Otolith Microchemical Fingerprints of Age-0 Red Snapper, *Lutjanus campechanus*, from the Northern Gulf of Mexico

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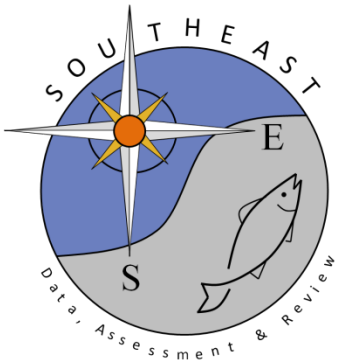

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Otolith Microchemical Fingerprints of Age-0 Red Snapper, *Lutjanus campechanus*, from the Northern Gulf of Mexico

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Red snapper, *Lutjanus campechanus*, in the northern Gulf of Mexico (Gulf) are believed to constitute a single stock. However, tagging and genetics studies suggest there is little mixing between populations of red snapper in the northern Gulf, and little is known about mixing rates of adult fish. The long-term goal of our work is to determine if age-0 red snapper from different nursery areas have unique microchemical fingerprints in their sagittal otoliths, and if so, can the microchemical fingerprints at the core of adult otoliths be used to determine retrospectively nursery area of origin. Ultimately, we hope to use the microchemical fingerprints at the core of adult snapper otoliths to estimate adults' mixing rates and movement patterns. In this study, the objective was to determine if age-0 red snapper collected from different northern Gulf nursery areas in summer and fall 1995 did contain unique microchemical fingerprints. Sagittal otoliths of age-0 red snapper collected off the coasts of Alabama/Mississippi, Louisiana, and Texas were analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES). Twelve elements in the sagittae of age-0 snapper were analyzed with ICP-AES. Of these, eight were put into a stepwise discriminant function analysis with the best-fitted model including Mg, Se, As, Fe, and Al, entered in that order (MANOVA, $P < 0.001$). Cross-validated classification accuracies were 92% for Texas fish, 91% for Louisiana fish, and 92% for Alabama/Mississippi fish. Therefore, it appears that otolith microchemistry can be used to infer nursery area of age-0 red snapper. Future work will focus on (1) establishing the temporal stability of age-0 red snapper otolith microchemical fingerprints and (2) inclusion of analyses of age-structured samples from adult red snapper otolith cores to estimate their nursery area of origin and mixing rates.

Red snapper, *Lutjanus campechanus*, are demersal reeffish that are distributed along the continental shelf throughout the Gulf of Mexico (Gulf). Young red snapper spend most of their first year of life over sand and mud bottom on the shrimping grounds in the northern Gulf, after which they move offshore to reef environments. Adult snapper are found in deeper offshore waters near coral, rocks, banks, outcrops, and manmade submerged objects such as oil and gas platforms and artificial reefs (Moran, 1988; Workman and Foster, 1994).

Historically, red snapper have supported important commercial and recreational fisheries in the Gulf. Fisheries management for this species currently operates under the paradigmatic assumption that red snapper constitute one homogenous or unit stock (Goodyear, 1995). While this assumption is paramount to almost every fisheries management plan (Ricker, 1975; Gulland, 1977), tagging and population genetics studies have shown that there may be little, if any, mixing between adjacent red snapper populations in the Gulf; the life history stage at which mixing may occur is unknown (Beaumariage, 1969; Fable, 1980; Camper et al., 1993; Szedlmayer and Shipp, 1994; Chapman et al., 1995; Gold et al., 1997; Szedlmayer, 1997). Nevertheless, it is hypothesized that while young adult snapper are relatively stationary, large adults found on offshore reef sites may make long migrations (Beaumariage, 1969; Goodyear, 1995). Management of Gulf red snapper is based on this hypothesis; however, due to the lack of adequate natural or practical artificial markers, this hypothesis remains untested.

The ultimate goal of our research is to quantify movement and mixing rates of adult red snapper in the Gulf by using otolith microchemical fingerprints as tags of snapper from different geographic regions. Traditionally, otoliths (ear stones) have been used as a hardpart with which to age fish, but they also contain natural biogeochemical markers of fish populations (Campana and Gagne, 1995; Campana et al., 1995). Otoliths are calcium carbonate (aragonite) and protein matrices that are formed as a fish grows (Casselman, 1987) and

are metabolically inert once formed (Campana and Neilson, 1985; Mugiya et al., 1991). Within the matrix of an otolith are trace elements that are derived from the water in which a fish lives (Simkiss, 1974; Kalish, 1989; Campana et al., 1994; Campana and Gagne, 1995). Because trace elemental composition of seawater varies between water bodies (Johnson et al., 1992; Campana and Gagne, 1995), the elemental composition of otoliths reflects the elemental composition of the water body of residence. Therefore, analysis of otolith chemistry has allowed researchers to differentiate fish from separate populations or stocks, even those within relatively close geographic proximity to one another (Edmunds et al., 1989, 1991, 1992, 1995; Kalish, 1990; Gunn et al., 1992; Sie and Thresher, 1992; Campana et al., 1994; Thresher et al., 1994; Campana and Gagne, 1995).

Because otoliths are metabolically inert once formed and accrete throughout the life of a fish, the microchemical fingerprint derived from the nursery habitat of age-0 fish will remain with fish throughout life. Several studies have employed this idea to determine retrospective nursery habitat of adult fish by analyzing the core of adult otoliths (e.g., Gillanders and Kingsford, 1996; Kalish, 1990; Rieman et al., 1994; Secor, 1992; Secor et al., 1995; Secor and Piccoli, 1996). The long-term goal of our work is to use otolith microchemical fingerprints of age-0 red snapper from historically important snapper nursery areas in the northern Gulf of Mexico as natural biogeochemical tags of adult fish found on offshore reefs. If successful, this approach will allow us to estimate from which nursery areas year classes of Gulf red snapper were derived, as well as allow us to look at movement and mixing of red snapper populations in the Gulf on large temporal and spatial scales. To achieve this long-term goal, this initial study was conducted to determine if age-0 snapper otoliths from different nursery areas in the northern Gulf of Mexico contain unique microchemical fingerprints. Our approach was to collect age-0 snapper from historically important nursery areas and to analyze the microchemistry of whole dissolved sagittal otolith samples from the age-0 fish using inductively coupled plasma–atomic emission spectrometry (ICP-AES). Lastly, univariate and multivariate statistical techniques were employed to determine the otolith microchemical fingerprints of age-0 red snapper from different nursery areas.

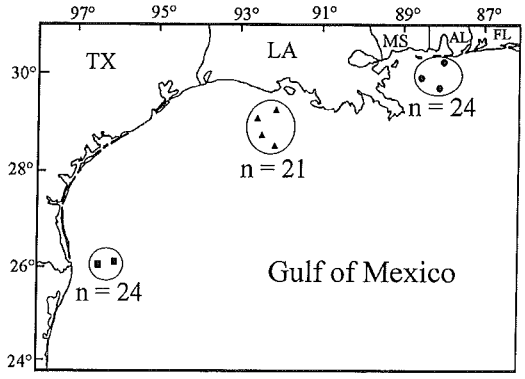

Fig. 1. Map of Gulf of Mexico showing age-0 red snapper sampling sites and sample sizes.

Materials and Methods

Age-0 red snapper collection.—In the summer and fall of 1995, age-0 red snapper were collected from offshore Alabama/Mississippi (n = 24, depth = 15–31 m), southwest Louisiana (n = 21, depth = 22–57 m), and south Texas (n = 24, depth = 46–49 m) nursery areas (Fig. 1). Collections were made cooperatively via the National Oceanographic and Atmospheric Administration (NOAA) Summer SEAMAP survey, the National Marine Fisheries Service Fall Groundfish Survey, and the Dauphin Island Sea Lab. All fish were collected using otter trawls and frozen shortly after collection.

Otolith sample preparation.—In the laboratory, fish were thawed, measured to the nearest mm total length (TL), and weighed to the nearest 0.2 g. Both sagittae were removed from each fish using glass probes. Otoliths always were handled with acid-washed glass probes or Teflon forceps to avoid contamination. Once removed, otoliths were scrubbed with a synthetic bristle brush and rinsed with Milli-Q water (18 MΩ polished water) to remove any adhering tissue. Otoliths were air dried in a laminar flow cabinet and placed in acid-washed high-density polypropylene vials. Sample preparation for ICP-AES took place under class-100 clean hoods. Otoliths were placed in baths of 3% ultrapure hydrogen peroxide in Milli-Q water and ultrasonically cleaned for 15 min; this process oxidized and removed any remaining tissue or surface contamination. After ultrasonic cleaning and rinsing with Milli-Q water, cleaned otoliths were air dried, weighed to the nearest 1×10^{-5} g, and dissolved in 10% ICP-grade HNO_3 at 0.2 ml 10% HNO_3 per mg otolith.

TABLE 1. ICP-AES operating parameters during analysis of age-0 red snapper otolith microchemistry.

	ICP-AES operating parameters
Power	0.46 DC amperes
Coolant	4.75 psi Ar, flow rate 14 L/min
Auxilliary	5 psi, flow rate 0.2 L/min
Nebulizer	42 psi, flow rate 0.4 L/min
Pump	1.2 ml/min

TABLE 2. ICP-AES limits of detection for 12 elements considered in analysis of age-0 red snapper otolith microchemistry.

Element	Detection limit (ppm)	Element	Detection limit (ppm)
Al	0.0519	mg	0.001
As	0.0556	Mn	0.001
Ba	0.001	Na	0.002
Ca	0.0016	Se	0.295
Fe	0.001	Sr	0.001
K	0.2406	Zn	0.0013

Chemical analysis.—All dissolved otolith samples were analyzed using a Leeman Labs Plasma Spec III with both simultaneous and sequential multielement analysis capabilities (Table 1). The elements Al, As, Ba, Ca, Fe, K, Mg, Mn, Na, Se, Sr, and Zn in were detected in otolith solutions with the ICP-AES, all of which were well above detection limits of the instrument (Table 2). The ICP-AES was calibrated daily with a multielement standard from Leeman Labs containing these elements. Calibration updates were run when necessary and samples repeated until all fell between sets of acceptable standards. All standards were required to stay within ± 10% of known values with the exceptions of As, Se, K (all ± 15%), and Zn (± 20%). In practice, however, all standards were within ± 6% of known values, and most were within ± 2%. Samples were run undiluted for elements present in low concentrations and then diluted appropriately so that each analyte fell within its linear calibration range. Background subtraction was used for Al, Ca, K, and Zn. Integration time was 5 sec for each element. Data for all elements were obtained in simultaneous multielement mode using an inorganic torch, a modified Scott spray chamber, and Hildebrand grid nebulizer.

Because of the high concentration of Ca in the dissolved otolith samples, rinse-out time between samples was long. A regimen of rinsing with distilled water for 5 min, 25% aqua regia rinse for 5–10 min, followed by 2% ICP-grade HNO_3 for 10 min was established. The plasma then was scanned to be certain that it was rinsed clean of calcium. The injector tip of the torch and sample and drain tubings were visually monitored for buildup of salts. The torch and spray chamber were cleaned daily and pump tubing was replaced daily. Generally, only four or five samples and their dilutions could be run per day.

Statistical analysis.—Otolith elemental data obtained from ICP-AES analyses were analyzed statistically to determine the microchemical fingerprints from each nursery area. Because the red snapper collected were not the same size and (presumably) age, we first tested for ontogenetic effects on elemental concentrations. To test for differences in the relationship between total length and otolith weight between nursery areas an analysis of covariance (ANCOVA) was performed. Differences in otolith elemental concentrations between nursery areas were tested using analysis of variance (ANOVA) and Tukey's multiple comparison test on means. Lastly, unique fingerprints of nursery areas were determined by discriminant function analysis and multivariate analysis of variance (MANOVA), with the most influential elements as response variables.

RESULTS

To minimize the potential effect of fish size or otolith weight in our analyses, we attempted to sample age-0 snapper of similar size ranges from all three nursery areas. However, Texas (TX) fish sampled were significantly larger (mean TL ± SE = 130.38 ± 2.41 mm) than Alabama/Mississippi (AL/MS) fish (mean TL ± SE = 114.41 ± 3.90 mm) and Louisiana (LA) fish (mean TL ± SE = 110.91 ± 4.30 mm) (ANOVA, df = 2, MS = 5136.14, $F = 8.08$, $P = 0.048$). Otoliths from TX fish were also significantly larger (mean weight ± SE = 37.50 ± 2.41 mg) than otoliths from AL/MS fish (mean weight ± SE = 31.64 ± 2.13 mg) and otoliths from LA fish (mean weight ± SE = 30.48 ± 2.03 mg) (ANOVA, df = 2, MS = 500.84, $F = 3.19$, $P < 0.001$). Because otolith growth rates of larval and juvenile fish may effect otolith elemental microchemistry (Fowler et al., 1995; Thorrold et al., 1997), we tested for differences in the relationship between TL and otolith weight between nursery areas (Fig. 2) and found that there was no significant difference (ANCOVA test for homogeneity of

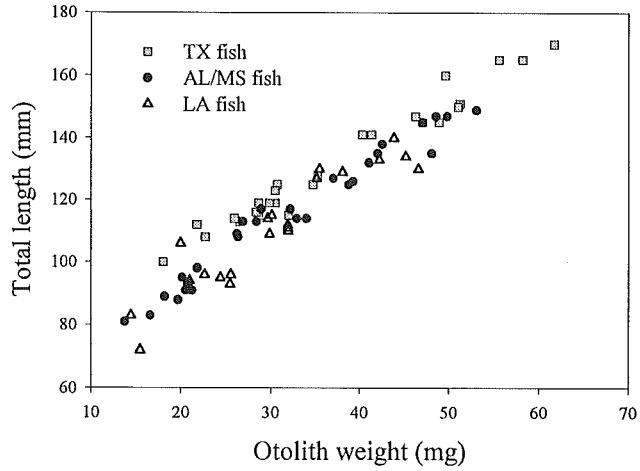

Fig. 2. Relationship between total length and otolith weight for age-0 red snapper sampled for analysis of otolith microchemical fingerprints.

slopes, df = 2, MS = 15.26, $F = 1.18$, $P = 0.3127$; ANCOVA test for equal intercepts, df = 2, MS = 29.65 $F = 1.63$, $P = 0.2331$). Based on the ANCOVA results, it was assumed that otoliths from fish from all three nursery areas were growing at similar rates despite differences in fish size (Thorrold et al., 1997).

Ten of the 12 elements detected in the otoliths were significantly different between nursery areas (ANOVA, $P < 0.05$) (Table 3). However, only Al, As, Ca, Fe, Mg, Mn, Se, and Zn were used in subsequent multivariate analyses to determine otolith microchemical fingerprints of the three nursery areas. K and Na were excluded because incorporation of these elements into accreting otoliths is most likely under physiologic control and thus may not result from true differences in water chemistry (Campana and Gagne, 1995; Edmonds et al., 1995; Kalish, 1989, 1991). For the eight remaining elements, there was no clear pattern in Tukey's groupings between nursery areas (e.g., no area was constistently low or high in otolith elemental concentrations relative to the others). These eight elements then were entered in a stepwise discriminant function analysis with the best-fitted model including Mg, Se, As, Fe, and Al entered in that order (Table 4).

The centroids of these five elements (Al, As, Fe, Mg, and Se) were significantly different between nursery areas (MANOVA, numerator df = 14, denominator df = 120, Wilk's lambda = 0.1375, $F = 14.55$, $P < 0.001$) (Fig. 3). Because TL and otolith weight were significantly different between nursery areas, correlation analyses were performed between otolith elemental concentrations and otolith weight to test for potential ontogenetic effects on otolith element concentrations. Pearson's product moment correlations were not significant between Al, Fe, and Mg and otolith weight. The correlations between As and Se and otolith weight were significant ($P < 0.05$); however, correlation coefficients were low for each element ($r = -0.35$ for As; $r = -0.27$ for Se), indicating weak and perhaps spurious relationships between these elements and otolith weight. Therefore, differences in Al, As, Fe, Mg, and Se otolith concentrations between nursery areas were perceived as true differences and not due to ontogenetic effects.

The discriminant function computed with Al, As, Fe, Mg, and Se as response variables and nursery area as the classification variable yielded clear discrimination in otolith microchemical fingerprints between nursery areas (Fig. 4). Classification accuracies based on the probability of an individual fish being correctly classified into the nursery area from which it was sampled were over 90% for each nursery area (Fig. 5). Of the misclassified fish, 8% of TX fish and 9% of LA fish were misclassified as AL/MS fish, and 4% of AL/MS fish were misclassified to TX and 4% to LA. Interestingly, no LA fish were misclassified as TX fish or vice versa.

Discussion

The results of the chemical and statistical analyses in this study show that 1995 age-0 red snapper in the northern Gulf of Mexico had otolith microchemical fingerprints unique to

TABLE 3. ANOVA table showing differences between nursery areas for each of the 12 elements considered in the analysis of age-0 red snapper otolith microchemistry using ICP-AES (note: $Ae^y = A \times 10^y$).

Source	df	SS	MS	F	$P > F$	Site	Mean concentration (ppm)	Tukey's grouping
Al								
Site	2	$2.648e^4$	$1.324e^4$	6.36	0.0030	TX	691.00	A
Error	66	$1.375e^5$	$2.083e^3$			LA	655.53	B
						AL/MS	646.20	B
As								
Site	2	$1.366e^3$	$6.814e^2$	6.87	0.0019	TX	93.43	A
Error	66	$6.544e^3$	$9.794e^1$			LA	104.51	B
						AL/MS	98.05	A
Ba								
Site	2	3.444	1.722	0.34	0.7162	TX	7.511	A
Error	66	$3.387e^2$	5.141			LA	8.058	A
						AL/MS	7.778	A
Ca								
Site	2	$2.904e^{10}$	$1.452e^{10}$	5.23	0.0078	TX	$3.903e^5$	A
Error	66	$1.833e^{11}$	$2.778e^9$			LA	$4.262e^5$	B
						AL/MS	$3.765e^5$	A
Fe								
Site	2	3.954	1.975	12.02	0.0001	TX	2.505	A
Error	66	$1.085e^1$	$1.644e^{-1}$			LA	1.999	B
						AL/MS	1.994	B
K								
Site	2	$6.882e^4$	$3.441e^4$	6.45	0.0028	TX	600.81	A
Error	66	$3.519e^5$	$5.333e^3$			LA	536.15	B
						AL/MS	608.30	A
Mg								
Site	2	6.453^{-2}	$3.225e^{-2}$	44.26	0.0001	TX	52.76	A
Error	66	$4.811e^{-2}$	$7.288e^{-4}$			LA	38.64	B
						AL/MS	41.11	B
Mn								
Site	2	$2.465e^1$	$1.232e^1$	6.06	0.0038	TX	6.686	A
Error	66	$1.342e^2$	2.036			LA	5.636	B
						AL/MS	5.313	B
Na								
Site	2	$4.292e^5$	5.259	4.16	0.0198	TX	$3.345e^3$	A
Error	66	$3.403e^6$	1.264			LA	$3.154e^3$	B
						AL/MS	$3.292e^3$	A
Se								
Site	2	$2.880e^5$	$1.440e^5$	23.83	0.0001	TX	472.96	A
Error	66	$3.988e^6$	$6.043e^3$			LA	612.30	B
						AL/MS	470.88	A
Sr								
Site	2	$7.375e^4$	$3.687e^4$	1.74	0.1838	TX	$1.833e^3$	A
Error	66	$1.400e^6$	$2.122e^4$			LA	$1.797e^3$	A
						AL/MS	$1.755e^3$	A
Zn								
Site	2	$6.978e^1$	$3.412e^1$	6.26	0.0033	TX	1.050	A
Error	66	$3.672e^2$	5.549			LA	3.555	B
						AL/MS	2.161	A and B

TABLE 4. Standardized discriminant function coefficients from stepwise discriminant function analysis of age-0 red snapper nursery area microchemical fingerprints.

Element	Standardized discriminant coefficients	
	Canonical variable 1	Canonical variable 2
Al	0.5388	−0.2772
As	−0.7044	−0.9154
Fe	0.6596	−0.1185
Mg	0.8892	0.9137
Se	−0.8500	1.7798

their nursery area. The success of this first attempt to distinguish age-0 red snapper from different nursery areas in the northern Gulf is demonstrated by clear discrimination between nursery areas resulting from discriminant function analysis of the otolith elemental fingerprints. The discriminant function classification accuracies being greater than 90% for all nursery areas is even more promising given that the analytical error of the ICP-AES for the elements detected in the otoliths was between 2 and 6%. Also, despite the relatively low precision of the ICP-AES, several elements when tested individually were significantly different between nursery areas.

Gillanders and Kingsford (1996) were able to classify age-0 wrasse, *Achoerodus viridis*, to reef nursery habitat (estaurine or rocky reef) with greater than 90% accuracy based on their otolith microchemistry. Then, they used a discriminant function derived from otolith elemental concentrations of age-0 wrasse to estimate the nursery habitat of adults based on otolith elemental concentrations in the cores of adult otoliths. Scatterplots of otolith elemental concentrations showed that some adults did not resemble either rocky reef or

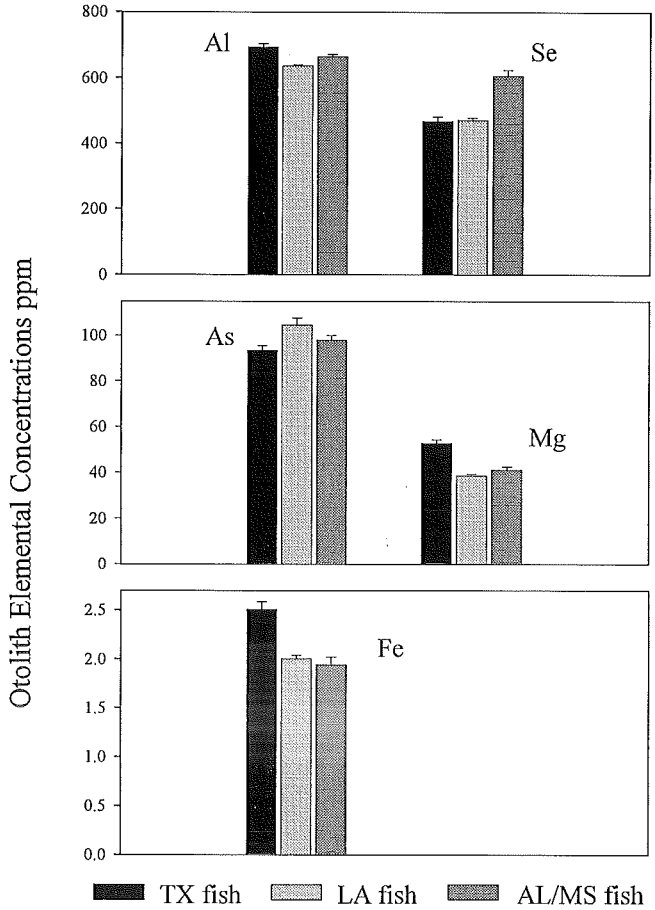

Fig. 3. Mean (± SE) otolith elemental concentrations of elements from the stepwise discriminant function analysis of age-0 red snapper otolith microchemical fingerprints.

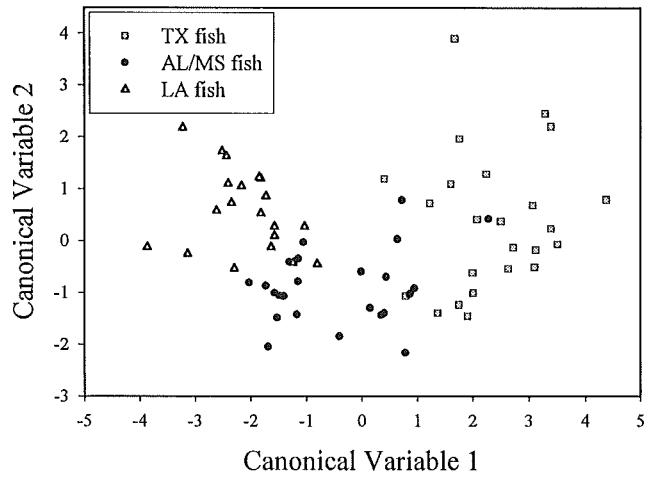

Fig. 4. Scatterplot of first and second canonical variables from discriminant function analysis of age-0 red snapper otolith microchemical fingerprints from each nursery area.

estuarine seagrass recruits, but algorithms used to classify the adults forced them into one of these two groups. Gillanders and Kingsford (1996) suggested that these results may have been attributable to using only one year class to estimate the microchemical fingerprints of the age-0 wrasse, and some potential nursery areas may not have been sampled. Despite this shortcoming, their study demonstrates the potential of using otolith microchemical fingerprints of age-0 fish from different nursery habitats to determine relative contributions of different nursery areas to year class strength. However, it also highlights the importance of ensuring that all potential nursery areas are sampled, as well as establishing the temporal stability of otolith microchemistry fingerprints.

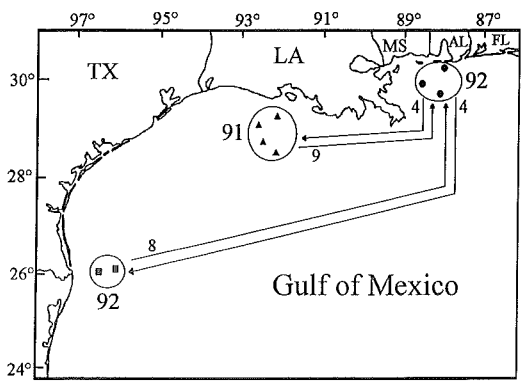

Fig. 5. Map of sampling sites showing classification accuracies and misclassification errors (arrows in direction of error) from discriminant function analysis of otolith microchemical fingerprints of age-0 snapper from each nursery area.

In this regard, some research using otolith microchemistry as a means to distinguish between fish stocks has examined the temporal stability of adult otolith microchemical fingerprints. Edmonds et al. (1995) showed that pink snapper, *Pagrus auratus*, and pilchard, *Sardinops sagax*, from southwest Australia had otolith microchemical fingerprints that differed between nursery areas; however, interannual variability in the otolith microchemical fingerprints was as great as that between sites in a given year. Most of the interannual differences were due to temporal variation in P and S concentrations, elements whose incorporation into otoliths is probably under physiologic control (Kalish, 1989, 1991). In contrast, Campana et al. (1995) reported that otolith microchemical fingerprints of adult cod *Gadus morhua* collected off of Canada in the northwest Atlantic showed only small interannual variation. They attributed the meager interannual differences to slight differences in environmental exposure of cod sampled in different years. The findings of Campana et al. (1995) illustrate that otolith microchemical fingerprints based on relatively heavy elements may better estimate true environmental signatures than those based on lighter elements that are presumably under physiologic control (Kalish, 1989, 1991). Therefore, otolith microchemical fingerprints based on heavier or trace elements may have a higher probability of demonstrating temporal stability, as well as demonstrating true environmental differences.

In estimating the otolith microchemical fingerprints of age-0 red snapper from the northern Gulf of Mexico, elements were excluded

from analyses that were felt more likely to be under physiologic control and not indicative of true environmental differences between nursery areas. The next step in establishing otolith microchemical fingerprints that can be used to estimate nursery area residency of adult snapper is to test for temporal stability in the fingerprints found in 1995 age-0 fish. Equally as important, efforts must be made to collect age-0 fish across a broader geographic area to increase the spatial resolution between areas sampled in 1995.

If otolith microchemical fingerprints of age-0 red snapper prove to be temporally stable, this technique may provide a tool by which to answer many questions about red snapper population biology and movement that heretofore have been unsolveable. If the core of adult otoliths can be analyzed successfully, estimation of the relative contribution of different nursery areas to snapper populations found on offshore reefs (both natural and artificial) throughout the northern Gulf will be possible. Also, microchemical analysis of otolith cores from progressively older fish collected from different offshore areas in the Gulf will allow estimation of long-term movement patterns and mixing rates of red snapper. This approach seems ideally suited for reeffish such as red snapper that spend some part of their early life history apart from the adult population, and then recruit to offshore reefs later in life. Moreover, estimation of fish movement rates and natural migration patterns, as well as source of recruits, is of paramount importance in artificial reef fisheries in which managers often are asked if artificial reefs affect production, or merely aggregate adults. In all of these potential applications, this powerful technique is superior to artificial tagging for long-term movement estimation because tagging studies of the magnitude necessary to permit tag returns 50 yr in the future are both economically and temporally unrealistic (note: northern Gulf red snapper can live for more than 50 yr). Otolith core microchemistry, on the other hand, provides a natural tag that can be sampled over a suite of ages at an instant in time, because each fish possesses a permanent biomarker that is present throughout its life.

Acknowledgments

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