Winter 2014

The Impact of Multiple Nursery Areas on the Population Structure of Atlantic Menhaden, *Brevooria tyrannus*

Kristen A. Anstead
Old Dominion University

THE IMPACT OF MULTIPLE NURSERY AREAS ON THE POPULATION STRUCTURE OF ATLANTIC MENHADEN, *BREVOORIA TYRANNUS*

by

Kristen A. Anstead
B.S. May 2002, Bates College

A Dissertation Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

OCEANOGRAPHY

OLD DOMINION UNIVERSITY
December 2014

Approved by:

Cynthia M. Jones (Director)

Alexander Bochdansky (Member)

Norou Diawara (Member)

ABSTRACT

THE IMPACT OF MULTIPLE NURSERY AREAS ON THE POPULATION STRUCTURE OF ATLANTIC MENHADEN, *BREVOORIA TYRANNUS*

Kristen A. Anstead
Old Dominion University, 2014
Director: Dr. Cynthia M. Jones

Understanding the population structure and patterns of connectivity in marine fishes is essential when making predictions about a species' resiliency and persistence in an increasingly changing environment. The Atlantic Menhaden *Brevoortia tyrannus* is a clupeid that plays a critical role in the marine food web and supports one of the largest fisheries on the US East Coast. In addition to a decrease in overall numbers and spawning stock biomass, recruitment levels have remained low since the 1990s. Menhaden use numerous estuaries along the Atlantic coast for juvenile development before recruiting to the adult population and the contribution of each of these nursery grounds is currently unknown. The Chesapeake Bay is believed to contribute 69% of the total recruits, although this estimate has never been quantitatively verified and is 25 years old, predating current low recruitment levels and increased development along the coastline. This study investigated the potential of trace element (Li, Mg, Mn, Rb, Sr, Y, Ba and Pb) and stable isotope ($\delta^{13}C$ and $\delta^{18}O$) signatures in otoliths to distinguish between coast-wide nursery grounds of menhaden for 2009-2011. Using geochemical signatures specific to each year, juvenile menhaden collected from Connecticut to South Carolina were classified to regional nursery grounds at nearly 90% accuracy. The geochemical signatures were applied to adult menhaden of unknown natal origin that corresponded to these year-classes to determine which nursery is producing the most recruits to the

fishable stock. The results indicate that while the Chesapeake Bay still dominates the proportion of age-1 recruits, the Bay's contribution has declined by 16-65% of the earlier estimate. Additionally, an evaluation of older age classes (ages 2-4), the spawning stock, offered a more complete assessment with nearly 70% of adults originating from the Northeast and Southeast nursery grounds rather than the Mid-Atlantic as previously believed. This study successfully evaluates historical estimates of nursery contribution for menhaden, identifies regions that are currently essential for survivorship of this population, and, thus, provides critical information to future stock assessments for this species.

ACKNOWLEDGEMENTS

There are numerous people who contributed to the completion of this research project. First I would like to thank my advisory and dissertation committee: Dr. Cynthia M. Jones, Dr. Alexander Bochdansky, and Dr. Norou Diawara, for their guidance, support, and patience during my time at Old Dominion University.

I am grateful to my advisor, Dr. Cynthia M. Jones, for all of the opportunities she offered me, including this degree and engaging research project. She always demanded that I be more thorough, bold, thoughtful, and inquisitive as a student, scientist, and person. I am grateful to have had such a good role model for those qualities in an advisor.

I would also like to thank Dr. Jason J. Schaffler who guided me through every step of this research project. Dr. Schaffler spent countless hours patiently answering my questions and discussing challenges I faced regarding anything from statistical analyses to the nuances of using a dremmel tool. I sincerely thank him for all his support and knowledge and I am inspired by his generosity in everything he does.

My fellow graduate students and colleagues at the Center for Quantitative Fisheries Ecology have been critical to the completion of this research. I would like to thank Michael Schmidtke, Kathleen Kirch, Dr. Renee Reilly, Dr. Stacy Baez, Jason Ferguson, Antranik Kajajian, James Davies, Dr. Hongsheng Liao, Dr. Julian Ashford, and Dr. William Persons. I am grateful for the support I have received from each of them in the form of processing samples, discussing scientific methods, or just having a good laugh in the lab.

This work would not have been possible without the field collections made by multiple state agencies and individuals along the Atlantic coast including the Connecticut Department of Marine Fisheries, Rhode Island Marine Fisheries, New York Department of Environmental Conservation, New Jersey Department of Fish and Wildlife, Maryland Department of Natural Resources Fisheries Service, Chris Newsome, Jacques van Monfrans, National Marine Fisheries Service, North Carolina Division of Marine Fisheries, South Carolina Department of Natural Resources, and Florida Fish and Wildlife.

I am thankful to my friends and family who supported and encouraged me throughout this degree program. Thank you to my dog, T Rex, because without him I would have never left the lab or gone outside for these last few years. Thank you to my husband, William Boll, for changing the path of my life in so many unexpected and exciting ways. And finally, there is no greater personal or professional inspiration to me than my mother, Alicia, and I thank her every day for everything.

Funding for this study was provided by a grant from the National Science Foundation. Project data will be archived online through the Biological and Chemical Oceanography Data Management Office (BCO-DMO).

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CHAPTER I

INTRODUCTION

Background

Despite considerable progress in fisheries science and management, understanding how population structure, dispersive larval stages, and connectivity influences the success and persistence of marine fishes remains a challenge. This is particularly important for species that use multiple coast-wide estuaries as nursery areas, since the resiliency of estuarine-dependent fishes depends on the productivity and health of these habitats. One of these species is the Atlantic Menhaden *Brevoortia tyrannus* and understanding the role that multiple nursery areas play in their population structure remains a central challenge to long-term sustainable management of this species. Menhaden comprise the largest landings by volume on the US East Coast and support the marine food web (ASMFC 2012), yet low population numbers, decreased recruitment, and a declining spawning stock biomass has plagued this species for several decades. Currently, the contribution and productivity of each of the nursery grounds along the Atlantic coast to the adult stock is unknown, but this information would provide critical insight for management and current stock assessments. To evaluate the persistence and resiliency of menhaden and to identify areas that are essential for survivorship, the relationship between population structure, larval supply, and recruitment along the Atlantic coast needs to be fully understood.

Menhaden, an estuary dependent, filter-feeding member of the Clupeidae family, are of significant economic value on the US Atlantic Coast. Commercially fished for the last few centuries, developments in purse-seine construction and oil reduction technologies in the 1950s increased the quantity of the catch and decreased the expended effort in finding and processing menhaden. Records for purse-seine landings have been recorded regularly since 1955, when 23 reduction plants operated 150 vessels landing 641,000 metric tons annually. In the following decades, increased fishing pressure lead to decreased landings. Currently, this species makes up both a bait and a reduction fishery and is used for fertilizer, oil, fish bait and meal. Only one reduction plant -- based in Reedville, Virginia -- continues to operate 10 vessels landing approximately 140,000 metric tons annually that generate millions of dollars annually (Kirkley 2011). The Atlantic States Marine Fisheries Commission (ASMFC) manages the stock by imposed quotas and limits on by-catch. Menhaden are not considered overfished and overfishing in not occurring, according to the current assessment and based upon recently updated reference points, although overfishing may have occurred in recent years. Even with the thorough assessment and recommendations by the ASMFC, the population continues to suffer from low recruitment and a decreased spawning stock biomass (ASMFC 2010).

Aside from their commercial value, menhaden play a large role in the marine food web. Menhaden are a key prey species for several predators with commercial and recreational value, such as Bluefish (*Pomatomus saltatrix*), Weakfish (*Cyoscion regalis*) and Striped Bass (*Morone saxatilis*), as well as seabirds and marine mammals (Ahrenholz 1991; Uphoff 2003; ASMFC 2010). Menhaden are one of the most abundant fish in the Chesapeake Bay and are major consumers of phytoplankton and zooplankton (Ahrenholz

1991). Although the importance of their role in affecting water quality is uncertain, as is whether lowered population numbers would contribute significantly to the health of the ecosystem, these filter feeders are still valuable as consumers of primary production (Kirkley 2011). Yet despite the importance of menhaden, much still remains understudied about menhaden, including the causes of low recruitment and the reduction in spawning stock biomass.

Understanding the life history and population structure of the menhaden is the first step in managing this fishery at a sustainable size for continued economic and environmental benefits. Much about the life history of menhaden has been known for several decades, including their distribution, migration patterns, and fecundity. Menhaden are distributed from Nova Scotia to Florida and undergo extensive seasonal migrations along the coast. In the summer, the population distributes by size and age so that the largest, older fish are in the northern part of the range while younger, smaller fish are relegated to the south (Nicholson 1978). In the fall, menhaden begin a coast-wide migration to North Carolina's Cape Hatteras where they aggregate and spawn as a population in the winter months (Nicholson 1978; Lewis et al. 1987). Menhaden are multiple spawners and become sexually mature between ages 1 and 2 and fecundity increases with age, and the oldest, largest females produce the most eggs (Lewis et al. 1987).

After spawning, menhaden rely on ocean currents to deliver larvae to multiple inshore nursery grounds where juveniles develop before recruiting to the adult stock and become subjected to the commercial fishery. Depending on physical processes and ocean circulation, larvae can be transported hundreds of kilometers from the spawning ground

to estuaries (Epifanio and Garvine 2000). The productivity of each of these nursery grounds in contributing both new recruits and recruits that survive to the older age classes is unknown. Nursery ground productivity may vary annually due to environmental factors such as the amount of larvae near estuarine mouths, changes in the Gulf Stream, and winter storms, just to name a few (Nelson et al. 1977; Checkley et al. 1988; Quinlan et al. 1999). Additionally, much of the coastline is heavily developed, influencing the stability and health of the ecosystem. Human activities and environmental change have significant impacts on estuarine systems, resulting in hypoxia, habitat loss, and increased sea temperatures (Kemp et al. 2005). These conditions have the potential to affect estuarine health and cause habitat degradation, potentially altering fish recruitment or changing the productivity of nursery grounds.

Apart from physical conditions and human activities, divergent migrations by size and age classes may increase the spatial and temporal placement of eggs and larvae (Secor 1999). While the majority of menhaden spawning occurs off of North Carolina, some spawning does takes place along the migratory tract of the older, larger, more fecund fish in northern regions, possibly ensuring recruitment from a variety of locations (Ahrenholz 1991). With the most fecund fish having a larger migratory pattern, the menhaden increases its spawning potential coast-wide and spawning in alternative locations could function as a bet-hedging strategy for the population. Small deviances in spawning behavior provide connectivity within the larger population, resupplying contingents to various patches (Secor et al. 2009) and yet the role that spawning outside of Cape Hatteras plays on recruitment and stock size is not well understood.

The contribution of multiple nurseries to the adult population of menhaden remains understudied even though it may provide insight to the low recruitment and decreased spawning stock biomass for menhaden in recent decades. The Chesapeake Bay is estimated to contribute approximately 69% of the total recruits to the adult menhaden population (Ahrenholz et al. 1989; ASMFC 2004a), but that number is dated, has not been quantitatively validated, and predates current climate conditions and development along the coast. The ASMFC identifies several areas of concern for the menhaden population in the most recent stock assessment update including the declining health of nursery areas, particularly in the Chesapeake Bay (ASMFC 2012). The Bay is flushed by freshwater inputs from its 168,00 km^2 watershed, but as the land around the estuary becomes more developed, these freshwater inputs introduce many pollutants and nutrients. Much of the threats in the Mid- and Lower Bay occur on land, including excessive waste from sewage and landfill facilities, nutrient and chemical overloading of pesticides, fertilizers, and livestock waste from farmland, and the proximity of estuaries to runoff from major roadways (Allan 2004; Goetz et al. 2004; Jantz et al. 2005; Kemp et al. 2005). These inputs influence many physical properties of the estuary, such as lowering dissolved oxygen concentrations, raising temperatures, changing salinities, and altering habitats that many fish species rely on (Goetz et al. 2004). The ASMFC (2012) names the Mid- and Lower Chesapeake Bay as the most threatened regions because of their role as nursery grounds and how this has altered recruitment in recent years remains a concern. The ability of other coast-wide nursery grounds to maintain nursery productivities or increase their productivity from historical estimates also is unknown and understudied. With climate change and industrial development along the Atlantic Coast,

it would be beneficial to reassess historical estimates of nursery productivity to see which regions are thriving and which contribute fewer recruits.

Fisheries scientists have many tools available to them for evaluating population structure, movement, and connectivity. These methods range from applied tags, such as those used in mark-recapture studies, to natural tags, such as the use of parasites, genetics, or otolith chemistry (Pine et al. 2003; Elsdon et al. 2008). While these tools are successful for many applications, several provide challenges for determining the natal origin of adults (Thorrold et al. 2001). Mark-recapture studies are proven effective for estimating abundance or survival in many fish species, but are inefficient for establishing nursery origin because of the high mortality rate of juvenile fishes and small number of tag returns (Nicholson 1978; Pine et al. 2003). Juveniles can become infected with a parasite unique to their nursery region so that when they join the adult population, natal origin can be inferred, but not all individuals may have the infection and parasites can change with time (MacKenzie and Abaunza 1998). Genetic markers are also commonly used where mitochondrial DNA, for example, shows differences for localized subpopulations, but this can be challenging for panmictic species, such as menhaden, where all members of the population mix during spawning so that regional loci are not preserved (Pertoldi et al. 2007; Lynch et al. 2010). The use of otolith chemistry to evaluate movements and natal origin in fishes has been widely applied in recent years and avoids many of the challenges faced by the other methods (Campana 1999; Gillanders et al. 2003). It has proven successful for addressing natal origin for menhaden in the Chesapeake Bay (Schaffler et al. 2014a) and is likely to be useful for addressing similar issues coast-wide.

Use of otolith chemistry

Otoliths are metabolically inert, paired calcium carbonate structures located in the inner ear of the fish. Much like the rings on a tree, new material is deposited daily, building out from a central nucleus. Both daily increments and annuli can be detected on an otolith, so they are effective tools for aging purposes (Campana 2001), but chemical information is also captured in its matrix. Deposited new material contains trace elements and stable isotopes that reflect the chemistry of the ambient water and because otoliths are inert, this material remains unchanged over the life of the fish (Campana et al. 1995; Fowler et al. 1995; Thorrold et al. 1997). The levels of elements in the water where the fish resides, as well as ambient temperatures and salinity, affect the isotopic ratio of elements or stable isotopes and these are incorporated into the structure of the otolith in varying quantitates (Dorval et al. 2005). Therefore, otoliths act as a natural tag by providing a chemical chronology of where the fish has been during its lifetime (Fowler et al. 1995). Otolith chemistry analyses isolate signatures that discriminate between locations, since water chemistries vary, so that information can be gained about the life history or movement of fishes.

Otolith chemistry studies are often comprised of two analyses: trace element and stable isotope. Each analysis provides different information for building geochemical signatures that will differentiate between bodies of water where a fish resides. For example, barium and strontium are two trace elements that have been proven effective for discriminating between regions because of they vary predictably with salinity and temperature (Fowler et al. 1995; Dorval et al. 2005). Additionally, lithium varies depending on offshore-inshore environments (Campana et al 2000; Gillanders 2002) and

elements such as manganese and magnesium have also proven to reflect regional differences (Campana 1999; Elsdon et al. 2008). In addition to trace elements, a significant amount of information resides in the carbon and oxygen stable isotope ratios in the otolith. The carbon isotope ($\delta^{13}C$) is deposited under non-equilibrium conditions and is influenced by diet, metabolic rate and environmental factors. The oxygen isotope ($\delta^{18}O$) is controlled by temperature, salinity, and the ratio of $\delta^{18}O$ to $\delta^{16}O$ in the ambient water (Thorrold et al. 1997; Campana 1999). Stable isotope signatures provide markers that indicate whether the fish used marine, estuarine, or freshwater habitats and can trace fish movement and migration between different habitats (Campana 1999). Combined, trace element and stable isotope analyses can be used to build chemical signatures that are unique to different locations.

Otolith elemental analysis can be implemented to evaluate the contribution of various natal habitats to the adult population, including for the menhaden population. Collecting juveniles from coast-wide nursery grounds and applying the use of otolith chemistry can identify signatures that distinguish between regions. To gain the most insight, juvenile signatures should be collected over several years to establish annual variability as well as spatial variability in signatures. Gillanders (2002) found that variability in temperature, salinity and freshwater inputs in estuarine systems affected the stability of the elemental fingerprints of juvenile fishes over a 3-year study. She argued that to accurately assign adults to natal regions, a library of should be built for several year-classes. Some research has shown inter-annual variability in elemental fingerprints (Schaffler and Winkelman 2008; Walther and Thorrold 2009) while others have not, particularly in freshwater systems (Wells et al. 2003; Munro et al. 2005). Therefore,

establishing temporal stability of the elemental fingerprints for menhaden is necessary when using otolith chemistry for classification purposes over several year-classes.

Once juvenile signatures have been established as effectively distinguishing between nursery grounds, adult fish of unknown origin can be assigned to their nursery region. After capture, a fish can be aged using otoliths and its juvenile cohort can be determined. By isolating and analyzing the juvenile core of the adult otolith, the chemical signature can be matched to that of a nursery ground, thus determining its natal origin. Assigning intermingling adults to various nursery grounds using these signatures assesses different area's contribution to the adult population and identifies which areas are most essential to preserving that stock.

Objectives

This dissertation research aims to quantify how each of the major estuarine nursery grounds along the Atlantic Coast contributes to the adult menhaden population, considering both new recruits and older age-classes. Three main questions are addressed in this work:

1) Can otolith chemistry be used to differentiate between coast-wide nursery grounds for juvenile Atlantic Menhaden?
2) Which nursery grounds along the US Atlantic coast proportionally produce the most new recruits to the Atlantic Menhaden population and how has this changed from historical estimates of nursery ground contribution?

3) Do coast-wide nursery grounds have differential survival through multiple age-classes and which are contributing to the spawning stock biomass?

In this dissertation, I address the three questions in our objectives in Chapters II, III, and IV, respectively, by using otolith trace element and stable isotope analysis for juvenile and adult menhaden collected coast-wide from 2009-2012. To obtain the highest accuracy for assigning adults to natal grounds and to be able to analyze multiple age-classes, as well as establish temporal stability of juvenile signatures, I analyzed juveniles from coast-wide nursery grounds over several years from 2009-2011. This research is summarized in Chapter II. In Chapter III, I assigned new recruits of unknown natal origin to their coast-wide nursery regions and reassessed historical estimates of nursery contribution. In Chapter IV, I evaluated the proportional nursery contribution through older age classes that comprise the commercial fishery and spawning stock biomass. Methods for each approach are discussed in detail within individual chapters. Chapter V summarizes the major findings of this dissertation and its significance as the first research project to evaluate the coast-wide menhaden population using otolith chemistry.

CHAPTER II

COAST-WIDE JUVENILE OTOLITH SIGNATURES OF THE ATLANTIC MENHADEN, BREVOORTIA TYRANNUS, 2009-2011

Introduction

Understanding the population spatial structure of marine fishes is essential when making predictions about a species' resilience and persistence. This is especially important for the Atlantic Menhaden *Brevoortia tyrannus*, hereafter referred to as menhaden. Menhaden are a clupeid that play a critical role in the ecosystem, support the largest fishery in the Chesapeake Bay, and suffer from overfishing (ASMFC 2012). As a filter feeder and consumer of primary production, menhaden contribute to water quality and nutrient cycling. Additionally, menhaden are a key prey species for several commercially and recreationally valued predators including Bluefish *Pomatomus saltatrix*, Weakfish *Cyoscion regalis* and Striped Bass *Morone saxatilis*. The most recent stock assessment by the Atlantic States Marine Fisheries Commission (ASMFC) indicates that along with a decrease in overall numbers and spawning stock biomass, menhaden recruitment levels have remained low since the 1990s (ASMFC 2012). The cause of declining recruitment is currently unknown, although overfishing, habitat degradation, and climate change are considered significant factors (Lozano et al. 2012). Particularly in critical nursery areas such as the Chesapeake Bay, much of the coastline has become heavily developed and vast regions suffer from hypoxia, habitat degradation, and decreased productivity (Kemp et al. 2005). Fully assessing the relationship of

population structure, larval supply and recruitment along the Atlantic coast will aid in evaluating the persistence and resiliency of menhaden and identify areas that are essential for survivorship.

The life history of menhaden is directly influenced by the spatial structure of the population. Adult menhaden undergo extensive seasonal migrations along the Atlantic Coast, distributing from Nova Scotia to Florida. The population segregates by size and age during the summer months, with older (age-3+), larger fish capable of migrating to the northern part of the species' range while younger, smaller fish remain in the southern part of the range (Nicholson 1978). Menhaden are multiple spawners and become sexually mature between ages 1 and 2. Fecundity increases with age, so the oldest, largest females produce the most eggs (Lewis et al. 1987). While spawning occurs from late fall through early spring along the migratory route, the majority of spawning takes place in the winter when the population aggregates off Cape Hatteras, NC (Nicholson 1978; Lewis et al. 1987). After spawning on the coastal shelf, menhaden rely on ocean circulatory patterns to supply larvae to the juvenile nursery grounds in estuaries. Research indicates that oceanic patterns influence the dispersal of larvae to estuaries along the US Atlantic coast so that some spawning locations contribute more to the adult stock than others (Page et al. 1999). These more favorable locations are likely to change on an annual basis depending on oceanic circulatory patterns, varying levels of local productivity and the overall health of the estuaries (Dias 1996). Research regarding coast-wide nursery use by juvenile menhaden and subsequent survival and recruitment is necessary for describing connectivity in the population and for properly managing the stock.

Otoliths have proven to be highly effective tools for studying population spatial structure and connectivity in fishes (Campana 1999; Elsdon et al. 2008). These paired calcium carbonate structures are located in the inner ear of the fish and compositionally reflect the chemical and physical properties of the surrounding water (Fowler et al. 1995; Thorrold et al. 1997b; Thorrold et al. 2001; Dorval et al 2005; Dorval et al 2007). New material is laid down on the otolith as the fish ages, building layers out from a central nucleus. Because otoliths are metabolically inert, the chemical composition remains unchanged once material is deposited, thus providing a spatial and temporal record of where the fish has been during specific stages of its life (Fowler et al. 1995). This record of environmental and migratory history makes the otolith an effective natural tag that can be used to accurately classify many species of fish to nursery grounds (Thorrold et al. 1998; Dorval et al. 2005; Walther et al. 2008). Because different regions can impart unique signatures on otoliths and because ambient water chemistry can vary over time, the inter-annual stability of these natural tags should be considered, especially for dynamic habitats like estuaries where there is variability in temperature, salinity and freshwater inputs (Gillanders 2002). Geochemical signatures in the otolith tend to be stable over short time periods (1 year), but not for longer time periods (4-13 years) (Campana et al. 2000). Therefore, establishing temporal stability of the geochemical fingerprints and building a multi-year library for signatures is necessary when using otolith chemistry for classification purposes over several year-classes.

To construct geochemical fingerprints, both trace element and stable isotope analyses prove to be effective tools for isolating region-specific signatures in fish from various water masses. Elements such as strontium and barium reflect regional differences

in salinities and temperatures whereas lithium, manganese and others have been shown to add meaningful information when establishing regional elemental signatures (Campana 1999; Elsdon et al. 2008). In addition to these trace elements, a significant amount of information regarding environmental variability resides in the carbon and oxygen stable isotope ratios. Oxygen isotopes are deposited on the otolith nearly in equilibrium with the ambient waters and reflect a relationship with temperature and water source, while carbon isotopes are deposited under non-equilibrium conditions and are influenced by environmental factors, diet and metabolic rate (Campana 1999; Smith and Jones 2006). An analysis of trace elements and stable isotopes provides information for identifying chemical signatures of otoliths and distinguishing between geographic regions.

This study evaluates the chemical signatures in juvenile menhaden otoliths collected from the major nursery grounds along the United States Atlantic coast. If juvenile signatures are distinct between nurseries, adult menhaden caught in the fishery could be assigned back to their region of origin, thus quantifying the spatial contribution of each of the major nurseries to the adult stock. This would be of great value to menhaden management, as it is believed that the Chesapeake Bay contributes 69% of the recruits to the fishable stock, but that estimate is over 20-years-old and predates current low recruitment levels (Ahrenholz et al. 1989). Currently the coast-wide juvenile contribution of each of the major estuaries to the adult stock is unknown, and therefore the impact of a changing environment and fishing practices cannot be predicted. To identify areas that are essential for the persistence and resiliency of menhaden, the relationship between population structure, larval supply and recruitment along the Atlantic coast needs to be fully assessed. As a first step toward that goal, the objectives of

this study were to identify sagittal otolith chemical signatures of the major nursery areas used by menhaden, to evaluate these fingerprints over several years to provide information on the inter-annual variability of nursery locations and to make recommendations about how often signatures should be collected in order to correctly classify adults.

Materials and methods

Fish collection

Juvenile menhaden were collected in 2009-2011 from the Thames and Essex Rivers in Connecticut, Hudson River in New York, Delaware Bay in Delaware, Potomac, Patuxent, Choptank and Nanticoke Rivers in Maryland, James River in Virginia, Albemarle Sound in North Carolina and Charleston Harbor in South Carolina. All samples were collected by United States state natural resource agencies from July to October with the goal of obtaining at least 30 samples annually from each area for the 3 years of this study. Because samples were collected from multiple rivers during different times of the season and in different quantities, the multiple collection sites were grouped into four regions: the Northeast, Delaware Bay, Chesapeake Bay and the Southeast (Figure 1). These groups are similar to other studies of this scope based on physical differences in water chemistries of these regions (Thorrold et al. 1998; Schaffler et al. 2009).

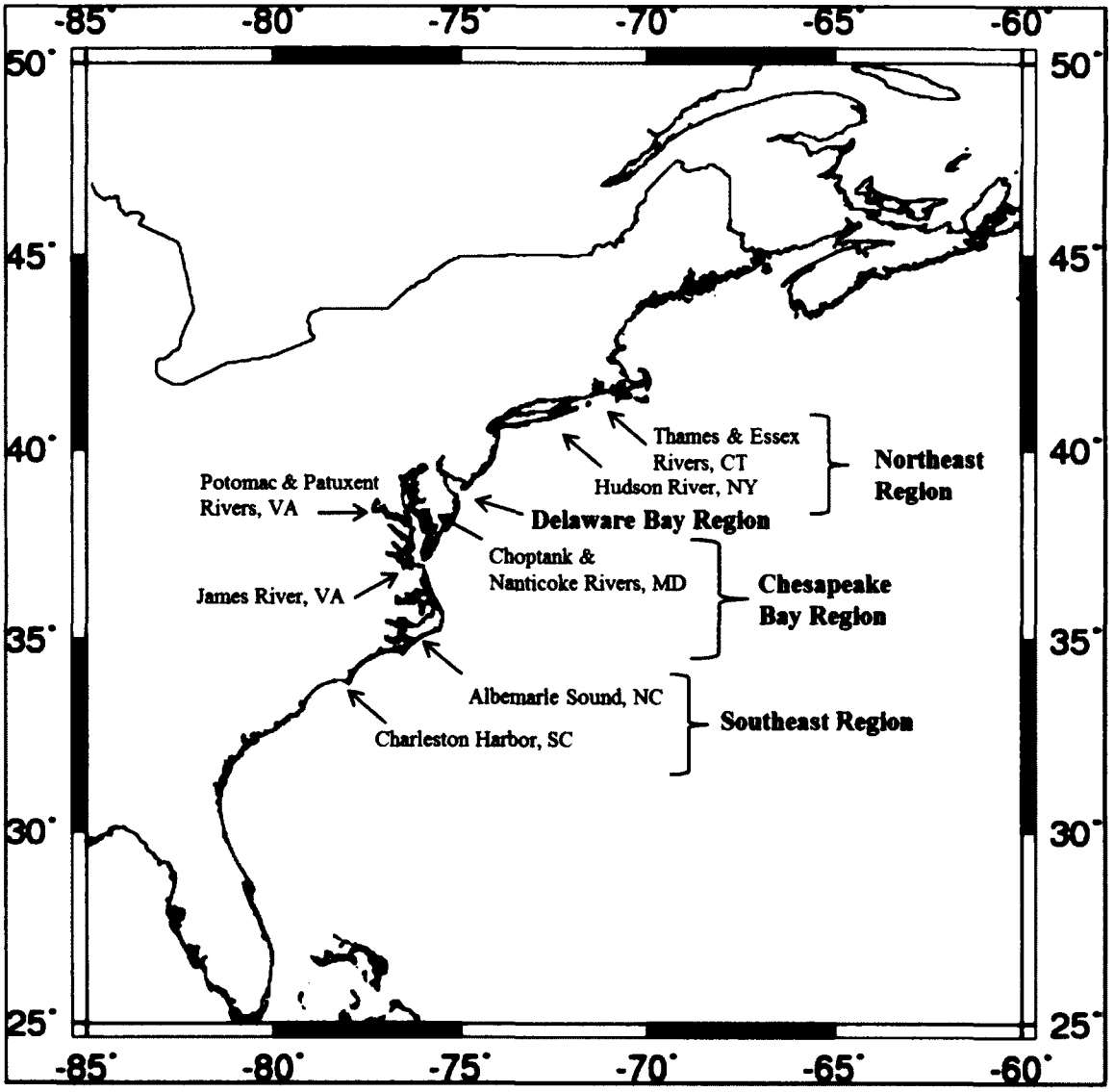


Figure 1. Locations of juvenile Atlantic Menhaden collection on the US East Coast.

Sample preparation and analysis

Juvenile menhaden were frozen after capture and transported to the laboratory. In the laboratory, we measured fork length (mm) and removed sagittal otoliths, ranging in length from 0.62 mm to 2.59 mm, in a class-100 clean room using acid-washed glass probes. Excess tissue was cleaned from the surface by rinsing with ultrapure hydrogen peroxide for 1 minute followed by triple rinsing with ultrapure Milli-Q water. Cleaned samples dried for 24 hours under a laminar flow hood and stored in acid-washed polyethylene vials. One sagittal otolith from each pair was selected randomly for trace element and one for stable isotope analysis.

Otoliths selected for stable isotopes were homogenized with a mortar and pestle and the resulting powder was placed in a clean sample cup. Samples were analyzed for carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) concentrations with a Finnigan Delta Plus with Kiel III Carbonate Device (Thermo Fisher Scientific, Waltham, MA) using standard procedures (Coplen et al. 1983; Coplen 1996; Ostermann and Curry 2000) at the University of Washington Stable Isotope Laboratory. Both oxygen and carbon were measured and corrected relative to Vienna Pee Dee belemnite. The accuracy of these measurements was made by averaging precision of the samples analyzed for each year of this study.

Samples selected for trace element composition were mounted sulcal side up on a glass slide using crystal bond and polished with 30 μm lapping film to expose growth rings followed by 0.3 μm lapping film to produce a smooth surface for laser ablation. Age verification was also made at this point and fish less than 1-year-old were considered to be juveniles. We mounted otoliths in blocks of 20 on a petrographic slide in a randomized order for each year-class. Each petrographic slide was sonicated in Milli-Q

(18 $M\Omega \cdot cm^{-1}$) water for 10 minutes to remove contaminants from the surface and allowed to dry under a laminar flow hood. Otoliths were analyzed using a thermo Finnegan Element 2 (Thermo-Fisher Scientific, Bremen, Germany) inductively-coupled plasma mass spectrometer (ICP-MS) with a New Wave 193 nm excimer laser ablation system (New Wave Research, Sunnyvale, CA) at the Woods Hole Oceanographic Institute's plasma facility. Otolith material was ablated using a laser beam with a 25 μm spot size, 10 μm s^{-1} scan speed, 70% power, and a 10 Hz frequency. We ablated and analyzed a transect from the core to the edge of the otolith that resulted in a trench that was approximately 25 μm wide and 30 μm deep in order to capture the juvenile signature. For each transect we collected counts for ^{7}Li, ^{25}Mg, ^{55}Mn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{137}Ba and ^{208}Pb in low-resolution mode (R=300) (Schaffler and Winkelman 2008). Elemental concentration was calibrated using two reference materials and multi-element standards prepared from ultrapure stock solutions (Yoshinaga et al. 1999; Sturgeon et al. 2005). All elements were normalized to Ca and expressed as element-to-calcium molar ratios (Schaffler and Winkelman 2008). Standards were run twice a slide, at the beginning and the end, to account for machine drift. Blanks were analyzed after every 5 samples and limits of detection (LODs) were calculated as mean blank values plus three standard deviations (Thorrold et al. 1997b) and expressed as a percent of the average sample intensity.

Data analysis

Trace element and stable isotope data were combined to identify natal signatures. Data were normalized using Box-Cox transformations (Box and Cox 1964; Schaffler et

al. 2014a). We assessed normality based on Kolmogorov–Smirnov test and equality of variance using O'Brien's test. Assumptions of multivariate normality were evaluated using tests based on Mardia's multivariate skewness and kurtosis measures (Khattree and Naik 2000; Schaffler et al. 2009) and graphically using Q-Q plots of squared Mahalanobis distances. We performed a multivariate analysis of variance (MANOVA) to detect differences in the multivariate elemental natal signatures in each of the nursery regions and for all cohorts. Pillai's trace statistic quantified significant differences in otolith chemistries between nursery areas and years (Scheiner 2001; Schaffler et al. 2014a). Following these analyses, we used univariate analyses of variance (ANOVA) to determine which elements exhibited differences. When nursery grounds were shown to exhibit statistically significant differences, we used a quadratic discriminant function analysis because of the unequal variance-covariance matrices as indicated by Bartlett's test (Khattree and Naik, 2000; Schaffler et al. 2009; Schaffler et al. 2014a) to assign juvenile menhaden to their nursery area. We tested this classification using a jackknife leave-one-out cross-validation approach (Schaffler and Winkelman 2008; Schaffler et al. 2009; Schaffler et al. 2014a) within years and between years to assess the annual signatures and combined signatures ability to predict other year classes. Additionally, we tested the classification success based solely on either trace element or stable isotope data. Canonical discriminant analysis (CDA) was used to visualize difference among locations (Walther et al. 2008). All statistical tests were performed at a α=0.05 significance level.

Results

We analyzed a total of 312 juvenile menhaden in 2009, 237 in 2010 and 161 in 2011 (Table 1). We obtained at least 30 samples per region for all years with the exception of Delaware Bay in 2009 (n=26). Average fork length was 80.2 mm for all juveniles collected, but varied between region and year due to sample size and the timing of collection. Samples from the Northeast, Delaware Bay and the Southeast regions were typically collected over a short period of time early in the season, were smaller in size and sizes were not significantly different from each other (ANOVA: $F_{2, 403}$=0.06, P = 0.99). The more numerous Chesapeake Bay juvenile samples were collected over a more extended time frame, were larger in size and were significantly different from the other regions (ANOVA: $F_{3, 706}$=40.45, $P < 0.001$). Overall, menhaden collected in 2010 were significantly larger (ANOVA: $F_{2, 707}$=23.87, $P < 0.001$) than those collected in 2009 and 2011. Average fork lengths were not different between 2009 and 2011 (t-test: t=1.47, df=471, P = 0.1415).

More than 86% of all samples were above the LODs for all trace elements with the exception of Pb in 2011 and Mn in 2009-2011 (Table 2). Because of the large number of samples below the LODs, both Pb and Mn were eliminated from further analyses. The precision of the measured stable isotopes of $\delta^{13}C$ and $\delta^{18}O$ was high and the standard deviation was low for the 3 years (Table 2), so carbon and oxygen were used in all analyses. Raw data did not exhibit normal distributions, so Box-Cox transformations were used to normalize the data and homogenize variances. The resulting lambda values of the transformation were highly variable (Table 3), which was expected because of the fluctuating nature of these elements in the environment both spatially and temporally.

Table 1. Juvenile Atlantic Menhaden sample size and mean fork length with standard error for each of the four sampling regions for 2009-2011.

Year	Region	N	Mean Fork Length ± SE (mm)
2009	Northeast	77	68.9 ± 2.2
	Delaware Bay	26	65.2 ± 2.9
	Chesapeake Bay	134	90.7 ± 1.8
	Southeast	75	64.7 ± 2.3
	Total	312	77.4 ± 1.3
2010	Northeast	49	88.6 ± 4.9
	Delaware Bay	32	80.1 ± 3.0
	Chesapeake Bay	113	92.1 ± 1.4
	Southeast	43	82.0 ± 3.2
	Total	237	88.2 ± 1.4
2011	Northeast	40	60.1 ± 2.6
	Delaware Bay	32	70.8 ± 2.8
	Chesapeake Bay	57	84.5 ± 2.5
	Southeast	32	75.5 ± 4.9
	Total	161	74.0 ± 1.7
	Grand Total	710	80.2 ± 0.9

Table 2. Limits of detection (LOD) for the trace element and stable isotope juvenile Atlantic Menhaden otolith data. Detection limits were calculated as mean blank value plus 3 standard deviations of elements analyzed by LA-ICPMS in low-resolution mode. Trace element concentrations were calibrated using Japanese (JPN) and NRC reference standards. For carbon and oxygen isotopes, RSD is the average precision of all samples within each year.

		2009				2010				2011			
Element	Unit	JPN RSD	NRC RSD	LOD	% >LOD	JPN RSD	NRC RSD	LOD	% >LOD	JPN RSD	NRC RSD	LOD	% >LOD
Li	ppb	6.0	7.3	44.4	93	3.9	5.3	67.9	86	3.7	3.9	21.1	95
Mg	ppm	6.5	7.3	6.3	100	2.5	3.0	17.5	100	4.1	3.4	17.1	96
Ca	ppm	7.0	8.1	0.4	100	2.5	3.2	0.8	100	3.2	4.0	1.0	100
Mn	ppb	7.0	7.8	90.1	81	2.2	2.9	94.3	66	3.0	4.3	92.7	75
Rb	ppb	7.0	7.8	21.4	96	2.1	2.6	47.2	95	2.4	3.8	51.8	91
Sr	ppm	7.3	8.2	0.9	100	2.2	2.8	1.0	100	2.9	3.7	1.3	100
Y	ppb	7.1	8.2	27.6	99	2.3	2.7	57.7	98	6.6	7.8	38.7	100
Ba	ppm	7.3	8.1	5.6	100	1.8	2.5	6.6	100	2.4	3.4	7.8	100
Pb	ppb	6.9	7.6	39.2	92	1.4	1.7	49.0	96	3.8	2.4	70.0	71
C	‰	0.0076		--	--	0.0029		--	--	0.0043		--	--
O	‰	0.0095		--	--	0.0055		--	--	0.0072		--	--

Because of this variability, some of the variables still did not meet univariate normality or the assumption of equality of variances even after being transformed. The transformations of Mg achieved normality for the 2009-2011 data sets, but Rb, Sr and C violated the assumption of normality for 1 out of the 3 years, while Li, Y, Ba and O violated normality for 2 of the 3 years of this study. Mardia's test was used to evaluate multivariate skewness ($P < 0.001$) and kurtosis ($P = 0.164$), indicating the data set was skewed and deviated from multivariate normality. Although these data may not be

normal, they were close to normal after transformation and the tests are robust enough to handle this.

Statistical analysis was applied to all stable isotope data and trace elements that were above the LOD. MANOVAs indicated a significant year effect (Pillai's trace = 1.1178, $F_{22,1390}$=80.06, $P < 0.001$) and a significant regional effect (Pillai's trace = 0.9795, $F_{33,2088}$=30.67, P <0.001) for juvenile menhaden collected in 2009-2011. The results of the ANOVAs indicated that for all years, all elements analyzed were significantly different between regions with the exception of Rb in 2009 (ANOVA: $F_{3, 308}$=1.95, P =0.083) and Rb in 2010 (ANOVA: $F_{3, 233}$=1.95, P = 0.122) (Figure 2).

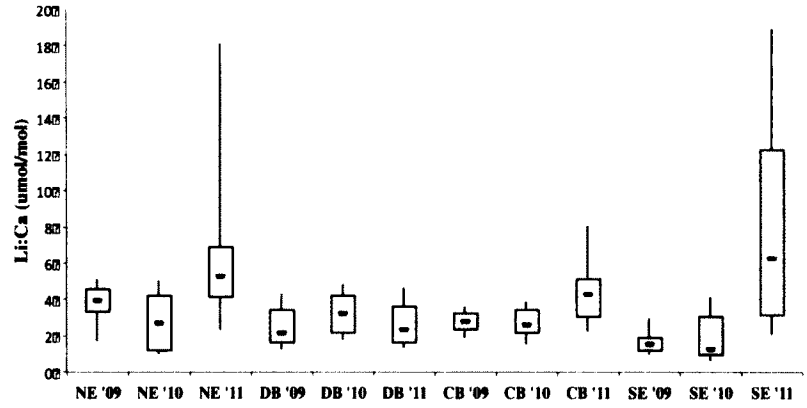
For all three years, a quadratic discriminant function was employed to build the classification function (2009: χ^2_{30}=212.67, $P < 0.001$; 2010: χ^2_{30}=193.39, $P < 0.001$; 2011: χ^2_{84}=350.02, $P < 0.001$). We identified the combination of trace elements and stable isotopes for the geochemical fingerprints with the highest classification rates using a stepwise variable selection procedure. Both carbon and oxygen isotopes contributed to regional separation for all three years, whereas the trace elements used in the discriminant function varied. In 2009, the elements identified as achieving the highest accuracy for classification of the 2009 juveniles were $\delta^{13}C$, $\delta^{18}O$, Li and Ba. In 2010, the elements used to build the multivariate signatures were $\delta^{13}C$, $\delta^{18}O$, Mg and Sr whereas $\delta^{13}C$, $\delta^{18}O$, Mg, Sr, Rb, Li and Ba were used in 2011. Using these respective signatures, juvenile menhaden were correctly assigned to their nursery grounds at a rate of 87% in 2009, 88% in 2010 and 89% in 2011 (Table 4).

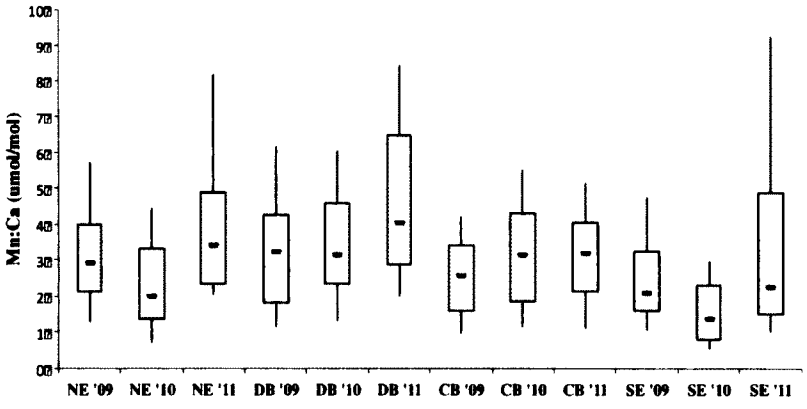
Table 3. Lambda values from Box-Cox transformations. These were used to address assumptions of equality of variance (O'Brien's test) and univariate normality (Kolmogorov–Smirnov test) for assessing otolith chemistry between regions for juvenile Atlantic Menhaden caught from 2009-2011, with a significance level of α=0.05 assumed for all tests.

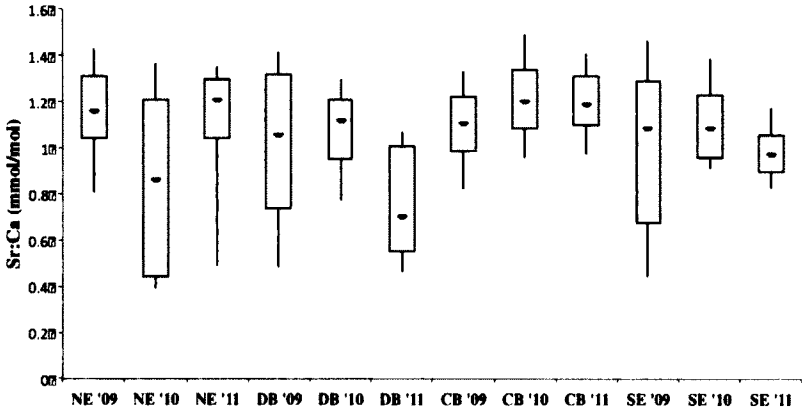
	2009			2010			2011		
Element	lambda	O'Brien's	K-S	lambda	O'Brien's	K-S	lambda	O'Brien's	K-S
Li	0.111	0.060	<0.01	0.096	<0.001	0.03	-0.174	0.078	0.12
Mg	-0.510	0.472	>0.15	-0.388	0.093	>0.15	-0.205	0.141	0.06
Mn	0.076	0.502	>0.15	0.425	0.657	>0.15	0.228	0.061	>0.15
Rb	-0.057	<0.001	0.23	0.046	0.028	0.09	-0.214	0.000	<0.01
Sr	1.709	<0.001	0.54	1.175	0.013	<0.01	1.890	0.028	>0.15
Y	0.424	0.007	<0.01	0.591	<0.001	0.10	1.359	0.415	<0.01
Ba	-0.289	<0.001	<0.01	-0.169	0.028	0.04	-0.428	0.000	>0.15
Pb	-0.985	0.128	>0.15	-0.244	0.016	>0.15	0.071	0.501	>0.15
C	1.217	0.000	<0.01	1.116	<0.001	0.12	1.406	0.000	0.15
O	1.531	<0.001	0.02	0.965	<0.001	0.07	1.091	0.001	<0.01

Table 4. Correct classification and misclassifications of Atlantic Menhaden natal signatures for juveniles caught in the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB) and the Southeast (SE) for 2009-2011. Classifications were based on trace element and stable isotope concentrations, with bold values on the diagonal indicating the number of correctly classified fish and percent (%) represent correct classification.

	2009					2010					2011				
Region	NE	DB	CB	SE	%correct	NE	DB	CB	SE	%correct	NE	DB	CB	SE	%correct
NE	**66**	2	9	0	86	**42**	3	3	1	86	**32**	0	7	0	82
DB	2	**23**	0	1	88	3	**27**	2	0	84	1	**30**	1	0	94
CB	21	5	**104**	4	78	5	6	**96**	6	85	6	0	**50**	1	88
SE	1	0	1	**73**	97	0	0	1	**42**	98	1	0	1	**30**	94
Total				312	87				237	88				160	89


Li:Ca (umol/mol)
NE '09
NE '10
NE '11
DB '09
DB '10
DB '11
CB '09
CB '10
CB '11
SE '09
SE '10
SE '11


Mn:Ca (umol/mol)
NE '09
NE '10
NE '11
DB '09
DB '10
DB '11
CB '09
CB '10
CB '11
SE '09
SE '10
SE '11


Sr:Ca (mmol/mol)
NE '09
NE '10
NE '11
DB '09
DB '10
DB '11
CB '09
CB '10
CB '11
SE '09
SE '10
SE '11

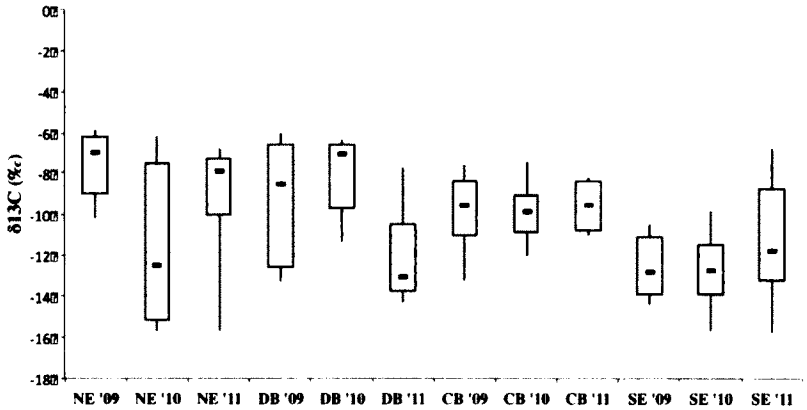

δ13C (‰)
NE '09
NE '10
NE '11
DB '09
DB '10
DB '11
CB '09
CB '10
CB '11
SE '09
SE '10
SE '11

Figure 2. (Continued)

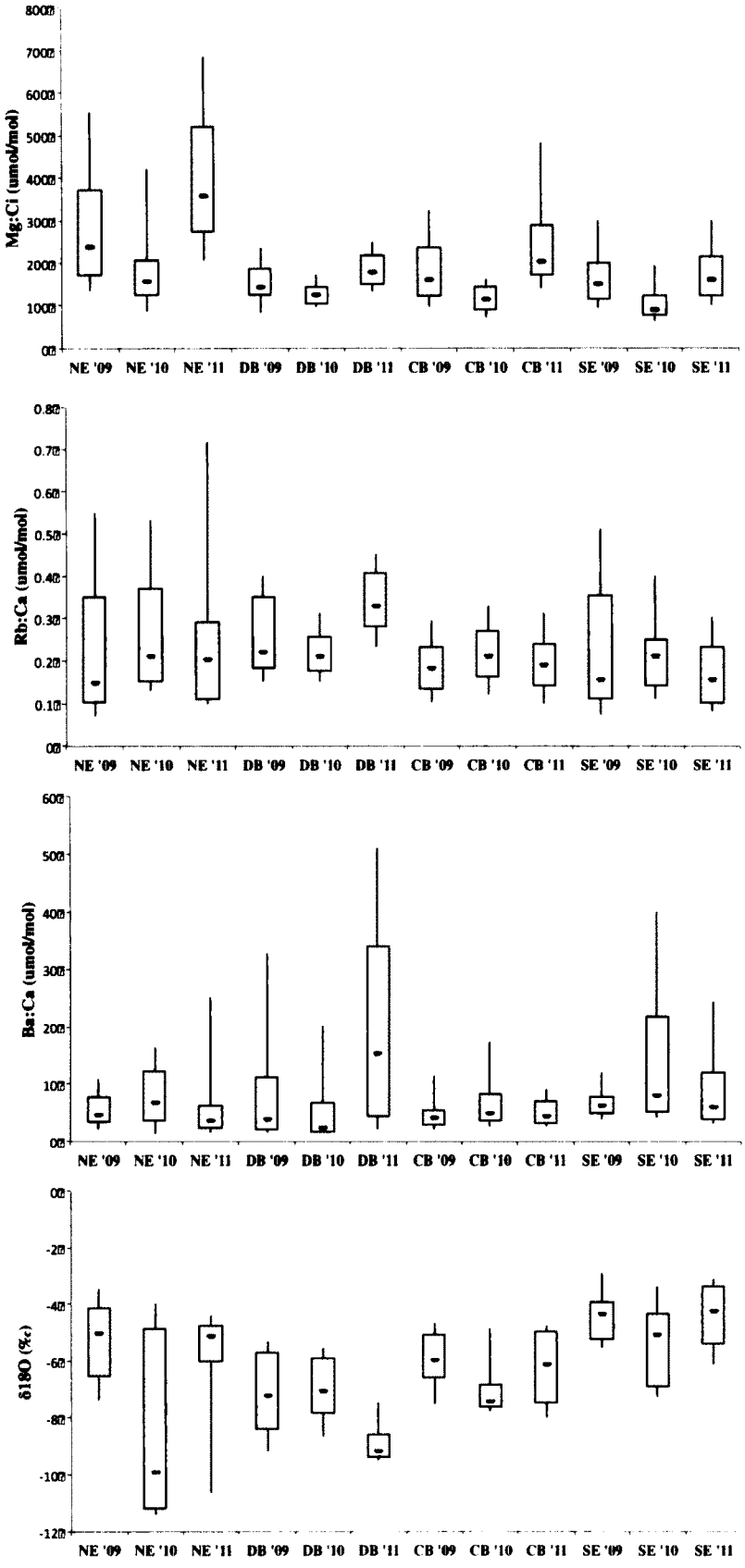


Figure 2. (Continued) Boxplots of juvenile Atlantic Menhaden otolith elemental concentrations. Boxplots represent the untransformed juvenile Atlantic Menhaden otolith elemental concentrations of Li, Mg, Mn, Rb, Sr and Ba expressed as element to Ca ratios and mean carbon and oxygen isotope concentrations. Plots are for the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB) and the Southeast (SE) regions from 2009-2011. Boxes represent the 25th and 75th percentiles, the whiskers represent the 10th and 90th percentiles, and the dash identifies the median.

The accuracy and stability of this model was tested using several approaches. Using stable isotopes alone decreased the accuracy of classification to 77% in 2009, 79% in 2010 and 73% in 2011. Conversely, eliminating stable isotope data and attempting to classify juveniles based only on trace elements decreased the accuracy of classification to 61% in 2009, 64% in 2010 and 67% in 2011. Classification success also decreased to 68% when using geochemical fingerprints from one year to predict the natal location of a different year. Combining all years to create a single classification function resulted in a higher classification of 82%, but it still resulted in a lower classification than the use of the year-class-specific classification functions at approximately 90% (Table 5). Therefore, the year-specific signatures identified by the discriminant analyses are in fact the most accurate way to classify juveniles.

Using the year-specific chemical signatures, the most common misclassifications occurred between Chesapeake Bay and the Northeast region juveniles, particularly in 2009. Both Delaware Bay and the Southeast juveniles were consistently classified correctly through all 3 years. The findings of CDA reinforce these findings. CDA shows separation between the four regions for 2009-2011 (Figure 3). The first two canonical axis of the CDA plot indicate that Delaware Bay and the Southeast regions are particularly well separated but that there is some spatial overlap between the Chesapeake Bay and the Northeast regions, confirming the conclusions from the discriminant analysis that indicated that the most errors occur between these two regions.

Table 5. Correct classification percentages for 2009-2011 juvenile Atlantic Menhaden. Menhaden were classified using functions developed from each year-class individually and all year-classes combined, with bold indicating the highest classification rates for each year.

Classification function	Collection year	Percent Correct (%)
2009	2009	**87**
	2010	58
	2011	62
2010	2009	68
	2010	**88**
	2011	54
2011	2009	59
	2010	63
	2011	**89**
Combined	2009	82
	2010	86
	2011	78

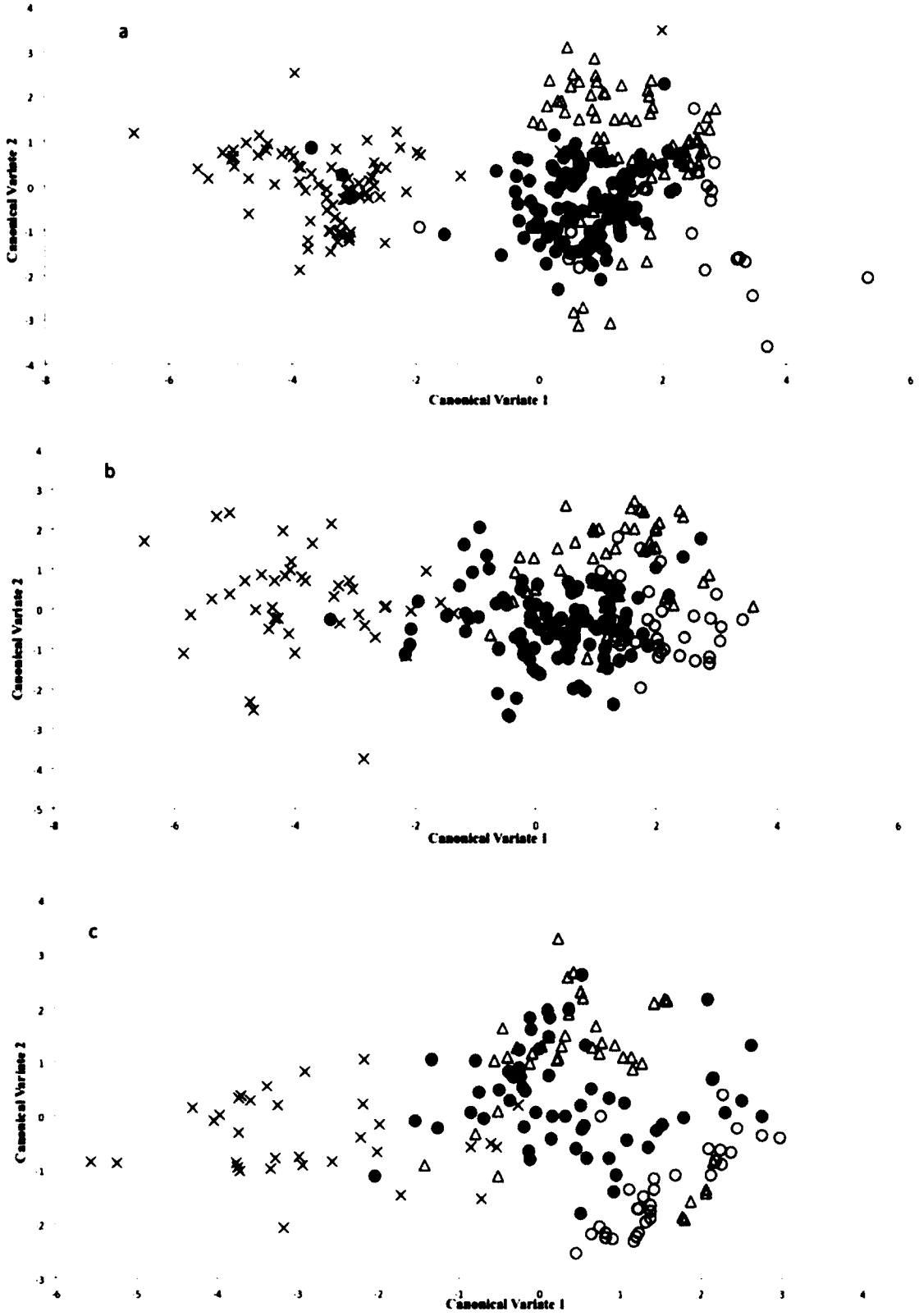

a
Canonical Variate 2
Canonical Variate 1
b
Canonical Variate 2
Canonical Variate 1
c
Canonical Variate 2
Canonical Variate 1

Figure 3. (Continued) Canonical variate plots for juvenile Atlantic Menhaden. Canonical variates 1 and 2 for (a) 2009 (b) 2010 (c) 2011 summarizing variations in trace element and carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotope signatures from juvenile Atlantic Menhaden otoliths. Juvenile menhaden were from the Northeast (Δ), Delaware Bay (o), Chesapeake Bay (•) and the Southeast (x) nursery grounds.

Discussion

Juvenile Atlantic Menhaden from distinct nursery regions can be differentiated based on their otolith chemistry. By combining trace element and stable isotope analysis, we were able to establish statistically distinct regional signatures and to build successful classification systems. For 2009-2011, juvenile menhaden could be classified to Northeast, Delaware Bay, Chesapeake Bay or Southeast nurseries at nearly 90% accuracy. Because of these distinct signatures, adult menhaden collected along the Atlantic coast that correspond to one of these year-classes can be assigned to the region of origin in future studies. This will allow us to assess which nursery area is producing the most recruits to the coastal population and whether this production is consistent annually.

Few studies have attempted to build a multiple-year library of coast-wide signatures for a single species. Thorrold et al. (1998) analyzed the coast-wide elemental signatures of juvenile Weakfish *Cynoscion regalis* with similar regional groupings and classification rates as this study and Schaffler et al. (2009) established distinct otolith signatures in larval Atlantic Croaker *Micropogonias undulates* from Delaware to North Carolina, but neither analyzed multiple year-classes. There have been several studies that have established inter-annual variability of otolith chemistry, but most of these have focused on localized areas or stocks (Campana et al. 2000; Gillanders 2002; Walther and Thorrold 2009; Schaffler et al. 2014a). Walther et al. (2008) evaluated the stable isotope and elemental signatures of American Shad *Alosa sapidissima* from New Hampshire to Georgia over three years to determine natal signatures and estimate the rates of straying between rivers. Using signatures that varied from year-to-year, their analysis allowed

them to correctly classify fish to their natal river approximately 91% of the time. Schaffler et al. (2014a) used otolith chemistry to classify juvenile menhaden to the upper, mid, and lower Chesapeake Bay nursery grounds with 85% and 95% accuracy in 2005 and 2006, respectively. We could not achieve this regional specificity with our data set due to our sample collection methods, but, much like Walther et al. (2008) did for Shad, our research provides a multiple year library of geochemical fingerprints for the Atlantic Menhaden across its range. Additionally, it could be combined with the results of Schaffler et al. (2014a) in future studies for more accurate adult classification.

We found that geochemical fingerprints vary year-to-year and no one group of elements is responsible for discriminating between nursery regions along the Atlantic coast. The four nursery regions studied are vastly different in their size, drainage basins, and responses to environmental influences and while there was some overlap in chemical signatures, these regions were statistically different from each other for all 3 years. The variation in elements is consistent with other studies that have evaluated spatial and temporal otolith composition and most likely reflects changes in ambient water chemistry (Gillanders 2002; Schaffler and Winkelman 2008; Walther and Thorrold 2009). For juvenile menhaden during the 3 years of this study, all trace elements and stable isotopes analyzed, with the exception of Rb in 2009 and 2010, were significantly different between regions and years. Discriminant analysis and stepwise variable selection showed that using all of the trace element and stable isotope data did not provide as high of a classification rate as focusing on a reduced set of variables. Therefore, combinations of C, O, Li, Ba, Sr, Mg and Rb data were important contributors to classifying menhaden for 2009-2011 and the signatures resulted in classification rates of nearly 90% for all 3 years.

The trace elements used in this study to differentiate along latitudinal gradients are consistent with the findings of previous research. Sr and Ba often prove to be valuable elements when classifying juveniles to distinct locations (Gillanders 2002; Wells et al. 2003; Brazner et al. 2004; Munro et al. 2005; Ludson et al. 2006; Schaffler and Winkelman 2008; Walther et al. 2008). The reliability of Sr and Ba in building chemical signatures in these coastal nursery grounds is due to these elements' relationship with both salinity and temperature. Fowler et al. (1995) show a positive correlation between Sr and salinity while Dorval et al. (2005) found that Ba in the otolith decreases with increasing salinity. Temperature also influences the incorporation of Sr into the otolith, although there is a debate about this in the literature (Townsend et al. 1989; Fowler et al. 1995; Dorval et al. 2005). However, because both Ba and Sr vary between habitats and salinities, these elements provide valuable information to establishing fingerprints (Campana 1999). The use of Mg is also useful in otolith chemistry research (Gillanders 2002; Brazner et al. 2004), but the relationship between Mg uptake into the otolith and its concentrations in the seawater is not clear (Thorrold et al. 1997b). Additionally, Li has also been found to fluctuate between years and regions due to its variation between onshore and offshore locations (Campana et al. 2000; Gillanders 2002). Therefore the combination of these specific trace elements for providing meaningful separation between nursery areas was expected. And while these elements contributed to chemical signatures, it is worth noting that using trace elements without stable isotopes resulted in a decrease of classifications rates from 88% to 64% on average from 2009-2011.

Stable isotopes of oxygen and carbon were identified by the discriminant function as contributing significantly to geochemical fingerprints for all 3 years of this study.

Similar to the findings of Walther et al. (2008), we found that oxygen isotopes were significantly different between regions and appear more depleted in northern nurseries and more enriched in the southern nurseries, indicating a latitudinal gradient. Due to the metabolic and environmental influences on the incorporation of carbon isotopes into the otolith (Thorrold et al. 1997a; Smith and Jones 2006), the results of the carbon analysis are more difficult than oxygen to interpret but still appear to contribute to regional separation. For juvenile menhaden during the 3 years of this study, both oxygen and carbon isotopes were valuable when correctly classifying juvenile menhaden, but classification accuracy was lowered from 88% to 76% on average when the stable isotopes were considered without trace elements. Using stable isotopes alone resulted in Chesapeake Bay juveniles being misclassified to the Northeast region and the Northeast juveniles misclassified to the Delaware Bay region at higher rates than when using stable isotopes and trace elements together. Therefore, trace elements are useful along with stable isotopes for increasing classification rates and distinguishing between natal grounds in juvenile menhaden, particularly for the Northeast and Chesapeake Bay.

Due to the variability in the chemical signatures from year-to-year, borrowing signatures from adjacent year classes decreases accuracy significantly. We showed very low accuracy in classification when using, for example, the 2009 natal signatures to classify 2010 or 2011 juveniles. This decrease of classification from 88% to 60% on average was consistent throughout this study, indicating sufficient temporal variability to obscure classification. Additionally, combining elemental data from all 3 years also resulted in a decline in accuracy from using single year-classes from 88% to 82% on average. This inter-annual variability was expected, as otolith chemistry reflects changing

environmental conditions and variations in temperature, precipitation, storm events and land use. Previous studies have documented the temporal variability of elemental signatures, particularly for estuarine or marine species (Gillanders 2002; Schaffler and Winkelman 2008; Walther and Thorrold 2009; Schaffler et al. 2014a). There is some support for pooling years together for juveniles when attempting to classify individuals (Walther and Thorrold 2009; Schaffler et al. 2014a) and this study showed an approximately 6% decrease in classification using a combined signature for juvenile menhaden. Therefore, a combined signature provides little decrease in accuracy and could prove to be useful particularly in data poor situations. Regardless, geochemical fingerprints specific to a year-class are still the most accurate approach when attempting to classify the nursery origin of an adult menhaden with the highest accuracy.

This is the first coast-wide quantitative study that exhibits the use of otolith elemental analysis to discriminate between nursery regions for menhaden and provides a 3-year library for juvenile geochemical fingerprints (Appendix Table 14). This research builds the foundation for a comprehensive estimate on recruitment rates from each of the major nursery areas, as well as the identification of essential areas for the menhaden population. This information is of vital importance to the effective management of this fishery that has suffered from low recruitment, low spawning stock biomass and overall numbers in recent decades.

CHAPTER III

COAST-WIDE NURSERY CONTRIBUTION OF NEW RECRUITS TO THE ADULT POPULATION OF ATLANTIC MENHADEN, BREVOORTIA TYRANNUS

Introduction

The Atlantic menhaden *Brevoortia tyrannus*, hereafter menhaden, is a commercially and environmentally critical prey species along the US East coast. Menhaden are fished as part of a bait and oil reduction fishery and comprise the largest amount of landings by volume in the region (ASMFC 2012). Apart from the millions of dollars that the fishery generates, menhaden play a vital role as the primary source of food for many other commercial and recreational species such as Striped Bass *Morone saxatilis*, Bluefish *Pomatomus saltatrix*, and Weakfish *Cycoscion regalis* (Uphoff 2003; ASMFC 2012). In addition to their role as a prey species for other fish and seabirds, they also are essential to the food web as filter feeders and consumers of phytoplankton and zooplankton. Menhaden are managed by the Atlantic States Marine Fisheries Commission (ASMFC) and are not considered overfished, although overfishing has occurred during recent years (ASMFC 2012). One of the major concerns that plague the menhaden population is the low recruitment levels since the 1990s (ASMFC 2010, 2012). Life history and migration patterns of menhaden are well known, but understanding the differential contribution of nursery grounds for producing new recruits is essential for evaluating this critical population's persistence and success for future years.

Menhaden are estuarine-dependent, coastal-spawning fish that use multiple nursery grounds along the US East Coast before recruiting to the adult population. The life history of menhaden has evolved to be timed with physical conditions in the ocean that are optimal for the transport of its larvae to nursery grounds along the coast. In the summer, adult menhaden are found in the open coastal waters and bays from Maine to Florida. They are segregated by size and age, with the largest, oldest, and possibly more fecund menhaden in the northern part of the range and smaller, younger adults in the southern part of the range (Lewis et al. 1987). Menhaden are mature between ages 1 and 2 years and can spawn nearly year-round in temperatures above 15°C. The larger, older, and highly fecund adults may spawn along the migratory route but the majority of spawning occurs in the fall when adults congregate off Cape Hatteras, North Carolina, where the entire population spawns from October through March (Nicholson 1971; Lewis et al. 1987). Larvae enter various estuaries along the coast, although the amount of larvae received and the success of these nursery grounds can vary. Interannual variation can be caused by many factors such as the number of eggs produced, the amount of larvae accumulated in nearshore or coastal zones, wind-driven Ekman transport, proximity to upwelling regions, temperature variation, winter storms, and the accessibility of the estuarine mouths (Nelson et al. 1977; Miller et al. 1984; Checkley et al. 1988; Quinlan et al. 1999; Werner et al. 1999). Therefore, some nursery locations receive more larvae or contribute more recruits to the adult population than others based on the physical characteristics of the ocean and the health of the estuaries (Dias 1996; Page et al. 1999). An evaluation of nursery contribution to any adult fish stock can provide insights to the resilience of a species, particularly in a changing climate.

There has been limited research on the contribution and productivity of nursery grounds for menhaden despite the continued need for this information to address the decline in recruitment in recent decades. The Chesapeake Bay has long been considered to be the main contributor of new recruits to the adult population but this estimate is over 20 years old and was based on juvenile abundance surveys and regional weightings that were derived from estuarine drainage areas and menhaden productivities of steams along the Atlantic coast (Ahrenholz et al. 1989; ASMFC 2004a). The estimates used regional groupings of nursery grounds along the coast and assigned the following estimates of nursery contribution: New England (Maine to Connecticut) – 2%, Middle Atlantic (New York to Coastal Maryland) – 12%, Chesapeake Bay (including coastal Virginia) – 69%, and South Atlantic (North Carolina to Florida) – 17%. These values have never been verified nor has any further quantitative work been done to see how these proportions may have shifted in the last 25 years. Since the work of Ahrenholz et al. (1989), large portions of the Atlantic coastline have become more developed, particularly in the Chesapeake Bay - an area that has suffered from eutrophication, habitat destruction, and decreased productivity (Kemp et al. 2005). It is critical to understand how environmental variability and anthropogenic factors may alter recruitment for menhaden and an evaluation of nursery contribution to new recruits could offer insight to answer this question.

This study uses otolith chemistry to evaluate the coast-wide nursery contribution of age-1 menhaden collected from the commercial fishery from 2010-2012. Previous research has demonstrated the utility of otoliths for evaluating spatial and temporal variability and connectivity in fish populations (Thorrold et al. 1998; Campana 1999;

Gillanders 2002) as well as their use classifying adults of unknown origin to their nursery grounds (Brown 2006; Hobbs et al. 2007; Walther et al. 2008; Schaffler et al. 2014b). Two recent studies for the Atlantic Menhaden used otolith chemistry to accurately classify juveniles to their nursery grounds in upper, mid, and lower Chesapeake Bay (Schaffler et al. 2014a) and then, using similar techniques, to coast-wide nursery grounds (Anstead et al. 2014, Chapter II). Using the chemical signatures of the latter paper, this study will build on that research and assign three cohorts of age-1 menhaden collected from the fishery and of unknown natal origin to the nursery regions of the Northeast, Delaware Bay, Chesapeake Bay, and the Southeast, mirroring those of the Ahrenholz et al. (1989) and ASMFC (2004a) studies. This approach will evaluate whether the Chesapeake Bay is still the primary source of new recruits to the menhaden population and provide quantitative estimates of nursery contribution coast-wide. The methods used here are different from Ahrenholz et al. (1989), so while comparisons between nursery contribution estimates are not direct, they each provide valuable information regarding recruitment challenges for stock assessment and fisheries scientists.

Materials and methods

Fish collection

Juvenile menhaden were collected in 2009-2011 from multiple sites along the US Atlantic coast, prepared, and analyzed as described in Anstead et al. (2014, Chapter II). Adult menhaden were collected in 2010-2012 from the coastal waters from Cape Cod to the Hudson River, Delaware Bay, Chesapeake Bay, Pamlico Sound, and Charleston

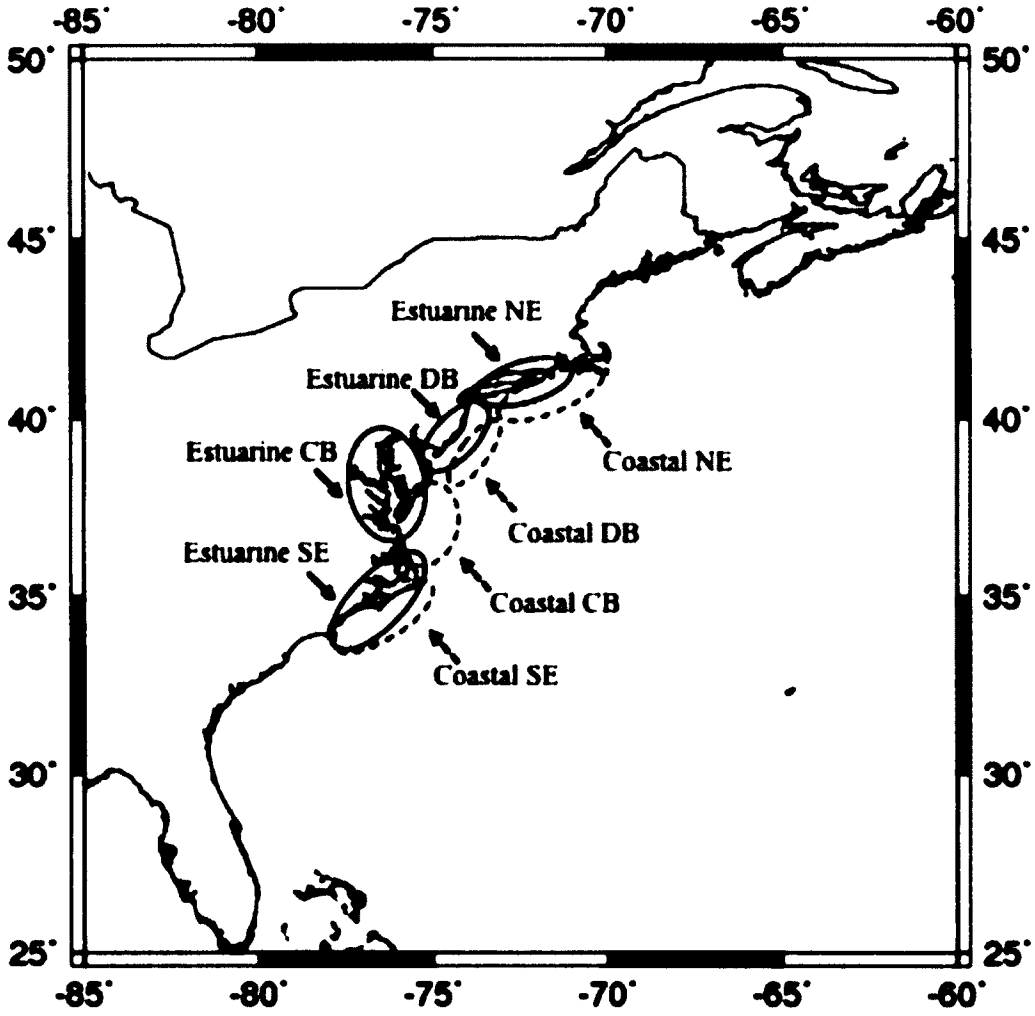


Figure 4. Approximate locations of juvenile and adult Atlantic Menhaden sample collections along the US East Coast. Juveniles were collected from estuarine nursery grounds and grouped into four regions: the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB), and Southeast (SE) as indicated by bold circles. Adult collections were made in the coastal waters of the same regions, as indicated by dashed circles.

Sound (Figure 4). Our goal was to obtain 100 age-1 samples per area, although we anticipate that this will be challenging since age-1s are rarely found in abundance north of Delaware Bay (Nicholson 1971; Kroger and Guthrie 1973) and because we are reliant on the commercial purse seine and pound net fisheries for our sampling. All adult menhaden samples were collected by state agencies from August to October during the three years of the study.

Sample preparation and analysis

Samples were frozen after capture and transported to the laboratory where fork length (mm) was recorded and sagittal otoliths were removed in a class-100 clean room using acid-washed glass probes. Excess tissue was cleaned from the surface by rinsing with ultrapure hydrogen peroxide for 1 minute followed by triple rinsing with ultrapure Milli-Q water. Cleaned samples dried for 24 hours under a laminar flow hood and stored in acid-washed polyethylene vials. One sagittal otolith from each pair was selected randomly for trace element and the other was used for stable isotope analysis.

One-year-old otoliths for both analyses were mounted on a glass slide using crystal bond and polished on both sides using 30 μm lapping film followed by 0.3 μm lapping film. This procedure removed the age-1 material leaving only the juvenile core for analysis as well as exposed growth rings for age verification purposes. For stable isotope analysis, the core material corresponding to the juvenile year was removed from the otolith and ground into powder using a handheld dremel tool and placed in acid washed vials for analysis. Samples were analyzed for carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) concentrations with a Finnigan Delta Plus with Kiel III Carbonate Device (Thermo Fisher

Scientific, Waltham, MA) using standard procedures (Coplen et al. 1983; Coplen 1996; Ostermann and Curry 2000) at the University of Washington Stable Isotope Laboratory. Both oxygen and carbon were measured and corrected relative to Vienna Pee Dee Belemnite (VPDB) and accuracy was determined by averaging precision of the samples within each year.

Polished otolith sections for trace element analysis were transferred to petrographic slides in blocks of 20 in a randomized order within collection years. Each petrographic slide was sonicated in Milli-Q (18 $M\Omega \bullet cm^{-1}$) water for 10 minutes to remove contaminants from the surface and allowed to dry under a laminar flow hood. Otoliths were analyzed using a thermo Finnegan Element 2 (Thermo-Fisher Scientific, Bremen, Germany) inductively-coupled plasma mass spectrometer (ICP-MS) with a New Wave 193 nm excimer laser ablation system (New Wave Research, Sunnyvale, CA) at the Woods Hole Oceanographic Institute's plasma facility. The juvenile core of the otoliths was ablated using a laser beam with a 25 μm spot size, 10 $\mu m\ s^{-1}$ scan speed, 70% power, and a 10 Hz frequency. We ablated and analyzed a transect from the core to the first annulus that resulted in a trench that was approximately 25 μm wide and 30 μm deep in order to capture the juvenile signature. For each transect we collected counts for ^{7}Li, ^{25}Mg, ^{55}Mn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{137}Ba, and ^{208}Pb in low-resolution mode (R=300) (Schaffler and Winkelman 2008; Anstead et al. 2014, Chapter II). Elemental concentration was calibrated using two reference materials and multi-element standards prepared from ultrapure stock solutions (Yoshinaga et al. 1999; Sturgeon et al. 2005). All elements were normalized to Ca and expressed as element-to-calcium molar ratios (Schaffler and Winkelman 2008). Standards were run twice a slide, at the beginning and

the end, to account for machine drift. Blanks were analyzed after every 5 samples and limits of detection (LOD) were calculated as mean blank values plus three standard deviations (Thorrold et al. 1997) and expressed as a percent of the average sample intensity. Age-1 slides were run a year after the juvenile slides were run, so a previously-run juvenile slide was rerun every third or fourth adult slide to correct for machine drift (Thorrold et al. 2001; Vasconcelos et al. 2008). Correction factors were calculated by dividing the original juvenile slide trace element data by rerun slide trace element data and averaging these values over the entire slide for each element. Correction factors were then applied to the age-1 data so that juvenile and age-1 data were comparable. To confirm that only juvenile core data was analyzed, slides were examined after analysis to ensure no age-1 data was captured and data was truncated when necessary.

Data analysis

Juvenile trace element and stable isotope data were combined previously to identify geochemical signatures unique to each of the four nursery grounds coast-wide (Anstead et al. 2014, Chapter II). Juvenile data were not normally distributed, so Box-Cox transformations (Box and Cox 1964; Anstead et al. 2014, Chapter II; Schaffler et al. 2014a) were used and the resulting lambda values were applied to the adult data for each element in each collection year. The quadratic discriminant analysis (QDA) function from the juvenile data set was then applied to the age-1 data set to predict the nursery origin of the new recruits and to estimate proportional contribution from each of the nursery areas over the three years of this study (Schaffler et al. 2014b). Additionally, we used canonical discriminant analysis (CDA) to confirm the presence of four clusters in

the adult data representing the four assumed nursery regions and to visualize the adults in relation to the juveniles using the average linkage method in Ward's minimum variance cluster procedure (Schaffler et al. 2014b). Pseudo t^2 statistic, pseudo F statistic, and the cubic clustering criterion (CCC) were used to determine the number of clusters present in the age-1 data (Milligan and Cooper 1985; Shima and Swearer 2009). All statistical tests were performed at a α=0.05 significance level.

Results

We analyzed a total of 157 samples from age-1 menhaden in 2010, 139 in 2011, and 85 in 2012 from multiple collection sites (Figure 4). As anticipated, we were not able to collect 100 age-1 samples from each of the regions due to sampling restrictions and menhaden's life history strategy where most age-1s are found in the southern part of the range and rarely north of the Chesapeake Bay. We received at least 100 adult samples from each of the regions, but after age verification we did not obtain the desired age-1 sample sizes. Although we did not anticipate collection region to have an effect, we did test for a state effect between collection regions since the cluster samplings estimates based on regional groupings as proposed by Ahrenholz were not the same. The weights were selected as the estimates based on regional groupings as proposed by Ahrenholz. We found no significant state effect for menhaden collected in 2010 (Pillai's trace=0.094, $F_{8, 304}$=1.86, P=0.065) or 2011 (Pillai's trace=0.016, $F_{4, 134}$=0.54, P=0.706). We did find a significant state effect for age-1 menhaden collected in 2012 (Pillai's trace=0.267, $F_{7, 77}$=3.9, P=0.001), but when we used the weights in the QDA, classification probabilities using proportional priors were not significantly different than assuming equal priors (*t*-

test: t<0.001, df=339, P=0.999). Therefore, there was no overall state effect detected based on collection region.

Average fork length for all age-1 menhaden was 182 mm, although it varied between region and year (Table 6). Age-1 menhaden from Chesapeake Bay were significantly larger than those from the Northeast and Southeast (ANOVA: $F_{2, 378}$ = 183.38, $P < 0.001$). Menhaden from the Northeast and Southeast were not significantly different from each other in length (t-test: t=1.438, df=72, P = 0.155), although this is likely due to the low sample sizes from these regions. Menhaden collected in 2012 were significantly larger (ANOVA: $F_{2, 378} = 24.57$, $P < 0.001$) than those collected in 2010 and 2011 which were not significantly different in length from each other (t-test: t=1.433, df= 294, $P = 0.153$).

The majority of samples were above the LODs (> 88%) for all trace elements in the analysis, with the exception of Pb in 2011 and 2012 and Mn for all three years (Table 7). The LODs for these two elements were also below detection limits for the juvenile analysis, so Pb and Mn had already been removed from consideration for the classification function and therefore it will not affect the use of the juvenile signatures for adult classification. The precision of stable isotopes was high for the three years, therefore carbon and oxygen isotopes were used for all analyses.

CDA shows age-1 data overlapping the juvenile data primarily in the middle of the four juvenile clusters (Figure 5). As age-1 data does not exhibit any obvious clustering in any of the three years, deciding on the number of clusters in this data set provides somewhat of a challenge as clustering procedures do not provide conclusive

Table 6. Sample size and mean fork length of new recruits to the Atlantic Menhaden population with standard error. In total, 381 samples were collected for the three years.

Collection Year	Region	N	Mean Fork Length ± SE (mm)
2010	Northeast	3	139.7 ± 5.5
	Chesapeake Bay	109	192.1 ± 0.7
	Southeast	45	151.6 ± 1.6
2011	Chesapeake Bay	124	180.5 ± 1.6
	Southeast	15	139.4 ± 2.1
2012	Chesapeake Bay	74	200.1 ± 1.8
	Southeast	11	163.9 ± 5.1

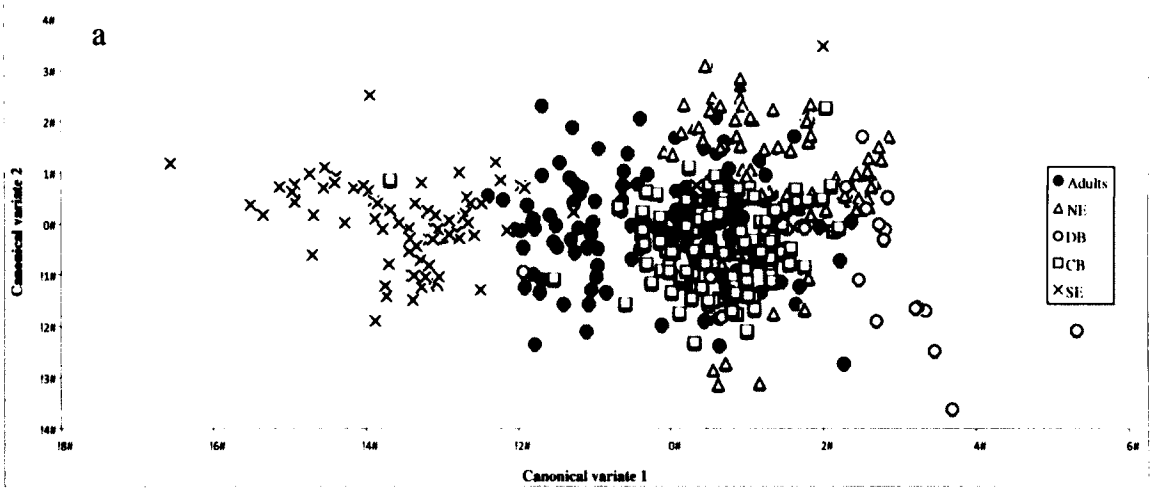
Table 7. Limits of detection (LOD) for otolith trace element and stable isotope data from new recruits to the Atlantic Menhaden population. Detection limits were calculated as mean blank value plus 3 standard deviations of elements analyzed by LA-ICPMS in low-resolution mode for age-1 Atlantic Menhaden in 2010-2012. Trace element concentrations were calibrated using Japanese (JPN) and NRC reference standards. For carbon and oxygen isotopes, RSD was calculated as the average precision of all samples within each year.

		2010				2011				2012			
		JPN	NRC		%>	JPN	NRC		%>	JPN	NRC		%>
Element	**Unit**	**RSD**	**RSD**	**LOD**	**LOD**	**RSD**	**RSD**	**LOD**	**LOD**	**RSD**	**RSD**	**LOD**	**LOD**
Li	ppb	3.2	4.9	28.5	95	2.7	3.5	28.6	100	2.9	3.8	44.7	99
Mg	ppm	2.6	3.4	14.1	100	2.7	3.1	25.4	98	3.7	4.2	14.2	100
Ca	ppm	2.8	3.9	0.6	100	3.0	3.6	1.0	100	3.7	4.3	1.0	100
Mn	ppb	3.2	4.1	95.2	73	2.7	3.4	93.8	78	3.7	3.9	92.2	85
Rb	ppb	2.5	3.6	49.8	100	2.7	3.7	74.3	88	3.5	3.9	34.6	100
Sr	ppm	2.4	3.7	0.9	100	2.8	3.4	1.2	100	3.5	4.1	1.7	100
Y	ppb	3.9	5.2	45.2	100	3.5	4.2	74.7	99	3.8	4.5	58.8	100
Ba	ppm	2.1	3.3	8.3	100	2.5	3.3	8.8	100	3.4	3.8	10.9	100
Pb	ppb	1.8	2.7	46.4	93	1.8	2.5	67.7	83	4.9	5.0	88.8	60
C	‰	0.0058		--	--	0.0075		--	--	0.0098		--	--
O	‰	0.0123		--	--	0.0112		--	--	0.0214		--	--

information about the number of clusters present (Milligan and Cooper 1985). Using the metrics of pseudo t^2 statistic, pseudo F statistic, and the CCC, there are two, three, or four clusters in the age-1 data depending on the year and the metric used. The pseudo t^2 and the pseudo F statistic both indicate four clusters in the age-1 data, while CCC indicates two or three depending on the year (Table 8).

The pseudo t^2 statistic was the smallest and the pseudo F statistic was the highest for four clusters, indicating four clusters in all three years of the age-1 data set. This clustering is also consistent with previous studies (Ahrenholz et al. 1989; ASMFC 2004a; Anstead et al. 2014, Chapter II) and allows for the most accurate comparisons among data sets. Age-1 menhaden were assigned to their nursery region with the QDA based on the juvenile data from the same cohort using the four nursery regions. For age-1 menhaden of unknown natal origin collected in 2010 that correspond to the 2009 cohort, 50 (32%) were from the Northeast, 13 (8%) were from Delaware Bay, 51 (33%) were from Chesapeake Bay, and 43 (27%) were from the Southeast nursery grounds (Table 4). For the 2010 cohort, 36 (26%) were classified as originating from nurseries in the Northeast, 39 (28%) from Delaware Bay, 50 (36%) from Chesapeake Bay, and 14 (10%) from the Southeast. For the 2011 cohort, 29 (34%) were classified as originating from nurseries in the Northeast, 3 (4%) from Delaware Bay, 21 (25%) from Chesapeake Bay, and 32 (37%) from the Southeast.

Previous research indicates that new recruits are unlikely to originate from the northern estuaries (Ahrenholz et al. 1989; ASMFC 2004a) and therefore three nursery areas may be more justifiable for applying the QDA. For all three years of the study, the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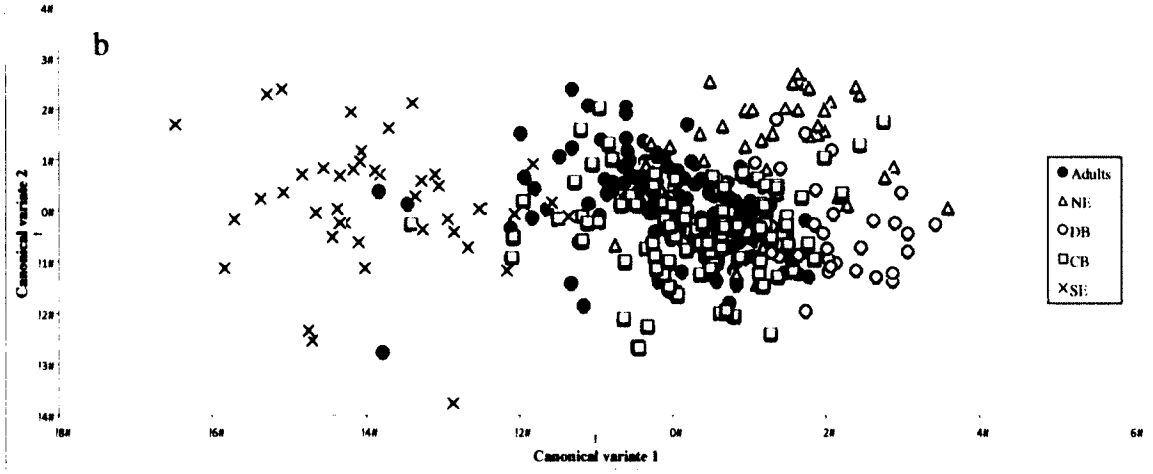
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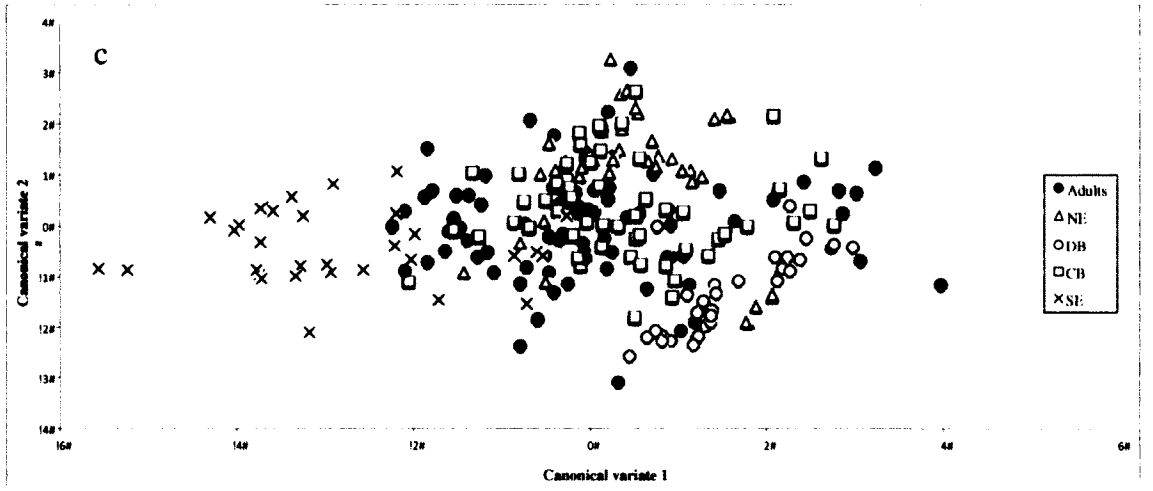
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Figure 5. (Continued) Canonical variate plots for age-1 Atlantic Menhaden. Canonical variates 1 and 2 summarizing differences in trace element and carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes for age-1 Atlantic Menhaden. The signatures from juvenile otoliths from the Northeast (Δ), Delaware Bay (o), Chesapeake Bay (□) and the Southeast ( ) nursery grounds and age-1 otoliths (•) of unknown natal origin for the cohorts from (a) 2009, (b) 2010, and (c) 2011.

Table 8. Values of the cubic clustering criterion (CCC), pseudo F, and pseudo t^2 statistics that were used to determine the number of clusters in the age-1 Atlantic Menhaden data from 2009-2011 cohorts. Bold indicates the amount of clusters in each data set based on statistic used.

Cohort	# of Clusters	CCC	*F*	t^2
2009	2	-6	37.1	42.2
	3	**-6.7**	44.3	61.2
	4	-5.5	**50.6**	**17.6**
2010	2	-4.4	196	56.5
	3	**-6.2**	128	214
	4	-6	**287**	**42.8**
2011	2	**-1.2**	174	66.8
	3	-0.48	189	121
	4	0.72	**341**	**12.8**

Table 9. Coast-wide nursery contribution of new recruits to the Atlantic Menhaden population using three successive cohorts and two different clustering strategies. The classification of new recruits from unknown origins to their nursery ground was based on juvenile otolith chemical signatures from 2009-2010 with associated probabilities from the quadratic discriminant function.

		Classification				Probability			
Cohort	# Clusters	NE	DB	CB	SE	NE	DB	CB	SE
2009	4	50 (32%)	13 (8%)	51 (33%)	43 (27%)	76%	80%	83%	95%
2010	4	36 (26%)	39 (28%)	50 (36%)	14 (10%)	76%	76%	73%	88%
2011	4	29 (34%)	3 (4%)	21 (25%)	32 (37%)	86%	81%	85%	88%
2009	3	0	23 (15%)	88 (56%)	46 (29%)	-	87%	92%	97%
2010	3	0	38 (27%)	82 (59%)	19 (14%)	-	80%	89%	86%
2011	2	0	0	49 (58%)	36 (42%)	-	-	94%	92%

CCC indicates two or three clusters, although the values are not that different from those for four clusters (Table 8). For the 2009 and 2010 juvenile cohorts, the CCC indicates three clusters. For the 2011 cohort, the CCC indicates two clusters although all CCC values are between 0 and 2 indicating that clusters should be interpreted cautiously (Sarle 1983). Decreasing the number of clusters in the age-1 data set based on the CCC leads to a different classification result using QDA. Assuming three clusters and no Northeast nursery origin, age-1 menhaden that correspond to the 2009 juvenile cohort had 23 (15%) originating from Delaware Bay, 88 (56%) from Chesapeake Bay and 46 (29%) from the Southeast (Table 9). Assuming three clusters and no Northeast nursery origin menhaden in the 2010 juvenile cohort results in 38 (27%) age-1 menhaden originating from Delaware Bay, 83 (59%) from Chesapeake Bay, and 19 (14%) from the Southeast. Assuming two clusters and no age-1 menhaden originating from the Northeast or Delaware Bay nurseries, 49 (58%) of menhaden were classified to the Chesapeake Bay and 36 (42%) were classified to the Southeast nurseries.

Discussion

The proportion of juvenile menhaden that recruit to the adult stock from the major nursery grounds along the US Atlantic coast has previously been unknown, although the Chesapeake Bay was believed to be the major contributor. This study used otolith chemistry to evaluate the contribution of new recruits from the major nursery regions coast-wide and the possible shift from historical estimates due to the effects of fishing pressure, a changing climate, habitat degradation, or anthropogenic impacts. We showed

that there has been a change in nursery contribution over the last 25 years since estimates were made using state surveys of juvenile indices, estuarine drainage areas, and regional productivities (Ahrenholz et al. 1989; ASMFC 2004a). Using the four nursery regions based on previous estimates and juvenile menhaden otolith data (Ahrenholz et al. 1989; ASMFC 2004a; Anstead et al. 2014, Chapter II), we found that the Northeast estuaries and Chesapeake Bay each contribute 31% of the age-1 menhaden, with Delaware Bay contributing 13% and the Southeast contributing 25% on average for the three years of this study. While the methods used in these estimates and the historic estimates of nursery recruitment are two distinct methodologies for obtaining nursery contribution, the continued use of historic estimates in the stock assessment demands that the values are reevaluated quantitatively.

Use of cluster analysis

The application of cluster analysis on the set of age-1 menhaden of unknown origin provides insight to the population's connectivity and nursery production to the adult stock. One of the limitations of cluster analysis is the lack of statistical significance testing and a variety of interpretable results (Hartigan 1975, 1985). We used three different statistics to determine the number of clusters in the age-1 data: the CCC, pseudo F, and pseudo t^2. These three statistics were shown to perform the best when estimating the number of clusters in a data set for hierarchical models (Milligan and Cooper 1985). The CCC is based on minimizing the within-cluster sum of squares and the assumption that clusters come from a uniform distribution. Inflection points in the data indicate the

number of clusters and these varied between the values of 2 and 3 in the age-1 data, although peaks between 0 and 2 should be interpreted with caution (Sarle 1983). The CCC can be used in combination with the pseudo F and t^2 statistics to obtain additional information about the amount of clusters in the data. The pseudo F statistic uses a ratio of the within-cluster variance and attempts to capture the tightness of the groupings (Calinski and Harabasz 1974), whereas the t^2 indicates whether the mean vectors between clusters lead to the most accurate combination of clusters (Duda and Hart 1973). Large pseudo F values indicate well-separated clusters whereas small t^2 values indicate more distinct clustering. The reported statistics are guidelines for interpreting the results of the cluster analysis and of the three statistics used in this study, there are two interpretable results.

Nursery contribution of new recruits

The psuedo F and t^2 statistics from cluster analysis indicated four clusters in the new recruit data. The use of four regions is also supported by previous research documenting coast-wide menhaden nursery grounds (Kroger and Guthrie 1973; Ahrenholz et al. 1989; ASMFC 2012a; Anstead et al. 2014, Chapter II). This analysis resulted in a surprisingly large contribution of age-1 menhaden from the Northern nursery regions. Conversely, it resulted in a much smaller contribution from the Chesapeake Bay region than anticipated. Previous research indicated that New England and Chesapeake Bay were likely to contribute 2% and 69% of new recruits respectively (Ahrenholz et al. 1989; ASMFC 2004a) whereas the classification based on otolith chemistry indicated an

average of 31% from both the Northeast and the Chesapeake Bay over the three years of this study. The average of the Delaware Bay (13%) and the Southeast region nurseries (25%) was fairly consistent with the previous estimates of 12% and 17% respectively. Granted, the highest errors in juvenile classification were between the Northeast and Chesapeake Bay estuaries, but the classification function was still accurate at nearly 90% (Anstead et al. 2014, Chapter II) so large errors in age-1 classification are not anticipated. Indeed, probability rates for these estimates range from 73-95% depending on the region and year so there is reason to believe that the New England nurseries may be producing more recruits than previously estimated.

Supporting the conclusion that the Chesapeake Bay may no longer be the major contributor of new recruits for menhaden is recent reports that recruitment from the Bay has been on the decline since the mid-1980s (Love et al. 2006; ASMFC 2010) due to a reduced numbers of larvae reaching the Bay from offshore waters (ASMFC 2004b; Lozano and Houde 2013). Lozano and Houde (2013) found that the ingress of menhaden larvae into the Chesapeake Bay varied 9-fold inter-annually and modeling efforts of the Mid and South Atlantic Bights indicated a shift from north-to-south spawning areas during the fall and winter spawning event so that the Southeast nursery grounds, not the Chesapeake Bay, were receiving an increased number of larvae (Quinlan et al. 1999; Werner et al. 1999). Additionally, it has been suggested that depending on the year and circulation patterns, larvae spawned off Cape Hatteras enter the warm Gulf Stream waters and get advected north, thus explaining an increased presence of larvae in Northern estuaries (Stegmann and Yoder 1996). The decreased amount of new recruits from the Bay during the three years of this study may be due to this year-to-year variability of

ingress to the region, the increase of larvae in other regions, or it could be indicative of the declining ability of the Chesapeake Bay to produce adult menhaden. Human activities and climate change make estuarine systems such as the Chesapeake Bay vulnerable to changes in water quality and quantity, nutrient and sediment fluxes, temperature, salinity, dissolved oxygen, species composition, pollution levels, and many more (Najjar et al. 2010). While each estuary along the Atlantic coast will respond differently to anthropogenic pressures, the Chesapeake Bay is the largest estuary in the United States and has a well-documented history of the deterioration of water quality (Officer et al. 1984; Hagy et al. 2004; Kemp et al. 2005), habitat loss (Rothschild et al. 1994; Kemp et al. 2005), and changes in food web dynamics (Diaz and Rosenberg 2008; Ludsin et al. 2009). In nationwide evaluations of estuarine health, nitrogen loads were higher in the mid-Atlantic region than in the estuaries north of New York and the Chesapeake Bay has the most eutrophic conditions (Bricker et al. 2008), as well as an increase in harmful algal blooms in the past few decades (Anderson et al. 2008). Undoubtedly this has an effect on the ability of the Chesapeake Bay to produce the amount of recruits that it once may have 25 years ago.

Previous estimates of nursery contribution for menhaden found that the Northeastern estuaries were unlikely to make significant contributions to the adult stock based on different methods using areal extent and productivities (Ahrenholz et al. 1989; ASMFC 2004a). In the clustering analysis, the CCC indicates fewer (2-3) clusters in the age-1 menhaden data, although this reduction in clusters makes comparisons with other studies challenging. Based on previous assumptions and using a very conservative analysis, eliminating the Northeast in the 2009-2010 cohorts and additionally the

Delaware Bay in the case of the 2011 cohort resulted in classifications that show changes from previous estimates of nursery contribution, albeit at less severity. The reduced clusters, on average for the three years, classified 14% of age-1 recruits as originating from the Delaware Bay, 58% from the Chesapeake Bay, and 28% from the Southeast nursery region. These results still indicate a 16% relative decline in the contribution of the Chesapeake Bay from 69% to 58%, but are closer to previous assumptions. Additionally, they have slightly higher probabilities (80-97%) than the four-cluster model. This analysis is not a direct comparison to the Ahrenholz and provides an upper bound of an estimate at best, as reducing the amount of clusters guarantees larger contributions from the remaining regions. Yet even with this estimate, the Chesapeake Bay is likely to play a less significant role as a productive nursery ground than previously believed.

There are many factors that can influence which nursery ground along the Atlantic coast can produce the new recruits to the adult menhaden population, e.g., ocean circulation, Ekman transport, and food availability. Body size of juveniles has also been recognized as a factor that can influence recruitment and play a significant role in the survival of juvenile fishes where larger and faster growing fish have increased survivorship (Houde 1987; Miller et al. 1988; Sogard 1997). Age-1 menhaden were significantly larger in 2012 than in 2010 and 2011 and although this may be due to sampling, it may also indicate some size-selective mortality in the juveniles during these years. There are many mechanisms that can cause juvenile size-selective mortality and recruitment variability, such as predation (Werner and Gilliam 1984; Post and Prankevicius 1987), starvation (Lasker 1975), or extremes in physical factors such as

temperature, salinity, or dissolved oxygen (Brett 1979; Sogard 1997; Hurst and Conover 1998). The most recent stock assessment update indicated that 2011 had the second lowest recruitment value in the entire time series beginning in 1955 (ASMFC 2012). Additionally, it has been shown that there is a significant inverse relationship between mean fork length and VPA estimates of year-class strength (Reish et al. 1985; Ahrenholz et al. 1989), indicating that 2012 may also not be a strong year-class based on its larger average fork length.

While some size-selective mortality may have been captured during the three years of this study, the focus was to quantitatively estimate nursery contribution using otolith chemistry. This approach has been successful, if not widely applied, for evaluating the nursery origins of unknown adults in other fish species. Hobbs et al. (2007) used trace elements and a discriminant function analysis to classify adult Delta Smelt to their nursery origin within the San Francisco Bay. They determined that a majority of adults were spawned in one region for the one year of the study. Using trace elements and stable isotopes, Walther et al. (2008) determined the natal origin of spawning adult American Shad in the York River, Virginia. They collected geochemical signatures of juveniles from New Hampshire to Georgia over 3 years that did not correspond to the year-classes of the adults. They used an abbreviated signature to determine the origins of the unknown adults and found that only 6% of spawning adults were strays from other rivers although there appeared to be no fidelity to tributaries within the York River. Schaffler et al. (2014b) used otolith chemistry to determine the natal origin of adult Alabama Shad returning to spawn in the Apalachicola-Chattahoochee-Flint River system to evaluate the fish passage efforts in that region. Using this approach, they were able to show that

juvenile Alabama Shad could in fact emigrate successfully through a lock and dam to contribute to the adult population. All of these studies demonstrated the utility of otoliths in determining nursery contribution and this study expands the scope to a coast-wide evaluation for multiple year-classes.

This study developed the first quantitative estimate of nursery contribution of new recruits to the adult population of the Atlantic Menhaden. By using the juvenile otolith geochemical signatures that differentiated between nursery ground regions by nearly 90% accuracy, we successfully assigned age-1 menhaden to their region of origin along the Atlantic coast. While the clustering techniques offer two distinct scenarios for recruitment, both conclude that the Chesapeake Bay provides significantly smaller proportion of new recruits than previously estimated using another methodology, with the Southeast and Delaware Bay potentially playing a larger role in producing age-1 menhaden. The role of the Northeast estuaries is a little less conclusive and depends very much on which clustering metric is used: the four-cluster analysis or the conservative three-cluster analysis that apportions each group higher. Regardless, nursery contribution along the Atlantic coast has shifted in the last 25 years and this study offers insight for fisheries managers and stock assessment scientists as they move forward with menhaden management and struggle to address declining recruitment in recent years.

CHAPTER IV

THE EFFECT OF MULTIPLE NURSERY AREAS ON THE POPULATION STRUCTURE OF ADULT ATLANTIC MENHADEN, BREVOORTIA TYRANNUS

Introduction

Understanding the role of multiple nursery areas in the population structure and dynamics of estuarine-dependent fishes is fundamental for effective management. The Atlantic Menhaden *Brevoortia tyrannus*, hereafter menhaden, is of critical importance as a prey species in the marine food web for many fish and seabirds, a consumer of primary production, and the largest fishery by volume on the Atlantic coast (ASMFC 2012). Even with the commercial and environmental importance of this species, there are still many aspects of the population that remain understudied and provide challenges to effective management. The fishery is managed by the Atlantic States Marine Fisheries Commission (ASMFC) and while menhaden are not considered overfished, low recruitment and decreased spawning stock biomass have plagued this fishery since the 1990s, with 2011 experiencing the second lowest recruitment since 1955 (ASMFC 2012). Knowledge about the effect of multiple nursery areas on the population structure of menhaden is needed to identify which regions produce not only new recruits but, more importantly, recruits that persist through multiple age-classes and contribute to the spawning stock biomass.

Migratory patterns, spawning behavior, and fecundity of menhaden are well studied and documented (Nicholson 1978; Ahrenholz et al. 1987; Lewis et al. 1987;

Ahrenholz 1991). Adult menhaden are distributed from Nova Scotia to Florida and undergo extensive seasonal migrations along the Atlantic Coast. The population segregates by size and age during the summer months, with older, larger fish migrating northward while younger, smaller fish reside in the southern part of the range (Nicholson 1978). Menhaden are multiple spawners and 50% are sexually mature between ages 1 and 2. Fecundity increases with age, so the oldest, largest females produce the most eggs (Lewis et al. 1987), and these are the fish that migrate the furthest north. While spawning can occur from late fall through early spring along the migratory route, it typically takes place in the winter when menhaden aggregate off the coast of North Carolina's Cape Hatteras and spawn as a population (Nicholson 1978; Lewis et al. 1987). After spawning, larvae are transported to multiple nursery grounds along the Atlantic coast where juveniles develop inshore before recruiting to the coastal adult stock.

Many variables that can influence which nursery grounds receive larvae coast-wide, such as time and location of spawning, wind-driven Ekman transport, the amount of larvae produced, and the accessibility of estuarine mouths (Nelson et al. 1977; Miller et al. 1984; Berkeley et al. 2004). While these factors are frequently acknowledged when evaluating recruitment variability and year-class strength, the age structure and spatial distribution of the population may be as important as the physical conditions of the ocean and the size of the spawning biomass (Murawski et al. 2001; Berkeley et al. 2004). Fully mature menhaden (ages 2+) undergo coast-wide seasonal migrations that potentially deliver larvae to nursery grounds outside of the main spawning event in the winter. This temporal and spatial variation in spawning could serve as a bet-hedging strategy to ensure multiple nursery grounds receive larvae and contribute to the stability or persistence of a

population (Secor 2009). And yet, the influence of age structure and size-dependent migration on the annual recruitment variability and the success of coast-wide nursery grounds in producing adults to the fishable stock remains understudied in most estuarine-dependent fishes including menhaden.

Historically, the Chesapeake Bay was believed to contribute 69% of the new recruits to the menhaden population based on nursery productivities and juvenile indices (Ahrenholz et al. 1989; ASMFC 2004a). We evaluated this 25-year-old estimate in a previous study for the 2009-2011 cohorts using different methods (Anstead et al. *in prep*, Chapter III) and found that, on average, most new recruits were still produced in Chesapeake Bay nurseries but at much lower proportions (25-36%) than previously believed based on four coast-wide nursery regions. While an evaluation of differential contribution of nursery grounds for producing new recruits is necessary to understanding the population dynamics of menhaden, it does not address how this affects the spawning stock biomass since age-1 menhaden have not reached full maturity (Lewis et al. 1987; ASMFC 2010, 2012). Additionally, exploring the stability of proportional nursery contribution through older age-classes provides an opportunity to understand the survivorship through the age-classes and possible affects of commercial fishing on menhaden, which focuses mainly on ages 1-3 years (ASMFC 2010, 2012). Together, this information would identify regions that produce the greatest abundance of fully mature adults that are responsible for replacing the population as part of the spawning stock biomass and evaluate the proportional contribution of nursery grounds through multiple age-classes.

The panmictic nature of menhaden spawning excludes the use of genetic approaches for evaluating the population structure of menhaden and nursery contribution (Lynch et al. 2010), but it does provide an opportunity to use otolith chemistry. We previously established the utility of otolith chemistry for building geochemical signatures that distinguished between coast-wide nursery grounds for menhaden with nearly 90% accuracy (Anstead et al. 2014, Chapter II). We showed that in addition to using year-specific signatures for classification in 2009-2011, a combined signature could be employed with little decrease in accuracy (from 90% to 86%) for data-poor years or when juvenile data has not been collected. In this study, we use the 2009-2010 juvenile signatures and the combined signature to classify older menhaden (ages 2-4) from the commercial fishery that correspond to the 2008-2010 year-classes to explore the stability of nursery contributions through older age-classes. This will identify which nursery regions are essential to the persistence of the menhaden population, not for producing the most new recruits but rather for producing adults that are mature. This component is necessary, as it is these adults that will comprise the spawning biomass that replaces the population and supports a large portion of the commercial fishery and marine food web.

Material and methods

Fish collection

Juvenile menhaden were collected in 2009-2011 from multiple sites along the US Atlantic coast, prepared, and analyzed as described in Anstead et al. (2014, Chapter II). Adult menhaden (ages 2+) were collected in 2010-2012 from the coastal waters from

Cape Cod to the Hudson River, Delaware Bay, Chesapeake Bay, Pamlico Sound, and Charleston Sound (Figure 6). The adult collections were grouped into four coastal regions to mimic those of the juvenile menhaden collections and previous studies (Kroger and Guthrie 1973; Ahrenholz et al. 1989; ASMFC 2004a; ASMFC 2012; Anstead et al. 2014, Chapter II): the Northeast, Delaware Bay, Chesapeake Bay, and Southeast. We relied on the state agencies and the commercial purse seine and pound net fisheries along the coast for our sampling. Adults were collected from August to October during the 3 years of this study and were frozen at capture and transported to the laboratory where they were measured for fork length (mm) and sagittal otoliths were removed in a class-100 clean room. Excess tissue was cleaned from the surface with acid washed glass probes and drops of ultrapure hydrogen peroxide followed by triple rinsing with ultrapure Milli-Q water. Cleaned samples dried under a laminar flow hood for 24 hours and stored in acid-washed polyethylene vials. One otolith from each pair was selected randomly for trace element analysis and the other was used for stable isotope analysis.

Sample preparation and analysis

Adult otoliths for trace element and stable isotope analyses were mounted on glass slides using crystal bond. Otoliths were polished on both sides using 30 μm lapping film followed by 0.3 μm lapping film to remove adult material, expose the juvenile core for analysis, and verify ages by counting growth rings. For otoliths selected for stable isotope analysis, the juvenile cores were removed and ground into a fine powder using a handheld dremel tool. Milled cores were placed in acid washed vials and analyzed for carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) concentrations with a Finnigan Delta Plus with Kiel III

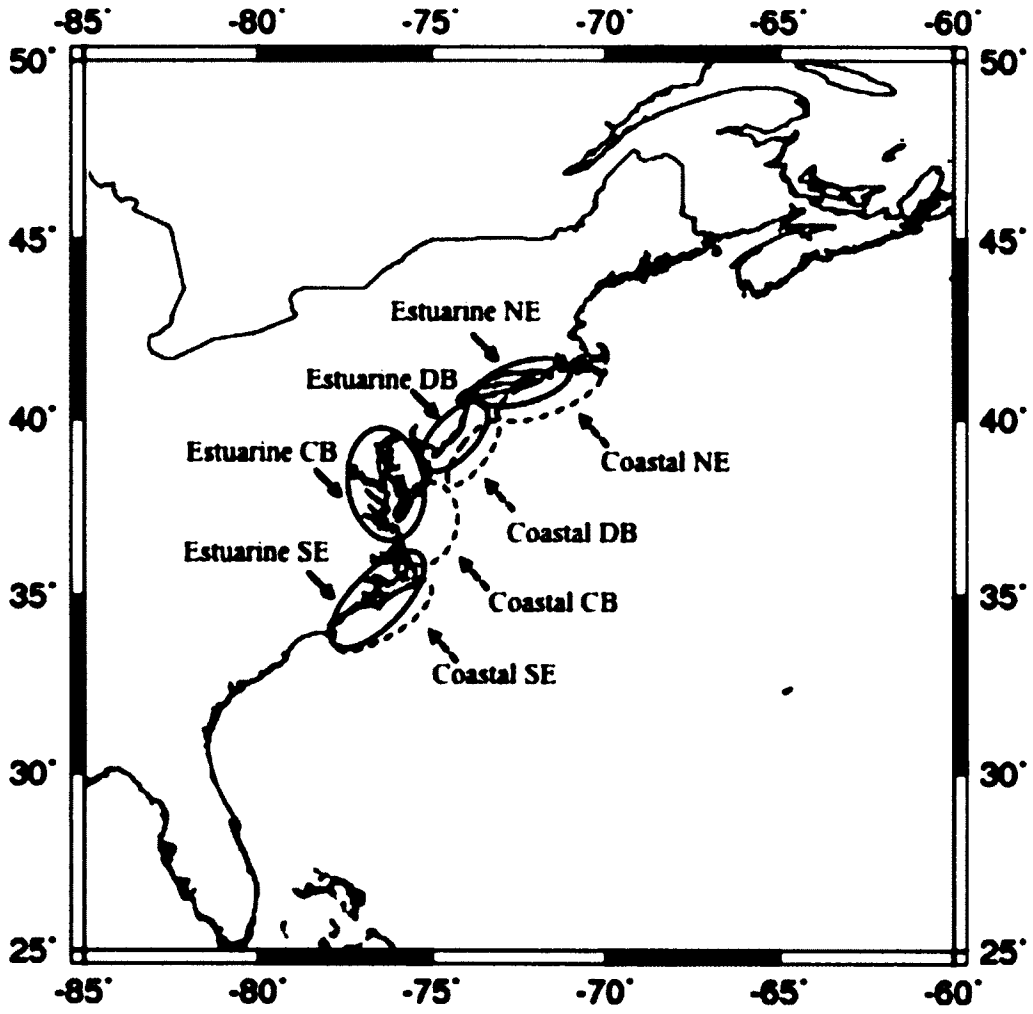


Figure 6. Approximate locations of juvenile and adult Atlantic Menhaden sample collections along the US East Coast. Juveniles were collected from estuarine nursery grounds and grouped into four regions: the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB), and Southeast (SE) as indicated by bold circles. Adult collections were made in the coastal waters of the same regions, as indicated by dashed circles.

Carbonate Device (Thermo Fisher Scientific, Waltham, MA) using standard procedures (Coplen et al. 1983; Coplen 1996; Ostermann and Curry 2000) at the University of Washington Stable Isotope Laboratory. Carbon and oxygen was measured and corrected relative to Vienna Pee Dee Belemnite (VPDB) and accuracy was determined by averaging precision of the samples within each year-class.

Polished otoliths selected for trace element analysis were transferred in groups of 20 onto petrographic slides using crystal bond. Otoliths were randomized on the slides within age and year-classes, sonicated in Milli-Q (18 $M\Omega \bullet cm^{-1}$) water for 10 minutes to remove contaminants from the surface, and allowed to dry under a laminar flow hood. Adult otolith slides were analyzed at the Woods Hole Oceanographic Institute's plasma facility using a thermo Finnegan Element 2 (Thermo-Fisher Scientific, Bremen, Germany) inductively-coupled plasma mass spectrometer (ICP-MS) with a New Wave 193 nm excimer laser ablation system (New Wave Research, Sunnyvale, CA). For all samples, we ablated a transect from the juvenile core to the first annulus using a laser beam with a 25 μm spot size, 10 $\mu m\ s^{-1}$ scan speed, 70% power, and a 10 Hz frequency that resulted in a trench that was 25 μm wide and 30 μm deep. Counts were collected for the trace elements of ^{7}Li, ^{25}Mg, ^{55}Mn, ^{85}Rb, ^{88}Sr, ^{89}Y, ^{137}Ba, and ^{208}Pb in low-resolution mode (R=300) (Schaffler and Winkelman 2008; Anstead et al. 2014, Chapter II and III). Two reference materials and multi-element standards prepared from ultrapure stock solutions were used to calibrate elemental concentrations (Yoshinaga et al. 1999; Sturgeon et al. 2005) and elements were normalized and expressed as element-to-calcium ratios (Schaffler and Winkelman 2008). Standards were run at the beginning and end of each slide to account for machine drift and blanks were run every 5 samples. Limits of

detection (LOD) for the collected elements were calculated as mean blank values plus three standard deviations (Thorrold et al. 1997) and expressed as a percent of the average sample intensity. Because juvenile and adult slides were run on separate research trips, a juvenile slide was analyzed a second time for every 4 adult slides run to correct for machine drift (Thorrold et al. 2001; Vasconcelos et al. 2008). Dividing the elemental concentration from the originally run slide by the value of the rerun slide and averaging the values over the entire slide for each element calculated correction factors for the juvenile slides. These correction factors were then applied to the adult slide trace element data so that comparisons could be more accurately made. At the completion of each research trip, adult slides were examined to confirm that only the juvenile core was analyzed. Data was truncated in the case when the transect extended past the first annulus.

Data analysis

Adult trace element and stable isotope data was combined and the lambda values from the juvenile data Box-Cox transformations (Box and Cox 1964) were applied to each year-class. Geochemical signatures that accurately distinguished between nursery regions within each year were developed from the juvenile data using quadratic discriminant analysis (QDA) for the 2009-2011 cohorts. We chose the number of clusters in each data set based on three metrics: the pseudo t^2 statistic, pseudo F statistic, and cubic clustering criterion (CCC) (Milligan and Cooper 1985; Shima and Swearer 2009; Anstead et al. *in prep*, Chapter III). We used the number of clusters in the adult data to determine how many nursery regions were present in the adult data and applied our

classification function to assign the adults to their nursery region of origin. Classification functions specific to 2009 and 2010 were used to assign adults that belonged to those cohorts to their nursery region. For the 2008 adults, a combined signature from the 2009-2011 juvenile data was applied, as its utility in correctly classifying adjacent year classes has been shown when year-specific data is not available (Anstead et al. 2014, Chapter II). We used canonical discriminant analysis (CDA) to visualize the adult data from each age and year-class using the average linkage method in Ward's minimum variance cluster procedure (Walther et al. 2008). All statistical tests were performed at a α=0.05 significance level.

Results

We analyzed a total of 585 adult menhaden otoliths collected from the commercial fishery of ages 2-4 that corresponded to the 2008-2010 cohorts (Table 10). We found 190 menhaden samples of ages 2-4 that were assigned to the 2008 year-class, 241 samples of ages 2-3 for the 2009 year-class, and 154 of age-2 to the 2010 year-class. Average fork length was significantly different between the age-classes (ANOVA: $F_{2, 583} = 126.07$; $P < 0.001$). The average fork length for age-2 menhaden was 229.3 mm, 235.1 mm for age-3 menhaden, and 265.7 mm for age-4 menhaden for all years combined. Age-2 menhaden were not significantly different between the 3 cohorts in fork length (ANOVA: $F_{2, 371} = 0.41$; $P = 0.667$), although age-3 menhaden were significantly different between the 2008 and 2009 year-classes (t-test: t=-2.876, df=171, $P = 0.005$) with age-3 menhaden more in 2009 being longer on average than those from 2008. This

Table 10. Adult Atlantic Menhaden sample size and mean fork length by juvenile cohort and age with standard error for the sampling regions. In total, 585 adult menhaden were analyzed for the three year-classes of this study.

Cohort Year	Age	Region	N	Mean Fork Length ± SE (mm)
2008	2	Delaware Bay	44	236.7 ± 3.8
	2	Chesapeake Bay	49	224.7 ± 1.9
	3	Northeast	4	266.0 ± 3.9
	3	Delaware Bay	31	245.4 ± 1.0
	3	Chesapeake Bay	23	252.6 ± 2.0
	4	Northeast	4	287.0 ± 3.3
	4	Delaware Bay	22	262.5 ± 1.4
	4	Chesapeake Bay	9	265.0 ± 2.8
	4	Southeast	4	263.5 ± 1.2
2009	2	Delaware Bay	40	241.9 ± 0.9
	2	Chesapeake Bay	80	218.8 ± 2.6
	2	Southeast	6	154.0 ± 9.0
	3	Northeast	22	277.9 ± 2.9
	3	Delaware Bay	39	266.8 ± 1.4
	3	Chesapeake Bay	45	240.9 ± 2.3
	3	Southeast	9	254.1 ± 8.0
2010	2	Northeast	5	275.2 ± 7.6
	2	Delaware Bay	13	248.9 ± 4.5
	2	Chesapeake Bay	125	226.1 ± 1.6
	2	Southeast	11	218.5 ± 8.6

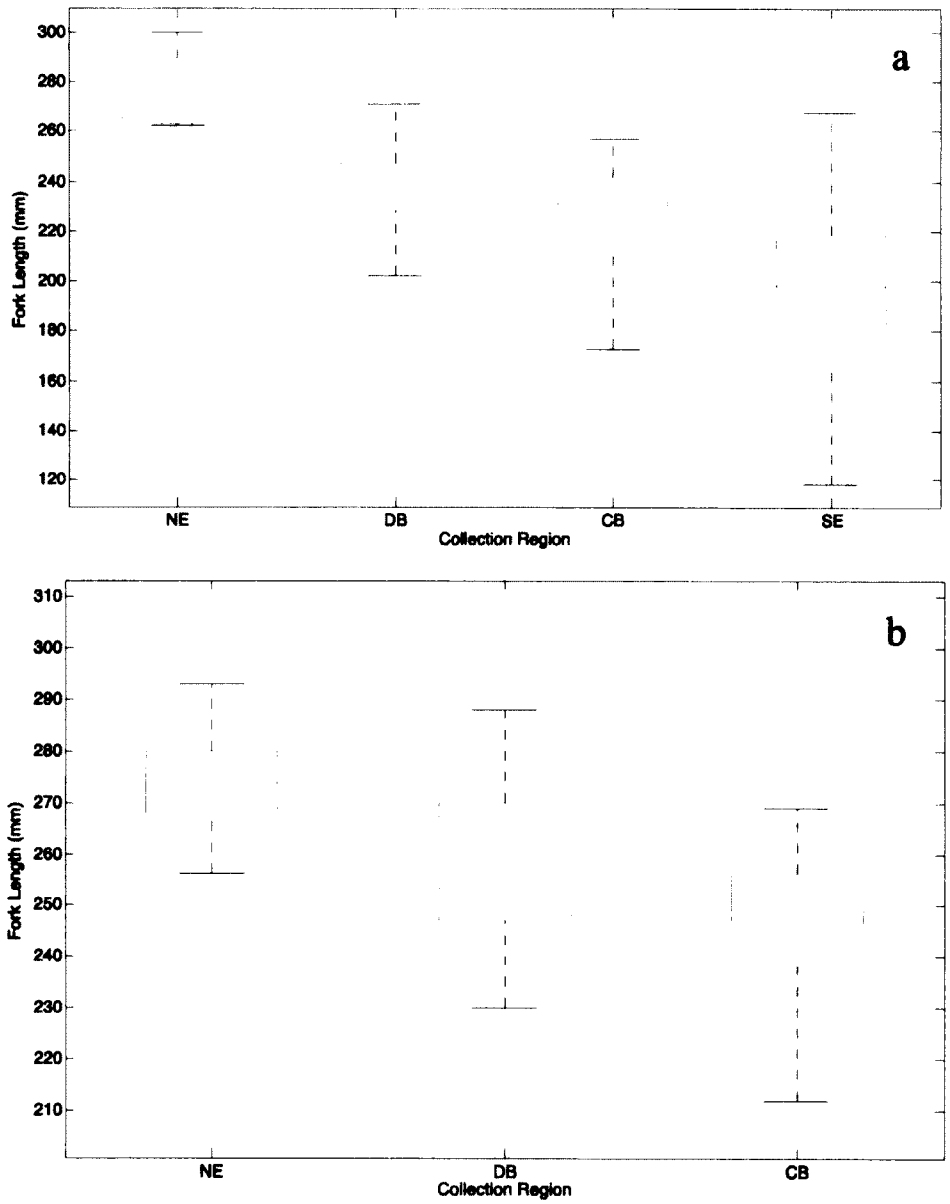


Figure 7. Boxplots showing a latitudinal gradient in average fork length (mm) of (a) age-2 (b) age-3 Atlantic Menhaden caught in the coastal waters of the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB), and the Southeast (SE) for the 2008-2010 year-classes. Boxes indicate upper and lower quartiles with median values expressed as horizontal lines, whiskers indicating variability outside the quartiles, and plus signs representing outliers. All regions are significantly different from each other (pairwise ANOVA, α=0.05).

Table 11. Limits of detection (LOD) for otolith trace element and stable isotope analysis for adult Atlantic Menhaden otoliths. Detection limits calculated as mean blank value plus 3 standard deviations of elements analyzed by LA-ICPMS in low-resolution mode for adult Atlantic Menhaden from the 2008-2010 cohorts. Trace element concentrations were calibrated using Japanese (JPN) and NRC reference standards. For carbon and oxygen isotopes, RSD was calculated as the average precision of all samples within each year.

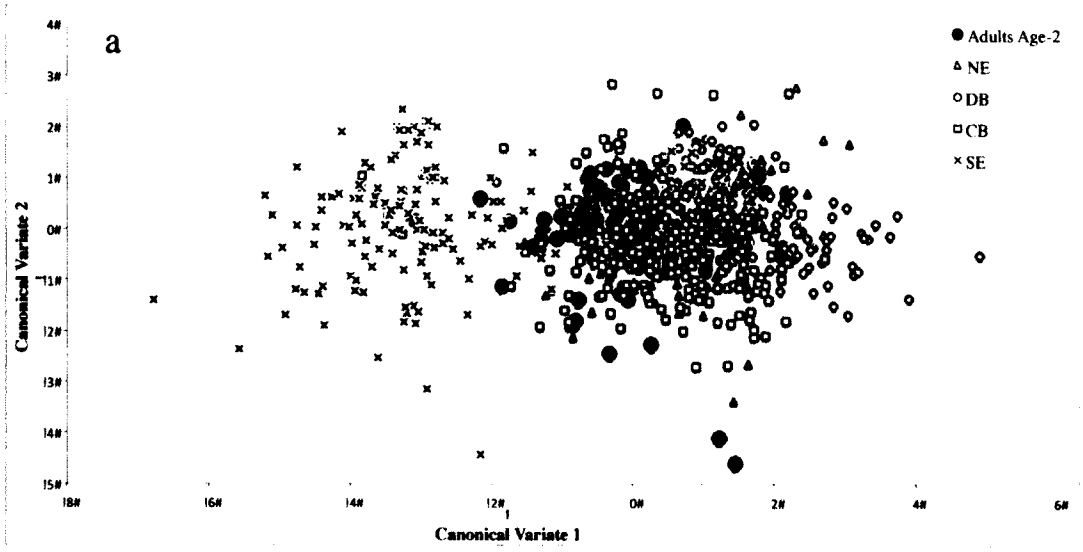
		2008				2009				2010			
Element	**Unit**	**JPN RSD**	**NRC RSD**	**LOD**	**%>LOD**	**JPN RSD**	**NRC RSD**	**LOD**	**%>LOD**	**JPN RSD**	**NRC RSD**	**LOD**	**%>LOD**
Li	ppb	10.4	10.4	1146.4	85	12.5	5.3	24.5	100	9.4	10.1	24.2	95
Mg	ppm	12.4	13.7	422763.3	75	17.5	5.2	105.8	100	9.0	10.7	296.8	97
Ca	ppm	12.0	12.4	126406.5	100	16.4	5.1	4718.6	100	8.6	9.7	5738.3	100
Mn	ppb	12.3	16.4	55551.5	71	5.1	4.9	1075.1	100	7.2	8.3	1963.7	100
Rb	ppb	10.4	8.8	9595.7	65	11.5	4.6	20.9	100	7.8	13.7	90.6	93
Sr	ppm	11.7	12.3	173688.0	100	16.1	4.7	12720.4	100	7.9	9.0	12360.8	100
Y	ppb	12.8	16.1	957.0	64	15.7	3.3	5.1	100	5.1	5.1	7.8	100
Ba	ppm	11.7	13.1	16128.0	83	15.3	4.3	113.1	100	7.1	8.4	144.6	100
Pb	ppb	13.0	17.6	91448.1	40	15.3	3.5	207.4	100	6.0	6.4	366.4	93
C	‰	0.0242		--	--	0.0337		--	--	0.0311		--	--
O	‰	0.0580		--	--	0.0569		--	--	0.0516		--	--

is likely due to the fact that age-3 menhaden were caught in the Northeast and Delaware Bay regions in 2009 and these fish, on average, tended to be longer than those caught in the southern regions. Age-2 menhaden caught in the four regions were significantly different from each other (ANOVA: $F_{3,370} = 29.66$; $P < 0.001$), with a latitudinal gradient where age-2 menhaden caught in the Northeast were the longest and age-2 menhaden caught in the Southeast were the shortest (Figure 7). The equivalent comparison could not be made for age-3 fish since there were no samples collected from the Southeast in 2008, although the remaining three regions were significantly different from each other

(ANOVA: $F_{2,161} = 48.55$; $P < 0.001$) and, as expected, exhibited the same latitudinal gradient as the age-2 menhaden.

All trace element samples were above the LODs (>93%) for the 2009 and 2010 year-classes (Table 11). The 2008 year-class had many trace elements below acceptable LODs (<83%). Therefore, for the 2008 age class, only Li, Sr, and Ba were detectable trace elements that can be included in the discriminant function for classification. The precision of stable isotopes of carbon and oxygen was high for all 3 years, so carbon and oxygen isotopes were used for all analyses. Juvenile signatures for the QDA were therefore usable for the 2009 and 2010 year-classes, but the use of the combined signature to classify the 2008 adults had to be adjusted to eliminate the elements below the LODs. Previously, the combined signature of the 2009-2011 juveniles used Li, Mg, Mn, Rb, Sr, Ba, and the carbon and oxygen isotopes to correctly classify the juveniles of known origin to 86%. Eliminating Mg, Mn, and Rb reduced the accuracy of this classification function to 83%, but allowed this combined classification function to be used to evaluate the 2008 year-class.

Much like the age-1 data (Anstead et al., *in prep*, Chapter III), CDA plots show a clumping of age 2-4 data in the middle of the four juvenile clusters regardless of age or year-class (Figure 8). We used the clustering metrics of the pseudo t^2 statistic, pseudo F statistic, and CCC to determine the number of clusters in the adult data. Depending on the metric used, we found either two or four clusters in all cohorts and age classes (Table 12). The CCC indicated two clusters in all adult data for all years of the study whereas the pseudo t^2 and the pseudo F statistics indicated four clusters for all ages and years except for age-2 in 2009 and 2010 where it indicates two clusters. We proceeded with the QDA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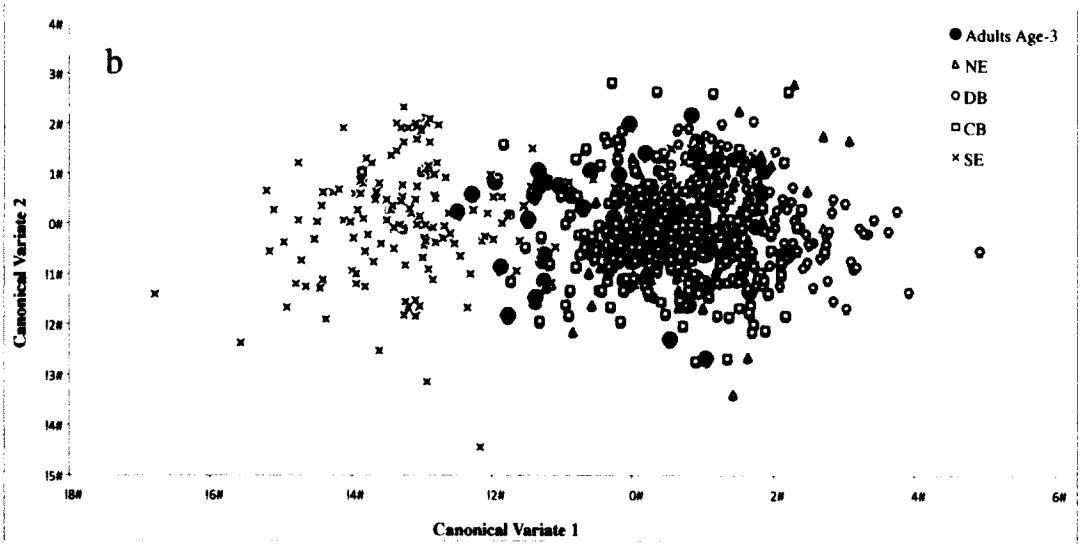
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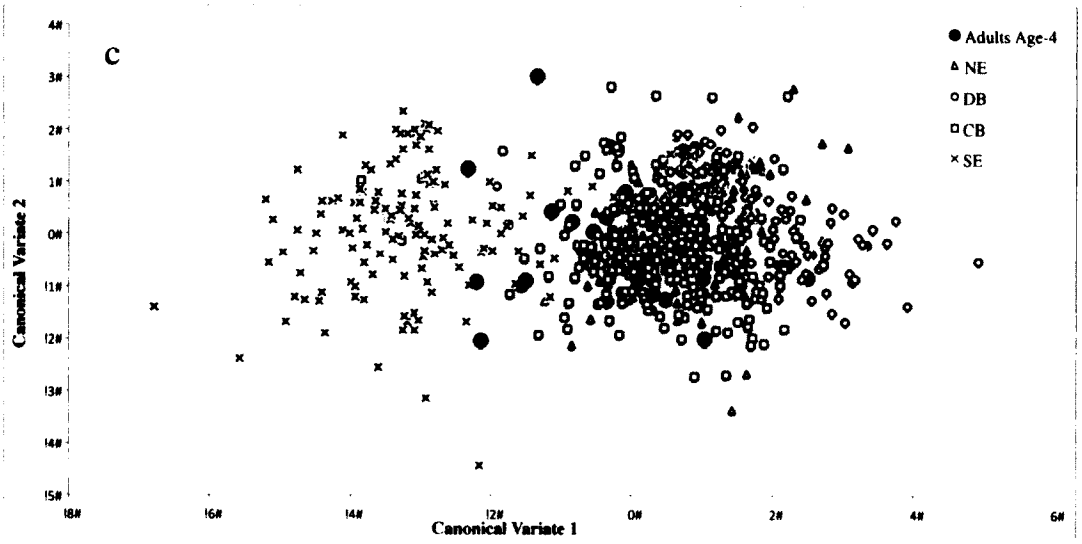
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Figure 8. (Continued)

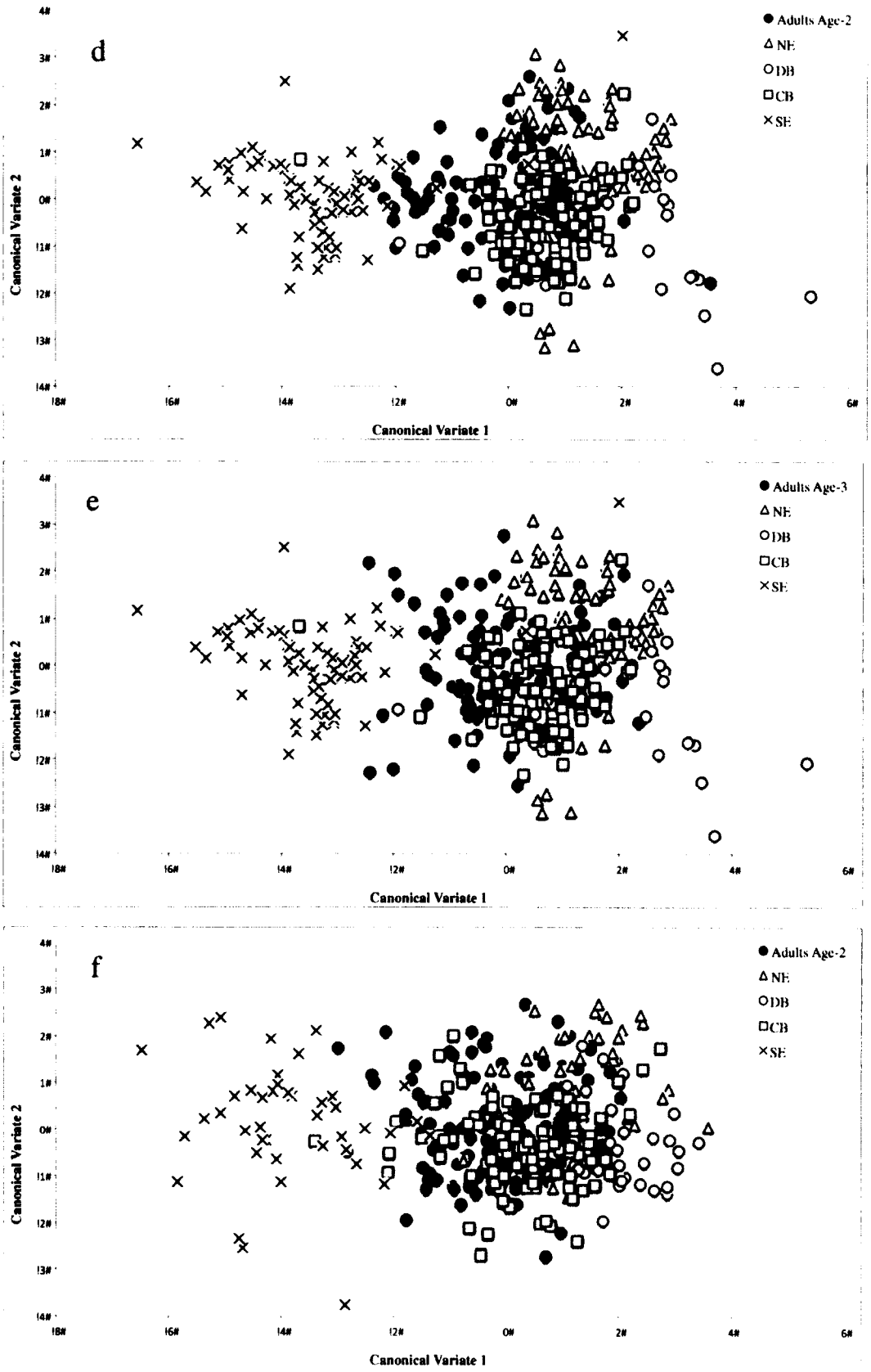

Figure 8. (Continued) Canonical variate plots for adult Atlantic Menhaden. Canonical variates 1 and 2 summarizing the differences in trace element and carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$) stable isotopes for adult Atlantic Menhaden. Plots show juvenile Atlantic Menhaden otoliths from the Northeast (Δ), Delaware Bay (o), Chesapeake Bay (□) and the Southeast ( ) nursery grounds and adults Atlantic Menhaden otoliths (•), ages 2-4, of unknown natal origin for the cohorts from (a-c) 2008, (d-e) 2009, and (f) 2010.

using four and two clusters to evaluate the two scenarios, although it should be noted that the latter model is not comparable with historical estimates of nursery contribution that assumed four nursery regions and, because of the reduced regions, it guarantees higher probabilities for each remaining region.

Proceeding with four clusters in the adult data based on previous studies (Kroger and Guthrie 1973; Ahrenholz et al. 1989; ASMFC 2004a; Anstead et al. 2014, Chapter II) and supported by the small pseudo t^2 statistics and large pseudo F statistic, we assigned adults of unknown origin to their nursery ground using the QDA. We found that, on average, 40% of age-2 menhaden originated in the Northeast nursery region, 8% from Delaware Bay, 22% from Chesapeake Bay, and 30% from the Southeast for the 2008-2010 year-classes (Table 13). For the age-3 menhaden in 2008 and 2009, on average, 29% were from the Northeast nursery region, 6% from Delaware Bay, 26% from Chesapeake Bay, and 40% from the Southeast. For the age-4 menhaden from the 2008 year-class, 18% originated in the Northeast, 5% from Delaware Bay, 59% from Chesapeake Bay, and 18% from the Southeast. These estimates had various probabilities associated with them, ranging from 53% to 100% depending on the age-class and the nursery region. Using all available data for the various age-classes for the 2008-2010 year-classes, the largest nursery contribution of age-1 menhaden comes from the Northeast and Chesapeake Bay (Anstead et al. *in prep*, Chapter III), the Northeast and Southeast nurseries contribute the most age-2s, and the Southeast contribution dominates age-3s (Figure 9). Although menhaden from the Chesapeake Bay nurseries predominantly make up the age-4 adults in 2008, there are no other year-classes to compare this figure to for observing trends in the data.

Table 12. Values of the cubic clustering criterion (CCC), pseudo F, and pseudo t^2 statistics that were used to determine the number of clusters in the adult Atlantic Menhaden data from 2008-2010 cohorts. Bold indicates the amount of clusters in each data set based on statistic used.

Cohort	Age	# of Clusters	CCC	*F*	t^2
2008	2	2	**-2.6**	21.9	16.1
		3	-1.8	25	17.9
		4	0.58	**28.9**	**12.1**
2008	3	2	**-2.9**	13.8	24.8
		3	-2.7	14.9	29.9
		4	-1.7	**16.5**	**17.2**
2008	4	2	**-2.2**	9.5	10.5
		3	-2.2	11.2	15.8
		4	-1.9	**16**	**3.1**
2009	2	2	**-4.2**	37.9	**48.6**
		3	-2	50.6	73.3
		4	1.87	**62.6**	58.6
2009	3	2	**-4.1**	35.1	35.1
		3	-3.3	39.7	37.2
		4	-2.3	**42.9**	**28.8**
2010	2	2	**-4.6**	41.5	**30.3**
		3	-4.5	45.7	30.5
		4	-4.2	**46.2**	33.6

Table 13. Coast-wide nursery contribution of Atlantic Menhaden using three successive cohorts. Adult Atlantic Menhaden collected in 2010-2012 of unknown natal origin were assigned to their nursery ground based on juvenile otolith chemical signatures with associated probabilities from the quadratic discriminant function.

Year of			Classification				Probability			
Cohort	Age	# Clusters	NE	DB	CB	SE	NE	DB	CB	SE
2008	2	4	29 (31%)	7 (8%)	25 (27%)	32 (34%)	76%	65%	62%	99%
2008	3	4	13 (22%)	4 (7%)	16 (28%)	25 (43%)	69%	70%	68%	100%
2008	4	4	7 (18%)	2 (5%)	23 (59%)	7 (18%)	74%	68%	69%	100%
2009	2	4	50 (40%)	7 (5%)	29 (23%)	40 (32%)	76%	63%	72%	98%
2009	3	4	40 (35%)	5 (4%)	28 (24%)	42 (37%)	75%	53%	72%	97%
2010	2	4	75 (49%)	15 (10%)	27 (17%)	37 (24%)	77%	73%	69%	93%
2008	2	2	-	-	65 (70%)	28 (30%)	-	-	98%	96%
2008	3	2	-	-	35 (60%)	23 (40%)	-	-	97%	98%
2008	4	2	-	-	32 (82%)	7 (18%)	-	-	98%	96%
2009	2	2	-	-	84 (67%)	41 (33%)	-	-	98%	97%
2009	3	2	-	-	71 (62%)	44 (38%)	-	-	97%	98%
2010	2	2	-	-	107 (69%)	47 (31%)	-	-	94%	93%

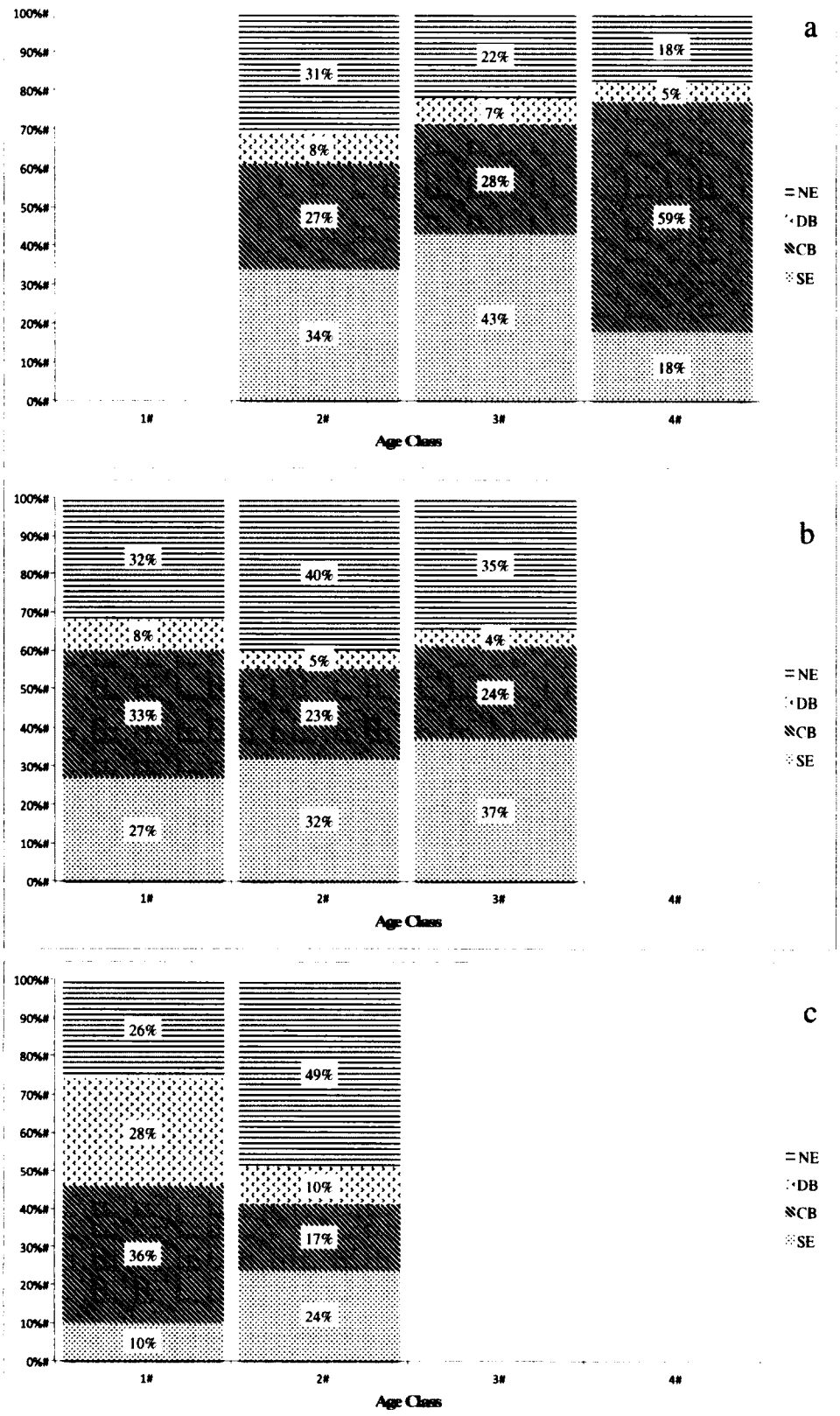
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Figure 9. (Continued) The stability of nursery contribution through multiple age-classes. Figure represents the (a) 2008, (b) 2009, and (c) 2010 cohorts for Atlantic Menhaden. Age-1 data was from previous research (Anstead et al. *in prep*, Chapter III), although age-1 data for the 2008 cohort was not available.

The CCC metric indicates two clusters for all ages and years in the adult menhaden data and we evaluated this scenario using the QDA. Previous research indicates that northern estuaries (Ahrenholz et al. 1989; ASMFC 2004a), such as those from the Gulf of Maine to Delaware, are unlikely to contribute significantly to the adult population. Therefore, we eliminated the Northeast and Delaware Bay from the classification function. Using only Chesapeake Bay and the Southeast nursery grounds, we found that for age-2 menhaden from the 2008-2010 cohorts, 69% originated from Chesapeake Bay and 31% originated from the Southeast, on average. For age-3 menhaden, 61% originated in the Chesapeake Bay and 39% were from the Southeast, on average for the 2008 and 2009 cohorts. For age-4 menhaden in the 2008 cohort, 82% were from Chesapeake Bay and 18% were from the Southeast. These results have very high probabilities associated with them (93-98%), which was expected since reducing the number of clusters guarantees larger contributions from the remaining regions. Regardless, the increased contribution of the Chesapeake Bay to 70% is much more consistent with previous estimates, even though those historical estimates were made based on four nursery regions, not two.

Discussion

Our estimates of coast-wide nursery contribution to the adult Atlantic Menhaden population offer critical information for evaluating survivorship and spawning stock biomass for multiple age-classes of this species. We applied otolith chemistry from a previously researched library of geochemical signatures to assess historical estimates of nursery contribution and provided updated values for the 2008-2010 year-classes. We

found that the Chesapeake Bay does not produce the highest proportion of adults in the population, but rather the Northeast and Southeast nursery regions combined contributed, on average, approximately 70% of adults ages 2-4 for the cohorts analyzed. Particularly in the case of the Northeast estuaries, this may indicate an important shift in the demographic of adult spawners where the productive nurseries are related to age and size-dependent migration as well as physical conditions in the ocean. Additionally, this identifies disproportionate survivorship in adults produced from coast-wide nursery grounds, as well as suggests that those from the Northeast and Southeast comprise a larger proportion of the spawning stock biomass then previously realized.

Spatial distribution and spawning behavior are related to the population's age and size structure for many fish species, including menhaden, and this study was consistent with observations on average size-at-age and the latitudinal gradient in body size (Ahrenholz et al. 1987; ASMFC 2012). We found that the average size-at-age was larger for those fish caught in the north Atlantic than those in south Atlantic for the 2008-2010 cohorts. This may have implications for the structure of the menhaden population and offer an explanation to the increased proportional contribution of the northern estuaries in older age-classes. Menhaden are prolific spawners that become sexually mature between ages 1 and 2, with some age-2 (40%) and nearly all age-3 (90%) adults considered to be fully mature (Higham and Nicholson 1964; Lewis et al. 2987; ASMFC 2010, 2012). Additionally, female menhaden with larger body sizes mature faster and produce a higher number of ova (Lewis et al. 1987), therefore increasing the spawning potential of larger adult menhaden in the northern regions. While the majority of spawning takes place when menhaden congregate as a population off of Cape Hatteras in the fall and winter months,

these larger, more fecund fish in the northern part of the range may be seeding the nurseries in the Northeast over the summer months and during their migration to the spawning grounds. Both Lewis et al. (1987) and Ahrenholz (1991) noted that menhaden spawned over their vast geographical range during nearly every month of the year and the ASMFC (2010) suggested that the importance of spawning outside of North Carolina coast in the winter might be underestimating the population's spawning potential. Therefore, larger age-2 and 3 adults in the north may be providing more larvae to northern nurseries than previously acknowledged, increasing their ability to proportionally contribute to older age-classes.

The role of adult body size and the potential of larger, more fecund adults spawning along the migration route may alter nursery ground productivity, but the structure of the commercial fishery could contribute to the differential survivorship observed along the coast. When menhaden landings were first recorded in 1955, 23 reduction plants operated 150 vessels along the coast. Currently, only one reduction plant based in Reedville, Virginia, continues to operate 10 vessels (Kirkley 2011; ASMFC 2012). The reduction fishery, which now accounts for nearly 80% of menhaden landings, focuses their activity in Virginia with fleets ranging from New Jersey in the summer to North Carolina in the fall. Therefore, adult menhaden, particularly ages 3+, that are seasonally migrating to the northern part of the range are unavailable to the fishery because of their spatial distribution. Additionally, the commercial catch of menhaden in 2009-2011 was comprised of a higher proportion of age-1 fish (40-48%) than the preceding years (ASMFC 2012). We previously showed that the Chesapeake Bay produced the most age-1s for the 2009-2010 cohorts (Anstead et al. *in prep*, Chapter III),

and yet these high proportions did not persist into older age-classes. Increasing the proportions of age-1 menhaden in the catch during these years may have disproportionally targeted menhaden from the Chesapeake Bay nursery grounds so that survivorship was decreased for these new recruits when compared to other regions. The decreased survivorship into older age-classes may be due to the spatial structure of the fishery, particularly if the new recruits moving from their Chesapeake Bay nursery grounds to the coast of Virginia were targeted.

In addition to changing the population structure, localized depletion of new recruits and disproportionate nursery contribution in older age-classes also affects the spawning stock biomass of menhaden. We found that the majority of age-2 and 3 menhaden were produced in the Northeast and Southeast nursery grounds for the 2008-2010 cohorts. For the single year of data we have on age-4, the Chesapeake Bay nursery region contributed the highest proportion of menhaden but whether this trend holds for the oldest age-classes is difficult to predict since we only have one year of age-4 data. Regardless, the question of nursery contribution in older age-classes may be moot since fish older than age-6 have been uncommon since 1965 and those older than age-4 are rarely caught in the fishery (ASMFC 2010, 2012). This suggests that the majority of the spawning stock biomass is comprised of menhaden ages 2-3, since few age-1 menhaden are fully mature (Lewis et al. 1989; ASMFC 2010, 2012) and older age-classes are increasingly absent from the population. Because the Northeast and Southeast nursery regions proportionally contribute the most age 2-3 menhaden, they comprise the majority of the spawning stock biomass and therefore are primarily responsible for replenishing the population. This could provide insight to the decline in spawning stock biomass in

recent decades if the Chesapeake Bay has experienced decreased survivorship to these older age-classes and no longer comprises as much as the spawning stock biomass as it did historically. These are difficult conclusions to make without relative abundances, but it is an important consideration nonetheless.

The results from the otolith chemistry data from this study indicate a substantial shift in our previous assumptions about which nursery grounds are contributing to the adult spawning population. Studies such of these are critical to evaluating the resiliency and persistence of fish species in coming years, as well as identifying areas that are essential to their success. The methods used in this study have previously proven to be effective for evaluating population structure and nursery contribution in several fish species such as the English Sole *Parophrys vetulus* (Brown 2006), Delta Smelt *Hypomesus transpacificus* (Hobbs et al. 2007), American Shad *Alosa sapidissima* (Walther et al. 2008), and Alabama Shad *Alosa alabamae* (Schaffler et al. 2014b). This is the first study to evaluate coast-wide nursery contribution through multiple year and age-classes and explore the stability of the proportion of adults from each nursery region from new recruits to fully mature adults (age 2-4). The use of the combined signature in the 2008 cohort also exhibits the utility of this approach for classifying adjacent year classes, so that a library of signatures could continue to be used for classifying adults outside of the 2009-2011 cohorts for further studies.

Even with the proven utility of an otolith chemistry approach in evaluating population structure and nursery contribution, there are some potential sources of error in this analysis from both the CDA and the classification function. Deciding on the number of clusters in the adult data set offers a challenge (see in Anstead et al. *in prep*, Chapter

III, for a more thorough discussion of this topic), as there was support for either two or four clusters. Two of the three statistics in CDA support the use of four clusters in all age and year-classes in this data set, as does previous research on the coast-wide nursery use of menhaden (Kroger and Guthrie 1973; Ahrenholz et al. 1989; ASMFC 2004a; ASMFC 2012). To be thorough, we proceeded with both a two and four-cluster model, but the use of the four-cluster model is essential for an evaluation of historical estimates regarding nursery contribution. For the four-cluster model, the use of the classification function could introduce error when assigning unknown adults to their nursery region, particularly for the Northeast and Chesapeake Bay. While the juvenile signatures accurately assigned menhaden to their known nursery ground at nearly 90% for three cohorts, the most overlap and classification errors in the juvenile data occurred between the Northeast and Chesapeake Bay nurseries. This overlap in regional signatures may also explain some of the decreased probabilities associated with the adult classifications in the four-cluster model from the two-cluster model. For both juvenile and adult data sets, the Southeast nursery region consistently separated from the other regions, so it is expected that assignments to this region come with high probabilities, especially when the Northeast and Delaware Bay are removed, as is the case of the two-cluster model. For the 2008 cohort, the low LODs for some of the trace elements and subsequent removal of those elements from analysis decreased the accuracy of the classification function, although only by 3%, and could be an additional source of error in the analysis. Yet even with these potential sources of error, the four-cluster model still had high probabilities with an average of 77% for all ages and years.

By evaluating older age-classes, regions that are essential to the population and perseverance of a sustainable menhaden population have been identified. Additionally, the proportional stability of nursery contributions through age-classes suggests that the age and size-dependent migrations of menhaden may have a larger role in the persistence of this population than previously believed. We found that coast-wide nursery grounds contribute adults with disproportionate survivorship rates so that ages 2-3 were predominately produced in the Northeast and Southeast. Without data regarding changes in relative abundance from nursery grounds to the fishery, it is difficult to assess the cause of reduced survivorship in some regions, although the spatial structure of the commercial fishery could be a factor, as could changes in climate. Regardless, the Northeast and Southeast are producing a large fraction of the older age-classes and thus these regions contribute the adults that make up the spawning stock biomass for menhaden. This study offers insight that could be valuable to fisheries scientists and habitat conservationists for the assessment and management of this environmentally and economically important species.

CHAPTER V

CONCLUSIONS

Understanding the role of multiple nursery ground and the spatial and temporal population structure of marine fishes remains fundamental to the effective management and persistence of many estuarine-dependent species. Addressing these issues for the Atlantic Menhaden has long been recommended by its stock assessment and yet the scope and methods of accomplishing this task has proven to be challenging. Through this research, I evaluated juvenile menhaden otolith signatures from nursery grounds that encompass the species range, assessed the historical estimates of nursery contribution of new recruits, and determined the contribution of coast-wide nursery grounds through multiple age-classes, including the spawning stock. I demonstrated that otolith chemistry is an effective and useful tool for these purposes and it can provide valuable information to the assessment, understanding, and management of species such as menhaden.

The first objective in this research project was to determine if otolith chemistry could be used to differentiate between nursery grounds for menhaden. I addressed this by using trace element and stable isotope analysis to evaluate juvenile otolith signatures from nursery grounds over the species' range. By using regional groupings for the nursery grounds, I was able to successfully classify the juveniles to their known nursery region at nearly 90% accuracy over three years and show the utility of a combined signature for adjacent year-classes when data is not available. I found the greatest overlap in regional nursery grounds between the Northeast and Chesapeake Bay regions, an overlap that would provide challenges for classifying adults, but that overall, regions

separated reliably on an annual basis. These results emphasized the effectiveness of otolith chemistry approaches for discriminating between nursery regions and laid the foundation for analyzing the adult data set. Additionally, it was a significant contribution to the literature regarding coast-wide natal signatures, as few studies have attempted to do so over an extensive geographical range for multiple years.

Low recruitment has plagued the menhaden population since the 1990s and remains a fundamental challenge to properly managing this fishery. Historical estimates suggest that the Chesapeake Bay is primarily responsible for contributing new recruits, but the health of the Bay and anthropogenic activity in recent decades may have altered the ability of the Bay to maintain its contribution. By using otolith chemistry and the juvenile signatures established in Chapter II, I determined the nursery contribution of new recruits along the Atlantic coast. This study used different methods from the historical estimates, so the comparison between the two studies is not direct. Regardless, the stock assessment relies heavily on these historical estimates, so it was critical to update them quantitatively. This research suggests that the Chesapeake Bay does in fact contribute less than historical estimates, although the proportion varied slightly from year to year and depended on the number of clusters in the data set. The conclusions suggest that the Northeast may contribute almost as many new recruits as the Chesapeake Bay depending on the year, which suggests a dramatic shift from the historical belief that it produced less than 2%. The Southeast nursery region was also a significant contributor of new recruits, although this finding is less controversial and surprising than those of the Northeast contribution. The cause of this shift in nursery productivity cannot be determined in this study alone, but undoubtedly it is tied to environmental variability,

climate change, and habitat health, particularly in the Chesapeake Bay. Without absolute numbers of juveniles and new recruits in the population, we can only discuss these relative proportions of nursery contribution. It is possible that, in fact, the Northeast contributes the same number of recuits as it did historically but that the survivorship and contribution of Chesapeake Bay fish has decreased dramatically. Regardless, what we now know because of this research is that coast-wide nursery grounds are critical to recruitment of juvenile to the adult stock of menhaden and Chapter III identified nearly all of the nursery regions as being necessary for the persistence of this critical fish population.

New recruitment to the population of menhaden is the first step to ensuring a sustainable future for this population, but it does not tell the whole story. Adults must survive to reach maturity and join the spawning stock biomass so that the population is replenished. By evaluating older age-classes of menhaden, as we did in Chapter IV, a more complete picture of recruitment and spawning was revealed. The Chesapeake Bay was an important contributor of new recruits to the population, but this contribution did not hold for older age classes. Either the survivorship of Chesapeake Bay recruits has decreased or this study provided evidence that the structure of the commercial fishery is changing the adult structure of the population. The latter theory is supported by the absence of older fish in the menhaden population over the last several decades, the increased presence of age-1 fish in the commercial catches, and, possibly, the findings of this project. For the years analyzed, the Chesapeake Bay was not a significant contributor of adults ages 2-3. In fact, the Northeast and Southeast nursery grounds proportionally contributed the most adults. This is significant because ages 2+ make up the spawning

stock biomass. Therefore, the Northeast and Southeast are essential nursery areas to the continuation of this stock.

Efforts have been made to preserve estuaries and critical fish habitats all along the Atlantic coast, although most success stories come from regions outside of the Chesapeake Bay. The Bay continues to suffer from dead zones, decreased water quality, and habitat degradation. This research project provides more evidence that these are wide-reaching effects and almost no species in the region will go unaffected by human activity or climate change. The key to increasing recruitment and spawning stock biomass for the menhaden population may rest in the health of the Chesapeake Bay. And, as primary locations of juvenile development decrease their productivity, the role of spawning along the migratory route in seeding alternative nursery locations may ensure the future of the population, as evidenced by this research, which relies on preservation of age structure.

The power of these findings would be greatly enhanced with a paired physical oceanographic model of the Atlantic coast during the years of this study. By modeling the physical conditions such as the currents, temperature and salinity distribution, and the Gulf Stream during the winter spawning event and migratory months, the dispersion of larvae to nursery grounds along the coast could be estimated. A decreased likelihood of larvae reaching the Chesapeake Bay for the 2008-2011 cohorts could explain some of the trends observed in this research project. But if in fact physical conditions indicate that larvae is disproportionately advected toward the Bay rather than other regions, the issue of recruitment lies with either the structure of the fishery or the health of our estuaries

along the coast. Additionally, more thorough juvenile and adult abundance surveys in multiple regions could provide invaluable information to this discussion.

To our knowledge, this is the first study of this scope. Otolith chemistry is widely applied, particularly in the case of juvenile classification papers. Not only is this the first coast-wide application of otolith chemistry for menhaden, it is one of the few that exist for any species over its range. Additionally, there is no other study evaluating juveniles and adults through multiple age-classes for several years in a row. But apart from showing the utility of otolith chemistry for this analysis, this approach addressed critically needed information for an essential fish of environmental and economic value. The study reevaluated historical estimates and provided updated information regarding areas that are necessary for the continuation of this population, as well as indicated a substantial shift in previous assumptions about its population structure. Considerations regarding estuarine health, coastal development, and climate change, as well as the impact of the fishery on the age and size-structure on the menhaden population, remain central issues for the effective management of this species and this research provided insight for these necessary discussions.

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APPENDIX

LIBRARY VALUES

Table 14. Library values of trace element and stable isotopes for juvenile Atlantic Menhaden for the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB) and the Southeast (SE) nursery regions from 2009-2011. Values are expressed as mean value ± standard deviation.

Year	Region	Trace Elements & Stable Isotopes							
		Li (μmol)	Mg (μmol)	Rb (μmol)	Sr (mmol)	Y (μmol)	Ba (μmol)	δ13C (‰)	δ18O (‰)
2009	NE	3.85±1.14	313.41±255.69	0.23±0.19	1.15±0.24	0.02±0.01	6.27±5.45	-7.98±2.49	-5.43±1.87
	DB	2.55±1.21	157.43±53.86	0.27±0.13	1.01±0.33	0.02±0.00	11.05±14.40	-9.39±3.06	-7.19±1.70
	CB	2.89±1.43	266.93±889.13	0.20±0.14	1.08±0.22	0.02±0.00	5.28±4.06	-9.86±2.08	-5.96±1.06
	SE	1.88±1.47	182.32±102.97	0.25±0.21	1.02±0.38	0.02±0.01	6.93±3.25	-12.51±1.80	-4.42±0.94
2010	NE	2.98±1.84	198.31±130.63	0.28±0.15	0.84±0.40	8.01±5.36	0.15±0.13	-11.55±3.98	-8.34±3.10
	DB	3.32±1.38	131.20±41.39	0.22±0.08	1.07±0.21	6.90±9.23	0.38±0.59	-8.17±2.15	-6.98±1.23
	CB	2.75±0.89	123.38±75.66	0.22±0.09	1.18±0.26	8.63±11.39	0.21±0.58	-9.87±1.71	-6.88±1.12
	SE	2.83±4.15	107.96±49.79	0.24±0.15	1.14±0.31	14.55±13.33	0.23±0.27	-12.72±2.42	-5.27±1.41
2011	NE	7.26±6.46	410.30±208.75	0.29±0.26	1.11±0.30	7.27±9.00	0.15±0.20	-9.19±3.06	-5.93±2.10
	DB	3.01±2.31	188.59±52.90	0.34±0.09	0.76±0.26	20.97±20.96	0.09±0.07	-11.93±2.37	-8.83±0.85
	CB	4.84±3.35	256.15±139.96	0.22±0.16	1.18±0.19	5.68±4.59	0.17±0.26	-9.63±1.25	-6.18±1.26
	SE	8.70±7.10	187.15±105.71	0.19±0.13	0.99±0.15	10.11±9.75	0.11±0.11	-11.41±3.13	-4.38±1.19

VITA

Kristen Anstead was raised in Maine where she attended Bates College in Lewiston. She graduated in 2002 with a B. S. in Biology with a secondary concentration in Anthropology. In 2011, she joined the Center for Quantitative Fisheries Ecology at Old Dominion University as a doctoral candidate in the Oceanography degree program (4600 Elkhorn Ave., Norfolk, VA 23529) under the advisement of Dr. Cynthia M. Jones.