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ARTICLE

## Discrimination of Juvenile Red Snapper Otolith Chemical Signatures from Gulf of Mexico Nursery Regions

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### Abstract

Age-0 red snapper *Lutjanus campechanus* from the 2005–2007 year-classes were sampled in six regions across the Gulf of Mexico (Gulf) to develop nursery signatures from otolith element : Ca ratios (Ba:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Li:Ca) and stable isotope delta values ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ). Element : Ca ratios were analyzed with sector field inductively coupled plasma mass spectrometry on dissolved right sagittae; isotope ratio mass spectrometry was employed to analyze pulverized left otoliths for  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ . Otolith chemical signatures were significantly different among regions in each year. Year-class-specific quadratic discriminant function analysis (QDFA) distinguished nursery regions with an accuracy of 82% for 2005, 70% for 2006, and 72% for 2007. However, samples were not obtained from all six study regions in 2005 and 2006. A QDFA of all year-classes combined produced an overall classification accuracy of 70%, thus indicating that region-specific otolith chemical signatures from adjacent sampling years could be used as surrogates for regions where samples were not obtained in a given year.

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The U.S. Gulf of Mexico (Gulf) stock of red snapper *Lutjanus campechanus* has been estimated as overexploited since the 1980s, with the major sources of fishing mortality being commercial and recreational fisheries as well as bycatch mortality caused by Gulf shrimp trawling (SEDAR 2005). Although it is estimated that the red snapper stock in the U.S. Gulf is no longer undergoing overfishing (SEDAR 2009), fishery managers are still tasked with balancing these three sources of fishing mortality and rebuilding the stock by 2032 (GMFMC 2010). Genetic evidence has failed to reject the null hypothesis that Gulf red snapper constitute a single panmictic stock (Camper et al. 1993; Pruett et al. 2005; Gold and Saillant 2007); however, demographic differences in red snapper size at age, maturation rates, and genetic effective population size occur across the northern Gulf (Fischer et al. 2004; Saillant and Gold 2006; Jackson et al. 2007). In fact, catch statistics suggest that there are two centers of stock abundance: one in the northwestern Gulf off the coast of Louisiana and a smaller one off the coasts of Alabama and northwest Florida (Goodyear 1995). Based upon these findings, the red snapper population has been categorized into eastern and western substocks, which are divided by the Mississippi River (SEDAR 2005). However, the information is recombined to estimate a Gulf-wide annual catch limit, and little is known about patterns of mixing between substocks.

An understanding of larval exchange rates and population connectivity of marine organisms is crucial to the analysis of marine population dynamics and management of fishery stocks (Cowen et al. 2000). Larval dispersal can be difficult to study, although based upon shelf currents, Johnson et al. (2009) estimated that red snapper larvae could be carried 480 km during the 4-week planktonic stage. However, only a small percentage of the western substock larvae were projected to cross the Mississippi River plume; in such an event, the larvae would most likely be transported away from the continental shelf and would have a low probability of survival. Conditions might be more favorable for western transport of larvae produced by the eastern substock (Johnson et al. 2009).

Conventional tagging has been used to examine postsettlement movement of juvenile and adult red snapper. Estimates of red snapper site fidelity range from 25% to 60% per year (Patterson and Cowan 2003; Schroepfer and Szedlmayer 2006; Strelcheck et al. 2007); several tagged fish have been recovered more than 100 km from the original tagging location, but only one fish (out of >1,000 recaptures) tagged in the eastern Gulf has been recaptured west of the Mississippi River mouth (Patterson et al. 2001; Strelcheck et al. 2007; Addis et al. 2008). However, problems associated with conventional tagging, such as external tag loss and low reporting rates, may result in the underestimation of red snapper movement (see Patterson 2007 for a review).

The use of otoliths as natural tags has become a popular tool among fishery scientists to distinguish fish from distinct nursery areas and to examine movement patterns of adults (Gillanders and Kingsford 1996; Thorrold et al. 1997). Otoliths

are calcium carbonate structures located within the acoustico-lateralis system of teleost fish. Otoliths are acellular, are metabolically inert, and precipitate as the fish grows, which allows elements and stable isotopes that are accreted onto the growing surface to be permanently retained (see Campana 1999 for a review). Therefore, elements that are deposited during the juvenile phase can act as natural markers for the nursery area of origin. The ability of otoliths to act as a natural tag has permitted the development of a more efficient technique for studying natal origin and population connectivity than conventional tagging methods as a result of the large number of fish that must be tagged to produce a useful number of tag returns. However, chemical signatures in otoliths can differ among years due to temporal variability in water mass characteristics and elemental composition (Gillanders and Kingsford 2000; Rooker et al. 2001), thus requiring cohort-specific signatures to be identified.

While the majority of marine studies utilizing otolith chemistry to estimate natal origin have focused on estuarine and nearshore nursery habitats (Thorrold et al. 1998; Gillanders and Kingsford 2000; Dorval et al. 2005), this technique also has been successfully used to identify nursery origins of highly migratory pelagic species (Rooker et al. 2001). In fact, otolith chemistry has been utilized previously to examine temporal and spatial variability in otolith elemental signatures of northern Gulf red snapper. Patterson et al. (2008) were able to distinguish among three nursery regions of the northern Gulf by using elemental signatures, with mean classification accuracy of 80% for four of the five cohorts examined. Elemental variability in otolith chemical signatures was attributed to hydrologic and oceanographic differences among regions. However, these same oceanographic processes may have been the source of poor discrimination in one of the cohorts examined. Thorrold et al. (1998) reported an improvement in accuracy of weakfish *Cynoscion regalis* classification to estuarine nurseries along the Atlantic coast when stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) ratios were combined with trace elemental data. Furthermore, Rooker et al. (2008) were able to determine the nursery of origin for Atlantic bluefin tuna *Thunnus thynnus* over a larger spatial scale by using only otolith  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  ratios. Thus, the addition of stable isotope ratios to otolith elemental signatures may increase the accuracy of nursery origin classifications for Gulf red snapper.

The purpose of this study was to examine otolith chemical signatures in age-0 red snapper from six regions within the Gulf. Specifically, natural tags derived from otolith element : Ca ratios and stable isotope ratios (collectively, “constituents”) were used to discriminate red snapper nursery regions on the continental shelf of U.S. and Mexican portions of the Gulf. Region-specific nursery signatures were developed for three consecutive year-classes to determine whether discriminant classifications were strong enough to validate the use of nursery signatures for estimating the source of recruits to Gulf regions.

**METHODS**

*Sample collection.*—Age-0 red snapper representing the 2005–2007 year-classes were sampled from six regions in the Gulf (Figure 1): the eastern Gulf along the west Florida shelf (EG); north-central Gulf (NCG); northwestern Gulf (NWG); southwestern Gulf (SWG); southern Gulf shelf between Tampico and Veracruz, Mexico (MEX1); and Campeche Banks (MEX2). Boundary lines between NCG, NWG, and SWG follow the delineation detailed by Patterson et al. (2008). The objective was to collect 30 juveniles from each region for each year-class ( $n = 540$ ). Samples from NCG, NWG, and SWG were collected in the fall (October and November) during the National Marine Fisheries Service (NMFS) Fall Groundfish Survey using otter trawls deployed from either the *R/V Oregon II* or the *R/V Gordon Gunter*. Juvenile fish were subsampled from a trawl catch with systematic random sampling, which targeted fish smaller than 150 mm TL. Immediately after selection, the fish were placed in plastic bags and frozen. Upon arrival at the dock, fish were transferred to the Fisheries Laboratory at the University of West Florida (UWF) for processing.

The collection of samples from EG, MEX1, and MEX2 was opportunistic and difficult to achieve. Juvenile red snapper from EG were collected in the fall of 2005 and 2007 from observers employed by the Gulf and South Atlantic Fisheries Foundation and the NMFS Galveston Laboratory, respectively. Red snapper were stored in plastic bags, frozen, and transported to UWF. Juvenile red snapper from the MEX1 and MEX2 regions were collected in the winter (December–March) of 2007 and 2008

from shrimp trawl bycatch. Fish TL was measured and otoliths were extracted before samples were shipped to Louisiana State University (LSU) for further processing. Unfortunately, samples were not obtained from the MEX1 or MEX2 regions for the 2005 year-class or from the EG region for the 2006 year-class (Figure 2).

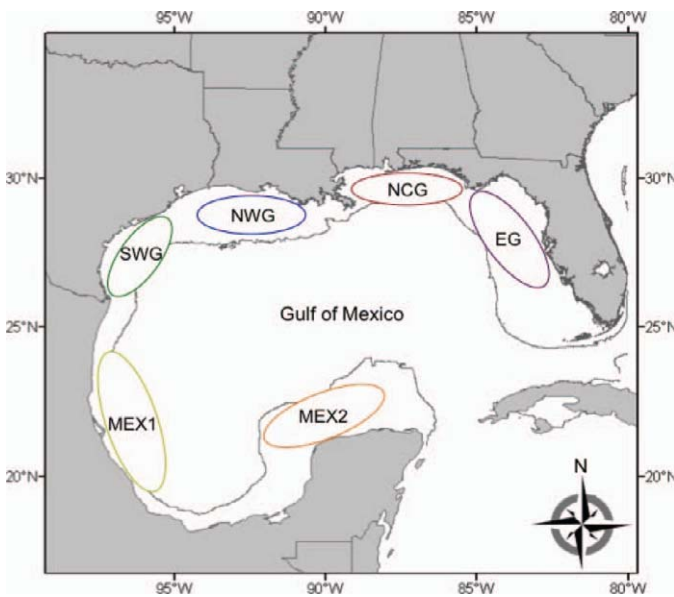


FIGURE 1. Nursery regions along the continental shelf of the Gulf of Mexico (Gulf), where age-0 red snapper representing the 2005–2007 year-classes were sampled. The 200-m depth contour indicates the continental shelf edge (regions: EG = eastern Gulf; NCG = north-central Gulf; NWG = northwestern Gulf; SWG = southwestern Gulf; MEX1 = Mexico region 1; MEX2 = Mexico region 2).

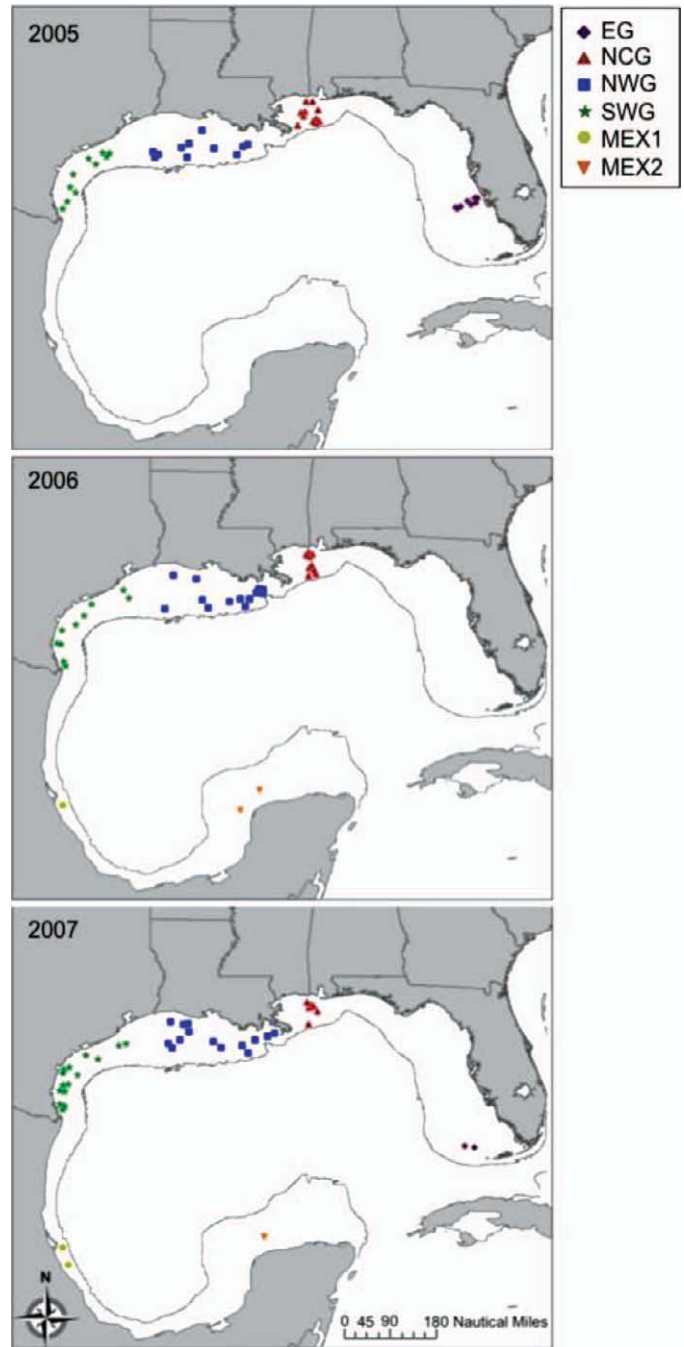


FIGURE 2. Specific locations where age-0 red snapper were sampled during fall 2005–2007. The 200-m depth contour indicates the continental shelf edge. Nursery region codes are defined in Figure 1.

Frozen age-0 fish that were collected within U.S. Gulf waters were thawed in the laboratory at UWF; weight was determined to the nearest 0.01 g, and TL was measured to the nearest millimeter. Sagittal otoliths were removed with glass probes and polyethylene tweezers; all materials that came into contact with extracted otoliths were acid-leached and triple-rinsed with double-deionized water (DDIH<sub>2</sub>O; ultrapure, 18-M $\Omega$ /cm water). Sagittae were cleaned with a synthetic-bristle brush to remove any adhering tissue, were rinsed with DDIH<sub>2</sub>O, and were placed in polyethylene vials to air-dry under a class-100 clean hood.

*Otolith preparation and analysis.*—Both right and left otolith samples were cleaned prior to elemental or stable isotope analysis under class-100 clean hoods in the laboratory at either UWF or LSU. Before and after cleaning, dry otoliths were weighed to the nearest 0.01 mg. Whole otoliths were immersed in a 1% solution of ultrapure nitric acid (HNO<sub>3</sub>) for 30 s to oxidize any material that was adhering to the surface; the solution was then flooded with DDIH<sub>2</sub>O to remove the acid. Otoliths were dried under a class-100 clean hood for at least 24 h.

All right otoliths were prepared for elemental analysis at UWF. Otoliths were dissolved in high-density polyethylene vials by adding 1% ultrapure HNO<sub>3</sub> until a dilution factor of approximately 1,000-fold was achieved. Although total dissolution typically occurred within 1 h, samples were not manipulated for at least 24 h once acid digestion began. Aliquots (5 mL) of otolith solutions were sent to the University of Southern Mississippi for trace elemental analysis with a Thermo Fisher Element2 sector field (SF) inductively coupled plasma (ICP) mass spectrometer. Otolith solutions were spiked with indium at a concentration of 2 ng/mL as an internal standard and then were analyzed for <sup>137</sup>Ba, <sup>48</sup>Ca, <sup>7</sup>Li, <sup>55</sup>Mn, <sup>25</sup>Mg, and <sup>88</sup>Sr. Three of the elements (Li, Mn, and Ba) were determined in low resolution, while the other elements were determined in medium resolution. Blanks were prepared from 1% ultrapure HNO<sub>3</sub> and were processed through the same stages of sample preparation as the otolith sample solutions. Blanks were analyzed concurrently with otolith sample solutions to determine instrument limits of detection, which were estimated as three SDs of mean blank values. Instrument performance and matrix effects were checked by assaying elemental concentrations of an otolith standard reference material (SRM) that was prepared from adult red snapper otoliths (Sturgeon et al. 2005). Solutions of the SRM were prepared and analyzed in a manner similar to that used for age-0 red snapper otolith samples.

All left otoliths were prepared for stable isotope analysis at LSU. Otoliths were pulverized with an agate mortar and pestle, and the resulting otolith powder was transferred into 2-mL microcentrifuge tubes. Subsamples (>1 mg) of homogenized, pulverized otoliths were sent to the Stable Isotope Laboratory (Department of Geology, University of California, Davis) for stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) analysis with a Finnigan MAT 251 isotope ratio mass spectrometer. The instrument was calibrated against the International Atomic Energy Agency's carbonate

TABLE 1. Sample size and size range of age-0 red snapper (2005–2007 year-classes) collected from six nursery regions in the Gulf of Mexico (Gulf; regions: EG = eastern Gulf; NCG = north-central Gulf; NWG = northwestern Gulf; SWG = southwestern Gulf; MEX1 = Mexico region 1; MEX2 = Mexico region 2).

Year-class	Region	Sample size	Size range (mm TL)
2005	EG	20	76–106
	NCG	30	65–146
	NWG	30	83–150
	SWG	30	61–145
	MEX1		
	MEX2		
2006	EG		
	NCG	30	70–148
	NWG	30	71–149
	SWG	30	71–150
	MEX1	30	95–140
	MEX2	29	160–230
2007	EG	29	68–150
	NCG	30	65–146
	NWG	30	63–141
	SWG	30	53–149
	MEX1	22	75–220
	MEX2	30	60–230

standard, NBS-19. Accuracy of analytical runs was measured through routine analysis of a check standard that had been stringently calibrated against NBS-19. Method precision based on long-term monitoring of the NBS-19 standard was  $\pm 0.02\%$  for  $\delta^{13}\text{C}$  and  $\pm 0.06\%$  for  $\delta^{18}\text{O}$ . The isotopic composition of otoliths is reported in standard delta ( $\delta$ ) notation relative to the Vienna Pee Dee belemnite reference standard:

$$\delta_{\text{sample}} (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3,$$

where  $R$  represents the ratio of heavy to light isotope ( $^{13}\text{C}/^{12}\text{C}$  or  $^{18}\text{O}/^{16}\text{O}$ ).

*Statistical analysis.*—There were minor differences in fish size among regions and among years (Table 1). Thus, to examine relationships between constituent concentrations and otolith weight (used as an alternative for fish size), a correlation analysis was performed to test whether significant relationships existed within and among nursery regions and years. Significant relationships were detected for Ba, Mg, Mn, and C. To ensure that differences in fish size did not confound any regional differences in otolith chemical signatures, the size effect was removed by subtracting the product of otolith weight and common within-group linear slope from each observed concentration of Ba, Mg, Mn, and C.

Multivariate ANOVA (MANOVA) and two-way ANOVA were used to test for differences in otolith elemental and stable isotope signatures among regions and year-classes. Pillai's

trace was used as the test statistic because it is the most robust to violations of homogeneity of variance (Wilkinson et al. 1996). However, only the regions that were sampled during each year of the study (i.e., NCG, NWG, and SWG) were examined with the MANOVA and two-way ANOVA. To determine whether otolith chemical concentrations varied among year-classes within the regions surveyed, one-way ANOVAs were performed on mean elemental and stable isotope ratios for each region, with year-class as the random factor. Variance components from each ANOVA were included to evaluate the percentage of total variance in each constituent ratio that was caused by variation among year-classes within a region. Constituents that did not have homogeneous variance across year-classes were either  $\log_e$  transformed or subjected to reciprocal transformation, and statistical analyses were recalculated. However, the transformations did not alter the significance of ANOVA and MANOVA, and therefore only the results of the untransformed data are presented.

A stepwise discriminant analysis (SDA) was computed to determine which constituents were the most significant in discriminating among regions within year-classes. An SDA is used to find a set of the original quantitative variables that best discriminate samples among sites or groups. To test the ability of otolith chemical signatures to distinguish red snapper nursery regions, we performed year-class-specific quadratic discriminant function analyses (QDFAs) as well as a QDFA model in which all year-classes were combined. Jackknifed cross validation classification accuracies were computed to estimate the success of classification to respective regions by year-class and with all year-classes combined. A canonical discriminant analysis (CDA) was used to visualize region-specific multivariate otolith chemical signatures for each year-class and for all year-classes combined. A CDA is computed to determine (1) the best linear combination of quantitative variables for which the means of the groups are most different and (2) whether this difference varies by year-class. All statistical analyses were performed by using the Statistical Analysis System (SAS 2006) with a significance level  $\alpha$  of 0.05.

## RESULTS

In total, 430 age-0 red snapper collected from six nursery regions across the Gulf were analyzed for otolith chemical signatures (Table 1). For all samples, concentrations of the six elements (Ba, Ca, Li, Mn, Mg, and Sr) were at least two orders of magnitude above detection limits. The SRM samples were within 5% of certified values for elements analyzed with SF-ICP mass spectrometry. Chemical signatures differed significantly among nursery regions (MANOVA:  $F_{14, 512} = 9.8$ ,  $P < 0.001$ ) and among year-classes (MANOVA:  $F_{14, 512} = 11.8$ ,  $P < 0.001$ ), and the region  $\times$  year-class interaction was significant (MANOVA:  $F_{28, 1,032} = 6.4$ ,  $P < 0.001$ ). When only the NCG, NWG, and SWG regions were considered, most of the element: Ca and stable isotope ratios differed significantly (ANOVA:  $P \leq 0.05$ ) among nursery regions and among year-classes and

demonstrated a significant region  $\times$  year-class interaction. The exceptions were Mg:Ca for the region  $\times$  year-class interaction (ANOVA:  $P = 0.085$ ), Sr:Ca for the region effect (ANOVA:  $P = 0.356$ ), and  $\delta^{13}\text{C}$  for the year-class effect (ANOVA:  $P = 0.692$ ).

Mean concentrations of element : Ca and stable isotope ratios varied among nursery regions and among year-classes (Figure 3). When present, fish sampled from EG tended to have constituent values that were either lower or higher than the values for the other regions. The same was true of fish sampled from MEX2. In fact, for the 2007 year-class, samples from EG and MEX2 had similar element : Ca and stable isotope ratios, with the only exception being Li:Ca. Fish collected from SWG tended to have higher values for Mg:Ca and Mn:Ca across all year-classes, and their Sr:Ca steadily decreased across years. Overall, NCG and NWG tended to have similar constituent values for each year-class (Figure 3). Significant differences in otolith chemical signatures among year-classes varied among the regions (Table 2). The only constituent that showed significant interannual variation in samples from MEX2 was Mg:Ca, but the variability of this ratio only accounted for 16% of the total variance within this region. For NWG and MEX1, almost all constituents were significantly different among year-classes; the exceptions were Mg:Ca for NWG and Mn:Ca for MEX1. In addition, Mn:Ca did not display significant interannual variation for EG or MEX2. All regions except MEX2 showed significant year-class variation in  $\delta^{13}\text{C}$ , which accounted for 8–38% of the total variance within a region. The largest proportion of total variance was attributable to significant mean  $\delta^{18}\text{O}$  (29–81%), followed by Li:Ca (9–62%) and Mg:Ca (12–58%).

The SDA retained all element : Ca and stable isotope ratios for each year-class. Mean jackknifed classification accuracies of the QDFA models were 81.7% for 2005, 69.9% for 2006, 71.6% for 2007, and 70.3% for all year-classes combined (Figure 4). Combining all year-classes resulted in a slightly higher classification success than was observed for the 2006 year-class. The lowest classification success was for MEX1 samples from the 2007 year-class (36.4%), and the highest classification success was for EG samples from the 2005 year-class (100%). With the exception of the especially low classification success for 2007 MEX1 samples, red snapper collected from NWG typically had the lowest classification success for each year-class, and the majority of misclassifications were to the NCG region. Samples from NCG had the next-lowest classification success for each year-class, with misclassifications to NWG being most prevalent. For the 2007 year-class, which was represented in samples from all nursery regions, EG and MEX2 had the highest classification success, and misclassification error was similar for EG and MEX2.

The high classification success for the 2005 year-class was apparent in its CDA, and CDA plots generally showed a trend of overlap among chemical signatures for the NCG, NWG, and SWG nursery regions for all year-classes (Figure 5). The CDA plot from the model in which all year-classes were combined closely resembled the plot for the 2007 year-class

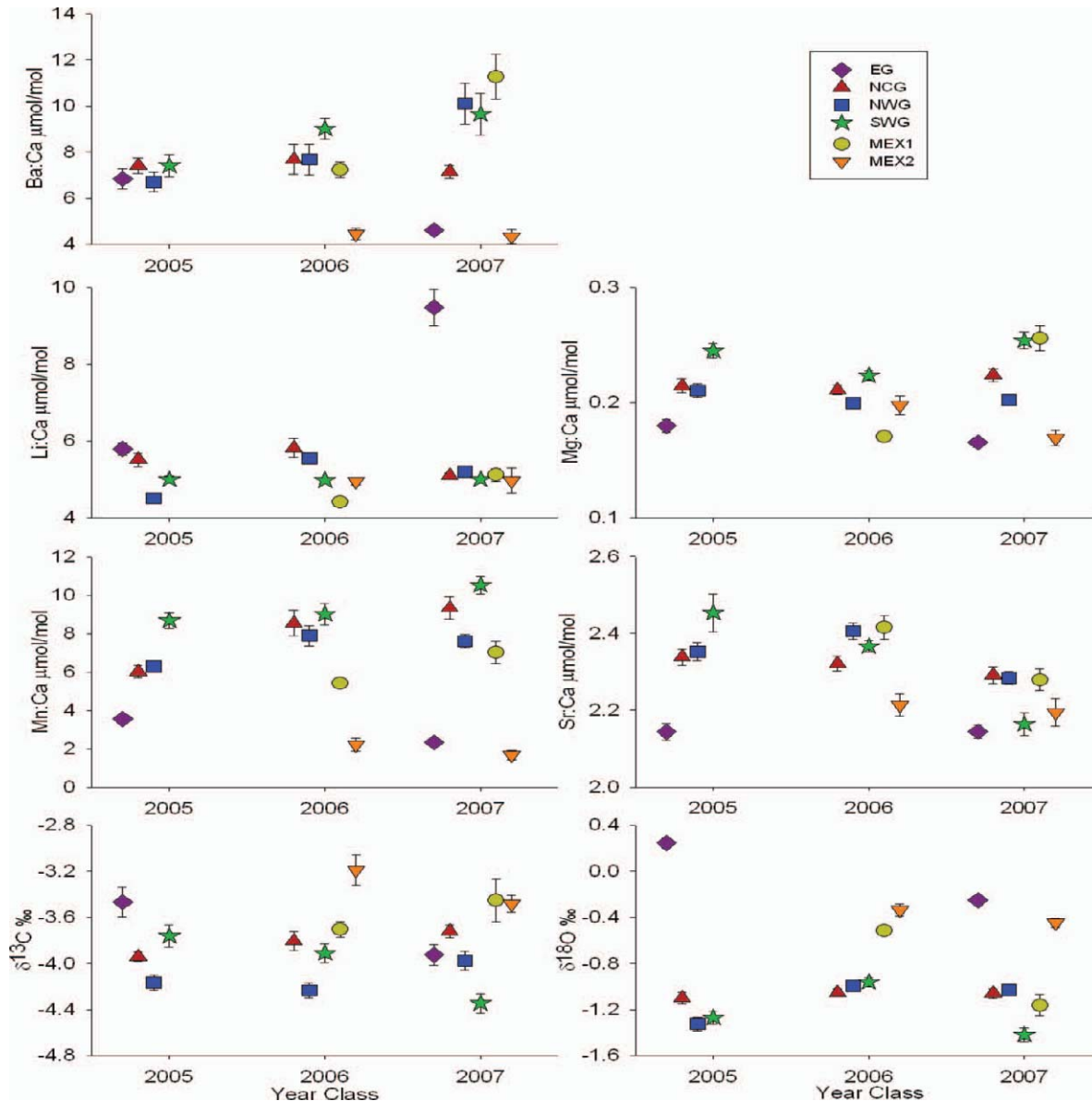


FIGURE 3. Mean ( $\pm$ SE) region- and year-class-specific otolith constituents for age-0 red snapper sampled from six Gulf of Mexico nursery regions in 2005–2007. Nursery region codes are defined in Figure 1.

(the only year-class that was sampled in all six nursery regions). Canonical structure coefficients are provided in Table 3 to demonstrate the importance of each elemental and stable isotope ratio in separating otolith chemical signatures among regions (as described by Walther and Thorrold 2008). The most notable trend was that the first canonical variate for each year-class was primarily influenced by  $\delta^{18}\text{O}$ , generally separating EG and MEX2 from the other regions (Figures 3, 5). For the 2005 year-class, the Mn:Ca ratio contributed to the high loadings on the second canonical variate, most likely reflecting the separation of SWG from the other three regions. However, for the other year-classes, Mn:Ca ratios began to contribute to

loadings on the first canonical variate, again reflecting the separation of EG and MEX2 from the other regions (Figures 3, 5). For the 2007 year-class, Li:Ca ratios contributed to separation along the second canonical variate, which appeared to correlate with the separation of EG from the other regions.

## DISCUSSION

The discriminant classifications of region-specific nursery signatures for the three consecutive year-classes studied validate the utility of natural otolith tags for estimating the source of recruits to Gulf regions and for examining red snapper

TABLE 2. Single-factor ANOVA results for year-class variation in otolith elemental and stable isotope ratios for age-0 red snapper collected from six Gulf of Mexico (Gulf) nursery regions (MS = mean square;  $\% \omega^2$  = variance component percentage of the total variance for each ANOVA; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ ).

Region or factor	df	Ba:Ca			Li:Ca			Mg:Ca			Mn:Ca			Sr:Ca			$\delta^{13}\text{C}$			$\delta^{18}\text{O}$		
		MS	F	$\% \omega^2$	MS	F	$\% \omega^2$	MS	F	$\% \omega^2$	MS	F	$\% \omega^2$	MS	F	$\% \omega^2$	MS	F	$\% \omega^2$	MS	F	$\% \omega^2$
Eastern Gulf																						
Year-class	1	31.99	16.85**	40	160.94	39.46***	62	$3.73 \times 10^{-6}$	0.01	4	0.83	1.75	3	$3.21 \times 10^{-6}$	0	4	3.72	15.46**	38	2.92	99.25***	81
Residual	47	1.90		60	4.08		38	$5.57 \times 10^{-4}$		96	0.48		97	$8.75 \times 10^{-3}$		96	0.24		62	0.03		19
North-central Gulf																						
Year-class	2	6.93	1.02	0	3.92	3.97*	9	$2.24 \times 10^{-4}$	0.26	2	91.26	11.58***	26	$1.70 \times 10^{-2}$	1.35	1	0.47	4.49*	10	0.02	0.34	2
Residual	87	6.82		100	0.99		91	$8.59 \times 10^{-4}$		98	7.88		74	$1.26 \times 10^{-2}$		99	0.11		90	0.05		98
Northwestern Gulf																						
Year-class	2	93.51	7.01**	17	8.51	19.17***	38	$1.22 \times 10^{-3}$	1.71	2	19.89	5.18**	12	$1.11 \times 10^{-1}$	8.49**	20	0.48	3.61*	8	0.98	13.45***	29
Residual	87	13.34		83	0.44		62	$7.12 \times 10^{-4}$		98	3.84		88	$1.31 \times 10^{-2}$		80	0.13		92	0.07		71
Southwestern Gulf																						
Year-class	2	36.81	3.06	6	$3.96 \times 10^{-3}$	0.02	3	$5.38 \times 10^{-3}$	5.27**	12	20.73	3.86*	9	$6.61 \times 10^{-1}$	18.65***	37	2.49	12.58***	28	1.62	20.48***	39
Residual	87	12.02		94	0.18		97	$1.02 \times 10^{-3}$		88	5.37		91	$3.55 \times 10^{-2}$		63	0.20		72	0.08		61
Mexico region 1																						
Year-class	1	116.62	11.5**	29	6.43	16.87***	38	$4.78 \times 10^{-2}$	35.87***	58	0.06	0.02	4	$2.35 \times 10^{-1}$	9.9**	26	2.37	6.38*	17	5.31	56.38***	69
Residual	50	10.14		71	0.38		62	$1.33 \times 10^{-3}$		42	3.65		96	$2.37 \times 10^{-2}$		74	0.37		83	0.09		31
Mexico region 2																						
Year-class	1	0.01	0	3	$3.58 \times 10^{-3}$	0	3	$1.03 \times 10^{-2}$	6.74*	16	2.64	0.96	0	$5.33 \times 10^{-3}$	0.17	3	1.40	4	9	0.18	2.64	5
Residual	57	2.37		97	1.74		97	$1.53 \times 10^{-3}$		84	2.74		100	$3.22 \times 10^{-2}$		97	0.35		91	0.07		95



population connectivity. Patterson et al. (2008) also demonstrated the potential for using otolith chemical signatures to discriminate among red snapper nursery regions, but those authors reported an overall higher mean classification success (80%) than was seen in the current study. However, Patterson et al. (2008) only examined three nursery regions (NCG, NWG, and SWG), whereas six regions were analyzed in the present study. The 2005 year-class had the highest classification success and the lowest number of regions sampled in the current study, whereas the 2007 year-class had the lowest classification success and the highest number of sampled regions. Thus, it would appear that the increase in regions resulted in a decrease in classification accuracy. However, misclassifications in the NCG, NWG, and SWG regions were mainly distributed among those regions, and removal of EG, MEX1, and MEX2 did not significantly alter the classification errors.

The circulation patterns of the Gulf can affect the way elements, stable isotopes, and nutrients are dispersed across the continental shelf. For instance, the Mississippi–Atchafalaya River system discharge, which accounts for 90% of the freshwater input into the Gulf (Rabalais et al. 1996), forms a stratified coastal current that usually flows westward along the Louisiana coast and can extend as far as the south Texas coast (Justić et al. 1995). In close proximity is the Mobile River basin (Alabama), which is the fourth-largest source of freshwater discharge in the nation (Warner et al. 2005). During autumn, winter, and spring, alongshore easterly winds create an exchange of river and shelf waters between the Louisiana–Texas and Mississippi–Alabama shelves, with maximum exchange occurring during northeast wind events (Walker et al. 2005). Thus, it is not surprising that otolith chemical signatures were similar among the NCG, NWG, and SWG regions. Although interannual differences were present, NCG and NWG samples tended to have similar ratios of Mg:Ca, Sr:Ca, and  $\delta^{18}\text{O}$ . Red snapper that were collected from SWG differed more than fish from the other two regions, mostly owing to higher ratios for Mg:Ca and Mn:Ca. Although not all of the present results are consistent with the results of Patterson et al. (2008), some similarities are evident. For instance, in both studies, the SWG samples had higher Mg:Ca than samples from the other two regions, and NWG samples had lower Mn:Ca, which was similar between NCG and SWG samples. Patterson et al. (2008) concluded that red snapper otolith chemical signatures were a reflection of oceanographic and hydrological differences among regions. Environmental parameters (e.g., salinity and temperature) and physiological regulations can affect the assimilation of elements into otoliths (see Elsdon and Gillanders 2003 for a review). Therefore, differences between the studies would be expected due to changes in water elemental concentrations, temperature, and salinity over time (as noted by Elsdon et al. 2008), whereas similarities are possibly caused by persistent hydrological and oceanographic processes within the NCG, NWG, and SWG regions.

Although red snapper samples from EG, MEX1, and MEX2 were not collected for all year-classes, when samples were avail-

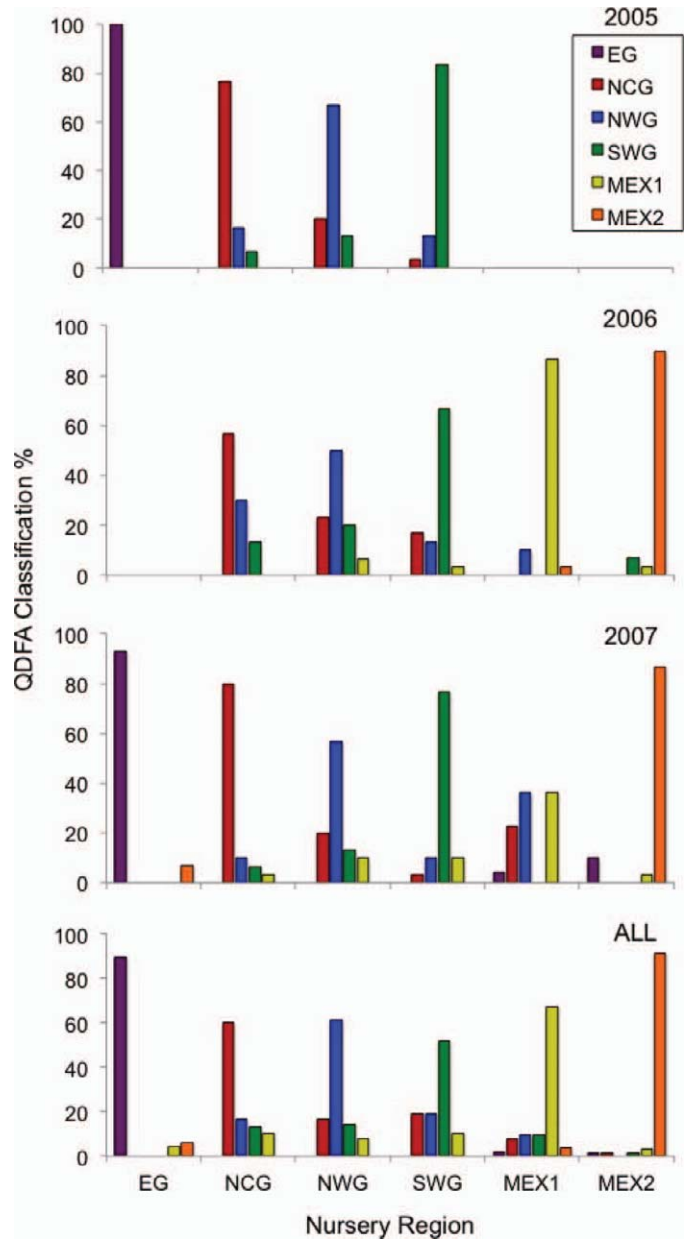


FIGURE 4. Jackknifed classification percentages estimated with quadratic discriminant function analysis (QDFA) of otolith chemical signatures from age-0 red snapper that were sampled in six Gulf of Mexico nursery regions during 2005–2007 (bar colors represent QDFA-assigned regions; x-axis shows the region of sample collection). “ALL” indicates all three year-classes combined. Nursery region codes are defined in Figure 1.

able these regions consistently had otolith chemical concentrations that differed from those of the NCG, NWG, and SWG regions. The exception was MEX1 samples, which tended to have concentrations similar to those of the NCG, NWG, and SWG samples, primarily for the 2007 year-class. The southern Gulf coastal waters are influenced by river runoff from the Grijalva–Usumacinta River system, which produces the second-largest volume of freshwater discharge into the Gulf (Signoret

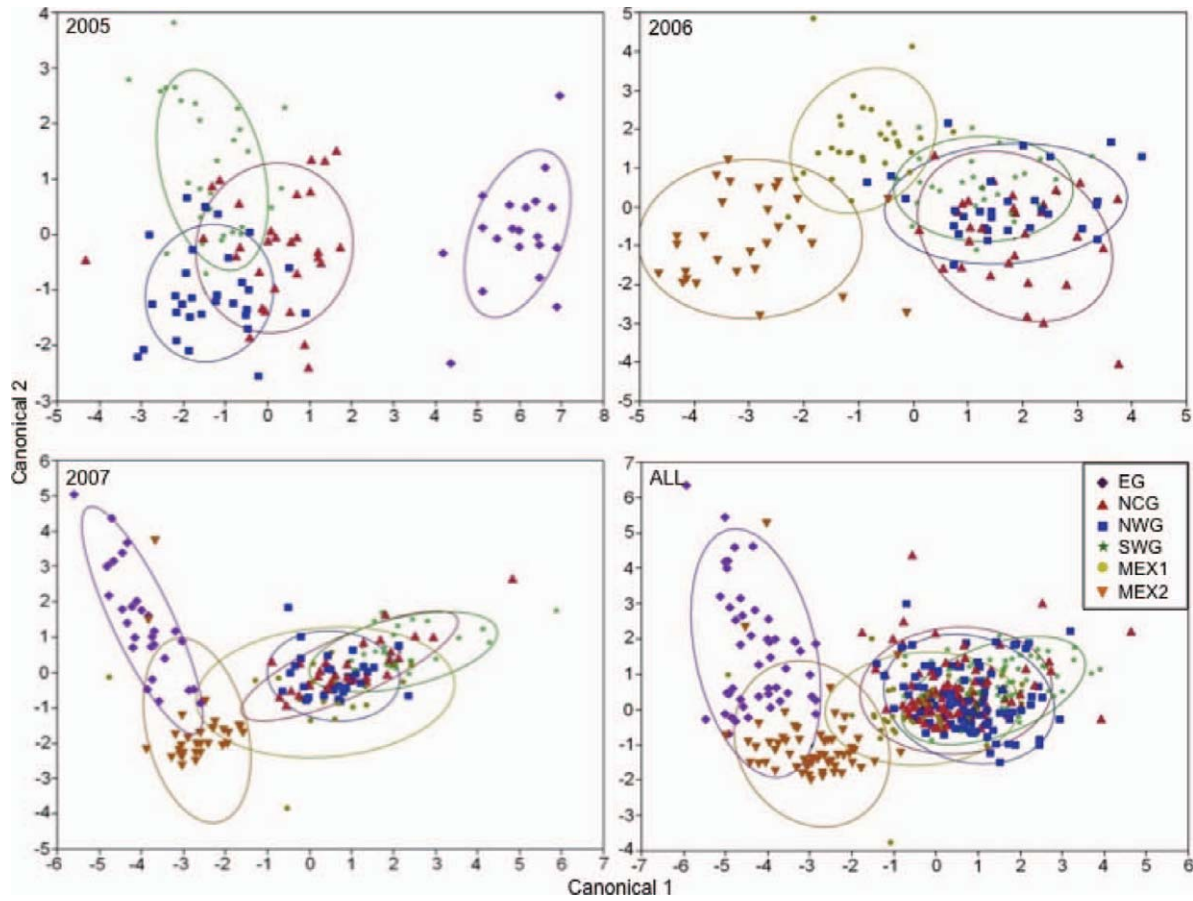


FIGURE 5. Canonical plot scores of otolith chemical signatures for age-0 red snapper (2005–2007 year-classes) sampled from six nursery regions in the Gulf of Mexico (canonical = canonical variate). Ellipses indicate 95% confidence levels; “ALL” indicates all three year-classes combined. Nursery region codes are defined in Figure 1.

et al. 2006). The river plume is displaced westward towards the Tamaulipas–Veracruz (TAVE) shelf; this displacement is caused by a westward coastal current. In the spring and summer, there is an upcoast current on the TAVE shelf that reaches the southern Texas continental shelf, where it encounters a down-

coast current favoring offshore transport. The TAVE shelf current reverses to a downcoast current during the fall and winter and extends to the southern Bay of Campeche, where it meets an opposing alongshelf current, generating seasonal offshore transport. The reversal of the current allows water from the

TABLE 3. Total canonical structure coefficients (canonical variates [var] 1 and 2) for discriminant function analyses comparing region- and year-class-specific otolith chemical signatures of age-0 red snapper for the 2005–2007 year-classes.

Constituent	2005		2006		2007		All year-classes	
	Var 1	Var 2	Var 1	Var 2	Var 1	Var 2	Var 1	Var 2
Ba:Ca	0.488	−0.133	0.252	0.185	−0.038	−0.240	−0.048	0.235
Li:Ca	0.311	0.367	0.365	−0.840	−0.658	1.251	−0.527	0.983
Mg:Ca	−0.143	0.380	−0.128	−0.107	0.110	0.225	0.013	0.154
Mn:Ca	−0.556	1.036	0.680	0.693	1.041	0.751	0.888	0.574
Sr:Ca	−0.303	0.163	−0.084	0.569	0.131	0.075	0.283	−0.014
$\delta^{13}\text{C}$	0.356	0.851	−0.500	−0.426	−0.239	−0.311	−0.357	−0.231
$\delta^{18}\text{O}$	2.078	0.489	−1.243	0.810	−1.059	0.017	−1.095	0.333

Mississippi and Atchafalaya rivers to reach the TAVE shelf (Zavala-Hidalgo et al. 2003). Hence, the high freshwater inflow from the Grijalva and Usumacinta rivers, along with seasonal inflow from the Mississippi–Atchafalaya River system, likely contributes to the similarities between samples from MEX1 and samples from the NCG, NWG, and SWG regions.

Prevailing upwelling winds cause circulation on the western Campeche Bank to flow westward along the coast throughout the entire year (Zavala-Hidalgo et al. 2003). These circulation patterns likely prevent mixing between MEX1 and MEX2 coastal waters, as evidenced by the differences in otolith chemical signatures between the two regions. The EG and MEX2 region samples were greatly enriched in  $\delta^{18}\text{O}$  compared with samples from the other nursery regions. Evaporation and freshwater input can alter  $\delta^{18}\text{O}$  values, resulting in heavier isotopes being associated with seawater and lighter isotopes being deposited into freshwater systems via precipitation (Lenanton et al. 2003). Since lighter isotopes are associated with freshwater, the present trend in  $\delta^{18}\text{O}$  most likely reflects the riverine influence on the NCG, NWG, SWG, and MEX1 regions. Furthermore, EG and MEX2 samples had lower Ba:Ca values. Barium follows a nutrient-type profile, with higher concentrations in riverine and near-coastal waters (Thorrold et al. 1997); thus, our Ba:Ca results further confirm the dominant riverine influence on the NCG, NWG, SWG, and MEX1 regions. Another notable difference was that EG and MEX2 had significantly lower Mn:Ca than the other regions. Hanson et al. (2004) reported that the Mn concentration in otoliths of gags *Mycteroperca microlepis* increased with latitude, corresponding to the same trend in coastal sediment Mn concentration along the west Florida shelf. Thus, latitudinal differences, an absence of heavy freshwater input, and a lack of water mixing as a result of circulation patterns likely contributed to the distinctness of EG and MEX2 chemical signatures relative to those of the other regions.

The efficacy of using otolith chemical concentrations as natural tags is partially dependent upon the temporal stability of the signature. Studies of the temporal stability of otolith chemical signatures have shown either differences between two consecutive years (Patterson et al. 1999; Gillanders and Kingsford 2000) or negligible differences over 2-year intervals, with drastic changes occurring after 4–13 years (Campana et al. 2000). Even though interannual differences are present, studies have shown that separation patterns among regions can still be similar and that regional differences are the cause of variability in otolith chemical signatures (Edmonds et al. 1992; Campana and Gagné 1995). Although thorough statistical testing of temporal stability was not possible in this study because of the unbalanced design, the NCG, NWG, and SWG regions demonstrated significant year-class differences, which were also reported by Patterson et al. (2008). However, it is interesting to note that when otolith chemical concentrations were combined for all three year-classes, the classification success for the combined year-classes was not much lower than that observed for the 2007 year-class and was a slight improvement over that observed

for the 2006 year-class. Therefore, although development of cohort-specific otolith chemical signatures would be appropriate for Gulf red snapper, the unbalanced design of this study suggests that it may be worthwhile to examine the usefulness of a signature developed from all three year-classes combined.

The results of this study indicate that element : Ca and stable isotope ratios can be used as natural tags to distinguish age-0 red snapper originating from different Gulf nursery regions. The ultimate goal of this research is the application of these natural tags to estimate the source of recruits among Gulf regions. Specifically, more work should be undertaken to (1) estimate the source of recruits to the west Florida shelf, (2) examine the connectivity between populations of the western Gulf and northeast Mexico, and (3) further explore mixing dynamics between populations east and west of the Mississippi River. The use of natural tags to study postsettlement movement and population connectivity could be beneficial to the management and recovery of red snapper stocks.

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