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ARTICLE

Coastwide Otolith Signatures of Juvenile Atlantic Menhaden, 2009–2011

Kristen A. Anstead*
Center for Quantitative Fisheries Ecology, Old Dominion University, 800 West 46th Street, Norfolk, Virginia 23508, USA

Jason J. Schaffler
Center for Quantitative Fisheries Ecology, Old Dominion University, 800 West 46th Street, Norfolk, Virginia 23508, USA; and Virginia Marine Resources Commission, 2600 Washington Avenue, Newport News, Virginia 23607, USA

Cynthia M. Jones
Center for Quantitative Fisheries Ecology, Old Dominion University, 800 West 46th Street, Norfolk, Virginia 23508, USA

Abstract

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 U.S. East Coast. Along with a decrease in overall numbers and spawning stock biomass, recruitment levels have remained low since the 1990s. Atlantic Menhaden use numerous estuaries along the Atlantic coast for juvenile development before recruiting to the adult population, but the contribution of each of these nursery grounds is currently unknown. Chesapeake Bay is thought to contribute 70% of the total recruits, although this estimate is over 20 years old and predates current low recruitment levels. We investigated the potential of trace element (Li, Mg, Mn, Rb, Sr, Y, Ba, and Pb) and stable isotope ratio ($\delta^{13}C$ and $\delta^{18}O$) signatures in otoliths to distinguish among Atlantic Menhaden collected from various nursery grounds along the U.S. Atlantic coast (Connecticut to South Carolina) during 2009–2011. Juveniles were classified to four regional nursery areas with nearly 90% accuracy. Due to significant interannual variation in the chemical signatures, our attempts to classify juveniles from adjacent year-classes or combined year-classes resulted in lower accuracy. However, this study provides a 3-year library of geochemical fingerprints for assigning adults to their regions of origin. This research builds the foundation for a comprehensive estimate of Atlantic Menhaden recruitment rates from each of the major nursery areas along the U.S. Atlantic coast for 2009–2011.

An understanding of population spatial structure is essential when making predictions about a marine fish species' resilience and persistence. This is especially important for the Atlantic Menhaden *Brevoortia tyrannus*, a clupeid species that plays a critical role in the ecosystem, supports the largest fishery in Chesapeake Bay, and suffers from overfishing (ASMFC 2012). As filter feeders and consumers of primary production, Atlantic Menhaden contribute to water quality and nutrient cycling. Additionally, the Atlantic Menhaden is a key prey species for several commercially and recreationally valued predators, including the Bluefish *Pomatomus saltatrix*, Weakfish *Cynoscion regalis*, and Striped Bass *Morone saxatilis*. The most recent stock assessment by the Atlantic States Marine Fisheries Commission (ASMFC 2012) indicates that along with a decrease in overall numbers and spawning stock biomass, Atlantic Menhaden recruitment levels have remained low since the 1990s. The cause of declining recruitment is currently unknown, although overfishing, habitat degradation,

*Corresponding author: kanstead@odu.edu
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and climate change are considered to be significant factors (Lozano et al. 2012). In critical nursery areas, such as Chesapeake Bay, much of the coastline has become heavily developed, and vast regions are characterized by hypoxia, habitat degradation, and decreased productivity (Kemp et al. 2005). A full assessment of the relationships among population structure, larval supply, and recruitment along the Atlantic coast will aid in evaluating the persistence and resilience of Atlantic Menhaden and in identifying areas that are essential for survivorship.

The life history of Atlantic Menhaden is directly influenced by the spatial structure of the population. Adults undergo extensive seasonal migrations along the Atlantic coast and are distributed from Nova Scotia to Florida. The population segregates by size and age during the summer months: older (age $\geq$ 3), larger fish are capable of migrating to the northern part of the species' range, whereas younger, smaller fish remain in the southern part of the range (Nicholson 1978). Atlantic 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). Spawning occurs from late fall through early spring along the migratory route; however, the majority of spawning takes place in the winter, when the population aggregates off Cape Hatteras, North Carolina (Nicholson 1978; Lewis et al. 1987). After spawning on the coastal shelf, Atlantic Menhaden rely on ocean circulatory patterns to supply their larvae to the juvenile nursery grounds in estuaries. Research indicates that oceanic patterns influence larval dispersal to estuaries along the U.S. Atlantic coast such that some spawning locations have greater contributions 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 on coastwide nursery use by juvenile Atlantic Menhaden as well as their 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 fish population spatial structure and connectivity (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, 2001; Dorval et al. 2005, 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 the 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, interannual 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 periods (4–13 years; Campana et al. 2000). Therefore, establishing the temporal stability of geochemical fingerprints and building a multiyear library for signatures are necessary when using otolith chemistry for classification of several year-classes.

When constructing geochemical fingerprints, both trace element and stable isotope analyses are effective tools for isolating region-specific signatures in fish from various water masses. Elements such as Sr and Ba reflect regional differences in salinity and temperature, whereas Li, Mn, and others have been shown to add meaningful information for 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 stable isotope ratios of carbon ($\delta^{13}C$) and oxygen ($\delta^{18}O$). Oxygen isotopes are deposited on the otolith nearly in equilibrium with the ambient waters and reflect a relationship with temperature and water source; carbon isotopes are deposited under nonequilibrium 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 the chemical signatures of otoliths and distinguishing between fish from different geographic regions.

We evaluated the chemical signatures in otoliths of juvenile Atlantic Menhaden collected from the major nursery grounds along the U.S. Atlantic coast. If juvenile signatures are distinct among nurseries, then adult Atlantic Menhaden that are caught in the fishery could be assigned back to their regions of origin, and each major nursery's contribution to the adult stock could be quantified. Such information would be of great value for management of Atlantic Menhaden. It is believed that Chesapeake Bay contributes 70% of the recruits to the fishable stock of Atlantic Menhaden, but that estimate is over 20 years old and predates current low recruitment levels (Ahrenholz et al. 1989). The coastwide juvenile contribution of each major estuary to the adult stock is unknown; therefore, the impacts of a changing environment and fishing practices cannot be predicted. To identify areas that are essential for the persistence and resilience of Atlantic Menhaden, the relationship between population structure, larval supply, and recruitment along the Atlantic coast must be fully assessed. As a first step toward that goal, the objectives of our study were to (1) identify the chemical signatures of sagittal otoliths collected from the major nursery areas used by Atlantic Menhaden; (2) evaluate these chemical fingerprints over several years to provide information on interannual variability among fish sampled from the nursery locations; and (3) make recommendations

about how often signatures should be collected to obtain correct classification of adults.

METHODS

Juvenile Atlantic Menhaden were collected during 2009–2011 from the Thames and Essex rivers in Connecticut; the Hudson River in New York; Delaware Bay in Delaware; the Potomac, Patuxent, Choptank, and Nanticoke rivers in Maryland; the James River in Virginia; Albemarle Sound in North Carolina; and Charleston Harbor in South Carolina. All samples were collected by state natural resource agencies (see Acknowledgments) from July to October, with the goal of obtaining at least 30 samples annually from each area for the 3 years of the 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: Northeast, Delaware Bay, Chesapeake Bay, and Southeast (Figure 1). The regions are similar to those used in other studies of this scope based on physical differences in water chemistry (Thorrold et al. 1998; Schaffler et al. 2009). Juvenile Atlantic Menhaden were frozen after capture and were transported to the laboratory; we then measured FL (mm) and removed sagittal otoliths (length = 0.62–2.59 mm) in a class-100 clean room using acid-washed glass probes. Excess tissue was cleaned from the otolith surface by rinsing with ultrapure hydrogen peroxide for 1 min followed by triple rinsing with ultrapure Milli-Q water. Cleaned samples were dried for 24 h under a laminar-flow hood and were stored in acid-washed polyethylene vials. One sagittal otolith from each pair was selected randomly for trace element analysis, and the other otolith was used for stable isotope analysis.

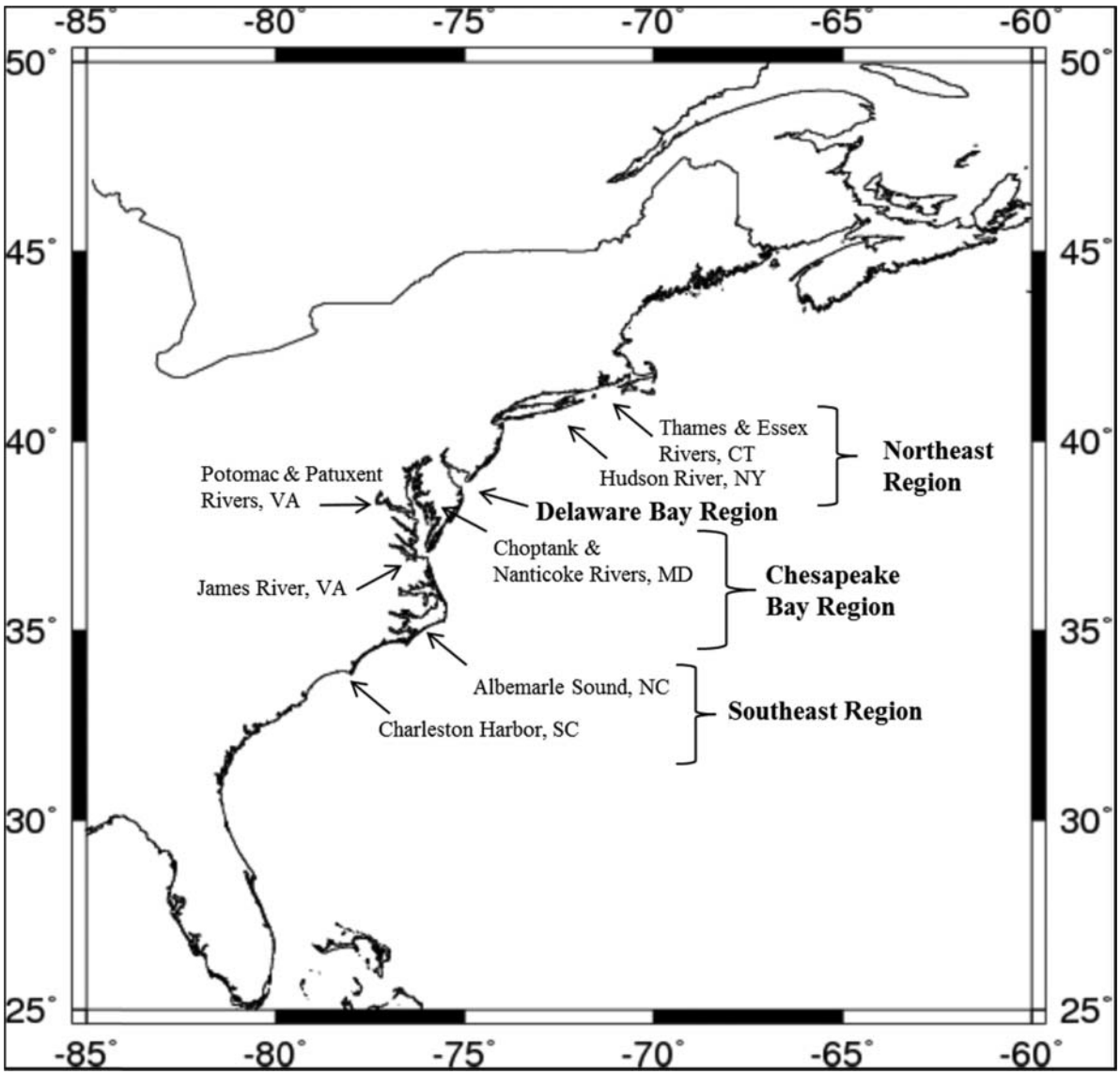

FIGURE 1. Locations of juvenile Atlantic Menhaden collection along the U.S. East Coast.

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

Samples that were selected for analyses of trace element composition were mounted sulcal side up on a glass slide with Crystal Bond and were polished with 30-μm lapping film to expose growth rings, followed by polishing with 0.3-μm lapping film to produce a smooth surface for laser ablation. Fish age was also verified 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 water (18 MΩ/cm) for 10 min to remove contaminants from the surface, and the slide was allowed to dry under a laminar-flow hood. Otoliths were analyzed using a Thermo Finnegan Element 2 (Thermo-Fisher Scientific) inductively coupled plasma mass spectrometer with a New Wave 193-nm excimer laser ablation system (New Wave Research, Sunnyvale, California) at the Plasma Mass Spectrometry Facility, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. Otolith material was ablated using a laser beam with a 25-μm spot size, 10-μm/s scan speed, 70% power, and 10-Hz frequency. To capture the otolith signature, we ablated and analyzed a transect from the otolith core to the otolith edge, resulting in a trench that was approximately 25 μm wide × 30 μm deep. 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 by 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 : Ca molar ratios (Schaffler and Winkelman 2008). Standards were run twice per slide (at the beginning and the end) to account for machine drift. Blanks were analyzed after every fifth sample; limits of detection (LODs) were calculated as the mean blank value plus 3 SDs (Thorrold et al. 1997b) and were expressed as a percentage of the average sample intensity.

Trace element and stable isotope data were combined to identify natal signatures. Data were normalized using Box–Cox transformations (Box and Cox 1964). We assessed normality by using the Kolmogorov–Smirnov test, and we examined for equality of variances by using O'Brien's test. Assumptions of multivariate normality were evaluated with tests based on Mardia's multivariate skewness and kurtosis measures (Khattree and Naik 2000) and were assessed graphically using *Q*–*Q* plots of squared Mahalanobis distances. We performed multivariate ANOVAs (MANOVAs) to detect differences in the multivariate elemental signatures for each nursery region and for all cohorts. Pillai's trace statistic was used to quantify significant differences in otolith chemistry among nursery areas and among years. After completing these analyses, we used univariate ANOVAs to determine which elements exhibited differences. When significant differences among nursery grounds were observed, a quadratic discriminant function analysis was used to assign juveniles to their nursery areas because of the unequal variance–covariance matrices as indicated by Bartlett's test. We tested this classification using a jackknife leave-one-out cross-validation approach within years and among years to assess the ability of annual signatures and combined signatures to predict the signatures of other year-classes. Additionally, we tested the classification success based solely on either trace element data or stable isotope data. Canonical discriminant analysis (CDA) was used to visualize differences among nursery locations.

RESULTS

We analyzed 312 juvenile Atlantic Menhaden sampled in 2009, 237 juveniles sampled in 2010, and 161 juveniles sampled in 2011 (Table 1). We obtained at least 30 samples/region for all years, with the exception of the Delaware Bay region in 2009 ($n = 26$). Average FL was 80.2 mm for all juveniles collected, but FL varied among regions and among years due to differences in sample size and the timing of collection. Fish

TABLE 1. Sample size and mean FL ± SE of juvenile Atlantic Menhaden collected from each of the four sampling regions along the U.S. Atlantic coast, 2009–2011.

Year	Region	*N*	FL (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
All years		710	80.2 ± 0.9

TABLE 2. Limits of detection (LODs; calculated as mean blank value plus 3 SDs) for each element analyzed with laser ablation inductively coupled plasma mass spectrometry in low-resolution mode. Trace element concentrations were calibrated by using Japanese (JPN) and National Research Council (NRC) reference standards. For carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$), RSD is the average precision of all samples within each year.

		2009				2010				2011			
Element or isotope	Units	JPN RSD	NRC RSD	LOD	% > LOD	JPN RSD	NRC RSD	LOD	% > LOD	JPN RSD	NRC RSD	LOD	% > LOD
Li	μmol	6.0	7.3	44.4	93	3.9	5.3	67.9	86	3.7	3.9	21.1	95
Mg	mmol	6.5	7.3	6.3	100	2.5	3.0	17.5	100	4.1	3.4	17.1	96
Ca	mmol	7.0	8.1	0.4	100	2.5	3.2	0.8	100	3.2	4.0	1.0	100
Mn	μmol	7.0	7.8	90.1	81	2.2	2.9	94.3	66	3.0	4.3	92.7	75
Rb	μmol	7.0	7.8	21.4	96	2.1	2.6	47.2	95	2.4	3.8	51.8	91
Sr	mmol	7.3	8.2	0.9	100	2.2	2.8	1.0	100	2.9	3.7	1.3	100
Y	μmol	7.1	8.2	27.6	99	2.3	2.7	57.7	98	6.6	7.8	38.7	100
Ba	μmol	7.3	8.1	5.6	100	1.8	2.5	6.6	100	2.4	3.4	7.8	100
Pb	μmol	6.9	7.6	39.2	92	1.4	1.7	49.0	96	3.8	2.4	70.0	71
$\delta^{13}C$	‰	0.0076		–	–	0.0029		–	–	0.0043		–	–
$\delta^{18}O$	‰	0.0095		–	–	0.0055		–	–	0.0072		–	–

sampled from the Northeast, Delaware Bay, and Southeast regions were typically collected over a short period early in the season; these fish were smaller in size, and FLs were not significantly different among the three regions ($P = 0.99$). Samples of Chesapeake Bay juveniles were more numerous, were collected over a more extended time frame, and were larger in size; the FLs of these fish were significantly different from the FLs of fish from the other regions ($P < 0.001$). Overall, juveniles collected in 2010 were significantly larger ($P < 0.001$) than those collected in 2009 and 2011. Average FLs were not different between 2009 and 2011 ($P = 0.688$).

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). Due to the large number of samples below the LODs, both Pb and Mn were eliminated from further analyses. The precision of $\delta^{13}C$ and $\delta^{18}O$ measurements was high and the SD was low for the 3 years (Table 2), so $\delta^{13}C$ and $\delta^{18}O$ were used in all analyses. Raw data did not exhibit normal distributions, so Box–Cox transformations were used to normalize the data and homogenize the variances. The resulting lambda values of the transformations were highly variable (Table 3), which was expected based on the fluctuating nature of these elements (both spatially and temporally) in the environment. Because of this variability, some of the variables still did not meet univariate normality or the assumption of equal variances even after being transformed. The transformation of Mg data achieved normality for the 2009–2011 data sets; however, Rb, Sr, and $\delta^{13}C$ data violated the assumption of normality for 1 of the 3 years, while Li, Y, Ba, and $\delta^{18}O$ data violated the normality assumption for 2 of the 3 years. Mardia's test was used to evaluate multivariate skewness ($P < 0.001$) and kurtosis ($P = 0.164$) and indicated

TABLE 3. Lambda values from Box–Cox transformations used to address assumptions of equality of variance (O'Brien's test) and univariate normality (Kolmogorov–Smirnov [K–S] test) in otolith chemistry data for juvenile Atlantic Menhaden collected in four nursery regions, 2009–2011. O'Brien's and K–S test results are presented as *P*-values. A significance level α of 0.05 was used for all tests.

	2009			2010			2011		
Element or isotope	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
$\delta^{13}C$	1.217	0.000	<0.01	1.116	<0.001	0.12	1.406	0.000	0.15
$\delta^{18}O$	1.531	<0.001	0.02	0.965	<0.001	0.07	1.091	0.001	<0.01

that the data set was skewed and deviated from multivariate normality. Although these data were nonnormal, they were close to normal after transformation, and the tests were robust enough to accommodate this.

Statistical analyses were applied to all stable isotope data and trace elements that were above the LODs. Multivariate ANOVAs indicated a significant year effect (Pillai's trace = 1.1178; $F_{22,\ 1,390} = 80.06$; $P < 0.001$) and a significant regional effect (Pillai's trace = 0.9795; $F_{33,\ 2,088} = 30.67$; $P < 0.001$) for juvenile Atlantic Menhaden collected during 2009–2011. The ANOVA results indicated that in 2009 and 2010, all elements analyzed were significantly different among regions ($P < 0.001$), with the exception of Rb (2009: $P = 0.083$; 2010: $P = 0.122$). In 2011, all elements analyzed were significantly different among regions ($P < 0.001$; Figure 2).

For all 3 years, a quadratic discriminant function was employed to build the classification function (2009: $\chi^2 = 212.67$, df = 30, $P < 0.001$; 2010: $\chi^2 = 193.39$, df = 30, $P < 0.001$; 2011: $\chi^2 = 350.02$, df = 84, $P < 0.001$). 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 $\delta^{13}C$ and $\delta^{18}O$ contributed to regional separation for all 3 years, whereas the trace elements used in the discriminant function varied. The elements identified as achieving the highest accuracy for classification of juveniles sampled in 2009 were $\delta^{13}C$, $\delta^{18}O$, Li, and Ba. For 2010 samples, the elements used to build the multivariate signatures were $\delta^{13}C$, $\delta^{18}O$, Mg, and Sr. For juveniles sampled in 2011, the multivariate signature was based on $\delta^{13}C$, $\delta^{18}O$, Mg, Sr, Rb, Li, and Ba. Using these respective signatures, we were able to correctly assign juveniles to their nursery grounds at a rate of 87% for 2009 samples, 88% for 2010 samples, and 89% for 2011 samples (Table 4).

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

When using the year-class-specific signatures, the most common misclassifications occurred between juveniles from the Chesapeake Bay and Northeast regions, particularly for the 2009 samples. Juveniles from the Delaware Bay and Southeast regions consistently had correct assignments for all 3 years. The results of CDA reinforced these findings. The CDA showed separation among the four regions for 2009–2011 (Figure 3). The first two canonical axes of the CDA plot indicated that the Delaware Bay and Southeast regions were particularly well separated but that there was some spatial overlap between the Chesapeake Bay and Northeast regions, confirming the conclusions from the discriminant analysis that the most errors occurred between these two regions.

DISCUSSION

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

Few studies have attempted to build a multiple-year library of coastwide signatures for a single species. Thorrold et al. (1998) analyzed the coastwide elemental signatures of juvenile Weakfish and found regional groupings and classification rates similar to those presented here. Schaffler et al. (2009) established distinct otolith signatures in larval Atlantic Croaker *Micropogonias undulatus* collected from Delaware to North Carolina. However, neither Thorrold et al. (1998) nor Schaffler et al. (2009) analyzed multiple year-classes. Several studies have established interannual variability in otolith chemistry, but most of those studies focused on localized areas or stocks (Campana et al. 2000; Gillanders 2002; Walther and Thorrold 2009; Schaffler et al. 2014). Walther et al. (2008) evaluated the stable isotope and elemental signatures of American Shad *Alosa sapidissima* in samples from New Hampshire to Georgia over 3 years to determine natal signatures and estimate the rates of straying between rivers. Using signatures that varied from year to year, Walther et al. (2008) were able to correctly classify American Shad to their natal rivers approximately 91% of the time. Schaffler et al. (2014) used otolith chemistry to classify juvenile Atlantic Menhaden to upper, middle, and lower Chesapeake Bay nursery grounds with 85% accuracy for 2005 samples and with 95% accuracy for 2006 samples. Due to our sample collection methods, we could not achieve this regional specificity with our data set; however, much like Walther et al. (2008) did for American Shad, our research provides a multiple-year library of geochemical fingerprints for Atlantic Menhaden across the species' range (Table A.1). Additionally, this library could be

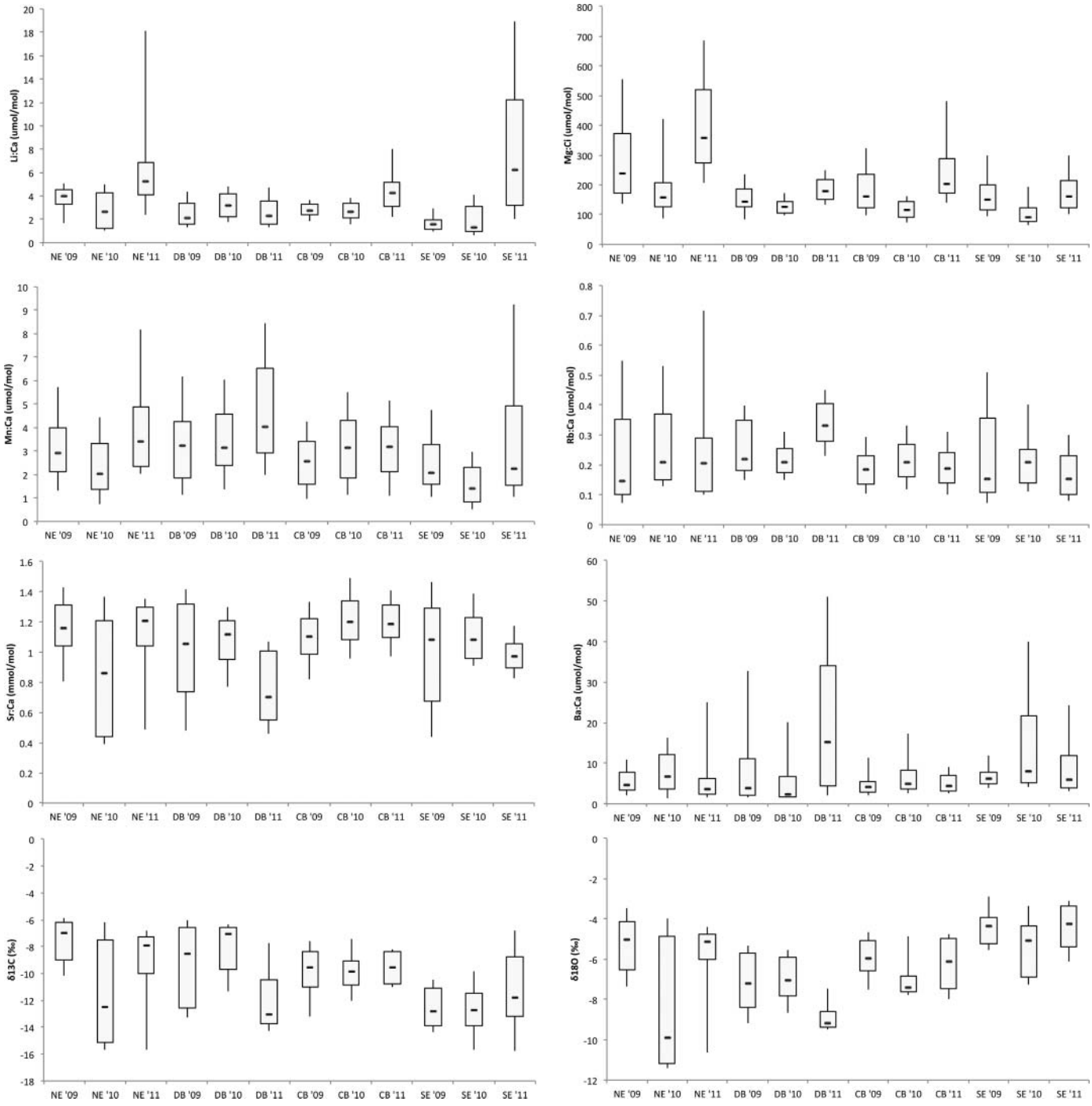

FIGURE 2. Box plots of untransformed concentrations of Li, Mg, Mn, Rb, Sr, and Ba (expressed as element : Ca ratios) and mean carbon and oxygen stable isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) in otoliths of juvenile Atlantic Menhaden sampled from the Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB), and Southeast (SE) regions, 2009–2011 (dash within box = median; ends of box = 25th and 75th percentiles; whiskers = 10th and 90th percentiles).

combined with the results of Schaffler et al. (2014) for more accurate adult classification in future studies.

We found that geochemical fingerprints vary from year to year and that no one group of elements is responsible for discriminating among nursery regions along the Atlantic coast. The four nursery regions are vastly different in size, drainage basins, and responses to environmental influences; although there was some overlap in otolith chemical signatures, juvenile Atlantic Menhaden from these regions exhibited significantly different signatures in all 3 years. The variation in elements is consistent with the results of other studies that have evaluated the spatial and temporal composition of otoliths and most

TABLE 4. Correct classifications and misclassifications based on otolith chemical signatures (trace element and stable isotope concentrations) for juvenile Atlantic Menhaden sampled from four nursery regions during 2009–2011: Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB), and Southeast (SE). Values in bold italics (on the diagonal) indicate the number of correctly classified fish.

	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

likely reflects changes in ambient water chemistry (Gillanders 2002; Schaffler and Winkelman 2008; Walther and Thorrold 2009). All trace elements and stable isotopes analyzed from the otoliths (except Rb in 2009 and 2010) were significantly different among regions and among the 3 years. Discriminant analysis and stepwise variable selection showed that using all of the trace element and stable isotope data resulted in a lower classification rate relative to that obtained by focusing on a reduced set of variables. Combinations of $\delta^{13}C$, $\delta^{18}O$, Li, Ba, Sr, Mg, and Rb data were important contributors to classifying Atlantic Menhaden for 2009–2011, and the signatures resulted in classification rates of nearly 90% for all 3 years.

The trace elements that were effective for differentiating along latitudinal gradients in this study were consistent with the findings of previous research. Strontium and Ba often prove to be valuable for classifying juveniles to distinct locations (Gillanders 2002; Wells et al. 2003; Brazner et al. 2004; Munro et al. 2005; Ludsin et al. 2006; Schaffler and Winkelman 2008; Walther et al. 2008). The reliability of Sr and Ba in building chemical signatures for coastal nursery grounds is due to their relationship with both salinity and temperature. Fowler et al. (1995) demonstrated a positive correlation between Sr and salinity, while Dorval et al. (2005) found that Ba in the otolith decreased with increasing salinity. Temperature also influences the incorporation of Sr into the otolith, although this has been debated in the literature (Townsend et al. 1989; Fowler et al. 1995; Dorval et al. 2005). However, because both Ba and Sr vary among habitats and in relation to salinity, they provide valuable information for establishing fingerprints (Campana 1999). Magnesium is also useful in otolith chemistry research (Gillanders 2002; Brazner et al. 2004), but the relationship between Mg uptake in otoliths and the Mg concentration in 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. Although these elements contributed to the chemical signatures in otoliths of juvenile Atlantic Menhaden, it is worth noting that the use of trace element data without stable isotope data resulted in a decrease of classification rates from 88% to 64%, on average, for 2009–2011.

TABLE 5. Correct classification percentages for juvenile Atlantic Menhaden sampled along the U.S. Atlantic coast during 2009–2011. The fish were classified using functions developed from each year-class individually and all year-classes combined; values in bold italics are 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

The discriminant function identified $\delta^{13}C$ and $\delta^{18}O$ as contributing significantly to geochemical fingerprints for all 3 years. Similar to the findings of Walther et al. (2008), we found that $\delta^{18}O$ was significantly different among regions and appeared 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 $\delta^{13}C$ analysis are more difficult to interpret, but $\delta^{13}C$ still appeared to contribute to regional separation. For juvenile Atlantic Menhaden sampled during the 3 years of this study, both $\delta^{18}O$ and $\delta^{13}C$ were valuable in correctly classifying the fish to nursery regions, but classification accuracy was lowered from 88% to 76%, on average, when the stable isotope data were considered without the trace element data. The use of only stable isotopes resulted in Chesapeake Bay juveniles being misclassified to the Northeast region and Northeast juveniles being misclassified to the Delaware Bay region at higher rates

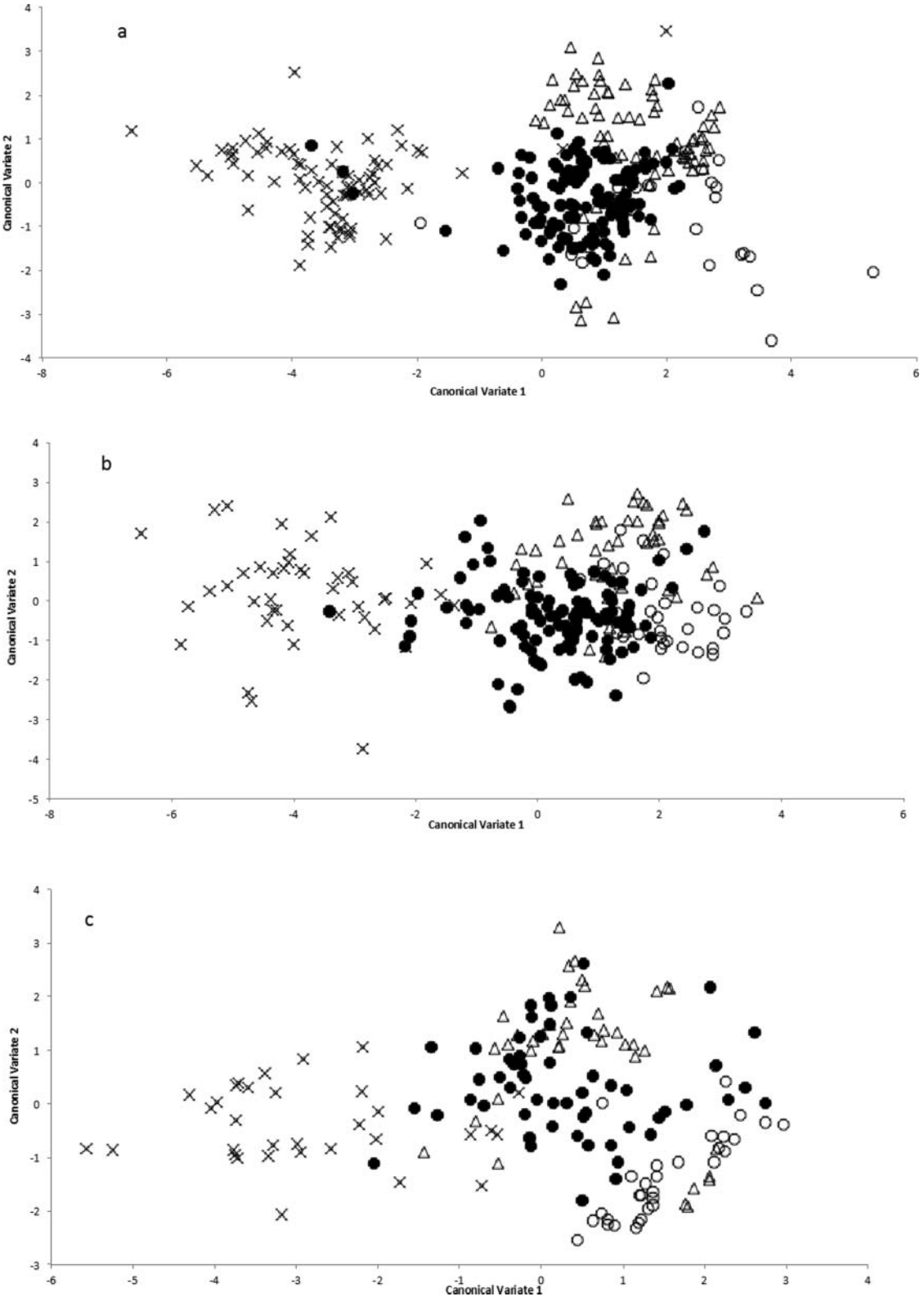

FIGURE 3. Canonical variates 1 and 2 summarizing variation in trace element and stable isotope ($\delta^{13}C$ and $\delta^{18}O$) signatures for otoliths of juvenile Atlantic Menhaden collected from the Northeast (open triangles), Delaware Bay (open circles), Chesapeake Bay (shaded circles), and Southeast (× symbols) regions in **(a)** 2009, **(b)** 2010, and **(c)** 2011.

than observed when stable isotopes and trace elements were used together. Therefore, analysis of trace elements in combination with stable isotopes is useful for increasing classification rates and distinguishing among nursery grounds, particularly for the Northeast and Chesapeake Bay regions.

Due to the variability in otolith chemical signatures from year to year, borrowing signatures from adjacent year-classes decreased accuracy significantly. For example, we found very low accuracy in classification when using the 2009 signatures to classify juveniles collected in 2010 or 2011. This decrease in classification from 88% to 60%, on average, was consistent throughout the study, indicating sufficient temporal variability to obscure classification. Additionally, the use of combined elemental data from all 3 years also resulted in a decline in accuracy from 88% to 82%, on average, relative to the use of single year-classes. This interannual variability was expected, as otolith chemistry reflects changing environmental conditions and variation 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. 2014). There is some support for pooling years together for juveniles when attempting to classify individuals (Walther and Thorrold 2009; Schaffler et al. 2014), and this study showed an approximately 6% decrease in classification accuracy when a combined signature was used for juvenile Atlantic Menhaden. Therefore, a combined signature provides little decrease in accuracy and could prove useful, particularly in data-poor situations. Nevertheless, the use of year-class-specific geochemical fingerprints is still the most accurate approach when attempting to classify the nursery origin of an adult Atlantic Menhaden with the highest accuracy.

This is the first quantitative coastwide study to demonstrate the utility of otolith elemental analysis for discriminating among Atlantic Menhaden nursery regions and provides a 3-year library for the geochemical fingerprints of juveniles. Our research builds the foundation for comprehensive estimation of recruitment rates from each of the major nursery areas as well as identification of essential areas for the Atlantic Menhaden population. This information is of vital importance to the effective management of this fishery, which has suffered from low recruitment, low spawning stock biomass, and low overall numbers in recent decades.

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APPENDIX: LIBRARY OF TRACE ELEMENT AND STABLE ISOTOPE RATIO VALUES FOR JUVENILE ATLANTIC MENHADEN

TABLE A.1. Library of trace element and stable isotope ratio values (mean ± SD) from the otoliths of juvenile Atlantic Menhaden sampled in four nursery regions during 2009–2011: Northeast (NE), Delaware Bay (DB), Chesapeake Bay (CB), and Southeast (SE).

		Trace elements						Stable isotope ratios	
Year	Region	Li (μmol)	Mg (μmol)	Rb (μmol)	Sr (mmol)	Y (μmol)	Ba (μmol)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)
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