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Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*)

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Abstract

Minor and trace elements incorporated into otoliths during growth may permanently record environmental conditions experienced by fishes. To determine the validity of this approach, we used laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) to assay sectioned otoliths from juvenile Atlantic croaker (*Micropogonias undulatus*) collected from each of three sites in the Neuse River, North Carolina, and the Elizabeth River, Virginia. Elemental concentrations at the center of the otoliths did not differ between locations, although both Mg:Ca and Ba:Ca were significantly higher at the edge of otoliths from the Neuse River than from the Elizabeth River. Three of the elements (Mg:Ca, Sr:Ca, and Ba:Ca) showed significant variation across otoliths. Sr:Ca, and to a lesser extent Mg:Ca, showed progressive decreases as the fish moved from offshore spawning sites to estuarine nursery areas. The opposite pattern was shown by Ba:Ca. We hypothesize that these patterns were related to the elemental concentrations within oceanic and estuarine water masses. Although both Sr:Ca and Ba:Ca seem to be useful tracers of offshore–inshore migration of estuarine-dependent species, the sensitivity of the technique to more subtle changes in water chemistry remains to be determined.

Otolith microchemistry may prove a valuable tool in studies of larval fish ecology and behavior. A powerful application of the technique utilizes otolith microchemistry to retrospectively track the migration of larvae among different water masses through time. Unlike some invertebrate taxa (Olsen and McPherson 1987), it is generally not practical to follow individual fish larvae throughout their larval life. However, by combining the time-keeping properties of the daily increments in otoliths (Jones 1986) with geochemical information on the incorporation of minor and trace elements into aragonite, it may be possible to track the movements of individual larvae through waters with differing physicochemical properties. The technique relies upon the metabolically inert nature of the otolith that ensures the aragonite mineralogy remains unaltered after deposition (Mugiya et al. 1991). To date, this approach has been applied predominantly to Sr:Ca levels within the otolith. Kalish (1990) and others have found that the deposition rate of Sr is reduced in freshwater environments, presumably owing to the lower ambient concentrations of Sr compared to marine waters. In a more ambitious application, Sr:Ca thermometry has been used to infer temperature histories of herring (Townsend et al. 1989) and cod larvae (Townsend et al. 1995) from the Georges Bank. However, Sr is only one of a suite of elements in otoliths at minor and trace levels that may provide information on environmental conditions experienced during larval life (Campana and Gagne 1994).

The small size of fish otoliths, at least compared to other aragonitic structures such as coral skeletons (Shen et al. 1992), presents formidable analytical problems for trace element analysis. Individual daily growth increments in otoliths typically range from 1 to 20 μm in width. Electron probe microanalysis (EPMA) provides adequate spatial resolution (<5 μm) and has been used extensively in the study of otolith microchemistry (*see* Gunn et al. 1992). However, because of the relatively high detection limits of EPMA, only six major and minor elements can be routinely quantified with this technique. The proton microprobe is more sensitive than EPMA, but detection limits are still close to the concentrations of many of the trace elements found in otoliths (Sie and Thresher 1992). Most recently, laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) has been applied to otoliths with promising results (Campana et al. 1994; Fowler et al. 1995*a*). This technique uses a narrow laser beam to probe solid samples. The resulting vaporized material is swept by an argon carrier gas into a plasma torch and analyzed by mass spectrometry. The technique combines the low detection limits (0.1–0.01 μg g^{-1}), wide dynamic range (ng g^{-1} to %), and isotopic discrimination of conventional ICPMS with spatial resolution of <10 μm (Wang et al. 1994).

Acknowledgments

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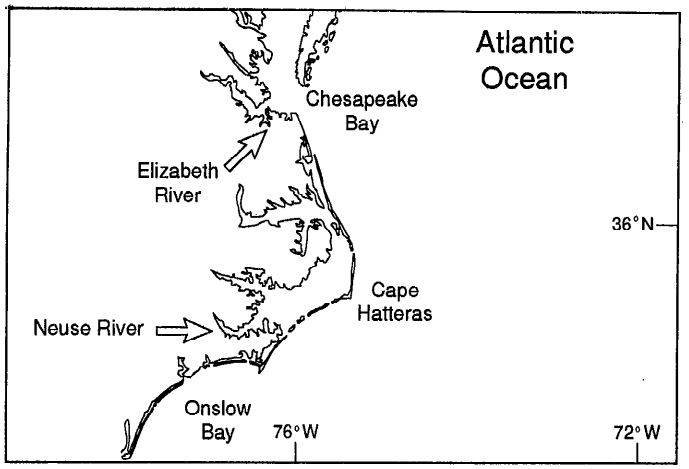

Fig. 1. Map showing sampling locations in the Neuse River (North Carolina) and the Elizabeth River (Virginia).

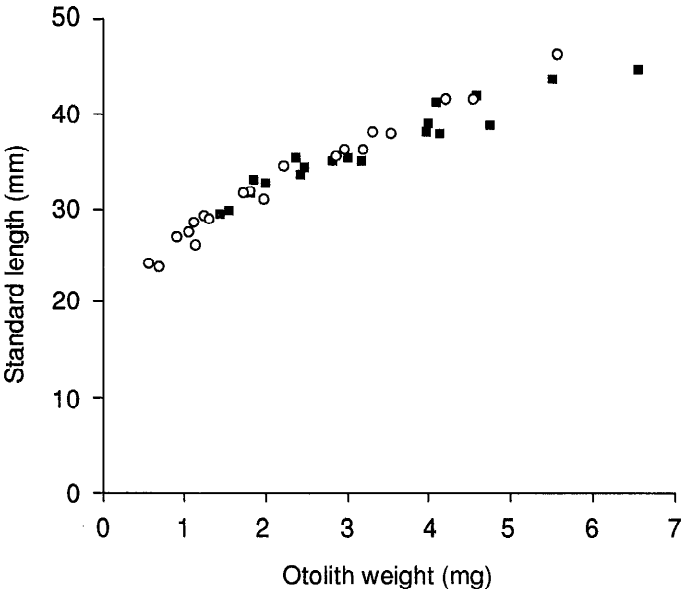

Fig. 2. Relationship between otolith weight and standard length for those Atlantic croaker from the Neuse River (○) and the Elizabeth River (■) used in the analysis of otolith microchemistry.

The objective of this study was to use LA-ICPMS to probe the trace element composition of wild-caught juvenile Atlantic croaker (*Micropogonias undulatus*) otoliths. Atlantic croaker are a common inshore demersal fish found along the Atlantic and Gulf of Mexico coasts of the United States. Adult croaker spend spring and summer months inshore before migrating onto the continental shelf to spawn and overwinter in the late fall. After spawning, larvae are thought to be advected inshore by wind-driven transport mechanisms. Post-larvae then migrate to estuarine and freshwater nursery grounds (Norcross 1991). Atlantic croaker juveniles have therefore been exposed to waters with very different water chemistries and are an excellent model species for testing ecological hypotheses with otolith microchemistry. Previous laboratory studies on this species confirmed that otolith elemental composition is indicative of water mass residency over the range of temperatures and salinities likely to be encountered by larval and juvenile Atlantic croaker (Fowler et al. 1995*a*,*b*).

Juveniles collected from the Neuse River, North Carolina, and from the Elizabeth River, Virginia, allowed us to test several specific hypotheses. First, do juveniles from the two systems originate from a common spawning location? If they do, we would expect no differences in microchemistry at the otolith center, which corresponds to the time of hatching. Second, are there differences in microchemistry from samples taken at the edge of the otoliths? This section of the otolith corresponds to the time just before capture, when the fish from the two locations were separated by several hundred kilometers. Finally, are elemental trajectories across the otolith different between locations? If so, this would imply that larvae recruiting into the two systems occupied coastal and estuarine waters at different times of their early life history. We hoped to identify factors that potentially influence larval survival and transport from spawning sites to juvenile nursery areas and to gain insight into the geographic extent of self-recruiting populations or "stocks" of Atlantic croaker in the Mid-Atlantic and South Atlantic Bights (MAB and SAB). Resolving these questions is significant for fisheries management (individual stocks are the logical unit of fisheries management) and, more generally, for increasing our understanding of the dispersal and metapopulation structure of marine organisms.

Methods

Juvenile Atlantic croaker were collected from the Elizabeth River, Virginia, on 23 March 1994 and from Hancock Creek, a tributary of the Neuse River, North Carolina, on 21 April 1994 (Fig. 1). Fish were collected with an otter trawl fitted with 10-mm (stretched) mesh in water depths from 2 to 8 m. Several trawls were made in three haphazardly chosen sites at each location. Otoliths (sagittae and lapilli) were removed from seven randomly selected juveniles from each of the six sites, weighed to the nearest 0.01 mg, and mounted on glass slides with cyanoacrylic glue. Because otolith growth rate may influence otolith microchemistry in Atlantic croaker (Fowler et al. 1995*b*), we first ensured there were no significant differences between locations in terms of otolith weight (ANOVA, df = 1, MS = 2.46×10^{-5}, F = 2.92, P = 0.16), standard length (ANOVA, df = 1, MS = 165, F = 2.47, P = 0.2), or the otolith weight : standard length relationship (ANCOVA test for homogeneity of slopes, df = 1, MS = 1.36, F = 0.69, P = 0.45, ANCOVA test for difference in intercepts, df = 1, MS = 7.59, F = 2.94, P = 0.16; Fig. 2). We assumed that any differences in otolith growth rates would be reflected in the otolith weight : standard length relationship because slower growing otoliths are typically larger for a given fish size (Fowler et al. 1995*b*).

Dissection and otolith decontamination methods followed those outlined by Fowler et al. (1995*a*). Samples were maintained under a class 100 laminar flow hood whenever possible, and otoliths were always handled with acid-washed,

Table 1. Operating conditions of the LA-ICPMS system used for the analysis of otoliths in this study.

ICPMS	
Acquisition mode	Scan
Acquisition time	30s
Coolant gas flow rate	1.4 liters min^{-1}
Auxiliary gas flow rate	1.4 liters min^{-1}
Carrier gas flow rate	1.6 liters min^{-1}
Dwell time	320 ms
Channels/amu	20
Mass range	6–208
Laser probe	
Laser type	Nd: YAG pulse
Laser mode	Q-switched
Flashlamp voltage	500–520 V
Frequency	355 nm
Repetition rate	2 Hz
Beam size	~10 μm
Crater size	~50 μm
Focus condition	Focus

nonmetallic equipment. Sagittal otoliths were then ground to the midplane with 30-μm and 3-μm lapping paper, ultrasonically cleaned in ultrapure (Milli-Q) water for 5 min, triple rinsed with ultrapure water, and air-dried under the laminar flow hood. After drying, otoliths were sealed in an acid-washed plastic container for subsequent transportation to the laser ablation facility.

LA-ICPMS assays—All LA-ICPMS assays were conducted with a custom-built laser ablation unit coupled to a VG Elemental PQ2+ ICPMS. The major difference in operating conditions from our earlier work (Campana et al. 1994; Fowler et al. 1995*a*) was the use of the 355-nm harmonic frequency of a 1,064-nm Nd:YAG laser. The 355-nm laser has better spatial resolution, with a nominal beam diameter of ~10 μm compared to 30 μm for the 1,064-nm laser. Ablation characteristics of materials that do not absorb strongly at 1,064 nm are also improved (Jenner et al. 1994). Operating conditions of both the ICPMS and laser unit are given in Table 1.

To assess the performance of the LA-ICPMS unit, we constructed a series of four multielement glass bead standards. Although a complete description of the glass bead production will be given elsewhere, a brief summary follows. The beads consisted of a 1:1 ratio of Atlantic croaker otolith powder (ground to micron-sized particles) and ultrapure lithium tetraborate flux. We added small quantities of 10 elements, in either carbonate or oxide form, to the resulting mix to generate four concentrations of each element, randomly assigned to each of the tablet types. Powders were heated on a 0.13-mm-thick tungsten filament in a nitrogen atmosphere until the powder melted. Two beads from each of the four standards were then prepared for LA-ICPMS analysis.

Concentrations of individual elements within each of the bead types were determined independently by analyzing a subsample of each bead type with either isotope dilution ICPMS (Ba, Sr, and Zn) or conventional ICPMS calibrated with standard additions (Ca and Mg). The beads were first dissolved in ultrapure HNO_3. After the acid digestion, enriched isotope spikes of ^{67}Zn, ^{86}Sr, and ^{135}Ba were added to the bead solutions and the resulting solution was then aspirated directly into a Finnigan MAT ICPMS. Of the 10 elements spiked in the beads, 4 (Mg, Zn, Sr, and Ba) were detectable in Atlantic croaker otoliths (*see below*), and only these elements will be considered further.

Instrument drift during LA-ICPMS assays is unavoidable owing to Ca deposition on nebuliser tips, sampler cones, and skimmer cones. To minimize the effect of drift, we blocked samples by site so that an otolith from each site was sampled in turn. The order of sites within a block was randomized. Otoliths were ablated sequentially from the center to the anterior edge at ~200-μm intervals. The precise axis of sampling was chosen so that all the growth increments were focused in the grinding plane. The distance from the otolith center to each ablation crater was then measured with an image analysis system mounted on a binocular microscope. This distance was standardized to the maximum diameter of each otolith so that otoliths that were sampled along different growth axes could be compared to one another. Mean ratios between the sampled and maximum axes were 84±1.1% (SD) for Neuse River fish and 86±0.9% (SD) for fish from the Elizabeth River.

Data analysis—A total of 28 isotopes representing some 13 elements were logged on each sampling occasion. Counts were corrected for blank values recorded before each sample was acquired. Of 28 isotopes, ^{7}Li, ^{24}Mg, ^{25}Mg, ^{46}Ca, ^{48}Ca, ^{64}Zn, ^{68}Zn, ^{87}Sr, ^{88}Sr, ^{137}Ba, and ^{138}Ba were determined to be above background (mean count >2 SD above mean blank count). Isotopic counts were converted to elemental counts by multiplying by percentage natural occurrence of the isotope. When elemental counts were available from more than one isotope, values were averaged among isotopes to improve precision. All data were then standardized to Ca to account for variability in laser energy and in the weight of ablated material (Campana et al. 1994).

Statistical analysis—All elemental data were initially examined for normality and homogeneity of variance by means of residual analysis and were found to conform to the assumptions of ANOVA without any data transformation. Elemental differences between locations at both the primordium and edge of the otoliths were compared by ANOVA. Location was treated as a fixed factor, whereas site was considered a random factor nested within location. Comparisons of differences in trajectories across otoliths were made with repeated measures ANOVA. This approach requires that adjacent points on the trajectories be equidistant. Samples were therefore assigned to a distance category at intervals of 200 μm (0–200, 200–400, 400–600, 600–800, and 800–1,000 μm) across the otolith, to a distance of 1,000 μm from the otolith center. Values were averaged when more than one sample was made within a distance category on the same otolith. Missing values compromised the design because on several occasions no sample was taken within one of the distance categories. Therefore, a split-plot ANOVA approach was used (Littell et al. 1991). Mauchly's criterion for the

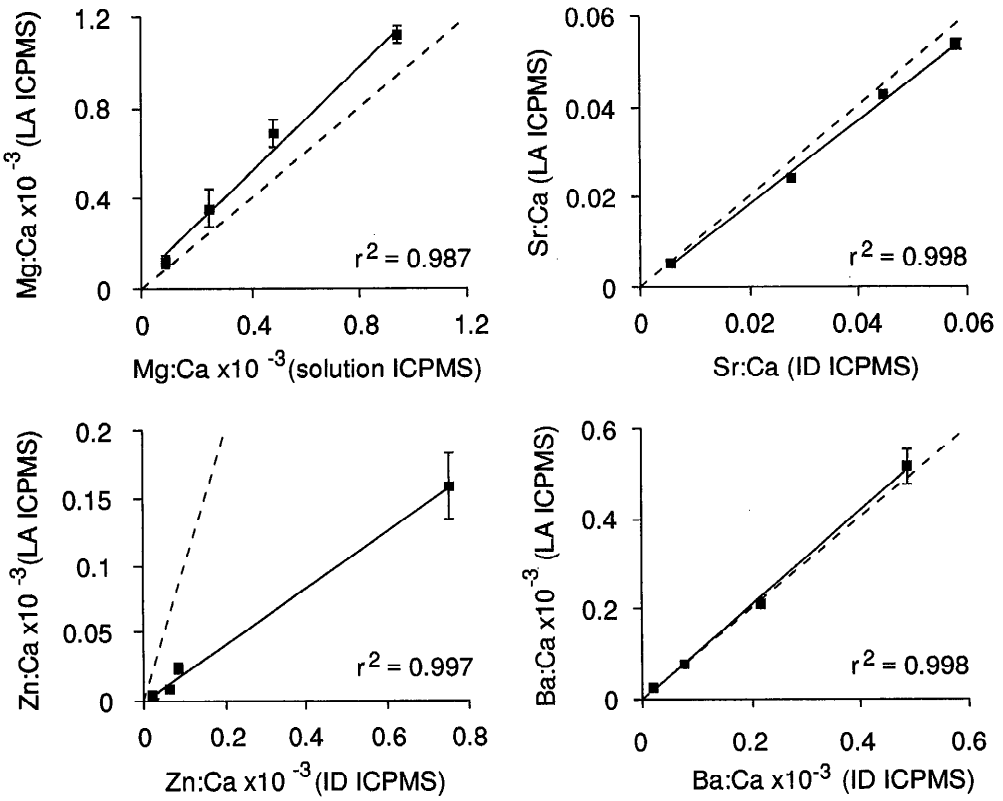

Fig. 3. Regressions of data from solution-based ICPMS and LA-ICPMS of multielement glass beads. Individual points indicate mean (±SE) of eight samples from each of two beads. Dashed lines indicate line of 1 : 1 correspondence between the two techniques.

hypothesis of sphericity of orthogonal components was rejected for all elements from the subset of those observations with records for each position on the otolith by using the χ^2 approximation ($P < 0.05$). Therefore, the Huynh-Feldt adjustment was made to the degrees of freedom for the appropriate tests (Huynh and Feldt 1976).

Results

Glass beads—Four of the five elements detected in the wild-caught croaker otoliths were also sampled in the glass beads. Eight points were assayed in each of two beads nested within the four bead types, for a total of 64 samples. Concentrations of the elements, as determined by ID and solution-based ICPMS, were 18–222 μg g^{-1} for Mg, 5–163 μg g^{-1} for Zn, 1,150–10,500 μg g^{-1} for Sr, and 4.6–106 μg g^{-1} for Ba. All four element ratios (Mg : Ca, Zn : Ca, Sr : Ca, and Ba : Ca) showed strong linear relationships with the solution-based ICPMS data (Fig. 3). The slopes of the relationship for Mg : Ca (slope = 1.15, SE = 0.09), Sr : Ca (slope = 0.93, SE = 0.03), and Ba : Ca (slope = 1.05, SE = 0.04) did not differ significantly from 1 ($P > 0.05$). However, the slope between the two techniques for Zn (slope = 0.21, SE = 0.01), although still linear, was significantly different from 1 ($P < 0.05$). Given the linearity of the results for all four elements over the range of concentrations likely to be found in otoliths, we were confident that LA-ICPMS data could be safely applied to the analysis of otoliths for these elements.

Otolith centers and edges—We found little evidence for differences in trace element composition between the centers of otoliths from the Neuse and Elizabeth Rivers. All ANOVA results were nonsignificant (Table 2), as were all site-nested-within-location effects (Fig. 4). More variation between locations was seen in assays from the otolith edge. Again, none of the site terms were significant, suggesting little variation within the two locations sampled. However, significant differences between locations were found for both Mg : Ca and Ba : Ca. Both elements were higher in the otoliths from the Neuse River than in those from the Elizabeth River (Fig. 5).

Across-otolith trajectories—Repeated measures ANOVA was used to assess variability in elemental composition across the otolith. Although the analysis provides tests of a number of hypotheses (Table 3), we were particularly interested in three terms in the model: the position term, the location term, and the position–location interaction. Other terms in the model are also provided so that the ANOVA table may be accurately reconstructed. A significant position effect was shown by Mg : Ca, Sr : Ca, and Ba : Ca. That is, these ratios showed significant variation from the center to

Table 2. ANOVA table showing differences in the elemental compositions at the center and edge of otoliths from the Neuse and Elizabeth Rivers ($\alpha = 0.05$).

Source	df	Type 3 SS	MS	F	$P > F$
		Center			
Mg					
Location	1	4.9×10^{-4}	4.9×10^{-4}	0.94	0.3880
Site(location)	4	2.1×10^{-3}	5.2×10^{-4}	1.51	0.2225
Error	31	1.0×10^{-1}	3.5×10^{-4}		
Zn					
Location	1	1.78×10^{-4}	1.78×10^{-4}	0.84	0.4100
Site(location)	4	8.41×10^{-4}	2.1×10^{-4}	1.08	0.3811
Error	31	6.0×10^{-3}	1.9×10^{-4}		
SR					
Location	1	0.549	0.549	5.54	0.0781
Site(location)	4	0.392	0.098	0.17	0.9522
Error	31	17.88	5.8×10^{-1}		
Ba					
Location	1	1.44×10^{-5}	1.44×10^{-5}	3.64	0.1289
Site(location)	4	1.58×10^{-5}	3.95×10^{-6}	0.20	0.9348
Error	31	6.0×10^{-4}	1.9×10^{-5}		
		Edge			
Mg					
Location	1	3.29×10^{-3}	3.29×10^{-3}	14.66	0.0186
Site(location)	4	8.96×10^{-4}	2.24×10^{-4}	0.59	0.6705
Error	33	1.2×10^{-2}	3.8×10^{-4}		
Zn					
Location	1	3.57×10^{-6}	3.57×10^{-6}	0.03	0.8626
Site(location)	4	4.2×10^{-4}	1.05×10^{-4}	0.65	0.6285
Error	33	5.3×10^{-3}	1.6×10^{-4}		
Sr					
Location	1	0.211	0.211	0.76	0.4335
Site(location)	4	1.115	0.279	0.68	0.6100
Error	33	13.5	0.409		
Ba					
Location	1	2.06×10^{-3}	2.06×10^{-3}	10.86	0.0301
Site(location)	4	7.58×10^{-4}	1.9×10^{-4}	1.03	0.4059
Error	33	6.1×10^{-3}	1.8×10^{-4}		

the edge, averaged over fish, sites, and location (Fig. 6). Mg:Ca ratios in otoliths from Neuse River fish increased at the edge of the otolith, whereas in Elizabeth River fish, Mg:Ca ratios declined from high values at the center to lower values at the edge. Sr:Ca ratios declined from high values at the center to lower values at the edge at both locations. Ba:Ca values at the center of the otoliths were relatively low compared to those at the edge. None of the four element ratios was consistently higher or lower across all positions at either location.

The interaction between position and location tested the hypothesis that elemental trajectories across the otolith differed between locations. Although trajectories across the otolith were very similar between locations for Zn:Ca and Sr:Ca, a significant interaction term was found for both Mg:Ca and Ba:Ca. The Mg:Ca interaction was related to a decline from the center to the edge in Elizabeth River fish, whereas the ratios generally increased at the position closest to the edge in Neuse River fish. Ba:Ca trajectories from both locations showed a general increase from the center to ~600 μm across the otoliths. However, although the Ba:Ca levels from the Neuse River increased gradually from the center to the edge of the otoliths, peak Ba levels from Elizabeth River fish occurred between 400 and 600 μm from the center before decreasing toward the edge. Finally, significant differences were also found among sites within the rivers for Ba:Ca, and significant interactions between site and position were detected for both Mg:Ca and Ba:Ca.

Discussion

The use of laser ablation to introduce small amounts of a solid sample into an ICPMS has only recently been developed (Denoyer et al. 1991). Although the approach offers tantilizing sensitivity and accuracy, with detection limits of ~0.01 μg g^{-1} and accuracies approaching 5% (Lichte 1995),

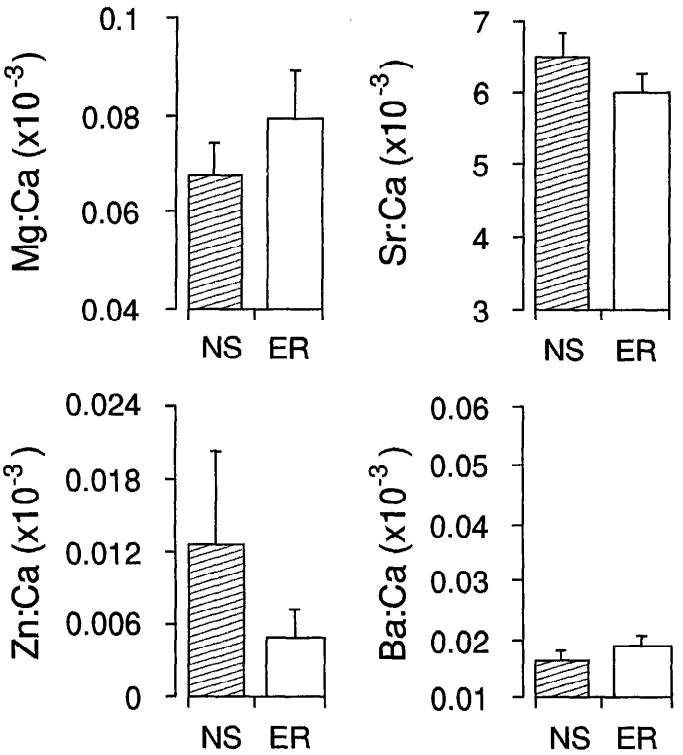

Fig. 4. Mean (±SE) abundance of four elements (sampled with LA-ICPMS and expressed as ratios to Ca) from the center of otoliths from fish collected in the Neuse River (NS) and the Elizabeth River (ER).

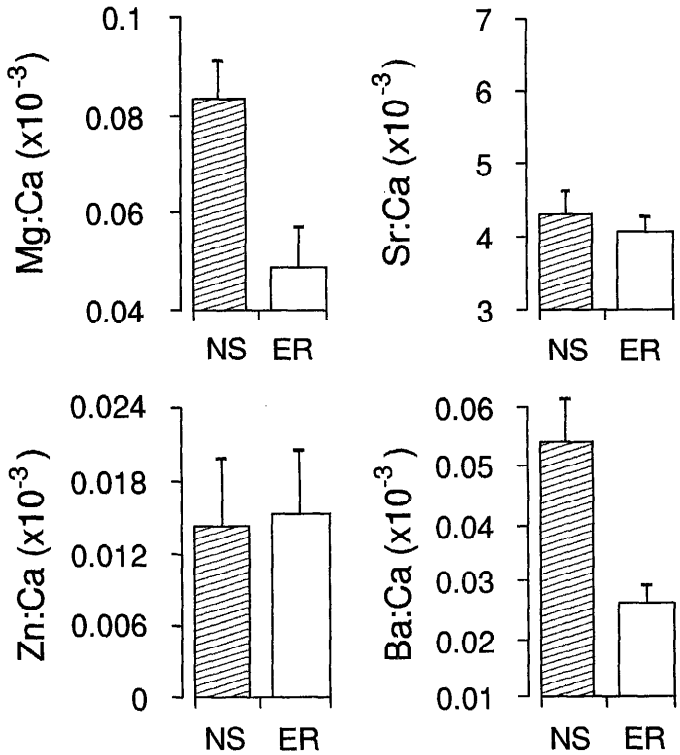

Fig. 5. Mean (±SE) abundance of four elements (sampled with LA-ICPMS and expressed as ratios to Ca) from the edge of otoliths from fish collected in the Neuse River (NS) and the Elizabeth River (ER). Note y-axis scale is the same as Fig. 4.

there is a lack of primary standards on which to assess the performance of the system for any given sample matrix. By using fused glass beads made from otolith powder and a lithium tetraborate flux, we hoped to get a more realistic appraisal of the performance of LA-ICPMS for fish otoliths than had been achieved through the use of the more traditional NBS glass standards (Campana et al. 1994). We did have to mix flux with the otolith powder to get reasonable fusion of the beads and therefore could not obtain perfect matrix matching between the beads and the juvenile croaker otoliths. However, recent studies have suggested that exact matrix matching is not necessary to obtain quantitative data from LA-ICPMS systems (Feng 1994). A strong linear response was found for the four elements considered in this study, confirming the performance of the LA-ICPMS unit over the range of concentrations likely to be found in otoliths. The 355-nm laser also gave improved spatial resolution compared to the IR (1,064 nm) laser used in our earlier work (Campana et al. 1994; Fowler et al. 1995*a*). Beam width and resulting ablation craters in the otoliths were ~3× smaller than with the IR laser. Specimen damage was similarly reduced, presumably because of a large reduction (as much as 2 orders of magnitude) in average power during UV laser ablation compared with the IR laser (Kelley et al. 1994).

LA-ICPMS assays of juvenile Atlantic croaker otoliths revealed significant variations in minor and trace element chemistry, both spatially across the otolith and between geographic localities. We were, however, unable to reject the hypothesis that croaker larvae from north and south of Cape Hatteras originated from different spawning sites. This may indicate that the larvae were spawned in close geographic proximity, and strengthens arguments that Atlantic croaker in the MAB and SAB represent a single spawning stock (Barbieri et al. 1994). Alternatively, the characteristics of the oceanic environment may have been unsuitable for the induction of significantly different elemental fingerprints. Water temperatures and salinities in the MAB are similar to shelf water in the SAB through late summer and fall, which represents the time of peak spawning of Atlantic croaker in these regions (Warlen 1982; Nixon 1993). Although Atlantic croaker are thought to spawn at mid- and outer-shelf locations in both the MAB and the SAB (Norcross 1991; Govoni and Pietrafesa 1994), the discovery of postovular follicles in adult Atlantic croaker collected from within Chesapeake Bay suggests that there may be significant nearshore spawning in the MAB (Barbieri 1993). Given the large temperature and salinity differences between the waters within Chesapeake Bay and those in adjacent shelf locations, the lack of distinct otolith elemental fingerprints between locations argues against a significant shift in the offshore extent of spawning between the MAB and SAB.

Elemental signatures at the edge of otoliths in fish from the Neuse River and the Elizabeth River showed significant differences in both Mg:Ca and Ba:Ca. These differences suggest that elemental compositions may reflect environmental variability among geographic localities. Several other

Table 3. Results from repeated-measures ANOVA of the trajectories of four elements (grouped at 200-μm intervals from the center to 1,000 μm) across otoliths from juvenile Atlantic croaker collected at locations in the Neuse and Elizabeth Rivers ($\alpha = 0.05$). Degrees of freedom for position effects, along with position–location (pos×loc) and position–site [pos×site(loc)] interactions, were adjusted according to Huynh and Feldt (1976).

Source	df	Type 3 SS	MS	*F*	*P* > *F*
Mg					
Position	4	3.1×10^{-3}	7.66×10^{-4}	3.71	0.0255
Location	1	2.7×10^{-3}	2.7×10^{-3}	17.67	0.1488
Site(location)	1	1.53×10^{-4}	1.53×10^{-4}	0.32	0.5750
Fish(site)	33	9.97×10^{-3}	3.02×10^{-4}	1.82	0.0120
Pos×loc	4	5.19×10^{-3}	1.3×10^{-3}	6.28	0.0031
Pos×site(loc)	16	3.31×10^{-3}	2.07×10^{-4}	1.77	0.0448
Error	105	1.22×10^{-2}	1.17×10^{-4}		
Zn					
Position	4	7.88×10^{-4}	1.97×10^{-4}	1.51	0.5 > *P* > 0.25
Location	1	7.61×10^{-5}	7.61×10^{-5}	12.74	0.1739
Site(location)	1	5.97×10^{-6}	5.96×10^{-6}	0.02	0.8890
Fish(site)	33	9.97×10^{-3}	3.02×10^{-4}	1.82	0.012
Pos×loc	4	8.39×10^{-4}	2.1×10^{-4}	1.61	0.5 > *P* > 0.25
Pos×site(loc)	16	2.09×10^{-3}	1.3×10^{-4}	0.78	*P*>0.75
Error	105	1.75×10^{-2}	1.66×10^{-4}		
Sr					
Position	4	25.73	6.43	29.89	0.0001
Location	1	3.41	3.41	6.54	0.2374
Site(location)	1	0.522	0.522	0.21	0.6488
Fish(site)	33	81.57	2.47	12.25	0.1108
Pos×loc	4	1.18	0.295	1.37	0.2873
Pos×site(loc)	16	3.44	0.215	1.07	0.3965
Error	105	21.19	0.202		
Ba					
Position	4	5.31×10^{-3}	1.32×10^{-3}	8.43	0.0007
Location	1	1.8×10^{-4}	1.8×10^{-4}	0.04	0.8714
Site(location)	1	4.3×10^{-3}	4.3×10^{-3}	12.11	0.0014
Fish(site)	33	1.17×10^{-2}	3.55×10^{-4}	4.3	0.0001
Pos×loc	4	2.34×10^{-3}	5.86×10^{-4}	3.72	0.0251
Pos×site(loc)	16	2.52×10^{-3}	1.57×10^{-4}	1.9	0.0278
Error	105	8.69×10^{-3}	8.26×10^{-5}		

factors may also influence deposition rates of trace elements in otoliths. Kalish (1991) suggested that differences in otolith microchemistry may be determined by the presence of calcium-binding proteins in the blood plasma. The concentrations of metal ions in the endolymph available for precipitation onto the otolith surface are hypothesized to be a function of the concentration of these Ca-binding proteins and the degree of discrimination of the proteins against other metal ions for Ca. Support for the role of physiological effects on otolith microchemistry comes from studies that show a relationship between Sr:Ca and fish growth rates (Sadovy and Severin 1992). Although we cannot rule out a physiological basis for the differences in otolith microchemistry that we observed, the similarity between otolith weight–standard length relationships argues that the physiological effects were not manifested in differential otolith growth rates. An alternative explanation that the differences in otolith microchemistry may have a genetic basis (Thresher et al. 1994) seems unlikely given that elemental differences were not consistent across the otolith. Regardless of the mechanism, the data suggest that the fish could be separated on the basis of their elemental composition. It may therefore be possible to build up a library of otolith elemental signatures characteristic of different estuaries along the MAB and SAB. These signatures could then be used to generate discriminant functions to determine the nursery areas used by adults that have successfully survived to reproductive age. The temporal stability of signatures would in turn determine the temporal scale at which the functions would need to be recalibrated (Campana et al. 1995).

If otoliths are to be reliable recorders of environmental variability, elements within the otolith must be deposited in proportion to concentrations in the surrounding waters, perhaps as modified by temperature (Fowler et al. 1995*b*). Unfortunately, there is remarkably little direct evidence for this relationship in fish otoliths. We found conflicting evidence for the hypothesis that uptake into the otolith is proportional to either the elemental concentration or the element:Ca ratio in the ambient water. Sr concentration in seawater is relatively constant at approximately 90 μM throughout the

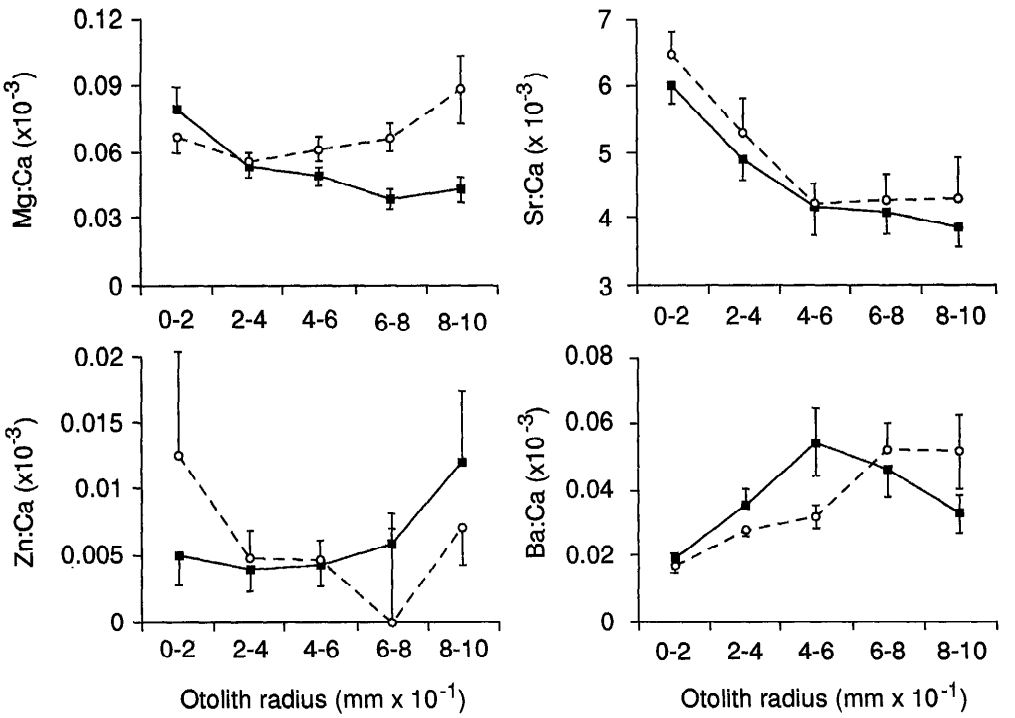

Fig. 6. Trajectories of the abundance of four elements (sampled with LA-ICPMS and expressed as ratios to Ca) in the otoliths of fish collected from the Neuse River (○) and the Elizabeth River (■). Individual points are mean (±SE) values grouped at 200-μm intervals.

world's oceans, which is more than an order of magnitude greater than in freshwater (Limburg 1995). The observed Sr:Ca trajectories, with high values at the center of the otolith and lower values toward the edge, is consistent with larval migration from (high Sr) shelf waters into (low Sr) estuarine or freshwater systems. This pattern has been documented for a number of diadromous fishes (e.g. Radtke et al. 1988; Secor et al. 1995). Mg is also a common constituent of seawater, with concentrations in riverine waters generally at least 2 orders of magnitude lower than in oceanic waters (Spaargaren 1991). Mg should therefore show a similar pattern to that of Sr if indeed otolith Mg:Ca ratios reflect concentrations in the ambient water. This pattern was not convincing in either location, which suggests the need for caution in attributing the differences in Mg:Ca ratios between the rivers to a concomitant difference in Mg concentrations in the two river systems.

Ba and Zn follow nutrient-type distributions in seawater. Concentrations are relatively high in riverine and near-coastal areas compared to slope and oceanic water masses (Shen and Stanford 1990). Ba:Ca trajectories from both locations generally showed an increase from the center to the edge of the otolith. This pattern is consistent with a movement from (low Ba) shelf water to (high Ba) nearshore waters. Although Sr:Ca and Ba:Ca were negatively correlated at most distances on the otoliths, we observed greater variability between locations across the Ba:Ca transects. If this variability reflects greater spatial variations in Ba concentrations throughout the waters of the region, Ba may prove to be a useful tracer of water mass residency. Zn concentrations increase from <0.1 nM in oceanic waters of the western North Atlantic to ~2.4 nM in coastal waters of the MAB (Bruland and Franks 1983). Levels in a North Carolina estuary and in the lower Chesapeake Bay are ~5–10 nM (Sunda et al. 1990). Although temporally variable, Zn levels at our collection sites in the Elizabeth River can rise as high as 1,550 nM, presumably due to anthropogenic inputs. Based on these data, we predicted that Zn would be an excellent tracer of migration from offshore waters to nearshore nursery areas. However, we found no indication that Zn:Ca levels in the otoliths tracked increasing Zn concentrations in nearshore waters or that the otoliths recorded a significant pollution signal from the Elizabeth River.

Otolith microchemistry will ultimately be determined by osmoregulatory processes across the gill membrane, the abundance of metal-binding macromolecules in the plasma, ionic exchange between the blood plasma and the endolymph, and the partition coefficients of metal species at the growth surface of the otolith (Kalish 1991). If otolith microchemistry is to represent a useful record of environmental conditions, the degree of physiological regulation of an element must be independent of the element's concentration in seawater. Levels of metal-binding proteins within the blood plasma must also be constant throughout the life history stages of interest. Although levels of these proteins may change seasonally in adult fish (*see* Kalish 1991), it is not known whether larval or juvenile fish show similar changes over shorter time scales. Finally, ions must be precipitated in the otolith in proportion to their concentrations within the

endolymph, which would appear likely only for those elements that substitute for lattice-bound Ca^{2+} ions (Lea and Boyle 1993). Our results for both Sr:Ca and Ba:Ca are consistent with the above conditions being met in larval and early juvenile Atlantic croaker. The lack of consistent differences across the otolith for either Mg:Ca or Zn:Ca argue that such relationships may not be universal. Exposing individuals to different levels of trace elements under controlled experimental conditions would appear the only way to directly corroborate the utility of otolith microchemistry for recording environmental variations experienced by larval and juvenile fish.

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