# Elemental Signatures of Red Drum (*Sciaenops ocellatus*) Otoliths from the Gulf of Mexico and Western Atlantic

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ABSTRACT

The red drum (*Sciaenops ocellatus*) is an estuarine-dependent species that supports recreational fisheries in the Gulf of Mexico and western Atlantic Ocean. In the mid-1980s, concerns about overfishing of red drum resulted in recreational catch limits and a ban on commercial fishing in Florida. Recent studies offshore of Tampa Bay, Florida, suggest that at least in this area the species is recovering. However, it is not known if this recovery is occurring throughout the entire Gulf of Mexico. Genetic studies have shown that there are weakly differentiated sub-populations in the Gulf. This could mean that a local recovery would not affect the entire region. We examined otolith chemistry as an independent measure of stock discreteness or connectivity. Otolith chemistry is a useful natural tag for examining the stock structure of fishes because it can reflect the elemental composition of the water in which the fish resides. Juvenile red drum otoliths from Texas, South Carolina, and Georgia and from three sites in Florida--Tampa Bay, Cedar Key, and Indian River-- were analyzed using solution-based inductively coupled plasma-mass spectrometry (ICP-MS). Five elements (Mg, Mn, Zn, Sr, and Ba) were routinely detected above background levels. A MANOVA (multiple analysis of variance) indicated significant differences in the otolith chemistry of the red drum collected at different sites (Wilk's Lambda = 15.80, p = 0.0001). Using a cross-validation procedure, we were able to correctly classify otoliths from the Florida sites with an accuracy rate of up to 95%. This research demonstrates that otolith chemistry may be useful in delineating the stock structure of red drum throughout their range. We plan to use this method to examine sources of recruitment to the Tampa Bay area and thereby examine the connectivity of Gulf stocks in general.

KEY WORDS: ICP-MS, otolith chemistry, *Sciaenops ocellatus*

## INTRODUCTION

The red drum, *Sciaenops ocellatus*, is an estuarine-dependent sciaenid found in temperate and subtropical waters, primarily in the Gulf of Mexico (Gulf) and western Atlantic Ocean. Although the commercial fishery for red drum in the Gulf was closed during the mid-1980s because of concerns about overfishing, the species still supports important recreational fisheries throughout the Gulf and southeastern U.S. Characteristics of the species such as age, growth, reproduction, and mortality are well studied (Peters and McMichael 1987, Beckman et al. 1988, Murphy and Taylor 1990, Wilson and Nieland 1994). Recently, Murphy and Crabtree (1999) examined the age structure of adult red drum from the waters offshore of Tampa Bay, Florida. They found that reduced rates of fishing mortality resulted in a measurably abrupt increase in the survival rates of red drum beginning with the 1985 or 1986 year-class. This increase in survival rates was the result of strict fishing regulations imposed on the fishery throughout the southeastern U.S. and contributed to increased abundance of adult offshore stocks in west Florida.

Whether this recovery affected other areas of the Gulf or Atlantic is central to the purpose of this study. There is significant genetic variation (mitochondrial [mt] DNA) to suggest that two separate populations are present in Gulf and Atlantic waters (Bohlmeyer and Gold 1991, Gold and Richardson 1991, Gold et al. 1993, 1994). The geographic and oceanographic features of Florida that may preclude gene exchange between Gulf and Atlantic populations of red drum would also presumably limit increases in Gulf population size from affecting the size of Atlantic stocks of red drum. In a recent genetic study, Gold et al. (1999) were able to distinguish different groups of red drum within the Gulf. They found significant variations in the mtDNA that were consistent with an isolation-by-distance effect, wherein fish from geographically closer areas are more genetically similar than fish from more distant locations. Gold et al. (1999) noted that this genetic pattern may be due to behavioral characteristics of female red drum such as philopatry to their natal estuaries and limited coastwise movement. These processes could also restrict the effects of local increases in fish abundance in Tampa Bay to adjacent areas within the Gulf.

A method of examining stock structure that does not directly examine gene flow is otolith chemistry. Otolith chemistry of individuals will differ between areas within the Gulf where dispersal and movements of fish are limited and between areas of different water chemistry (e.g., Edmunds et al. 1989, 1991, 1992, Campana and Gagne 1995, Thorrold et al. 1997, 1998a, 1998b, Patterson et al. 1999). The elements that are deposited in the aragonite matrix of the otolith appear to be derived mainly from water (Farrell and Campana 1996) and will remain unaltered because the otolith is metabolically inert (Campana and

Neilson 1985). Thus, the chemistry of the otolith will reflect, to some degree, the chemistry of the water in which the fish resides.

The objective of this study was to examine the elemental signatures of red drum otoliths from the Gulf and western Atlantic to determine if differences in otolith chemistry could be detected and whether such differences were consistent with recent genetic data. The data presented here are part of a larger study to be published separately; our ultimate objective is to identify the source of recruitment of red drum to Tampa Bay.

## MATERIALS AND METHODS

Young-of-the-year and juvenile red drum otoliths were collected in mid-to-late 1998 through early 1999 from sites in Texas, Georgia, and South Carolina and from three sites in Florida: Tampa Bay, Cedar Key, and Indian River (Figure 1; Table 1). The red drum otoliths collected at the Florida sites were collected by staff of the Florida Marine Research Institute (FMRI) field labs specifically for this study, whereas the otoliths from sites in the three other states had been previously collected by other researchers who donated them to the study. The red drum from Florida were collected for specific size, spatial, and temporal parameters, and the handling of these otoliths was controlled from the time the otoliths were removed from the fish (e.g. teflon forceps used to remove otoliths, and otoliths rinsed in Milli-Q water). Otoliths from other states were not collected for these specific parameters or handled in a controlled manner prior to their donation.

**Table 1.** Summary information of the red drum, *Sciaenops ocellatus*, used in this study including collection site, collection date (month/year), the number of otoliths analyzed (n), and minimum and maximum total length (TL [mm], mean).

| Location | Date | n | Min-Max TL (mean) |
|---|---|---|---|
| Cedar Key | 1/99-2/99 | 20 | 69 - 166 (120.4) |
| Indian River | 2/99 | 20 | 46 - 52 (49.0) |
| Tampa Bay | 2/99-3/99 | 20 | 66 - 147 (112.7) |
| Georgia | 8/98-12/98 | 20 | 310 - 364 (350.6) |
| South Carolina | 10/98-12/98 | 20 | 338 - 447 (405.2) |
| Texas | 8/98-11/98 | 20 | 205 - 307 (280.5) |

The weight of whole otoliths varied between sites (3.6 - 57.3 mg), so heavier otoliths were sectioned with a Buehler Isomet low-speed saw to fit within a weight range of 9.0 - 9.9 mg. Otoliths from Indian River were not sectioned because they already fell below this range (3.6 - 4.5 mg). Sections of whole otoliths were cut approximately 1 - 1.5 mm thick around the core and then polished evenly on all sides using grit size 220 lapping paper until the

sections were in the desired weight range. Before they were acid-washed, the polished sections and Indian River otoliths were cleaned ultrasonically for 15 minutes and triple rinsed in Milli-Q water. We acid-washed the otoliths by dipping them in 1% trace-metal-grade nitric acid for 15 seconds and triple rinsing them with Milli-Q water. The otoliths were allowed to dry under a Class 100 laminar flow hood for 24 hours and then were weighed to the nearest 10 µg. The otolith sections were then dissolved in 0.5 or 0.25 ml (depending on otolith weight) of 70% trace-metal-grade nitric acid, and Milli-Q water was added in a ratio equal to 9x the acid volume. Blanks were similarly prepared so that we could calculate detection limits and for blank corrections.


**Figure 1.** Map showing sampling locations including Texas (TX), Georgia (GA), and South Carolina (SC), and three Florida sites including Indian River (IR), Cedar Key (CK), and Tampa Bay (TB).

Elemental concentrations of the otoliths were determined using a Perkin-Elmer Elan 5000 ICP-MS. Preliminary tests had indicated that several elements were detectable and suitable for ICP-MS analysis (Ba, Ca, Mg, Mn, Sr, and Zn).

Samples were blocked so that one 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 $^{159}Tb$. In order to avoid the problem of instrument drift due to build up of Ca on the cones, the cones were conditioned with a solution of 1000 mg/L Ca and 10 µg/L Ba. This solution was analyzed five times and the Ba signal monitored for stability. This process allowed an equilibrium between evaporation and deposition on the cones to be reached, so the cones were conditioned to the matrix, which allowed for a consistent signal. Calcium and Sr were analyzed with a 10x online dilution using a crossflow nebulizer and a double-pass spray chamber. The remaining elements were analyzed undiluted with a Meinhard high-efficiency nebulizer and a baffled cyclonic spray chamber. The limits of detection for each element were calculated from the prepared blanks as 3σ plus the mean blank value and were as follows (in µg/g): Ca 280, Sr 12.1, Ba 0.04, Mg 0.32, Mn 0.04, and Zn 0.07. The limits of detection were well below the observed values for all elements.

Elemental data were converted to molar concentrations and expressed as ratios to Ca because the elements we were working with substitute for Ca in the aragonite matrix of the otolith. Statistical analysis was carried out using both univariate and multivariate approaches. To test for overall significance at all the sites and including all five elemental ratios (Ba/Ca, Mg/Ca, Mg/Ca, Sr/Ca, and Zn/Ca), we performed a MANOVA (multivariate analysis of variance) using the GLM and MANOVA statements in SAS (Littell et al. 1991). This was followed by individual ANOVAs for each of the five elemental ratios to determine which elements were significantly different and by unplanned contrast comparisons (least significant difference method).

To visualize differences between the sampling sites in canonical space, we used the CANDISC function in SAS (SAS 1989) to perform a canonical discriminant analysis. This procedure derives linear combinations of the variables that summarize between-class (i.e., sampling site) variation. Pearson correlation coefficients determined which elemental ratios loaded significantly on each axis. Finally, the DISCRIM procedure (SAS 1989) was used to determine classification accuracy. We used the cross-validation option, which removes each sample in turn from the data set and then classifies it based on the remaining observations.

## RESULTS

The MANOVA (Wilks' Lambda = 15.8006; p = 0.0001) showed that there was an overall significant difference between the sites based on otolith chemistry. This difference was examined more closely in the individual ANOVAs of each elemental ratio (Figure 2). Three of the elemental ratios we analyzed were significantly different between sites: Ba/Ca, Mn/Ca, and Sr/Ca.

Elemental ratios of Mg/Ca and Zn/Ca were not significantly different between sites based on univariate analysis, but were left in the remaining multivariate analyses described below and were found to contribute significantly.

The first three canonical axes accounted for 70%, 53%, and 40% of the variation between sites. The only elemental ratio to load significantly on canonical axis 1 was Sr/Ca ($P = 0.0001$), which loaded in a positive direction. Both Ba/Ca ($p = 0.0001$) and Zn/Ca ($p = 0.0012$) loaded significantly in a positive direction on canonical axis 2, and Mn/Ca ($p = 0.0001$) loaded significantly in a negative direction. Lastly, Ba/Ca ($p = 0.0001$) and Mn/Ca $p = 0.0001$) both loaded positively on canonical axis 3, and Mg/Ca ($p = 0.0006$) loaded in a negative direction on this axis. A plot of the first two axes depicts the separation of the sites (Figure 3). The three Florida sites form distinct groups, whereas the other sites are less clearly defined.

The results of the cross-validation procedure show that the classification accuracy of the otoliths from the three Florida sites ranged from 90% to 95%. South Carolina and Texas otoliths had marginal recoveries with 45% and 55%, respectively, while Georgia had a classification accuracy of only 25%. The South Carolina and Georgia otoliths were primarily misclassified with each other, whereas Texas otoliths were scattered throughout the classification (Table 2).

**Table 2.** Results of the cross-validation procedure showing the classification accuracy for each group based on the elemental signatures of otoliths. The locations are Tampa Bay, FL (TB), Cedar Key, FL (CK), Georgia (GA), Indian River, FL (IR), South Carolina (SC), and Texas (TX). Both the number and percentage of classified otoliths are shown. The number of samples analyzed for each group was 20.

| Site | TB | CK | GA | IR | SC | TX |
|---|---|---|---|---|---|---|
| TB | 19 | 0 | 1 | 0 | 0 | 0 |
| | 95% | 0% | 5% | 0% | 0% | 0% |
| CK | 1 | 19 | 0 | 0 | 0 | 0 |
| | 5% | 95% | 0% | 0% | 0% | 0% |
| GA | 0 | 0 | 5 | 1 | 12 | 2 |
| | 0% | 0% | 25% | 5% | 60% | 10% |
| IR | 0 | 1 | 1 | 18 | 0 | 0 |
| | 0% | 5% | 5% | 90% | 0% | 0% |
| SC | 0 | 2 | 8 | 0 | 9 | 1 |
| | 0% | 10% | 40% | 0% | 45% | 5% |
| TX | 2 | 1 | 2 | 2 | 2 | 11 |
| | 10% | 5% | 10% | 10% | 10% | 55% |

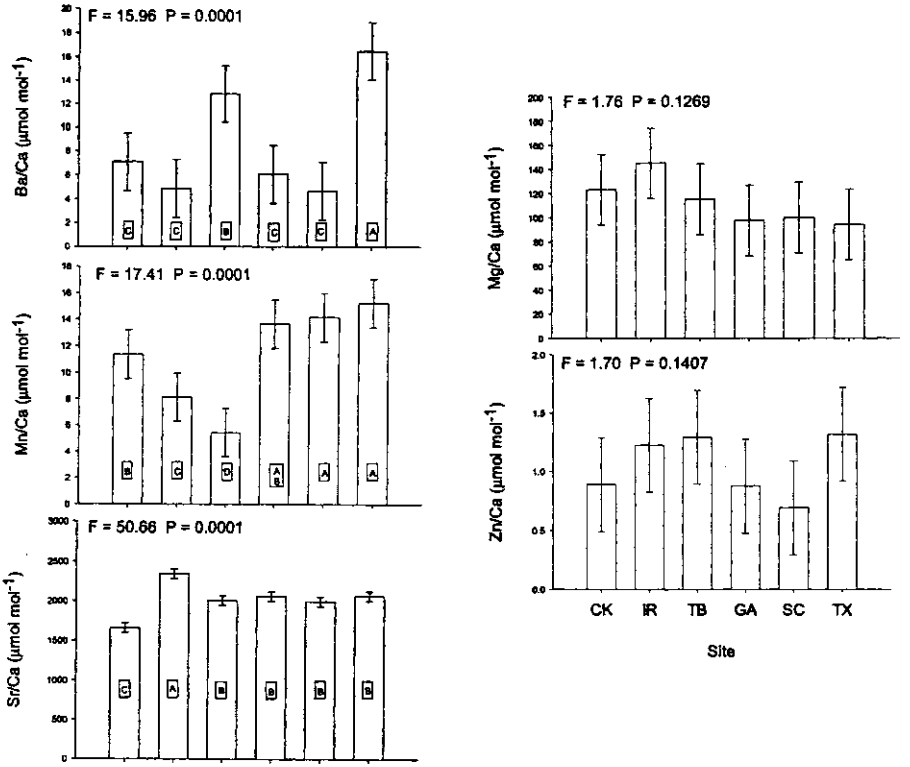


**Figure 2.** Mean concentrations expressed as a molar ratio to Ca (± SE) of the five elements analyzed in *Sciaenops ocellatus* otoliths (see Figure 1 for sample locations). The F and P values were determined by one-way ANOVAs and are indicated on each graph. The least significant difference contrast groupings are indicated as letters (i.e. A, B, C, D) where overall differences were significant ($p < 0.05$).

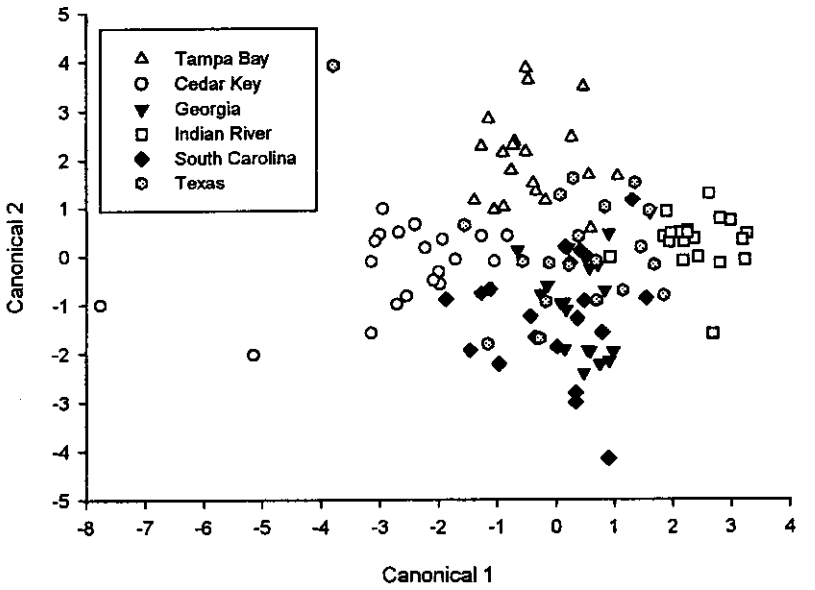


**Figure 3.** Canonical discrimination of elemental ratios for *Sciaenops ocellatus* otoliths collected from five sampling sites in the Gulf of Mexico and Atlantic Ocean. Only the first two canonical axes are shown here. Tampa Bay is indicated by open triangles, Cedar Key open circles, Indian River open squares, Georgia filled inverted triangles, South Carolina filled diamonds, and Texas gray hexagons.

## DISCUSSION

The elemental concentrations of the red drum otoliths we analyzed varied significantly by site. Otoliths from the three sites in Florida were correctly classified more often than otoliths from any other site and formed distinct multivariate groups. Based on the postulations of Gold et al. (1999) concerning the causes of an isolation-by-distance genetic structure for red drum in the Gulf (e.g., limited coastwise movement and female philopatry to natal estuaries), we expected significant differences between the chemistry of the otoliths from the different sites if significant variation in water chemistry existed. Indeed, we were able to find differences between sites on a finer scale than genetic studies have found. Gold et al. (1999) found a geographic neighborhood size relative to genetic migration from an estuary to be 500 to 600 km, but we were able to

find a significant difference in otolith chemistry between Tampa Bay and Cedar Key, which are approximately 150 km apart.

Regarding sampling procedures, only Florida otoliths were collected specifically for this study and were collected as young-of-the-year in discrete sampling periods. The South Carolina, Georgia, and Texas samples introduced variability into the analysis that appears to have confounded separation of stocks. The otoliths we were sent from South Carolina and Georgia were collected in several locations, although in each of these states, the sites appear to have been relatively close to each other. It has been demonstrated that otoliths collected only a small distance from each other (i.e., sites within the same river) can differ chemically (Thorrold et al. 1998a), and Texas otoliths, which were collected at sites that spanned the entire coast of that state, were a poor fit to the cross-validation procedure. We concluded that the large spatial distribution of the Texas sites where the otoliths were collected (i.e., Port Aransas, East Matagonda Bay, Sabine Pass, Corpus Christi Bay, Galveston Bay, and Upper/Lower Laguna Madre) introduced variation that reflects very different bay systems within this state.

In this study we have demonstrated that red drum from different sites can be distinguished based on their otolith chemistry, and the results are largely consistent with the genetic models of red drum. Results that deviated from this conclusion were related to sampling and handling methods. Our continuing research with red drum otoliths will focus on whether individuals or consecutive year-classes of fish residing in the same estuary maintain a consistent elemental signature over time. The ultimate objective of this research is to use laser ablation ICP-MS to look at the core region of otoliths from adult red drum from the waters offshore of Tampa Bay and see if their elemental signatures can be used to determine the origin of these fish. Although genetic models have provided a framework for our study, genetic methods might not be sufficient for identifying the source of recruitment to Tampa Bay because only limited genetic exchange is necessary to produce genetic homogeneity (Allendorf and Phelps 1981). If these adult red drum are indeed from waters near Tampa Bay, as research has suggested (Gold et al. 1999), then recovery of the population in this area may have little impact on other sub-populations in the Gulf of Mexico. Such information will be important in deciding future management strategies for this economically important species.

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LITERATURE CITED

Allendorf, F.W. and S.R. Phelps. 1981. Use of allelic frequencies to describe population structure. *Can. J. Fish. Aquat. Sciences.* 38:1507-1514.

Beckman, D.W., C.A. Wilson and A. L. Stanley. 1988. Age and growth of the red drum, *Sciaenops ocellatus*, from offshore waters of the northern Gulf of Mexico. *Fish. Bull.* **87**:17-28.

Bohlmeyer, D.A. and J.R. Gold. 1991. Genetic studies in marine fishes. II. A protein electrophoretic analysis of population structure in the red drum *Sciaenops ocellatus*. *Mar. Biol.* **108**:197-206.

Campana, S.E. and J.A. Gagne. 1995. Cod stock discrimination using ICPMS elemental assays of otoliths. Pages 671-691 in: D.H. Secor, J.M. Dean and S.E. Campana, (eds.) *New developments in fish otolith research.* Univ. of South Carolina.

Campana, S.E. and J.D. Neilson. 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* **42**:1014-1032.

Edmunds, J.S., N. Caputi and M. Morita. 1991. Stock discrimination by trace-element analysis of otoliths of orange roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. *Aust. J. Mar. Freshwater Res.* **42**:1014-1032.

Edmunds, J.S., R.C.J. Lenanton, N. Caputi and M. Morita. 1992. Trace elements in the otoliths of yellow-eye mullet (*Aldrichetta forsteri*) as an aid to stock identification. *Fish. Res.* **13**:39-51.

Edmunds, J.S., M. J. Moran, N. Caputi and M. Morita. 1989. Trace element analysis of sagittae as an aid to stock identification: Pink snapper (*Chrysophrys auratus*) in Western Australian waters. *Can. J. Fish. Aquat. Sci.* **46**:50-54.

Farrell, J. and S.E. Campana. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comp. Biochem. Physiol.* **115**:103-109.

Gold, J.R. and L.R. Richardson. 1991. Genetic studies in marine fishes. IV. An analysis of population structure in the red drum (*Sciaenops ocellatus*) using mitochondrial DNA. *Fish. Res.* **12**:213-241.

Gold, J.R., L.R. Richardson and T.F. Turner. 1999. Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in the

red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico. *Mar. Biol.* **133**:593-602.

Gold, J.R., L.R. Richardson, C. Furman and T.L. King. 1993. Mitochondrial DNA differentiation and population structure in red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. *Mar. Biol.* **116**:175-185.

Gold, J.R., T.L. King, L.R. Richardson, D.A. Bohlmeyer and G.C. Matlock. 1994. Allozyme differentiation within and between red drum (*Sciaenops ocellatus*) from the Gulf of Mexico and Atlantic Ocean. *J. Fish. Biol.* **44**:567-590.

Littell, R.C., R.J. Freund and P.C. Spector. 1991. *SAS Systems for Linear Models*, 3rd ed. SAS Institute Inc., Cary, NC. 329 pp.

Murphy, M.D. and R.E. Crabtree. 1999. Age structure of offshore red drum populations in nearshore waters off west-central Florida. MARFIN Final Report NA57FF0296.

Murphy, M.D. and R.G. Taylor. 1990. Reproduction, growth, and mortality of red drum *Sciaenops ocellatus* in Florida waters. *Fish. Bull.* **88**:531-542.

Patterson, H.M., S.R. Thorrold and J.M. Shenker. 1999. Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from the Bahamas and Belize using solution-based ICP-MS. *Coral Reefs* **18**(2):171-178.

Peters, K.M. and R.H. McMichael Jr. 1987. Early life history of *Sciaenops ocellatus* (Pisces: Sciaenidae) in Tampa Bay, Florida. *Estuaries* **10**(2):92-107.

SAS Institute Inc. 1989. *SAS/STAT User's Guide*, version 6, 4th ed., Cary, NC. 943 pp.

Thorrold, S.R., C.M. Jones and S.E. Campana. 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnol. Oceanogr.* **42**(1):102-111.

Thorrold, S.R., C.M. Jones, S.E. Campana, J.W. Mclaren and J.W.H. Lam. 1998a. Trace element signatures in otoliths record natal river of juvenile American shad (*Alosa sapidissima*). *Limnol. Oceanogr.* **43**(8):1826-1835.

Thorrold, S.R., C.M. Jones, P.K. Swart and T.E. Targett. 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.

Wilson, C.A. and D.L. Nieland. 1994. Reproductive biology of the red drum, *Sciaenops ocellatus*, from the neritic waters of the northern Gulf of Mexico. *Fish. Bull.* **92**:841-850.