Vol. 371: 243–252, 2008
doi: 10.3354/meps07604

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 19

Otolith chemistry of juvenile spotted seatrout *Cynoscion nebulosus* reflects local natal regions of coastal Mississippi, USA

Bruce H. Comyns[1,*], Chet F. Rakocinski[1], Mark S. Peterson[1], Alan M. Shiller[2]

[1]Department of Coastal Sciences, The University of Southern Mississippi, 703 East Beach Drive, Ocean Springs, Mississippi 39566, USA

[2]Department of Marine Science, The University of Southern Mississippi, Stennis Space Center, Mississippi 39529, USA

ABSTRACT: Early juvenile spotted seatrout *Cynoscion nebulosus* (n = 199) were collected during late summer and autumn 2001 from shoreline habitats within 9 coastal regions bordering Mississippi Sound in the north-central Gulf of Mexico to ascertain how well fish could be spatially classified based on otolith chemistry. Left otoliths were assayed for trace element:Ca ratios of Ba, Li, Mg, Mn and Sr, and right otoliths for $\delta^{13}C$ and $\delta^{18}O$. Significant overall differences in otolith chemistry existed among the 9 regions; 61% of the joint variance in the 7 otolith chemistry variables was explained by the regional factor in a multivariate analysis of variance (MANOVA). All 7 otolith chemistry variables differed significantly among the 9 regions. The isotopes $\delta^{13}C$ and $\delta^{18}O$ showed the highest regional affinities, and Li showed the strongest regional association of all the trace elements. Canonical discriminant function analysis (CDFA) maximally separated regional groups of early juvenile fish. The first 3 of 7 discriminant functions accounted for 97.5% of the cumulative variance in the 7 otolith chemistry variables. CDF 1 was influenced primarily by $\delta^{18}O$ and Li, CDF 2 by Mn and $\delta^{13}C$, and CDF 3 by Mg and Ba. In the all-inclusive CDFA, 93.4% of cases were classified correctly, and classification success among regions ranged from 83.3 to 100%. The influence of freshwater discharge from 7 rivers along the Mississippi coastline likely made it possible to detect the relatively fine-scale spatial differences seen in this study, as defined by a mean interregional distance of only 25 km.

KEY WORDS: Otolith chemistry · Spotted seatrout · Gulf of Mexico · Juvenile

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Nursery habitats are essential for the growth, survival, and recruitment of many estuarine-dependent species (Beck et al. 2001, Minello et al. 2003), and there is a pressing need to delineate important nursery source areas at both microhabitat and regional landscape scales. Recent studies have shown that otolith chemistry can be used as an environmental indicator to address difficult fishery recruitment issues, including stock identification, the determination of migration pathways, the reconstruction of previous habitat information, and age validation (Campana et al. 1995, Campana 1999, Thorrold et al. 1998a,b, 2001, Secor et al. 2001, Rooker et al. 2003, Patterson et al. 2004, Dorval et al. 2005).

Otolith chemistry has proved especially useful in providing a natural tag of ambient conditions experienced by early stages of estuarine-dependent sciaenids. By combining assays of trace elements and carbon (^{13}C) and oxygen (^{18}C) stable isotopes, Thorrold et al. (1998b) could clearly distinguish regional groups of juvenile weakfish *Cynoscion regalis* originating from 3 adjacent river systems within Chesapeake Bay, and Thorrold et al. (2001) also found evidence of natal homing for this sciaenid. Using otolith chemistry, Dorval et al. (2005) recently identified natal source areas of juvenile spotted seatrout (*C. nebulosus*) from seagrass habitats within 5 regions of Chesapeake Bay on a fairly small spatial scale. Coastal Mississippi is physiographically different from Chesapeake Bay; the 7 watersheds

*Email: bruce.comyns@usm.edu

© Inter-Research 2008 · www.int-res.com

which empty along the 117 km coastline of Mississippi should provide sufficient heterogeneity in water chemistry to distinguish different natal regions on an especially fine spatial scale.

Spotted seatrout *Cynoscion nebulosus* is a valuable sciaenid species of special concern for resource managers in Mississippi, where coastal habitats are rapidly disappearing and stock enhancement efforts for this species are currently underway. The spotted seatrout is one of the most highly prized inshore game fish throughout the northern Gulf of Mexico (GOM) (Perret et al. 1980, Hettler 1989, Deegan 1990). The only sciaenid that spawns primarily in shallow inshore waters (Johnson & Seaman 1986, Peebles & Tolley 1988), spotted seatrout remain inshore throughout life. Juveniles require shallow marsh-edge or seagrass habitats (McMichael & Peters 1989), but we do not know where the most important nursery source areas are located in Mississippi. However, if juveniles from regions influenced by different watersheds can be distinguished by the elemental 'fingerprints' of their otoliths, then natal source regions of surviving adults can be determined as part of stock assessment, and those natal source regions protected. The goal of this study was to determine how well early juvenile spotted seatrout can be classified into 9 *a priori* natal regions within coastal Mississippi based on spatial patterns of otolith chemistry.

MATERIALS AND METHODS

Collection of juveniles. Early juvenile spotted seatrout (n = 199) were collected during late summer and autumn 2001 from marsh-edge and seagrass habitats in 9 *a priori* nursery regions bordering Mississippi Sound, from Grand Bay at the Alabama border to the Louisiana (LA) marshes east of the Mississippi River (Fig. 1). These regions were chosen to encompass the entire geographic range of source areas potentially contributing to the stock structure of spotted seatrout in Mississippi. Regions encompass major physio-geographic features including Horn Island, Cat Island, Chandeleur Islands, LA marshes, Grand Bay, St. Louis Bay, Biloxi Bay, and the mouths of the Pearl and Pascagoula Rivers. Collections were made using a 15.2 m bag seine with 3.17 mm mesh. Juvenile spotted seatrout were obtained from 32 of the sites sampled within the 9 regions, and the number of sites represented in each region ranged from 2 to 7. Juveniles ranged in size from 30 to 101 mm standard length (SL), and each region except the Pascagoula River (n = 9) was represented by 22 to 24 individuals. After collection, juveniles were stored on ice, returned to the laboratory, and frozen.

Otolith analyses. To prepare sagittal otoliths for elemental and isotopic analyses, they were removed from both the left and right sides of each specimen using acid-washed teflon-coated forceps (rinsed with ultrapure Milli-Q water) and stored in sterile 24-well cell culture clusters. On a laminar flow bench in a Class 100 clean room, each otolith was placed into an acid-washed, preweighed (µg precision), microcentrifuge tube using acid-washed teflon forceps. Centrifuge tubes were filled with 0.001 N ultrapure nitric acid (Seastar Baseline) using a metal-free polyetheylene pipette tip that had been triple-rinsed with 0.1 N ultrapure nitric acid, followed by triple-rinsing with Milli-Q water. Otoliths were washed with the dilute acid to remove any remaining contaminants

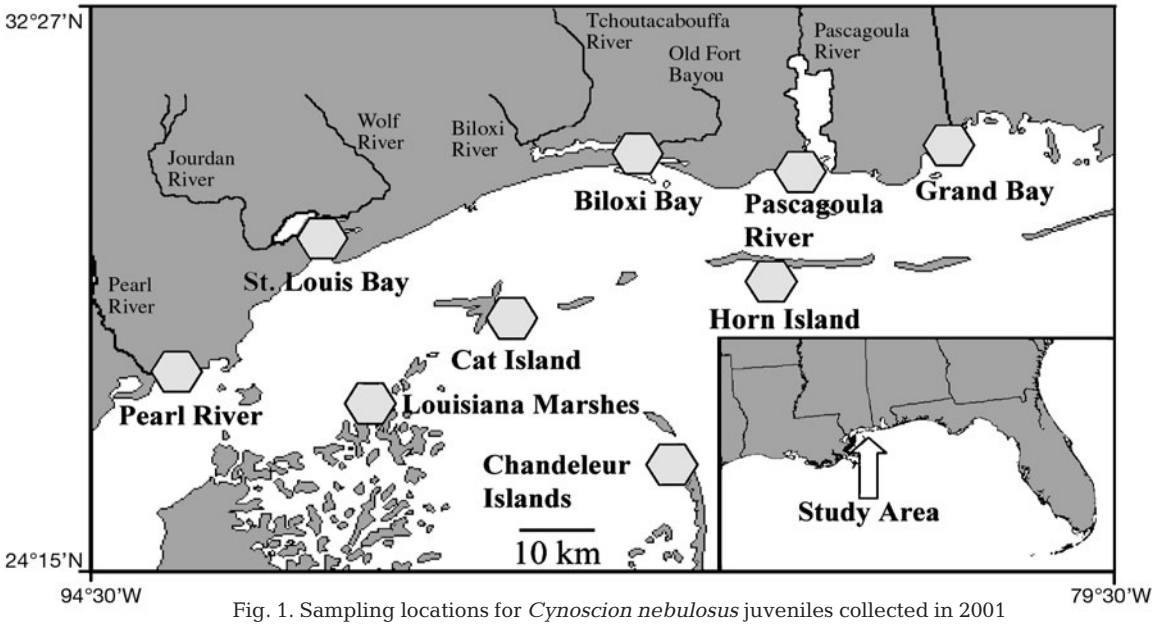

Fig. 1. Sampling locations for *Cynoscion nebulosus* juveniles collected in 2001

(metal ions) from the otolith surface. After 1 to 2 min, the acid was removed from the centrifuge tubes with a clean pipette tip, and the otoliths were triple-rinsed 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 re-weighed to obtain otolith weights (µg). Elemental analysis was performed using a method similar to that described by Rosenthal et al. (1999) for determining element:calcium ratios in foraminiferal calcite. Cleaned left otoliths were dissolved in 0.15 N ultrapure HNO_3 (Seastar) containing 20 nM In as an internal standard. In order to minimize possible matrix effects during analysis, the quantity of acid in which the samples were dissolved was adjusted to produce a final Ca concentration of approximately 4 mM. Standards were likewise prepared to be 4 mM in Ca.

Elemental analysis was performed using a Thermo Electron Element-2 sector field inductively coupled plasma–mass spectrometer (ICP–MS). The sample inlet system consisted of a self-aspirating Teflon 100 µl min^{-1} micronebulizer, a teflon spray chamber, and a sapphire injector (all from Elemental Scientific). Ni cones were utilized. The following isotopes were monitored in medium resolution ($m/\Delta m$ = 4000): Li-7, Mg-24, Ca-43, Mn-55, Sr-88, Ba-138, and In-115 (as added internal standard). Standards were prepared by diluting concentrated stock solutions (High Purity Standards) in 0.15 N ultrapure HNO_3 containing 4 mM Ca and 20 nM In. The high-concentration Ca standard contributed negligibly to the blank for the elements considered here. Although Rosenthal et al. (1999) found it adequate to use just one standard, we utilized both high- and low-concentration standards, but noted no difference in calibration. The In internal standard served as a preliminary check for instrument drift and sensitivity changes. Instrument sensitivity varied by ≤15% during any 6 h analytical run. A blank and a standard were run between every 9 samples as a check on detection limits and to correct for possible calibration drift. The order of otolith analysis was randomized to preclude possible confounding effects of instrument drift (Campana & Gagné 1995). The precision of the analysis, based on the relative standard deviation derived from the repeated analysis of a standard with elemental concentrations similar to a typical otolith sample, was better than 5% for all element:Ca ratios, except for the Li:Ca ratio where the relative standard deviation was 11%. The lower precision of the Li:Ca ratio can be attributed to low absolute count rates for Li, resulting in the median sample having Li:Ca ratios only 9 times higher than the detection limit (detection limit = 3 × the standard deviation of the blank); although the lowest sample still had a Li:Ca ratio 3 times higher than the detection limit. For other element:Ca ratios, the typical otolith sample had element concentrations up to 2 orders of magnitude or greater than the detection limit.

Cleaned otoliths from the right sides of juveniles were powdered and analyzed for $\delta^{13}C$ and $\delta^{18}O$. Analyses were conducted in the stable isotope laboratory at the University of California, Davis, USA. Otoliths were powdered with an agate mortar and pestle, pre-rinsed with Milli-Q water. Powdered otoliths were transferred to acid-washed microcentrifuge 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, 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 Vienna PeeDee Belemnite (VPDB). Mean precision was (1 sigma) ±0.04‰ for $\delta^{13}C$ and ±0.06‰ for $\delta^{18}O$.

Data analyses. Otolith chemistry variables included $\delta^{13}C$ 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 5 successive stages using SPSS 11.0.

First, mean otolith weights for each region were compared to determine whether differences in body size might influence subsequent analyses. Kolmogorov-Smirnov (K-S) 1-sample tests were made against the normal distribution for both otolith weight and $\log_{10}$ otolith weight for each of the 9 regions. Subsequently, a 1-way 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. Two specimens qualifying as outliers (i.e. ≥3 SD units) were excluded from ontogenetic relationships and subsequent multivariate analyses.

Third, because trace element:Ca ratios for several elements differed by an order of magnitude, otolith chemistry variables were scaled to a mean of 0 ± 1 SD 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 dis-

criminant function analysis (CDFA) to eliminate possible regional bias due to fish size. Alternatively, for those otolith variables without significant ontogenetic relationships, *Z*-scores (i.e. difference between observation and overall mean divided by the standard deviation) were used.

Fourth, a MANOVA was performed to determine whether the 7 otolith chemistry variables differed among the 9 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 7 otolith variables differed among the 9 regions. Levene's tests addressed whether error variances were homogeneous among regions. Because variances among regions were mildly to moderately heterogeneous for both the 1-way ANOVA on otolith weight and the MANOVA on the otolith chemistry variables, overall regional differences were confirmed through the multi-response 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 2001 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 assumptions 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 2-dimensional space formed by the first 2 canonical functions. Reliability of the CDFA was evaluated in 3 ways: (1) through classification success within the CDFA in which all specimens were included; (2) through cross-validation using the 'jack-knife' 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 & Salkind 2000).

RESULTS

A total of 199 early juvenile spotted seatrout were used to characterize 9 regions of coastal Mississippi (Fig. 1). Salinities at the collection sites (Table 1) were highest at the Chandeleur Islands (32.2 to 32.3 psu), intermediate at the barrier islands and Grand Bay (16.6 to 22.2 psu), and lowest at the LA marshes and remaining inshore locations (1.3 to 3.0 psu). Otolith chemistry variables included $\delta^{13}C$ and $\delta^{18}O$, and molar concentrations of Li, Mg, Mn, Sr and Ba, standardized by molar Ca concentrations (Table 2).

Regional differences in otolith weight

Mean (±SE) otolith weight ranged from 4.69 ± 1.66 to 14.80 ± 1.34 mg among the 9 regions. Although otolith weight distributions from several of the 9 regions were non-normal (K-S tests; $p < 0.05$), $\log_{10}$-transformed otolith weight distributions did not differ from the normal distribution across the 9 regions (K-S tests; $p = 0.17$ to 0.97). In a 1-way ANOVA, $\log_{10}$ otolith weight differed significantly among the 9 regions ($F = 15.936$; $p < 0.001$), notwithstanding heterogeneous variance in $\log_{10}$ otolith weight ($F = 7.479$; $p < 0.001$). The MRPP test of an overall regional difference in log otolith weight yielded a T of −22.6 ($p \ll 0.0001$), confirming that body sizes of fish differed greatly among regions. Post-hoc Games and Howell tests implied that fish from St. Louis Bay were largest ($p < 0.05$ for 4 of 8 tests), while those from the Pearl River ($p < 0.05$ for 6 of 8 tests) and the LA marshes ($p < 0.05$ for 6 of 8 tests) were smallest. Mean SL of juveniles from St. Louis Bay was 77.5 mm (60.5 to 95.0 mm), whereas mean SL of juveniles from the Pearl River and the LA marshes was 45.8 mm (30.2 to 110.0 mm) and 49.6 mm (30.5 to 77.9 mm), respectively.

Table 1. Range and mean of salinity and temperature recorded at time of collection of *Cynoscion nebulosus* juveniles

	No. of stations (positive)	Salinity (psu) Range	Salinity (psu) Mean	Temperature (°C) Range	Temperature (°C) Mean
Pascagoula River	4	1.3–12.3	5.2	22.7–31.2	26.8
Pearl River	3	3.2–8.3	5.8	27.7–30.4	28.8
St. Louis Bay	3	3.5–11.1	6.6	28.4–29.5	28.9
Biloxi Bay	4	5.5–11.9	8.9	27.8–31.3	29.4
Louisiana marshes	2	10.6–13.0	11.8	30.1–31.2	30.6
Cat island	3	16.6–20.4	18.1	29.0–30.5	29.5
Grand Bay	3	17.2–19.9	18.9	31.3–32.7	32.0
Horn Island	2	21.9–22.2	22.0	21.6–22.7	22.2
Chandeleur Islands	2	32.2–32.3	32.2	26.6–27.1	26.8

Table 2. *Cynoscion nebulosus.* Molar concentrations (mean ± SD) of otolith microchemical variables (standardized by molar calcium concentration), and $\delta^{13}C$ and $\delta^{18}O$ in otoliths of juveniles collected in 9 regions of the north-central Gulf of Mexico

Site	$\delta^{13}C$ (‰)	$\delta^{18}O$ (‰)	Li (μg g^{-1})	Mg (mg g^{-1})	Mn (μg g^{-1})	Sr (mg g^{-1})	Ba (μg g^{-1})
Biloxi Bay	−6.59 (0.71)	−3.84 (0.39)	1.60 (0.68)	0.172 (0.021)	44.4 (12.3)	2.51 (0.18)	18.7 (5.7)
St. Louis Bay	−7.80 (1.27)	−3.96 (0.36)	1.01 (0.56)	0.186 (0.022)	52.8 (16.0)	2.70 (0.22)	33.9 (7.9)
Cat Island	−3.82 (0.35)	−2.30 (0.09)	3.00 (0.61)	0.193 (0.027)	31.2 (4.8)	2.25 (0.17)	23.6 (6.0)
Chandeleur Islands	−1.73 (0.56)	−0.657 (0.28)	4.19 (0.53)	0.155 (0.019)	53.6 (10.3)	2.05 (0.18)	8.72 (2.47)
Grand Bay	−5.12 (0.55)	−2.39 (0.070)	2.88 (0.47)	0.173 (0.029)	42.8 (9.2)	2.20 (0.17)	12.2 (4.0)
Horn Island	−3.03 (0.49)	−2.30 (0.05)	3.17 (0.55)	0.185 (0.023)	17.3 (2.3)	2.20 (0.14)	20.6 (3.8)
Louisiana marshes	−5.15 (0.39)	−2.69 (0.16)	2.27 (0.56)	0.208 (0.031)	39.9 (6.9)	2.35 (0.20)	30.0 (8.7)
Pascagoula River	−7.89 (0.99)	−4.39 (0.23)	1.18 (0.35)	0.147 (0.019)	45.9 (8.1)	2.61 (0.27)	27.3 (12.0)
Pearl River	−8.21 (0.80)	−3.27 (0.33)	1.19 (0.37)	0.175 (0.017)	86.2 (27.8)	2.57 (0.21)	42.9 (11.3)

Ontogenetic relationships

Four of the 7 otolith chemistry variables were significantly related to $\log_{10}$ otolith weight, including Mg, Mn, Ba, and $\delta^{13}C$ (Table 3). The 3 significant relationships involving trace metals were inversely related to otolith weight, whereas $\delta^{13}C$ increased with otolith weight. One outlier (≥3 SD units) was excluded from the ontogenetic relationship for both Li and Ba; consequently, a total of 197 cases were complete for all 7 otolith variables.

MANOVA

A highly significant overall difference in otolith chemistry among the 9 regions was conveyed by a Wilk's Λ of 0.001 and the associated *F*-value of 42.170 ($p < 0.001$). The accompanying η^2 indicated that 61% of the joint variance in the 7 otolith chemistry variables was explained by the regional factor. Although MANOVA covariance matrices were heterogeneous (Box's $M = 750.57$; $p < 0.001$), the test was likely oversensitive as the associated *F*-value was fairly low and the degrees of freedom very high ($F = 2.815$; df1 = 224, df2 = 16112). Nevertheless, a follow-up MRPP test of an overall regional difference in otolith chemistry yielded a T of −52.7 ($p \ll 0.0001$), confirming very significant variability in the otolith chemistry of spotted seatrout early juveniles among the 9 regions.

Subsequent univariate ANOVAs elucidated which otolith chemistry variables differed among the 9 regions. Notwithstanding moderately heterogeneous variance among regions for 5 otolith chemistry variables ($F = 2.835$ to 7.465; $p < 0.01$), all 7 individual otolith variables differed significantly among the 9 regions ($F = 15.18$ to 398.5; $p < 0.001$) (Table 4). Indeed, the 7 otolith variables were still significantly different when stringently controlled for Type I error (i.e. all $p < 0.007$; 0.05/7). Thus, all 7 otolith variables were potentially useful for regional classification within the CDFA.

Table 3. *Cynoscion nebulosus.* Ontogenetic relationships for 7 otolith chemistry variables. Otolith variables regressed against $\log_{10}$ otolith weight. **Bold** font indicates a significant difference

Otolith variable	Slope	n	*F*-value	p
Log Li	−0.0363	198	2.709	0.101
Log Mg	−0.0284	199	24.162	**<0.001**
Log Mn	−0.0732	199	17.227	**<0.001**
Log Sr	0.0045	199	0.976	0.324
Log Ba	−0.0670	198	10.451	**0.001**
$\delta^{13}C$	0.5400	199	7.507	**0.007**
$\delta^{18}O$	−0.1180	199	1.628	0.203

Table 4. *Cynoscion nebulosus.* Univariate ANOVA results following significant MANOVA of 7 otolith chemistry variables over 9 *a priori* Mississippi coastal regions. Dependent variables are scaled to a mean of 0 ± 1 SD either as standardized residuals with respect to otolith weight or *Z*-scores. All 7 otolith variables differ significantly among the 9 regions 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

Source Dependent variable	Type III SS	df	Mean square	*F*-value	p-value	Partial η^2
Region						
Log Li	146.04	8	18.37	70.02	<0.001	0.749
Log Mg	76.59	8	9.57	15.18	<0.001	0.393
Log Mn	145.37	8	18.17	66.62	<0.001	0.739
Log Sr	107.70	8	13.46	29.56	<0.001	0.557
Log Ba	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

Partial η^2 values indicated that from 39 to 94% of the variance in the individual otolith variables were explained by the region factor. The $\delta^{13}C$ and $\delta^{18}O$ values showed the highest regional affinities, and Li showed the strongest association of all the trace elements ($\eta^2 = 0.75$).

CDFA

The first 3 of 7 discriminant functions accounted for 97.5% of the cumulative variance in the 7 otolith chemistry variables: 73.5% for CDF 1, 16.7% for CDF 2, and 7.3% for CDF 3. Serial chi-square tests of residual discrimination effectiveness after removing the effects of preceding discriminant functions were all significant at $p < 0.002$ (Wilk's $\Lambda = 0.001$ to 0.894), except for CDF 7 (Wilk's $\Lambda = 1.0$; $p = 0.99$). Again, the *F*-value of 2.815 associated with Box's *M*-test in the MANOVA indicated mildly heterogeneous covariance matrices. The CDFs can be interpreted by jointly considering standardized CDF coefficients and correlations with the otolith chemistry variables (Table 5). Accordingly, CDF 1 was influenced primarily by $\delta^{18}O$ and Li, CDF 2 by Mn and $\delta^{13}C$, and CDF 3 by Mg and Ba. The function which was least important for distinguishing region within the CDFA, CDF 7, was most influenced by Sr.

Considerable separation of the 9 regional groups within the first 2 CDFA dimensions is apparent in a plot of the 197 specimens and regional centroids (Fig. 2). As implied by the arrangement of group centroids, CDF 1 likely reflects differences in discharge regimes, rather than geographical affinity. Thus, otoliths of specimens from high salinity locations generally had high $\delta^{18}O$ values and low amounts of Li. Accordingly, Chandeleur Island specimens fell at the high end of the CDF 1.

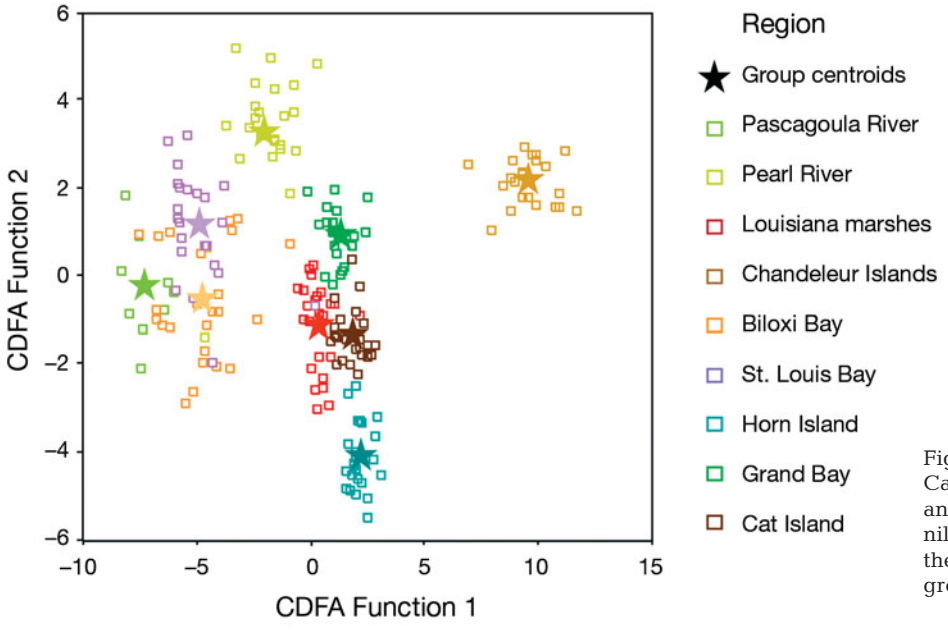

Fig. 2. *Cynoscion nebulosus.* Canonical discriminent function analysis (CDFA) plot of 197 juveniles collected from 9 regions along the Mississippi coast, along with group centroids, within the first 2 canonical dimensions

Table 5. *Cynoscion nebulosus.* Correlation coefficients (Corr) and standardized coefficients (SC) for the 7 otolith chemistry variables within the canonical discriminant function (CDF) analysis for classifying specimens according to their respective coastal regions. **Bold** values indicate the highest correlations for a particular otolith variable. Influences of variables on CDFs determined by joint consideration of correlation and standardized coefficients

Variable	CDF 1		CDF 2		CDF 3		CDF 4		CDF 5		CDF 6		CDF 7	
	Corr	SC	Corr	SC	Corr	SC	Corr	SC	Corr	SC	Corr	SC	Corr	SC
$\delta^{18}O$	**0.901**	1.261	−0.031	0.578	−0.053	0.536	0.214	0.225	−0.211	−0.170	0.308	−0.560	−0.010	0.120
$\delta^{13}C$	**0.600**	0.136	−0.532	−0.773	−0.194	−0.281	−0.168	−0.675	0.159	0.740	0.512	0.448	0.060	−0.139
Mn	−0.079	−0.107	**0.740**	0.735	−0.060	−0.147	−0.017	−0.143	0.539	0.340	0.377	0.692	0.093	−0.086
Ba	−0.241	−0.111	−0.016	0.023	**0.746**	1.105	−0.338	−0.370	0.025	−0.315	0.119	0.557	0.506	−0.068
Mg	−0.028	0.027	−0.193	−0.300	0.308	0.387	**0.763**	0.712	0.499	0.592	0.169	−0.136	−0.082	−0.089
Li	0.337	−0.563	−0.283	−0.039	−0.208	−0.444	0.432	0.496	−0.361	−0.696	**0.609**	0.763	0.273	0.570
Sr	−0.227	0.272	0.127	−0.051	0.160	−0.447	−0.235	0.201	0.323	0.385	−0.241	−0.720	**0.830**	1.071

When all 197 specimens were included within the CDFA, 93.4% of cases were classified correctly. Classification success among regions ranged from 83.3 to 100% (Table 6). The lowest value resulted from the misclassification of 4 of the 24 Biloxi Bay specimens. Cross-validation through the 'jack-knife' procedure estimates how well the CDFA should be able to correctly classify specimens from a new sample. According to this procedure, 90.9% of the 197 specimens were correctly classified, and classification success among regions ranged from 70.8 to 100% (Table 6). Although misclassification rates were somewhat higher within the cross-validation, misclassified specimens were often attributed to proximate or adjacent regions. For example, 5 of 7 misclassified Biloxi Bay specimens were assigned to the Pascagoula River, and conversely, the only 2 misclassified Pascagoula River specimens were assigned to Biloxi Bay. All 23 Grand Bay and all 22 Chandeleur Island specimens were initially classified and cross-validated correctly. The Kappa index, which corrects for chance agreement within the CDFA, was 0.925 ($p < 0.001$), where the value +1 indicates perfect prediction.

DISCUSSION

Potential source areas of early juvenile spotted seatrout from 9 *a priori* regions bordering Mississippi Sound were clearly distinguished based on the chemical composition of their otoliths. The regional groups were maximally separated within multivariate space by CDFA, which also provided a quantitative tool for identifying natal source areas of adult spotted seatrout from the same year class. Indeed, cross-validation of the 197 specimens was 90.9% accurate, and classification success ranged from 70.8 to 100% among regions. In subsequent work, regional classification of adults could be made even more accurate by prudent grouping of selected regions, given that misclassified specimens were generally misplaced into adjacent regions. For example, 5 of 7 specimens misclassified from Biloxi Bay were from the Pascagoula River; conversely, both of the specimens misclassified from the Pascagoula River were from Biloxi Bay. These 9 specimens comprised 53% of all the misclassified specimens in the cross validation.

It was necessary to adjust 4 (Mg, Mn, Ba, and $\delta^{13}C$) of the 7 otolith microchemistry variables for significant ontogenetic effects. Although only early juveniles were examined, wet weights of fish used for our study still ranged over an order of magnitude, from 0.22 to 29.04 g (4.76 ± 0.29, mean ± SE). Furthermore, differences in otolith weight did exist among regional samples. By removing ontogenetic effects on otolith chemistry, biases due to such inter-regional differences in size distributions were removed. Dorval et al. (2005) also found ontogenetic relationships between otolith

Table 6. *Cynoscion nebulosus.* Regional classification summary. Top portion of table based on inclusion of all 197 juveniles; bottom portion represents cross-validation through the 'jack-knife' procedure. LA: Louisiana. **Bold** font indicates the number and percentage of juveniles that were correctly classified regarding their collection location

Region	Cat Island	Grand Bay	Horn Island	St. Louis Bay	Biloxi Bay	Chandeleur Islands	LA marshes	Pearl River	Pascagoula River	Total
Original count										
Cat Island	**22(92%)**	2	0	0	0	0	0	0	0	24
Grand Bay	0	**23(100%)**	0	0	0	0	0	0	0	23
Horn Island	1	0	**23(96%)**	0	0	0	0	0	0	24
St. Louis Bay	0	0	0	**22(96%)**	0	0	1	0	0	23
Biloxi Bay	0	1	0	1	**20(83%)**	0	0	0	2	24
Chandeleur Islands	0	0	0	0	0	**22(100%)**	0	0	0	22
LA marshes	1	0	1	0	0	0	**22(92%)**	0	0	24
Pearl River	0	0	0	2	0	0	0	**22(92%)**	0	24
Pascagoula River	0	0	0	0	1	0	0	0	**8(89%)**	9
Cross-validation count										
Cat Island	**21(88%)**	2	0	0	0	0	1	0	0	24
Grand Bay	0	**23(100%)**	0	0	0	0	0	0	0	23
Horn Island	1	0	**23(96%)**	0	0	0	0	0	0	24
St. Louis Bay	1	0	0	**22(96%)**	0	0	0	0	0	23
Biloxi Bay	0	1	0	1	**17(71%)**	0	0	0	5	24
Chandeleur Islands	0	0	0	0	0	**22(100%)**	0	0	0	22
LA marshes	1	0	1	0	0	0	**22(92%)**	0	0	24
Pearl River	0	0	0	2	0	0	0	**22(92%)**	0	24
Pascagoula River	0	0	0	0	2	0	0	0	**7(78%)**	9

weight and trace elements of juvenile spotted seatrout in Chesapeake Bay, but only during 1 of the 2 years of their study. Other careful studies have also found it necessary to adjust for ontogenetic trends (Mulligan et al. 1987, Campana et al. 2000, Rooker et al. 2001, Hanson et al. 2004), while some studies of otolith chemistry have failed to account for ontogenetic effects.

Based on the joint products of associated CDF correlations, CDF standard coefficients, and CDF variances accumulated across the 7 CDFA functions, the order of decreasing influence for distinguishing regions was $\delta^{18}O$, Li, $\delta^{13}C$, Mn, Ba, Sr, and Mg. It is notable that Li was the most effective of the 5 trace elements used in this study because the use of Li as a geochemical tracer in the GOM is relatively novel. Indeed, Li was also prominent in the regional MANOVA, as reflected by the highest percent of variance (74.9%) attributed to the region factor in a follow-up ANOVA, and was closely followed by Mn (73.9%), and Ba (70.6%).

Moreover, Li did not require any ontogenetic correction, whereas both Mn and Ba required ontogenetic correction prior to their use in analyses of regional affinities. The combined use of stable isotopes and trace elements was also warranted, given the prominent roles played by $\delta^{18}O$ and $\delta^{13}C$. A full 94.4% of variance in $\delta^{18}O$ and 90.0% of variance in $\delta^{13}C$ was explained by region in follow-up ANOVAs. In addition, $\delta^{18}O$ did not require any ontogenetic correction, whereas $\delta^{13}C$ required ontogenetic correction prior to multivariate analyses. Previous studies have found the combined use of stable isotopes and trace elements as regional tags to be superior to the sole use of trace elements. For example, by combined use of trace elements and stable isotopes, Thorrold et al. (1998b) clearly distinguished regional groups of juvenile weakfish *Cynoscion regalis* from 3 adjacent river systems, and Thorrold et al. (2001) even demonstrated natal homing in this sciaenid.

The relative positions of centroids relative to CDF 1 implied the influence of an underlying riverine discharge gradient, as the coordinates were arrayed better with respect to presumed freshwater discharge than to geographic proximity. For example, the Pearl River, St. Louis Bay, Biloxi Bay, and Pascagoula River regions had lower salinities than the remaining 5 locations, and these regions also had corresponding low values with respect to CDF 1. The first CDF was driven largely by the isotope ^{18}O, the trace element Li, and the isotope ^{13}C. It may be recalled that otoliths from high salinity locations generally had high $\delta^{18}O$ values and low amounts of Li, and these 2 variables did not require ontogenetic correction.

Strontium largely drove CDF 7, which only had a negligible influence on the overall discriminant function. Nevertheless, the Sr:Ca ratio was surprisingly high in early juvenile otoliths from regions associated with relatively low salinity (i.e. Pearl River, St. Louis Bay, Biloxi Bay, and Pascagoula River). For example, the Sr:Ca ratio was about 10 to 30% lower in fish otoliths at locations farther from the mainland, including Horn Island, Cat Island, and the Chandeleur Islands. Salinities at these 3 locations at the time of collection of early juveniles ranged from 16.6 to 32.3 psu, whereas salinities at the more inshore locations ranged from 1.3 to 12.3 psu. Higher Sr:Ca ratios in fish otoliths from regions of lower salinity were unexpected, because Sr concentration is known to be much greater in seawater than in freshwater (Rosenthal et al. 1970, Bagenal et al. 1973, Bruland 1983, Kalish 1990). Sr is substituted for Ca within the aragonite calcium carbonate lattice (Kinsman & Holland 1969, Secor 1992), and the amount of Sr incorporated into an otolith is typically directly proportional to the amount of Sr within the endolymph, at least for salmonids (Kalish 1989). Moreover, studies of anadromous behavior of various fishes, including salmonids (Kalish 1990), striped bass (Secor 1992) and American shad *Alosa sapidissima* (Limburg 1995), are predicated on the usual direct relationship between Sr:Ca ratios in otoliths and salinity. This approach has also been used to study up-estuary movements in bay anchovy *Anchoa mitchilli* (Kimura et al. 2000), and offshore-inshore migrations of larval and juvenile Atlantic croaker *Micropogonias undulatus* (Thorrold et al. 1997). However, the lack of a definite relationship between otolith Sr:Ca ratio and salinity for spotted seatrout from Chesapeake Bay was also reported by Dorval et al. (2005), who found that variation of Sr:Ca ratios could not be explained by salinity. It is unclear why the typical direct relationship between Sr:Ca ratio and salinity was not apparent in otoliths of early juvenile spotted seatrout in Mississippi, but possible explanations include the use of earlier life-stages of fish in our study, species-specific differences in Sr uptake mechanisms, or species-specific metabolic pathways.

Unlike the Sr:Ca ratio, the Ba:Ca ratio, which largely drove CDF 3, did conform as expected with the salinity gradient. Concentrations of Ba are often higher in coastal regions influenced by riverine input than farther offshore (Thorrold et al. 1997), just as we found for coastal Mississippi. For example, the Ba:Ca ratio was highest in otoliths of fish collected in the vicinity of the Pearl River, and lowest in fish from the most offshore region, the Chandeleur Islands.

The influence of freshwater discharge from 7 rivers along the Mississippi coastline likely made the delineation of source areas on such a small geographic scale as was seen in this study possible. Indeed, the mean interregional distance was only 25 km. Using otolith chemistry, Dorval et al. (2005) also recently distin-

guished juvenile spotted seatrout from seagrass within 5 regions of the Chesapeake Bay on a comparatively small spatial scale (15 km). Dorval et al. (2005) did not consider Li or Mg, but they did assess the rare earth element, La, for the first time.

Our results imply that early juvenile spotted seatrout remain within the same estuarine nursery region in Mississippi, and thus, source areas for adults of this species can be accurately determined using otolith chemistry. Contrary to our expectation, preliminary analysis of Year 1 spotted seatrout (same year class as juveniles described in the present study) indicates that the Grand Bay National Estuarine Research Reserve, located at the Mississippi-Alabama border, contributes disproportionately to the spotted seatrout stock in eastern Mississippi waters (authors' pers. obs.). Coastal Mississippi is currently undergoing extensive development, and the ability to determine spotted seatrout source regions will be essential for justifying the conservation of key regions containing valuable nursery habitats.

Acknowledgements. Prior to this study, S. Baker and W. Patterson generously provided much needed technical advice. Numerous graduate students participated in field sampling, including P. Grammer, G. Waggy, G. Zapfe, N. Cotton (Crochet), S. Griffith and C. Woodley. P. Grammer contributed many tedious hours carefully preparing otoliths for chemical analyses. D. Winter of the University of California at Davis provided invaluable assistance with analyzing otoliths for isotope ratios, and L. Gross from the University of Southern Mississippi's Department of Marine Science analyzed otolith trace elements. This work was sponsored by the US Department of Commerce, National Oceanic and Atmospheric Administration, through the Mississippi-Alabama Sea Grant Consortium (MASGC) (grant R:SP-4). The US Government and the MASGC are authorized to produce and distribute reprints notwithstanding any copyright notation that may appear hereon. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies.

LITERATURE CITED

Bagenal TB, Mackereth FJH, Heron J (1973) The distinction between brown trout and sea trout by the strontium content of their scales. J Fish Biol 5:555–557

Beck MW, Heck KL Jr, Able KW, Childers DL and others (2001) The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. Bioscience 51:633–641

Bruland KW (1983) Trace elements in sea-water. In: Riley JP, Chester R (eds) Chemical oceanography, Vol 8. Academic Press, London, p 157–220

Cai L. (2006) Multi-response permutation procedure as an alternative to the analysis of variance: an SPSS implementation. Behav Res Methods 38:51–59

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188:263–297

Campana SE, Gagné JA (1995) Cod stock discrimination using ICPMS elemental assays of otoliths. In: Secor DH, Dean JM, Campana SE (eds) Recent developments in fish otolith research. University of South Carolina Press, Columbia, SC, p 671–691

Campana SE, Gagné JA, McLaren JW (1995) Elemental fingerprinting of fish otoliths using ID-ICPMS. Mar Ecol Prog Ser 122:115–120

Campana SE, Chouinard GA, Hanson JM, Fréchet A, Brattey J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. Fish Res 46:343–357

Deegen F (1990) Mississippi saltwater angler attitude and opinion survey. Mississippi Department of Wildlife, Fisheries and Parks, Bureau of Marine Resources, Biloxi, MS, p 22

Dorval E, Jones CM, Hannigan R, van Montfrans J (2005) Can otolith chemistry be used for identifying essential seagrass habitats for juvenile spotted seatrout, *Cynoscion nebulosus*, in Chesapeake Bay? Mar Freshw Res 56:645–653

Green SB, Salkind NJ (2000) Using SPSS for Windows and Macintosh: analyzing and understanding data, 3rd edn. Prentice Hall, NJ, p 443

Hanson PJ, Koenig CC, Zdanowicz VS (2004) Elemental composition of otoliths used to trace estuarine habitats of juvenile gag *Mycteroperca microlepis* along the west coast of Florida. Mar Ecol Prog Ser 267:253–265

Hettler WF Jr (1989) Food habits of juveniles of spotted seatrout and grey snapper in western Florida Bay. Bull Mar Sci 44:155–162

Johnson DR, Seaman W Jr (1986) Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (south Florida)–spotted seatrout. US Fish Wildl Serv Biol Rep 82(11.43)

Kalish MK (1989) Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. J Exp Mar Biol Ecol 132:151–178

Kalish MK (1990) Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fish Bull US 88:657–666

Kimura R, Secor DH, Houde ED, Piccoli PM (2000) Up-estuary dispersal of young-of-the-year bay anchovy *Anchoa mitchilli* in the Chesapeake Bay: inferences from microprobe analysis of strontium in otoliths. Mar Ecol Prog Ser 208:217–227

Kinsman DJJ, Holland HD (1969) The co-precipitation of cations with $CaCO_3$-IV. The co-precipitation of Sr^{2+} with aragonite between 16° and 96°C. Geochim Cosmochim Acta 33:1–17

Limburg KE (1995) Otolith strontium traces environmental history of subyearling American shad *Alosa sapidissima.* Mar Ecol Prog Ser 119:25–35

McMichael RH Jr, Peters KM (1989) Early life history of spotted seatrout, *Cynoscion nebulosus* (Pisces: Sciaenidae), in Tampa Bay, Florida. Estuaries 12:98–110

Mielke PW, Berry KJ, Johnson ES (1976) Multi-response permutation procedures for *a priori* classifications. Comm Statist Theory Methods 5:1409–1424

Minello TJ, Able KW, Weinstein MP, Hays CG (2003) Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. Mar Ecol Prog Ser 246:39–59

Mulligan TJ, Martin FD, Smucker RA, Wright DA (1988) A method of stock identification based on the elemental composition of striped bass *Morone saxatilis* (Walbaum) otoliths. J Exp Mar Biol Ecol 114:241–248

Patterson HM, McBride RS, Julien N (2004) Population structure of red drum (*Sciaenops ocellatus*) as determined by otolith chemistry. Mar Biol 144:855–862

Peebles EB, Tolley SG (1988) Distribution, growth and mortality of larval spotted seatrout, *Cynoscion nebulosus*: a comparison between two adjacent estuarine areas of southwest Florida. Bull Mar Sci 42:397–410

Perret WS, Weaver JE, Williams RO, Johansen PL, McIlwain TD, Raulerson RC, Tatum WM (1980) Fishery profiles of red drum and spotted seatrout. Gulf States Marine Fisheries Commission, No. 6, Ocean Springs, MS

Rooker JR, Secor DH, Zdanowicz VS, Itoh T (2001) Discrimination of northern bluefin tuna from nursery areas in the Pacific Ocean using otolith chemistry. Mar Ecol Prog Ser 218:275–282

Rooker JR, Secor DH, Zdanowicz VS, De Metrio G, Orsi Relini L (2003) Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. Fish Oceanogr 12:75–84

Rosenthal HL, Eves MM, Cochran OA (1970) Common strontium of mineralized tissues from marine and sweet water animals. Comp Biochem Physiol 32:445–450

Rosenthal Y, Field MP, Sherrell RM (1999) Precise determination of element/calcium ratios in calcareous samples using sector field inductively coupled plasma mass spectrometry. Anal Chem 71:3248–3253

Secor DH (1992) Application of otolith microchemistry analysis to investigate anadromy in Chesapeake Bay striped bass *Morone saxatilis.* Fish Bull US 90:798–806

Secor DH, Rooker JR, Zlokovitz E, Zdanowicz VS (2001) Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Mar Ecol Prog Ser 211:245–253

SPSS (2002) SPSS for Windows. Release 11.0. SPSS, Chicago, IL

Thorrold SR, Jones CM, Campana SE (1997) Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). Limnol Oceanogr 42:102–111

Thorrold SR, Jones CM, Campana SE, McLaren JW, Lam JWH (1998a) Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). Limnol Oceanogr 43:1826–1835

Thorrold SR, Jones CM, Swart PK, Targett TE (1998b) Accurate classification of juvenile weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. Mar Ecol Prog Ser 173:253–265

Thorrold SR, Latkoczy C, Swart PK, Jones CM (2001) Natal homing in a marine fish metapopulation. Science 291: 297–299

Editorial responsibility: Cynthia Jones, Norfolk, Virginia, USA

Submitted: February 14, 2006; Accepted: June 6, 2008
Proofs received from author(s): October 28, 2008