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ARTICLE

Validation of Oligohaline Elemental Otolith Signatures of Striped Bass by Use of In Situ Caging Experiments and Water Chemistry

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Abstract

The spatiotemporal variability in strontium (Sr), barium (Ba), magnesium (Mg), and manganese (Mn) elemental signatures of water and fish otoliths was assessed from July to October 2008 across river habitats of Albemarle Sound, North Carolina. We examined whether relationships in these signatures exist and the potential of otoliths to serve as innate chemical tags. Hatchery-reared age-0 striped bass *Morone saxatilis* were placed in cages at four different locations to test development of habitat-specific otolith signatures. Dissolved elemental water and otolith signatures exhibited spatial variability but did not vary temporally. Chemical water signatures classified habitats with 76–81% accuracy, and otolith signatures of caged fish displayed 59–63% total classification accuracy depending on the classification method used. The elements Sr, Ba, and Mn were the main habitat discriminators, as their concentrations in otoliths were significantly correlated with concentrations in the water. Otolith Mg was not related to water chemistry and did not vary among habitats. Natural physiochemical gradients, geochemical processes, and possibly anthropogenic inputs influenced the trace elemental signatures of Albemarle Sound habitats. The unique chemical signals of the sound's river habitats validate the use of otolith signatures for determining striped bass habitat utilization in this system. Use of otolith elemental signatures as natural tags provides a quantitative method to determine the proportion of juvenile striped bass recruiting to the adult spawning stock from specific habitats, thus aiding resource managers in identifying habitats that should receive priority in restoration and conservation decisions.

Accurate assessment of the ecological role of juvenile fish habitats is essential for identifying vital nursery areas to be protected and conserved. Juvenile nursery habitats vary in terms of fish growth potential, survival, and recruitment, and thus influence adult fish populations. Obtaining reliable information about the habitats fish utilize as juveniles is difficult with traditional tagging approaches. The trace element content of fish otoliths has been widely used as a natural tag to indicate habitat utilization in freshwater (Wells et al. 2003; Schaffler and Winkelman 2008), estuarine (Thorrold et al. 1997; Fodrie and Herzka 2008), and marine systems (Rooker et al. 2001; Humphreys et al. 2005). Otolith chemistry provides details of fish habitat use because elements from the environment are incorporated into the calcified structure, providing a permanent chronological record of encountered water masses and food intake (Campana 1999).

Otoliths grow daily as calcium carbonate precipitates onto a protein matrix, forming aragonite crystals that are deposited with diel periodicity (Panella 1971). Because otoliths are located in the ear canal and are not in direct contact with water, there are biological barriers that influence otolith chemistry, including the endolymph–crystal, blood–endolymph, and water–gill interfaces (Campana 1999). Many trace elements are biologically regulated, which would result in discrimination of a specific element at a given biological barrier (Miller et al. 2006). The most commonly investigated elements in otolith chemistry studies include strontium (Sr), barium (Ba), magnesium (Mg), and manganese (Mn). These elements are believed to directly substitute for calcium (Ca) cations during otolith crystallization (Melancon et al. 2005), or they can become included at crystal defects (De Vriesa et al. 2005). Thus, the inclusion of these elements into otoliths is proportional to the availability of dissolved elements in the inner ear of the fish, which is influenced by both ambient water and fish physiology.

Use of otolith elements to suggest habitat utilization requires an understanding of the chemical variability in the ambient water. Estuaries are dynamic mixing zones of freshwater and ocean waters that can be influenced by geochemical and biochemical processes occurring along a salinity gradient (Church 1986; Zwolsman and van Eck 1999). The dissolved load of elements depletes along the salinity gradient through adsorption and desorption processes, and the elements Sr, Ba, and Mg display relatively predictable behavior (Zwolsman and van Eck 1999; Dorval et al. 2005). Manganese, a redox-sensitive element, exhibits unpredictable behavior and can be released from the sediments during suboxic and anoxic conditions (Laslett 1995; Klinkhammer and McManus 2001). Trace elements also are added to aquatic systems through anthropogenic pollution, such as domestic wastewater effluent, fertilizers, and dumping of agricultural wastes (Nriagu and Pacyna 1988). The small-scale temporal and spatial variation that occurs in water chemistry because of natural gradients and human inputs typically is not quantified in otolith element studies and can confound interpretation of chemical signals (Elsdon and Gillanders 2006).

The relationship between otolith chemistry and water chemistry has been investigated for numerous species in controlled laboratory settings (Secor et al. 1995; Geffen et al. 1998; Bath et al. 2000; Milton and Chenery 2001; Martin et al. 2004; Martin and Thorrold 2005). Although consistent patterns between otoliths and water chemistry have been described by laboratory studies, the conditions experienced by laboratory fish deviate from natural conditions. Placing fish in holding cages in the natural environment provides a method of validating the chemical signals produced by a specific habitat (Chittaro et al. 2004; Kraus and Secor 2004; Forrester 2005; Fodrie and Herzka 2008). Fodrie and Herzka (2008) held wild juvenile California halibut *Paralichthys californicus* in cages within southern California embayments and then compared their otolith signatures with those of wild-caught fish. They found no difference in otolith signatures between the caged and wild-caught fish from the Punta Banda habitat, and significant spatial variation in the otolith signatures suggested development of habitat-specific chemical tags. In reciprocal field transplant caging study of juvenile longjaw mudsuckers *Gillichthys mirabilis*, Forrester (2005) detected strong correlations between dissolved Mn in the water and copper (Cu) in the sediments and between Mn:Ca and Cu:Ca concentrations in otoliths of caged fish. Field caging studies have provided evidence that otolith chemistry reflects the ambient water, but the exact relationships differ among species and elements (Kraus and Secor 2004; Forrester 2005; Fodrie and Herzka 2008).

We stocked hatchery-reared juvenile striped bass *Morone saxatilis* in cages within Albemarle Sound, North Carolina, for 6 weeks to investigate the development of habitat-specific signatures of recently deposited otolith material. Dissolved concentrations of trace elements in water were also measured to investigate the relationship between otolith chemistry and ambient water chemistry.

Although researchers have investigated chemical tag development by caging fish in mesohaline and polyhaline estuarine systems where physiochemical properties are influenced by lunar tides (Forrester 2005; Dorval et al. 2007; Fodrie and Herzka 2008), no studies have examined the utility of this technique in a shallow, oligohaline, wind-driven system such as the Albemarle Sound, the second-largest lagoonal complex on the U.S. East Coast.

METHODS

Sampling locations.—We selected four sampling locations in Albemarle Sound: three riverine habitats (Alligator River [ALLG]; Pasquotank River [PASQ]; and Perquimans River [PERQ]) and one shoreline habitat (Batchelor Bay [BATC]; Figure 1). Sample locations were chosen based upon depth to place fish cages (1.2–2.1 m). The three sites within each river represented a sampling gradient of upriver, midriver, and downriver habitats near the open sound. The four sampling locations were chosen to represent a salinity gradient from the oligohaline (western sound) to the mesohaline (eastern sound). Each sample location was sampled in a single day, and all four locations were
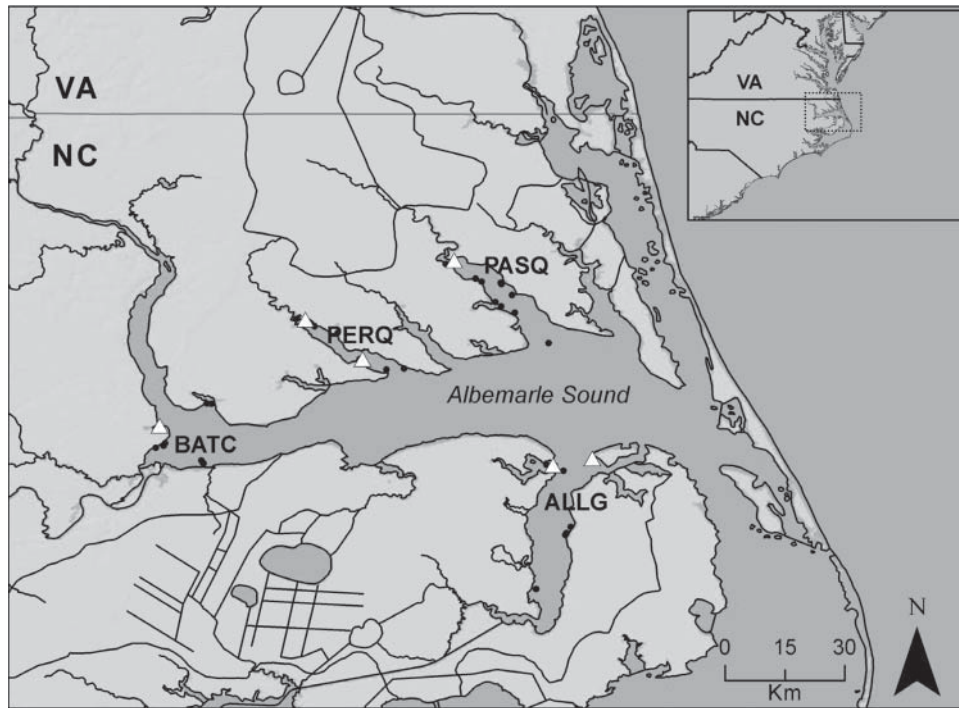
FIGURE 1. Map of the study area in Albemarle Sound, North Carolina, showing age-0 striped bass cage locations (white triangles) and water sample sites (black dots) within four habitats: Batchelor Bay (BATC), Perquimans River (PERQ), Pasquotank River (PASQ), and Alligator River (ALLG). Two cages (one in BATC and one in PASQ) that were lost during storms are not depicted.

sampled over four consecutive days. All locations were sampled once per month from July to October 2008.

Water sample collection and analysis.—We planned to take 48 water samples (4 habitats × 3 sites/habitat × 4 months [July–October]), but on some sampling days the weather, boat trouble, or both prevented us from sampling a particular site; thus, the total number of samples was 43. The 43 water samples were collected at a depth of 80 cm (within 2 m of the cages) by use of a Masterflex peristaltic pump. The pump collected and filtered water inline (Whatman glass microfiber filters: grade GF/D = 1.5 μm; grade GF/F = 0.7 μm) into new 125-mL, high-density fluorinated Nalgene bottles, each rinsed with three sample volumes before the sample was collected. Water samples were stored on ice during transport to the laboratory and were acidified to a pH less than 2.0 with trace-metal-grade nitric acid. Each sample was then filtered by using a 0.2-μm syringe filter (Supor) to remove particulate fractions while retaining colloidal and dissolved fractions. Ambient water quality parameters (temperature, salinity, conductivity, dissolved oxygen [DO], pH, and Secchi depth) were recorded for each sample.

In the laboratory, samples were stored at 4°C until elemental analysis. A Perkin Elmer inductively coupled plasma (ICP) optical emission spectrometer (Optima 2100 DV) was used to measure elemental concentrations of Ca (ppm), Mg (ppm), Sr (ppb), Ba (ppb), and Mn (ppb) with the standards and calibration methods described hereafter. Samples collected at salinities less than 3‰ were diluted with 10 parts of ultrapure water (18.5 Ω)

to one part of sample. A stock standard solution (1,000 mg/L in 2% HNO_3) for each element was diluted to create an element-specific calibration curve with five standards (lowest low, low, medium, high, and highest high). The combined stock solution was analyzed before sample measurements, and quality control checks requiring greater than 90% recovery were issued after every nine samples. Concentrations from water samples were normalized by dividing the concentration of each element by the concentration of Ca to account for the role of Ca in otolith element uptake and to allow direct comparison between otolith and water chemistries.

Caging study.—Eight cylindrical cages (2 cages/habitat) were constructed by using two 1-m-diameter plastic hoops and three 1-m vertical polyvinyl chloride support pipes. The plastic cylindrical frame was covered on the sides, top, and bottom with high-density polyethylene plastic netting (1-cm mesh) attached with cable ties and twine. To minimize contamination of otolith elements, no metal materials were used on the cages. Cinderblocks were used to anchor cages on the bottom (1.2–2.1-m depths); each cage was marked with surface floats. Empty cages were deployed in August, allowing time for development of epiphytic communities (isopods, amphipods, copepods, crabs, shrimp, etc.) that would provide a natural food source for caged fish (Kraus and Secor 2004).

Hatchery-reared age-0 striped bass (10–15 cm total length) from the Edenton National Fish Hatchery (ENFH; U.S. Fish and Wildlife Service, Edenton, North Carolina) were used as

experimental stock. From 15 to 19 September 2008, 15 fish were placed in each cage, where they were held for 6 weeks. Extra fish were transported into the field but were not caged; these individuals were sacrificed to serve as a control group (ENFH: $N = 25$). Extreme weather events (Tropical Storm Hanna on 6 September 2008 and a Nor'easter on 24 September 2008) resulted in the loss of one BATC cage and both PASQ cages. One new cage was deployed empty in PASQ and allowed to biofoul for 2 weeks, after which five hatchery fish were added. Two weeks into the experiment, the single cage remaining in BATC experienced 93% mortality, leaving one fish. The single survivor was harvested, and five additional hatchery fish were added. Only five fish were added instead of 15 because it was hypothesized that overcrowding might have caused the mortality. Five fish were subsequently removed from each cage every 2 weeks, in case additional cage loss occurred. Each fish was measured for total length, fork length, and weight to determine a relative condition factor (K_n), which measures the deviation of an individual fish from the average population weight based on length ($K_n = [W/W'] \times 100$, where W is the actual weight of the fish and W' is the predicted fish weight based on length–weight regression coefficients that were estimated from $\log_{10}$-transformed data collected from the Albemarle Sound striped bass population; Le Cren 1951).

Otolith preparation and elemental analysis.—Sagittal otolith pairs were extracted by using plastic forceps; scrubbed to remove surface tissue; cleaned with distilled, deionized water; and stored in open, 1.5-mL microcentrifuge polypropylene vials to dry in an oven (60–70°C for 12 h). Both otoliths were weighed and stored in individual microcentrifuge tubes. No difference in mass was detected between left and right otoliths; therefore, one otolith was randomly selected for chemical analysis.

A subsample of 41 otoliths from caged fish was sent to the Mass Spectrometry Laboratory at the University of Manitoba (Winnipeg, Canada) for chemical analysis by using laser ablation (LA) ICP mass spectrometry (MS). Otoliths were embedded in an epoxy resin (Buehler Epoxicure), and a 2-mm-thick dorsoventral transverse section (including the core) was cut by using a diamond-blade Isomet saw (Buehler Model 646) at low speed. The dorsoventral section exposes increments with a geometry that dips into the plane of the section, allowing penetration of the laser beam to sample discrete increments (Palace et al. 2007; Halden and Friedrich 2008). The cut sections were then re-embedded in 25-mm-diameter Plexiglas ring mounts (typically 4 otolith sections/mount). The orientation and identity of each section within each ring mount were diagrammed for sample reference. To expose the nucleus region and otolith core, sections were ground down with 320-, 400-, and 600-grit wet sandpaper and were then ultrasonically cleaned for 2 min. Scratches on the otolith surface were removed by polishing with Buehler diamond polishing suspensions (9.00 and 0.05 μm) on a polishing wheel to achieve the completely smooth surface required for laser ablation. Polished and mounted otoliths were given a final ultrasonic cleaning with ultrapure water and were

then digitally photographed to create an illustrated reference for LA-ICP-MS analysis.

Otolith elements were quantified by using a Thermo-Finnigan Element 2 ICP mass spectrometer coupled to a Merchantek LUV 213 neodymium : yttrium aluminum garnet laser. The LA-ICP-MS operating parameters were as follows: beam size was 15 μm; scan speed was 2 μm/s; repetition rate was 20 Hz; power was set at 75% using low-resolution mode. The isotopes counted (^{44}Ca, ^{25}Mg, ^{88}Sr, ^{138}Ba, ^{55}Mn, ^{63}Cu, ^{66}Zn, and ^{208}Pb) were selected based on criteria to reduce molecular interference. Calcium (56% molecular weight calcium oxide) was used as the internal standard to account for variability in laser energy and mass of ablated material. National Institute of Standards and Technology 610 glass was used for external calibration and to monitor any instrument drift. Laser scans were initiated beyond the nucleus and conducted through the core, continuing along the longest axis of otolith growth to the outer edge. Isotope counts were converted to parts per million and plotted versus laser distance. Concentrations of ^{63}Cu, ^{66}Zn, and ^{208}Pb were below detection limits and excluded from subsequent analysis.

The elemental concentration of the otolith outer edge was used to examine the chemical signatures related to the experimental caging period. The amount of otolith edge material used to represent the experimental period was 20 μm for 2 weeks, 40 μm for 4 weeks, and 60 μm for 6 weeks. It was assumed that caged fish deposited 1.4 μm of otolith material per day (Kline 1990).

Statistical analysis.—Nonparametric tests were chosen because some elemental measurements did not meet the assumptions of normality and because of low numbers of fish from the BATC ($N = 6$) and PASQ ($N = 5$) sites. Kruskal–Wallis tests were used to examine the spatial variability (habitat effect) and temporal variability (month or cage time effect) of water and otolith elemental chemistries. When a significant difference was found with the Kruskal–Wallis test, multiple comparisons were conducted with an appropriately adjusted α, controlling the type I error rate to further elucidate the differences, and were subsequently illustrated with box plots. Quadratic discriminant function analysis (QDFA) was used to explore how chemical signatures could be used to correctly classify the water samples or fish otoliths to the habitat of collection. This analysis does not require the data to meet the assumptions of homogeneity of variance and multivariate normality. A linear discriminant function analysis (LDFA), which does require the assumptions of multivariate normality and constant variance, was also conducted to compare with the QDFA classifications. To assess the degree of classification accuracy due to random chance, a random number generator was used to assign elemental measurements of water and otoliths to habitats randomly, and the QDFA and LDFA were then conducted. This was repeated 10 times to create 95% confidence intervals based on random correct classifications (White and Ruttenberg 2007). Canonical discriminant functions were plotted to visualize the separation of habitats in multivariate

TABLE 1. Results of Kruskal–Wallis tests exploring the spatial and temporal variability of Albemarle Sound water chemistry and striped bass otolith chemistry. The effects of habitat (see Figure 1) and month on salinity, temperature, dissolved oxygen (DO), and element : Ca ratios of the water were investigated, as were the effects of habitat and duration of caging (cage time) on otolith element concentrations. For each one-way comparison, α was set at 0.05.

Variable	Effect	χ^2	df	P-value
Salinity	Habitat	32.65	3	<0.001
	Month	4.52	3	0.211
Temperature	Habitat	1.79	3	0.617
	Month	32.77	3	<0.001
DO	Habitat	0.54	3	0.909
	Month	23.20	3	<0.001
pH	Habitat	12.66	3	0.005
	Month	6.68	3	0.083
Water Sr:Ca	Habitat	24.83	3	<0.001
	Month	0.80	3	0.850
Water Ba:Ca	Habitat	11.87	3	0.008
	Month	6.43	3	0.093
Water Mn:Ca	Habitat	14.27	3	0.003
	Month	1.65	3	0.648
Water Mg:Ca	Habitat	26.75	3	<0.001
	Month	1.09	3	0.778
Otolith Sr	Habitat	12.93	3	0.005
	Cage time	2.33	2	0.311
Otolith Ba	Habitat	13.50	3	0.004
	Cage time	1.02	2	0.602
Otolith Mn	Habitat	27.61	3	<0.001
	Cage time	0.62	2	0.735
Otolith Mg	Habitat	1.86	3	0.602
	Cage time	3.11	2	0.211

space. To test the hypothesis that there is a positive relationship between otolith chemistry and water chemistry, the otolith and water chemistry data were matched site-to-site and Spearman's rank correlation coefficient (Spearman's ρ), which is a nonparametric measure of the relationship between two variables, was calculated for each element.

RESULTS

Salinity, Temperature, Dissolved Oxygen, and pH

The physiochemical properties of Albemarle Sound habitats displayed complex relationships. Salinity and pH exhibited significant differences among habitats but not among months, while DO levels and temperature differed among months but not among habitats (Table 1). Temperature decreased steadily from July through October, and DO gradually increased through September and October (Figure 2A, C). Salinity was consistently lowest in BATC (mean = 1.42‰, SD = 0.77), and the mean salinity ranking (from lowest to highest salinity) for the other habitats was PERO, PASQ, and ALLG (range = 3.6–

8.1‰) in all months except October, during which PASQ displayed higher salinity than ALLG (Figure 2B).

Dissolved Elemental Ratios

We found that Sr:Ca, Mg:Ca, and Mn:Ca ratios exhibited mid-salinity concentration maxima between salinities of 4‰ and 6‰ (Figure 3A, C, D), and the Ba:Ca ratio decreased as salinity increased (Figure 3B). Kruskal–Wallis tests revealed only spatial variability of dissolved element : Ca ratios and no significant month effects for any element (Table 1). The Sr:Ca ratio was lowest in BATC, highest in PERQ and PASQ habitats, and intermediate in ALLG (Figure 4A). The Ba:Ca ratio was significantly higher in BATC than in the other habitats (Figure 4B). The Mg:Ca and Mn:Ca ratios tended to be higher in the PERQ and PASQ habitats than in the BATC and ALLG habitats (Figure 4C, D).

Chemistry of Caged Fish Otoliths

In total, 61 hatchery fish were recovered from the caging study. Caged fish exhibited similar lengths and weights after the experimental period, which resulted in similar K_n values among habitats (Table 2). However, the mean K_n of caged fish after the caging study (K_n = 95.96) was considerably lower than that of noncaged control ENFH fish (K_n = 110.63; Table 2). Although somatic growth was slowed, otoliths of caged fish exhibited greater mass (mean = 16.75 mg, SD = 1.06) than control fish otoliths (mean = 14.17 mg, SD = 2.89; Table 2).

Except for Mg, otolith chemistry varied among all habitats, but the duration of caging did not affect otolith chemistry (Table 1). Examining individual element trends with Kruskal–Wallis tests revealed that Sr, Mn, and Ba in otoliths of caged fish were different among habitats (Figure 5). Specifically, Ba was highest in BATC and PERQ otoliths and lowest in ALLG otoliths (Figure 5B); Sr and Mn were highest in PERQ otoliths and lowest in ALLG otoliths (Figure 5A, D). The patterns of Sr, Ba, and Mn were similar among habitats for both water chemistry and otolith chemistry (Figures 4, 5).

Correlations between Water and Otolith Chemistries

Spearman's ρ values revealed that the strongest relationship between otolith chemistry and water chemistry occurred for Mn (Spearman's ρ = 0.77, P < 0.0001), followed by Ba (ρ = 0.60, P < 0.0001) and Sr (Spearman's ρ = 0.59, P < 0.0001). Only Mg displayed a nonsignificant and weak relationship between otoliths and water (Spearman's ρ = 0.25, P = 0.1143).

Multivariate Signature Classification

The degree of classification success varied between QDFA and LDFA; LDFA consistently produced higher classification rates than QDFA. Total classification success of the LDFA was 81% for water and 63% for otoliths, while total classification success of the QDFA was 76% for water and 59% for otoliths. Both QDFA and LDFA produced correct classification rates that were better than random chance (Table 3). For water chemistry
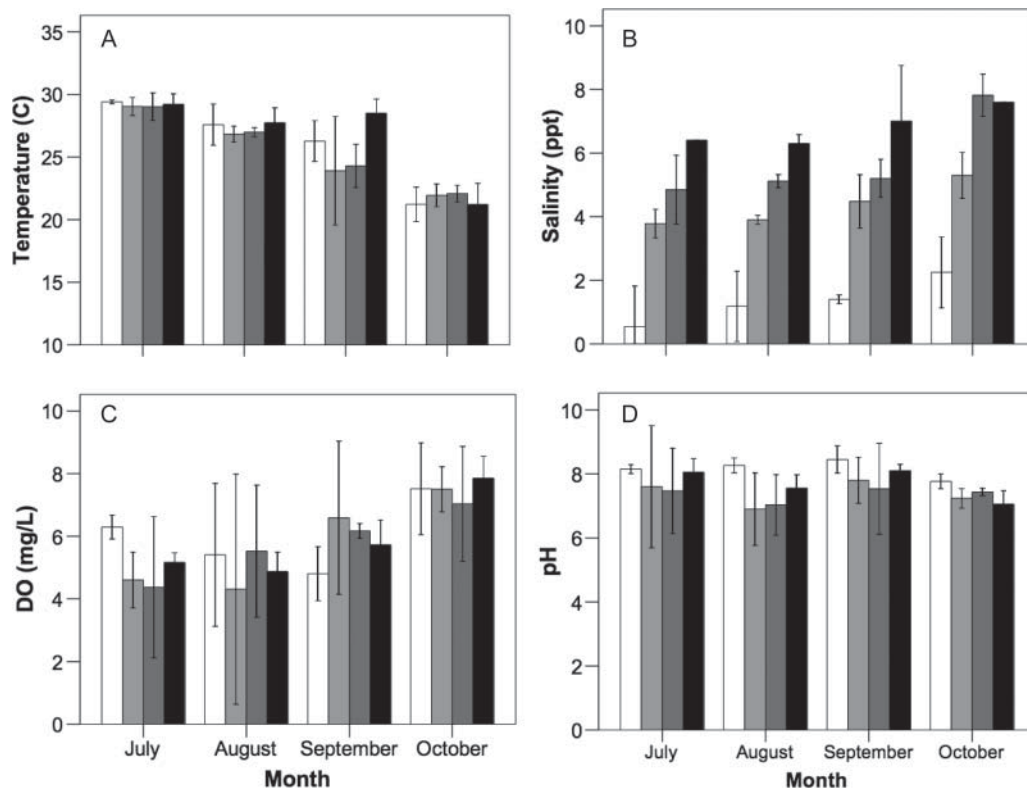
FIGURE 2. Spatial and temporal (July–October 2008) variation (mean $\pm$ 2SD) in (A) temperature ($^{\circ}$C), (B) salinity (ppt [‰]), (C) bottom dissolved oxygen (DO) level, and (D) pH measured at the four Albemarle Sound habitats (abbreviations are defined in Figure 1: white bars = BATC; light-gray bars = PERQ; dark-gray bars = PASQ; black bars = ALLG).

signatures analyzed with QDFA, the highest correct classification occurred for PERQ (82%) and the lowest was for PASQ (67%). The LDFA for water chemistry also classified the PASQ with exactly 67% accuracy, but PERQ had the lowest accuracy (64%); both ALLG and BATC had 100% classification success (Table 3). In general, the water chemistry signatures were classified more accurately than otolith chemistry signatures.

The QDFA for otolith chemistry correctly classified 73% of the PERQ otoliths and 80% of the ALLG otoliths (both habitats:

$N = 15$). However, QDFA classification success was low (17%) for BATC otoliths ($N = 6$) and was 0% for PASQ otoliths ($N = 5$). The LDFA also produced exactly 0% classification success for PASQ otoliths, matched the QDFA exactly for PERQ otoliths (73% accuracy), and was within 1 percentage point for ALLG otoliths (80%). For BATC otoliths, the LDFA produced very high (67%) correct classification, while the random LDFA 95% confidence interval produced no classification (0%). The 95% confidence intervals for random classification were always

TABLE 2. Mean ($\pm$ SD) fork length, weight, otolith weight, and relative condition factor (K_n) of hatchery-reared striped bass placed in cages at four Albemarle Sound habitats (habitat abbreviations are defined in Figure 1). Control fish from the same hatchery source as caged fish (Edenton National Fish Hatchery [ENFH]) were transported into the field but were not caged.

| Habitat or group	Cage	N	Fork length (mm)	Fish weight (g)	Otolith weight (mg)	K_n
BATC	1	6	131.66 $\pm$ 5.47	28.46 $\pm$ 6.09	18.00 $\pm$ 1.31	94.86
PERQ	1	14	131.36 $\pm$ 13.75	29.16 $\pm$ 11.66	17.11 $\pm$ 3.11	95.36
PERQ	2	10	131.50 $\pm$ 12.22	29.43 $\pm$ 9.08	17.21 $\pm$ 2.55	96.44
PASQ	1	5	119.20 $\pm$ 3.63	21.12 $\pm$ 2.40	14.95 $\pm$ 1.41	97.16
ALLG	1	13	126.46 $\pm$ 15.50	27.19 $\pm$ 12.18	16.16 $\pm$ 3.35	98.37
ALLG	2	9	127.70 $\pm$ 19.25	26.66 $\pm$ 10.28	17.07 $\pm$ 2.63	93.56
Pooled habitats	All	57	128.77 $\pm$ 5.16	27.37 $\pm$ 3.16	16.75 $\pm$ 1.06	95.96
ENFH control		25	127.27 $\pm$ 15.48	30.88 $\pm$ 12.24	14.17 $\pm$ 2.89	110.63
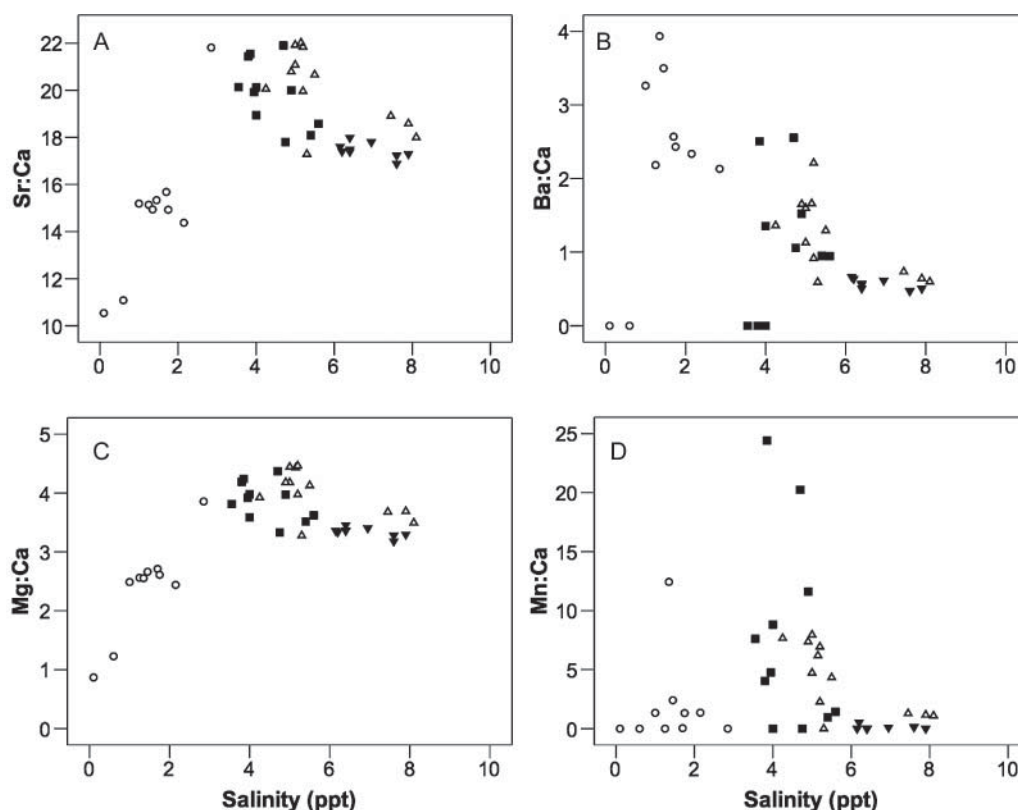
FIGURE 3. Water column element : Ca ratios, **(A)** Sr:Ca, **(B)** Ba:Ca, **(C)** Mg:Ca, and **(D)** Mn:Ca, presented in relation to salinity (ppt [‰]) at the four Albemarle Sound habitats (abbreviations are defined in Figure 1: open circles = BATC; black-shaded squares = PERQ; open triangles = PASQ; inverted black-shaded triangles = ALLG).

higher for otolith chemistry signatures except those from the BATC and PASQ habitats, which had low sample sizes and low random classification success.

Canonical plots showed that the behaviors of the water and otolith signatures were similar, as BATC was clearly separated from the other habitats (Figures 6, 7). The 95% confidence ellipses for PERQ, PASQ, and ALLG overlapped with each other based on otolith chemistry (Figure 7), but only the confidence intervals of PASQ and ALLG overlapped based on water chemistry (Figure 6). The clear separation

TABLE 3. Accuracy of Albemarle Sound habitat classifications (habitat abbreviations are defined in Figure 1) from quadratic discriminant function analysis (QDFA) and linear discriminant function analysis (LDFA) of elemental signatures from water and striped bass otoliths (CI = confidence interval).

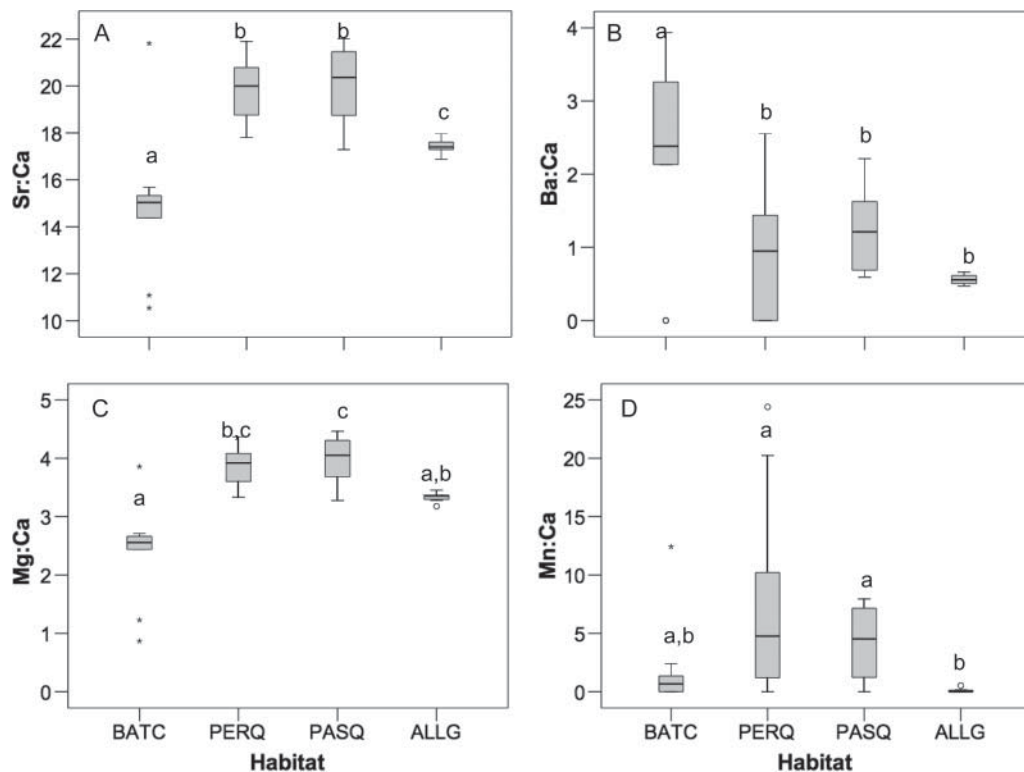| Habitat	Signature	N	Correct classification (%)		Random classification (%) ± 95% CI	
			QDFA	LDFA	QDFA	LDFA
BATC	Water	10	80	100	34 ± 13	28 ± 14
	Otolith	6	17	67	2 ± 3	0 ± 0
PERQ	Water	11	82	64	33 ± 13	35 ± 17
	Otolith	15	73	73	43 ± 9	52 ± 10
PASQ	Water	12	67	67	9 ± 7	30 ± 18
	Otolith	5	0	0	0 ± 0	9 ± 11
ALLG	Water	10	78	100	25 ± 9	9 ± 5
	Otolith	15	80	81	45 ± 16	52 ± 10
All	Water	43	76	81	25 ± 5	26 ± 7
	Otolith	40	59	63	33 ± 6	39 ± 6

FIGURE 4.   Box-and-whisker plots showing element: Ca ratios, **(A)** Sr:Ca, **(B)** Ba:Ca, **(C)** Mg:Ca, and **(D)** Mn:Ca, in water sampled from four Albemarle Sound habitats (habitat abbreviations are defined in Figure 1; horizontal line within box = median; lower and upper edges of box = 25th and 75th percentiles; ends of whiskers = 10th and 90th percentiles; open circles and asterisks = outliers). Within each panel, differing letters (above bars) indicate significant differences between habitats (multiple comparison test after Kruskal–Wallis test).

of BATC was primarily driven by Ba, which followed the same direction in canonical space for both water and otoliths (Figures 6, 7). Manganese followed the direction of PERQ, and Mg pointed towards PASQ for both otoliths and water. Strontium followed opposite directions in canonical space for the chemical signatures of water (towards BATC) and otoliths (towards PERQ, PASQ, and ALLG; Figures 6, 7).

DISCUSSION

The results of this study provide validation of site-specific elemental signatures in otoliths of juvenile striped bass that were caged in four habitats of Albemarle Sound. We hypothesized that the rivers draining into Albemarle Sound would have unique water chemistries due to sediment geology and the existence of physiochemical gradients that control dissolved element behavior. Gradients of ionic strength, pH, oxidation potential, and suspended particulate matter occur in estuaries and change the phase of elements, altering adsorption and desorption processes that affect the mass transport of elements to the ocean (Coffey et al. 1997). The mixing of fluvial and marine water masses leads to the depletion of adsorbed elements on particulate matter and subsequent increases in dissolved elements along the salinity gradient, a common pattern observed in estuarine systems (Zwolsman and van Eck 1999; Dorval et al. 2005). The unique

physiochemical properties of the water masses examined in this study created distinct distributions of trace elements throughout Albemarle Sound.

Spatiotemporal Variability

Salinity varied among habitats and temperature varied among months. Salinity was lowest in the BATC habitat, which receives freshwater input from the Roanoke and Chowan rivers. Among the remaining habitats, PERQ had the lowest salinity, PASQ had intermediate salinity, and ALLG had the highest salinity; these rankings were related to habitat distance from marine water and mixing rates. The only anomaly occurred in October, when salinities at PASQ exceeded those at ALLG (Figure 2B). This anomaly could have resulted from prevailing south winds that transported the salt wedge into PASQ. Wind is the primary factor affecting water circulation patterns within Albemarle Sound; lunar tides and precipitation are of secondary importance (Giese et al. 1979). Temperature steadily decreased each month and was lowest in October, although a temperature anomaly occurred in September at ALLG (Figure 2A). Dissolved oxygen was highest in October when temperatures were lowest (Figure 2A, C) and was consistently above 4 mg/L, which concurs with the observations of Giese et al. (1979). They reported sufficient DO levels in Albemarle Sound year-round, which they attributed
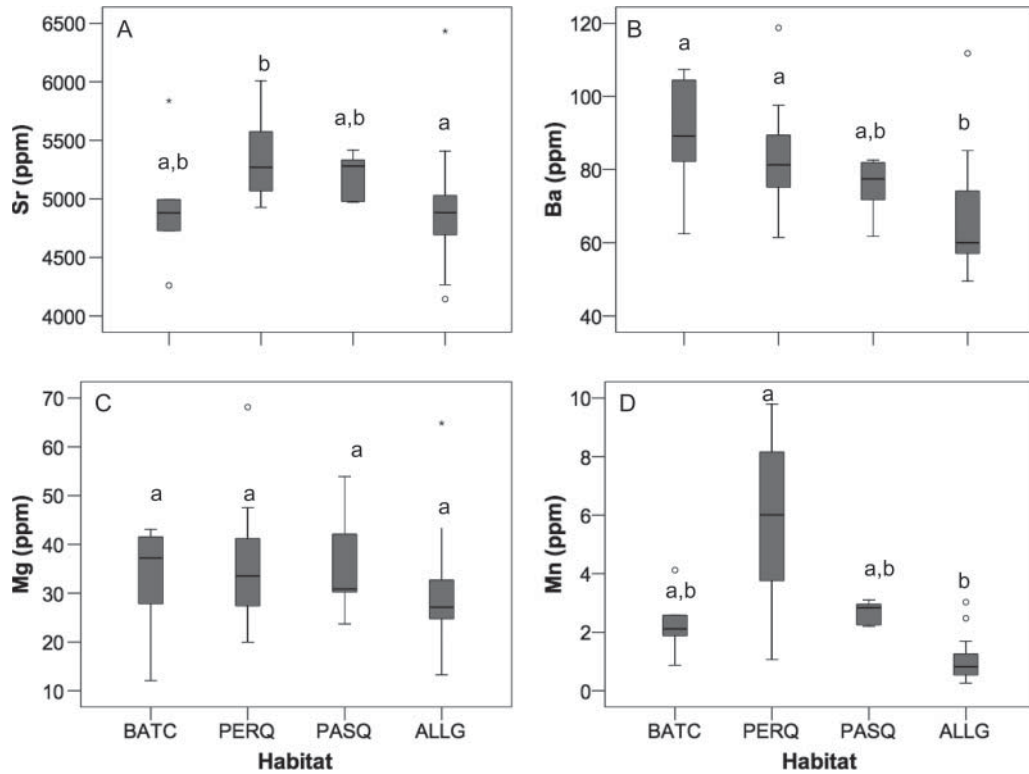
FIGURE 5. Box-and-whisker plots showing otolith edge concentrations (ppm) of **(A)** Sr, **(B)** Ba, **(C)** Mg, and **(D)** Mn from striped bass held in cages at four Albemarle Sound habitats (habitat abbreviations are defined in Figure 1; horizontal line within box = median; lower and upper edges of box = 25th and 75th percentiles; ends of whiskers = 10th and 90th percentiles; open circles and asterisks = outliers). Within each panel, differing letters (above bars) indicate significant differences between habitats (multiple comparison test after Kruskal–Wallis test).
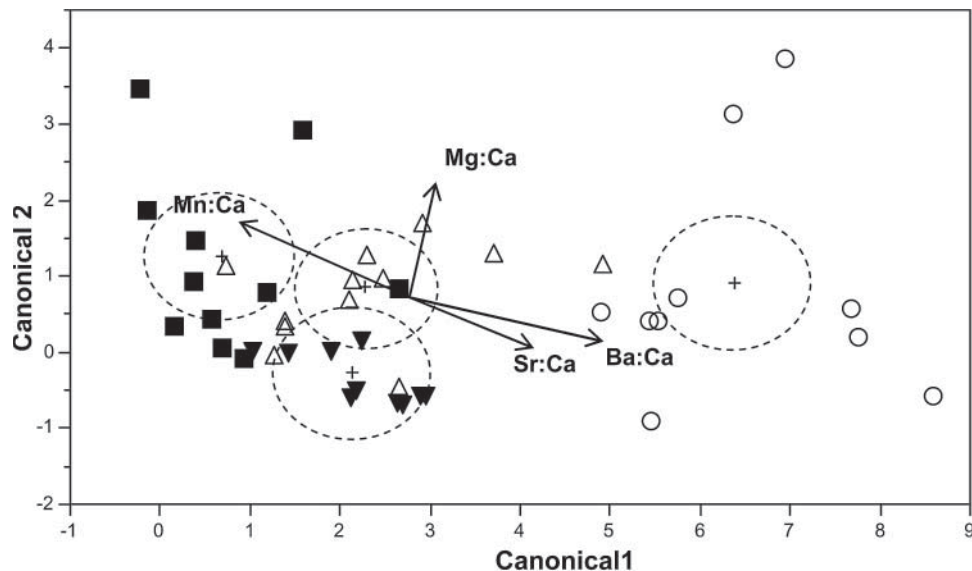

FIGURE 6. Canonical discriminant function plot of water chemical signatures (dependent variables are Sr:Ca, Ba:Ca, Mg:Ca, and Mn:Ca). Plus signs represent the means and dashed circles represent 95% confidence intervals for each of four Albemarle Sound habitats (habitat abbreviations are defined in Figure 1: open circles = BATC; black-shaded squares = PERQ; open triangles = PASQ; inverted black-shaded triangles = ALLG). The direction of each variable in canonical space is displayed as an arrow projecting from the center (grand multivariate mean).
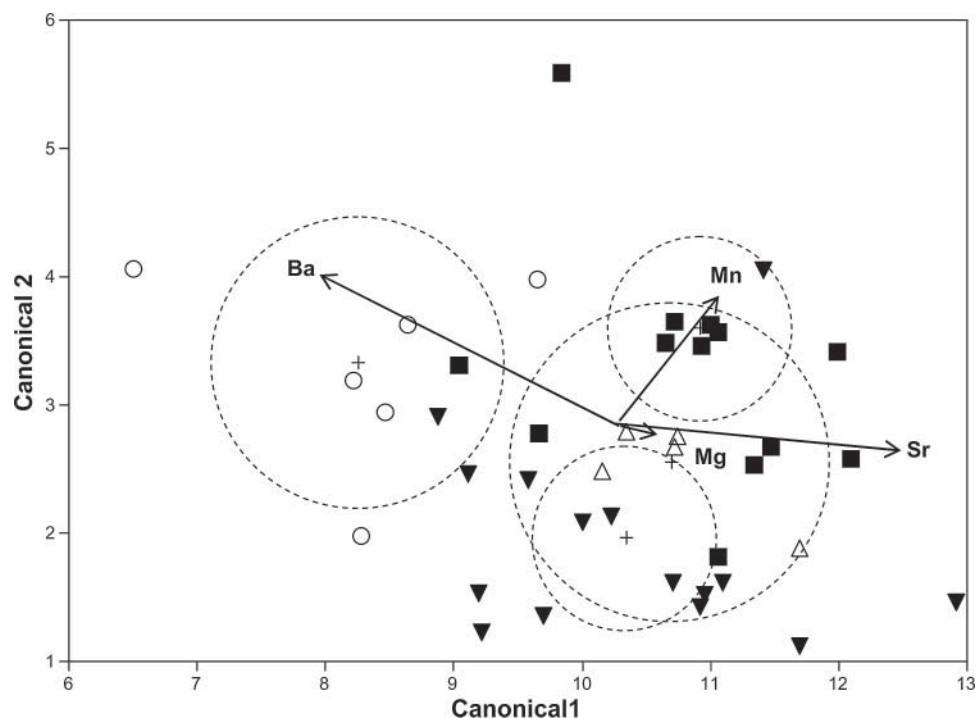
FIGURE 7. Canonical discriminant function plot of striped bass otolith chemical signatures (dependent variables are Sr, Ba, Mg, and Mn). Plus signs represent the means and dashed circles represent 95% confidence intervals for each of four Albemarle Sound habitats (habitat abbreviations are defined in Figure 1: open circles = BATC; black-shaded squares = PERQ; open triangles = PASQ; inverted black-shaded triangles = ALLG). The direction of each variable in canonical space is displayed as an arrow projecting from the center (grand multivariate mean).

to sufficient wind mixing. Although pH remained stable for all months, it was higher in BATC, which receives freshwater and organic material inputs from the Roanoke and Chowan rivers, whereas the PERQ and PASQ habitats drain acidic blackwater swamps with low sediment loads (Riggs 1996).

The dissolved chemical signals remained stable within each habitat through time. The concentration of dissolved elements displayed considerable spatial variation among habitats, but none of the dissolved elements varied by month. Dorval et al. (2005) also reported significant spatial variability of dissolved elemental water chemistry that could be resolved independent of time for five seagrass habitats of the Chesapeake Bay. The habitats examined by Dorval et al. (2005) were separated by distances (10–50 km) and sampled over a time period (July–September) similar to the distance and sampling period in our study, albeit the lack of detection of temporal variation in multielement water chemistry in this study may be attributable to sampling frequency. Using a nested sampling design, Elsdon and Gillanders (2006) detected variation in salinity and elemental water chemistry occurring over the scale of days or weeks that was greater than monthly or seasonal variation. The water circulation patterns of the Australian estuary (Gulf St. Vincent) they examined were influenced by diurnal lunar tides, which could have contributed to the fine-scale variation that was detected (Elsdon and Gillanders 2006). Short-term fluctuation of water circulation in Albemarle Sound is controlled by wind patterns

rather than tidal cycles, as with the Chesapeake Bay (Dorval et al. 2005) and Gulf St. Vincent (Elsdon and Gillanders 2006). A finer-scale sampling design would be needed to investigate the influence of wind direction, frequency, and magnitude on trace element distributions in Albemarle Sound.

Barium

Our data support the use of otolith Ba to indicate habitat utilization of juvenile striped bass in Albemarle Sound. In freshwater, Ba is removed from the dissolved load and is bound to particulate matter. As salinity increases in the estuarine mixing zone, Ba is released, resulting in a mid-salinity concentration maximum (Coffey et al. 1997). The highest dissolved Ba concentrations occurred at the low-salinity BATC sites, which were located at the mouths of the Roanoke and Chowan rivers. Because Roanoke Rapids Reservoir provides approximately 87% of the flow to the Albemarle Sound coastal watershed (Rulifson et al. 1993), the high particulate matter input in the western sound probably resulted in Ba enrichment at the river mouths (mixing zone); temporal trends were related to extreme rainfall events in September (i.e., Tropical Storm Hanna), when Ba concentration was highest in all habitats.

The positive relationships between otolith Ba and ambient Ba have been supported by controlled laboratory experiments (Bath et al. 2000; Milton and Chenery 2001; Martin and Thorrold

2005; Walther and Thorrold 2006) and field studies (Elsdon and Gillanders 2005a; Dorval et al. 2007; Hamer and Jenkins 2007; Comyns et al. 2008; Fodrie and Herzka 2008). Barium displays low biological toxicity and is not physiologically regulated by organisms (Hope et al. 1996). The Ba^{2+} ions could directly substitute for Ca^{2+} ions at lattice binding sites during otolith crystal formation (Campana 1999; Bath et al. 2000) or could attach to nonlattice sites due to crystal defects (De Vriesa et al. 2005). Thus, Ba is incorporated into otoliths in proportion to Ba availability in the water and is useful for tracing freshwater habitat use.

Manganese

Excess concentrations of Mn occurred at mid-salinities (Klinkhammer and McManus 2001). Dissolved Mn is produced in suboxic environments when microbes reduce solid Mn oxides to soluble Mn(II) (Laslett 1995; Klinkhammer and McManus 2001). We found elevated dissolved Mn in both of the mid-salinity habitats, PERQ and PASQ. During our daytime sampling, we observed no hypoxic events that could be related to in situ production of dissolved Mn. Suboxic conditions could have occurred during the night, when respiration exceeds photosynthesis and when oxygen is consumed (Rulifson et al. 1992). The slow kinetics of Mn(II) would allow it to accumulate and persist in the water column into the daytime (Laslett 1995).

Otolith Mn displayed the highest correlation with water Mn:Ca (Spearman's $\rho = 0.77$). Positive correlations between otolith Mn and water Mn as reported by Dorval et al. (2007) were related to redox reactions, which regulate dissolved Mn in Chesapeake Bay. Thorrold and Shuttleworth (2000) suggested that increased Mn:Ca levels detected at the edges of otoliths from Atlantic croakers *Micropogonias undulatus* collected in the Neuse River, North Carolina, could have been related to reducing conditions in the bottom sediments during hypoxic events. Reductive dissolution of particulate MnO_x enhances the dissolved Mn level that is bioavailable to Atlantic croaker otoliths, and thus the otolith is a potential proxy for hypoxic conditions occurring in estuaries (Thorrold and Shuttleworth 2000). Forrester (2005) measured Mn concentrations in water, sediments, and otoliths of longjaw mudsuckers and found that otolith Mn was correlated with ambient water concentrations but not with sediment concentrations. In addition to natural redox cycles that control dissolved Mn concentration, anthropogenic activities could be a source of Mn, including sewage outfall and agricultural practices. For example, Geffen et al. (2003) found elevated Mn in otoliths of plaice *Pleuronectes platessa* collected near a sewage dumping ground. Manganese was highest in otoliths and water from the PERQ, PASQ, and BATC habitats, which experience commercial, residential, and agricultural development upstream and on the shorelines. In contrast, the ALLG habitat is surrounded by the Alligator River National Wildlife Refuge and displayed the lowest water and otolith Mn concentrations.

Strontium and Magnesium

Both Sr:Ca and Mg:Ca behaved similarly; the highest ratios occurred in the PERQ and PASQ habitats. This was unexpected because Sr and Mg typically display predictable behavior and increase along a salinity gradient (Dorval et al. 2005). We expected the highest ratios to occur in the highest salinity habitat (ALLG) because Sr:Ca has been demonstrated to increase positively with salinity (Kraus and Secor 2004). In the Albemarle Sound system, the Sr:Ca and Mg:Ca ratios reached concentration maxima at salinities between 4‰ and 6‰. Chang et al. (2004) found significant differences in the Sr:Ca ratio between 0‰-salinity water and 5‰-salinity water, but the ratio remained constant for water with salinities from 5‰ to 35‰. In our study, the elevated levels of Sr:Ca and Mg:Ca in PERQ and PASQ could be related to the underlying geology of these watersheds. Both PERQ and PASQ lie on the north shore of Albemarle Sound, where bottom sediments consist of 75% mud and 25% sand (Riggs 1996). The ALLG habitat, located on the southern shore of the sound, contains more chemically inert sand (47%), and the differences in sediment types may relate to the difference in elemental distributions for these river systems.

Otolith Mg was not significantly different among habitats, and we did not detect a significant correlation between otolith Mg and water Mg (Spearman's $\rho = 0.25$, $P = 0.1143$). Otolith Sr was significantly correlated with water Sr:Ca (Spearman's $\rho = 0.59$, $P < 0.0001$), but the only significant habitat difference was between PERQ and ALLG; both of these habitats had 15 fish that survived the caging period. In fact, the caged fish from PERQ and ALLG had significantly different otolith concentrations for all elements except Mg.

Although we did not find high Sr in the habitat with the highest salinity (ALLG), dissolved Sr:Ca ratios could be higher in freshwater than in seawater because of the sediment geology of the watershed (Kraus and Secor 2004; Comyns et al. 2008; Howland et al. 2009). Morris et al. (2003) found elevated levels of Sr:Ca in otolith nuclei (i.e., reflecting the juvenile life stage) of adult striped bass collected from the Roanoke River, which concurs with the high dissolved Sr:Ca we detected in water and otoliths of striped bass caged in Albemarle Sound habitats.

Many studies have reported consistent relationships between otolith Sr:Ca and salinity (Secor et al. 1995; Kraus and Secor 2004; Dorval et al. 2007). However, a higher-than-expected otolith Sr:Ca ratio was detected by Comyns et al. (2008), who examined juvenile spotted seatrout *Cynoscion nebulosus* collected in low-salinity water in Mississippi. Juvenile and earlier life stages use different Sr uptake mechanisms or metabolic pathways, which might account for discrepancies in Sr:Ca and salinity relationships between juvenile and adult fish (Comyns et al. 2008). If Sr:Ca ratios become stable at low salinities, then Sr might not be useful as a tracer of habitat use in systems that lack steep salinity gradients (e.g., Albemarle Sound). It is necessary to understand the spatiotemporal variability of dissolved element : Ca ratios in ambient waters before using corresponding otolith chemistries as tags of habitat use.

Habitat Classification based on Multi-Element Signatures

By use of dissolved elemental water signatures, habitats were identified with 81% accuracy by LDFA and with 76% accuracy by QDFA. The separation of BATC from the other habitats in canonical space was presumably dictated by the influx of freshwater to BATC from the Roanoke and Chowan rivers on the west side of the sound. The ALLG habitat, located in the eastern portion of the sound, displayed a high degree of separation from the other habitats, and it is the only river with minimal human development (i.e., it is surrounded by the Alligator River National Wildlife Refuge). The chemical signature of ALLG might remain distinct by natural physiochemical processes if anthropogenic influence remains low. The two habitats in the central sound exhibited less-distinct signals: the classification accuracy was 64% (LDFA) to 82% (QDFA) for PERQ and was 67% for PASQ (QDFA and LDFA). The PERQ and PASQ locations displayed no statistically different water element : Ca ratios or otolith concentrations for any of the examined elements, possibly due to their similar sediment geology (Riggs 1996) or similar anthropogenic inputs, as both rivers are proximal to urban areas and animal farming operations (Clermont 2008). The similarity of the PERQ and PASQ habitats explains their lower classification success based on water chemistry.

The total classification success of the otolith signatures was slightly lower than that of water signatures. When sample sizes where large, the classification success for otolith signatures was similar to that observed for water signatures, regardless of whether QDFA or LDFA was used. However, for PASQ (where $N = 5$) neither QDFA nor LDFA could classify the otolith signatures with any accuracy. For BATC, the LDFA resulted in 67% correct classification, when random chance classification success was 0%. These results suggest that LDFA is more sensitive to sample size than QDFA and may result in inflated classification accuracy. Thus, to prevent false classification success, QDFA is the preferred classification method when sample sizes are low.

Caging Experiment

Development of chemical signals did not depend on the duration of caging (2, 4, or 6 weeks). Elsdon and Gillanders (2005b) found that 20 d of exposure were required for otoliths of juvenile southern black bream *Acanthopagrus butcheri* to become saturated with a chemical signal. Using enriched heavy stable isotopes injected into the peritoneal cavity of yellow perch *Perca flavescens*, S. Melancon and B. Fryer (University of Windsor, unpublished data) found that Sr and Ba were incorporated into the otoliths within 3–5 d. The minimum number of days for which fish were caged in the present study (range = 12–16 d) was sufficient for signature development.

Although caged fish had macroinvertebrate food available on the biofouled cages, several findings indicated that they fed minimally throughout the duration of the study. Caged fish were observed to have empty stomachs upon dissection and might not have eaten the natural food available on the cages

because they were accustomed to feeding on commercially prepared pelleted diets at the hatchery. Caged fish had lower total weights but similar fork lengths as control fish, resulting in considerably lower K_n values. The overall effect of minimal natural diet and low K_n on the deposition of otolith signatures in our study is unclear. Starvation would slow somatic growth and reduce the amount of otolith material deposited (Kline 1990), which was evident in the decreases we observed for all element concentrations, including Ca, along the otolith edge. The low K_n and empty stomachs indicate that caged fish did not experience somatic growth; however, the caged fish did gain otolith weight, suggesting some otolith growth. Otolith growth has been demonstrated to be uncoupled from somatic growth, and daily increments are deposited even during starvation (Secor and Dean 1989; Kline 1990; Wright et al. 1990). Atlantic menhaden *Brevoortia tyrannus* that experienced short-term starvation still deposited daily rings, although increment width was significantly reduced (Maillet and Checkley 1990). Fodrie and Herzka (2008) detected no difference in otolith chemistry between caged and wild California halibut in the Punta Banta estuary, Mexico, even though some caged fish had no discernible daily increment growth bands. Slow growth was compensated for in our study by using a conservative estimate of increment width (1.4 μm/d) to calculate the amount of otolith edge material that represented the fish's time spent in the cage.

Conclusions

We detected significant spatial variability in water chemistry signals of Albemarle Sound habitats, which resulted in overall habitat classification accuracy of 76–81% depending on the classification method. Habitats into which the caged striped bass were placed could be discriminated with 59% accuracy (QDFA) and 63% accuracy (LDFA) based on otolith chemical signatures. Classification success would probably increase with higher sample sizes. Manganese, Ba, and Sr were important elements for differentiating habitats because concentrations in the otoliths reflected the varying concentrations in the water. The unique chemical signals of Albemarle Sound riverine habitats validate the use of otolith signatures for determining habitat utilization by juvenile striped bass and their contribution to the coastal migratory stock in this system.

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