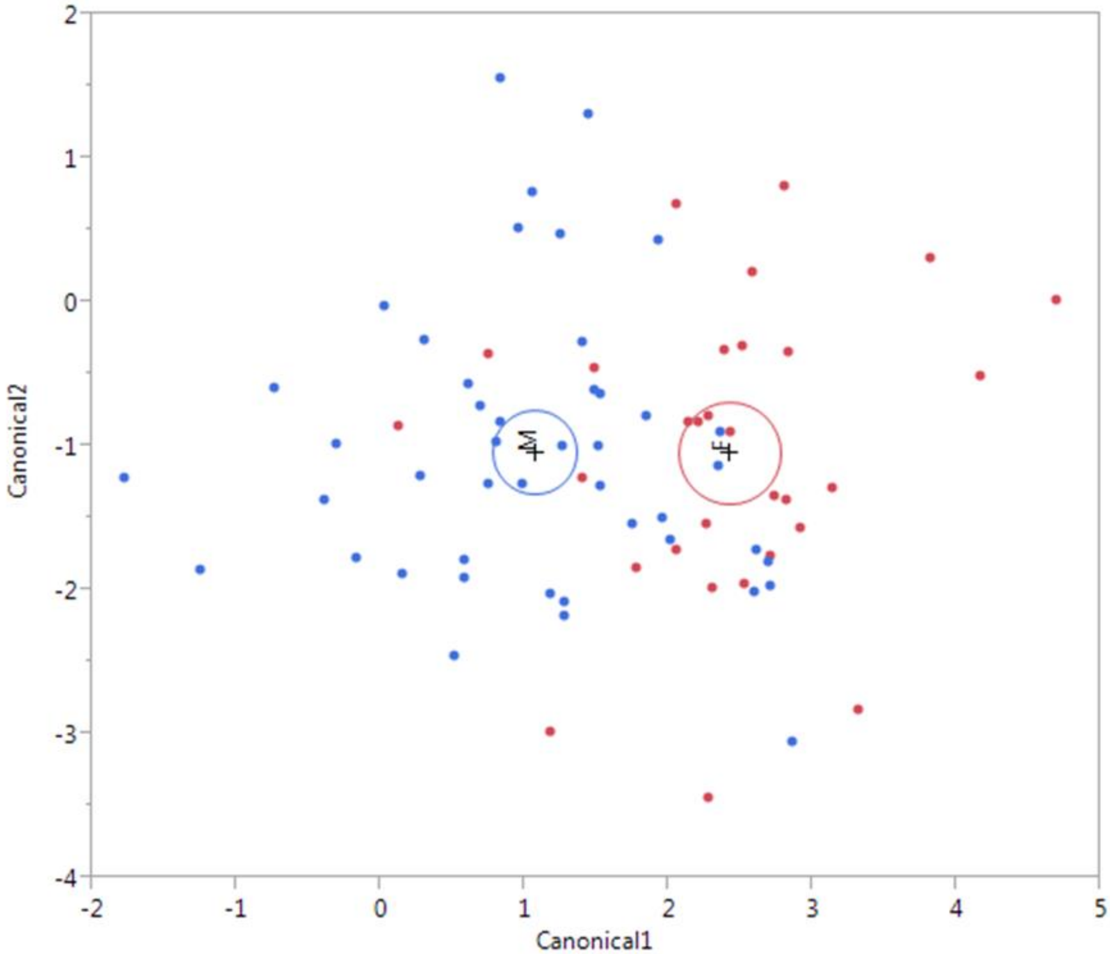

Figure 16. Plot of the first two canonical variates obtained from linear discriminant analysis of Elliptical Fourier Descriptors (EFDs) used to classify adult male (M) and female (F) Blueback Herring caught in 2014. Group centroids are indicated by (+) marks and ellipses represent the 95% confidence region for each location.

Table 1. The 10 morphological landmarks digitized on each River Herring sample. Landmarks were digitized using tps-Dig2 software.

Measurement Number	Landmark
1	Top of operculum
2	Anterior insertion of dorsal fin
3	Posterior insertion of dorsal fin
4	Dorsal insertion of caudal fin
5	Center of caudal peduncle
6	Posterior insertion of anal fin
7	Anterior insertion of anal fin
8	Insertion of pelvic fin
9	Insertion of pectoral fin
10	Bottom of operculum

Table 2. Number of juvenile and adult Alewife and Blueback Herring caught in various water bodies associated with the Albemarle Sound system that were used for geometric morphometric analysis. A small sample of adult Alewife and Blueback Herring from the Metedeconk River in New Jersey was gathered to serve as an outgroup for the study.

	Juveniles			Adults
	Alewife	Blueback Herring		Blueback Herring
Location	2013	2013	2014	2014
Chowan	14	104	29	84
Currituck	3	0	0	0
Metedeconk (NJ)	0	0	0	22
North	8	0	0	0
Pasquotank	10	3	0	0
Scuppernong	13	0	0	0
W. Albemarle	9	69	68	0
Yeopim	10	0	12	6
Total	67	176	109	112

Table 3. Classification matrix based on linear discriminant analysis of Procrustes coordinates derived from Age 0 Alewife and Blueback Herring caught in 2013 and 2014.

Location	n	Chowan	Currituck	North	Pasquotank	Scuppernong	W. Albemarle	Yeopim	% Correct	Bootstrap %
Alewife – 2013										
Chowan	14	**10**	1	1	1	0	1	0	71.4	59
Currituck	3	0	**3**	0	0	0	0	0	100	89.7
North	8	0	0	**5**	1	1	0	1	62.5	66.1
Pasquotank	10	1	1	2	**3**	0	1	2	30	26.2
Scuppernong	13	0	0	2	2	**8**	1	0	61.5	51.9
W. Albemarle	9	2	1	0	1	0	**5**	0	55.6	62.8
Yeopim	10	0	0	2	2	2	1	**3**	30	39
Blueback – 2013										
Chowan	104	**62**			6		36		59.6	53.0
Pasquotank	3	0			**3**		0		100	96.3
W. Albemarle	69	20			1		**48**		69.6	64.2
Blueback – 2014										
Chowan	29	**29**					0	0	100	94.0
W. Albemarle	68	2					**55**	11	80.9	75.8
Yeopim	12	0					2	**10**	83.3	77.2

Table 4. Classification matrix based on linear discriminant analysis of Procrustes coordinates derived from male and female adult Blueback Herring caught in 2014. Males and females were compared and found to be significantly different (Pillai's trace: P =0.0002).

Sex	n	Female	Male	% Correct	Bootstrap %
Female	40	**28**	12	70	65.2
Male	72	26	**46**	63.8	67.9

Table 5. Classification matrix based on linear discriminant analysis of Procrustes coordinates derived from adult male Blueback Herring caught in the Chowan and Yeopim rivers during the 2014 season.

Location	n	Chowan	Yeopim	% Correct	Bootstrap %
All males					
Chowan	56	**49**	7	87.5	75.7
Yeopim	4	2	**2**	50.0	29.7
All Age 3					
Chowan	41	**37**	4	90.2	79.6
Yeopim	4	1	**3**	75.0	62.6

Table 6. Classification matrix based on linear discriminant analysis of Procrustes coordinates derived from adult male Blueback Herring caught in North Carolina (Chowan and Yeopim rivers) and New Jersey (Metedeconk River) during the 2014 season.

Location	n	NC	NJ	% Correct	Bootstrap %
All males					
NC	58	**48**	10	82.8	80.0
NJ	14	1	**13**	92.9	75.4
All Age 3					
NC	45	**37**	8	82.2	80.0
NJ	11	1	**10**	90.9	66.0

Table 7. Number of adult Blueback Herring caught in various water bodies associated with the Albemarle Sound system that were utilized for otolith shape analysis. A small sample of adult Alewife and Blueback Herring from the Metedeconk River in New Jersey was gathered to serve as an outgroup for the study.

Location	2010	2014
Chowan	35	56
Metedeconk (NJ)	0	16
Perquimans	18	0
Scuppernong	27	0
Yeopim	0	3
Total	80	75

Table 8. Classification matrix based on linear discriminant analysis of Elliptical Fourier Descriptors (EFDs) associated with adult male and female Blueback Herring caught during the 2010 and 2014 seasons. Otolith shape did not significantly differ between males and females (Pillai's trace: 2010, P = 0.3803; 2014, P = 0.342).

Sex	n	Female	Male	% Correct	Bootstrap %
2010					
Female	26	17	9	65.4	62.8
Male	54	21	33	61.1	62.1
2014					
Female	30	25	5	83.3	70.2
Male	45	11	34	75.6	71.9

Table 9. Classification matrix based on linear discriminant analysis of Elliptical Fourier Descriptors (EFDs) associated with adult Blueback Herring caught in the Chowan, Perquimans, and Scuppernong rivers during the 2010 season, and Chowan and Yeopim rivers during the 2014 season.

Location	n	Chowan	Perquimans	Scuppernong	Yeopim	% Correct	Bootstrap %
2010							
Chowan	35	**16**	7	12		45.7	53.1
Perquimans	18	2	**12**	4		66.7	49.1
Scuppernong	27	7	8	**12**		44.4	46.0
2014							
Chowan	56	**55**			1	98.2	94.8
Yeopim	3	0			**3**	100	66.7

Table 10. Classification matrix based on linear discriminant analysis of Elliptical Fourier Descriptors (EFDs) derived from adult Blueback Herring in North Carolina (Chowan and Yeopim rivers) and New Jersey (Metedeconk River) during the 2014 season.

Location	n	NC	NJ	% Correct	Bootstrap %
All adults[1]					
NC	59	47	12	79.9	81.9
NJ	16	3	**12**	75.0	68.5
All Females[2]					
NC	32	25	7	73.5	79.8
NJ	8	2	**6**	75.0	63.1

[1]Pillai's trace: P=0.499
[2]Pillai's trace: P = 0.0487

Appendix Table 1. Total length (TL), weight, and Fulton's condition factor (K) of juvenile Alewife caught in the Albemarle Sound system during the 2013 season. Fish from the Western (W.) Albemarle were caught at various locations between the west of the mouth of the Scuppernong River and east of the mouth of the Chowan River.

River of Capture	Month of Capture	n	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
Chowan	June	18	46.25±2.55	1.00±0.24	0.94±0.05
	July	5	45.00±2.12	0.79±0.14	0.74±0.02
Currituck	July	4	39.00±1.15	0.38±0.04	0.63±0.04
North	July	8	67.00±2.63	3.23±0.36	1.04±0.02
Pasquotank	July	12	57.00±2.91	1.70±0.30	0.89±0.05
	August	3	72.67±1.76	3.81±0.39	0.99±0.05
Scuppernong	June	10	50.00±1.40	1.14±0.08	0.88±0.01
	July	1	47.00	1.01	0.97
W. Albemarle	June	10	42.10±1.22	0.61±0.07	0.78±0.03
Yeopim	July	7	58.17±2.33	2.28±0.25	1.08±0.02
	August	2	83.00±3.00	5.42±0.11	0.95±0.08

Appendix Table 2. Total length (TL), weight, and Fulton's condition factor (K) of juvenile Blueback Herring caught in the Albemarle Sound system during the 2013 and 2014 seasons. Fish from the Western (W.) Albemarle were caught at various locations between the west of the mouth of the Scuppernong River and east of the mouth of the Chowan River.

River of Capture	Month of Capture	n	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
2013					
Chowan	September	4	49.67±1.45	1.12±0.13	0.82±0.01
	October	117	56.53±0.28	1.29±0.02	0.71±0.01
Currituck	October	1	59	1.39	0.68
Pasquotank	September	3	45.67±0.88	0.74±0.01	0.78±0.03
Scuppernong	September	1	70	2.51	0.73
W. Albemarle	June	52	46.15±0.64	0.82±0.04	0.81±0.01
	August	18	52.33±0.57	1.18±0.04	0.82±0.01
	September	13	57.15±0.47	1.10±0.03	0.76±0.01
	October	49	52.38±0.48	1.30±0.04	0.69±0.01
2014					
Chowan	July	28	54.79±0.89	1.50±0.09	0.89±0.01
	August	1	65	2.63	0.96
W. Albemarle	August	66	48.76±0.34	0.86±0.02	0.75±0.01
	September	2	59.50±3.50	1.60±0.23	0.76±0.02
Yeopim	July	11	45.45±1.36	0.80±0.05	0.86±0.04

Appendix Table 3. Total length (TL), weight, and Fulton's condition factor (K) of adult Blueback Herring caught in the Albemarle Sound system during the 2010 season.

River of Capture	n	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
Chowan	35	241.04±2.57	92.32±4.21	0.65±.02
Perquimans	18	267.88±2.59	171.69±6.23	0.87±0.02
Scuppernong	27	288.67±2.56	174.72±6.71	0.88±0.02

Appendix Table 4. Total length (TL), weight, and Fulton's condition factor (K) of adult Blueback Herring caught in the Albemarle Sound system during the 2014 season. Fish from the Metedeconk River were caught in New Jersey.

River of Capture	Month of Capture	n	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
Chowan	April	83	254.35±1.81	145.74±3.90	0.87±0.01
	May	3	256.67±5.21	151.84±10.22	0.90±0.04
Metedeconk	April	5	233.50±6.59	139.73±18.06	1.02±0.07
	May	17	231.8±3.37	113.24±5.76	0.90±0.01
Yeopim	March	1	256.00	169.80	1.01
	April	4	263.00±4.71	171.11±18.36	0.95±0.07

Appendix Table 5. Total length (TL), weight, and Fulton's condition factor (K) of adult male and female Blueback Herring caught in the Albemarle Sound system during the 2014 season. Fish from the Metedeconk River were caught in New Jersey.

River of Capture	Sex	n	Age ± S.E.	TL ± S.E. (mm)	Weight ± S.E. (g)	K ± S.E.
Chowan	M	54	3.60±0.12	250.36±2.27	135.66±4.26	0.85±0.01
	F	35	3.59±0.14	261.09±2.29	162.17±6.12	0.90±0.02
Metedeconk	M	14	3.58±0.23	226.50±2.36	104.95±3.72	0.90±0.01
	F	8	3.29±0.18	243.43±5.52	144.30±11.86	096±0.05

Appendix Table 6. Results of Kruskal-Wallis tests examining spatial and temporal differences in total length (TL), weight, and Fulton's condition factor (K) of juvenile Alewife and Blueback Herring caught in the Albemarle Sound system during the 2013 season.

Variable	Species	Effect	chi-square	df	p-value
TL	Alewife	Location	47.14	6	<0.0001
		Month	23.41	2	<0.0001
	Blueback	Location	25.29	4	<0.0001
		Month	53.33	2	<0.0001
Weight	Alewife	Location	48.92	6	<0.0001
		Month	22.24	2	<0.0001
	Blueback	Location	22.3	4	<0.0001
		Month	19.61	2	<0.0001
K	Alewife	Location	39.68	6	<0.0001
		Month	6.96	2	0.0309
	Blueback	Location	9.84	4	0.0432
		Month	63.06	2	<0.0001

Appendix Table 7. Results of Kruskal-Wallis tests examining spatial and temporal differences in total length (TL), weight, and Fulton's condition factor (K) of juvenile Blueback Herring caught in the Albemarle Sound system during the 2014 season.

Variable	Effect	chi-square	df	p-value
TL	Location	45.49	2	<0.0001
	Month	20.24	2	<0.0001
Weight	Location	54.68	2	<0.0001
	Month	30.84	2	<0.0001
K	Location	51.4	2	<0.0001
	Month	45.22	2	<0.0001

Appendix Table 8. Results of Kruskal-Wallis tests examining spatial and temporal differences in total length (TL), weight, and Fulton's condition factor (K) of male and female adult Blueback Herring caught in the Albemarle Sound system during the 2010 season.

Variable	Sex	chi-square	df	p-value
TL	M	25.75	2	<0.0001
	F	7.10	2	0.0288
Weight	M	34.70	2	<0.0001
	F	16.14	2	0.0003
K	M	28.13	2	<0.0001
	F	6.13	2	0.0466

Appendix Table 9. Results of Kruskal-Wallis tests examining spatial and temporal differences in total length (TL), weight, and Fulton's condition factor (K) of male and female adult Blueback Herring caught in the Albemarle Sound system during the 2014 season.

Variable	Sex	Effect	chi-square	df	p-value
TL	M	Location	21.46	2	<0.0001
		Month	10.54	2	0.0051
	F	Location	6.61	2	0.0367
		Month	7.10	1	0.0077
Weight	M	Location	13.24	2	0.0013
		Month	7.87	2	0.0196
	F	Location	2.53	2	0.2827
		Month	4.69	1	0.0303
K	M	Location	7.13	2	0.0283
		Month	4.40	2	0.1109
	F	Location	4.78	2	0.0917
		Month	1.82	1	0.1774

Appendix 10. North Carolina Division of Marine Fisheries sample approval letter

NCDENR

North Carolina Department of Environment and Natural Resources

Pat McCrory
Governor

Donald R. van der Vaart
Secretary

MEMORANDUM

TO: Graduate School, Dr. Thomas McConnell, Associate Dean
East Carolina University, Greenville, NC 27858

FR: Charlton Holloman Godwin, Biologist Supervisor
Division of Marine Fisheries, NCDENR, Elizabeth City, North Carolina 27909

DA: 23 July, 2015

RE: River herring (A*losa pseudoharengus and Alosa aestivalis)* samples collected for Mr. Walt Rogers

This memorandum confirms that in 2013 and 2014 North Carolina Division of Marine Fisheries staff in the Elizabeth City office saved adult and juvenile river herring for Mr. Walt Rogers that we collected during our routine river herring monitoring programs.

If there are any questions please feel free to contact me directly.

Regards,

Charlton Holloman Godwin
Biologist Supervisor NCDMF
Elizabeth City, NC 27909
252-264-3911
charlton.godwin@ncdenr.gov

7/23/2015

Charlton Holloman Godwin
Biologist Supervisor
Signed by: Charlton Godwin