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Spatial variation in otolith chemistry of Atlantic croaker larvae in the Mid-Atlantic Bight

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ABSTRACT: Larval Atlantic croaker *Micropogonias undulatus* (1 to 7 mm in standard length) were collected on the east coast of the United States from North Carolina to Delaware during 2000. We defined 3 water-mass boundaries for potential groups of spawning Atlantic croaker using temperature and salinity measured at each sampling station. We tested the hypothesis that distinct otolith chemistries existed among 3 groups of larval Atlantic croaker collected from these water masses using solution-based inductively coupled plasma-mass spectrometry. Multivariate analysis of variance indicated that otolith chemistry differed significantly among water masses. Using a quadratic discriminant function, we were able to correctly classify fish from the Mid-Atlantic Bight (MAB) 73% of the time, South Atlantic Bight (SAB) 53% of the time, and Chesapeake Bay plume 36% of the time. The correct reclassification rates observed were significantly better than random. Results from this study indicate that it is possible to obtain measurable elemental concentrations from otoliths much smaller than previously analyzed (weight 0.015 to 1.976 µg). Moreover, contrary to previous studies, our results indicate that it is possible to distinguish natal signatures among larvae on different spawning grounds in the MAB and SAB. Further, this new information could benefit investigations of dispersal from offshore spawning grounds to estuaries or other nursery habitats.

KEY WORDS: Larvae · Otolith chemistry · Microchemistry · Connectivity · Spatial variability · Solution · Laser · ICP-MS

INTRODUCTION

Atlantic croaker *Micropogonias undulatus* are common inshore demersal fish distributed along the Atlantic and Gulf of Mexico coasts of the United States (Chao & Musick 1977), which support important commercial and recreational fisheries (Mercer 1987). In the Mid-Atlantic Bight (MAB), peak spawning occurs from August to September over the continental shelf (Nixon & Jones 1997) where oceanographic processes result in larval transport to bays and estuaries (Cowan & Shaw 1988, Norcross 1991). Because transport distances for Atlantic croaker larvae may exceed 100 km and may take more than 60 d (Warlen 1981), there is a high probability of mixing among discrete groups of larvae spawned over the continental shelf. Indeed, the low heterogeneity of mitochondrial DNA among Atlantic croaker collected along the Atlantic coast of the United States suggests this is the case (Lankford et al. 1999).

Genetic studies have been very useful for determining the population structure of many species; however, for species with planktonic larvae, genetics have shown limited value. Likewise, genetic studies often underestimate the degree of population structure present in species that have discrete nursery areas but where adults are widely ranging (Ferguson & Danzmann 1998). This is because relatively little gene flow between groups of organisms is required to maintain genetic homogeneity in populations that are otherwise ecologically distinct (Kimura & Maruyama 1971). Measuring connectivity in these populations has been problematic because estimates of connectivity are most effective when genetic subdivisions are pronounced (Hedgecock et al. 2007). In contrast, otolith chemistry has shown spatial structure when genetics

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have failed (Thorrold et al. 2001), thus allowing measurements of demographic connectivity despite limited genetic divergence. Spatial structure shown by otolith chemistry may indicate incipient reproductive isolation and genetic structure that is not detectable in neutral markers or spatially discrete spawning in distinct water masses within a panmictic population (because there is considerable movement of larvae or adults among spawning locations). Otolith chemistry is a rapidly growing field of research that relies on an otolith to chronologically record exposure to environmental differences in a metabolically inert form (Campana 1999, Gillanders 2002). Otolith chemistry makes no assumption about the genetic structure of a population, but merely relies on the otolith to record differences in the chemical environment to which different portions of the population of interest have been exposed.

Otolith chemistry is proving to be a valuable tracer of fish stocks because of the relationship between trace element concentrations in the otolith and the environment (Wells et al. 2003, Dorval et al. 2007). Thorrold et al. (1997) demonstrated that the elemental signatures in otolith cores of juvenile Atlantic croaker did not differ between juveniles collected in the Elizabeth River in Chesapeake Bay and juveniles collected in the Neuse River in Pamlico Sound. This result may indicate that larvae in these 2 systems originated from the same water mass on the continental shelf, that chemical differences between the water masses where these 2 groups of larvae originated was not sufficiently different to leave detectable differences in the otolith chemistries of each group (e.g. Stransky et al. 2005), or that the technique used could not resolve subtle differences that were imprinted to otoliths from larvae in each region. Here we attempted to resolve these 3 possibilities by sampling larvae closer to the time of spawning and analyzing their otoliths using a technique that can resolve more subtle differences.

Many studies have used laser-ablation inductively coupled plasma-mass spectrometry (ICP-MS) to examine natal signatures in the core regions of juvenile and adult otoliths with varying degrees of success (Campana et al. 1994, Thorrold et al. 1997, Stransky et al. 2005). One of the limitations of these studies is the assumption that collections of adults or juveniles that are in spatially discrete patches resulted from spawning events in spatially discrete areas. However, this is not necessarily true, as spatially discrete patches of juveniles or adults could have resulted from the transport of larvae from multiple source locations. Therefore, determination of nursery areas is complicated by the fact that population mixing can occur between natal areas and juvenile or adult capture locations, thus distorting the true natal signature and hindering our ability to make inferences about processes occurring during the larval stage (e.g. Thorrold et al. 1997). As analytical precision with laser-ablation systems has progressed (Jones & Chen 2003), the ability to analyze smaller otoliths with increased temporal resolution has resulted in a better ability to discriminate populations (FitzGerald et al. 2004, Warner et al. 2005, Ludsin et al. 2006). Sampling larval fish on their natal grounds has resulted in a greater ability to discern the true natal signature, because fish are captured before dispersal occurs. Therefore, the potential for analyzing otolith material not associated with spawning areas that was incorporated near the core is eliminated. Most studies have relied on laser-ablation ICP-MS for these analyses and have not investigated the use of solution-based ICP-MS (see Ludsin et al. 2006). However, solution-based ICP-MS is generally considered to be superior to laser-ablation ICP-MS in terms of both precision and sensitivity (Fowler et al. 1995, Campana 1999).

The objective of this study was to determine if the otolith chemistry of larval Atlantic croaker collected from their natal waters in the southern MAB and northern South Atlantic Bight (SAB) shows spatial variation using solution-based ICP-MS. Four major water masses are found on the Mid-Atlantic shelf off Virginia and the South Atlantic shelf off North Carolina. Chesapeake Bay plume (CB) water is characterized by salinity <30‰ (Boicourt et al. 1987, Reiss & McConaugha 1998), Mid-Atlantic shelf water is characterized by salinities of 32 to 34‰ (Manning 1991), and Mid-Atlantic Cold-Pool Water (CPW) is characterized by temperatures <10°C (Houghton et al. 1982). CPW is found below the pycnocline on the outer shelf throughout the MAB. South of Cape Hatteras, North Carolina, a water mass distinctly different from MAB shelf water is SAB water, which is characterized as warmer and more saline then MAB water (Pietrafesa et al. 1994). If successful, our method will represent a powerful tool to identify source locations, and thus connectivity, for many marine fishes whose larvae are spawned offshore and transported to bays and estuaries along coastlines.

MATERIALS AND METHODS

Atlantic croaker larvae were collected during September 2000 from south of Beaufort Inlet to the mouth of Delaware Bay (Fig. 1). At each station, the temperature and salinity of the water column was measured using a Model 19 CTD (Sea-Bird) probe. Larval fish were collected with a 1 m^2 Tucker trawl equipped with three 333 µm mesh nets. The tucker trawl was deployed to the top of the pycnocline as determined from the CTD cast. The first net of the tucker trawl

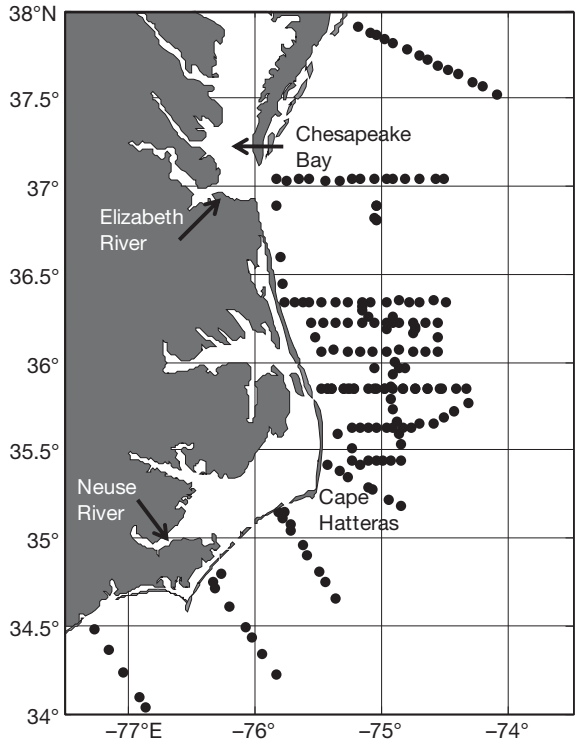

Fig. 1. Locations sampled to collect larval fish off the east coast of the US

sampled from the sea surface to the pycnocline (~15 m). At the pycnocline, the first net was closed and the second net opened. The second net fished from the pycnocline to half the distance to the surface where it was then closed, and the third net fished to the surface. Mean temperature and salinity of the upper mixed layer (surface to pycnocline), the bottom half of the tow, and the surface layer were calculated.

Larval croaker (up to 20) were immediately picked from each net and frozen in vials. When fewer than 20 croaker were collected, additional tows were collected at the station. Because we used larval croaker from multiple tows, we averaged all salinity and temperature measurements for each station and performed a cluster analysis of station hydrographic properties to assign a water mass identity for each station. We used Ward's minimum variance method to partition our data into clusters and verified the observed number of clusters with a temperature-salinity (T-S) plot. This served to define the boundaries for potential groups of spawning Atlantic croaker. For each cluster, we determined whether there was a difference in the mean temperature and salinity among the water masses.

Larval Atlantic croaker ranged from 1 to 7 mm in standard length. All otoliths were extracted, using stainless steel insect pins, in a drop of Milli-Q water on an acid-cleaned microscope slide in a clean room. Due to the delicate nature of the otoliths and increased chances for contamination, insect pins were not used to scrape tissue from the otolith, but rather were used to facilitate extraction and movement of the otolith. We measured otholith diameter (OD) with an ocular micrometer before gently transferring it into a drop of ultra pure hydrogen peroxide to remove any adhering tissue. The otolith was allowed to soak until all visible tissue was removed and was then transferred through 3 consecutive drops of Milli-Q water to remove hydrogen peroxide and any other contaminants on the surface. After cleaning, the otolith was transferred to an acid-cleaned vial and allowed to dry under a Class-100 laminar-flow hood.

We analyzed a total of 150 otolith samples from 18 stations. Otolith samples were prepared for analysis using 2 methods. In the first method, we pooled 2 otoliths per sample (1 otolith each from separate fish) to increase the chances of detecting elements above the analytical limits of our equipment. Otoliths that were combined were similar in size and were from the same sample station and tow. In total, 119 otolith samples were prepared in this manner. In the second method, we prepared 1 otolith per sample. In total, 31 otolith samples were prepared in this manner. All otolith samples were prepared for solution-based ICP-MS analysis by dissolving each sample in 20 µl of concentrated ultra pure nitric acid. Otolith samples were allowed to digest for 1 h, and the otolith-acid solution was brought to volume by adding 480 µl of 1% ultra pure nitric acid solution for a total volume of 500 µl. All samples, blanks, and standards were spiked with an internal standard (indium 2.0 ng g^{-1}) to correct for instrumental drift. Detection limits were calculated as mean blank value (14 blank samples) +3 SD (Table 1). Prior to analysis, sample order was randomized.

Analysis was completed using a MAT Element 2 double-focusing sector-field ICP-MS (Finnegan). Sam-

Table 1. *Micropogonias undulatus.* Detection limits (DL) of elemental concentrations and percent of larval Atlantic croaker otoliths from each sample area that were above DLs. DLs are expressed in parts per million. MAB and SAB: Mid- and South Atlantic Bight, respectively; CB: Chesapeake Bay

	DL	% > DL		
		MAB	CB	SAB
Mg	1.25E–05	100	100	100
Ca	2.27E–03	100	96	100
Mn	5.09E–06	100	100	100
Rb	1.20E–07	100	100	100
Sr	2.77E–05	100	99	100
Y	4.08E–08	97	95	100
Ba	1.15E–06	100	100	100
Pb	1.59E–07	100	100	100

ple introduction was accomplished with a self-aspirated Perfluoroalkyoxy microflow nebulizer (50 µl min^{-1}). A preliminary analysis of larval Atlantic croaker otoliths suggested that 9 elements in addition to Ca were detectable. Mg, Rb, Y, Ba, and Pb were measured in low resolution mode, and Na, P, Ca, Mn, and Sr were measured in medium resolution mode. Na and P were not included in any analysis because both elements are likely physiologically regulated and therefore may not be appropriate as a natural tag (Campana 1999, Campana et al. 2000). Elemental concentrations were calculated by linear interpolation based on known concentration multi-element solutions synthesized from stock single element standards. However, we were unable to obtain the absolute elemental concentrations because the otoliths were too small to accurately weigh.

Due to the small size (mean OD = 16.94 µm) of the otoliths we examined and the difficulty in moving each otolith from a slide into a sample vial, we needed a method to determine whether an otolith was present in the sample or had been accidentally lost in transfer. These otoliths were too small to be seen once transferred. The mean detection limit for calcium in blank samples was 0.002 ppm. Therefore, we considered samples with a Ca concentration above this value to have had an otolith present; below this concentration, we considered the otolith to have been lost in transfer. Three samples that contained 1 otolith and 1 sample that contained 2 otoliths had Ca concentrations below the detection limit and were omitted from further analysis.

Ca concentrations and otolith weights (OWs) are highly correlated above a certain threshold (Brazner et al. 2004). Below this threshold, there is seemingly no relationship between OW and Ca concentration. This is because errors associated with weighing otoliths increase as OW decreases. If we use OD as a proxy for weight, we find a strong relationship between OD and Ca concentration even at very low mass (Fig. 2; C. Jones unpublished data). Therefore, if we assume that the otolith matrix is nearly pure calcium carbonate and approximately 40% Ca by weight (Campana 1999), we can calculate a weight for otoliths too small to directly weigh based on the Ca content present in the otolith. To make this calculation of weight-proxy, we can use the following formula:

$$OW = [(Ca \times SW)/40\%] \quad (1)$$

where OW is estimated OW (hereafter referred to as OW; µg), Ca is calcium concentration (µg g^{-1}), SW is the weight of the otolith-acid solution (g), and the 40% accounts for the fraction of calcium carbonate matrix occupied by Ca.

We examined OD and OW among sites while using fish length as a covariate to determine whether differences existed and found significant differences in OD and OW of fish collected among sites. Therefore, we examined plots of OW or OD versus elemental concentration. Plots of OW versus elemental concentration indicated a nonlinear relationship, whereas plots of OD versus elemental concentration indicated a linear relationship (Fig. 3). A correlation between Ca and OW could not be made because OW was calculated as a linear product of the measured Ca content. We used OD as a covariate to remove size effects from our data because we were able to evaluate the relationship between OD and all elemental concentrations. OD was highly correlated with P ($r = 0.45$, $p < 0.0001$), Ca ($r = 0.66$, $p < 0.0001$), and Sr ($r = 0.67$, $p < 0.0001$). We detrended the concentrations of these elements by subtracting the product of OD and the common within-group linear slopes obtained with analysis of covariance (P: $b_1 = 0.026$; Ca: $b_1 = 0.037$; Sr: $b_1 = 0.044$) from each sample point to detrend the elemental concentrations (Campana et al. 2000). After detrending, elemental concentrations no longer possessed significant effects due to differences in OD. All subsequent statistical analyses are based on the detrended elemental concentrations.

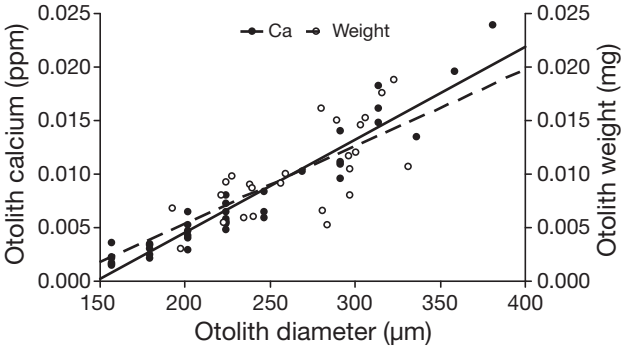

Fig. 2. *Micropogonias undulatus.* Relationship between otolith diameter (OD) and Ca concentration (●) and OD and otolith weight (○). Lines are least squares prediction for each relationship

To detect broad-scale differences in the multivariate elemental fingerprint and concentrations of specific elements in the otoliths of larval Atlantic croaker that contributed to differences in otolith chemistry, we performed multivariate analysis of variance (MANOVA) followed by univariate analyses of variance (ANOVA) to determine which elements contributed to separation among areas. Pillai's trace statistic quantified the significance of overall differences in the otolith chemistries of larval Atlantic croaker. We checked the assumption of multivariate normality analytically, using tests based on Mardia's multivariate skewness and kurtosis measures (Khattree & Naik 1999) and graphically using Q-Q plots of squared Mahalanobis distances (d_i^2). To fulfill the as-

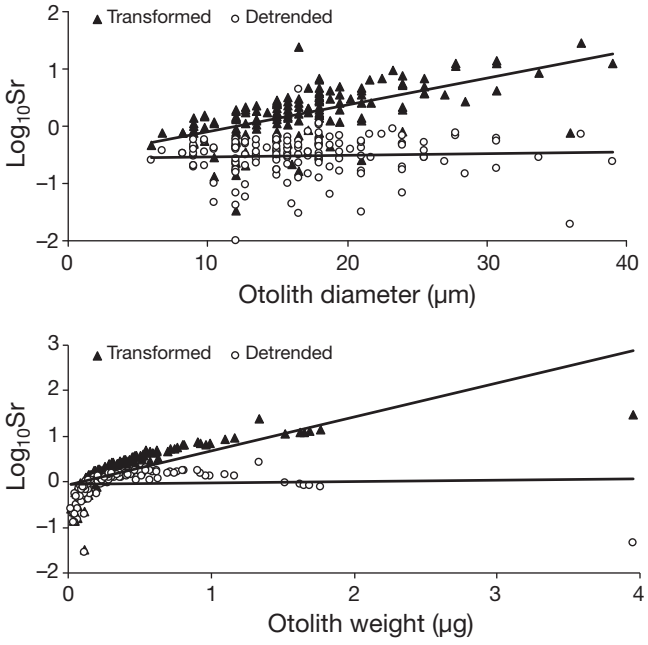

Fig. 3. *Micropogonias undulatus*. Detrended and undetrended Sr concentrations as an example of the effects of detrending elemental concentration data with the use of the within-group slope coefficients from analysis of covariance with otolith diameter or otolith weight as covariates

sumption of multivariate normality, we applied a $\log_{10}$ transformation to all elemental data. Sample skewness was 0.80 (p = 0.9380) and kurtosis was −0.53 (p = 0.5938). Q-Q plots did not indicate any departures from multivariate normality. Robust squared Mahalanobis distances (D_i^2) were used to identify multivariate outliers (Khattree & Naik 1999). Based on these criteria, we removed 1 observation from our data set. Our data were multivariate normal regardless of whether this data point was included in our skewness and kurtosis calculations. However, when this data point was removed, our ability to separate each group of larval Atlantic croaker was enhanced.

We examined larval Atlantic croaker captured in each of the broadly defined water masses for departures from the null hypothesis of no differences in elemental concentrations. We used a 2 factor MANOVA, where the first factor was the water mass and the second factor was a potentially confounding factor (day the sample was analyzed). Type 1 sums of squares were used to calculate all *F* values. We also analyzed the data using univariate ANOVAs and linear contrasts to identify which elements and water masses contributed most heavily to the multivariate signature.

Because there was a strong effect due to the potentially confounding factor, day (Pillai's trace = 1.0224, $F_{30, 396}$ = 6.82, p < 0.0001), we removed this effect from subsequent analyses using linear regression where day was the dependent variable and each elemental concentration was the independent variable. We used a stepwise selection procedure to select variables for inclusion in the discriminant function. Before proceeding with discriminant function analysis, we tested for equality of the variance-covariance matrices using a multivariate generalization of Bartlett's test for homogeneity of variances. After determining that the variance-covariance matrices were not equal (χ^2 = 268.32, df = 110, p < 0.0001), we applied a quadratic discriminant function (QDA) to classify larval Atlantic croaker to their natal water mass (Khattree & Naik 2000). Error rates were estimated using a cross-validation procedure with equal prior probabilities, and a randomization test was used to determine if the error rates were significantly different from random (Solow 1990, White & Ruttenberg 2007). We used 1000 random permutations of our data to determine whether the error rates we observed could have been due to chance alone. Multivariate separation was visualized with a nonmetric multidimensional plot.

RESULTS

Cluster analysis revealed 3 well defined water masses (Fig. 4a). Pseudo Hotelling's T^2 statistic was much smaller for 3 clusters (34.6) than it was for 2 clusters (58.4) or 4 clusters (78.0). We verified the number of clusters with a T-S plot (Fig. 4b). There were strong differences in the temperature ($F_{2, 51}$ = 61.41, p < 0.0001) and salinity ($F_{2, 51}$ = 59.13, p < 0.0001) of the 3 water masses (Table 2). SAB is warmer and more saline than the other 2 water masses. CB water is lower in salinity but warmer than MAB shelf water. These 3 regions were the basis of the groups of larval Atlantic croaker larvae in subsequent analyses.

For a given length, larval Atlantic croaker captured in the SAB had significantly larger ($F_{2, 146}$ = 51.79, p < 0.0001) and heavier ($F_{2, 146}$ = 10.85, p < 0.0001) otoliths than croaker in the CB or the MAB (Table 2). Otolith size and weight from larval Atlantic croaker captured in CB water were not different than those from fish captured in MAB shelf water. The relationship between OD and OW was nonlinear (Fig. 5). This relationship was best described by an exponentially increasing relationship where $OW = 0.0014\ e^{1.6616 \cdot OD}$ ($F_{1, 143}$ = 114.59, p < 0.0001, R^2 = 0.4449).

There were large differences in otolith chemistries based on water mass residency (Pillai's trace = 0.3084, $F_{20, 262}$ = 2.39, p = 0.0010). Linear contrasts showed that the otolith chemistry of Atlantic croaker collected from MAB shelf waters was significantly different from Atlantic croaker collected from the SAB (Pillai's trace = 0.1909, $F_{10, 130}$ = 3.07, p = 0.0016) or in CB water (Pillai's

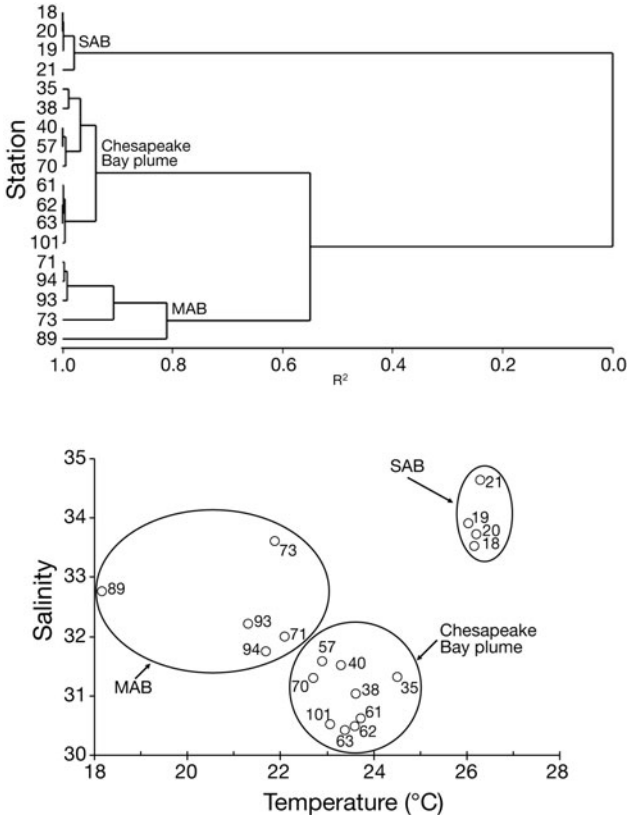

Fig. 4. Water mass classification using temperature (°C) and salinity measured at each larval fish collection site to construct a cluster tree (top) and validated with a T-S plot (bottom). SAB and MAB: South and Mid-Atlantic Bight, respectively

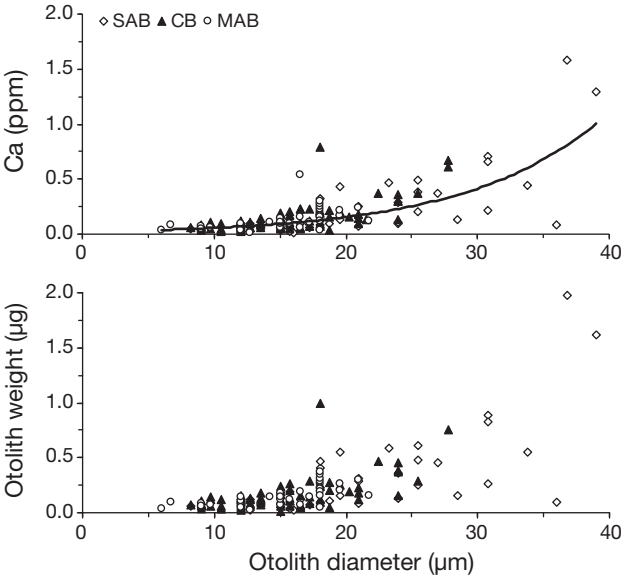

Fig. 5. *Micropogonias undulatus.* Relationship between Ca concentration and OW (top) and OD and OW (bottom) for larval Atlantic croaker. OW was calculated as a function of Ca concentration and solution weight, using solution-based ICP-MS. Abbreviations in Table 2

trace = 0.1308, $F_{10, 130}$ = 1.96, p = 0.0433). Larval Atlantic croaker collected from the SAB had significantly different otolith chemistry than Atlantic croaker collected in CB water (Pillai's trace = 0.1559, $F_{10, 130}$ = 2.40, p = 0.0119). Sr was significantly greater in the SAB and CB than MAB, but not different in the SAB or CB (Table 3, Fig. 6). Mn and Ba were marginally different ($0.05 < p < 0.10$), where CB showed elevated levels of both elements relative to MAB or SAB.

Sr and Mn were retained in the discriminant function through a stepwise variable selection procedure. We were able to separate fish from the MAB based on their lower otolith Sr compared to other water masses. Further, separation of fish from the SAB and CB water masses was possible because of the generally higher otolith Mn in fish from the CB water. Overall correct classification was 54% based on QDA. Our observed classification was significantly better ($p < 0.001$) than random based on a randomization test. However, correct classification for each water mass varied substantially (Table 4). Larval Atlantic croaker from the MAB were correctly classified to this water mass with 73% accuracy (Fig. 7), while those from the SAB were correctly classified to their water mass with 53% accuracy. For both of these groups, misclassification was most often to the other water mass and not to CB water. Larval Atlantic croaker from CB water were incorrectly classified to the MAB more often than they were correctly classified to the CB water mass.

Table 2. *Micropogonias undulatus.* Details of collection used to compare otolith elemental concentrations of larval Atlantic croaker collected in the Mid-Atlantic Bight (MAB), Chesapeake Bay plume water (CB), and South Atlantic Bight (SAB). Fish length, otolith diameter, otolith weight, temperature, and salinity values are mean ± 1 SE

Location	N	Stations	Fish length (mm)	Otolith diameter (µm)	Otolith weight (µg)	Temperature (°C)	Salinity (‰)
MAB	34	5	2.1 ± 0.6	15.1 ± 0.7	0.157 ± 0.016	21.0 ± 0.4	32.5 ± 0.2
CB	73	9	2.1 ± 0.5	15.3 ± 0.5	0.162 ± 0.018	23.4 ± 0.1	31.0 ± 0.1
SAB	38	4	2.8 ± 1.1	21.7 ± 1.2	0.344 ± 0.067	26.2 ± 0.1	33.9 ± 0.4

Table 3. *Micropogonias undulatus*. ANOVA showing differences in the elemental composition of larval Atlantic croaker otoliths

Source	df	Type 1 SS	MS	*F*	p > *F*
Mg					
Water Mass	2	0.2980	0.1490	1.54	0.2180
Error	142	13.4490	0.0968		
Mn					
Water Mass	2	0.4565	0.2283	2.63	0.0755
Error	142	12.0519	0.0867		
Rb					
Water Mass	2	0.0700	0.0350	0.73	0.4826
Error	142	6.6411	0.0478		
Sr					
Water Mass	2	0.4037	0.2018	3.50	0.0330
Error	142	8.0268	0.0577		
Y					
Water Mass	2	0.0572	0.0286	0.29	0.7469
Error	142	13.5958	0.0978		
Ba					
Water Mass	2	0.5933	0.2966	2.63	0.0756
Error	142	15.6700	0.1127		
Pb					
Water Mass	2	0.1204	0.0602	0.80	0.4516
Error	142	10.4694	0.0753		

DISCUSSION

Our results demonstrate that Atlantic croaker larvae captured in distinct water masses can be differentiated based on their otolith chemistries. This has important implications for determining migration histories and transport routes for offshore spawning species as well as determining rates of connectivity among nursery areas. This is in stark contrast to a previous study that found no significant differences in the chemistries of juveniles recruited to estuaries north (Elizabeth River) and south (Neuse River) of Cape Hatteras (Thorrold et al. 1997). We can now use this new information to determine the origin of juveniles recruited to MAB and SAB estuaries. Further, we can evaluate Cape Hatteras as a potential boundary separating Atlantic croaker stocks on the US east coast. This has potential to provide a resolution where researchers have found little evidence for stock structure in Atlantic croaker (Lankford et al. 1999, Lankford & Targett 2001) and other species such as summer flounder (Jones & Quattro 1999) using population genetics, but the existence of a zoogeographic barrier has often been hypothesized to exist in this region based on morphometric characteristics (Burke et al. 2000).

There are several possible reasons why we detected differences in otolith chemistries of croaker whereas Thorrold et al. (1997) found no differences. The first is a methodological issue. Thorrold et al. (1997) used a laser that produced a crater ~50 µm in diameter, 11 µm larger than the largest otolith analyzed in this study. Therefore, it is likely that Thorrold et al. (1997) ablated and analyzed otolith material in addition to what was present in the core region and altered the signal relative to what would have been detected had they been able to analyze less material (Jones & Chen 2003). Another possibility is spatial and/or temporal variation in water chemistries that may preclude otolith chemistries from showing distinct differences. As variations in the timings and magnitudes of freshwater inputs occur (Gillanders & Kingsford 2000, Dorval et al. 2005, 2007), upwelling dynamics (Patterson et al. 2004) and oceanographic circulation patterns (Patterson et al. 2004, Patterson & Kingsford 2005) change, and the chemical properties of water masses change. The magnitude of the chemical differences among water masses is directly related to the magnitude of the differences we are able to observe in fish otoliths from individuals exposed to a unique environment (Wells et al. 2003). Therefore, it is possible that prevailing conditions while Thorrold et al. (1997) sampled led to low differentiation among water masses where croaker originated. A third possibility is larval transport from multiple source locations. Thorrold et al. (1997) analyzed the larval portion of croaker otoliths after ingress to the Elizabeth and Neuse Rivers. If larvae transported to these systems originated from multiple and potentially overlapping source locations, the resulting signatures would be indistinguishable. Any combination of these factors could have biased previous research, but we were able to overcome these by sampling larvae closer to spawning locations and using solution-based ICP-MS on otoliths smaller than would have been possible with laser ablation.

We have shown that there are unique signatures associated with multiple water masses, and it is now possible to determine where those recruits originated. Some methodological details remain to be addressed before this technique can be used to measure connectivity between natal areas and juvenile nurseries or adult spawning areas. The first relates to characterization of the natural tags. We must be confident that we have adequately characterized the spatial extent of the groups in question and that there are no uncharacterized groups of fish that may overlap or otherwise be indistinguishable from a characterized group. Additionally, in croaker and many other species with protracted spawning seasons, we must be sure that there

are no differences among early and late spawned individuals (i.e. temporal effects on otolith chemistry) or that these groups can be separated based on another characteristic (e.g. daily rings). The second issue is how to extract the natural tag from the core region of juvenile or adult croaker recruits. We were able to distinguish croaker in multiple water masses because we analyzed a portion of the otolith that was developed before transport from natal areas. To sample recruits, we will need to be able to core the otolith using a micromilling procedure to extract the core from recruit otoliths and analyze it using solution-based ICP-MS or

Table 4. *Micropogonias undulatus.* Results of a quadratic discriminant function used to classify larval Atlantic croaker to their natal water mass. Results are expressed as percentages based on a cross-validation procedure. Rows: collection locations, columns: locations to which croaker were classified. Correct classifications are shown in **bold.** Abbreviations in Fig. 6

Water Mass	SAB	CB	MAB
SAB	**53**	18	29
CB	22	**36**	42
MAB	18	9	**73**

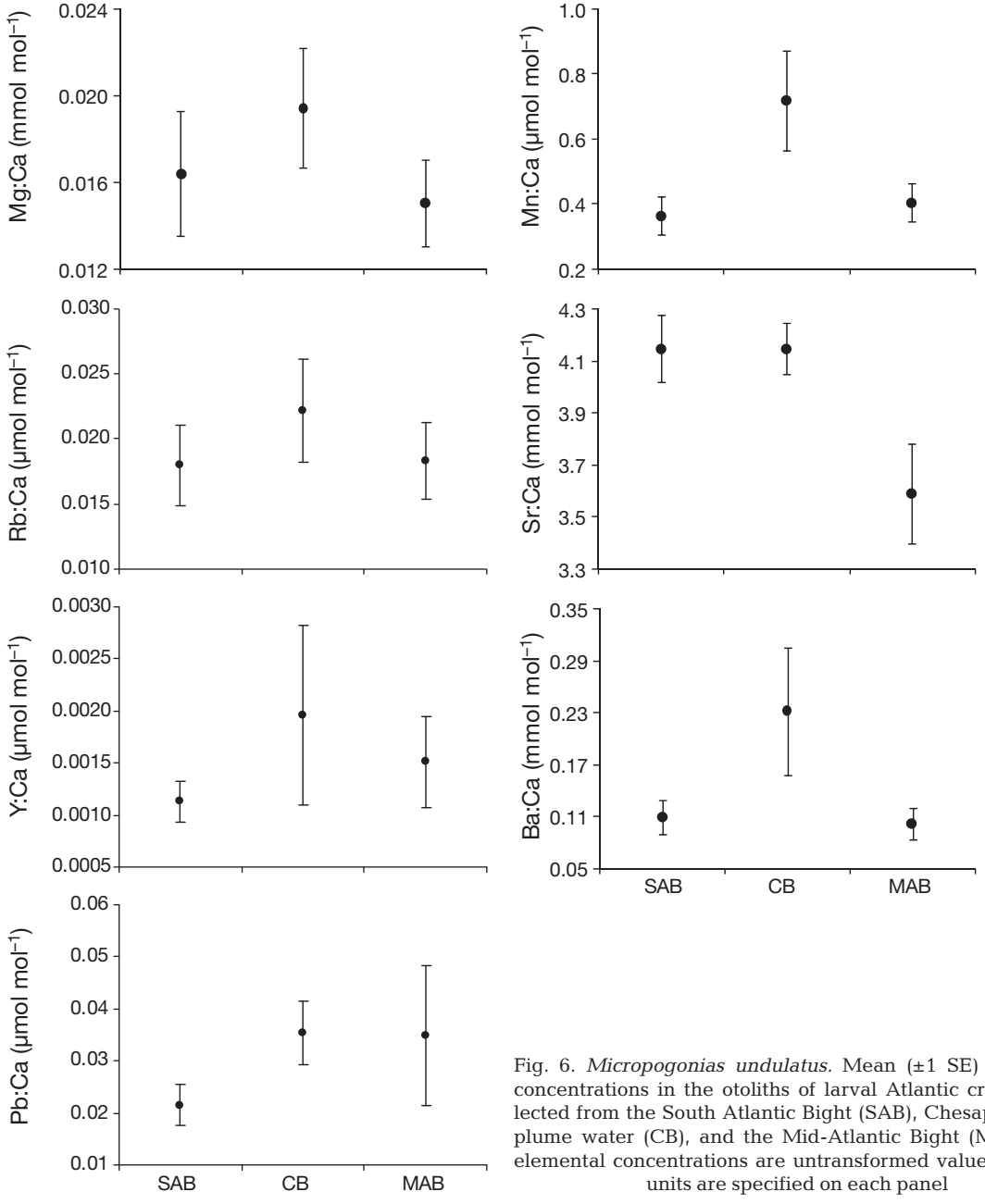

Fig. 6. *Micropogonias undulatus.* Mean (±1 SE) elemental concentrations in the otoliths of larval Atlantic croaker collected from the South Atlantic Bight (SAB), Chesapeake Bay plume water (CB), and the Mid-Atlantic Bight (MAB). The elemental concentrations are untransformed values, and the units are specified on each panel

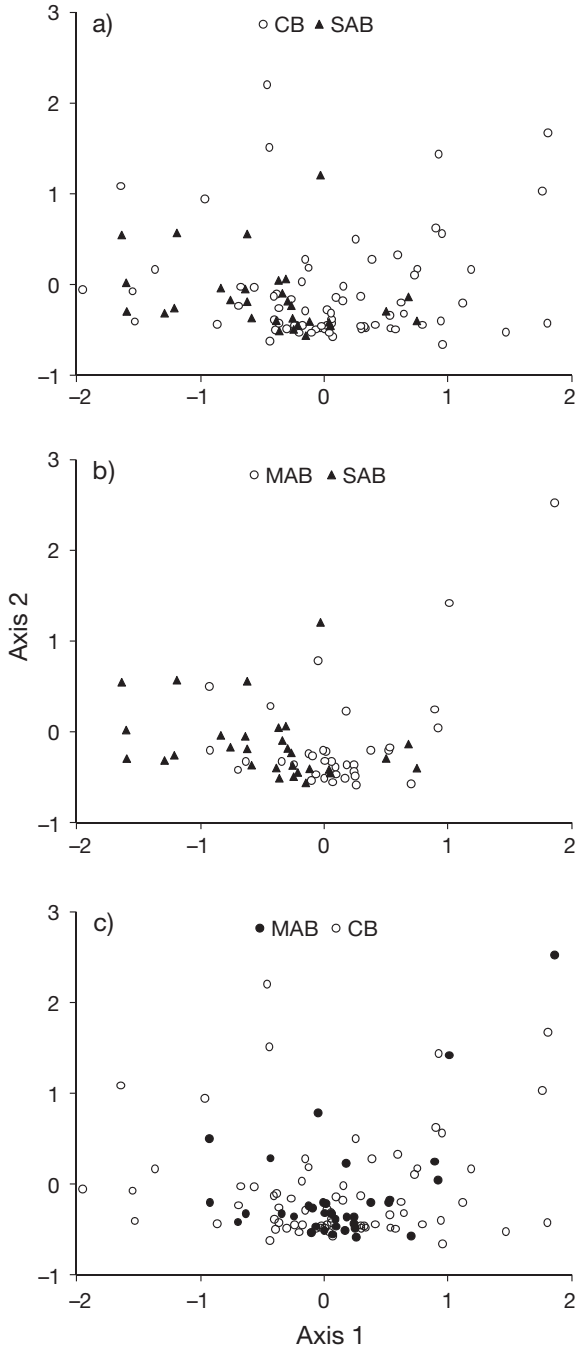

Fig. 7. *Micropogonias undulatus*. Relationship between individual fish taken in sampling areas off the US east coast using non-metric multidimensional scaling. Each panel contrasts 2 sampling locations. Abbreviations as in Fig. 6

alternatively use laser-ablation ICP-MS to match the dimensions of the larval otolith previously sampled (Thorrold et al. 2001). Both techniques present logistical constraints to overcome. For example, using a micromilling procedure to sample otolith cores this size has not been attempted. Conversely, lasers are regularly used with spot sizes that will match the ODs we analyzed (Ruttenberg et al. 2005, Warner et al. 2005, Ruttenberg & Warner 2006). Although we can match ODs to laser spot sizes, otoliths are 3-dimensional, and careful attention will be needed to match the thickness of the otolith with the ablation depth of the laser (Jones & Chen 2003).

Another interesting avenue to pursue that has shown promise in other species is otolith microstructure analysis (Guido et al. 2004, Smith et al. 2006). Otolith microstructure analysis has been successfully used to discriminate between fish that settled in different habitats (Guido et al. 2004) as well as between hatchery-reared and wild fish (Smith et al. 2006). The major reason for discriminatory success using this technique is the occurrence of differences in growth rates of fish that have led to detectable patterns in their otoliths at a later stage. We found that otoliths of fish collected in the SAB had significantly larger and heavier otoliths than fish of the same body length from other areas sampled. Conceivably, this could be due to differences in growth rates between areas. For example, Nixon & Jones (1997) showed that early spawned croaker have significantly larger otoliths for a given size than later spawned individuals. Additionally, these early spawned individuals grew faster than later spawned individuals. One of the main explanations for this observation was that early spawned fish were exposed to warmer temperatures. Although we cannot explicitly say that croaker in the SAB were growing faster than croaker at more northerly locations in our study area, croaker in the SAB were clearly exposed to warmer temperatures. This could potentially translate into faster growth and wider increment spacing during early life. Microstructure analysis in combination or on its own could potentially be used to discriminate natal origins of recruits to Chesapeake Bay.

This study represents one of the first attempts to use solution-based ICP-MS techniques on otoliths generally <1 µg to identify natal signatures in larvae from potentially different spawning areas. We were able to measure elemental concentrations in otoliths much smaller than previous researchers have analyzed (see Ludsin et al. 2006) and to characterize the otolith chemistry of larval Atlantic croaker that may recruit to Chesapeake Bay. Traditionally, researchers have used laser-ablation ICP-MS techniques to ablate the core region of juvenile otoliths (Thorrold et al. 1997, Thorrold & Shuttleworth 2000, Brophy et al. 2003) or the entire otolith from larval fish (Brophy et al. 2003, Warner et al. 2005, Ruttenberg & Warner 2006). Ludsin et al. (2006) evaluated the performance of solution-based and laser-ablation ICP-MS in quantifying the otolith chemistry of larval yellow perch whose otoliths ranged in size from 0.1 to 27.5 µg. Their results indi-

cated that laser-ablation ICP-MS is less sensitive to contamination but that both techniques could accurately discriminate among larvae produced in different spawning areas.

Collecting juveniles in estuaries and subsequently examining the natal origin (i.e. otolith core) is complicated by the possibility that larval Atlantic croaker ingress into the study areas may represent a combination of larvae from multiple source locations. We have demonstrated that solution-based ICP-MS is a viable technique for identifying regions where recruitment may be originating by directly analyzing the natal signature present in larval Atlantic croaker otoliths before dispersal can occur. We now have baseline elemental signatures that could be used to identify this cohort after it has recruited to juvenile or adult rearing habitats, thereby allowing us to determine the origins of croaker ingress into Chesapeake Bay. However, the low classification success we observed for some groups (i.e. CB) may prohibit subsequent classification of recruited juveniles in this instance. A more feasible approach we could take in this instance is to determine if groups maintain the observed level of distinction throughout the life cycle or become more mixed (and hence more similar) as fish get older. Further, if otolith signatures are combined with other methods (such as microstructure), identification of larval origin in individual juveniles or adults may be more reliable.

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