Discriminating Between Age-0 Red Snapper, *Lutjanus campechanus*, Nursery Areas in the Northern Gulf of Mexico Using Otolith Microchemistry

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ABSTRACT

Natural biogeochemical tags of age-0 red snapper, *Lutjanus campechanus*, nursery areas in the northern Gulf of Mexico were determined based on sagittal otolith microchemistry. Age-0 red snapper were collected in 1996 and 1997 from historically important nursery areas in north central, northwest and southwest areas of the United States' Gulf of Mexico. Otolith microchemistry of these fish was assayed using solution-based inductively coupled plasma-mass spectrometry (ICP-MS). In addition to Ca, four elements (Ba, Cd, Mg, Sr) were consistently detected in age-0 red snapper otolith solutions above ICP-MS limits of detection. For statistical analyses, otolith concentrations of these elements were expressed as ratios to Ca. In 1996 and 1997 there were significant differences in Ba:Ca, Cd:Ca, Mg:Ca, and Sr:Ca ratios between nursery areas. Multivariate analyses of variance, with all four element:Ca ratios as dependent variables, indicated that differences in nursery-specific elemental signatures were statistically significant in 1996 and 1997. Linear discriminant functions were computed from elemental data in each year as a tool to classify individual fish to their nursery areas of collection. In 1996, overall classification accuracy of age-0 fish to nursery area was 93%, while 1997 age-0 fish were correctly classified with an overall accuracy of 87%. Future research will focus on determining the otolith core microchemistry of adult red snapper from offshore reefs in the northern Gulf of Mexico, and on determining the nursery areas from which adults recruited based on the microchemical tags developed from age-0 red snapper otoliths.

KEY WORDS: ICP-MS, Otolith microchemistry, nursery areas

INTRODUCTION

Red Snapper are long-lived reef fish that occur in United States' waters as far north as Massachusetts, but generally are distributed from North Carolina to Florida in the Atlantic Ocean and from Florida to the Yucatan Peninsula in the Gulf of Mexico (Hoese and Moore 1977). In the northern Gulf of Mexico (hereafter Gulf), red snapper are distributed along the continental shelf out to the shelf's edge and demonstrate high affinity for vertical structure. Adults aggregate on or near coral reefs, gravel bottoms, or rock outcrops, as well as on artificial reefs, oil rigs, and ship wrecks (Moran 1988). Young red snapper spend their first year of life over the continental shelf on the shrimping grounds where they are concentrated in areas with vertical complexity, such as relic shell habitats (Moseley 1966, Szedlmayer and Howe 1998). Adult red snapper may display agonistic behavior toward young snapper (Bailey 1995), but as they grow, young fish recruit to the adult population on offshore reef structures (Moseley 1966, Szedlmayer and Howe 1998).

Red snapper are managed as a single stock in United States' waters of the Gulf. The conclusion that Gulf red snapper constitute a single stock is supported by population genetics studies that generally have reported no differences between red snapper from different geographic areas in the northern Gulf (Gold et al. 1997). Contrary to the genetic evidence, tagging studies of adult red snapper generally have shown that adult fish demonstrated high site fidelity and moved little (Beaumariage 1969, Fable, 1980, Szedlmayer and Shipp 1994). More recently, Watterson et al. (1998) and Patterson (1999) reported that red snapper demonstrated low rates of site fidelity to artificial reefs in the northern Gulf (20 - 40%/yr), and reported 17 tagged fish moved over 100 km away from their release sites. However, high rates of tag shedding reported by Patterson (1999) impede observation of long-term movement by tagged fish.

For observation of red snapper movement on longer temporal scales, a permanent tag of fish is needed. Recent studies of otolith microchemistry have shown that otolith microchemistry serves as an ideal natural biogeochemical tag of fish (Campana and Gagne 1995, Edmunds et al. 1995, Thorrold et al. 1997, 1998 a,b). Otoliths are calcium carbonate and protein structures that serve in the accustico-lateralis system of fishes. Their growth is directly related to fish growth and, traditionally, have been used as the hardpart of choice in aging studies of fishes. More recently, it has been shown that otoliths are metabolically inert once formed and incorporate minor and trace metals from surrounding water into their matrices as they accrete (Campana and Neilson 1985, Casselman 1987, Kalish 1989, Mugiya et al. 1991). Therefore, otolith microchemical analysis reveals the environmental history of fish and can be used as a natural tag (Kalish 1989, Patterson et al. 1998, Thorrold et al. 1998 a,b).

Patterson et al. (1998) reported significant differences in otolith microchemical fingerprints of age-0 red snapper collected from northern Gulf nursery areas in 1995. The purpose of the present study was to expand on this initial work by examining otolith microchemistry of age-0 red snapper collected in the northern Gulf in 1996 and 1997. Our objective was to develop natural tags of historically important red snapper nursery areas in the northern Gulf based on otolith microchemistry. Eventually, these natural tags will be used in long-term movement analysis of adult fish. If successful, this approach will allow us to address problems concerning stock mixing and stock structure in adult red snapper, as well as to determine the source of recruits to offshore reefs throughout the northern Gulf.

METHODS

Age-0 red snapper were collected from three different regions in the northern Gulf in October and November of 1996 and 1997 (Figures 1. A,B). Fish were collected using otter trawls aboard the United States' National Oceanographic and Atmospheric Administration's R/V Oregon II and the Dauphin Island Sea Lab's R/V Verril during the National Marine Fisheries Service's fall groundfish survey in each year. Fish were collected over the continental shelf in the north central Gulf off Alabama/Mississippi (NC Gulf), in the northwest Gulf off Louisiana and east Texas (NW Gulf), and in the southwest Gulf off southeast Texas (SW Gulf). Immediately following collection, fish were placed in plastic bags and frozen. Sample sizes and geographic range of sampling were increased in 1997 to better estimate region-specific otolith microchemical tags (Figures 1.A,B).

In the laboratory, fish were thawed, weighed to the nearest mg, and measured to total length (TL). Sagittae were extracted using acid-washed glass probes and acid-washed polyethylene tweezers; all materials that came in contact with extracted otoliths were acid-washed and triple-rinsed in ultrapure water (18 MΩ polished water). Extracted otoliths were scrubbed with a synthetic bristle brush, rinsed with ultrapure water, and placed in acid-leached polyethylene vials to air-dry. Further otolith cleaning and sample preparation took place in a class-100 clean room. Otoliths were cleaned with 2% ultrapure nitric acid for 10 sec, rinsed repeatedly with ultrapure water, and allowed to air-dry in cell wells. Dry otoliths were weighed to the nearest 1 x 10^{-5} g and placed in acid-leached polyethylene vials for dissolution. Otoliths were dissolved in 10% ultra-pure nitric acid at 1 ml acid per 0.2 mg otolith.

Otolith solutions were diluted 2.5 fold and analyzed for elemental composition using a Perkin/Elmer Elan 5000 inductively couple plasma mass spectrometer (ICP-MS) with an AS-90 autosampler and FIAS-400MS accessory. All analyses were performed using internal standards which were added online using the second pump on the FIAS-400MS unit. Ca and Sr were analyzed using

a standard crossflow nebulizer and Scott double pass spraychamber. The FIAS accessory was used to dilute the samples 100 fold for analysis of Ca and Sr. All other analytes were determined using a Meinhard high efficiency nebulizer and cyclonic spray chamber with a 1.25 fold dilution. Due to high concentrations of Ca in otolith solutions, nickel cones of the ICP-MS were cleaned every 15-20 samples, followed by recalibration with the internal standard. In addition to otolith solutions, blank solutions were analyzed to estimate detection limits (mean + 2σ in ppm) of elements of interest.

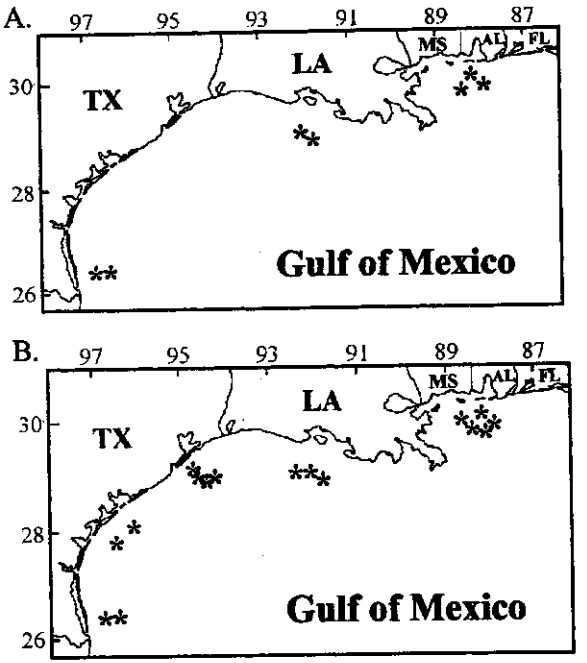

Figure 1. Maps of sampling sites for age-0 red snapper A.) in 1996 and B.) in 1997.

We were primarily interested in analyzing elements that substitute directly for Ca in otolith aragonite. Among these, Sr, Mg, Ba, Cd, Mn, Cr, Zn, Pb, and Ni were detected in otolith samples, but only Sr, Mg, Ba, Mn, and Cd concentrations were consistently above detection limits. Sr, Mg, Ba, and Mn concentrations always were well above detection limits; however, Cd concentrations were below detection limits in 26% of samples. In statistical analyses, Mn was not considered due to potential polyatomic interference from potassium oxide on mass 55.

Univariate and multivariate statistical techniques were employed to determine otolith microchemical tags unique to each nursery area. First, we tested for potential ontogenetic effects on elemental concentrations. Analyses of variance (ANOVAs) tested for differences in otolith weight and fish size between regions in 1996 and 1997. Additionally, analyses of covariance (ANCOVAs) tested for differences between regions in the relationship of otolith weight to total length in each year. Correlation analyses were performed to test if significant relationships existed between otolith weight and element:Ca ratios both within and among nursery areas. Differences in element:Ca ratios between nursery areas in each year were tested with ANOVAs. Unique otolith microchemical tags of nursery area in each year were determined using multiple analysis of variance (MANOVA) and linear discriminant function analysis (LDFA).

RESULTS

In all univariate statistical analyses, variables met the assumptions of normality and equal variances. Therefore, it was assumed that the assumptions of multivariate normality and equal variance/covariance matrices were met for multivariate statistics, although no tests of these assumptions were performed. All statistical tests were computed using SAS (SAS Institute Inc., 1990).

There was no significant difference in TL (ANOVA, $F_{2;82} = 1.51$, $p = 0.2216$) or otolith weight (ANOVA, $F_{2;82} = 1.88$, $p = 0.1595$) between nursery areas in 1996 (Figure 2A). There was a significant difference in TL (ANOVA, $F_{2;153} = 5.48$, $p = 0.005$) and otolith weight (ANOVA, $F_{2;153} = 8.77$, $p < 0.001$) in 1997 (Figure 2B). Because fish growth rate may affect incorporation of trace and minor elements into otoliths (Fowler 1995, Thorrold et al. 1997), and because fish sampled from the southwest Gulf were smaller than the other two regions in 1997, we tested for differences in the relationship of otolith weight and TL between areas in each year. In 1996 there was no difference in the relationship of otolith weight and TL between nursery areas (ANCOVA test for homogeneity of slopes, $F_{2;79} = 2.79$, $p = 0.0671$; ANCOVA test for homogeneity of intercepts, $F_{2;79} = 1.69$, $p = 0.1991$) (Figure 2A). There was

also no difference in the relationship of otolith weight and TL between areas in 1997 (ANCOVA test for homogeneity of slopes, $F_{2;145} = 0.51$, $p = 0.5986$; ANCOVA test for homogeneity of intercepts, $F_{2;145} = 1.49$, $p = 0.2282$) (Figure 2B).

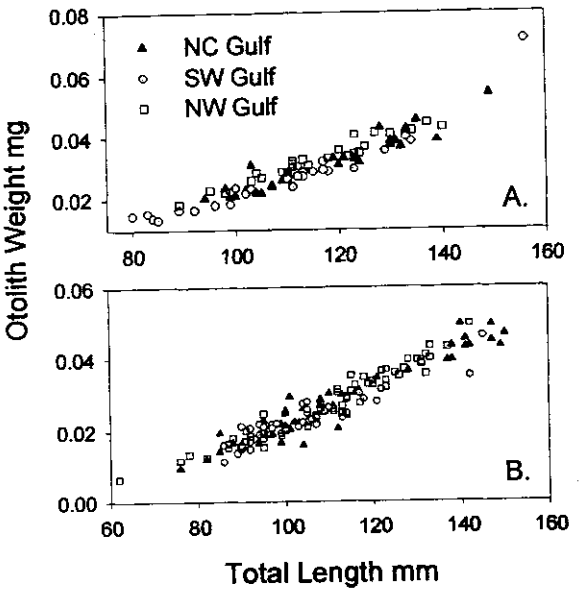

Figure 2. Relationship between otolith weight and TL for age-0 red snapper from the northern Gulf of Mexico nursery areas sampled in A.) 1996 and B.) 1997. The legend is the same for both years.

Few correlations between otolith weight and element:Ca ratios were statistically significant (Pearson's r, $p < 0.05$) within nursery areas in each year (Figure 3 A,B). For element:Ca ratios that were significant, the slopes of the relationships from different nursery areas often were opposite in direction. Therefore, although some correlations between otolith weight and element:Ca ratios pooled across nursery areas were significant, no correction for the effect of otolith weight on element:Ca ratios could be implemented via analysis of covariance (Thorrold et al. 1998b). Furthermore, because correlations between otolith weight and element:Ca ratios areas were weak and mostly non-significant when data were pooled across nursery areas, and because correlations for each element:Ca ratio within nursery areas differed in direction between areas, no

systematic effect of otolith weight on element:Ca ratios was perceived (Figure 3).

In 1996, Ba:Ca ratios were statistically significant between nursery areas (ANOVA, $F_{2;82} = 3.91$, $p = 0.0239$), and Cd:Ca, Mg:Ca, and Sr:Ca, ratios were all highly significant ($p < 0.001$) between nursery areas (Figure 4). All four element:Ca ratios were highly significant between nursery areas in 1997 (Fig. 4). MANOVAs with Ba:Ca, Cd:Ca, Mg:Ca, and Sr:Ca ratios as dependent variables were significant in 1996 (Pillai's Trace, $F_{8;160} = 25.079$, $p < 0.001$) and in 1997 (Pillai's Trace, $F_{8;302} = 43.936$, $p < 0.001$). Canonical discriminant function analysis was employed as a data reduction devise to aid visualization of multivariate differences among nursery areas in each year (Figure 5 A,B). In each year, the first canonical variate accounted for approximately 75% of the discrimination between nursery areas.

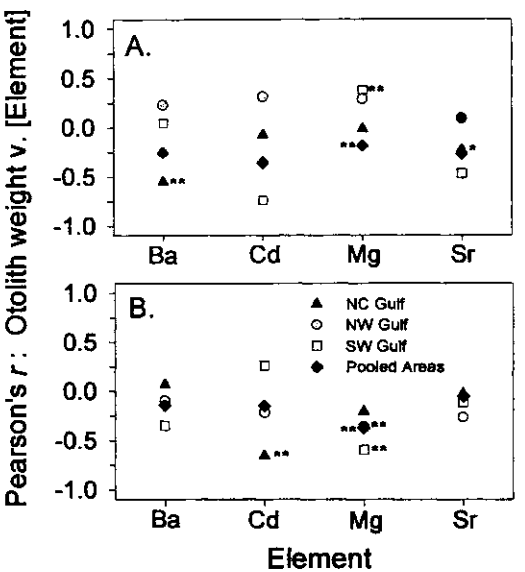

Figure 3. Correlation coefficients between element concentrations (ppm) in otoliths and otolith weight for fish sampled A.) in 1996 and B.) in 1997. One asterisk denotes $p < 0.05$ and two asterisks denote $p < 0.01$. The legend is the same for both years.

Linear discriminant function analyses computed with Ba:Ca, Cd:Ca, Mg:Ca, and Sr:Ca ratios as response variables and nursery area as the classification variable yielded clear discrimination between nursery areas in 1996 and 1997. Overall, the percentage of individual fish correctly classified to nursery area using the cross-validation algorithm in SAS was 93% in 1996 and 87% in 1997 (Tables 1 and 2) (SAS Institute Inc., 1985).

Table 1. Results of linear discriminant function analysis for classifying age-0 red snapper to nursery area in 1996 based on elemental signatures in otoliths. Bold numbers represent correct classification of individual fish to nursery area.

Area of Assignment	Area of Capture % (n)		
	NC Gulf	**NW Gulf**	**SW Gulf**
NC Gulf	**96.6 (28)**	10.0 (3)	0.0 (0)
NW Gulf	3.4 (1)	**86.7 (26)**	3.8 (1)
SW Gulf	0.0 (0)	3.3 (1)	**96.1 (25)**
Total	29	30	26
Error Rate	0.034	0.133	0.038

Table 2. Results of linear discriminant function analysis for classifying age-0 red snapper to nursery area in 1997 based on elemental signatures in otoliths. Bold numbers represent correct classification of individual fish to nursery area.

Area of Assignment	Area of Capture % (n)		
	NC Gulf	**NW Gulf**	**SW Gulf**
NC Gulf	**93.9(46)**	8.9 (5)	0.0 (0)
NW Gulf	6.1(3)	**85.7 (48)**	19.6 (9)
SW Gulf	0.0 (0)	5.4 (3)	**80.4 (37)**
Total	49	56	46
Error Rate	0.061	0.143	0.196

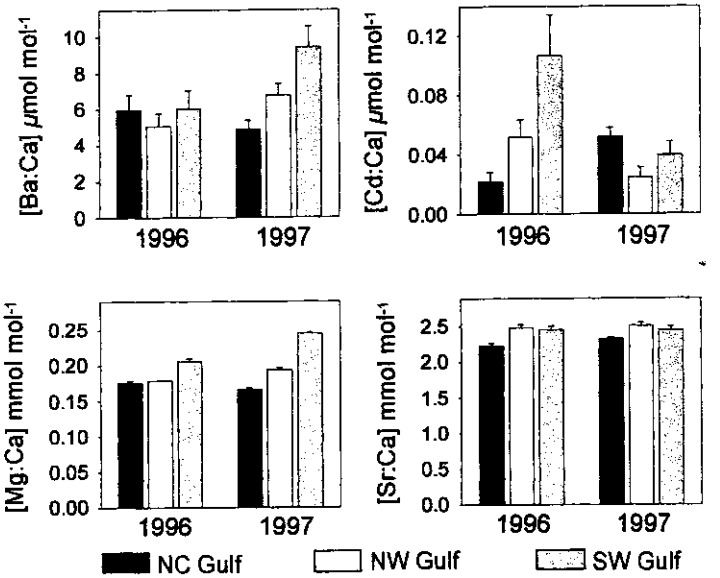

Figure 4. Plots of mean Element:Ca ratios in age-0 red snapper otoliths sampled in 1996 and 1987. Error bars are S. E. of the mean.

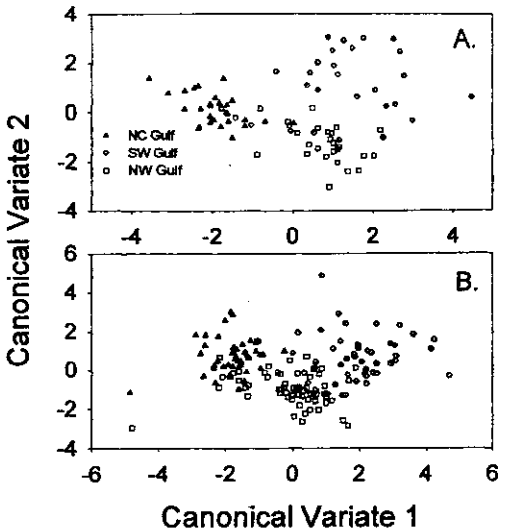

Figure 5. Scatterplots of first and second canonical variates from canonical discriminant function analysis of elemental signatures of age-0 red snapper otoliths for A.) 1996 and B.) 1997. The legend is the same for both plots.

DISCUSSION

Variability in elemental signatures was significant between nursery areas in 1996 and 1997, and differences in elemental signatures among nursery areas in both years were sufficient to allow for accurate classification of age-0 fish to northern Gulf nursery areas. There was also significant variability of individual element:Ca ratios within nursery areas between years. Statistical tests of elemental signatures were not performed between years, however, because of differences in sampling in 1996 and 1997. In 1998 and 1999, fish have been sampled from northern Gulf nursery areas similarly to those sampled in 1997. When elemental signatures of these fish are available, statistical tests of the temporal stability of nursery-specific elemental signatures will be made.

Patterson et al. (1998) reported that the relationship between otolith weight (sagittae) and TL of age-0 red snapper sampled from northern Gulf of Mexico nursery areas in 1995 did not differ among nursery areas. We have shown here that this relationship also did not differ among nursery areas for age-0 snapper sampled in 1996 and 1997. Fish sampled from the SW Gulf were smaller than the other two areas in 1997, but consistency in the relationship between otolith weight and TL among nursery areas indicates that fish from different nursery areas were growing at similar rates (Szedlmayer, 1998; Szedlmayer and Conti, 1999). Therefore, the smaller size of fish sampled from the SW Gulf in 1997 suggests that on average these fish were slightly younger than fish from the other two areas, which may partially explain some of the differences in elemental signatures between areas in 1997. However, differences in elemental signatures between nursery areas in 1997 were similar to differences in 1996 when there was no difference in mean size of fish.

One area in which we seek to improve is in our ability to quantify elements present in otoliths at trace levels. Cd was an important element in both 1996 and 1997 for discriminating between nursery areas, but concentrations of Cd in otolith solutions were often close to or below limits of detection. Among element:Ca ratios, Cd:Ca ratios showed the highest variability both within and among nursery areas, especially in 1996 when the concentrations of Cd in otolith solutions of many fish sampled from the NC Gulf were low. We feel it is not appropriate to rely on estimates of elemental concentrations that are below estimated detection limits for discrimination between nursery areas; however, Cd was included in statistical analyses here because Cd levels in most samples were above its detection limit. When LDFAs were performed without Cd:Ca ratio included as a dependent variable, overall classification accuracies were 87% in 1996 and 80% in 1997.

While the concentrations of Cd in otolith solutions were near or just above its detection limit, concentrations of Pb, Zn, Ni, and Cr were below detection limits in most samples. More reliable estimates of Cd concentrations will be

attained by decreasing the dilution factor of otolith solutions, and by doing so it is likely that we also will be able to quantify other elements present at trace levels. These analyses are currently being performed, but results were not available at the time of this presentation.

The initial results of this study suggest that elemental signatures in age-0 red snapper otoliths may provide ideal natural tags of red snapper nursery areas. Future directions of our work involve developing analytical protocols which will allow us to better estimate elements that may be present in otoliths at trace levels, and to begin to examine the microchemistry of adult snapper otolith cores. We hope to be able to discriminate between age-0 red snapper nursery areas routinely with greater than 90% accuracy. Eventually, our aim is to use natural tags of red snapper nursery areas based on otolith chemistry in studies of movement of adult snapper. If successful, our approach will allow us to address many aspects of red snapper population ecology that are presently allusive. For example, in the future we hope to be able to determine the source of adult fish on offshore reefs in different regions of the northern Gulf and to estimate trans-basin movement and stock mixing rates.

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