HICKORY SHAD *ALOSA MEDIOCRIS* (MITCHILL) STOCK IDENTIFICATION USING MORPHOMETRIC AND MERISTIC CHARACTERS

by

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The Hickory Shad *Alosa mediocris* is an anadromous fish species of the family Clupeidae. Little is known about its distribution, life history, and status. Adult Hickory Shad are found seasonally during spring spawning in estuaries and coastal watersheds along the East Coast of the United States from Maryland to Florida, and during late summer as far north as Connecticut. To provide information on stock identification and watershed fidelity the distributional patterns of 17 morphometric and four meristic characteristics of adult Hickory Shad were analyzed from spawning populations along its range. A total of 687 specimens were examined along the latitudinal gradient from the Susquehanna River, Maryland, to the Wekiva River, Florida. Due to low sample sizes ($n<13$) for some rivers as well as missing measurements or counts, some specimens had to be excluded from multivariate analysis. Prior to statistical analysis morphometric characters were corrected for size-dependent variation using an allometric formula. After correction morphometric characters were natural log transformed to better approximate multivariate normality. Correlation analysis on transformed measurements and SL confirmed the results obtained from the allometric method; yet showed fork length and total length were strongly correlated (>0.80) and therefore excluded. Multivariate analysis of variance of pooled morphometric and meristic characters showed a significant effect of sex ($P < 0.05$)

therefore, all analyses were separated by sex. Analysis of variance showed highly significant difference ($P < 0.003$) for 15 characters between 10 locations for males and 12 characters between 12 locations for females. Non-significant characters were excluded and only significant characters for males and females were used for subsequent analysis including Principle Components Analysis (PCA) and Discriminant Function Analysis (DFA). PCA extracted 6 and 4 components (eigenvalues > 1) cumulatively explaining 63.67% and 60.88% of the variance for males and females, respectively. Bartlett's Test of Sphericity was significant ($P < 0.05$) and Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.60 for males and 0.68 for females, which confirmed appropriateness of the data for PCA. Principal component 1 for males and females was most correlated (> 0.4) with the head region and fin lengths. Using Quadratic Discriminant Function Analysis (QDFA), 77.9% and 80.3% of males and females, respectively, were correctly classified to their rivers of collection using separate-groups covariance matrix and equal prior probabilities. Individual river classification varied between 58.6% - 100%. The Tar-Pamlico River had the lowest percent correct classification for both male (58.6%) and female (62.0%) QDFAs. Tributary level discrimination was achieved in two instances: the James and Appomattox rivers, and the Roanoke and Cashie rivers. Overall, results of this study suggest that meristic and morphometric characters are a viable and potentially lower cost method to identify separate spawning populations (stocks) of Hickory Shad. Fishery management agencies desire more basic life history information to better manage the species. One large gap in knowledge is the untested assumption of natal homing in Hickory Shad; though the results of this study cannot directly confirm this assumption, the significant variation between river populations provides support for natal homing. The results of this work offer foundational information for creating a unique management plan for Hickory Shad.

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Statement of the Problem

Historically, Hickory Shad *Alosa mediocris* (Mitchill, 1814), along with other anadromous species of the family Clupeidae, including American Shad *A. sapidissima* (Wilson, 1811), Alewife *A. pseudoharengus* (Wilson, 1811), and Blueback Herring *A. aestivalis* (Mitchill, 1814), have comprised very substantial fisheries in North Carolina (Smith 1907, Hightower et al. 1996) and other East Coast US states (ASMFC 2010). The anadromous life history strategy of these species, which concentrates the adults within a small area (i.e., watershed or tributary) for a brief-to-extended period during the spring spawning, makes them extremely vulnerable to multiple causes of mortality related to human activities near rivers and estuaries (ASMFC 2010). Problems with habitat destruction, dam construction limiting access to spawning grounds (Polk 1879; Limburg and Waldman, 2009; Harris and Hightower 2011; Smith and Rulifson 2015), commercial and recreational harvest, and other anthropogenic activities have reduced the viability of these species across coastal watersheds of the Atlantic coast (Rulifson 1994; Waldman and Limburg, 2003).

Smith and Rulifson (2015) addressed another potential problem facing Hickory Shad survival, which is climate change and the resultant altering of spawning phenology (timing), since ocean water temperature is thought to be the major driver of spawning for alosines. If seasonal spawning patterns are forced together in time (resulting from rising temperatures) and space (due to coastal dams limiting responses to sea level rise and saltwater intrusion), the end result is possibly limited freshwater spawning habitat unnaturally shared with the other anadromous and freshwater species (Smith and Rulifson, 2015).

The Hickory Shad has been referred to as "largely ignored scientifically" by some fisheries scientists due to the fact that there are gaps in our knowledge and little research has explored its life history (Waldman and Limburg, 2003). Because of this lack of knowledge, the life history of the American Shad *A. sapidissima*, a perceived close relative whose life history and biology has been extensively studied, is often applied to Hickory Shad (Harris et al. 2007). This is interesting because genetically Hickory Shad are more closely related to the River Herrings Alewife *A. pseudoharengus* and Blueback Herring *A. aestivalis* than American Shad (Bloom and Lovejoy 2014). The assumption of a life history similar to American Shad is necessary in order to include the Hickory Shad in fishery management plans, but a majority of the American Shad life history aspects applied to Hickory Shad have not been examined carefully and may not accurately represent true Hickory Shad life history. This can be problematic for fisheries management, and further research is needed to determine the life history of Hickory Shad instead of depending on information about another species. The Atlantic States Marine Fisheries Commission (ASMFC) Amendment 3 to the Interstate Fisheries Management Plan (IFMP) for Shad and River Herring (2010) identified the need for research on Hickory Shad life history, specifically relating to migratory behavior. This could provide information on the reasons behind the apparent increase in Hickory Shad populations while other alosines are experiencing decline across the Eastern Seaboard (ASMFC 2010).

One of the key assumptions by state fisheries agencies is that Hickory Shad practice natal homing, though it has never been explicitly determined for the species (Batsavage and Rulifson 1998; Harris et al. 2007). Hickory Shad are believed to home to their natal streams to spawn once they have accumulated adequate energy reserves and the environmental conditions are optimal (Stence et al. 2014). American Shad possess a homing tendency and show fidelity to

natal streams based on tagging studies (Hollis 1948; Nichols 1960; Melvin et at. 1986). It is necessary to understand if Hickory Shad exhibit fidelity to natal streams in order to determine stock size and spawning run size, which are two integral parts required to calculate the level of harvest mortality experienced by different populations.

The Hickory Shad is a multi-jurisdictional species for management purposes when in the Atlantic Ocean as well as in river habitats. Hickory Shad are at present managed under Amendment 3 of the ASMFC Interstate Fishery Management Plan (IFMP) for Shad and River Herring, yet this management plan only incorporates biological information on the American Shad (ASMFC 2010). As it stands, North Carolina as well as other Atlantic coast states require superior and more up to date information on Hickory Shad in order to properly manage the recreational and commercial harvests. Before this can be accomplished, we need to identify different Hickory Shad stocks and determine if they show fidelity to natal streams. If they do exhibit fidelity, then we expect to be able to identify anatomical traits combined with genetic information unique to that population. Once that is accomplished, we can then determine to what degree inter-mixing occurs among the different populations.

Goal and Objectives

The goal of this study was to assess whether Hickory Shad spawning stocks in different watersheds can be identified for use in fishery management plans. Five different methods were selected for use on all fish specimens collected: 1) genetics; 2) otolith shape; 3) otolith chemistry; 4) body shape analysis; and 5) meristic and morphometric analyses. This goal assumes some aspect of natal homing.

My study involved examining the meristic (counts of anatomical features) and morphometric characters (measurements) of the Hickory Shad, which was never fully described in the original species description (Mitchill, 1814). Therefore, anatomical features of Hickory Shad, and how those features may shift throughout the species range, are undocumented. It is possible that these characteristics might be usable as inexpensive diagnostic features for stock identification if they vary by latitudinal region or watershed.

Hypotheses:

My two hypotheses were:

The morphological characters vary throughout the range, and so therefore fish can be classified to certain watersheds and possibly to tributaries.

The meristic characters of this species are not fixed throughout the range, and the variability of those characters will allow classification of fish to certain watersheds and possibly tributaries.

The purpose of Chapter 1 (this chapter) is to describe the hypotheses and existing background information about the life history of Hickory Shad. Chapter 2 redefines the species characteristics of Hickory Shad completing the original description provided by Mitchill (1814) that was weak in morphometric characteristics and which may have contributed in part to several reclassifications of the species within the family Clupeidae. Chapter 3 uses the information from Chapter 2 to answer the question of whether meristic and morphological variation can be used as tools to separate spawning populations on a watershed or tributary level. Chapter 4 is a summary of the information gathered by my research, and provides recommendations for future work and management plan development for the species.

Background

The Hickory Shad *Alosa mediocris* was first described in 1814 by Samuel L. Mitchill (Mitchill 1814), presumably from a New York specimen (Jenkins and Burkhead, 1993). The Hickory Shad is considered an understudied fish species though it is found in coastal and inland waters on the East Coast of the United States from Maine to Florida (Hildebrand and Schroeder, 1928). Yet, the current northern limit of the Hickory Shad spawning is not precisely known and they are not commonly reported north of Connecticut (Rulifson 1994). The southernmost river with a spawning population of Hickory Shad is the St. Johns River in Florida (Harris et al. 2007). It is not known how these spawning populations along the Eastern Seaboard interact, and where they could be classified as independent spawning units (i.e., stocks). The term "stock," as used in fisheries, describes a management unit, which can include separate spawning populations of a species (Meng and Stocker, 1984). Therefore, determining the independence of these spawning populations will assist in defining the stock concept for the Hickory Shad, which is necessary for creating management plans that ensure and conserve the biodiversity of separate spawning populations (Khan et al. 2012).

The Hickory Shad is an anadromous schooling species of the family Clupeidae that enters coastal freshwater between February and June to spawn; the increasing latitudes correspond to later dates of entry into freshwater (Murauskas and Rulifson, 2011). Hickory Shad spawn in small freshwater streams but spend most of their adult lives in the Atlantic Ocean (Mansueti 1962; McBride and Holder 2008). As with other anadromous alosines, water temperature seems to be the largest controller of the annual timing of spawning (phenology) for Hickory Shad (Mansueti 1962). Aside from water temperature, other proposed factors for spawning include photoperiod, current velocity, and turbidity (Leggett and Whitney 1972).

Diadromy, the term used to encompass anadromous, catadromous, and amphidromous life-history behaviors, has long intrigued biologists (McDowall 1988). These impressive life-history behaviors, which involve migration between oceans and freshwater, can be found in a small number of described fish species, roughly 250 across the globe, which include important food and sport fishes (Bloom and Lovejoy 2014). Many diadromous fish species are subject to intense scientific investigation (e.g., salmon), yet the evolution of diadromy in fishes is largely unresolved (McDowall 1988). Diadromy in the family Clupeidae is very common, roughly 30 species, and the family Clupeidae is more speciose than all other families of diadromous fishes except Gobiidae and Salmonidae (Bloom and Lovejoy 2014). There are two main hypotheses for the evolution of diadromy, which are of great theoretical debate. 1) Gross et al. (1998) suggested that the productivity differences between marine and freshwater regions determined the various forms of diadromy, often referred to as 'productivity hypothesis'. 2) The 'safe-site hypothesis' proposes that freshwater environments provide the eggs and larvae of marine fish species greater protection from predation (Bloom and Lovejoy 2014).

Other anadromous members of the family Clupeidae include the American Shad *Alosa sapidissima* and river herrings, Blueback Herring *Alosa aestivalis* and Alewife *Alosa pseudoharengus*. The Hickory Shad is often confused with the American Shad, due to their morphological similarity, but can be differentiated by its projecting lower jaw; the jaw of the American Shad fits within a slot of the upper jaw (Mitchill 1814; Uhler and Lugger 1876; Smith 1907; Hildebrand and Schroeder 1928). Hickory Shad reach a maximum length of 600 mm Standard Length (SL) and mature between 2-4 years; a majority of fish live a maximum of 7 years (Jenkins and Burkhead, 1993; Batsavage and Rulifson 1998), although some fish in

spawning populations at the northern end of the range have been aged up to 9 years (MDDNR, 2016).

Hickory Shad are piscivorous, feeding on small fishes, invertebrates, and crustaceans (Hildebrand and Schroeder 1928, Harris et al. 2007; NCWRC 2010; Murauskas and Rulifson 2011). It is thought that once in freshwater systems Hickory Shad refrain from feeding. Murauskas and Rulifson (2011) found that for fish collected in North Carolina, there was a decreasing proportion of fish with prey items in their stomachs from those collected from ocean (97%), estuary (64%), and freshwater (6%) habitats. The diet consisted primarily of unidentified fish pieces, anchovies, and amphipods (Murauskas and Rulifson, 2011). In contrast, Hickory Shad in Florida's St. Johns River are known to forage on fish during their spawning run, potentially providing them the energy necessary to complete the long freshwater migration and spawn multiple times (Harris et al. 2007).

Presently Hickory Shad are a sought-after fish for recreational anglers during the spring time and in North Carolina represent a multimillion-dollar fishery (NCWRC, unpublished data). Yet, recreational and sometimes commercial landings are not well documented and there remains poor understanding of Hickory Shad stock status. In fact, in 1999 the Maryland Department of Natural Resources (MDNR) initiated stocking of hatchery-reared Hickory Shad into the Choptank, Patuxent, and Nanticoke rivers to restore self-sustaining populations and to serve as a substitute for the American Shad, which is more difficult to rear and has a longer time to maturity in the wild (Stence et al. 2014).

Not only do Hickory Shad support important recreational fisheries during the spawning run (Harris and Hightower, 2011), they serve the vital ecological function of bringing marine-derived nutrients into freshwater ecosystems (Garman and Macko 1998). The ASMFC does not

address Hickory Shad stock status because there has not been any coast-wide investigation into Hickory Shad stock status (ASMFC 2017). It is believed that the largest spawning populations of Hickory Shad are found between South Carolina and Delaware (Rulifson 1994). North Carolina has large Hickory Shad populations located in the Neuse, Tar-Pamlico, and Roanoke Rivers (Smith 1907; Murauskas and Rulifson, 2011). Hickory Shad are also found in other rivers in North Carolina, for example the Cape Fear, New, and Chowan rivers, yet there is little reference to these populations in the literature. The Cape Fear River has a highly variable presence of Hickory Shad from year to year, so it is unknown whether this is a separate spawning population or the wandering of fish from South Carolina rivers (e.g., Waccamaw River). The North Carolina Wildlife Resources Commission (NCWRC) in describing "Herring and Shad in North Carolina" briefly mention the Cape Fear and Meherrin (Chowan) rivers as possessing spawning runs for Hickory Shad (as well as American Shad), but do not list the New River (NCWRC 2010).

Even though Hickory Shad eggs have been collected from rivers in multiple states, and the development of egg and larvae have been studied and illustrated (Mansueti 1962), little research has investigated the specific micro- and macro-habitats necessary for spawning. Once in river systems, Hickory Shad spawn in both tributaries and main channels (Burdick and Hightower 2006). Typically spawning occurs when water temperatures are between 14.4°C – 16.6°C (NCWRC 2010) or 12.0°C – 14.9°C (Harris and Hightower 2011). Water velocity is also a significant component influencing spawning; in the Roanoke River, Hickory Shad preferred velocities >0.1 m/s with larger substrate. Eggs have been collected in water velocities up to 1.26 m/s, and dissolved oxygen levels between 6.76 to 11.27 mg/L (Harris and Hightower 2011). Mansueti (1962) initially described Hickory Shad eggs as transparent, spherical, mostly non-adhesive, and of medium size when compared to eggs of other fish species that spawn in similar

habitats. More recently hatchery staff have noted that Hickory Shad eggs are initially semi-adhesive (M. Odom, Harrison Lake National Fish Hatchery (HLNFH), pers. comm.). Under laboratory conditions fertilized and water hardened Hickory Shad eggs average 1.49 mm in diameter (Mansueti 1962). The eggs are initially semi-demersal under slow-moving water, yet will become buoyant under fast-moving water conditions (Mansueti 1962). After fertilization, hatching occurs in roughly 48-76 h, depending largely on temperature (Mansueti 1962). Hatching occurred in 96 h at 15°C at the HLNFH (R. Rulifson, pers. comm.). Few studies have examined Hickory Shad fecundity and the estimates are quite variable. Pate (1972) estimated total potential fecundity to be between 43,500 and 347,500 eggs per female in the Neuse River, North Carolina. A study by Street (1969) looked at Hickory Shad from the Altamaha River, Georgia, and determined fecundity ranged from 252,700 to 730,200. In Virginia coastal rivers, Watkinson and Garman (2003) estimated individual fecundity as between 46,600 and 847,300, which is slightly higher than other estimates. The relatively low fecundity compared to other anadromous fish, such as American Shad and Striped Bass *Morone saxatilis* combined with the removal of the larger and most fecund females for roe causes Hickory Shad to be vulnerable to overfishing (Batsavage and Rulifson 1998).

Hickory Shad utilize the reproductive strategy of iteroparity, distinguished by having multiple reproductive cycles over the life of a fish (Harris et al. 2007). Yet, it is not well understood whether Hickory Shad show latitudinal differences in reproductive attributes relating to repeat spawning, which is observed in the American Shad (Leggett and Carscadden 1978). Based on observations it is believed Hickory Shad are batch spawners (Harris et al. 2007), with indeterminate fecundity and group-synchronous development of oocytes, possibly spawning over 2-3 days (Murauskas and Rulifson 2011). After spawning, the duration of time spent in

freshwater by adults is unknown (Greene et al. 2009). Batch spawning is evolutionarily advantageous because it allows fish to disperse gametes over a greater spatial and temporal scale; this improves the likelihood offspring will face favorable conditions (Murphy 1968; Olney et al. 2001). Utilizing scale spawning mark analysis to determine spawning attempts, Stence et al. (2014) found that many Hickory Shad in Maryland's Patuxent and Susquehanna rivers had spawned up to 7 times in the oldest fish.

Hickory Shad larvae are often collected in downstream tributaries; Smith and Rulifson (2015) suggested they utilize these areas for food resources, refuge from predators, or to avoid non-ideal water quality. Generally, it is believed they become juveniles around 35 mm TL (Mansueti 1962). It is believed that young Hickory Shad leave fresh and brackish environments in early summer and emigrate to estuaries earlier than other juvenile *Alosa* species (Pate 1972; Batsavage and Rulifson 1998). Based on scale analysis and looking at the freshwater "zone" that forms when anadromous fish spend time in freshwater, Pate (1972) found this marking to be much less apparent for Hickory Shad than other clupeids. It has even been suggested that some young Hickory Shad may refrain from using estuarine waters and instead move straight to saltwater (Batsavage and Rulifson 1998).

Little information is available about Hickory Shad genetics, especially population genetics. The first such study conducted by Vishakha (2012) involved 12 neutral microsatellite loci to determine genetic diversity, as well as Hickory Shad population structure, in rivers of the lower Chesapeake Bay, Virginia. The rivers included in this study were the James (and tributaries Appomattox and Chickahominy Rivers), Rappahannock, and Pamunkey rivers. Results indicated that genetic diversity of these populations was very low and heterozygosity was below what was expected (Vishakha 2012). This led Vishakha (2012) to conclude that a

serious bottleneck or multiple bottlenecks likely had happened in the past, possibly over 30 years ago.

My research is only a part of a larger project utilizing multiple techniques in an effort to assess these critical life history aspects. Overall, we will use geometric morphometrics, otolith shape analysis, otolith elemental chemistry, genetics, and meristic and morphometric analyses. Using these five methods will help determine at what level population discrimination is possible. My portion of this large collaborative project involved the meristic and morphometric analysis of Hickory Shad. It has long been known that the body form of a fish can be affected by both environmental and genetic factors, which is the premise for differences in phenotypic expression (Melvin et al. 1992). Differences in environmental factors, such as temperature, salinity, light, and dissolved gases, during early development can yield substantial variation in meristic counts for individuals of the same species (Taning 1952). The idea of variation in meristic characters, specifically vertebral counts, has been around since the mid to late 1800s; Jordan (1891) described the inverse relationship between temperature and number of vertebrae found in many fish species. Morphological characters are also controlled by these same environmental factors. It is not known for every species at exactly what point in development environmental factors no longer can affect morphometric characters, but it is believed to be for an even longer length of time than meristic characters, leading to the possibility of larger differences (Martin 1949).

Analysis of morphometric and meristic characters of fish is straightforward, highly cost-efficient, and an often-used tool to identify and differentiate fish stocks and populations (Swain and Foote 1999; Siddik et al. 2016). I analyzed specimens collected from different North Carolina rivers, particularly the Roanoke, Neuse, Pungo, Tar, Cape Fear, New, and Chowan rivers. I also analyzed Hickory Shad samples from select river systems outside of North Carolina

in states including Maryland, Delaware, Virginia, South Carolina, Georgia, and Florida. The morphometric and meristic variation for this species, across its range has never been determined, and will be useful in future management plans for defining stock units.

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Chapter 2: The Anadromous Hickory Shad (Clupeiformes: Clupeidae, *Alosa mediocris* [Mitchill 1814]): Morphometric and Meristic Variation

Abstract

The anadromous Hickory Shad *Alosa mediocris* of the family Clupeidae is reviewed, specifically regarding morphometric and meristic variation. Few descriptions of Hickory Shad morphometric and meristic characters exist in the literature, though it was described in 1814. Most authors of the historic literature have failed to provided capture location for specimens, analyze large numbers of Hickory Shad, or document how morphometric and meristic characters of the species vary spatially. To address this information gap, a total of 717 mature Hickory Shad were collected from 23 different locations in Maryland, Delaware, Virginia, North Carolina, South Carolina, Georgia, and Florida using electroshocking, gill net, or rod and reel. All specimens were frozen and then thawed to examine for 17 morphometric characters and four meristic characters; a random subset (n = 463) were analyzed for an additional 4 meristic counts of gill rakers. Overall specimens ranged from 206-389 mm SL with a mean $\pm$ SD of 278.41 $\pm$ 27.69 mm, 232-435 mm FL with a mean of 310.98 $\pm$ 30.35 mm, and 272-508 mm TL with a mean of 365.62 $\pm$ 35.52 mm. The linear relationships between FL and TL, and FL and SL, were investigated and found to be: TL = 1.169*FL + 1.660 (n=705, r^2=0.995) and SL = 0.909*FL - 4.274 (n=717, r^2=0.992). Substantial differences in character means for many morphometric measurements were found between male and female specimens, suggesting the need to separate Hickory Shad by sex for analysis. However, meristic characters did not show differences in character means by sex. No one morphometric measurement could distinguish Hickory Shad from other morphologically similar Clupeids, but the meristic count of gill rakers on the lower limb of the first arch were important to separate Hickory Shad (19-22) from American Shad, Alewife, and Blueback Herring.

Introduction

No published study has examined and described an extensive set of morphometric and meristic characters of the Hickory Shad *Alosa mediocris*. The initial description of Hickory Shad by Samuel L. Mitchill, a professor at Columbia University in New York City, was published in 1814, yet his description is lacking some key information. He indicates that this is a species unknown to the system and proceeded to describe it from “fresh specimens,” though unfortunately there is no reference to the capture location of the fish nor quantity examined. It is possible that the description could have been based from one or several individuals. I speculate

that the likely watershed from which Mitchill collected his specimen(s) was the Hudson River due to its close proximity to Columbia University.

Few records exist of Mitchill's early attempts to describe New York fauna, including *A. mediocris*. Perhaps Professor Mitchill took students to the shores of the Hudson River to observe fauna from pulling small seines; unless more early writings of Professor Mitchill are discovered, the locations and manner of these ichthyological collections will remain unknown. One or more of those specimens collected was an undescribed species of "Shad", which he presumably took back to his laboratory for examination and decided the specimen(s) fit within the family Clupeidae. Mitchill proceeded to designate the species *Clupea mediocris* – the "Staten Island Herring". In a presumably similar manner, Mitchill also described 11 other new species during that era (including *A. aestivalis,* the Blueback Herring) although all 12 new "Mitchillian" species, including the current-day Hickory Shad and Blueback Herring, were placed in different genera by subsequent authorities (Gill 1898).

Unfortunately, the original description of the Hickory Shad contained only a sparse description of the anatomical features. Mitchill (1814) included basic descriptions of the fish shape, color, size, and meristic counts for branchiostegal, pectoral, ventral, anal, dorsal, and caudal fin rays, but he did not include any information on morphological measurements or ratios of size between various body features. Interestingly, many researchers describing the few characteristics of this species did so citing other investigators, who in turn cited Mitchill (1814). Therefore, little additional meristic or morphological information has been recorded for the species since the original description.

In addition, no record can be located of the original museum specimen described, nor where or when the specimen was collected. During this time of budding taxonomy in America, it

was neither common nor required to keep holotype specimens for newly described species. Other taxonomists after Mitchill revised the taxonomic status of the Hickory Shad. Notably, the genus *Alosa* was divided into three genera by Regan in 1917: *Alosa*, *Caspialosa* [Berg], and *Pomolobus* [Rafinesque]; the Hickory Shad was classified under the genus *Pomolobus* along with the Alewife and the Blueback Herring (Bowen et al. 2007). Later work by Bailey et al. (1954) and Svetoviodov (1964) led to the combining of the genera *Pomolobus* and *Caspialosa* into the genus *Alosa*, thereby changing the scientific name of Hickory Shad from *Pomolobus mediocris* to *Alosa mediocris* [Mitchill 1814] (Bowen et al. 2007).

Mansueti (1962) examined the hypothesis that the Hickory Shad might be a hybrid between the American Shad *Alosa sapidissima* and one of the River Herrings, the Alewife *A. pseudoharengus* or the Blueback Herring *A. aestivalis.* He concluded that hybridization was unlikely and "not substantiated by any reliable evidence" (Mansueti 1962). Around this time, a few fish culturists experimented in hatcheries and actively pursued creating hybrids involving Hickory Shad and River Herring, though none of these attempts was successful (Mansueti 1962).

The objective of Chapter 2 of this thesis was to fully describe the various anatomical features, including meristic counts and morphological measurements, of the Hickory Shad across its range. The Hickory Shad is considered an understudied fish species though it spawns in rivers on the United States Eastern Seaboard from Maryland to Florida (Richkus and DiNardo 1984). The northern range limit of Hickory Shad is not precisely known and some authors purport the species occurring as far north as Maine (Hildebrand and Schroeder 1928). As for the southern range limit, it is well documented to be the St. Johns River in Florida (Harris et al. 2007). It is relatively uncommon to find Hickory Shad as far north as Maine or the Bay of Fundy, and many accounts of this are quite dated or anecdotal; no recent publications or reports can corroborate

this assertion. Rulifson (1994) reported that Connecticut is the northernmost state having a presence of Hickory Shad based on responses to questionnaires by respective state fisheries biologists. It is possible some of these northern accounts of Hickory Shad are either misidentifications with morphologically similar species, such as the American Shad *A. sapidissima,* or possibly wandering Hickory Shad collected in bays or the Atlantic Ocean, but not actively spawning. The Hickory Shad is a schooling species of the family Clupeidae and utilizes the life history strategy of anadromy, entering coastal freshwater between February and June to spawn; the higher latitudes correspond to later dates of entry into freshwater (Murauskas and Rulifson 2011).

Relatively few authors have included morphometric and meristic values for Hickory Shad (Uhler and Lugger 1879; Jordan and Evermann 1896; Smith 1907; Hildebrand and Schroeder 1928; Jones et al. 1978; Smith 1985; Menhinick 1991), but none investigated how these characters vary spatially. Most previous studies fail to provide capture location(s) for the specimens examined and cover many fewer characters than the present study. Furthermore, some authors provide only one value for various meristic counts and morphometric measurements, when in reality there is often considerable variation. No published study has described Hickory Shad specimens across such a large latitudinal gradient, covering the majority of the species range. Similar studies have been undertaken for the American Shad (Melvin et al. 1992), Alewife, and Blueback Herring (Rulifson et al. 1987).

Historically, morphometric and meristic analyses of fish have been valuable tools for early ichthyologists and naturalists alike (Swain and Foote 1999). Starting in 1894, the Royal Society of the United Kingdom created the "Committee for Conducting Statistical Inquiries into the Measurable Characters of Plants and Animals." One of the committees' chief tasks was to

investigate morphometric variation in Atlantic Herring *Clupea harengus* (Cadrin 2000). Analysis of morphological and meristic characters of fish is straightforward, cost-efficient, and an often used tool to identify and differentiate fish species, stocks, and populations (Siddik et al. 2016).

Methods

Hickory Shad specimens were collected during the 2016 and 2017 spawning runs from the Susquehanna and Patapsco rivers, Maryland; the Nanticoke River, Delaware; the Rappahannock, Appomattox, and James rivers, Virginia; the Chowan River headwaters (Meherrin, Nottaway, and Blackwater), also in Virginia; the Roanoke, Cashie, Pungo, Pamlico, Tar, Neuse, New, and Cape Fear rivers, North Carolina; Pamlico Sound, also in North Carolina; the Waccamaw and Santee rivers, South Carolina; the Altamaha River, Georgia, and the St. Johns River, Florida (Table 2-1). In addition, a few specimens (n=5) were obtained from the Atlantic Ocean close to shore, near Wrightsville Beach, North Carolina. Relative location of rivers as well as collection sites are depicted in Figure 2-1. All specimens were collected from the different locations by recreational angling (i.e., rod and reel), gill net, or electrofishing. Specimens from rivers outside of North Carolina were collected and donated to this study by the respective state or federal fisheries agencies. North Carolina fish came from the North Carolina Wildlife Resources Commission (NCWRC) or the North Carolina Division of Marine Fisheries (NCDMF). Additional sampling was conducted by the Rulifson Lab with electrofishing and rod and reel (Scientific Collection Permit Number 17-SFC00133; AUP #D330).

Initially all specimens were frozen in water to minimize freezer burn, and then eventually transferred to the Rulifson Lab at East Carolina University (ECU) for examination. Once received or collected, fish were identified to species based on projection of the lower jaw beyond the maxilla (as opposed to the American Shad, for which the lower jaw inserts into a slot in the

maxilla), weighed to the nearest 0.01 g, bagged individually without water, and given a unique identification number. After this step the fish were placed in freezers (-20°C or -0°C) on the ECU campus until analysis. Specimens were removed from the freezer and slowly allowed to thaw. A small tissue sample was taken from the dorsal fin, which was then placed in 95% ethanol (ETOH) and stored in a -80°C freezer for later genetic analysis.

A total of 17 morphometric measurements and 4 meristic characters (Table 2-2) were recorded generally following the methods outlined by Hubbs and Lagler (1947). All measurements were straight line distances from point to point on the left side of the body unless there was physical damage: standard length (SL) -- distance between most anterior portion of the head (lower jaw) to the last vertebrae; fork length (FL) -- the distance between the lower jaw to the fork of the caudal tail; total length (TL) -- the greatest distance between lower jaw and end of caudal fin when the caudal rays are pinched together; lower lip to nose (LLN) -- the distance of the projecting lower jaw to maxilla; snout to anal length (SAL) -- the distance between lower jaw and the anus; body depth (BD) -- greatest depth distance between anterior to dorsal fin and anterior of the ventral fin; head length (HL) -- the distance from lower jaw to the most distant point of the operculum (including membrane); eye length (EL) -- the greatest distance of the orbit; snout length (SNL) -- the distance from the most anterior point of the upper lip to the anterior margin of the orbit; head width (HW) -- the distance (width) across the head where the preopercle ends; interorbital width (IOW) -- distance between the eyes at the top of the cranium; maxillary length (ML) -- the distance from the tip of the upper jaw to the distal end of the maxillary; fin length dorsal base (FLD) -- the greatest distance of the structural base between the origin and insertion of the dorsal fin when the fin is erect; fin length anal base (FLA) -- the greatest distance of the structural base between the origin and insertion of the anal fin when the

fin is erect; longest ray dorsal fin (LRD) -- the distance from the structural base of the dorsal fin to the tip of the longest ray; longest ray pectoral fin (LRP) -- distance from the structural base of the pectoral fin to the tip of the longest ray; longest ray ventral (pelvic) fin (LRV) -- distance from the structural base of the ventral fin to the tip of the longest ray; longest ray anal fin (LRA) -- distance from the structural base of the anal fin to the tip of the longest ray when the fin is erect. A Hickory Shad illustration (Figure 2-2) depicts how most morphometric measurements were taken. IOW and HW were omitted on the illustration since they are width measurements and cannot be accurately depicted. The standard length, total length, and snout-to-anal length were measured to the nearest mm; all other measurements were taken by using Fisherbrand "Traceable" digital calipers (model number 06-644-16) to the nearest 0.01 mm.

External meristic counts were taken on the left side of the body, unless there was damage: post ventral (pelvic) scutes (PVS) -- count of scutes from the end of the ventral fin to the anus; anterior ventral scutes (AVS) -- count of scutes from the beginning of the operculum to the ventral fin, including the scute straddling the ventral fins; scale rows (SR) -- count of scales along the lateral line, beginning at the upper angle of the operculum and terminating at the end of the hypural plate as determined with a crease in the caudal peduncle by folding the tail; and longitudinal scale rows (LSR) -- count of scales from the origin of the dorsal fin to the origin of the ventral fin. A random subset of specimens (n = 463) were analyzed for an additional four internal meristic counts, including the left and right gill rakers of the upper first arch (L-GRU, R-GRU) -- count of all gill rakers on the upper arch of first gill raker, not including the raker straddling the angle; and left and right gill rakers lower (L-GRL, R-GRL) -- count of all first arch gill rakers from the raker straddling the angle to the end, regardless of size.

External meristic characters including the scale rows between the upper angle of gill opening and base of caudal fin, longitudinal scale rows between origin of ventral fin and origin of dorsal fin, post-ventral scutes, and anterior-ventral scutes, were all counted from the freshly-thawed specimens.

To the best of my knowledge, there are no references in the literature detailing specific methods for counting scutes of Clupeids. I chose to divide the scute count into two -- anterior and posterior -- of the ventral fin following Smith (1985), though Nichols (1966) and Melvin et al. (1992) choose to count total scutes for American Shad. All scutes were counted, regardless of size, from where the ventral surface reaches the operculum posterior to the anus. Special care was given to check for scutes obscured by the anus in all fish, specifically ripe females. Occasionally scales near the scutes had to be removed to fully expose all scutes, and then counts were obtained with the aid of a probe.

After external morphometric measurements and meristic counts were completed, fish were then dissected to remove the gonads, which were weighed to the nearest 0.01 g. Sex was determined for each specimen based on visual inspection of the gonad. Once features of each specimen were recorded, the data were compiled into one Microsoft Excel file for analysis.

Sample sizes for each state, watershed, and capture locations were not uniform, nor were the number of males and females the same, due to the various collection methods and availability at the time of collection. In addition, the number of fish analyzed for each character was not always equal because some of the specimens were damaged necessitating the omission of one or more characters. Also the timing of the collection for each watershed was not standardized; spawning often started prior to the typical timeline for state agency spring sampling. The morphometric and meristic data presented here are from frozen and thawed -- not fresh --

Hickory Shad and for purposes of the analyses I assumed that any bias caused by this process was the same across all specimens.

Results

Overall 717 Hickory Shad were analyzed for 17 morphometric measurements and four meristic characters from 23 different rivers and estuaries in Maryland, Delaware, Virginia, North Carolina, South Carolina, Georgia, and Florida following the methods outlined above. Results of descriptive statistics for all locations combined, separated by sex, for all measurements and counts are presented in Table 2-3. Results for each individual river and combined sex can be found in Table 2-4. The random subset of specimens (n = 463) analyzed for four internal meristic gill raker counts showed that Hickory Shad had between 8-11 rakers on L-GRU, 8-12 rakers on R-GRU, and 19-22 rakers on both L-GRL and R-GRL.

A basic review of the morphometric and meristic data showed sexual difference in many characters, namely morphometric measurements. All morphometric characters showed sexual difference in character means, yet some character differences were more substantial. For instance, the mean measurements (mm) of BD (Female: 91.44, Male: 79.03), FLD (Female: 63.41, Male: 39.77), SAL (Female: 218.99, Male: 197.51), and HL (Female: 81.40, Male: 74.72) were largely different between sexes. As for meristic counts (SR, LSR, PVS, and AVS), there was no observed difference between sexes and so the averages between males and females were similar. Of the four counts, the largest difference in the averages was found for the count of LSR where the averages were 17.81 and 17.71 for females and males, respectively. Due to the differences in some characters (i.e., morphometric) by sex, it was necessary to divide the morphometric and meristic data for males and females for accurate description and analysis.

Specimen Size

All Hickory Shad collected and included in this study were adults (sexually mature) participating in the annual spawning run and all morphometric and meristic data reported are for adult fish. Male specimens from locations combined ranged from 206 to 344 mm SL, the mean ± SD was 264.42 ± 20.52 mm. Female specimens ranged from 229 to 389 mm with a mean ± SD of 289.72 ± 24.71 mm. Sizes for sexes and locations combined ranged from 206 to 389 mm SL with a mean ± SD of 276.53 ± 26.31 mm. The linear relationships between FL and TL, and FL and SL, were:

$$TL = 1.169*FL + 1.660 \ (n=705, r^2=0.995); \text{ and}$$

$$SL = 0.909*FL - 4.274 \ (n=717, r^2=0.992).$$

The largest Hickory Shad were from the Waccamaw River, SC and the mean ± SD was 359.86 ± 13.92 mm SL with a range between 350 and 389 mm SL; average weight was 1281.13 ± 95.46 g and all 7 specimens from this river were female. On average the smallest Hickory Shad were collected from the New River, NC with a mean ± SD of 248 ± 24.92 mm SL and a range of 209 - 279 mm SL. However, the smallest Hickory Shad collected in this study (206 mm SL) was a male from the Tar River, NC. Specimen total body weight (n = 695), with sexes and locations combined ranged from 206.03 to 1488.28 g with a mean ± SD of 501.34 ± 187.52 g, and gonad weights (n = 691) from 0.38 to 266.03 g with a mean of 49.88 ± 45.78 g.

Sex

The sex ratio between male and female Hickory Shad varied among the locations, but substantial differences were noted in the larger size of females compared to the males of similar SL. The smallest male weighed 206.03 g and largest weighed 866.50 g. The smallest female

weighed 242.40 g and largest 1488.28 g. Gonads for females weighed from 0.38 to 266.03 g with a mean ± SD of 74.43 ± 51.15 g. Gonads for males weighed from 0.90 to 62.53 g with a mean ± SD of 22.86 ± 11.53 g. Variation in size and weight of female gonads were largely dependent on spawning status. Some gonad specimens had deteriorated so gonad weight measurements (n = 4) and sex determination (n = 22) were not possible. In addition, the sexing of some specimens was omitted on the data sheet during the examination process.

Missing data

Some of the 717 Hickory Shad could not be analyzed for the entire suite of 17 morphometric and four meristic characters due to specimen damage. This resulted in 146 missing values across all morphometric and meristic characters. Missing value analysis was performed in SPSS version 24 (IBM Corporation 2016) and the meristic character LSR had the most missing data (7.7%). Of the remaining characters only SR, LRA, and PVS had more than 1.0% missing: 4.3, 1.3, and 1.1%, respectively. Values for count and percent missing of each character are reported in Table 2-5.

Comparison between Hickory Shad and other Clupeids

Morphometric and meristic results of this study were compared to available literature values for morphologically similar Clupeids, including the American Shad, Alewife, and Blueback Herring (Table 2-6). Characters mentioned here represent the clearest difference between species: Hickory Shad have a larger body depth as a percent of total length (22.31-26.55) compared to American Shad (17.2-19.4) and Alewife (17.8-21.7), but body depth is similar to that of Blueback Herring (22.1-25.2). The upper portions of the variable ranges for

Hickory Shad scute and scale row counts (PVS, AVS, and SR) were less than that for American Shad, but LSR was greater for Hickory Shad. This is not surprising since the body depth as a percent of total length was greatest for Hickory Shad, and the LSR character is counted along the depth of the body. The range of interorbital width (IOW) as a percent of head length for Hickory Shad (16.24-19.28) was most similar to Alewife (15.7-21.6); the range for American Shad (18.6-21.6) was higher than for Hickory Shad but within the range for Alewife. Overall, Blueback Herring interorbital width as a percent of head length (21.1-26.4) is the largest. As for eye length as a percent of head length, the Hickory Shad has the smallest range (18.08-19.10), which is much less than that of American Shad (27.3-32.0), Blueback Herring (23.4-30.0) and Alewife (26.9-35.7).

Discussion

It is often difficult to discern the causes of morphological and meristic variations between fish populations (Cadrin 2000) though it is assumed they might be related to genetic differences or linked to phenotypic plasticity resulting from non-homogeneous environmental factors in each river (Melvin et al. 1992). However, reasons why there are variations in meristic and morphological characters were not an objective of this study.

Instead, my study provides foundational information on the morphometric and meristic variation of Hickory Shad across a large portion of the species range. To complement this study, further research is needed to investigate these characters of Hickory Shad from more southern rivers in Georgia and Florida. This would allow comparison of morphometric and meristic variation across the entire species range and determine if greater geographic distance corresponds to larger variation. It is more likely that adjacent rivers or watersheds share common

environmental characteristics compared to rivers separated by large distances, possibly leading to greater variation in morphometrics and meristics. For instance, we were able to obtain 22 samples from a small tributary of the Susquehanna, River Maryland at the mouth of Deer Creek (39.613358 N, -76.149024 W), which is near the northern end of the assumed Hickory Shad spawning range. Unfortunately, we were unable to obtain large sample sizes from the southernmost Hickory Shad spawning population of the St. Johns River, Florida, though we did obtain 3 specimens from the Wekiva River, a tributary of the St. Johns (28.8728226 N, -81.3689402 W). The Wekiva River, Florida, and Deer Creek, Maryland, are separated by roughly 1280 Km.

One limitation of this study is that equal sample sizes for each state and watershed could not be collected. Attempts were made to have between 25-50 fish per watershed and a 50:50 sex ratio, but as with most all fisheries work, success in sampling is often not reliable. Multiple factors influenced our ability to collect more samples, including early Hickory Shad spawning runs in some locations, foul weather, low river water levels prohibiting boat access, severe long-term flooding, and expense of traveling to distant locations. It is possible that the morphometric and meristic values presented here for rivers with small samples sizes may not accurately capture the true natural variation of the characters in those populations. Additionally, the timing of specimen collection was not standardized and often started after the spawning run had fully began, which could have potentially affected this study (i.e., size or sex distributions). Also, collection of an equal proportion of male and female Hickory Shad from each state and watershed was not possible. Overall, slightly more female specimens (n = 365) were collected than male (n = 330) representing 52.5% and 47.5% of the specimens included in this study, respectively. The difference in the number of males and females could be a product of gear bias

and not necessarily representative of the natural populations. For instance, gill nets used to collect some specimens in this study are more selective for larger female Hickory Shad than smaller males. Melvin et al. (1992) studying American Shad, also found gill nets to be selective for larger females. Furthermore, we experienced a willingness of sportfishers to provide specimens for our study, but reluctance to provide females since most fishers wanted the roe.

Sexual Differences

The difference observed in the averages of morphometric characters when compared by sex was not a surprising result and is relatively common in fish, though it has never been explicitly described for Hickory Shad. This has significant implications and suggests studies on Hickory Shad to be separated by sex and analyzed in that manner since there is substantial difference between male and female specimens. Melvin et al (1992) came to similar conclusions for morphometric and meristic characters of American Shad and so males and females were analyzed separately.

Specimen Size

It is important to note that the morphometric measurements presented in this study are of frozen and not freshly caught Hickory Shad. It is possible that the freezing and thawing process may slightly alter the shape and or size of some morphometric characters. Melvin et al. (1985) reported a significant difference ($P < 0.01$) between length measurements of live American Shad in the field compared to measurements of dead specimens in the laboratory. In the event American Shad were frozen prior to measurement, the length was multiplied by 1.021 to better approximate fresh length (Melvin et al. 1985). Though fish samples are often frozen by

biologists for later processing, future studies should investigate if there is a significant difference between morphometric measurements for fresh versus frozen Hickory Shad and, if so, which measurements are the most robust to the freezing and thawing process. Cronin-Fine et al. (2013) found 10 geometric morphometric measurements of Alewife that did not have a significant difference between fresh and frozen specimens. Generally for meristics, the act of freezing and thawing is not a problem since it does not change the counts of meristic features.

The freezing and thawing process could also have biased the weight of the fish, but similar to morphometric measurements, the bias is shared across all individuals. Also, gonad weight can be extremely dependent on spawning status (pre or post-spawn), especially for females. Spent females weigh less than ripe and ready-to-spawn individuals, but unfortunately spawning status was not recorded during dissections. There were a few instances of gonads that were unable to be weighed (or sexed) because they were no longer intact or starting to decompose. This was likely a result of freezer storage for an extended length of time, multiple freezing and thawing events, or the length of time from collection till initial freezing. This was not a serious problem; 26 specimens exhibited deterioration and this state was relatively random across rivers. Also, it was likely that some of the individuals not sexed was caused by human error instead of relating to the state of the gonads.

The regression equations for relationships between Hickory Shad FL and TL, and between FL and SL, provide a means for converting between the various measurements of fish size. This could be useful for biologists or fishery managers to accurately estimate one length from another in the instance that only one of the measurements was recorded.

Missing data

Though not a frequent problem in this study, missing data are quite common in morphometric (and meristic) studies (Clavel et al. 2014). Some of the specimens could not be analyzed for the entire 17 morphometric and four meristic characters due to damage including broken or missing fins, missing scales, and wounds from predation or gear-related injury. Missing scales are not surprising, since the Hickory Shad as well as other Clupeids are very susceptible to shedding scales. The frequency of missing values for all characters can be found in Table 2-5. In this study no imputation procedures (i.e., replacement or regression-based approaches) were used to estimate missing data; instead these values were simply omitted.

Comparison between Hickory Shad and other Clupeids

Most of the morphometric and meristic characters investigated in this study do not serve to easily differentiate Hickory Shad from American Shad, Alewife, or Blueback Herring though careful examination of certain characters can help narrow down the species. One common and definitive way to distinguish Hickory Shad from the other species is by gill raker counts. Though not directly incorporated into this study, a random subset of Hickory Shad specimens was analyzed for gill raker counts. It was determined that Hickory Shad had between 19-22 gill rakers on the lower limb of the first arch (n=463), which is considerably less than the other anadromous *Alosa* species. American Shad typically have 59-76 lower gill rakers on the first arch, Blueback Herring 41-52, and Alewife 38-46, all of which are higher counts (Hildebrand 1963) due to their diet being different than Hickory Shad, which are more piscivorous (Greene et al. 2009).

Conclusion

Mansueti (1962) described Hickory Shad as "The most enigmatic of all estuarine clupeoids" and the intent of my study was to expand the existing taxonomic knowledge of the species. Mitchill (1814) used six meristic characters in describing the species: branchiostegal, pectoral, ventral, anal, dorsal, and caudal rays. These six characters were not included in this study, due to the fact that the methods Mitchill used to count them are not available and would not allow direct comparison. Instead, 17 morphometric measurements and four meristic counts not included in the original description of the species were utilized. The information about the anatomical characteristics presented herein are lacking in the literature, though they are well known for most other anadromous fish species. These additional morphological and meristic characters may prove valuable for separating regions or watersheds in future studies (See Chapter 3). Geometric morphometric analysis may be another viable option to investigate body shape variability. In addition, there still remain many unanswered questions regarding Hickory Shad life history, biology, and stock status that should be addressed so that the species can be properly managed and all spawning populations sustained. Furthermore, the intraspecific variation of Hickory Shad described here could be used to discriminate the different populations using multivariate analysis (See Chapter 3).

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TABLES

Table 2-1. List of states, river (north to south), sex, and total number of Hickory Shad collected in 2016 and 2017

		Sex			
State	River	Female	Male	Unknown	Total
Maryland	Susquehanna R.	13	9		22
Maryland	Patapsco R.	11	39		50
Delaware	Nanticoke R.	16	6		22
Virginia	Rappahannock R.	23	21	3	47
Virginia	Appomattox R.	25	25		50
Virginia	James R.	26	37	2	65
Virginia	Chowan R. (Meherrin)		1		1
Virginia	Chowan R. (Nottaway)	7	11		18
Virginia	Chowan R. (Blackwater)	13	11	1	25
North Carolina	Roanoke R.	21	23		44
North Carolina	Cashie R.	17	17		34
North Carolina	Pamlico Sound	63	29	2	94
North Carolina	Pungo R.		2	1	3
North Carolina	Pamlico R.	39	24	1	64
North Carolina	Tar R.	31	20	1	52
North Carolina	Neuse R.	14	30	3	47
North Carolina	New R.	2	2	6	10
North Carolina	Atlantic Ocean*	3		2	5
North Carolina	Cape Fear R.	5	13		18
South Carolina	Waccamaw R.	7			7
South Carolina	Santee R.	2	4		6
Georgia	Altamaha R.	26	4		30
Florida	St. Johns R. (Wekiva)	1	2		3
		365	**330**	**22**	**717**

*denotes non-river or sound sampling location

Table 2-2. Morphometric measurements and meristic counts analyzed and acronyms used in this study

Morphometric	Acronym	Meristic	Acronym
Standard Length	SL	Posterior Ventral Scutes	PVS
Fork Length	FL	Anterior Ventral Scutes	AVS
Total Length	TL	Scale Rows	SR
Lower Lip-Nose	LLN	Longitudinal Scale Rows	LSR
Snout-to-Anal Length	SAL	Left Gill Raker Upper	L-GRU
Body Depth	BD	Right Gill Raker Upper	R-GRU
Head Length	HL	Left Gill Raker Lower	L-GRL
Eye Length	EL	Right Gill Raker Lower	R-GRL
Snout Length	SNL		
Head Width	HW		
Interorbital Width	IOW		
Maxillary Length	ML		
Fin Length-Dorsal Base	FLD		
Fin Length-Anal Base	FLA		
Longest Ray Dorsal Fin	LRD		
Longest Ray Left Pectoral Fin	LRP		
Longest Ray Left Ventral Fin	LRV		
Longest Ray Anal Fin	LRA		

Table 2-3. Descriptive data of morphometric and meristic characters for female and male specimens of Hickory Shad. See text for descriptions of each measurement or count. All measurements given in mm.

	Female						Male				
Character	Range	Mean	SD	% SL	n	Character	Range	Mean	SD	% SL	n
SL	229 - 389	292.41	26.09	-	365	SL	206 - 344	264.62	20.63	-	330
FL	260 - 435	326.36	28.57	111.61	365	FL	232 - 382	295.81	22.64	111.78	330
TL	306 - 508	383.36	33.55	131.10	364	TL	272 - 444	347.67	26.48	131.38	326
LLN	2.44 - 7.90	3.93	0.73	1.34	365	LLN	2.39 - 7.39	3.60	0.54	1.36	327
SAL	172 - 289	218.99	20.26	74.89	364	SAL	155 - 251	197.51	15.45	74.64	330
BD	65.74 - 134.89	91.44	13.32	31.27	365	BD	60.70 - 105.09	79.03	7.46	29.86	329
HL	64.73 - 108.43	81.40	6.96	27.84	364	HL	58.86 - 93.96	74.72	5.99	28.24	329
EL	11.82 - 19.60	14.80	1.26	5.06	363	EL	11.53 - 18.59	13.96	1.16	5.28	330
SNL	15.93 - 27.84	20.75	1.84	7.10	363	SNL	15.13 - 24.76	19.07	1.63	7.21	330
HW	23.67 - 47.07	31.33	3.38	10.71	363	HW	21.87 - 37.11	28.45	2.53	10.75	330
IOW	10.38 - 20.90	14.52	1.77	4.96	364	IOW	9.56 - 20.75	13.35	1.60	5.05	329
ML	26.51 - 41.90	33.98	2.52	11.62	362	ML	25.38 - 37.15	31.51	2.18	11.91	330
FLD	33.97 - 63.41	63.41	4.80	21.69	365	FLD	29.66 - 52.89	39.77	3.74	15.03	329
FLA	37.80 - 68.23	49.00	4.65	16.76	363	FLA	32.43 - 62.17	44.65	4.15	16.87	328
LRD	29.88 - 55.12	39.77	3.99	13.60	364	LRD	24.63 - 45.91	36.23	3.32	13.69	327
LRP	43.10 - 77.09	55.42	5.32	18.95	363	LRP	38.84 - 64.67	50.83	4.45	19.21	330
LRV	23.76 - 46.09	34.90	3.29	11.93	362	LRV	23.80 - 39.84	31.97	2.88	12.08	330
LRA	14.61 - 26.59	19.74	2.33	6.75	360	LRA	13.13 - 23.60	17.99	1.89	6.80	326
SR	49 - 56	51.65	1.23		349	SR	49 - 56	51.60	1.12		316
LSR	15 - 19	17.81	0.55		339	LSR	15 - 19	17.71	0.60		309
PVS	14 - 18	15.86	0.75		361	PVS	14 - 18	15.88	0.72		326
AVS	17 - 23	21.35	0.84		363	AVS	18 - 24	21.39	0.79		329

Table 2-4. Morphometric and meristic characters of Hickory Shad by river of collection (north to south). For each character; minimum - maximum, and mean values are presented.

Character	Susquehanna	Nanticoke	Patapsco	Rappahannock	Appomattox	James	Meherrin	Nottaway	Blackwater	Roanoke	Cashie	Pungo
SL	234 - 318	257 - 325	249 -309	257 - 336	234 - 300	221 - 331	265	224 - 306	235 - 334	221 - 334	234 - 326	234 - 268
	277.27	293.64	271.06	294.34	272.24	272.91		270.72	273.08	273.77	286.06	250.33
FL	260 - 353	285 - 361	281 - 347	288 - 373	263 - 336	250 - 369	302	252 - 341	266 - 375	249 - 369	266 - 368	264 - 302
	306.64	325.50	304.44	327.83	304.54	305.17		303.72	306.84	305.86	320.59	282.67
TL	302 - 414	336 - 421	328 - 403	338 440	310 - 395	294 - 431	353	298 -401	311 - 441	292 - 425	315 - 433	312 - 354
	359.00	382.14	356.62	385.11	357.34	357.89		357.56	361.44	359.44	376.73	332
LLN	2.44 -5.20	3.07 - 4.74	3.22 - 4.24	2.93 - 5.77	2.52 - 4.17	2.92 - 5.07	3.84	3.06 - 4.23	3.27 - 4.79	2.45 - 5.66	2.83 - 5.26	2.75 - 3.81
	3.63	4.00	3.79	4.10	3.35	3.70		3.65	3.77	3.46	3.74	3.28
SAL	171 - 234	186 - 243	188 - 235	190 - 255	175 - 227	167 - 254	202	169 - 228	171 - 256	165 - 249	176 - 251	176 - 198
	203.82	216.36	204.32	218.98	202.38	204.23		204.22	205.96	204.14	216.56	187.33
BD	63.79 - 94.86	71.55 - 97.23	70.19 - 93.96	75.72 - 114.74	65.74 - 97.68	66.19 - 111.24	77.60	66.58 - 101.63	69.49 - 101.80	61.29 - 101.53	67.12 - 112.66	69.22 - 77.97
	81.93	83.29	81.41	91.34	80.71	84.00		84.23	84.19	80.74	87.4	74.32
HL	65.45 - 90.54	72.56 - 89.36	70.13 - 86.79	73.19 - 94.29	67.35 - 86.34	64.42 - 91.91	77.94	65.27 - 83.91	66.72 - 92.33	62.15 - 87.45	68.15 - 90.30	67.93 - 75.88
	76.79	81.43	76.24	82.60	77.41	77.03		76.66	76.69	76.09	80.04	71.69
EL	12.61 - 17.27	13.46 - 17.25	12.75 - 15.80	13.34 - 17.54	12.31 - 15.41	11.59 - 17.74	13.97	13.11 - 15.31	11.92 - 15.88	12.12 - 16.34	12.97 - 15.93	12.97 - 14.53
	15.03	15.46	13.76	15.60	14.21	14.14		14.19	13.84	14.22	14.46	13.77
SNL	15.84 - 23.29	18.39 - 23.58	16.85 - 21.66	18.53 - 25.24	17.47- 22.72	16.74 - 23.30	19.28	16.45 - 21.64	16.51 - 24.56	16.23 - 22.60	16.85 - 23.11	17.82 - 19.08
	19.51	21.22	18.84	21.24	20.14	19.92		19.56	19.36	19.49	20.03	18.32
HW	24.64 - 34.82	27.52 - 34.63	25.74 - 31.43	27.64 - 36.87	25.37 - 33.31	24.34- 36.74	28.81	24.1 - 33.62	24.75 - 35.57	22.46 - 34.08	24.56 - 34.78	25.44 - 29.82
	29.96	31.33	28.12	31.94	29.47	29.59		29.36	29.11	29.18	30.13	27.52
IOW	12.06 - 18.16	12.87 - 16.93	12.42 - 15.79	12.62 - 18.27	12.24 - 16.32	10.59 - 17.96	13.08	10.62 - 14.96	10.31 - 14.94	10.28 - 16.64	10.84 - 15.37	10.93 - 14.28
	14.98	15.24	13.85	15.06	14.09	13.49		12.91	12.58	13.54	13.34	12.45
ML	26.57 - 37.15	30.79 - 37.94	29.36 - 35.82	29.84 - 38.15	29.04 - 35.73	27.02 - 38.54	32.79	29.53 - 35.75	28.78 - 37.48	26.1 - 36.92	29.36 - 37.59	28.70 - 30.97
	31.95	34.12	31.73	34.13	32.42	32.27		32.63	32.49	32.02	33.46	30.15
FLD	34.69 - 50.47	37.64 - 53.39	34.91 - 46.14	37.61 - 51.80	34.33 - 48.28	34.13 - 50.23	37.77	33.20 - 45.75	35.92 - 56.08	31.98 - 49.50	37.71 - 50.84	37.15 - 41.03
	41.71	45.14	39.77	45.18	41.17	41.02		40.73	41.30	41.33	43.39	38.48
FLA	38.03 - 57.49	44.93 - 55.16	35.26 - 52.49	43.73 - 57.52	38.93 - 51.78	37.22 - 53.10	43.73	37.09 - 50.92	39.43 - 53.58	37.20 - 54.42	40.05 - 55.36	41.30 - 48.69
	47.47	49.76	44.82	49.65	46.33	45.81		44.71	45.78	46.29	47.57	44.28
LRD	30.11 - 44.06	33.48 - 46.83	32.52 - 41.97	32.49 - 46.91	31.97 - 40.93	30.02 - 44.94	37.99	24.63 - 41.36	31.43 - 42.87	25.13 - 43.85	31.82 - 45.91	35.25 - 36.79
	37.67	39.04	37.38	39.85	36.76	37.02		36.18	37.07	35.96	38.54	36.23
LRP	41.66	48.74	46.33 - 57.53	48.69 - 64.12	44.77 - 57.83	41.35 - 64.92	52.07	44.70 - 58.36	45.27 - 64.07	41.38 - 59.11	47.17 - 62.22	48.18 - 51.50
	51.47	53.90	51.25	55.77	52.31	52.16		52.54	52.98	52.14	54.5	49.36
LRV	26.76 - 37.51	28.84 - 39.37	24.76 - 36.35	30.42 - 40.12	23.76 - 36.53	23.80 - 40.60	32.61	25.86 - 36.49	28.82 - 40.54	26.26 - 37.53	28.79 - 39.33	29.95 - 31.72
	32.61	34.54	32.17	35.64	33.35	32.92		32.52	33.36	32.80	34.21	30.68
LRA	14.53 - 22.32	16.36 - 21.59	13.84 - 20.94	16.35 - 23.90	14.61 - 23.35	14.44 - 23.99	19.16	15.76 - 22.11	15.25 - 23.07	14.60 - 23.53	16.18 - 23.60	17.34 - 18.26
	18.55	18.99	17.44	19.63	17.98	18.38		18.80	18.93	18.27	19.66	17.79
SR	51 - 55	52 - 55	49 - 52	50 - 56	50 - 53	50 - 56	51	50 - 52	50 - 53	51 - 55	51 - 54	50 - 52
	53.00	53.14	50.73	52.51	51.60	51.83		51.33	51.56	52.11	51.65	51
LSR	15 - 19	17 - 19	16 - 18	16 - 19	17 - 19	17 - 19	18	16 - 18	17 - 19	17 - 19	17 - 19	17
	18.00	17.73	17.74	18.02	17.88	17.94		17.61	17.92	17.73	18	17
PVS	15 - 17	15 - 18	15 - 17	15 - 17	15 - 17	14 -17	15	14 - 17	15 - 17	15 - 18	15 - 18	15
	15.71	16.05	16.08	15.81	15.86	15.59		15.83	15.96	16.14	16.33	15
AVS	20 - 22	21 - 22	18 - 23	17 - 23	21 - 23	20 - 24	24	21 - 23	21 - 23	18 - 23	21 - 23	22
	21.05	21.23	21.54	21.21	21.38	21.34		21.61	21.50	21.59	21.56	22
n	22	22	50	47	50	65	1	18	25	44	34	3

Table 2-4-2. Morphometric and meristic characters of Hickory Shad by river of collection (north to south). For each character; minimum - maximum, and mean values are presented.

Character	Pamlico	Pamlico Sound	Tar	Neuse	New	Cape Fear	Atlantic Ocean	Waccamaw	Santee	Altamaha	Wekiva	Grand Total
SL	232 - 361	216 - 349	206 - 375	230 - 330	209 - 279	234 - 333	243 - 313	350 - 389	243 - 354	255 - 365	315 - 344	206 - 389
	284.58	272.93	267.31	267.49	248.00	268.39	272.20	359.86	281.00	321.33	325.33	278.41
FL	260 - 402	243 - 393	232 - 417	258 - 363	234 - 312	261 - 371	275 - 350	389 - 435	272 - 394	286 - 406	348 - 382	232 - 435
	315.09	305.60	299.31	297.64	278.30	300.94	305.80	399.86	315.50	359.47	360.00	310.98
TL	305 - 472	288 - 460	272 - 487	301 - 427	274 - 369	306 - 436	324 - 413	445 - 508	323 - 464	340 - 479	412 - 444	272 - 508
	370.81	358.91	352.63	350.51	330.75	354.20	359.20	466.43	372.67	423.57	423.33	365.62
LLN	2.64 - 7.89	2.85 - 4.83	2.47 - 5.55	2.7 - 5.34	2.64 - 3.65	2.87 - 4.58	3.23 - 3.73	3.97 - 6.03	2.39 - 3.66	3.26 - 4.88	4.03 - 4.55	2.39 - 7.89
	4.48	3.61	3.73	3.70	3.15	3.65	3.52	4.47	2.96	3.89	4.30	3.76
SAL	174 - 272	166 - 271	155 - 281	172 - 240	158 - 210	174 - 251	182 - 231	257 - 289	182 - 255	194 - 279	229 - 251	155 - 289
	212.16	204.61	200.50	198.30	184.70	200.56	203.00	267.14	209.33	242.73	238.33	208.12
BD	65.13 - 124.05	65.97 - 106.01	60.7 - 121.04	63.55 - 111.30	60.95 - 91.72	73.25 - 110.60	70.74 - 85.62	125.57- 134.89	74.85 - 114.13	77.92 - 126.67	100.25 - 103.00	60.7 - 134.89
	89.68	82.08	83.11	80.53	74.38	84.43	76.54	128.58	87.40	110.53	101.48	85.21
HL	61.03 - 91.40	61.79 - 103.08	58.86 - 105.97	62.00 - 93.12	60.33 - 80.19	67.53 - 91.84	69.81 - 88.54	93.27 - 108.43	68.2 - 94.59	71.43 - 101.47	85.66 - 93.96	58.86 - 108.43
	76.74	77.03	76.37	75.06	70.29	76.70	79.27	98.25	79.67	88.90	88.83	78.02
EL	11.78 - 19.60	11.53 - 17.93	11.71 - 18.66	11.65 - 17.78	11.24 - 14.56	11.68 - 15.71	12.02 - 14.86	16.32 - 18.51	12.50 - 17.39	12.90 - 17.73	15.57 - 17.29	11.24 - 19.60
	14.62	13.93	14.11	14.25	12.95	13.35	13.56	17.17	14.68	15.56	16.37	14.38
SNL	16.58 - 27.03	15.13 - 27.84	15.25 - 25.70	16.60 - 22.91	15.47 - 20.37	16.46 - 22.88	15.93 - 22.08	23.31 - 26.81	16.70 - 24.30	17.92 - 25.05	22.06 - 24.76	15.13 - 27.84
	20.59	19.27	19.59	19.38	17.52	18.59	19.40	24.69	20.15	21.59	23.03	19.90
HW	24.91 - 47.07	22.72 - 39.21	21.87 - 42.18	23.88 - 35.00	21.51 - 30.44	23.75 - 34.77	24.22 - 31.63	39.06 - 42.41	27.37 - 38.21	28.09 - 37.84	34.63 - 37.11	21.51 - 47.07
	32.09	28.21	29.05	28.75	25.88	28.32	28.13	40.62	31.30	34.10	35.49	29.86
IOW	10.84 - 20.90	9.59 - 20.42	9.56 - 18.25	11.00 - 17.11	10.49 - 14.32	11.29 - 16.73	10.93 - 15.89	17.09 - 19.73	12.83 - 17.51	13.42 - 18.29	15.69 - 19.44	9.56 - 20.90
	15.05	13.12	12.94	13.50	12.24	13.52	13.33	18.09	14.69	16.16	17.28	13.92
ML	27.00 - 40.51	26.51 - 41.23	25.38 - 41.78	26.92 - 36.81	26.05 - 33.92	27.86 - 37.37	28.99 - 35.74	38.93 - 41.90	29.55 - 38.37	30.43 - 39.79	35.43 - 37.15	25.38 - 41.90
	33.28	32.13	32.24	31.66	29.95	31.91	32.03	39.78	33.18	36.33	36.09	32.72
FLD	34.16 - 55.88	30.34 - 52.33	29.95 - 61.11	29.66 - 50.86	29.70 - 42.66	33.97 - 47.30	36.89 - 48.09	54.32 - 63.41	39.06 - 51.82	38.97 - 57.88	43.56 - 52.89	29.66 - 63.41
	42.98	41.08	40.03	39.39	37.15	40.39	41.46	56.55	43.34	49.90	49.47	42.00
FLA	35.15 - 60.78	35.33 - 56.26	32.43 - 62.29	36.44 - 53.82	36.39 - 50.97	39.33 - 56.15	41.25 - 54.32	55.23 - 68.23	40.21 - 63.03	37.50 - 64.81	56.84 - 62.17	32.43 - 68.23
	47.42	46.10	44.67	44.79	43.13	46.25	46.71	60.17	48.14	53.90	58.98	46.85
LRD	31.67 - 55.12	29.88 - 47.24	27.09 - 48.73	28.43 - 45.81	25.49 - 39.65	29.57 - 42.92	32.58 - 41.35	44.32 - 53.94	33.18 - 50.27	34.85 - 50.25	44.92 - 45.82	24.63 - 55.12
	40.38	37.10	37.22	37.08	34.72	37.38	36.74	47.71	39.95	43.37	45.32	38.01
LRP	38.83 - 69.67	43.12 - 67.21	39.22 - 70.87	41.60 - 64.11	39.93 - 57.00	45.11 - 62.10	45.65 - 59.48	63.38 - 77.09	46.59 - 72.86	49.15 - 68.44	60.08 - 64.67	38.83 - 77.09
	53.29	52.15	51.90	50.89	47.95	52.76	51.88	69.32	56.07	61.49	61.75	53.09
LRV	26.67 - 43.48	25.12 - 43.07	24.49 - 43.28	25.75 - 40.06	23.99 - 33.80	26.78 - 38.25	29.10 - 38.04	39.25 - 46.09	29.76 - 41.98	24.87 - 43.45	36.84 - 39.84	23.76 - 46.09
	33.55	32.59	32.44	32.28	29.71	33.03	32.51	42.80	34.64	38.01	37.93	33.40
LRA	13.13 - 26.34	14.16 - 25.17	13.79 - 25.80	13.99 - 22.77	14.08 - 19.09	14.30 - 21.19	15.88 - 19.85	23.49 - 26.59	15.11 - 24.14	16.79 - 26.33	21.40 - 22.86	13.13 - 26.59
	19.65	18.42	19.06	18.48	16.89	18.30	18.16	24.81	18.76	21.85	22.16	18.85
SR	49 - 55	50 -55	50 - 54	50 - 54	50 - 52	50 - 51	50 - 51	51 - 53	51 - 52	49 - 52	52	49 - 56
	52.03	51.34	51.40	51.81	50.89	50.78	50.40	51.43	51.17	50.23	52.00	51.62
LSR	15 - 19	16 - 18	17 - 18	16 - 19	18	18	17 - 18	16 - 19	17 - 18	17 - 18	16 - 19	15 - 19
	17.51	17.63	17.79	17.36	18.00	18.00	17.60	17.86	17.67	17.67	17.33	17.77
PPS	14 - 18	15 - 17	14 - 17	14 - 17	14 - 17	14 - 17	15 - 16	14 - 16	16	14 - 17	15 - 16	14 - 18
	16.13	15.82	15.72	15.96	15.33	15.39	15.40	15.29	16.00	15.50	15.67	15.87
APS	17 - 23	20 - 23	20 - 23	19 - 23	21 - 22	21 - 23	21 - 22	21 - 22	21 - 22	20 - 22	21 - 23	17 - 24
	20.63	21.42	21.40	21.60	21.30	21.72	21.40	21.43	21.33	21.33	22.33	21.37
n	64	94	52	47	10	18	5	7	6	30	3	717

Table 2-5. Missing value analysis of 18 morphometric and four meristic characters of Hickory Shad

				Missing	
Character	N	Mean	Std. Deviation	Count	Percent
SL	717	278.41	27.69	0	0.0
FL	717	5.73	0.01	0	0.0
TL	710	5.89	0.01	7	1.0
LLN	714	1.31	0.14	3	0.4
SAL	716	5.33	0.02	1	0.1
BD	716	4.43	0.09	1	0.1
HL	715	4.35	0.03	2	0.3
EL	714	2.66	0.05	3	0.4
SNL	715	2.98	0.04	2	0.3
HW	715	3.39	0.04	2	0.3
IOW	715	2.62	0.07	2	0.3
ML	714	3.48	0.03	3	0.4
FLD	716	3.73	0.05	1	0.1
FLA	713	3.84	0.05	4	0.6
LRD	713	3.63	0.10	4	0.6
LRP	715	3.97	0.04	2	0.3
LRV	714	3.50	0.04	3	0.4
LRA	708	2.93	0.07	9	1.3
SR	686	51.62	1.17	31	4.3
LSR	662	17.77	0.58	55	7.7
PVS	709	15.86	0.73	8	1.1
AVS	714	21.36	0.81	3	0.4

Table 2-6. Comparison of morphometric and meristic characters for Hickory Shad, American Shad, Alewife, and Blueback Herring. Range given for each, if available; usual values reported in literature in parentheses.

Character	Hickory Shad	American Shad	Blueback Herring	Alewife
BD % TL	22.31-26.55	17.2-19.4 [a]	22.1-25.2 [a]	17.8-21.7 [a]
HL % TL	21.34-21.64	22.7-24.0 [a]	18.5-20.6 [a]	20.3-23.7 [a]
EL % HL	18.08-19.10	27.3-32.0 [a]	22.0-26.4 [a]	26.1-32.0 [a]
SNL % HL	25.68-25.71	26.9-32.0 [a]	23.4-30.0 [a]	26.9-35.7 [a]
IOW % HL	16.24-19.28	18.6-21.6 a	21.1-26.4 a	15.7-21.6 a
FLA % TL	11.92-13.43			10.3-12.0 [a]
PVS	14-18	12[b]-19[a]	12-16 [a]	12[d]-17[f] (14-15)[a]
AVS	17-24	19-25 [b]	18-21 [d]	17-21 (19-20) [a]
SR	49-56	52-64 [c]	46-54 [d]	42[d]-54[g]
LSR	15-19	15-16 [d]	13-14 [e]	14 [d]

[a] Scott and Crossman 1973, [b] Hill 1956, [c] Walburg and Nichols 1967, [d] Hildebrand 1963
[e] Thomson et al 197, [f] Leim and Scott 1966, [g] Miller 1957

FIGURES

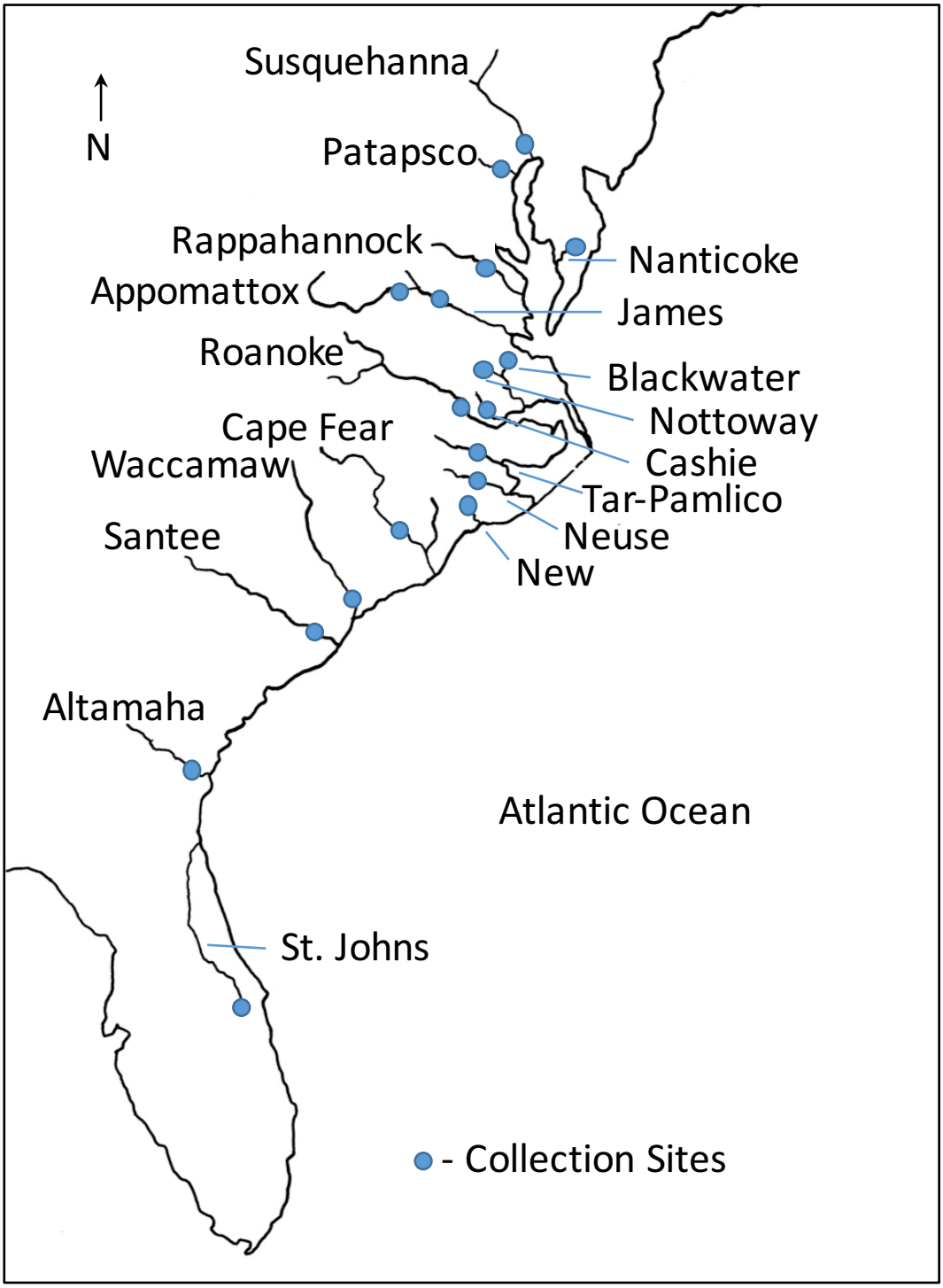

Figure 2-1. Map showing relative location of rivers included in this study as well as collection sites of Hickory Shad. Revised after Melvin et al. 1992.

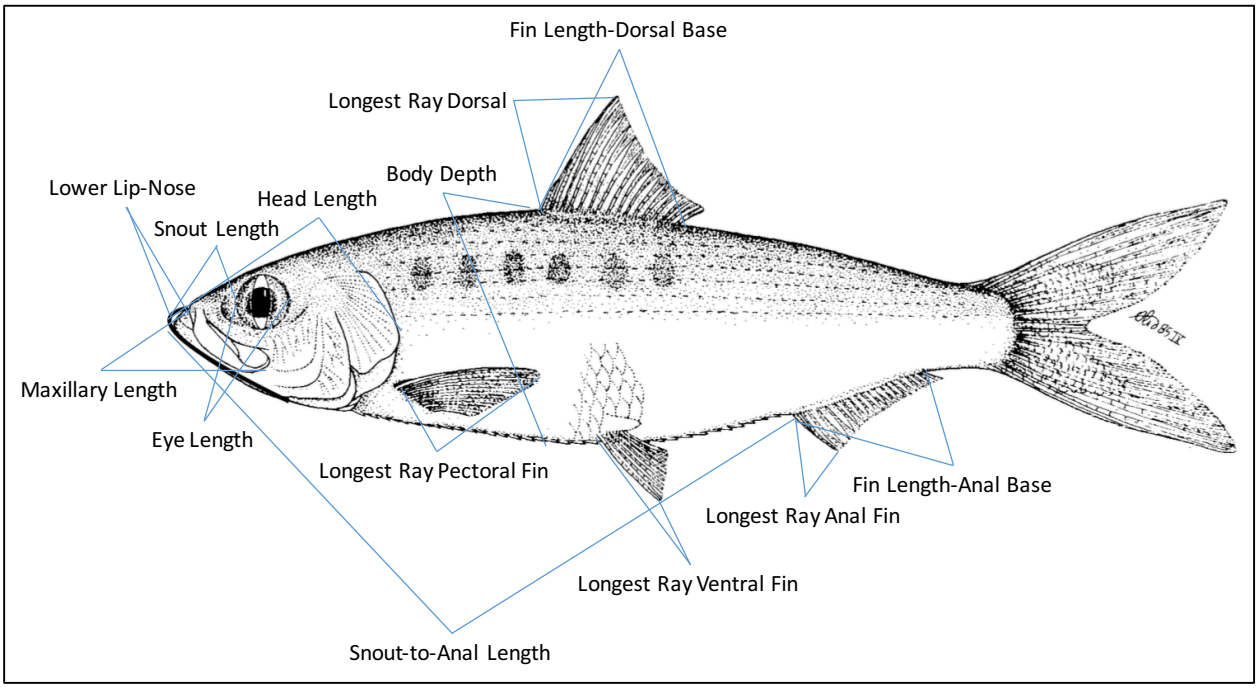

Figure 2-2. Hickory Shad illustration showing how morphometric measurements were taken. Reproduced from Whitehead 1985.

Chapter 3: Can Meristic Characters and Morphological Relationships be used to Identify Discrete Spawning Populations of the Hickory Shad *Alosa mediocris*?

Abstract

The Hickory Shad *Alosa mediocris* is an anadromous fish species of the Family Clupeidae. Little is known about its distribution, life history, and status. To provide information on stock identification and watershed fidelity, which are currently unknown, the distributional patterns of 17 morphometric and four meristic characteristics of adult Hickory Shad were analyzed using multivariate techniques. A total of 687 specimens were examined along the latitudinal gradient from the Susquehanna River, Maryland to the St. Johns River, Florida. Prior to analysis morphometric characters were corrected for size-dependent variation using an allometric formula and natural log transformed to better approximate multivariate normality. Multivariate analysis of variance of pooled morphometric and meristic characters showed a significant effect of sex ($P < 0.05$) therefore, all analyses were separated by sex. Analysis of variance showed highly significant difference ($P < 0.003$) for 15 characters between 10 locations for males and 12 characters between 12 locations for females. Non-significant characters were excluded and only significant characters for males and females were used for subsequent analysis. Principal Components Analysis (PCA) extracted 6 and 4 components (eigenvalues > 1) cumulatively explaining 63.67% and 60.88% of the variance for males and females, respectively. Bartlett's Test of Sphericity was significant ($P < 0.05$) and Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.60 for males and 0.68 for females, which confirmed appropriateness of the data for PCA. Principal component 1 for males and females was most correlated (> 0.4) with the head region and fin lengths. Using Quadratic Discriminant Function Analysis (QDFA), 77.9% and 80.3% of males and females, respectively, were correctly classified to their rivers of collection using separate-groups covariance matrix and equal prior probabilities. Individual river classification varied between 58.6% - 100%. The Tar-Pamlico River had the lowest percent correct classification for both male (58.6%) and female (62.0%) QDFAs. Tributary level discrimination was achieved in two instances: the James and Appomattox rivers, and the Roanoke and Cashie rivers. Results of this study suggest that morphometric and meristic analysis of Hickory Shad is effective and possibly more cost efficient than other stock identification methods (e.g., otolith analysis) to identify separate spawning populations (stocks) of Hickory Shad. Additional research is needed to identify whether this method exhibits stability over multiple years of collection.

Introduction

The current study was initiated to examine the intraspecific variation of Hickory Shad from spawning populations along the East Coast of the United States to provide information on stock differentiation and potentially identify individual fish to a watershed of origin. The

Hickory Shad *Alosa mediocris* is an anadromous species of the Family Clupeidae that enters coastal freshwater between February and June to spawn, depending on latitude (Murauskas and Rulifson 2011). Although it has not been specifically determined for the species, Hickory Shad are assumed to home to natal river systems to spawn (Batsavage and Rulifson 1998). This life history trait, if true for the species and characterized by low degrees of wandering, could produce distinct morphometric (i.e., measurements between morphological attributes) and meristic characters (i.e., countable structures) due to the effect of the specific river environment during ontogeny (Beachham et al. 1988; Melvin et al. 1992). Morphometric measurements and meristic characters of fish are concurrently regulated by both environmental and genetic components, and this is the basis for differences in phenotypic expression (Melvin et al. 1992; Begg and Waldman 1999).

For example, differences in environmental factors, such as temperature, salinity, light, and dissolved gases during early development can yield substantial variation in meristic counts for individuals of the same species (Taning 1952). Both morphometric and meristic attributes are typically fixed early during larval and juvenile development and remain unchanged throughout life (Begg and Waldman 1999). Therefore, these characters could serve as a record of the effect of the natal environment even though young fish may spend a relatively short period of time in that environment (Begg and Waldman 1999; Cadrin 2000). With morphometric and meristic analyses, it is possible to discriminate stocks even if there is little genetic difference because genetic differentiation likely occurs at a much slower rate (Begg and Waldman 1999). However, for stock identification researchers must assume that the environmental conditions producing these morphological shifts remain constant within the watershed over time (e.g., multiple years) to provide stability of these shifts for multiple generations.

Historically, researchers utilizing morphometric and/or meristic analysis simply used descriptive statistics and univariate methods independently on each character (Nichols 1966), but today the use of multivariate techniques is standard (Cadrin 2000; Patiyal et al. 2014). Morphometric and meristic analysis of fish can be a useful tool to identify stocks at the regional or watershed level and it has been utilized successfully for many fish species including clupeids (Carscadden and Leggett 1975; Gabriel et al. 1976; Meng and Stocker 1984; Melvin et al. 1992; Cronin-Fine et al. 2013). However, whether this analysis could be used to discriminate among Hickory Shad spawning populations remains untested.

It should be noted that differing degrees of success have been achieved using the analysis of morphometric measurements and meristic characters, depending on the species and geographic area studied (Melvin et al. 1992). The technique was used by Melvin et al. (1992) to discriminate between populations of American Shad *A. sapidissima* collected from 14 different spawning sites in rivers ranging from Canada to Florida. Melvin et al. (1992) used linear discriminant function analysis (LDFA) with pooled morphometric and meristic characters and assigned fish to regional groups following individual river classification, resulting in a mean of 87.2% and 82.4% correct classification for male and female American Shad, respectively. For the Pacific region, Meng and Stocker (1984) looked at 17 characters of Pacific Herring *Clupea harengus pallasi* and were able to correctly assign the herring into 6 groups 55.3% of the time and into 2 groups 85-90% of the time. Meng and Stocker (1984) used a stepwise discriminant function analysis to determine percent correct classification. Vatandoust et al. (2015) investigated morphometric variation in two river populations of the anadromous Caspian Lamprey *Caspiomyzon wagneri;* using DFA they were able to correctly classify males 77.1% and females 84.0% of the time.

The purpose of Chapter 3 of my study was to determine the viability of using multivariate analysis of morphometric and meristic characters to discriminate Hickory Shad spawning populations from different locations along the East Coast of the United States. Currently, there is no method to distinguish Hickory Shad populations, especially when they are found outside of riverine habitat, so the goal was to assess the ability to correctly classify groups of fish of known origin (samples from targeted watersheds) using discriminant function analysis. Assuming that the method is valid, and fish home to natal streams to spawn, the use of meristic and morphometric analysis may provide a relative estimate of how mixed a population might be, based on the percent of misclassified fish.

Methods

Specifics of Hickory Shad collection as well as detailed description of all measurements and counts were described previously (Smith, Chapter 2).

Before analysis the raw data for all fish were inspected graphically for outliers, which could be a result of human error or deformities of the fish. If outliers were found, the original data sheets were consulted to ensure the value was entered correctly. If the value was largely outside the range for that character, the value was excluded from future analysis. Some specimens were missing fins, possessed a deformity, or received damage during capture not allowing them to be accurately measured or counted. In these cases, no imputation method was used to estimate the missing morphometric or meristic value.

The overall size range (standard length [SL]) of the 669 samples that could be sexed was 206-389 mm. As a result, it was necessary to correct for size-dependent variation for all morphometric characters. This ensured that variation in morphometric measurements was related

to differences in body shape rather than fish length. Measurements were standardized by an allometric method described by Elliott et al. (1995) using the equation:

$$M_s = M_0(L_s/L_0)^b,$$

where M_s = standardized measurement, M_0 = measurement of the character, L_s = overall (arithmetic) mean SL for all fish in each analysis, L_0 = SL of specific specimen, and b is determined for each character from the observed data by using the slope of the regression of log M_0 on log L_0 (Elliott et al. 1995). After size adjustment, all morphometric measurements were natural log transformed in order to better approximate multivariate normality. Visual inspection of Q-Q Plots showed no large deviations from normality.

It was not necessary to mathematically adjust the meristic counts since meristic characters are independent of fish length after the early juvenile phase and therefore remain stable throughout life (Meng and Stocker, 1984; Swain and Foote, 1999). Furthermore, this study only examined mature Hickory Shad participating in the spawning run.

All statistical analyses were performed in SPSS version 24 (IBM Corporation 2016).

Results

Descriptive statistics for each of the morphometric and meristic variables were reported in Chapter 2 (Table 2-4). Correlations between all size-adjusted and natural log transformed measurements and meristic characters were also analyzed (Table 3-1). Only 2 characters, fork length (FL) and total length (TL), showed high correlation with each other (Pearson Correlation > 0.80), which is the statistical value suggested by Mertler and Reinhart (2017) as the threshold value for variable removal. The strong correlation (0.81) between FL and TL is not surprising

since they are directly related to SL, and therefore both were excluded. The variable SL also was excluded from the final analysis since it was used for morphometric size adjustment.

Character Variation

Each character was examined by sex using analysis of variance (ANOVA) and evaluated with a Bonferroni corrected alpha of 0.003 (Table 3-2). Significant differences by sex were identified for five characters: eye length (EL), head width (HW), interorbital width (IOW), longest ray dorsal fin (LRD), and longest ray pectoral fin (LRP). The remaining 10 morphometric and all four meristic characters analyzed by sex were not significantly different ($P > 0.0026$).

When all 19 of the variables were considered jointly, multivariate analysis of variance (MANOVA) resulted in a significant effect of sex ($P < 0.05$; Wilk's Lambda = 0.684, $F_{19, 564} = 13.693$, and partial eta squared = 0.316). Therefore, all multivariate analyses for morphometric and meristic characters were separated by sex.

Results of a One-way ANOVA showed that male Hickory Shad from the 10 locations differed significantly ($P < 0.003$) in 12 size-adjusted and natural log transformed morphological measurements, as well as three meristic characters. Maxillary length (ML), fin length anal base (FLA), longest ray pectoral (LRP), and posterior ventral scutes (PVS) were the non-significant variables (Table 3-3). As for female specimens, One-way ANOVA results showed that 12 of the 19 total characters were significantly different ($P < 0.003$) between the 12 locations. The characters not contributing to watershed discrimination for females were body depth (BD), head length (HL), fin length dorsal base (FLD), fin length anal base (FLA), longest ray ventral fin (LRV), longitudinal scale rows (LSR), and anterior ventral scutes (AVS, Table 3-4). Therefore,

only the 15 significant characters for males and 12 significant characters for females were used for all further tests, including principal component analysis (PCA) and discriminant function analysis (DFA).

Principal Component Analysis

PCA was performed by sex on the significant variables; watersheds were excluded if less than 13 specimens were available for analysis for each sex for that location. Bartlett's Test of Sphericity was significant ($P < 0.05$) for both sexes, and the Kaiser-Meyer-Olkin Measure of Sampling Adequacy was 0.603 and 0.678 for male and female specimens, respectively, which confirmed appropriateness of the data for PCA. Results yielded six principal components for males and four components for females, with eigenvalues > 1, cumulatively accounting for 63.67% and 60.88% of the total variation in males and females, respectively (Table 3-5). The first principal component (PC1) accounted for 18.83% and 21.40% of the variance in the data for males and females, respectively. PC1 for males revealed that the characters most important (|factor loadings| > 0.40) were related to the head region and longest fin ray lengths. These characters were HW (0.740), EL (0.699), head length [HL] (0.647), snout length [SNL] (0.645), LRV (0.449), interorbital width [IOW] (0.442), and longest ray anal fin [LRA] (0.429). PC1 results for females showed that the most important characters also corresponded to the head region and fin ray lengths, but also included the meristic count of scales along the lateral line. These characters for females were ML (0.768), LRP (0.745), LRA (0.588), HW (0.567), SNL (0.535), and scale rows [SR] (0.439). Principal component 2 (PC2) accounted for 12.93% of the variance for males and 18.46% for females. The most significant loadings on PC2 were from SAL, SR, IOW, and HL for male specimens (Table 3-6), and EL, IOW, SR, HW, and SAL for female specimens (Table 3-7). When the PC1 score was plotted against the PC2 score for males

(Figure 3-1) and females (Figure 3-2) no extreme visual separation was observed, yet certain rivers showed clustering. The male PCA plot showed the Appomattox, Cape Fear, and Cashie specimens exhibited the best clustering compared to the fish from other rivers, with the Neuse River fish showing the greatest spread. The female PCA plot showed that the Altamaha, Appomattox, Cashie, and Susquehanna fish were somewhat tightly clustered. Results for females collected from other watersheds were relatively scattered (e.g., the James, Rappahannock, and Tar-Pamlico rivers).

Discriminant Function Analysis

As with PCA, specimens were excluded from DFA due to unknown sex, missing values (list-wise deletion), or were excluded if less than 13 specimens were available of each sex for that location.

DFA was conducted separately for the same male and female specimens using only the significant variables included in the PCA. Box's M Test, which tests the null hypothesis of equal population covariance matrices of canonical discriminant functions, was significant ($P < 0.05$) for both male and female specimens. Therefore, separate-groups covariance matrix was used, which is equivalent to quadratic discriminant function analysis (QDFA). Also, prior probabilities for all groups were kept equal, since sample sizes were not extremely different between locations. QDFA results for males found that overall, 77.9% of specimens were correctly classified to their rivers of collection (Table 3-8). For females, 80.3% were correctly classified (Table 3-9). The highest percent correct classification (100%) for males was achieved for individuals collected from the Cape Fear and Cashie rivers, and the lowest (58.6%) classification for Tar-Pamlico River specimens. The model was able to classify male specimens from six

watersheds with higher accuracy than the overall (77.9%), including the Patapsco (89.2%), Appomattox (86.4%), Roanoke (78.3%), Cashie (100%), Pamlico Sound (85%), and Cape Fear (100%) rivers. Fish with correct classification lower than the overall for male specimens were from the Rappahannock (65%), James (77.1%), Tar-Pamlico (58.6%), and Neuse (60%) rivers.

For female specimens the highest percent correct classification (100%) was achieved from fish collected from the Nanticoke, Blackwater, Neuse, and Altamaha rivers, and the lowest classification (62%) was for Tar-Pamlico fish. The model was able to classify female specimens from eight locations with accuracy higher than the overall (80.3%) including the Susquehanna (92.3%), Nanticoke (100%), Rappahannock (90.5%), Blackwater (100%), Roanoke (82.4%), Cashie (93.3%), Neuse (100%), and Altamaha (100%) rivers.

Canonical discriminant functions 1 (DF1) and 2 (DF2) were plotted showing group centroids for male specimens (Figure 3-3) and female specimens (Figure 3-4). Similar to the plots of PC1 and PC2, visually there was no extreme separation between the rivers for the plots of male and female DF1 and DF2, although group centroids were separated. For example, the male specimens plot showed that the Cape Fear and Patapsco river specimens were very distinct in discriminant space from male individuals from the other rivers, which were more tightly clustered together. Watersheds were grouped by state except for the Cape Fear River, which was not grouped with other North Carolina rivers.

Based on the structure matrix and discriminant coefficients (loadings), DF1 for males (Table 3-10) was most highly correlated (|loadings| > 0.3) with HW (0.371), SNL (0.347), and SR (0.304). DF1 loadings for female specimens (Table 3-11) showed significant loadings (|loadings| > 0.3) of SR (0.507), EL (0.417), snout to anal length [SAL] (0.377), IOW (0.380), and SNL (0.380).

Discussion

The goal of this study was to investigate the viability of using morphometric and meristic characters to discriminate Hickory Shad from multiple spawning populations. Measurements and counts were based on fish that had been frozen and thawed, which is common and does not require samples to be processed immediately, making this practical to fishery managers and biologists. Based on 15 combined morphometric and meristic characters for males and 12 characters for female specimens, results indicate that there is a significant difference between Hickory Shad from the locations included in this study.

Principal Component Analysis

PCA is a commonly used multivariate method in morphometric and meristic studies that allows assessment of variance patterns in the data (Cadrin 2000). However, PC1 only accounted for 18.83% and 21.40% of the variance in the data for male and female Hickory Shad included in this study. These percentages are low compared to a study by Cronin-Fine et al. (2013) for Alewife, who found that found PC1 accounted for 90% of the variability based on 10 morphometric characters for 2,714 specimens.

The fact that PC1 for both Hickory Shad males and females revealed that the characters most important for each sex (|factor loadings| > 0.40) were related to the head region and longest fin ray lengths is very interesting and deserves further investigation.

Discriminant Function Analysis

The overall levels of correct classification for males (77.9%) and females (80.3%) achieved in this study are rather high considering the number of rivers involved in each analysis.

It suggests that the use of morphometric and meristic characters is a viable and perhaps better method at a finer detail for discriminating spawning populations of Hickory Shad when compared to other methods (i.e., otolith microchemistry or genetics). Also, this method could be used on marine-captured Hickory Shad to identify probable watershed of origin. This technique could prove valuable for investigating how different stocks distribute themselves in the Atlantic Ocean during the ocean migratory phase, which is currently unknown and essential for management.

The use of DFA to classify individuals (fish) of uncertain origin into a priori groups (rivers) is common, yet there are major assumptions (Melvin et al. 1992; White and Ruttenberg 2007). Using this method, individuals collected from one of the rivers are classified by the discriminant function as a member of that river (Melvin et al. 1992). For my study, all Hickory Shad included in each QDFA are assumed to have originated from one of the 10 locations for males or 12 locations for females, which is likely an impractical assumption. Another limitation is that DFA can classify some individuals correctly solely by chance, regardless of actual differences between groups (White and Ruttenberg 2007). In addition, using morphometric and meristic characters to distinguish Hickory Shad populations presumes consistent and significant variation among stocks (Gabriel et a. 1976) and stable environmental conditions with the watershed that causes shifts in morphological variables at critical early life stages. The decision to use separate-groups covariance matrix and equal prior probabilities regardless of group sizes undoubtedly influenced the results, but was the best option given the data. Additional research is recommended to determine the stability of these differences among watersheds over multiple years to validate the method as a viable population discriminator.

DF1 for both sexes showed that the majority of characters -- 2 out of 3 for males and 3 out of 5 for females -- showed high loadings (> 0.3) associated with the head region of the fish. This is similar to what was observed by examining the PC1 loadings. Differences in the head region, contributing the major portion of variability thereby assigning specimens to unique river populations, might be an effect of slight variation in the diet or turbidity of the water of developing Hickory Shad in various rivers. Turan (2004), investigating Mediterranean Horse Mackerel *Trachurus mediterraneus* morphometrics, also found significant differences in the characters of the head region and came to a similar conclusion. Alternatively, for Hickory Shad, there are many possible explanations for the differences in the head region between locations including other biotic and abiotic factors, which were not investigated in this study.

The plots of DF1 against DF2 for males and females allow visual inspection of the variation between the populations using the characters included in the analyses. When looking at the group centroids, these plots depict the relative similarity among the Hickory Shad populations sampled, with distance between centroids corresponding to the extent of similarity (or dissimilarity). The male DF1 and DF2 plot showed that most rivers within close geographic distance, or within a state, clustered together in discriminant space. The exception was the Cape Fear River, which was very distinct from the other five North Carolina rivers included in the male analysis. The reason(s) for the distinctness of the Cape Fear River population is unclear, though it could be related to unique environmental conditions (biotic or abiotic) within the river – the Cape Fear has a series of locks and dams that have a potential to alter environmental parameters.

Visually, the female discriminant functions plot did not show tight clustering of rivers located within the same state as observed in male specimens, yet distant rivers were distinct. One

possible explanation of the female plot clustering less tightly than the one for males might be due to an increased number of rivers included in the analysis (12) compared to 10 rivers in the male comparison.

Misclassification Rates

The Tar-Pamlico River, North Carolina, showed the lowest correct classification (58.6%) of any location in this study for the male QDFA. Interestingly, a majority of the misclassifications were to the Roanoke (10.3%) and James (10.3%) rivers. The Tar-Pamlico also had the lowest correct classification for female (62.0%) QDFA. One interpretation of this result is that the Hickory Shad population of the Tar-Pamlico is likely comprised of multiple populations. The model predicted fish originally collected in the Tar-Pamlico to be from eight other watersheds included in this study: Rappahannock, Appomattox, James, Blackwater, Roanoke, Cashie, Neuse, and Altamaha rivers. It should be noted that the Tar-Pamlico watershed had a relatively high number of samples for male (n=28) and female (n=50) analyses.

In contrast to the low correct classification of Tar-Pamlico fish, the model was able to distinguish between some rivers with 100% accuracy, including the Cashie and Cape Fear, for males, as well as the Nanticoke, Blackwater, Neuse, and Altamaha for female specimens. Fish collected from the Cashie River had high classification for both sexes, 100% for males and 93.3% for females, which is somewhat surprising since the Cashie River connects to Albemarle Sound within the Roanoke River distributary. The sample sizes for Cashie females (n=14) and males (n=16) were lower than the average, which could have affected the results.

For the most part, the rivers including in this study are independent of each other, with the exception of the Roanoke and its Cashie River tributary, and the James River with its

Appomattox River tributary. The male and female QDFAs were able to discriminate and correctly classify specimens from the Appomattox and James suggesting that the models, in some instances, can discriminate subpopulations at small spatial scales and within the same watershed. Unfortunately, due to low sample sizes, other watersheds containing tributary samples had to be excluded, so the ability to discriminate at the tributary level could not be tested further.

For other watersheds, it is possible that lower levels of correct classification at smaller spatial scales may be attributed to higher instances of Hickory Shad not returning to the natal tributary (i.e., wandering). Various levels of wandering from natal tributaries, or among watersheds, is likely not uniform across the species range, an observation exhibited in other anadromous species (McDowall 2001). Currently there is no information on the fidelity of Hickory Shad to natal watersheds, yet it is believed they practice natal homing (Batsavage and Rulifson 1998; Harris et al. 2007). American Shad do possess a homing tendency and show fidelity to natal streams based on tagging studies (Hollis 1948; Nichols 1960; Melvin et at. 1986). Such natal homing in Hickory Shad would force the same watershed environmental conditions on all members of the spawning population at the earliest life stage, but there is potential for shifts in meristic and morphometric characters when environmental conditions differ from year to year. This could lead to misclassification and false positives when using morphometric and meristic characters for stock identification.

Assuming there is some genetic element to Hickory Shad phenotype, specifically concerning morphometric and meristic characters, exchanges of genetic material between different river populations due to wandering could decrease the likelihood that morphometrics and meristics could be used to discriminate the stocks (Begg and Waldman 1999; Jorgensen et al.

2008). A large-scale tagging study would be very useful to investigate the degrees of wandering and overall fidelity of Hickory Shad to natal streams, which could complement this study. Answers to these questions, as well as understanding stock differentiation, may assist calculation of life history parameters, and population dynamics such as run class size and mortality estimates.

Research is also needed to explore more characters that can be used to increase classification success. Generally, in multivariate analysis the addition of variables will improve the overall DFA classification (White and Ruttenberg 2007). Meristic characters are well suited for this type of analysis due to the fact that they are normally fixed earlier in development than the morphological relationships, and remain constant regardless of future environmental differences (Begg and Waldman 1999; Swain and Foote 1999). Unfortunately, Hickory Shad early life history is not explicitly known, and the amount of time juveniles reside in natal freshwater habitats is not understood. It is believed young Hickory Shad leave fresh and brackish environments in early summer and emigrate to estuaries earlier than other juvenile *Alosa* species (Pate 1972; Batsavage and Rulifson 1998). This naturally leads to the question of how long Hickory Shad must remain in unique river environments for the conditions to force river-specific phenotypic variation. Taning (1952) referred to this time as "the plastic period," though he was experimenting with Sea Trout *Salmo trutta* and was only focusing on meristic characters. It was determined for *S. trutta* that the number of vertebrae was set exceptionally early during ontogeny, specifically the gastrulation period, and fin rays were set slightly later (Taning 1952).

Incorporating other stock structure identification methods, including genetics, could provide compelling evidence for stock differentiation. Little investigation has been conducted on Hickory Shad genetics, specifically in regard to population genetics. The first such study was

conducted by Vishakha (2012); 12 neutral microsatellite loci were used to determine genetic diversity and Hickory Shad composition in rivers of the lower Chesapeake Bay, Virginia. The rivers included in this study were the James (and tributaries Appomattox and Chickahominy rivers), Rappahannock, and Pamunkey rivers. Three of these rivers were also included in the current study: the James, Appomattox, and Rappahannock. Overall, Vishakha's (2012) results indicated genetic diversity of these populations was very low, the heterozygosity was below what was expected, and Analysis of Molecular Variance (AMOVA) showed a 9% molecular difference among populations and 91% within populations (Vishakha 2012). This led Vishakha (2012) to conclude that a serious bottleneck or multiple bottlenecks likely happened in the past, possibly greater than 30 years ago. Even so, it is possible that genetics may be useful in determining stock structure at smaller spatial scales (Cronin-Fine et al. 2013) than is possible with the 17 morphometric and four meristic characters used in my analysis.

My study provides foundational information on Hickory Shad, specifically the variability of anatomical characteristics across the species range. From a management perspective, the implications of such detectable variation hinge on the degree to which wandering occurs and spawning populations purportedly continue to exhibit natal fidelity, assuming the phenotypic variation found here is not arbitrary (Turan 2004). Information presented here will be useful in the development of management plans involving conservation of Hickory Shad spawning populations. The results of this work offer critical information for creating a unique management plan for Hickory Shad or for updating current management strategies for shad and river herring in which Hickory Shad are scarcely mentioned. Furthermore, the results presented here should be compared to that of future geometric morphometric, genetic, and otolith microchemistry investigations on Hickory Shad to confirm the existence of unique stocks.

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Table 3-1. Correlations between morphometric and meristic characters of Hickory Shad following size adjustment and natural log transformation.

Characters	SL	FL	TL	LLN	SAL	BD	HL	EL	SNL	HW	IOW	ML	FLD	FLA	LRD	LRP	LRV	LRA	SR	LSR	PVS	AVS
SL	1																					
FL	.108**	1																				
TL	.107**	.815**	1																			
LLN	-0.025	-.214**	-.194**	1																		
SAL	.105**	.441**	.384**	0.011	1																	
BD	-0.016	.105**	.170**	.080*	.137**	1																
HL	0.013	.418**	.457**	-.329**	.203**	0.038	1															
EL	-0.029	-.153**	-0.069	-.152**	-.175**	-0.046	.344**	1														
SNL	-.077*	0.066	.138**	0.074	-0.015	-0.03	.189**	.118**	1													
HW	-0.058	-0.007	.126**	.130**	-0.056	.219**	-0.052	.242**	.460**	1												
IOW	-0.012	-.145**	-.088*	0.013	-.209**	-.078*	-0.072	.320**	.303**	.443**	1											
ML	-0.008	.312**	.445**	0.012	.205**	.136**	.452**	.140**	.444**	.356**	0.038	1										
FLD	0.03	.150**	.189**	-.082*	-0.049	0.013	.124**	.116**	.087*	.177**	.099*	.088*	1									
FLA	0.002	0.061	.103**	-.122**	-.337**	-0.043	.100**	.120**	-0.007	-0.008	.115**	-0.053	.241**	1								
LRD	.753**	.083*	.147**	.136**	0.042	0.066	-0.043	-0.061	0.071	.109**	0.065	.132**	0.033	-0.027	1							
LRP	0.002	.454**	.595**	-.214**	.175**	.159**	.452**	.094*	.221**	.190**	-0.01	.465**	.179**	0.046	.143**	1						
LRV	0.005	.272**	.361**	-.196**	-0.009	0.059	.291**	.105**	.170**	.169**	-0.003	.320**	.167**	0.065	.133**	.547**	1					
LRA	0.008	.192**	.325**	-0.023	.078*	.077*	.119**	-0.003	.254**	.261**	0.03	.311**	.150**	0.04	.135**	.423**	.288**	1				
SR	.120**	-.488**	-.459**	.102**	-.297**	-.149**	-.282**	.309**	0.044	0.016	.227**	-.230**	-0.029	.126**	.101*	-.276**	-.104**	-.097*	1			
LSR	.126**	0.068	0.073	-0.01	.148**	.119**	0.042	-0.031	-0.058	-0.03	-0.078	0.02	0.019	-0.018	.107**	0.073	0.053	0.005	.144**	1		
PVS	0.049	-0.071	-.122**	.080*	0.047	-0.01	-.259**	-.080*	0.001	0.005	0.028	-0.065	-0.033	-.225**	0.068	-.103**	-0.055	0.028	.173**	0.074	1	
AVS	0	0.057	0.065	-.231**	-0.004	-0.011	.284**	0.072	-.127**	-.201**	-.173**	-0.006	-0.068	0.007	-.084*	.109**	.079*	-0.053	-0.019	-0.015	-.099*	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Table 3-2. ANOVA test of between-subject effects for 19 characters of Hickory Shad and sex.

Character	Mean Square	F	Sig.	Partial Eta Squared
LLN	0.124	9.088	0.003	0.015
SAL	0.002	8.578	0.004	0.015
BD	0.060	6.344	0.012	0.011
HL	0.002	3.447	0.064	0.006
EL	0.030	13.179	0.000*	0.022
SNL	0.011	7.058	0.008	0.012
HW	0.017	12.593	0.000*	0.021
IOW	0.084	21.235	0.000*	0.035
ML	0.005	7.364	0.007	0.012
FLD	0.000	0.216	0.642	0.000
FLA	0.000	0.067	0.795	0.000
LRD	0.679	96.460	0.000*	0.142
LRP	0.015	11.215	0.001*	0.019
LRV	0.009	5.942	0.015	0.010
LRA	0.005	1.041	0.308	0.002
SR	0.258	0.177	0.674	0.000
LSR	0.917	2.909	0.089	0.005
PVS	0.358	0.744	0.389	0.001
AVS	0.055	0.107	0.743	0.000

*Significant at $P < 0.003$, Bonferroni adjusted alpha

Table 3-3. Results of Oneway ANOVA for male Hickory Shad based on 15 size adjusted and natural log transformed morphometric characters and four meristic characters.

Character	df	F value	*P*-value
LLN	274	4.237	0.000*
SAL	277	5.671	0.000*
BD	276	3.596	0.000*
HL	276	3.360	0.001*
EL	277	5.348	0.000*
SNL	277	8.551	0.000*
HW	277	12.504	0.000*
IOW	276	5.626	0.000*
ML	277	0.859	0.562
FLD	276	3.278	0.001*
FLA	275	2.879	0.003
LRD	274	3.377	0.001*
LRP	277	2.126	0.028
LRV	277	3.586	0.000*
LRA	273	4.215	0.000*
SR	263	6.413	0.000*
LSR	256	3.388	0.001*
PVS	275	2.353	0.014
AVS	276	4.079	0.000*

*Significant value ($P < 0.003$)

Table 3-4. Results of Oneway ANOVA for female Hickory Shad based on 15 size adjusted and natural log transformed morphometric characters and four meristic characters.

Character	df	F value	*P*-value
LLN	326	8.066	0.000*
SAL	325	6.286	0.000*
BD	326	2.33	0.009
HL	326	2.482	0.005
EL	325	5.378	0.000*
SNL	325	9.815	0.000*
HW	325	11.238	0.000*
IOW	325	11.313	0.000*
ML	323	3.811	0.000*
FLD	326	2.15	0.017
FLA	324	1.529	0.120
LRD	325	5.842	0.000*
LRP	324	2.802	0.002*
LRV	323	0.99	0.455
LRA	321	4.121	0.000*
SR	310	13.04	0.000*
LSR	300	2.629	0.003
PVS	322	3.562	0.000*
AVS	324	2.183	0.015

*Significant value ($P < 0.003$)

Table 3-5. Principal component analysis results for male and female Hickory Shad specimens showing; eigenvalues, percentage of variance, and percentage of cumulative variance for components extracted with eigenvalues > 1.

	Eigenvalues		% of variance		% cumulative variance	
Component	Male	Female	Male	Female	Male	Female
1	2.82	2.57	18.83	21.40	18.83	21.40
2	1.94	2.22	12.93	18.46	31.76	39.85
3	1.36	1.39	9.06	11.58	40.83	51.43
4	1.29	1.13	8.61	9.45	49.44	60.88
5	1.12		7.48		56.91	
6	1.01		6.76		63.67	

Table 3-6. Component matrix from principal component analysis of male Hickory Shad, with six components extracted with eigenvalues > 1.

	Component					
Character	1	2	3	4	5	6
HW	0.740	-0.105	-0.383	0.066	-0.043	-0.134
EL	0.699	-0.347	-0.163	0.022	0.162	0.012
HL	0.647	0.470	-0.061	-0.347	0.157	0.018
SNL	0.645	-0.078	-0.060	-0.413	-0.261	0.036
LRV	0.449	0.320	0.432	0.214	-0.228	-0.174
SAL	-0.019	0.687	-0.240	-0.259	0.240	0.016
SR	0.173	-0.634	0.288	0.218	0.097	0.164
IOW	0.442	-0.495	-0.062	-0.172	0.421	-0.073
LRD	0.276	0.144	0.656	-0.048	0.269	-0.360
BD	0.135	0.370	-0.460	0.548	0.242	-0.069
FLD	0.336	-0.063	-0.168	0.500	-0.209	0.260
LSR	0.091	0.216	0.237	0.414	0.548	0.060
LRA	0.429	0.348	0.226	0.233	-0.440	0.080
AVS	-0.019	0.155	0.153	-0.164	0.191	0.759
LLN	-0.355	-0.085	-0.215	0.015	0.014	-0.379

Table 3-7. Component matrix from principal component analysis of female Hickory Shad, with 4 components extracted with eigenvalues > 1.

	Component			
Character	1	2	3	4
LLN	-0.032	0.145	0.738	-0.442
SAL	0.27	-0.439	0.28	0.355
EL	0.176	0.751	0.003	0.239
SNL	0.535	0.317	-0.075	-0.149
HW	0.567	0.491	-0.158	0.016
IOW	0.157	0.736	-0.252	0.172
ML	0.768	-0.081	0.079	-0.137
LRD	0.336	0.228	0.618	-0.138
LRP	0.734	-0.246	-0.039	0.191
LRA	0.588	-0.239	0.184	0.225
SR	-0.439	0.613	0.338	0.142
PVS	-0.224	-0.017	0.368	0.747

Table 3-8. Quadratic discriminant function analysis (QDFA) classification results for male Hickory Shad. Values given in percentages.

		Predicted River									
		Patapsco	Rappahannock	Appomattox	James	Roanoke	Cashie	Pamlico Sound	Tar-Pamlico	Neuse	Cape Fear
	Patapsco	**89.2**	0	0	2.7	0	0	2.7	0	0	5.4
	Rappahannock	0	**65**	10	5	0	10	5	0	5	0
	Appomattox	0	9.1	**86.4**	0	0	4.5	0	0	0	0
	James	0	2.9	5.7	**77.1**	5.7	0	0	8.6	0	0
River of	Roanoke	4.3	0	4.3	0	**78.3**	0	0	4.3	8.7	0
Collection	Cashie	0	0	0	0	0	**100**	0	0	0	0
	Pamlico Sound	0	0	0	0	5	5	**85**	0	5	0
	Tar-Pamlico	0	6.9	3.4	10.3	10.3	3.4	0	**58.6**	6.9	0
	Neuse	0	6.7	3.3	6.7	16.7	0	3.3	3.3	**60**	0
	Cape Fear	0	0	0	0	0	0	0	0	0	**100**

Table 3-9. Quadratic discriminant function analysis (QDFA) classification results for female Hickory Shad. Values given in percentages.

		Predicted River										
		Susquehanna	Nanticoke	Rappahannock	Appomattox	James	Blackwater	Roanoke	Cashie	Pamlico Sound	Tar-Pamlico	Neuse	Altamaha
	Susquehanna	**92.3**	7.7	0	0	0	0	0	0	0	0	0	0
	Nanticoke	0	**100**	0	0	0	0	0	0	0	0	0	0
	Rappahannock	0	0	**90.5**	0	0	0	4.8	0	4.8	0	0	0
	Appomattox	0	0	4.2	**79.2**	0	0	12.5	0	4.2	0	0	0
River of	James	4	0	4	8	**72**	0	4	4	0	0	4	0
Collection	Blackwater	0	0	0	0	0	**100**	0	0	0	0	0	0
	Roanoke	0	0	5.9	5.9	0	0	**82.4**	0	0	0	0	5.9
	Cashie	0	0	0	0	6.7	0	0	**93.3**	0	0	0	0
	Pamlico Sound	0	0	3.3	4.9	9.8	1.6	0	1.6	**67.2**	6.6	3.3	1.6
	Tar-Pamlico	0	0	10	6	6	4	2	2	4	**62**	2	2
	Neuse	0	0	0	0	0	0	0	0	0	0	**100**	0
	Altamaha	0	0	0	0	0	0	0	0	0	0	0	**100**

Table 3-10. Structure matrix from male Hickory Shad quadratic discriminant function analysis.

Character	Function								
	1	2	3	4	5	6	7	8	9
SNL	0.347	0.218	0.31	-0.105	0.043	-0.204	-0.229	-0.099	0.259
SR	0.304	0.099	-0.026	-0.187	0.3	0.078	-0.033	-0.102	-0.291
LSR	-0.009	0.448	-0.196	0.346	0.201	-0.032	0.094	0.152	0.053
IOW	-0.097	0.368	0.546	-0.189	-0.134	-0.169	0.39	-0.139	0.036
FLD	0.155	0.231	-0.267	0.078	0.032	0.245	0.227	0.083	-0.096
BD	-0.091	-0.034	0.263	0.46	-0.074	0.354	-0.148	0.422	-0.201
SAL	-0.22	-0.172	-0.261	0.458	0.122	-0.332	0.088	0.347	-0.045
HW	0.371	0.284	0.429	0.437	-0.101	-0.018	0.315	0.069	0.039
LRD	-0.088	0.28	-0.031	-0.285	0.573	0.143	0.155	0.175	0.44
LLN	-0.109	-0.196	0.291	0.243	0.437	0.384	0.008	-0.421	-0.204
LRV	0.099	0.321	-0.046	-0.204	-0.232	0.351	0.196	0.263	-0.02
EL	0.263	0.052	0.32	-0.2	0.147	-0.086	0.597	0.255	-0.255
AVS	-0.059	-0.155	-0.26	0.028	-0.128	0.201	0.13	-0.548	0.108
HL	-0.034	0.314	-0.013	0.027	0.036	0.083	-0.009	0.321	0.049
LRA	0.213	-0.16	-0.061	0.078	-0.163	0.418	0.216	0.458	0.587

Table 3-11. Structure matrix from female Hickory Shad quadratic discriminant function analysis.

Character	Function										
	1	2	3	4	5	6	7	8	9	10	11
SR	0.507	0.099	-0.289	-0.435	-0.183	-0.335	0.373	0.181	0.001	0.357	0.019
IOW	0.380	-0.560	0.505	-0.044	-0.058	0.012	-0.106	0.087	0.133	0.403	0.267
LLN	0.071	0.506	0.073	0.208	-0.427	-0.175	-0.167	0.130	0.439	0.259	-0.149
HW	0.286	0.118	0.670	0.050	0.606	0.042	0.023	-0.085	0.070	0.093	-0.249
SNL	0.380	0.069	0.046	0.670	0.301	-0.071	-0.066	-0.209	0.087	-0.030	0.489
ML	0.057	0.236	0.248	0.566	0.073	0.400	-0.095	0.276	-0.285	0.198	-0.105
LRA	-0.196	0.273	0.259	0.081	0.055	0.456	0.505	-0.063	0.218	0.165	0.469
LRD	0.153	0.354	0.178	-0.250	-0.084	-0.032	-0.421	0.200	0.386	0.043	0.203
EL	0.417	0.067	0.396	-0.267	-0.213	0.130	-0.082	-0.532	-0.372	0.312	0.077
PVS	0.060	-0.005	-0.522	-0.217	0.236	0.459	-0.162	-0.145	0.233	0.531	-0.071
SAL	-0.377	0.251	0.019	0.023	0.356	-0.114	-0.339	0.157	-0.321	0.510	0.372
LRP	-0.200	0.023	0.206	0.273	0.180	-0.033	0.153	-0.304	0.111	0.365	-0.029

FIGURES

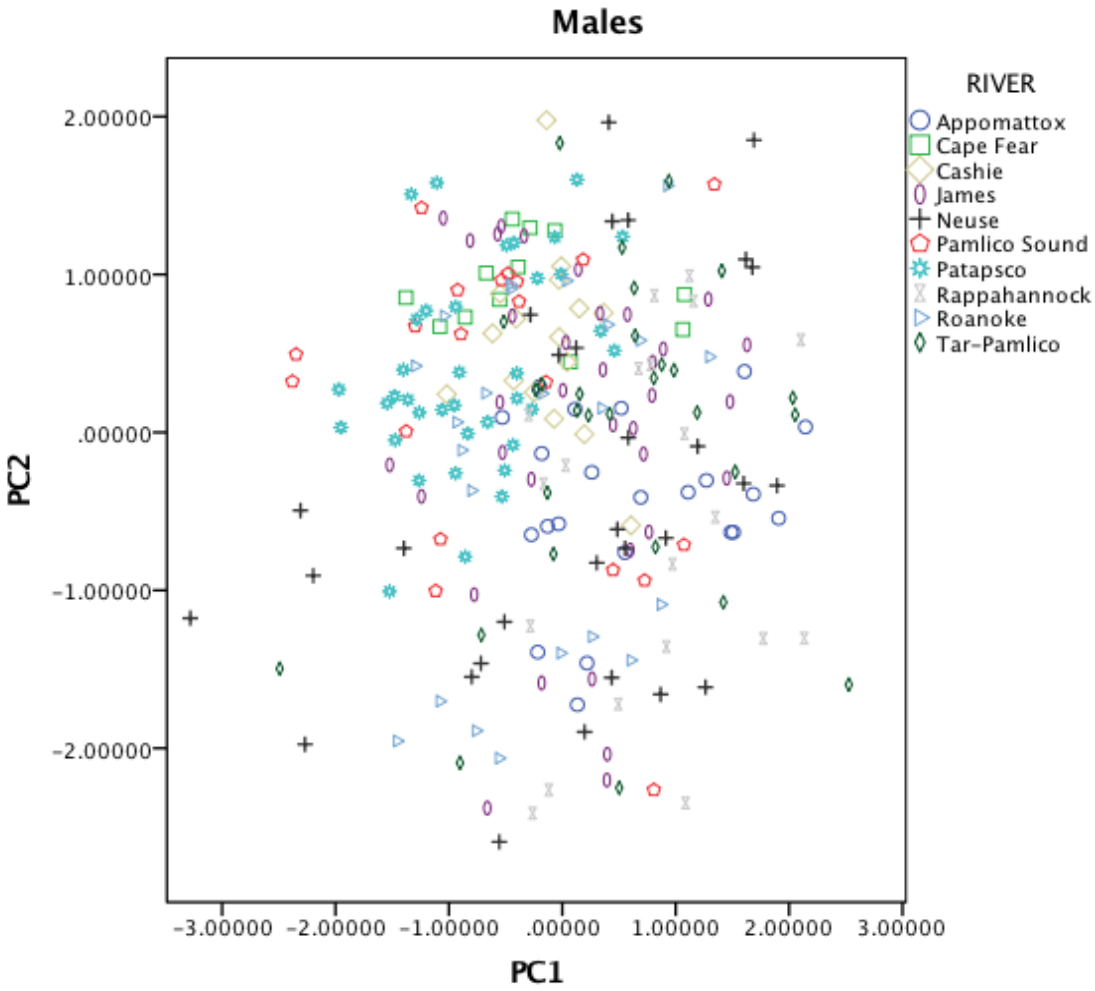

Figure 3-1. Scatter plot of principal component 1 (PC1) score plotted against principal component 2 (PC2) score for male Hickory Shad.

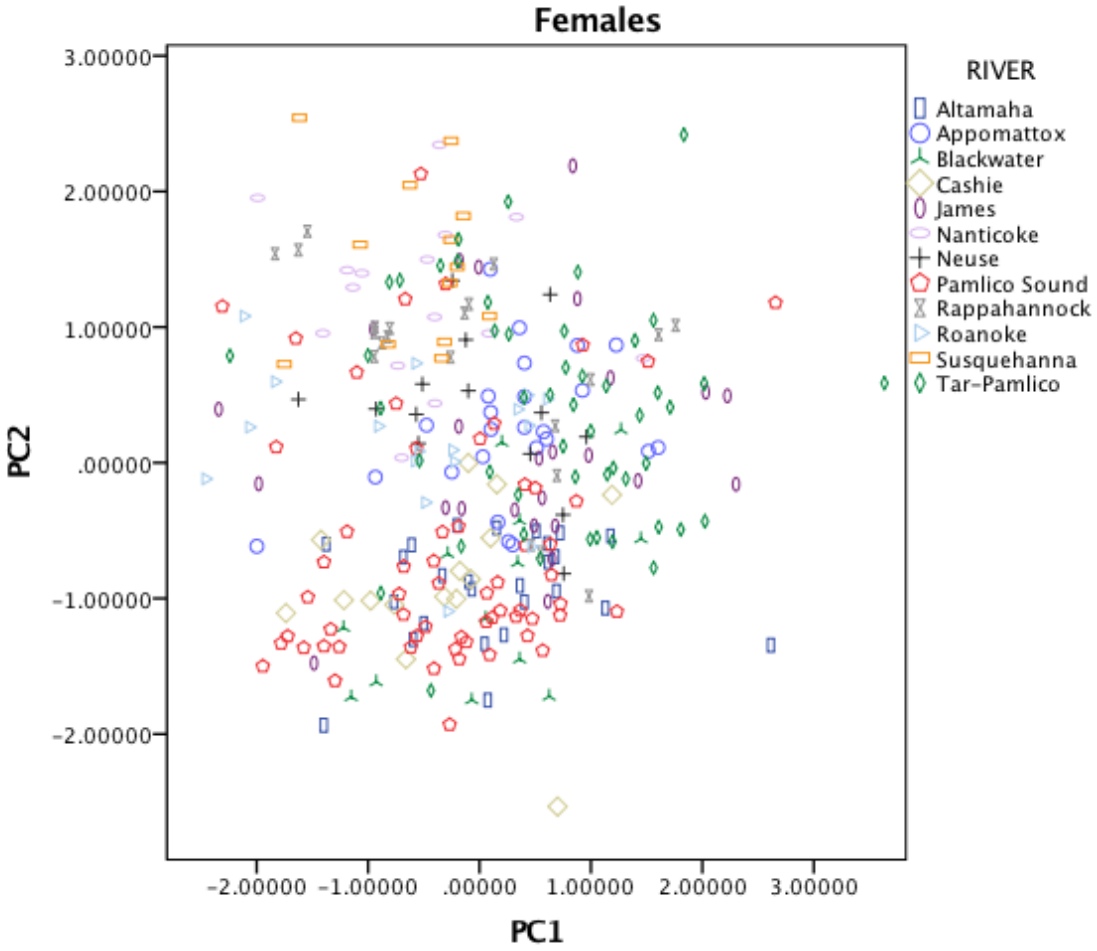

Figure 3-2. Scatter plot of principal component 1 (PC1) score plotted against principal component 2 (PC2) score for female Hickory Shad.

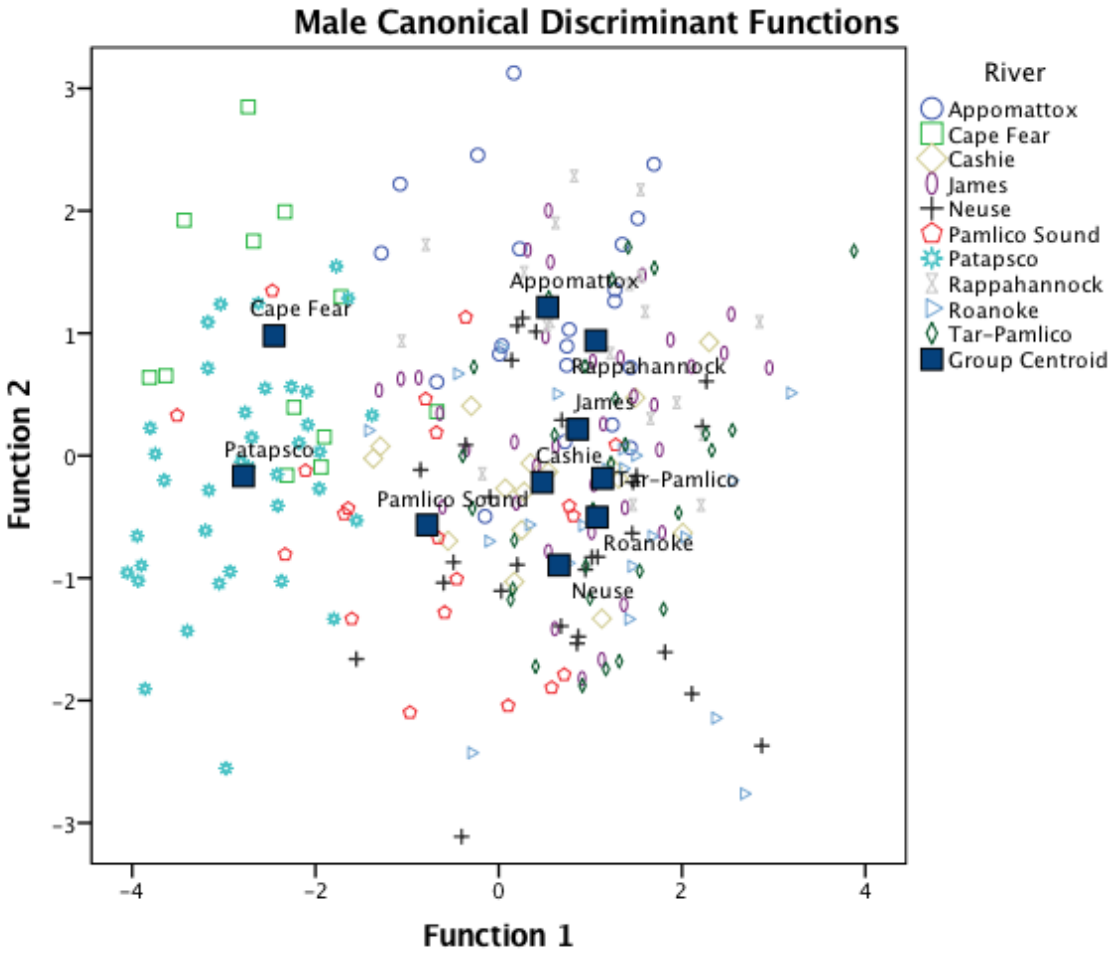

Figure 3-3. Scatter plot of male Hickory Shad canonical discriminant function 1 (DF1) and 2 (DF2) scores showing group centroids.

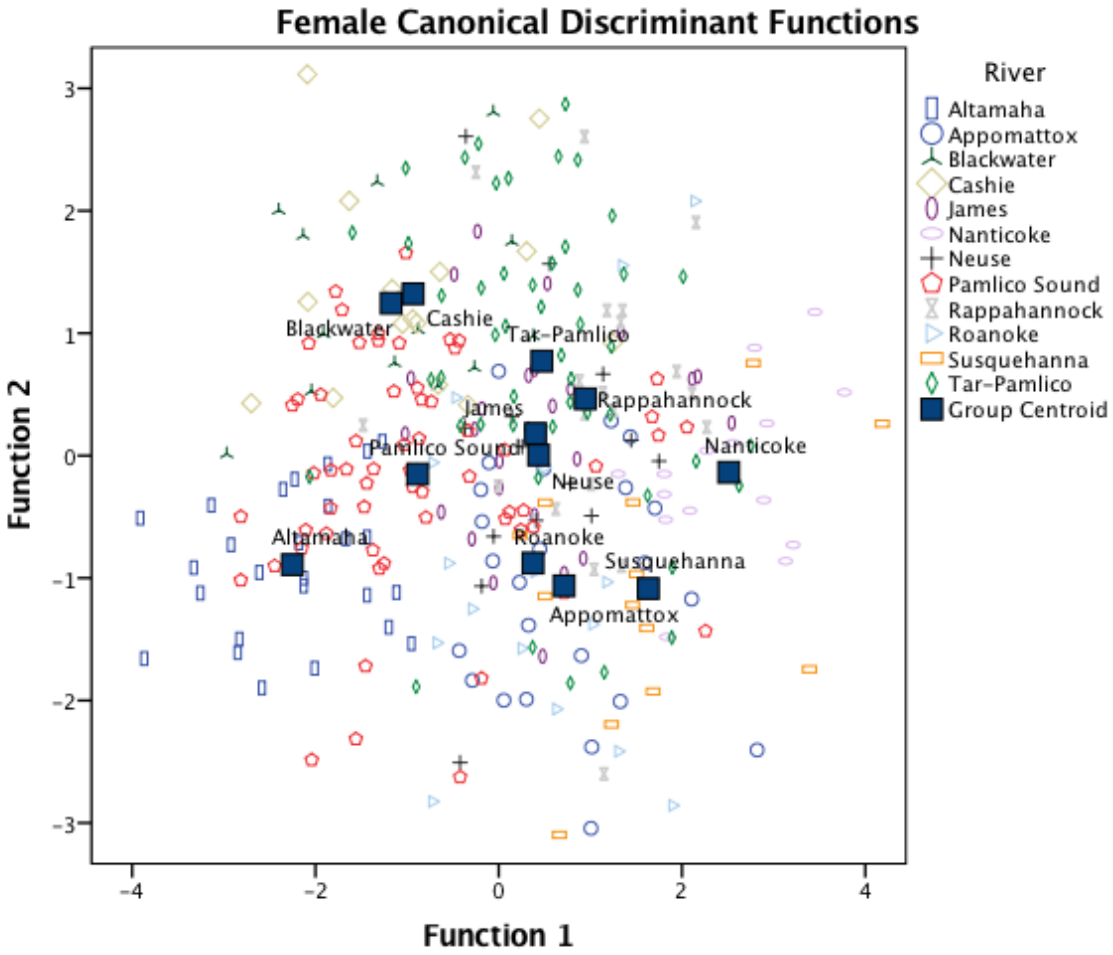

Figure 3-4. Scatter plot of female Hickory Shad canonical discriminant function 1 (DF1) and 2 (DF2) scores showing group centroids.

Chapter 4: Summary and Recommendations

The current study provides, to my knowledge, the first evidence for phenotypic differences of adult Hickory Shad among 14 different spawning populations from Maryland, Delaware, Virginia, North Carolina, and Georgia. The results of multivariate analysis, including discriminant function analysis (DFA) and principal component analysis (PCA), on 15 morphometric and four meristic characters of mature Hickory Shad show that these populations can be successfully discriminated with relatively low error (depending on river) utilizing this technique.

The Hickory Shad is currently the least studied of the four anadromous Clupeids that inhabit the East Coast of the United States. Presently, there does not exist a unique management plan for Hickory Shad; instead the Atlantic States Marine Fisheries Commission (ASMFC) has combined this species with American Shad and the River Herrings (Alewife and Blueback Herring) due to data limitations (ASMFC 2010). More investigation on Hickory Shad is greatly needed as there are still many unanswered questions that must be addressed before species-specific management can be implemented. To date, no research has investigated the assumption of natal homing in Hickory Shad (Batsavage and Rulifson 1998; Harris et al. 2007) and the results of this study cannot directly confirm this assumption, yet the significant variation between river populations provides support for natal homing in Hickory Shad. Wandering or straying behavior from natal rivers may vary between populations of Hickory Shad and is likely not uniform across the species range, an aspect shown for other anadromous species (McDowall 2001).

The results of this study, and the fact that Hickory Shad from multiple rivers show statistically significant differences in morphometric and meristic characters, suggest that these

spawning populations should be classified as independent spawning units, or stocks. Again, the term "stock," as used here, describes a management unit that can consist of separate spawning populations of a species (Meng and Stocker, 1984). Until my study the independent stock concept for Hickory Shad had not been defined or described, which is necessary for creating management plans that ensure and conserve the biodiversity of separate spawning populations.

Dams continue to be a primary threat to Hickory Shad, as well as virtually all diadromous fish species on the East Coast of the United States, by impeding and often barring access to historic spawning grounds in addition to altering physical and biological properties of the river (Limburg and Waldman, 2009). Fortunately for diadromous fish, dam removal has become a priority on many rivers, and the ASMFC (2010) lists this as one of their strategies to restore "shad" and river herring populations. The most recent American Shad stock assessment report determined that American Shad stocks were at "all-time lows" and that recovery to acceptable levels was not occurring (ASMFC 2007; ASMFC 2010). Stock declines were attributed to multiple factors including high mortality, decreased habitat, habitat degradation, and reduced access or barriers to migration (ASMFC 2007). Not only are American Shad at historically low numbers, they have been extirpated from many rivers and their populations have decreased from 138 to as few as 68 (Limburg et al. 2003), possibly less today. This has implications for Hickory Shad since purportedly they have a similar life history and experience many of the same negative anthropogenic factors (Rulifson 1994). Also, declining stocks of American Shad could affect Hickory Shad due to the recreational fishing effort switching to the more abundant Hickory Shad. If this is indeed true, it will require further work to determine Hickory Shad post-release mortality, due to the fact that a large portion of the recreational fishery is catch and release (ASMFC 2007).

One recommendation for state fisheries agencies is that they should actively sample and record occurrences of Hickory Shad in all rivers. One severe data limitation for the species is that the current number of Hickory Shad populations is unknown (Rulifson, 1994; Limburg and Waldman, 2009). During the course of this study and in contacting fish biologists to procure samples from various watersheds, it became apparent that the presence of Hickory Shad was occasionally found in watersheds long believed to no longer have Hickory shad spawning runs. An accurate identification of the number and location of spawning populations is essential. This ensures that Hickory Shad populations will not be extirpated from locations without our knowledge. For this study Hickory Shad were collected from 19 assumed spawning population along the east coast, yet likely there are more. Unfortunately, due to low sample sizes for some locations, only 12 locations could be included in the multivariate analysis. Rulifson (1994) reported a total of 64 rivers with Hickory Shad spawning runs, with four in New England, 44 in the Mid-Atlantic, and 26 in the South Atlantic. North Carolina's Hickory Shad runs were not included in the manuscript due to lack of information about the species in NC coastal rivers, so the total count of rivers with Hickory Shad likely underestimates the full extent of their distribution.

The coastal states further north than Maryland, including Delaware, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, New Hampshire, and Maine, should determine if Hickory Shad are present within their inland (or coastal) waters and, if present, determine the relative abundance, timing, and purpose of their presence. Historically some authors suggested the range of Hickory Shad extends as far north as Maine (Hildebrand and Schroeder, 1928). In a more recent investigation into the status of *Alosa* stocks along the east coast, Rulifson (1994) reported that according to answers from a questionnaire, state fishery personnel indicated the

presence of Hickory Shad only as far north as Connecticut, with runs in four rivers and unknown stock status.

Moving forward, aside from the research needs already listed, another critical component to Hickory Shad conservation will be understanding their ocean movements and habits as well as whether different spawning populations migrate to the same or different ocean feeding grounds. Hickory Shad could exhibit similar ocean distributions to American Shad, which exhibit mixed aggregations during the ocean habitation phase (Gabriel et al. 1976; Dadswell et al. 1986). The discriminant functions for male and female specimens created in this study can be used by other researchers and managers to determine the distribution of Hickory Shad stocks once they leave freshwater and emigrate back to the Atlantic Ocean. If Hickory Shad have unique ocean distributions based on river spawning populations, these areas need to be documented to ensure adequate protection of the species from offshore harvest.

Hickory Shad, along with other anadromous Cluepids, are culturally important fish species for east coast communities; many of these communities continue to cherish the spawning runs and celebrate with festivals (Limburg and Waldman, 2009). In addition to being culturally important, anadromous fish supply food to the people who inhabit coastal and inland areas and provide the vital ecosystem service of bringing marine-derived nutrients into freshwater ecosystems (Garman and Macko 1998). Anadromous fish are important prey items for many commercially (and recreationally) important inland and coastal piscivorous fish species (McDermott et al. 2015), in addition to birds and mammals. Yet, it is not well understood what role Hickory Shad play in this relationship; few studies have investigated predation of Hickory Shad. Pine et al. (2005) reported predation on Hickory Shad by introduced Flathead Catfish *Pylodictis olivaris* in the Neuse River, North Carolina. For the reasons listed here, Hickory Shad

should be considered a priority for research to address the knowledge gaps, and unique management strategies focused on Hickory Shad conservation that will guarantee continued survival of all stocks must be developed.

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APPENDIX A: IACUC APPROVAL

East Carolina University
Tomorrow starts here.

Animal Care and Use Committee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834-4354

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

December 12, 2017

Roger Rulifson, Ph.D.
Department of Biology
Flanagan 385
East Carolina University

Dear Dr. Rulifson:

The Amendment to your Animal Use Protocol entitled, "Hickory Shad 2015" (AUP #D330) was reviewed by this institution's Animal Care and Use Committee on December 12, 2017. The following action was taken by the Committee:

"Approved as submitted"

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

Sincerely yours,

Susan McRae, Ph.D.
Chair, Animal Care and Use Committee

SM/jd

Enclosure