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RESEARCH ARTICLE

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# Population structure of red drum (*Sciaenops ocellatus*) as determined by otolith chemistry

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**Abstract** To examine current genetic-based paradigms pertaining to the structure and possible philopatry of red drum populations, we used solution-based inductively coupled plasma-mass spectrometry (ICP-MS) to analyze the otolith chemistry of juvenile red drum (*Sciaenops ocellatus*) from eight different estuaries in the Gulf of Mexico (Gulf) and the North Atlantic Ocean. One estuary (Tampa Bay, Fla.) was sampled in three different years. Analyses of variance for five elemental ratios (Mg/Ca, Mn/Ca, Zn/Ca, Sr/Ca, Ba/Ca) were all significantly different between estuaries, as was a multi-element signature (MANOVA, Pillai's trace $F_{50,\,1020} = 19.41$, $P < 0.0001$). We also found that red drum from the Gulf could be distinguished from those taken from the Atlantic Ocean with 99.5% accuracy, likely due to differences in water chemistry between these water masses. A discriminant function developed using these elemental ratios was more than 80% accurate in assigning juvenile red drum to their natal estuary, or in the case of Tampa Bay, to the correct year of spawning. We also used laser ablation ICP-MS to examine the otolith core chemistry of adult red drum collected from spawning aggregations near Tampa Bay. Using a discriminant function analysis with a calibration data set derived from juvenile signatures, we found that 75% of the adult cores matched the juvenile signal established for Tampa Bay 1982. Although preliminary, the results presented here suggest that red drum may return to their natal estuary to spawn, which has been postulated from genetic data.

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## Introduction

The red drum, *Sciaenops ocellatus* Linnaeus, is a well-studied, estuarine-dependent species found in the Gulf of Mexico (Gulf) and in the waters off the southeastern coast of the United States. Although a commercial red drum fishery no longer exits in Florida, red drum is a highly prized sportfish regulated by size and daily catch limits (Murphy and Crabtree 2001). Previous studies using allozymes and mitochondrial (mt) DNA have concluded that Atlantic and Gulf red drum are two distinct subpopulations, likely due to oceanographic and geographic features in south Florida that limit genetic exchange between the two coastal groups (Gold and Richardson 1991, 1998; Gold et al. 1993, 1994; Seyoum et al. 1999).

Elucidating the population structure of red drum has proven more difficult within the Gulf than between Gulf and Atlantic waters. Early genetic studies in the northern Gulf were unable to identify significant differences in red drum collected from different locations (Gold et al. 1993). However, more recent studies using mtDNA have discerned significant patterns of heterogeneity in Gulf red drum consistent with an isolation-by-distance (IBD) pattern, whereby red drum were more genetically similar to conspecifics from adjacent estuaries than from more distant locations (Gold et al. 1993, 1999). In addition, Gold and Turner (2002) examined nuclear-encoded microsatellites from the red drum used in the 1999 study to compare genetic markers and again found the IBD pattern. Red drum behavior such as limited coastwise movement (i.e. indolence) and/or return migrations to a natal estuary (i.e. philopatry) could reconcile these genetic results (Gold et al. 1999; Gold and Turner 2002) with tagging study results that have demonstrated that juvenile red drum have limited dispersal but that adult red drum can travel considerable distances. Tagging studies have repeatedly demonstrated that adult red drum move tens and even hundreds of kilometers in the Gulf (Ingle et al. 1962; Osburn et al. 1982; Overstreet 1983) and the Atlantic (Ross et al. 1995; Johnson et al. 1999).

Genetic studies have been very useful for modeling the population structure of some species, such as red drum (Gold et al. 1999). However, because relatively little genetic exchange is required to maintain genetic homogeneity in populations that are otherwise ecologically distinct (Kimura and Maruyama 1971), genetic models may underestimate the degree of population structure present. This article investigates the population structure and possible philopatry of red drum by using elemental signatures of otoliths, which are independent of genetic data. Otolith chemistry has been shown to be a useful tool in distinguishing fish from geographically separated areas (Edmonds et al. 1992; Thorrold et al. 1997; Patterson et al. 1999; Rooker et al. 2001), and several studies have demonstrated the utility of this technique in distinguishing nursery areas and the potential for determining the contribution of specific nursery areas to adult stocks (Gillanders and Kingsford 1996, 2000; Thorrold et al. 1998a, 1998b, 2001; Gillanders 2002). These determinations are possible because the trace elements deposited in the aragonite matrix are principally derived from the water in which the fish resides (Farrell and Campana 1996) and thus enable the geochemical signature encoded in the otolith to act as a natural tag. Because red drum remain in their natal estuary until they reach sexual maturity, typically at 4 years of age (Wilson and Nieland 1994), the otolith signature formed during that time period should be indicative of their natal estuary.

A preliminary study by Patterson et al. (2001) demonstrates that it is possible to classify red drum to estuaries with a high degree of accuracy (up to 95%) using otolith chemistry. Here we extend these elemental data by including otoliths from more estuaries to examine variation in otolith chemistry at both large (i.e. Atlantic vs Gulf) and small (i.e. among estuaries) spatial scales. We also examined temporal variation in elemental signatures at a single site (Tampa Bay). In addition, we compared the otolith chemistry of juveniles collected in Tampa Bay in 1982 with the elemental signatures found in the core regions of adult otoliths from the 1980–1982 year-classes collected from spawning aggregations in coastal waters off Tampa Bay to determine if otolith signatures would be useful for distinguishing philopatry in red drum. Such behavior has rarely been documented for nonanadromous fish species (Buechner 1987), although it has been well studied in other animals (Nichols 1996) and is suggested by the genetic models of red drum (Gold et al. 1999; Gold and Turner 2002). We specifically tested four hypotheses regarding red drum: $H_0$(a): there is no difference in the otolith signatures of juveniles from Gulf and Atlantic estuaries; $H_0$(b): otolith signatures of juveniles collected from all geographically separate estuaries do not differ; $H_0$(c): otolith signatures of juveniles collected in Tampa Bay during different years do not differ; $H_0$(d): there is no affinity of elemental signatures from the otolith core regions of adults with any of the established signatures of juvenile otoliths.

## Materials and methods

### Sample collection and preparation

Juvenile red drum were collected in seine nets at various sites within Florida (Tampa Bay, Charlotte Harbor, Cedar Key, and Indian River). Tampa Bay was sampled in 1982, 1998, and 1999. When available, additional otoliths from locations outside Florida were included in the analysis (Alabama, South Carolina, Louisiana, and Georgia; Fig. 1). Table 1 provides a summary of the otoliths used in the study. Purse-seine fishermen collected adult red drum from spawning aggregations in the nearshore waters off Tampa Bay as

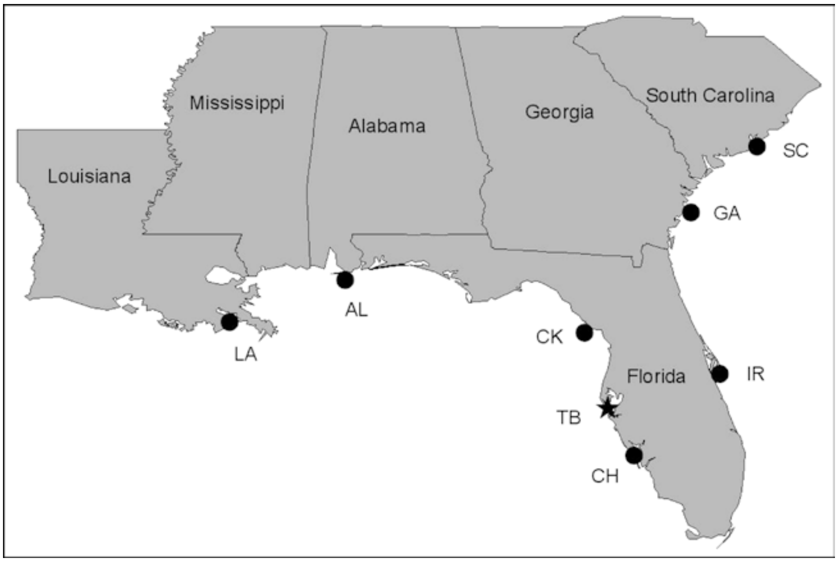

**Fig. 1** Map of the southeastern United States displaying sampling locations: Tampa Bay (*TB*; *star* indicates this site was sampled multiple times), Cedar Key (*CK*), Charlotte Harbor (*CH*), Indian River (*IR*), Alabama (*AL*), Louisiana (*LA*), Georgia (*GA*), and South Carolina (*SC*). Positions of the spawning aggregations where adult red drum were collected in the mouth of Tampa Bay can be found in Murphy and Crabtree (2001)

**Table 1** Red drum (*Sciaenops ocellatus*) collection information including collection sites and dates, size range and mean [total length (*TL*), mm], and sample size. Three juvenile collections in 3 years were made in Tampa Bay. *Tampa Bay-A* indicates spawning adults

| Site | Collection period | Range TL (mean) | Sample size |
|---|---|---|---|
| Tampa Bay | January 1982–June 1982 | 250–516 (367.3) | 19 |
| Tampa Bay | March 1998 | 119–173 (145.8) | 20 |
| Tampa Bay | February 1999–March 1999 | 66–147 (112.7) | 20 |
| Tampa Bay-A | May 1996–November 1998 | 911–1,061 (1,004.7) | 20 |
| Charlotte Harbor | February 1998–March 1998 | 76–136 (110.1) | 18 |
| Cedar Key | January 1999–February 1999 | 69–166 (120.4) | 20 |
| Indian River | February 1999 | 46–52 (48.9) | 19 |
| Alabama | March 1985 | 81–90 (84.5) | 20 |
| Louisiana | March 1998–April 1998 | 292–336 (317.8) | 19 |
| South Carolina | October 1998–December 1998 | 338–447 (405.2) | 20 |
| Georgia | August 1998–December 1998 | 310–364 (350.6) | 20 |

part of a separate study (Murphy and Crabtree 2001). Otoliths from adults of the 1980, 1981, or 1982 year-classes were used in the study ($n=5$, 7, and 8, respectively), because an adequate number of otoliths from the 1982 year-class alone could not be obtained (Table 1).

Juvenile red drum collected in Florida in 1998 and 1999 were frozen whole and the sagittae later removed in clean conditions. The otoliths were then triple-rinsed in Milli-Q water, dried under a laminar flow hood, and stored dry in clean microcentrifuge tubes. Otoliths from Louisiana were removed from the fish and rinsed with Milli-Q water and stored dry. All other juvenile otoliths were rinsed in tap water and were stored dry in paper envelopes. Adult red drum otoliths were removed from the fish shortly after capture, rinsed in tap water, and stored dry in vials.

To reduce the amount of otolith material and to ensure that only the core was included in the analysis, we used a Buehler Isomet low-speed saw with a diamond blade to cut ~1–1.5 mm-thick otolith sections containing the core from juvenile otoliths. A previous study using a diamond saw demonstrated that no contamination was introduced by sectioning the otoliths (Dove et al. 1996). These sections were polished with grit-size-220 lapping paper until only core material remained and the otolith weight was between 9 and 13 mg. Otoliths from Alabama and Indian River did not require polishing as they already fell in the designated weight category and were processed whole. These otolith sections or whole otoliths were then rigorously cleaned in a class-100 clean room. The cleaning protocol followed that of Patterson et al. (2001), which dictated that the sections be cleaned ultrasonically for 15 min and then triple-rinsed in Milli-Q water. The sections were then acid-washed in 1% ultrapure $HNO_3$ for 10 s and again triple-rinsed with Milli-Q water. The otoliths were allowed to dry under a class-100 laminar flow hood for 24 h and then were weighed to the nearest 10 µg. To dissolve the otolith sections for solution-based analysis, 0.5 ml of 70% ultrapure $HNO_3$ was added. After dissolution was complete, 4.5 ml of Milli-Q water was added to each vial. Blanks were similarly prepared so that we could calculate limits of detection (LOD) and for blank corrections.

Adult red drum otoliths were prepared by cutting a section ~2 mm thick to expose the core region. The sections were polished with lapping film (3–9 µm) to produce a smooth surface and were then cleaned in a protocol similar to the one described above, with the exception of a longer acid rinsing time of 30 s as these sections were much larger than those from juveniles. Adult otolith sections were stored in acid-washed tubes until analysis. The second sagittae was used to age the fish (see Murphy and Crabtree 2001 for a complete description of the aging process).

## Solution-based ICP-MS analysis

Elemental signatures of juvenile red drum otoliths were determined by solution-based inductively coupled plasma-mass spectrometry (ICP-MS) with a Perkin-Elmer Elan 5000 ICP-MS. Preliminary analysis of a single otolith indicated that six elements were detectable above background levels (Mg, Mn, Ca, Zn, Sr, Ba). Samples were blocked so that one individual from each location was sampled in turn, with the order within each block randomized. Internal standards for each element were used and referenced against $^{45}Sc$, $^{72}Ge$, $^{89}Y$, and $^{159}Tb$. To counter instrument drift caused by Ca accumulation on the cones, we used a method described in Patterson et al. (2001). Briefly, the cones were conditioned using a solution of 1,000 mg/l Ca and 10 µg/l Ba, which allowed equilibrium between evaporation and deposition to be reached. By conditioning the cones to the sample matrix it was possible to reduce instrument drift and thereby achieve a stable signal. We monitored instrument drift by analyzing a calibration-verification solution every 20 samples. Ca and Sr analyses were completed in a single run without exceeding the acceptance limits of ±10% of the expected value of the calibration solution. Previous experience with Ba, Mg, Mn, and Zn in otoliths indicated that even with the cone conditioning, these limits could not be maintained for extended periods. For these analytes, the instrument was recalibrated every 60 samples. Precision was typically $<5\%$ relative standard deviation (RSD) for Ca and Sr and $<10\%$ RSD for trace elements. In otoliths, Ca and Sr are orders of magnitude higher in concentration than other elements are, so these elements were analyzed with a 10× online dilution using a crossflow nebulizer and a double-pass spray chamber; Mg, Mn, Zn, and Ba were analyzed undiluted with a Meinhard high-efficiency nebulizer and a baffled cyclonic spray chamber. Elemental LODs were calculated from the prepared blanks as $3\sigma$ plus the mean blank value and were as follows (in micrograms per gram): Mg 0.29, Mn 0.04, Ca 146, Zn 0.04, Sr 0.51, and Ba 0.04. Observed concentrations of all elements were well above detection limits.

## Laser ablation ICP-MS

The laser we used was a CETAC LSX-100 (CETAC Technologies, Omaha, Neb.) and was used with the ICP-MS described above. This laser operated at a wavelength of 266 nm with an energy output of 3.3 mJ and produced a crater 20 µm in diameter. Multiple adult red drum otolith sections (up to ten) were chosen randomly and placed inside the sampling chamber. Each time the sampling chamber was opened to change samples, the chamber was flushed with Ar for ~20 min and the laser recalibrated using NIST 612 (National Institute of Standards and Technology, Gaithersburg, Md.). Samples were viewed via a camera located inside the sampling chamber while the chamber was mounted to a computer-controlled XYZ stage. Specific blast points were chosen and their positions on the otoliths recorded in the computer. The laser then moved in turn to each predetermined location. Two firing sequences of five shots per sequence were completed at each preset otolith location. The purpose of the initial firing sequence was to clean surface contamination from the otolith and expose clean material for analysis. The data from the initial firing sequence were therefore discarded. The transient signal profile from the second firing sequence was smoothed with a nine-point Savitsky-Golay moving average routine and then integrated to produce the actual sample data. The crater size after the second firing sequence was ~20 µm. Each otolith was sampled in five locations along an axis adjacent to the sulcal groove. The first location (A) was directly on the core and each additional location (B, C, D, E) was 400 µm farther along the axis toward the proximal margin of the otolith. The final point E generally fell just inside the first annulus. LOD values for the laser, based on Ar gas blanks with no attendant laser pulse, were (in micrograms per gram) Mg 1.1, Mn 0.12, Ca 273, Zn 0.21, Sr 0.22, and Ba 0.17. Mn and Zn values fell below detection limits after the third laser hit (C). We therefore did not include laser hits D and E in any further analysis.

### Statistical analysis

Elemental data were standardized to Ca and expressed as molar ratios because the elements we used were likely substituting for Ca in the aragonite matrix and standardizing these elements to Ca is considered more accurate than standardizing them to otolith weight (Thorrold et al. 1998a). Standardizing data to Ca is also a way to compensate for uneven topography of the otoliths and fluctuations in power that can affect the amount of material ablated by the laser (Sinclair 1999). The elemental data were $\ln(x+1)$ transformed to reduce heterogeneity of variances, which was detected with a Levene's test. We did not adjust for weight differences between samples because they were not significant (analysis of covariance, $P > 0.1$ for all elemental ratios; Patterson et al. 1999; Gillanders 2002).

One-factor (site and year combinations) univariate analyses of variance (ANOVAs) for each elemental ratio were performed, and Tukey's honestly significant difference (HSD) post hoc tests were used to examine differences in the Tampa Bay sites. A multivariate ANOVA (MANOVA) was used to examine an overall difference of the multi-element otolith signature. The number of dependent variables (i.e. elemental ratios) was reduced to two dimensions to visualize differences between sites using canonical discriminant analysis (CDA). A stepwise analysis was used to determine which elemental ratios were useful in discriminating the sites. This procedure retained all elemental ratios in the CDA except Zn/Ca.

Discriminant criteria were derived from juvenile red drum data, and these criteria are referred to hereafter as the calibration data set. Assumptions of normality and equal variances did not maximize the accuracy of classification results; instead, we used a nonparametric method (kernel) that normalized the mean values. The number of misclassifications was evaluated by using a jackknife cross-validation procedure, in which each observation was removed in turn from the data set and was then classified based on the remaining observations (Everitt and Der 1998). Due to differences in sample size, prior probabilities were adjusted to be proportional to sample size.

The chemistry of adult otolith cores was measured by laser ablation ICP-MS (LA-ICP-MS) to determine if adults returned to their natal estuary. Elemental ratios from the cores of the adults were calculated by pooling the second firing sequence from the first three laser-blast positions (A–C). Elemental ratios of the cores of adult red drum were tested against the calibration data set in a single discriminant analysis to determine the affinity, if any, of these spawning adults to juveniles collected on nursery grounds. All statistical analyses were done using SAS (SAS 1999).

## Results

Elemental concentrations varied greatly between sites (Table 2), and the univariate analyses of individual elemental ratios indicated that there were significant differences among site and year combinations (Table 3). Tukey's HSD post hoc tests demonstrated that for some of the elemental ratios examined, there were significant ($P < 0.05$) differences among Tampa Bay collections (Table 4). Most notably, Sr/Ca ratios differed in all the Tampa Bay collections, whereas no differences were observed for Mg/Ca or Zn/Ca.

A MANOVA of four elemental ratios (Sr/Ca, Ba/Ca, Mn/Ca, and Mg/Ca) revealed a significant difference among sites in these multi-element signatures (Pillai's trace $F_{50,\ 1020} = 19.41$, $P < 0.0001$). A CDA using all the juvenile otoliths was then performed to examine the separation between each estuary. The first three canonical axes accounted for 93.3, 64.3, and 35.5% of the variation, respectively. A plot of scores showed a definitive separation along the first canonical axis between Atlantic red drum and red drum collected from the Gulf in 1998 (Fig. 2). Another group containing red drum collected from the Gulf in 1999 (Tampa Bay and Cedar Key) was more closely related to, but distinct from, the Atlantic fish than the 1998 Gulf fish. All four elemental ratios loaded significantly ($P < 0.05$) along the first canonical axis: Mn/Ca and Mg/Ca were positively correlated with the first canonical axis, whereas Sr/Ca and Ba/Ca were negatively correlated with this axis. Only two elemental ratios loaded significantly along the second canonical axis: Sr/Ca was positively correlated and Ba/Ca was negatively correlated.

A jackknife cross-validation procedure resulted in a correct classification rate of 37–100% for each estuary

**Table 2** Mean concentrations (± 1 SE) of elemental ratios in micromoles per mole of the otoliths of red drum from each estuary and time sampled. All juvenile otoliths were analyzed using solution-based inductively coupled plasma-mass spectrometry (ICP-MS), whereas adult otoliths from Tampa Bay (*TB-A*) were analyzed using laser ablation ICP-MS and only the core area was sampled. Abbreviations of site names are the same as indicated in Fig. 1. Multiple collections in Tampa Bay are indicated by *TB82* (1982), *TB98* (1998), and*TB99* (1999)

| Site | Mg/Ca | Mn/Ca | Zn/Ca | Sr/Ca | Ba/Ca |
|---|---|---|---|---|---|
| AL | 123.50 ± 3.16 | 197.65 ± 8.57 | 1.76 ± 0.15 | 1,547 ± 23 | 6.01 ± 1.13 |
| TB82 | 109.07 ± 5.02 | 138.46 ± 11.85 | 1.52 ± 0.34 | 1,687 ± 46 | 3.11 ± 0.62 |
| TB98 | 123.92 ± 6.61 | 169.19 ± 10.64 | 1.12 ± 0.07 | 1,928 ± 48 | 4.40 ± 0.31 |
| TB99 | 115.18 ± 5.28 | 5.41 ± 0.40 | 1.29 ± 0.23 | 2,007 ± 22 | 12.81 ± 1.66 |
| TB-A | 40.14 ± 4.91 | 0.04 ± 0.009 | 3.78 ± 0.81 | 2,988 ± 50 | 2.96 ± 0.22 |
| CK | 123.13 ± 5.11 | 11.37 ± 0.70 | 0.89 ± 0.09 | 1,661 ± 48 | 7.00 ± 0.58 |
| LA | 100.35 ± 1.97 | 161.61 ± 15.19 | 1.31 ± 0.07 | 1,718 ± 26 | 10.32 ± 0.85 |
| SC | 99.96 ± 4.25 | 14.17 ± 1.08 | 0.69 ± 0.10 | 1,996 ± 30 | 4.65 ± 1.08 |
| GA | 97.51 ± 2.74 | 13.67 ± 0.95 | 0.87 ± 0.21 | 2,060 ± 18 | 6.07 ± 0.55 |
| CH | 123.91 ± 4.07 | 257.71 ± 68.39 | 1.16 ± 0.04 | 1,988 ± 69 | 3.08 ± 0.13 |
| IR | 110.50 ± 3.55 | 8.13 ± 0.58 | 0.92 ± 0.15 | 2,340 ± 19 | 4.89 ± 0.31 |

**Table 3** ANOVA results for individual elemental ratios in the otoliths of both juvenile and adult red drum collected from multiple sites and times. Analyses were made on ln($x+1$) data

| Source | *df* | Mg/Ca | | Mn/Ca | | Zn/Ca | | Sr/Ca | | Ba/Ca | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MS | *F* | MS | *F* | MS | *F* | MS | *F* | MS | *F* |
| Site | 10 | 2.75 | 41.12* | 62.56 | 403.1* | 0.52 | 3.10* | 0.64 | 52.47* | 3.01 | 20.81* |
| Residual | 204 | 0.067 | | 0.16 | | 0.17 | | 0.012 | | 0.14 | |

* $P < 0.01$

**Table 4** Summary of the Tukey's HSD tests for differences in each elemental ratio among the four Tampa Bay collections (1982, 1998, 1999, and adults). Collections that did not differ significantly by elemental ratio share the same letter, whereas collections that differ at the $P < 0.05$ level are denoted by a different letter

| | 1982 | 1998 | 1999 | Adults |
|---|---|---|---|---|
| Mg/Ca | A | A | A | A |
| Mn/Ca | A | A | B | C |
| Zn/Ca | A | A | A | A |
| Sr/Ca | A | B | C | D |
| Ba/Ca | A | A | B | A |

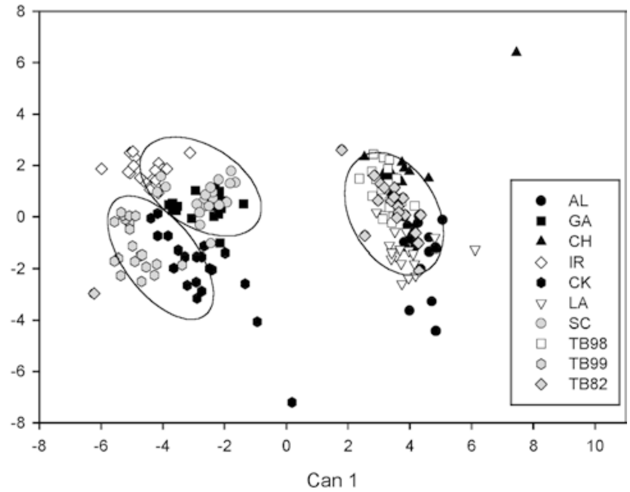

**Fig. 2** Canonical plot scores of juvenile red drum (*Sciaenops ocellatus*) otoliths by estuary using multiple elemental ratios (Mg/Ca, Mn/Ca, Sr/Ca, Ba/Ca). Three discrete groups demarcated by 95% confidence ellipses can be seen: Atlantic (*GA*, *SC*, *IR*), Gulf 1999 (*CK*, TB99), and Gulf (*AL*, *CH*, *LA*, *TB82*, *TB98*). Site abbreviations are the same as found in Fig. 1, with multiple collections in Tampa Bay noted by year

independently and an overall error rate of 19% (Table 5). The greatest misclassification was for juvenile otoliths from Tampa Bay 1982, which overlapped with the other collections from Tampa Bay, samples from neighboring Charlotte Harbor, and the nearly contemporaneous samples from Alabama 1985. South Carolina samples also overlapped heavily with those from Georgia (30%). Juvenile otoliths from Cedar Key were most distinctive; all 20 otoliths were correctly determined as having come from Cedar Key. In most misclassifications (47%) otoliths were assigned to an adjacent estuary, or in the case of Tampa Bay, to another year (41%). Only 1 (0.5%) otolith was classified to the wrong coast, suggesting that Gulf and Atlantic water masses are distinct in their chemistries.

When the adult otolith cores were classified using the discriminant function derived for the juvenile otoliths, 15 of the 20 otoliths (75%) were assigned to Tampa Bay 1982 (Table 5). Two adult otoliths were classified as having come from Tampa Bay in 1999, 1 of the adults was classified to Cedar Key, and 2 were classified to sites on the Atlantic coast (Indian River and South Carolina).

## Discussion

Our results show that variation in the otolith chemistry of juvenile red drum exists on both large and small spatial scales. Only 1 of 195 juvenile otoliths (0.5%) was misclassified at the level of coast. Thus, the otolith chemistries of red drum from the Gulf and Atlantic regions were distinct, even when a temporal component was introduced into the analysis (i.e. multiple collections at Tampa Bay). Such a distinction in otolith chemistry has been documented for other species collected in Atlantic and Gulf waters (Patterson et al., unpublished data) and likely stems from differences in the chemistry of the Atlantic and Gulf water masses. For example, freshwater flows into Gulf estuaries are higher than into Atlantic estuaries (Roman et al. 2000). In particular, massive freshwater and sediment inputs into the Gulf by the Mississippi River discharge plume ($\sim 1.83\times10^4$ km$^3$ per year; Gunther 1979) reach as far as the West Florida Shelf near Tampa Bay (Del Castillo et al. 2001).

At a finer spatial scale, the otolith elemental signatures were generally distinct for each estuary sampled and for each year Tampa Bay was sampled. Variation in otolith chemistry at such spatial scales has been noted in other studies working in coastal habitats (Gillanders and Kingsford 1996, 2000; Thorrold et al. 1998a; Gillanders and Kingsford 2003) and likely reflects variation in chemical composition of water mass, temperature, and salinity, which have all been shown to influence otolith chemistry (Fowler et al. 1995; Farrell and Campana 1996; Bath et al. 2000). The estuaries examined for this study ranged from industrialized ports with freshwater flows influenced by mining operations, agricultural practices, and urbanization (i.e. Tampa Bay and Charlotte Harbor; Bass and Cox 1985; McCain et al. 1996) to more rural areas with less freshwater input (i.e. Cedar Key). Thus, each estuary would be exposed to a unique

**Table 5** Results of a jackknife cross-validation procedure to classify otoliths to estuary based on a multi-element signature (Mg/Ca, Mn/Ca, Sr/Ca, Ba/Ca). Includes all juvenile otoliths analyzed by solution-based ICP-MS. Results are expressed as percentages; percentages of correct classifications shown in *bold*. Sites are abbreviated as described in Table 2and Fig. 1. Classification of the adult otoliths (*TB-A*) was analyzed by laser ablation ICP-MS using the discriminant function derived for the juvenile signatures

| Site: | TB82 | TB98 | TB99 | CK | CH | IR | AL | LA | GA | SC |
|---|---|---|---|---|---|---|---|---|---|---|
| TB82 | **37** | 21 | 5 | 0 | 11 | 0 | 26 | 0 | 0 | 0 |
| TB98 | 10 | **80** | 0 | 0 | 10 | 0 | 0 | 0 | 0 | 0 |
| TB99 | 0 | 0 | **95** | 5 | 0 | 0 | 0 | 0 | 0 | 0 |
| CK | 0 | 0 | 0 | **100** | 0 | 0 | 0 | 0 | 0 | 0 |
| CH | 0 | 28 | 0 | 0 | **72** | 0 | 0 | 0 | 0 | 0 |
| IR | 0 | 0 | 0 | 5 | 0 | **95** | 0 | 0 | 0 | 0 |
| AL | 5 | 5 | 0 | 0 | 0 | 0 | **85** | 5 | 0 | 0 |
| LA | 0 | 5 | 0 | 0 | 0 | 0 | 5 | **90** | 0 | 0 |
| GA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | **85** | 15 |
| SC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 30 | **70** |
| TB-A | 75 | 0 | 10 | 5 | 0 | 5 | 0 | 0 | 0 | 5 |

suite of trace elements derived from a myriad of sources (Cutter 1991), which would in turn influence otolith chemistry.

We also detected significant temporal variation among the Tampa Bay otoliths collected from multiple years. Temporal drift in otolith signatures has been less studied than spatial variation, although several researchers have reported significant differences in otolith chemistry both within (Thorrold et al. 1998b) and among years (Patterson et al. 1999; Gillanders 2002). Such temporal variation in estuaries likely reflects elemental fluxes and variable mixing rates with seawater (Church 1986; Peters 1999) and could potentially confound attempts to differentiate fish from different areas (Gillanders 2002). Thus, it may be necessary to recalibrate estuary signatures periodically to compensate for this drift. We found that several individual elemental ratios differed significantly among years for the Tampa Bay collections. However, the majority of the differences were for the Tampa Bay 1999 otoliths (Mn/Ca, Sr/Ca, Ba/Ca), while otoliths from 1982 and 1998 differed only in Sr/Ca. Using a multi-element signature, the cross-validation procedure indicated some interesting relationships. Both the 1982 and 1998 Tampa Bay otoliths showed some overlap with other Tampa Bay collections, indicating some degree of temporal persistence in the Tampa Bay signature; 41% of misclassifications in the Tampa Bay groups were to other Tampa Bay collections.

This difference in the Tampa Bay 1999 otoliths was noted in the multivariate analyses as well, and we found that otoliths collected from the Gulf in 1999 (Tampa Bay and Cedar Key) formed a distinct group. Although we were unable to identify conclusively the mechanism(s) driving this separation, it seems likely that the 1997–1998 El Niño Southern Oscillation (ENSO) was at least partially involved, because rainfall during the dry season (November–April) was ~60 cm, twice the average for the region (National Oceanic and Atmospheric Administration, National Weather Service, http://www.srh.noaa.gov). Gulf red drum collected in 1999 would have been spawned in September–October 1998 (Murphy and Taylor 1990) in waters that were influenced by the increased rainfall and associated river drainage from the previous months. Gulf red drum from 1998 appear to have been unaffected, because those fish grouped together with fish from Alabama 1985 and Tampa Bay 1982, presumably because they were collected before the heaviest rains occurred. Red drum from the Atlantic all grouped together irrespective of year, indicating that the Gulf may be more heavily influenced by such events, possibly because of the greater river discharge and longer water residence time.

There was a strong match between the core chemistry of adult red drum from the 1980–1982 year-classes collected in spawning aggregations in the mouth of Tampa Bay and the chemistry of juvenile red drum otoliths collected in Tampa Bay in 1982. Specifically, our analysis suggested that 75% of the adult red drum we examined might have originated near Tampa Bay. Adult red drum that were not classified to Tampa Bay 1982 consisted of both males and females from the 1981 and 1982 year-classes, thereby demonstrating no obvious patterns related to sex or year-class. In addition, the rate of adult misclassification was similar to the error rate of the calibration dataset of juveniles (25 and 19%, respectively), suggesting that misclassifications of adult fish may be due to measurement error alone.

Otolith chemistry studies do not always have the benefit of concomitant genetic data, as are available for red drum, and direct comparisons of these data are valuable for understanding and managing coastal fish populations. Our results are consistent with the results from mtDNA and nuclear microsatellite studies. These genetic studies have suggested philopatry in red drum and estimated a geographic neighborhood size of 700–900 km (i.e. little or no gene flow occurs between subpopulations separated by more than this distance; Gold et al. 1999, Gold and Turner 2002). Despite the genetic evidence, we acknowledge that our results do not conclusively demonstrate philopatry as we have examined only a single estuary, and we have likely introduced some degree of error into the analysis by combining different year-classes. Thus, our estimate of red drum returning to their natal estuary is likely over-stated.

However, we feel our results have demonstrated the utility of otolith chemistry in investigating behaviors of marine fish that have been difficult to address by more conventional means.

The concept of philopatry in terrestrial populations, such as birds, is well studied (Nichols 1996). However, documenting this behavior in aquatic systems has proven more difficult. Most studies examining natal homing in marine species have examined anadromous species such as American shad (*Alosa sapidissima*; Leggett and Carscadden 1978), Atlantic sturgeon (*Acipenser oxyrinchus*; Waldman et al. 1996), and striped bass (*Morone saxatilis*; Waldman et al. 1996). The best example of natal homing in a fish species is Pacific salmon (*Onchorynchus* spp.), which is well known for spawning-site fidelity, documented by tagging studies from hatchery-reared fish (Quinn 1993; Urwin and Quinn 1993). The logistical obstacles of following individuals in open systems have hindered the study of philopatry and other migratory behaviors in marine fish. Indeed, the rates of natal homing in a nonanadromous marine fish population have been determined for only one species. Thorrold et al. (2001) used otolith chemistry to calculate the natal homing rate of weakfish (*Cynoscion regalis*) in several estuaries along the eastern coast of the United States. Their estimates ranged from 60 to 81%, with most strays going to adjacent estuaries. This degree of spawning-site fidelity by weakfish was significant because genetic analyses had indicated only a single weakfish stock (Graves et al.1992), whereas the otolith chemistry data suggested that a metapopulation approach was more appropriate for managing this species. For red drum, limited dispersal of juveniles and a combination of adult indolence and philopatry appears to explain the genetic and otolith chemistry results. At this time red drum are managed within the constraints of a single stock model for the entire Gulf, but it may be appropriate to manage red drum using a metapopulation approach.

In conclusion, we have demonstrated variation in the otolith chemistry of red drum at both large and small spatial scales, that is, between Atlantic and Gulf populations and among individual estuaries. A more spatially structured sampling protocol may reveal differences within estuaries, although previous studies have shown that variation within estuaries is small compared to variation among estuaries (Gillanders and Kingsford 2003). In addition, we found significant temporal variation in otoliths from Tampa Bay. However, this variation was largely confined to the 1999 samples, possibly as a result of ENSO-driven rainfall, while the other collections had similar concentrations of most elemental ratios. Although our attempt to detect philopatry in red drum was preliminary, we feel it illustrates the potential of otolith chemistry to examine behaviors that have been difficult to document. Future research may be able to show philopatry conclusively by eliminating temporal variation that could introduce error and expanding the spatial scale of the study.

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