USE OF OTOLITH MICROCHEMISTRY OF SPOTTED SEATROUT TO IDENTIFY STOCK SOURCE-AREAS, REVEAL POPULATION MOVEMENTS, AND DETERMINE INTERANNUAL VARIABILITY IN REGIONAL PATTERNS OF OTOLITH SIGNATURES IN MISSISSIPPI COASTAL WATERS

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ABSTRACT

It is known that juvenile spotted seatrout require shallow marsh-edge or seagrass habitat, but in Mississippi we do not know where the most important nursery source areas for these young fish are located. In a previous Sea Grant study (Comyns et al. 2004) we collected juvenile spotted seatrout from nine coastal regions of Mississippi, and using otolith microchemistry we were able to correctly classify approximately 90% of the juveniles (n=199) with respect to the region from which they were collected in 2001. In addition, by subsequently analyzing the inner portion of otoliths from older fish (same year class) we found that fish that ostensibly developed as juveniles in Grand Bay were also found across much of the Mississippi coastline, indicating that this region may be an important source area of spotted seatrout. The present study furthers this investigation by examining interannual variability in the microchemistry of otoliths from juvenile spotted seatrout collected in 2006 from the same nine regions of coastal Mississippi. This research also enabled us to continue tracking the 2001 year class regarding the location of fish with respect to the region from which they originated. Cleaned otoliths from the left side of juveniles were assayed using inductively coupled plasma-mass spectrometry. Cleaned otoliths from the right side of the same juveniles were analyzed for the stable isotope ratios of carbon and oxygen using a gas ratio mass spectrometer. The same methodology was used for the extracted inner portion of adult otoliths that formed during the early juvenile life-stage. The suite of otolith microchemical variables thus included element/Ca ratios of Ba, Li, Mg, Mn, Sr, as well as $\delta^{13}C$ and $\delta^{18}O$. Six of the microchemical variables showed significant ontogenetic relationships, i.e. were significantly related to ln otolith weight. For these variables, standardized residuals from regressions of otolith variables on ln otolith weight were used in subsequent MANOVA and CDFA analyses. For strontium that showed no ontogenetic relationship, Z-scores relative to the overall mean were used in subsequent analyses. The overall percent of juveniles correctly classified with respect to their capture location was 93.0% when all 201 specimens were included within the CDFA. Cross-validation through leave-one-out procedure estimates how well the CDFA should be able to correctly classify specimens

from a new sample. Overall, 91.5% of the 201 specimens were correctly classified in the cross-validation. Misclassified specimens in were mainly placed into adjacent regions. For example, all 3 misclassified Bay Saint Louis specimens were classified by the cross-validation as having a Pearl River origin; and 4 of the 7 misclassified Pearl River specimens were classified by the cross-validation as having a Saint Louis Bay origin. The Kappa index corrects for chance agreement within the CDFA, and the notably strong Kappa value of 0.921 ($P < 0.001$) for the CDFA was very close to the value of +1, which indicates perfect prediction. The mean distance among sub-regions was only 25 km. Such discernable fine-scale differences in the microchemistry of juvenile spotted seatrout was likely made possible because the Mississippi coastline is influenced by freshwater discharge from seven rivers. Inter-annual differences in otolith chemical signatures between 2001 and 2006 show that caution must be used when using the otolith chemical signature from a particular year to track more than one year class. For instance, the order of decreasing influence of variables for distinguishing regions in 2006 was $\delta^{18}O$, Ba, $\delta^{13}C$, Mn, Li, Mg, and Sr, but in 2001 Li replaced Ba as the second most important variable. These differences were likely influenced by rainfall; mean monthly rainfall was almost twice as much during May through September in 2001 (18.5 cm) than in 2006 (10.2 cm). In this report we did make some inferences about the 2002 year class based on 2001 otolith chemical signatures, but feel this was worthwhile because mean monthly rainfall during this period in 2001 and 2002 was very similar, differing by less than 1one cm. During the second phase of this study we continued tracking the 2001 year class regarding the location of fish with respect to the region from which they originated. We found a general east-west movement of adults by differences between where fish were captured and where they were predicted to have originated. Frequency distributions of 105 adults were aggregated into three major east-west regions across the Mississippi coast: East (Grand Bay, Pascagoula River, Horn Island); Central (Biloxi Bay, Chandaleur Islands, Cat Island), and West (Saint Louis Bay, Pearl River, Marsh Islands). A chi-square test between frequency distributions of where fish were captured and where they were predicted to have originated confirmed that the supply of fish was disproportionate across the three major regions (chi-square = 13.174; df = 2; $P < 0.005$). Fish were

markedly better represented by eastern fish (i.e., 1.34 predicted vs. 1 captured) and less well represented by western fish (i.e., 0.35 predicted vs. 1 captured). Approximately 20% of two and three year-old fish collected across the Mississippi coast in 2004 were predicted to have come from Grand Bay. In contrast, no three-year-olds and only 3% of the two-year-olds were predicted to have come from either the Pearl River region or the eastern Louisiana marshes (Biloxi marsh) for any of the other sampling areas in Mississippi. Although no evidence was found to support the idea that either the Pearl River region or the eastern Louisiana marshes are important source areas of spotted seatrout for other areas of the Mississippi coast, this in no way diminishes the importance of these regions as habitat for juveniles, as evidenced by the significant population of spotted seatrout that they support. In addition, it is quite possible that these areas are important source areas of fish for more westerly sections of the coast. Coastal Mississippi is currently undergoing extensive coastal development, and the ability to determine spotted seatrout source regions will be essential for justifying the conservation of key regions containing valuable nursery habitats. In addition, considering the current strong interest in stock-enhancement of spotted seatrout in Mississippi, regional movements of adults should be taken into consideration for determining release locations for hatchery-reared young juveniles.

INTRODUCTION

Spotted seatrout (*Cynoscion nebulosus*) is a highly prized game fish in inshore waters throughout the Gulf states (Perret et al., 1980; Hettler, 1989; Deegan, 1990). This is the only species of the drum family (Sciaenidae) that spawns primarily in shallow inshore waters (Johnson and Seaman, 1986; Peebles and Tolley, 1988), and remains in inshore waters throughout life. It is known that juveniles require shallow marsh-edge or seagrass habitat (McMichael and Peters, 1989), but in Mississippi we need to learn more about the location of important nursery source areas for these young fish, and the extent to which fish move away from their natal region as they grow. This information is particularly important because the degradation of coastal ecosystems and habitats will

likely continue to increase with expanding coastal development. Otolith microchemistry is proving to be a tool that can help answer these questions.

The microchemistry of fish otoliths has been shown to be extremely useful as a biological tag. This microchemical "fingerprint" has been used as an environmental recorder to address various difficult fishery recruitment issues, including stock identification, the determination of migration pathways, the reconstruction of previous habitat information, age validation, and especially, for use as a natural tag of ambient conditions experienced during various life-history phases (Gunn et al. 1992; Campana et al. 1995, 1999; Thorrold et al. 1997, 1998a; Patterson et al., 2001).

Otoliths are already formed in newly-hatched fish larvae and continue to grow through concentric additions of alternating calcium carbonate and protein layers around a central nucleus. Also incorporated into the crystalline component of the otolith matrix are various trace elements, and the relative abundance of these elements in the otoliths is influenced by the chemical composition of the water in which the fish are growing. The elemental composition of otoliths is not just a reflection of the chemical composition of the water in which the fish are growing because of the metabolic and physiological pathways that elements follow to become incorporated into the otolith. Saltwater fish drink water to maintain their osmotic balance, and consequently many inorganic elements from this water first pass from the intestine into the blood plasma. From the blood plasma the elements then pass into the endolymph fluid that bathes the otoliths, and finally, some of the elements become part of the otolith during the otolith crystallization process (Campana 1999). Because the otoliths are not susceptible to dissolution or resorption, and because growth continues throughout life, these calcified structures provide a permanent record of the influence of exogenous factors on their calcium-protein matrices.

Environmental conditions during otolith growth can also be signified by particular carbon and oxygen stable isotope ratios within otoliths (Thorrold et al., 1998b). These stable isotope ratios also reflect the water chemistry during otolith growth, and are particularly sensitive to changes in salinity. By jointly considering trace element signatures and both carbon and oxygen stable isotope ratios, Thorrold et al. (1998b) were

able to clearly distinguish juvenile weakfish, *Cynoscion regalis*, originating from three adjacent rivers within the Chesapeake Bay estuarine system. In a previous Sea Grant study we used a similar methodology with the closely related spotted seatrout, (Comyns et al. 2004) collected in nine coastal regions of Mississippi. Using otolith microchemistry we were able to correctly classify approximately 90% of the juvenile spotted seatrout (n=199) with respect to the region from which they were collected in 2001. In addition, by subsequently analyzing the inner portion of otoliths from older fish (same year class) we found that fish that ostensibly developed as juveniles in Grand Bay were also found across much of the Mississippi coastline, indicating that this region may be an important source area of spotted seatrout.

The present study furthers this investigation by examining interannual variability in the microchemistry of otoliths from juvenile spotted seatrout collected in 2006 from the same nine regions of coastal Mississippi. This research also enabled us to continue tracking the 2001 year class regarding the location of fish with respect to the region from which they originated.

OBJECTIVES

(Year1)

1) During summer 2004 collect age 3+ spotted seatrout from the same nine coastal regions where juvenile fish were collected in 2001 for otolith microchemistry analyses. Collection sites will extend from Grand Bay, Alabama to the Louisiana marshes east of the Mississippi River.

2) Remove sagittal otoliths and analyze the inner portion (*i.e.*, the portion that formed during the larval and young juvenile period) to identify which nursery areas or regions these adult fish probably used as young juveniles. This will reveal regional patterns in stock-recruitment structure of adult reproductive spotted seatrout with respect to source areas of 2001 juveniles, and will provide information about the movement of these older fish.

(*Year 2*)

1) Collect juvenile spotted seatrout from the same nine coastal regions where we collected juvenile fish in 2001.

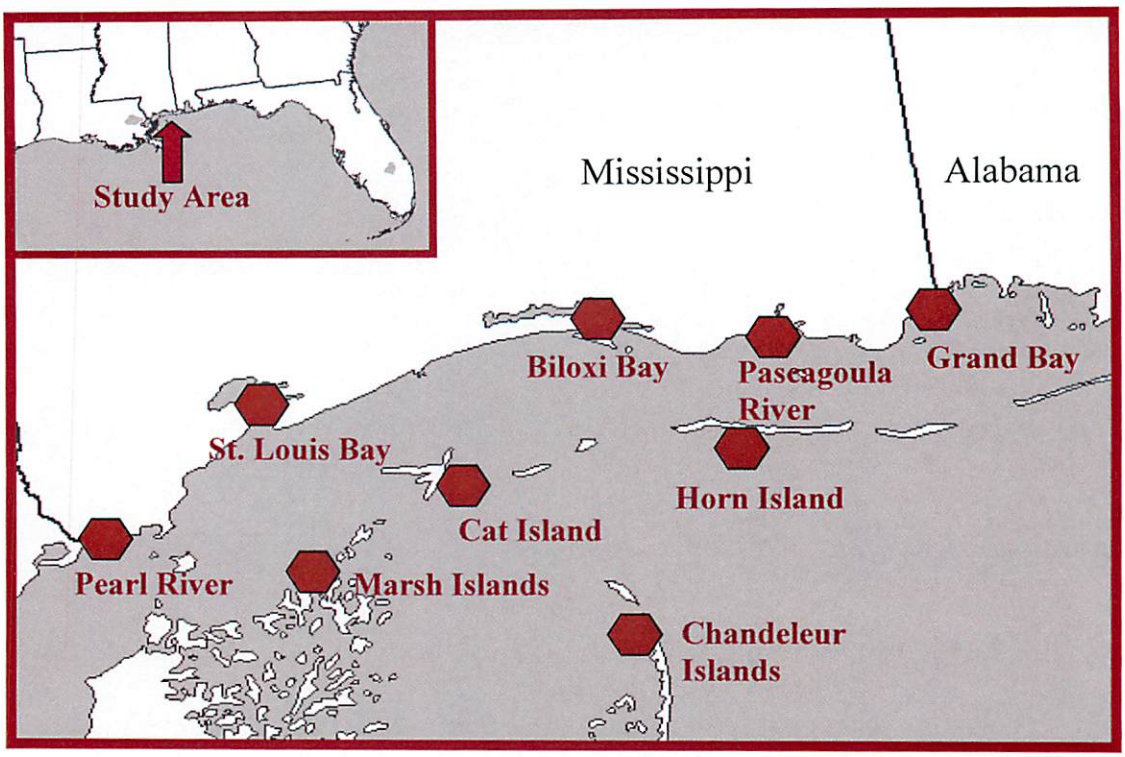
2) Remove sagittal otoliths and analyze for spatial patterns in otolith microchemistry. Compare regional patterns in otolith microchemistry between 2001 and 2006 cohorts of spotted seatrout to determine if particular chemical "fingerprints" are typical for our different estuarine areas, or if there is much inter-annual variability. This would need to be known for possible future studies of not only spotted seatrout, but also for other species that assuredly will be studied using these techniques.

METHODS

Collection of Juveniles and Adults.

During summer 2005 juvenile spotted seatrout were collected at five locations along the Mississippi shoreline but this effort came to an abrupt halt with Hurricane Katrina. All specimens had been stored frozen in the Research building of the Gulf Coast Research Laboratory (GCRL). This building was flooded, and thus all these specimens were destroyed. Efforts resumed in late summer 2006, and young juvenile spotted seatrout were collected from shoreline habitat in nine nursery sub-regions bordering Mississippi Sound from Grand Bay, Alabama to the Louisiana marshes east of the Mississippi River (Figure 1). Collections were taken with a 15.2 m bag seine with a bag mesh size of 3.17 mm. Juveniles were stored on ice, returned to the laboratory and frozen.

Figure 1. Sampling locations for juvenile spotted seatrout collected in 2006.



Age 3+ spotted seatrout, which range in length from approximately 300 to 550 mm TL (Warren 1998), were collected with two 91 m gill nets (7 cm stretch mesh) during summer and fall 2004, and spring 2005. The net was fished by anchoring one end to the shoreline and allowing a soak time of 30 min. In addition, specimens were collected by local anglers. Fish were stored on ice, returned to the lab, measured and sexed. Heads were frozen until the otoliths could be removed. Fish were collected from each of the same nine coastal regions as juveniles were obtained in 2001. The age three year class was targeted because the inner portion of otoliths from these fish was formed at the same time as the juveniles we collected in 2001 (same year class). The collection of fish continued into spring 2005 because relatively few three-year-old fish were collected in 2004 at several locations. Unfortunately, frozen heads from adults that were subsequently

collected from the Pascagoula River, St. Louis Bay, and the Louisiana marshes were destroyed during Hurricane Katrina.

Otolith analyses using solution-based elemental assays and isotope analyses of $\delta^{13}C$ *and* $\delta^{18}O$.

Some of this methodology was presented in a previous final report but is repeated for completeness. Juvenile spotted seatrout were thawed and measured prior to the removal of otoliths. Sagittal otoliths were used because they are the largest of the three types of otoliths and are conventionally used for otolith microchemistry work. Otoliths were removed from both the left and right sides of juveniles with acid-washed teflon-coated forceps, rinsed with ultrapure (Milli-Q) water, and temporarily stored in sterile 24-well cell culture clusters. In a Class 100 clean room using a laminar flow bench, each otolith was placed into an acid-washed, pre-weighed (μg), micro centrifuge tube using acid-washed teflon forceps. Centrifuge tubes were then filled with 0.001 N re-distilled nitric acid using a metal-free polyetheylene pipette tip that had been triple-rinsed with 0.1 N re-distilled nitric acid and triple-rinsed with Milli-Q water. Otoliths were washed with the dilute acid to remove any remaining contaminants (metal ions) from the otolith surface. After one to two minutes, the acid was removed from the centrifuge tubes with a clean pipette tip, and then the otoliths were triple-rinsed while in the centrifuge tubes with Milli-Q water, and air-dried in the laminar flow bench for 24 h. Centrifuge tubes containing cleaned otoliths were then re-weighed to obtain otolith weights (μg).

Cleaned otoliths that were removed from the left side of juveniles were dissolved in a measured quantity of 0.1 N re-distilled nitric acid, and otolith solutions were assayed with a magnetic sector ICP mass spectrometer (ThermoFinnigan Element 2) located at the Stennis Space Center (USM Department of Marine Science). Calibration was by external standards which were 4 mM in Ca, about the same Ca concentration as the otolith samples. All elements (Ba, Li, Mg, Mn and Sr) were measured at medium resolution on the ICP-MS and In was used as an internal standard to correct for instrument drift. In addition, selection of samples for analysis was random which precluded the confounding effects of instrument drift (Campana and Gagné, 1995). The

molar concentrations of different elements in the otoliths were standardized to the number of calcium ions in the otoliths and expressed as ratios to the molar concentration of Ca.

Cleaned otoliths from the right side of juveniles were powdered and analyzed for stable isotope ratios of $\delta^{13}C$ and $\delta^{18}O$. Otoliths were powdered with an agate mortar and pestle which was rinsed with Milli-Q water. Two mortars and pestles were used so that one could be dried under a heat lamp while the other was in use. Powdered otoliths were transferred to acid-washed micro centrifuge tubes. Samples were pretreated by heating in vacuo at 75 °C for 0.5 h, and analyzed on a Micromass Optima isotope ratio mass spectrometer. Carbon dioxide from each sample was generated by acidification with phosphoric acid in a heated (90°C) common acid bath. The resultant gas was purified and introduced into the mass spectrometer inlet system and compared against a standard reference gas of known isotopic value. Values of $\delta^{13}C$ and $\delta^{18}O$ were calculated against V-PDB. Mean precision was (one sigma) +/- 0.04 per mille for $\delta^{13}C$ and +/- 0.06 per mille for $\delta^{18}O$.

Adult spotted seatrout were thawed, measured and sexed prior to the removal of sagittal otoliths. Otoliths were removed from both the left and right sides of fish and embedded in epoxy-resin molds. The inner portion of otoliths that formed during the early juvenile life-stage was extracted with six precision cuts from each otolith using a low-speed Buehler Isomet saw. This methodology followed that reported in our previous SeaGrant final report (Comyns et al. 2004), but is repeated here for completeness.

Extraction accuracy was enhanced by observing otoliths using a large mounted magnifying-lens while making the cuts. The size of extracted cores, i.e. length, width and depth, was determined by comparison with otoliths from juveniles. Juvenile otoliths collected in 2001 ranged in weight from 2 mg to 48 mg (n=240, $\bar{x}$ = 8.9 mg). Otolith lengths, widths and depths (maximum thickness) were measured for 17 otoliths that ranged in weight from 4.7 to 20.9 mg (Table 1).

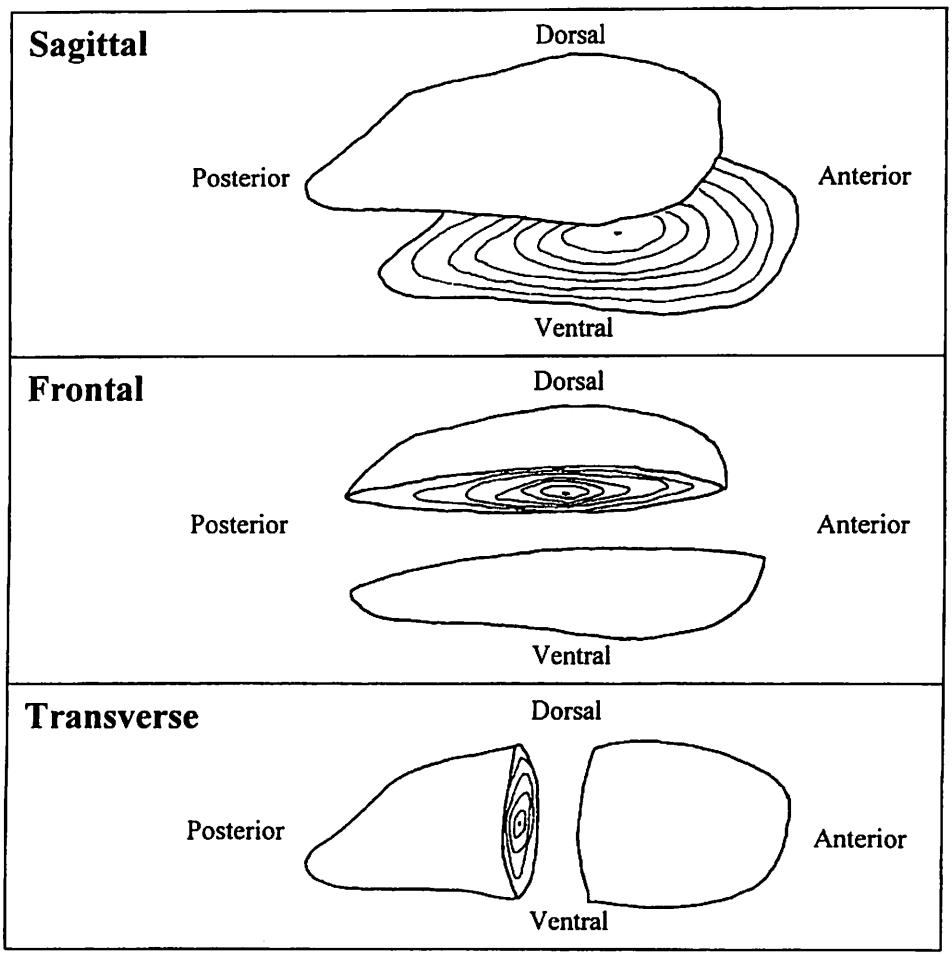
Table 1. Morphometric measurements of otoliths from juvenile spotted seatrout (collected in 2001) used to determine dimensions of core to be extracted from adult otoliths.

Otolith Weight (mg)	Otolith Length (mm)	Otolith Width (mm)	Otolith Depth (mm) (maximum thickness)
4.7	3.60	1.70	0.72
4.8	3.68	1.60	0.64
5.4	3.76	1.76	0.72
5.6	3.60	1.76	0.80
5.8	3.84	1.76	0.64
5.8	4.00	1.92	0.64
6.1	3.92	1.68	0.72
6.2	3.76	1.76	0.80
11.6	4.80	2.32	0.96
15.3	5.28	2.16	1.04
18.0	5.68	2.40	1.04
18.2	5.68	2.88	1.04
18.3	5.84	2.48	1.12
20.4	5.92	2.80	1.12
20.7	5.92	2.64	1.12
20.9	5.76	2.48	1.20
20.9	6.00	2.48	1.04

Otolith width averaged 45.5% of otolith length, and otolith depth averaged 19.0% of otolith length. Because the edges of otoliths are tapered, as opposed to the thicker edges of a rectangular block that is cut from the center of an adult otolith, the extracted portion was chosen to be shorter (3.6 mm) than the mean juvenile otolith length. Based on the otolith length/width/depth relationships of spotted seatrout otoliths, an otolith with a length of 3.6 mm would have a width of about 1.6 mm and a maximum depth of 0.7 mm. This pre-determined otolith length of 3.6 mm also provided a mean weight of 10.7 mg for the rectangular cores that were extracted from adult otoliths. This was similar to the mean weight of juvenile otoliths ($\bar{x}$ = 8.9 mg). As previously mentioned, these otolith measurements were from juveniles collected in 2001. This same methodology for extracting the core region of adult otoliths was followed for spotted seatrout collected in 2004 and 2005.

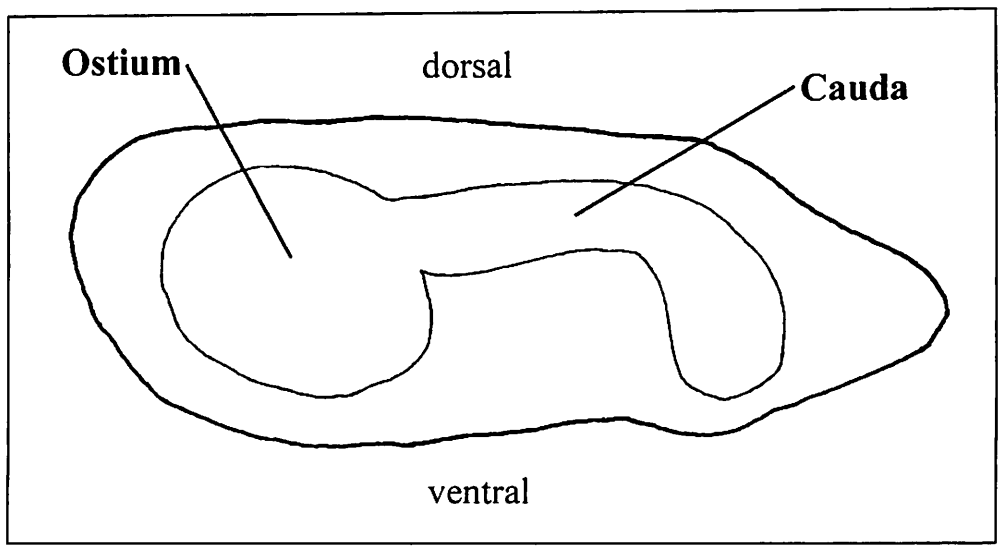
Embedded adult otoliths were first observed in the sagittal plane (Figure 2).

Figure 2. Orientation of cross section through otolith taken in the sagittal, frontal and transverse planes.



The saw blade was aligned over the otolith at the junction between the ostium portion of the sulcus acousticus, and the ventral edge of the cauda (Figure 3). This reference point was positioned over the otolith primordium.

Figure 3. Surface of spotted seatrout otolith showing the ostium and cauda portions of the sulcus acousticus.



A core length of 3.6 mm was obtained by making a transverse cut at 1.8 mm to each side of the primordium. The embedded otolith was re-positioned to expose a transverse cross section. The center of this section, which was positioned over the primordium and which was referenced by the base of a cross section of the sulcal groove, was lightly marked with an ultra fine-point pencil mark. This mark was needed to reposition the saw blade, and a frontal cut was then made at 0.8 mm on each side of the mark to establish the width of the removed core (1.6 mm). Finally, the depth of the core to be extracted was first determined by again re-positioning the otolith to expose a frontal cross section, and marking the center of this section across the width of the otolith with several fine pencil marks. A core depth of 0.7 mm was obtained by making a sagittal cut at 0.35 mm to each side of the marked center.

Prior to cutting the core from an otolith, a thin transverse slice was cut at the edge of the block to be extracted in order to age the adults. Annuli were counted following Bedee et al. (2003).

To initially clean otolith cores of the ultra-fine pencil marks, marked sides of the cores were lightly sanded using 1000 grit wet-or-dry sandpaper and cores were rinsed

with Milli-Q water. Otolith cores were then placed into acid-washed micro centrifuge tubes, and during the second stage of cleaning, the outer layer of the core was dissolved using 0.3 N re-distilled nitric acid. Acid was added to centrifuge tubes using a metal-free polyetheylene pipette tip that had been triple-rinsed with 0.1 N re-distilled nitric acid and triple-rinsed with Milli-Q water. After five minutes, the acid was removed from the centrifuge tubes with a clean pipette tip, and then the otolith cores were triple-rinsed while in the centrifuge tubes with Milli-Q water, and air-dried for 24 h. The weight of otolith cores was reduced by about 9% by this acid treatment. After the treatment, otolith cores remained as sharp-edged rectangular blocks without any visible pitting of the otolith surface. Final cleaning was conducted in a Class 100 clean room using a laminar flow bench. Secor et al. (2001) also used a more rigorous approach to remove surface contamination of otoliths than in most studies; otoliths were immersed for 5 min in 1% nitric acid, resulting in a mass loss of four to five percent. In studying the effect of such an acid treatment on the chemical composition of otoliths, Secor et al. (2001) found only small changes in the concentration of elements, and the effect of the acid treatment was consistent among elements. Campana et al. (2000) found no significant differences for concentrations of elements between acid rinsed and un-rinsed cod otoliths; elements examined were Li, Mg, Mn, Sr and Ba.

Otolith cores were treated the same way as juvenile otoliths. Each otolith core was placed into an acid-washed, pre-weighed, micro centrifuge tube using acid-washed teflon forceps. Centrifuge tubes were then filled with 0.001 N re-distilled nitric acid using a metal-free polyetheylene pipette tip that had been triple-rinsed with 0.1 N re-distilled nitric acid and triple-rinsed with Milli-Q water. Otoliths cores were washed with the dilute acid to remove any remaining contaminants (metal ions) from the otolith surface. After one to two minutes the acid was removed from the centrifuge tubes with a clean pipette tip, and then the otolith cores were triple-rinsed while in the centrifuge tubes with Milli-Q water, and air-dried in the laminar flow bench for 24 h. Centrifuge tubes containing cleaned otolith cores were then re-weighed to obtain core weights. Cleaned cores from left and right otoliths were analyzed for trace elements and both $\delta^{13}C$ and $\delta^{18}O$ in the same way as for juvenile otoliths.

Data Analyses

Otolith chemistry variables included stable isotope ratios of $\delta^{13}C$ and $\delta^{18}O$, and element/Ca ratios of Ba, Li, Mg, Mn, and Sr. Calcium-standardized lithium values were scaled up by 100 to attain proper precision for SPSS 11.0 (SPSS 2002). Data analysis of otolith chemistry proceeded through five successive stages using SPSS 15.0.

First, mean otolith weights for each region were compared to determine whether differences in body size might influence subsequent analyses. Kolmorgorov Smirnov One-Sample tests were made against the normal distribution for both otolith weight and $\log_{10}$ otolith weight for each of the nine regions. Subsequently, a One Way Analysis of Variance (ANOVA) was run using $\log_{10}$ transformed otolith weight with Region as a Fixed factor. Post-hoc Games and Howell tests that assume heterogeneous variance among groups were used to elucidate regional differences in fish-size distributions based on $\log_{10}$ otolith weight.

Second, in light of regional differences in body size distributions, ototlith chemistry variables were regressed against otolith weight to ascertain which variables might confound the subsequent regional classification due to ontogenetic variability. Trace metal variables (concentrations) were $\log_{10}$ transformed to stabilize their variances in linear regressions with $\log_{10}$ otolith weight.

Third, because element/Ca ratios for several elements differed by an order of magnitude, otolith chemistry variables were scaled to a mean of 0 and a standard deviation of 1 prior to multivariate analyses. For the otolith variables with significant regressions on otolith weight, standardized residuals were used in subsequent Multivariate Analysis of Variance (MANOVA) and Canonical Discriminant Function Analysis (CDFA) to eliminate possible regional biases due to fish size. Alternatively, for those otolith variables without significant ontogenetic relationships, Z-scores (i.e., with respect to the overall mean) were used.

Fourth, a MANOVA was performed to determine whether the seven otolith chemistry variables differed among the nine regions, and to identify which otolith variables should be included in a follow-up CDFA. Region was analyzed as a Fixed

factor. The homogeneity of covariance matrices assumption was tested using Box's M test. Subsequent univariate ANOVAs with Region as a Fixed factor examined the degree to which each of the seven otolith variables differed among the nine regions. Levene's tests addressed whether error variances were homogeneous among regions.

Because variances among regions were mildly to moderately heterogeneous for both the One-Way ANOVA on otolith weight and the MANOVA on the otolith chemistry variables, overall regional differences were confirmed through the Multiresponse Permutation Procedure (MRPP) (Mielke et al. 1976), using a SPSS macro written by Cai (2006). The standardized statistic T, reflects the difference between the mean of the MRPP null distribution and the observed parameter, δ, which depends only on the regional means. An exact probability is associated with any particular T value.

Fifth, a CDFA was carried out to develop a regional classification instrument for the 2006 year class of spotted seatrout. The reliability of the CDFA was evaluated via cross-validation and the Kappa index. As for the MANOVA, the homogeneity of covariance matrices assumption was tested using Box's M test. Selected CDFA options included: (1) the within-groups covariance matrix; (2) prior probabilities of group membership were considered equal across groups; and (3) all otolith variables were entered together into the analysis. Coordinates of specimens coded by their regional affiliation along with regional centroids were plotted within the two-dimensional space formed by the first two canonical functions. Reliability of the CDFA was evaluated in three ways: (1) through classification success within the CDFA in which all specimens were included; (2) through cross-validation using the "jackknife" procedure, which involves classifying each individual using a CDFA that excludes the "unknown" specimen; and (3) through the Kappa index, which adjusts for potential chance agreement within the CDFA (Green and Salkind 2000).

An algorithm was developed based on the CDFA parameters to predict original sub-regions for adult spotted seatrout based on their otolith microchemistry. The algorithm accepts input for each specimen: the sub-region where it was collected, $\log_{10}$ otolith weight, and raw values for the eight otolith variables. The raw values for the otolith variables were transformed as in the original CDFA, and standardized residuals

were calculated with respect to predicted values based on otolith weight using parameters from the otolith weight regressions. Using classification functions from the CDFA for each of the nine sub-regions, function values were calculated based on subregion-specific constants and weights applied to each otolith variable for each individual. The individual was subsequently assigned to the sub-region for which the highest classification function value was obtained.

RESULTS

Juvenile Spotted Seatrout

Two hundred and one juvenile spotted seatrout were collected from the nine sub-regions of coastal Mississippi. Otolith microchemical variables included stable isotope ratios of $\delta^{13}C$ and $\delta^{18}O$, and molar concentrations of Ba, Li, Mg, Mn and Sr, standardized by molar Ca concentrations (Table 2, Appendix 1). In our preceeding study we also incorporated Na, but this was probably not appropriate because this element is under strong physiological control and is not likely to show spatial differences in water chemistry. Most regions were represented by from 22 to 24 specimens; except for the Saint Louis Bay, which was represented by 11 cases.

Table 2. Mean (±SD) molar concentrations of otolith microchemical variables (standardized by molar calcium concentrations), and stable isotope ratios of $\delta^{13}C$ and $\delta^{18}O$ in otoliths of juvenile *Cynoscion nebulosus* collected in the northcentral Gulf of Mexico.

SITE	$\delta^{13}C$ (‰)	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	$\delta^{18}O$ (‰)	Li ($\mu g\ g^{-1}$)	Li ($\mu g\ g^{-1}$)	Mg ($mg\ g^{-1}$)	Mg ($mg\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Mn ($\mu g\ g^{-1}$)	Sr ($mg\ g^{-1}$)	Sr ($mg\ g^{-1}$)	Ba ($\mu g\ g^{-1}$)	Ba ($\mu g\ g^{-1}$)
	2006	2001	2006	2001	2006	2001	2006	2001	2006	2001	2006	2001	2006	2001
Biloxi Bay	-5.33 (0.61)	-6.59 (0.71)	-2.34 (0.17)	-3.84 (0.39)	3.19 (0.24)	1.60 (0.68)	0.204 (0.019)	0.172 (0.021)	44.3 (7.7)	44.4 (12.3)	2.46 (0.13)	2.51 (0.18)	28.1 (4.8)	18.7 (5.7)
St. Louis Bay	-5.77 (0.76)	-7.80 (1.27)	-1.78 (0.29)	-3.96 (0.36)	4.07 (0.79)	1.01 (0.56)	0.242 (0.047)	0.186 (0.022)	69.2 (7.1)	52.8 (16.0)	2.30 (0.09)	2.70 (0.22)	42.3 (23.5)	33.9 (7.9)
Cat Island	-4.66 (0.56)	-3.82 (0.35)	-1.76 (0.08)	-2.30 (0.09)	4.07 (0.32)	3.00 (0.61)	0.210 (0.015)	0.193 (0.027)	32.3 (7.1)	31.2 (4.8)	2.46 (0.11)	2.25 (0.17)	34.7 (5.6)	23.6 (6.0)
Chandeleur Islands	-1.99 (0.36)	-1.73 (0.56)	-1.34 (0.15)	-0.66 (0.28)	4.65 (0.31)	4.19 (0.53)	0.190 (0.021)	0.155 (0.019)	54.4 (14.5)	53.6 (10.3)	2.40 (0.28)	2.05 (0.18)	11.2 (3.42)	8.72 (2.47)
Grand Bay	-4.62 (0.69)	-5.12 (0.55)	-1.69 (0.15)	-2.39 (0.07)	3.72 (0.40)	2.88 (0.47)	0.164 (0.022)	0.173 (0.029)	41.4 (10.0)	42.8 (9.2)	2.24 (0.12)	2.20 (0.17)	7.5 (1.7)	12.2 (4.0)
Horn Island	-2.14 (0.57)	-3.03 (0.49)	-1.42 (0.16)	-2.30 (0.05)	4.01 (0.33)	3.17 (0.55)	0.182 (0.014)	0.185 (0.023)	19.8 (4.3)	17.3 (2.3)	2.31 (0.16)	2.20 (0.14)	17.0 (3.3)	20.6 (3.8)
LA Marshes	-4.26 (0.64)	-5.15 (0.39)	-0.99 (0.20)	-2.69 (0.16)	4.08 (0.55)	2.27 (0.56)	0.185 (0.020)	0.208 (0.031)	28.7 (5.7)	39.9 (6.9)	2.07 (0.13)	2.35 (0.20)	23.8 (6.4)	30.0 (8.7)
Pascagoula River	-5.62 (0.80)	-7.89 (0.99)	-3.00 (0.45)	-4.39 (0.23)	2.91 (0. 57)	1.18 (0.35)	0.216 (0.016)	0.147 (0.019)	39.8 (5.8)	45.9 (8.1)	2.31 (0.19)	2.61 (0.27)	13.8 (5.7)	27.3 (12.0)
Pearl River	-4.82 (0.98)	-8.21 (0.80)	-1.98 (0.30)	-3.27 (0.33)	2.25 (0.43)	1.19 (0.37)	0.182 (0.028)	0.175 (0.017)	51.4 (16.4)	86.2 (27.8)	2.23 (0.11)	2.57 (0.21)	27.6 (7.5)	42.9 (11.3)

Distribution of otolith weight across regions

One sample K-S tests implied that ln otolith weight was normal for all regions ($P > 0.1$), except the Pearl River (K-S $Z = 1.56$; $P = 0.016$). In a One-Way ANOVA, ln otolith weight differed significantly among the nine regions ($F = 13.404$; $P < 0.001$); notwithstanding heterogeneous variance in ln otolith weight ($F = 7.861$; $P < 0.001$). Post-hoc Games and Howell tests on differences in ln otolith weight implied that fish sizes were the most distinct from the Chandeleur Islands ($P < 0.06$ for 4 of 8 tests) and from Saint Louis Bay ($P < 0.05$ for 4 of 8 tests), where fish were relatively small; and from the Pearl River ($P < 0.05$ for 5 of 8 tests) and Horn Island ($P < 0.06$ for 6 of 8 tests), where fish were relatively large. These specific differences in the size of fish collected from different regions were not the same as were found in 2001 (Comyns et al. 2004), but the degree of significance in regional differences and heterogeneity in ln otolith weight were comparable to the 2001 data. The multi-response permutation test value confirmed significant overall regional differences in ln otolith weight (T value = -23.8; $P< 0.0001$). This test result was very similar to that found with the 2001data ($T = -22.6$).

Ontogenetic relationships with otolith microchemistry of juveniles

In 2001, four of the seven otolith chemistry variables were significantly related to ln otolith weight, including magnesium, manganese, barium, and $\delta^{13}C$ (Table 3). In 2006 these same four variables were significantly related to ln otolith weight, but lithium and $\delta^{18}O$ were also found to be significantly related to ln otolith weight (Table 3). In fact lithium showed the strongest ontogenetic relationship of any variable in either year. Strontium was unrelated to body size in both 2001 and 2006. Ontogenetic relationships with otolith microchemistry were indicated by log-log regressions of elemental concentrations and otolith weight, and although the magnitudes and significances of the slopes differed somewhat between years, the directionality of the relationships (i.e., signs of slopes) were the same between years for all of the otolith variables.

Table 3. Ontogenetic relationships for seven otolith chemistry variables in 2006 vs. 2001. Otolith variables regressed against $\log_{10}$ otolith weight.

Otolith Variable	Slope	n	F Value	P
2006				
Log Lithium	-0.067	201	139.548	**<0.001**
Log Magnesium	-0.023	201	29.754	**<0.001**
Log Manganese	-0.034	201	8.348	**0.004**
Log Strontium	0.001	201	0.042	0.839
Log Barium	-0.045	201	6.678	**0.010**
$\delta^{13}C$	0.255	201	6.389	**0.012**
$\delta^{18}O$	-0.100	201	5.477	**0.020**
2001				
Log Lithium	-0.036	198	2.709	0.101
Log Magnesium	-0.028	199	24.162	**<0.001**
Log Manganese	-0.073	199	17.227	**<0.001**
Log Strontium	0.004	199	0.976	0.324
Log Barium	-0.067	198	10.451	**0.001**
$\delta^{13}C$	0.540	199	7.507	**0.007**
$\delta^{18}O$	-0.118	199	1.628	0.203

Standardizing otolith microchemistry variables

Because ontogenetic variation of the microchemistry variables could potentially confound the geographic pattern of the otolith chemical signatures and collection locations, it was necessary standardize the otolith microchemistry variables. Variables were standardized to the same scale as defined by a mean of 0 and a standard deviation of 1 prior to multivariate analyses, both to eliminate biases due to differences in fish size as well as to standardize the variables. For 2006 data, the six otolith micro-chemicals with significant ontogenetic relationships, standardized residuals were used in subsequent MANOVA and CDFA analyses. For strontium that showed no ontogenetic relationship, Z-scores relative to the overall mean were used in subsequent analyses.

MANOVA and subsequent univariate ANOVA's

A preliminary MANOVA was done to confirm whether the seven otolith microchemistry variables differed among the nine regions, as well as to identify which otolith variables could be used in a follow-up CDFA. Overall (i.e., all regions combined) one-sample K-S tests inferred normality ($P > 0.1$) for all transformed trace element variables, except $\delta^{13}C$ and $\delta^{18}O$ (K-S $Z = 1.95$ and 1.78; $P = 0.001$ and 0.004, respectively). Covariance matrices were heterogeneous among the seven otolith variables for all nine levels of the regional factor (Box's $M = 662.09$; $P < 0.001$), but this test was likely oversensitive as the associated F value was fairly low and the degrees of freedom very high ($F = 2.521$; $df1 = 224$, $df2 = 23068.7$).

A highly significant multivariate difference in the otolith variables among the nine regions in 2006 was conveyed by a Wilk's Λ of 0.001 and the associated F value of 48.305 ($P < 0.001$). Indeed, the accompanying η^2 value indicated that 63% of the variance in the seven otolith variables was explained by the regional factor (Green and Salkind 2000). This is very similar to the value of 61% that was found for the 2001 data. The occurrence of very significant variability in spotted seatrout otolith chemistry among the regions was confirmed by a multi-response permutation test of the overall difference in the seven otolith microchemistry variables among regions; this test yielded a T value of -64.3 ($P << 0.0001$).

After the MANOVA showed that there were differences in the otolith variables among the nine regions, it was appropriate to use univariate ANOVA's to show whether individual otolith variables differed among the nine regions. Levene's tests indicated that error variances were heterogeneous among regions for all of the seven otolith variables ($P < 0.05$). However, significant F values for Levene's tests were modest, ranging from 2.188 for magnesium residuals to 8.277 for $\delta^{18}O$ residuals. Notwithstanding error variance differences, all seven otolith variables varied significantly among the nine regions ($F = 14.69$ -133.53; all $P < 0.001$) (Table 4). All seven otolith variables were still significantly different, even when stringently controlled for Type I error (i.e., all $P < 0.007$).

Table 4. Univariate ANOVA results following significant MANOVA of seven otolith chemistry variables over nine *apriori* Mississippi coastal regions in 2006 and 2001. Dependent variables are all scaled to a mean of zero ± 1 standard deviation either as standardized residuals with respect to otolith weight or Z-scores. All seven otolith variables differ significantly among the nine regions within each year, even when strictly corrected for repeated testing (i.e., all $P < 0.007$). Partial η^2 values convey the amount of variance explained by the Region factor. Between-year correlation in η^2 values for the seven otolith variables is 0.896 ($P < 0.0025$, 1-tailed).

Source	Dependent Variable	Type III SS	df	Mean Square	F-Value	P-Value	Partial η^2
REGION - 2006							
	Log lithium	142.20	8	17.77	60.08	< 0.001	0.715
	Log magnesium	84.41	8	10.30	16.96	< 0.001	0.414
	Log manganese	136.87	8	17.11	52.87	< 0.001	0.688
	Log strontium	75.94	8	9.49	14.69	< 0.001	0.380
	Log barium	159.75	8	19.97	97.68	< 0.001	0.803
	$\delta^{13}C$	163.05	8	20.38	108.84	< 0.001	0.819
	$\delta^{18}O$	168.68	8	21.08	133.53	< 0.001	0.848
REGION - 2001							
	Log lithium	146.04	8	18.37	70.02	< 0.001	0.749
	Log magnesium	76.59	8	9.57	15.18	< 0.001	0.393
	Log manganese	145.37	8	18.17	66.62	< 0.001	0.739
	Log strontium	107.70	8	13.46	29.56	< 0.001	0.557
	Log barium	136.62	8	17.08	56.47	< 0.001	0.706
	$\delta^{13}C$	174.96	8	21.87	210.71	< 0.001	0.900
	$\delta^{18}O$	185.20	8	23.15	398.52	< 0.001	0.944

Because all seven otolith variables varied significantly among the nine regions, all variables were potentially useful for regional classification within a CDFA, just as observed in 2001. Partial η^2 values indicated that from 38 to 85 % of the variance in the individual otolith variables was explained by the Region Factor. The isotopes, $\delta^{13}C$ and $\delta^{18}O$, showed the highest affinities with region, just like in 2001. Again as in 2001, lithium showed the strongest association of all the trace elements (i.e., $\eta^2 = 0.72$). Remarkably, the interannual correlation between η^2 values for the seven otolith variables was 0.896 ($P < 0.0025$, 1-tailed), showing that nearly equivalent amounts of variance in individual variables were explained by regional differences in both years (Table 4).

Canonical Discriminant Function Analysis (CDFA)

Following the MANOVA, a CDFA yielded a regional classification instrument for the 2006 year class of spotted seatrout that could be compared with the regional classification for the 2001 year class from coastal Mississippi. As shown above, the F-value of 2.521 associated with Box's M test implied that the covariance matrices were mildly heterogeneous. The first four of seven discriminant functions accounted for 97.1% of the cumulative variance in the seven otolith variables in 2006: 39.5% for CDF1, 32.6% for CDF2, 15.8% for CDF3, and 9.2% for CDF4 (Table 5). By contrast, CDF1 was much stronger in 2001, when the first three discriminate funtions accounted for 97.5% of the cumulative variance in the seven otolith variables: 73.5% for CDF1, 16.7% for CDF2, and 7.3% for CDF3 (Table 5).

Table 5. Correlation coefficients (Corr) and standardized coefficients (SC) for the seven otolith chemistry variables within the Canonical Discriminant Function (CDF) Analyses for classifying specimens according to their respective coastal regions in 2001 and 2006. Bold values indicate those correlations which are highest for a particular otolith variable.

2006	**CDF1** (39.5%)		**CDF2** (32.6%)		**CDF3** (15.8%)		**CDF4** (9.2%)		**CDF 5**		**CDF 6**		**CDF 7**	
Variable	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**
$\delta^{18}O$	0.196	-.393	**0.906**	0.938	-.111	-.676	0.221	0.017	0.061	0.042	0.139	0.008	0.237	0.613
$\delta^{13}C$	**0.570**	0.642	0.436	-.190	0.299	0.472	0.569	0.824	-.261	-.460	0.053	-.177	0.030	-.133
manganese	-.089	0.020	-.298	-.199	**-.564**	-.649	0.553	0.611	0.417	0.298	0.184	0.174	-.270	-.307
barium	**-.619**	-.742	0.120	0.276	0.484	0.576	0.398	0.317	0.398	0.126	-.209	-.065	-.083	-.462
magnesium	-.162	-.321	-.188	-.270	0.222	0.060	0.176	0.271	-.045	-.285	**0.826**	0.593	0.417	0.800
lithium	0.398	0.442	0.394	0.124	0.263	0.422	-.028	-.450	0.376	0.696	**0.683**	0.480	-.090	-.684
strontium	0.059	0.418	-.152	-.270	0.215	0.069	0.179	0.019	**0.714**	0.601	-.417	-.346	0.459	0.746
2001	**CDF1** (73.5%)		**CDF2** (16.7%)		**CDF3** (7.3%)		**CDF 4**		**CDF 5**		**CDF 6**		**CDF 7**	
Variable	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**	**Corr**	**SC**
$\delta^{18}O$	**0.901**	1.261	-.031	0.578	-.053	0.536	0.214	0.225	-.211	-.170	0.308	-.560	-.010	0.120
$\delta^{13}C$	**0.600**	0.136	-.532	-.773	-.194	-.281	-.168	-.675	0.159	0.740	0.512	0.448	0.060	-.139
manganese	-.079	-.107	**0.740**	0.735	-.060	-.147	-.017	-.143	0.539	0.340	0.377	0.692	0.093	-.086
barium	-.241	-.111	-.016	0.023	**0.746**	1.105	-.338	-.370	0.025	-.315	0.119	0.557	0.506	-.068
magnesium	-.028	0.027	-.193	-.300	0.308	0.387	**0.763**	0.712	0.499	0.592	0.169	-.136	-.082	-.089
lithium	0.337	-.563	-.283	-.039	-.208	-.444	0.432	0.496	-.361	-.696	**0.609**	0.763	0.273	0.570
strontium	-.227	0.272	0.127	-.051	0.160	-.447	-.235	0.201	0.323	0.385	-.241	-.720	**0.830**	1.071

Influences of variables on CDF's were determined by joint consideration of correlation and standardized coefficients. The order of decreasing influence for distinguishing regions across the seven CDFA functions in 2001 was $\delta^{18}O$, Li, $\delta^{13}C$, Mn, Ba, Sr, and Mg. In 2006, the order of decreasing influence was $\delta^{18}O$, Ba, $\delta^{13}C$, Mn, Li, Mg, and Sr; Ba had replaced Li as the most important element. Influences of individual otolith variables on CDF's were generally more diffuse in 2006 than in 2001. In 2006, CDF 1 was influenced primarily by barium and $\delta^{13}C$, CDF2 predominantly by $\delta^{18}O$, and CDF3 by manganese. In contrast, in 2001 CDF 1 was influenced primarily by $\delta^{18}O$ and lithium, CDF2 by manganese and $\delta^{13}C$, and CDF3 by barium and magnesium. In 2001, strontium was least important for classifying regional origins within the context of this CDFA as it most strongly influenced CDF7; however, strontium influenced CDF5 most strongly in 2006.

Considerable separation of the nine regional groups is illustrated by a plot of the 201 juvenile spotted seatrout collected in 2006, along with regional centroids within the first two CDFA dimensions (Figure 4). Like in 2001, the arrangement of group centroids along CDF1 apparently reflects differences in discharge regimes rather than geographical affinity, with Chandeleur Island specimens falling at the high end of the CDF1 axis in both years, but with less separation from the other regional groups in 2006. Thus, otoliths of specimens from high salinity locations mainly had lower barium and higher $\delta^{13}C$ concentrations in 2006 vs. higher $\delta^{18}O$ and lower lithium concentrations in 2001.

The overall percent of cases correctly classified in 2006 was 93.0% when all 201 specimens were included within the CDFA; classification success ranged from 70.8 to 100% among regions (Table 6). All Juveniles from Cat Island (n=24) and the Chandeleur Islands (n=23) were correctly classified with respect to their capture location. The lowest classification success was for the Pearl River region for which seven of the 24 Pearl River specimens were misclassified (2 as Louisiana Marsh, 4 as Saint Louis Bay, and 1 as Biloxi Bay).

Cross-validation through leave-one-out procedure estimates how well the CDFA should be able to correctly classify specimens from a new sample (Green and Salkind 2000). Overall, 91.5% of the 2006 specimens (n=201) were correctly classified in the cross-validation, with classification success ranging from 70.8 to 100% among regions (Table 6). Misclassified specimens in were mainly placed into adjacent regions. For example, all 3 misclassified Bay Saint Louis specimens were classified by the cross-validation as having a Pearl River origin; and 4 of the 7 misclassified Pearl River specimens were classified by the cross-validation as having a Saint Louis Bay origin.

The Kappa index corrects for chance agreement within the CDFA (Green and Salkind 2000). The notably strong Kappa value of 0.921 ($P < 0.001$) for the CDFA was very close to the value of +1, which indicates perfect prediction.

Table 6. Regional classification summary for 2006; top portion of table based on inclusion of all 201 juvenile seatrout cases, bottom portion represents cross-validation through the "jackknife" procedure.

Original	REGION	Cat Island	Grand Bay	Horn Island	St Louis Bay	Biloxi Bay	Chandeleur Islands	LA Marsh	Pearl River	Pascagoula River	Total
Count	Cat Island	24(100%)	0	0	0	0	0	0	0	0	24
	Grand Bay	0	23(96%)	1	0	0	0	0	0	0	24
	Horn Island	1	0	22(92%)	0	0	0	1	0	0	24
	St Louis Bay	0	0	0	10(91%)	0	0	1	1	0	11
	Biloxi Bay	0	0	0	0	23(96%)	0	0	1	0	24
	Chandeleur Islands	0	0	0	0	0	23(100%)	0	0	0	23
	LA Marsh	1	0	0	0	0	0	23(96%)	0	0	24
	Pearl River	0	0	0	4	1	0	2	17(71%)	0	24
	Pascagoula River	1	0	0	0	1	0	0	0	22(96%)	23
Cross Validation	REGION	Cat Island	Grand Bay	Horn Island	St Louis Bay	Biloxi Bay	Chandeleur Islands	LA Marsh	Pearl River	Pascagoula River	Total
Count	Cat Island	24(100%)	2	0	0	0	0	1	0	0	24
	Grand Bay	0	23(96%)	1	0	0	0	0	0	0	24
	Horn Island	1	0	22(92%)	0	0	0	1	0	0	24
	St Louis Bay	0	0	0	8(73%)	0	0	0	3	0	11
	Biloxi Bay	0	0	0	0	23(96%)	0	0	1	0	24
	Chandeleur Islands	0	0	0	0	0	23(100%)	0	0	0	23
	LA Marsh	1	0	1	0	0	0	22(92%)	0	0	24
	Pearl River	0	0	0	4	1	0	2	17(71%)	0	24
	Pascagoula River	1	0	0	0	0	0	0	0	22(96%)	23

Adult spotted seatrout

Three hundred and thirty nine adult spotted seatrout were collected from the nine areas of coastal Mississippi in 2004 (Appendix 2). Fish were also collected in 2005 from the Pascagoula River, St. Louis Bay, and the Louisiana Marshes to increase sample size, but these specimens (otoliths or frozen heads) were lost during Hurricane Katrina. Fish ranged in age from one to five years old; one year old (n=78), two years old (n=178), three years old (n=50), four years old (n=17), and five years old (n=1). Otolith microchemical variables measured from the inner portion of otoliths were the same as those measured for otoliths from juveniles and included stable isotope ratios of $\delta^{13}C$ and $\delta^{18}O$, and molar concentrations of Ba, Li, Mg, Mn and Sr, standardized by molar Ca concentrations (Table 7, Appendix 3).

An algorithm was developed based on the CDFA parameters determined for juveniles collected in 2001 to predict natal regions for adult spotted seatrout based on their otolith microchemistry (Table 8). Because we targeted the 2001 year class, of primary concern were three year old fish collected in 2004, all of which were used for otolith analyses. Two year old fish were also included for six of the regions because the sample size of three year olds was relatively small (Table 8). One year old fish were only included for the Grand Bay region. When subsequently interpreting the data, age groups remained distinct.

Table 7. Mean (±SD) molar concentrations of otolith microchemical variables (standardized by molar calcium concentrations), and stable isotope ratios of $\delta^{13}C$ and $\delta^{18}O$ in otolith cores of *Cynoscion nebulosus* from the 2001 and 2002 year-class collected during 2004 in the northcentral Gulf of Mexico.

Collection Location	$\delta^{13}C$ (‰)		$\delta^{18}O$ (‰)		Li ($\mu g\ g^{-1}$)		Mg ($mg\ g^{-1}$)		Mn ($\mu g\ g^{-1}$)		Sr ($mg\ g^{-1}$)		Ba ($\mu g\ g^{-1}$)	
	2001 year class	2002 year class	2001 year class	2002 year class	2001 year class	2002 year class	2001 year class	2002 year class	2001 year class	2002 year class	2001 year class	2002 year class	2001 year class	2002 year class
Biloxi Bay	-5.81 (1.95)	NA	-2.77 (1.17)	NA	1.52 (0.72)	NA	0.139 (0.020)	NA	32.8 (15.4)	NA	2.28 (0.34)	NA	21.9 (13.6)	NA
Cat Island	-3.33 (0.58)	-3.28 (0.96)	-2.43 (0.26)	-2.26 (0.24)	1.61 (0.53)	2.09 (0.58)	0.145 (0.014)	0.134 (0.022)	40.3 (19.1)	31.7 (14.4)	2.16 (0.17)	1.93 (0.20)	32.9 (11.6)	15.4 (13.7)
Horn Island	-4.79 (2.75)	-4.91 (2.20)	-2.81 (1.00)	-2.99 (0.85)	2.38 (0.79)	2.14 (1.04)	0.144 (0.016)	0.165 (0.016)	43.1 (19.3)	57.0 (35.4)	2.09 (0.24)	2.11 (0.23)	15.0 (6.4)	21.5 (22.6)
Chandeleur Islands	-2.38 (1.54)	NA	-1.89 (0.86)	NA	2.61 (0.97)	NA	0.128 (0.032)	NA	49.4 (25.8)	NA	2.07 (0.18)	NA	17.4 (21.6)	NA
Grand Bay	-5.01 (n=1)	-4.53 (1.72)	-1.71 (n=1)	-2.22 (0.42)	2.23 (n=1)	2.17 (0.59)	0.118 (n=1)	0.148 (0.022)	17.8 (n=1)	50.0 (30.6)	2.22 (n=1)	2.12 (0.19)	14.2 (n=1)	12.3 (13.3)
LA marshes	NA	-4.19 (0.58)	NA	-2.44 (0.27)	NA	2.43 (0.22)	NA	0.144 (0.024)	NA	34.5 (12.8)	NA	2.07 (0.32)	NA	13.3 (5.3)
Pascagoula River	NA	-6.95 (1.90)	NA	-3.24 (0.84)	NA	1.57 (0.74)	NA	0.148 (0.028)	NA	42.9 (19.3)	NA	2.16 (0.22)	NA	16.6 (16.0)
Pearl River	-5.27 (n=1)	-5.58 (1.49)	-2.95 (n=1)	-3.06 (0.57)	1.03 (n=1)	1.26 (0.41)	0.118 (n=1)	0.133 (0.017)	76.9 (n=1)	50.3 (19.4)	2.88 (n=1)	2.23 (0.18)	57.3 (n=1)	25.0 (13.3)

Table 8. Capture location and predicted location during juvenile life-stage for adult spotted seatrout collected in 2004. Additional specimens collected in 2005 from the Pascagoula River, St. Louis Bay and the Louisiana Marshes were lost during Hurricane Katrina.

Capture Location	Age	Predicted Location During Juvenile Life-Stage (number of fish)								
		Grand Bay	Pasc Riv	Biloxi Bay	St. Louis Bay	Pearl River	Horn Island	Cat Island	LA Marsh	Chand Islands
Grand Bay	1	3	1	2						1
	2	3					1			2
	3						1			
Pascaloula River	2	3	3	5		2		1		
Biloxi Bay	3	5	1	3			3			3
St. Louis Bay										
Pearl River	2	2		5			3	1	1	
	3								1	
Horn Island	2	1	1	3		1	3			
	3	3	3				4			
Cat Island	2	1					7	2		
	3						1	2		
Louisiana Marshes	2	1					3			
Chandeleur Islands	3	1					5	2	1	4

Biloxi Bay - All fifteen specimens were age three when collected in 2004 (Table 8), and so were from the 2001 year class for which the otolith chemical signatures were developed. Eight of the specimens were predicted to have come from either Biloxi Bay (n=3) or the Grand Bay region (n=5), and one specimen was ostensibly from neighboring Pascagoula River (Table 8). The remaining six specimens were predicted to have come from either Horn Island (n=3) or the Chandeleur Islands (n=3).

Pascagoula River - All 14 fish from this region were age two. Eight of these fish were predicted to have come from neighboring Grand Bay or Biloxi Bay, and three fish ostensibly came from the Pascagoula River region. Two fish were predicted to have come from the Pearl River region, and one fish was ostensibly from Cat Island. Fish collected in 2005 to augment the three year olds were lost during Hurricane Katrina.

Grand Bay - Only one of 14 fish from Grand Bay was age three. Six specimens were age two and seven specimens were age one. Six of the 14 fish were predicted to have come from Grand Bay, and three fish were ostensibly from neighboring Pascagoula River or Biloxi Bay. Three fish were predicted to have developed as juveniles at the Chandeleur Islands, and two specimens were predicted to have come from Horn Island.

St. Louis Bay - No fish were predicted to have come from St. Louis Bay. Unfortunately otoliths from fish collected in St. Louis Bay were lost during Hurtricane Katrina, and it is quite likely that some of these fish would have developed here as young juveniles.

Horn Island - Of the 10 three-year-old fish collected at Horn Island, four were predicted to have come from Horn Island, and three fish were ostensibly from both Grand Bay and the Pasgagoula River. Nine two-year-old fish were collected at Horn Island; three were predicted to have come from both Horn Island and Biloxi Bay, and one fish was predicted to have come from each of Grand Bay, the Pascagoula River, and the Pearl River region.

Cat Island - Of the three three-year-old fish collected at Cat Island, two were predicted to