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Variability in red snapper otolith microchemistry among Gulf of Mexico regions

Michelle Zapp Sluis
Louisiana State University and Agricultural and Mechanical College

VARIABILITY IN RED SNAPPER OTOLITH MICROCHEMISTRY AMONG GULF OF MEXICO REGIONS

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Michelle Zapp Sluis
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DEDICATION

To my brother Michael Alan Zapp, who can do anything he puts his mind to…even if it does not involve the ocean.

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ABSTRACT

Red snapper, *Lutjanus campechanus*, has been an economically important reef fish in the Gulf of Mexico (Gulf) for over 150 years and is currently overfished. Catch statistics and demographic differences have lead to the population being categorized into eastern and western substocks divided by the Mississippi River, but data is recombined to set a Gulf-wide annual catch limit. The two objectives of this study were to apply otolith nursery chemical signatures to estimate red snapper mixing dynamics in the western Gulf, and to determine if signatures based upon trace metals associated with oil and gas platforms could discriminate between region and habitat of origin to further examine population connectivity. Nursery otolith signatures were developed from age-0 red snapper belonging to the 2005 - 2007 year classes and collected from six nursery regions in the Gulf (Florida, Alabama, Louisiana, Texas, Veracruz, and Campeche Banks). Year class-specific quadratic discriminant function analyses (QDFAs) distinguished nursery regions with 71 – 84% accuracy. Maximum likelihood analyses identified sources of sub-adult and adult red snapper sampled during the summer of 2006 - 2008 from the western Gulf and Mexico regions based on year class-specific otolith core chemical concentrations. Locally derived and Louisiana recruits were apparent among red snapper collected off Texas, but data were inconclusive to estimate connectivity between the western Gulf and Mexico regions. Otoliths of red snapper collected from platforms and other habitats off Alabama, Louisiana and Texas during the summer of 2007 and 2008 were analyzed to determine if platforms impart detectable signatures based on seventeen trace metals. Mean jackknifed classification accuracies from QDFAs indicated higher success for discriminating among regions (86%) than habitats (79%). Maximum likelihood analyses estimated region and habitat of origin of red snapper collected from natural habitats off Florida, Louisiana and Texas during the summer of 2009.

Platform signatures were evident in otoliths from red snapper collected off Florida, a region devoid of platforms, possibly reflecting a western Gulf contribution to the eastern substock. The microchemical otolith signatures of western Gulf red snapper in this study demonstrated discrete regional populations with some interpopulation mixing, further supporting a metapopulation structure.

GENERAL INTRODUCTION

The red snapper, *Lutjanus campechanus*, fishery in the Gulf of Mexico (Gulf) began over 150 years ago off the coast of Pensacola, Florida, but due to severe overfishing the stock became depleted between 1865 and 1883 (Camber 1955). This caused the fishery to shift to the western Gulf from the mouth of the Mississippi River to the Galveston Lumps off Texas and even as far south as the Campeche Banks off the coast of Yucatan, Mexico. Substantial red snapper landings continued until the early 1980s when the US fishing fleet was banned from Mexican waters, restricting the fleet to the western Gulf from Mississippi/Alabama to Texas (Gallaway et al. 1998). Catches continued to decline due to overexploitation by the directed fisheries and bycatch mortality from the Gulf shrimp fishery. In 1984 the Gulf of Mexico Fishery Management Council (GMFMC) developed a reef fish fishery management plan to manage the Gulf red snapper stock. The plan has been modified over the years to comply with regulations set by the Magnuson-Stevens Fishery Conservation and Management Act to end overfishing and rebuild the red snapper stock by 2032. Gulf red snapper are currently overfished, although populations in the western Gulf appear to be recovering from overfishing (GMFMC 2010).

Red snapper is a long-lived, reef-associated species that can grow to about 1000 mm total length (TL) and has been observed to live for more than 50 years (Wilson and Nieland 2001; Allman and Fitzhugh 2007). Spawning occurs throughout the summer with a peak lasting from June through August (Beaumariage and Bullock 1976; Woods et al. 2003; Jackson et al. 2007; Porch et al. 2007). It has been estimated that red snapper larvae could be carried 480 km by currents during the four-week planktonic stage (Johnson et al. 2009). Newly settled juveniles are attracted to low-profile reefs, relic-shell habitats and adjacent mud/sand bottom habitats (Rooker et al. 2004; Szedlmayer and Howe 1997; Wells et al. 2008). As red snapper mature, a natural

ontogentetic shift in habitat occurs resulting in movement to more complex natural and artificial reef habitats with increasing vertical dimension, including oil and gas platforms (see Patterson 2007 for review). As red snapper continue to mature, larger individuals are less dependent on structured habitat and can be found on outer shelf-edge reefs (Render 1995; Mitchell et al. 2004).

While genetic evidence has confirmed red snapper as a single stock (Camper et al. 1993; Pruett et al. 2005), demographic variations in growth rates and size-frequency distributions may indicate the existence of isolated units of red snapper in the northern Gulf. Red snapper collected off Texas and Florida are significantly smaller at age and reach smaller maximum size than red snapper collected off Louisiana and Alabama (Fischer et al. 2004; Saari 2011). Deegan et al. (1986) reported that fish capture per unit area was positively correlated with river discharge. Thus, it has been hypothesized that both the nutrient-rich, productive waters of the northern Gulf and the Mississippi River discharge may be the reason Alabama and Louisiana red snapper reach a greater maximum size than snapper from areas less affected by river influence, such as south Texas and central Florida (Fischer et al. 2004).

Catch statistics suggest there are two centers of stock abundance: one in the northwestern Gulf off Louisiana and a smaller one off the coast of Alabama (Goodyear 1995). Based upon these findings and demographic differences, the red snapper population has been categorized into eastern and western substocks divided by the Mississippi River (SEDAR 7 2005). However, plans to rebuild red snapper biomass are applied Gulf-wide and not on the individual management sub-units. Gold and Saillant (2007) determined that the genetic effective population size of red snapper off the coast of Louisiana was an order of magnitude larger than that of red snapper off the coasts of Alabama and Texas, alluding to spatial differences in viable adults able to produce surviving offspring. The 2009 red snapper stock assessment indicated that

the recent increase in red snapper spawning potential ratio (SPR; the average fecundity of a recruit over its lifetime when the stock is fished divided by the average fecundity of a recruit over its lifetime when the stock is unfished) has been attributed to the western Gulf, which will likely continue into the near future. The assessment also determined that reducing fishing mortality uniformly Gulf-wide, in combination with the higher stock biomass of the western substock, and the higher fishing mortality of the eastern substock is expected to result in the western substock recovering faster than the eastern substock (SEDAR 2009). Thus, without reconfiguring management techniques, the western substock may continue to be larger than the eastern substock.

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), with several tagged fish being recovered more than 100 km from the original tagging location (Patterson et al. 2001). A consistent pattern seen in red snapper tagging studies is that most fish only move short distances (<10 km; Patterson 2007), with larger fish more likely to travel greater distances than smaller fish (Patterson et al. 2001). However, problems associated with conventional tagging, such as external tag loss and low reporting rates, may cause red snapper movement to be underestimated (see Patterson 2007 for review).

The use of otolith (ear stone) microchemistry to develop natural tags has become a popular tool among fishery scientists to distinguish fish from distinct nursery areas and to examine movement patterns of adult fish (Gillanders and Kingsford 1996; Thorrold et al. 1997, 2001; Rooker et al. 2008). Otoliths are calcium carbonate structures occurring in three pairs (sagittae, asterisci and lapilli) located within the acoustico-lateralis system of teleost fish. The

otolith is acellular, metabolically inert and accretes as the fish grows, which allows chemicals from surrounding seawater absorbed onto the growing surface to be permanently retained (Campana 1999). Therefore, elements that are deposited during the juvenile phase can act as natural markers for the nursery of origin. Additionally, elements associated with known anthropogenic sources may be used in natural markers to reconstruct region and habitat of origin (Spencer et al. 2000; Nowling et al. 2011). The otolith's ability to act as a natural tag has become a more efficient technique for studying natal origin and population connectivity than conventional tagging methods due to the large number of fish that must be tagged to result in 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), requiring cohort specific signatures to be identified.

The overall objectives of this study were twofold: 1) to develop otolith nursery signatures for six regions in the Gulf to estimate red snapper mixing dynamics in the western Gulf, and 2) to determine if signatures based upon trace metals associated with oil and gas platforms could discriminate between region and habitat of origin to further examine population connectivity of Gulf red snapper. The nursery signature portion of this dissertation was part of a collaborative project with Dr. William Patterson, III and Beverly Barnett of the University of West Florida (UWF). Once nursery signatures were developed, Ms. Barnett focused on the eastern Gulf to examine the source of recruits to the west Florida shelf, while I focused on the western Gulf to determine the source of recruits to the Texas shelf and whether connectivity existed between Texas and Mexico red snapper populations.

Understanding the rates of larval exchange and population connectivity of marine organisms is crucial to the development of marine population dynamics and management of

fishery stocks (Cowen et al. 2000). Previous studies have demonstrated that it is possible to distinguish red snapper nursery regions within the Gulf with otolith chemical signatures (Patterson et al. 2008), as well as develop otolith signatures based on elements associated with oil and gas platforms (Nowling et al. 2011). Thus, such signatures could serve as an effective tool to examine recruitment dynamics and population connectivity of Gulf red snapper. Chapter 1 focuses on the use of chemical signatures in otoliths of age-0 red snapper from six regions within the Gulf to determine if elemental concentrations differed enough to discriminate among nursery regions of origin. Signatures were based upon otolith elemental concentrations of ^{137}Ba, ^{7}Li, ^{55}Mn, ^{25}Mg and ^{86}Sr because these elements are incorporated into otoliths relative to ambient water conditions and are not strongly affected by physiological processes (Campana 1999). Additionally, stable isotope ratios (δ^{13}C and δ^{18}O) were analyzed to increase classification accuracies of nursery origins for Gulf red snapper, as isotopes have been used to successfully determine nursery origins over large spatial scales (Rooker et al. 2008). In Chapter 2, the otolith chemical nursery signatures described in Chapter 1 are used to estimate population structure and connectivity in the western Gulf. I was specifically interested in the source of recruits to the Texas continental shelf, as well as examining any potential mixing dynamics between Texas and Mexico. Chapter 3 results determine whether or not oil and gas platforms impart detectable chemical signatures that are temporally and geographically stable in red snapper otoliths. Otolith elemental concentrations of ^{11}B, ^{138}Ba, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{7}Li, ^{55}Mn, ^{98}Mo, ^{206}Pb, ^{120}Sn, ^{205}Tl, ^{51}V, ^{64}Zn, ^{66}Zn were used to develop signatures. These elements were chosen based upon a pilot study (Nowling et al. 2011) to determine which metals associated with platforms may be incorporated into otoliths. Several of these metals have been detected at significantly higher levels than natural marine sediments and seawater (Neff et al. 1987).

Finally in Chapter 4, the otolith chemical signatures described in Chapter 3 are used to estimate region and habitat of origin for adult red snapper collected from regions devoid of platforms to further examine Gulf red snapper population connectivity.

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CHAPTER 1: DISCRIMINATION OF JUVENILE RED SNAPPER OTOLITH CHEMICAL SIGNATURES FROM GULF OF MEXICO NURSERY REGIONS

Introduction

The Gulf of Mexico red snapper, *Lutjanus campechanus*, stock has been estimated to be overexploited since the 1980s, with the chief sources of fishing mortality being commercial and recreational fisheries, as well as bycatch mortality caused by Gulf of Mexico (Gulf) shrimp trawling (SEDAR 7 2005). Although the red snapper stock is estimated to be no longer undergoing overfishing in the western Gulf (SEDAR 2009), fishery managers are still tasked with balancing these three sources of fishing mortality (F) and rebuilding the stock by 2032 (GMFMC 2010). While 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), demographic differences in size at age, maturation rates, and genetic effective population size of red snapper occur across the northern Gulf (Fischer et al. 2004; Jackson et al. 2007; Saillant and Gold 2006). In fact, catch statistics suggest there are two centers of stock abundance, one in the northwestern Gulf off Louisiana and a smaller one off the coast of Alabama (Goodyear 1995). Based upon these findings, the red snapper population has been categorized into eastern and western substocks divided by the Mississippi River (SEDAR 7 2005), but the information is recombined to estimate a Gulf-wide annual catch limit. Yet, little is known about mixing patterns between substocks.

Understanding the rates of larval exchange and population connectivity of marine organisms is crucial to the development of marine population dynamics and management of fishery stocks (Cowen et al. 2000). Larval dispersal can be difficult to study, though based upon shelf currents it has been estimated that red snapper larvae could be carried 480 km during the four-week planktonic stage (Johnson et al. 2009). However, only a small portion of the western

substock larvae would be able to cross the Mississippi River plume, and in such an event would most likely be transported away from the continental shelf with a low probability of survival. Conditions are estimated to be more favorable for western transport of the eastern substock (Johnson et al. 2009). Conventional tagging has been used to examine postsettlement movement of juvenile and adult red snapper. Although 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 more than 1,000 recaptures) has been observed moving east to west across the Mississippi River (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 cause red snapper movement to be underestimated (see Patterson 2007 for review).

The use of otoliths (ear stones) 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. 1997a). Otoliths are calcium carbonate structures located within the acoustico-lateralis system of teleost fish. They are acellular, metabolically inert and precipitate as the fish grows, which allows chemical signatures from surrounding seawater accreted onto the growing surface to be permanently retained (Campana 1999). 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 become 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), requiring cohort specific signatures to be identified.

The majority of marine studies utilizing otolith chemistry to estimate natal origin focuses on estuarine and near-shore nursery habitats (Thorrold et al. 1998; Gillanders and Kingsford 2000; Dorval et al. 2005), but 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 using elemental signatures, with mean classification accuracies of 80% for four out of the five cohorts examined. Elemental variability was attributed to hydrologic and oceanographic differences among regions, with otolith chemistry most likely reflecting ambient water elemental concentrations. Yet, these same oceanographic processes may be the cause of poor discrimination in one of the cohorts examined. Thorrold et al. (1998) reported an improvement in classification accuracies of weakfish to estuarine nurseries along the Atlantic coast when combining stable isotope ($\delta^{13}C$ and $\delta^{18}O$) ratios to trace element data. Further, Rooker et al. (2008) were able to determine the nursery of origin of Atlantic bluefin tuna over a larger spatial scale using only otolith $\delta^{13}C$ and $\delta^{18}O$ ratios. Thus, the addition of stable isotope ratios to otolith elemental signatures may increase classification accuracies of nursery origins for Gulf red snapper.

The current study is part of a collaborative project to examine red snapper population connectivity and mixing across the Gulf. 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 were used to discriminate

red snapper nursery regions on the continental shelf of US and Mexican portions of the Gulf. Region-specific nursery signatures for three consecutive year classes were developed to determine if discriminant classifications were strong enough to validate the use of nursery signatures to estimate the source of recruits to regions among the Gulf.

Methods

Sample Collection

Age-0 red snapper from the 2005, 2006 and 2007 year classes were sampled from 6 regions in the Gulf (Figure 1.1), including the west Florida shelf (FL), north central Gulf (AL), northwestern Gulf (LA), western Gulf (TX), southwestern Gulf off Veracruz, Mexico (MEX1), and the Campeche Banks (MEX2). The objective was to collect thirty juveniles from each region for each year class (n = 540). Samples from AL, LA and TX were collected in the fall (October and November) during the National Marine Fisheries Service's (NMFS) Fall Groundfish Survey using otter trawls aboard either the *R/V Oregon II* or *R/V Gordon Gunter*. Juvenile fish were sub-sampled from a trawl catch with systematic random sampling, targeting fish < 150 mm in total length (TL). Immediately following selection, 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) as part of a collaborative study.

Collecting samples from FL, MEX1 and MEX2 was opportunistic and difficult to achieve. Juvenile red snapper from FL were collected in the fall of 2005 and 2007 from the Florida Fish and Wildlife Research Institute's (FWRI) Baitfish Survey, NMFS's Small Pelagic Survey, and Shrimp Observers employed by the Gulf and South Atlantic Fisheries Foundation (GSAFF). Red snapper were stored in plastic bags, frozen, and transported to UWF. Unfortunately, FL samples were unavailable for the 2006 year class. A trip was made

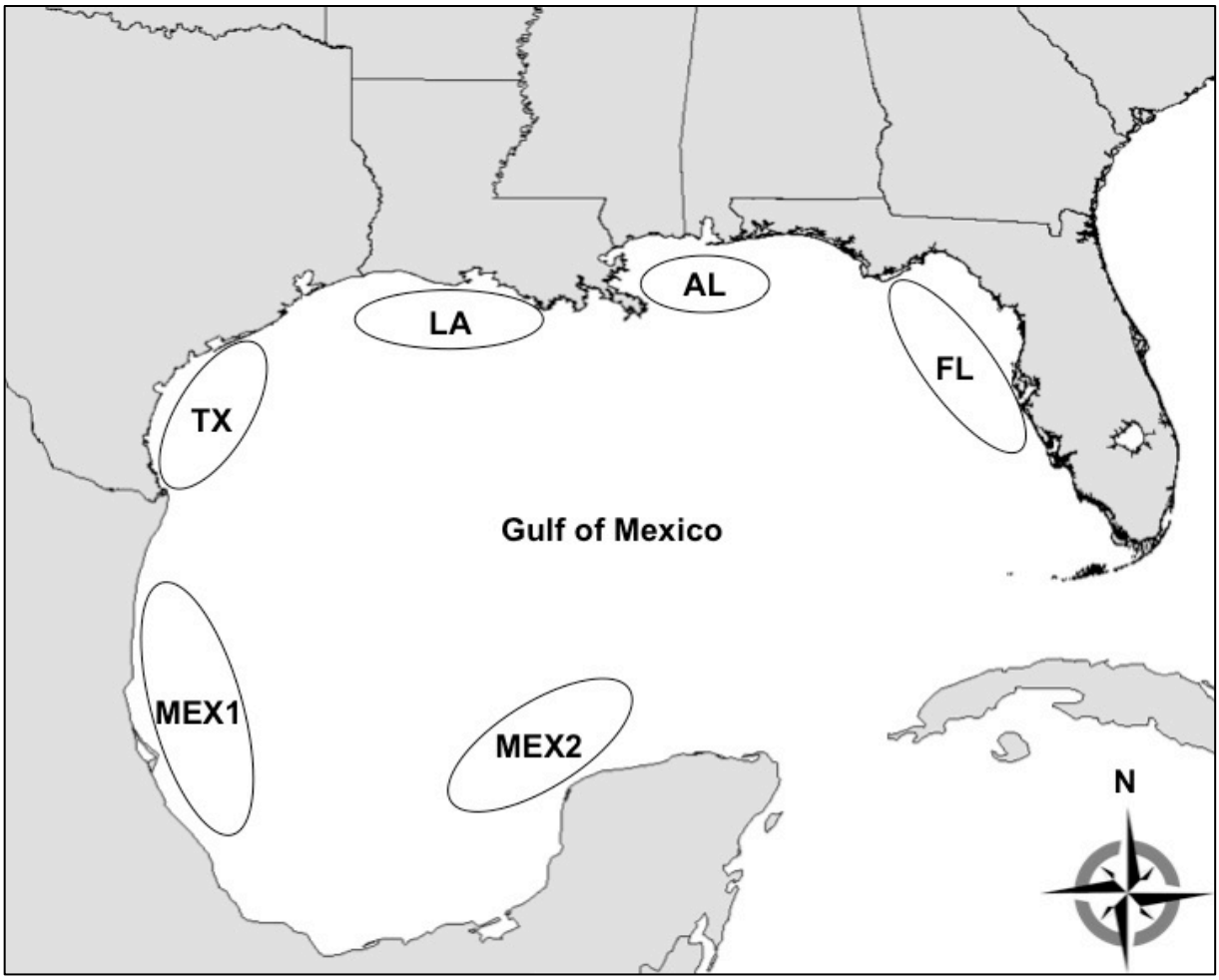

Figure 1.1. Nursery regions along the continental shelf of the Gulf of Mexico where age-0 red snapper, *Lutjanus campechanus*, corresponding to the 2005, 2006 and 2007 year classes were collected.

to Merida, Mexico in March 2006 to collect age-0 red snapper from the Campeche Banks corresponding to the 2005 year class, and to enlist help for future sample collections. However, no juvenile samples were obtained at that time. A Mexican colleague collected juvenile red snapper in the winter (December through March) of 2007 and 2008 from shrimp trawl bycatch on the Campeche Banks (MEX2) and along the Mexican shelf between Tampico and Vera Cruz (MEX1). Fish TL was measured and otoliths were extracted before samples were shipped to Louisiana State University (LSU).

Frozen age-0 fish collected within US Gulf waters were thawed in the laboratory at UWF, weighed to the nearest 0.01 g, and TL was measured to the nearest mm. 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 (ultra-pure 18 MΩ cm^{-1} water; $DDIH_2O$). Sagittae were cleaned with a synthetic bristle brush to remove any adhering tissue, rinsed with $DDIH_2O$, and placed in polyethylene vials to air-dry under a class-100 clean hood.

Otolith Preparation and Analysis

Otoliths samples were cleaned prior to elemental or stable isotope analysis under class-100 clean hoods. Dry otoliths were weighed before and after cleaning to the nearest 0.01 mg. Whole otoliths were immersed in 1% ultrapure nitric acid (HNO_3) for 30 seconds to oxidize any material adhering to the surface, and then flooded with $DDIH_2O$ to remove the acid. Otoliths were dried under a class-100 clean hood for at least 24 hours.

All otoliths from the right side of the fish were prepared at UWF as part of the collaborative study. Otoliths were dissolved in high-density polyethylene (HDPE) vials by adding 1% ultrapure HNO_3 until a dilution factor of approximately 1,000-fold was achieved. Although total dissolution typically occurred within 1 hour, samples were not manipulated for at least 24 hours once acid digestion began. Aliquots (5 ml) of otolith solutions were sent to the University of Southern Mississippi for trace elemental analysis with a Finnigan MAT Element2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS). Otolith solutions were spiked with Indium at a concentration of 2.5 parts per billion (ppb) as an internal standard and then analyzed for ^{137}Ba, ^{48}Ca, ^{7}Li, ^{55}Mn, ^{25}Mg and ^{86}Sr. Blanks were prepared from 1% ultrapure HNO_3 and processed through the same stages of sample preparation as sample

solutions. Blanks were analyzed concurrently with otolith sample solutions to estimate instrument limits of detection (LOD), which were estimated as three standard deviations of mean blank values. Instrument performance and matrix effects were checked by assaying elemental concentrations of an otolith standard reference material (SRM) prepared from adult red snapper otoliths (Sturgeon et al. 2005). Solutions of the SRM were prepared and analyzed similarly to age-0 red snapper otolith samples.

All otoliths from the left side of the fish were sent to LSU where they were prepared for stable isotope analysis. Otoliths were pulverized with an agate mortar and pestle, and transferred into 2 ml microcentrifuge tubes. Subsamples (>1 mg) of homogenized pulverized otoliths were sent to the Stable Isotope Laboratory in the Department of Geology at the University of California at Davis for stable isotope ($\delta^{13}C$ and $\delta^{18}O$) analysis with a Finnigan MAT 251 isotope ratio mass spectrometer (IR-MS). The instrument was calibrated against the International Atomic Energy Agency's carbonate standard, NBS-19. Accuracy of analytical runs was measured through routine analysis of a check standard, which had been stringently calibrated against NBS-19. The isotopic composition of otoliths are reported in standard δ notation relative to the Vienna Peedee belemnite (V-PDB reference standard for $\delta^{13}C$ and standard mean ocean water for $\delta^{18}O$) using the standard equation:

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

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

Statistical Analysis

To meet parametric assumptions, element:Ca ratios were ln transformed prior to statistical analysis. Although fish size differed among regions (Table 1.1), data were not length corrected because elements and stable isotopes that were significantly correlated with length

Table 1.1. Sample size and size range of age-0 red snapper, *Lutjanus campechanus*, collected from six nursery regions across the Gulf of Mexico corresponding to the 2005, 2006 and 2007 year class. FL = Florida; AL = Alabama; LA = Louisiana; TX = Texas; MEX1 = Veracruz, Mexico; MEX2 = Campeche Banks, Mexico.

Year Class	Region	Sample Size	Size Range (mm TL)
2005	FL	20	76 – 106
	AL	30	65 – 146
	LA	30	83 – 150
	TX	30	61 – 145
	MEX1	-	-
	MEX2	-	-
2006	FL	-	-
	AL	30	70 – 148
	LA	30	71 – 149
	TX	30	71 – 150
	MEX1	30	95 – 140
	MEX2	29	160 – 230
2007	FL	29	68 – 150
	AL	30	65 – 146
	LA	30	63 – 141
	TX	30	53 – 149
	MEX1	22	75 – 220
	MEX2	30	60 – 230

varied among year classes and no systematic bias was present (Figure 1.2A). When only analyzing the three regions that were sampled each year of the study (AL, LA and TX), again no systematic bias was present and elements that correlated with length differed compared to the results when all regions were analyzed (Figure 1.2B). Thus, it is uncertain if the correlations were an effect of length or changes in ambient water chemistry for the years studied. Multivariate analysis of variance (MANOVA) was used to test for differences in otolith elemental and stable isotope signatures among regions and year classes, with Pillai's trace (V) as

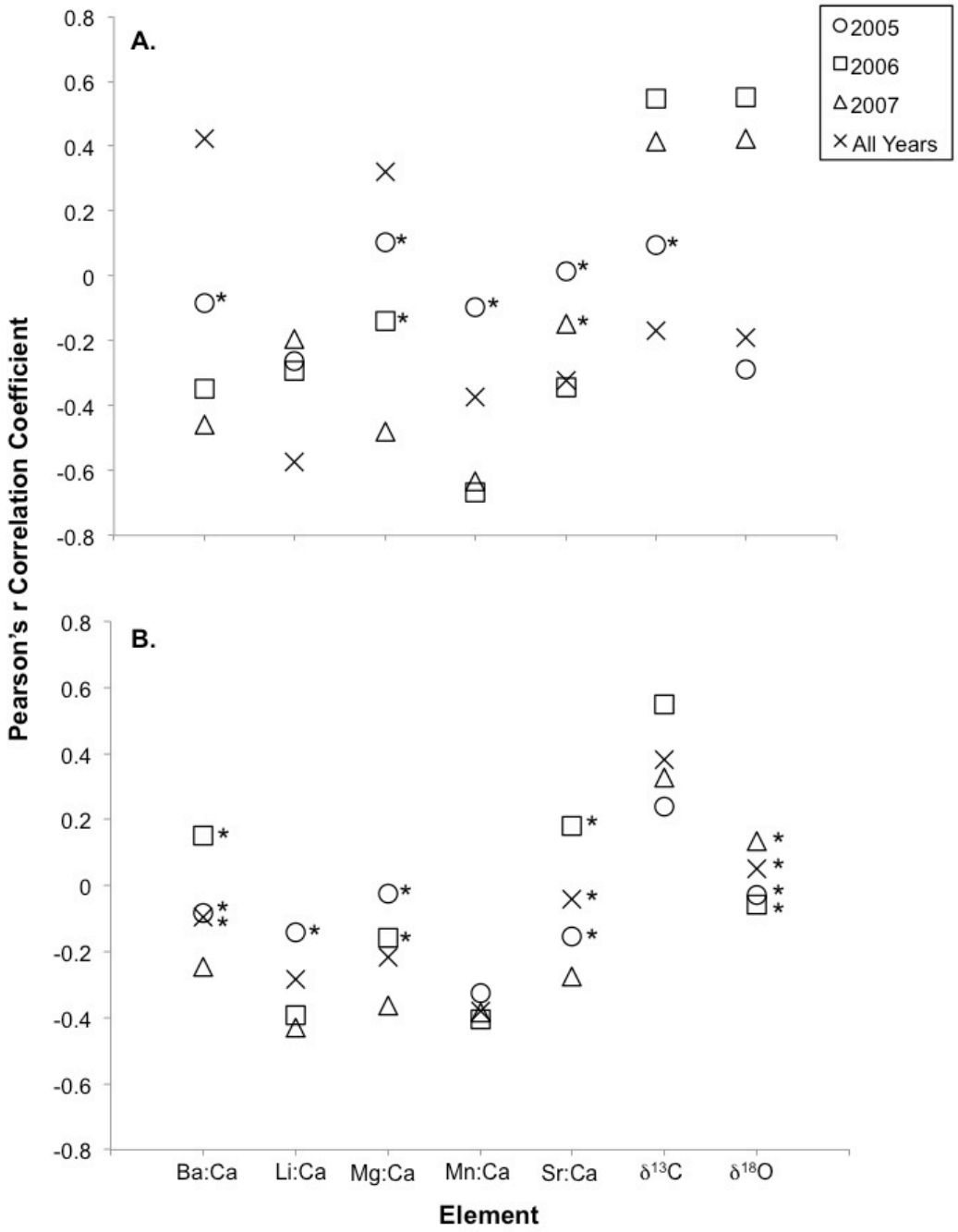

Figure 1.2. Linear correlations between otolith elemental and stable isotope concentrations and total length (TL) of age-0 red snapper, *Lutjanus campechanus*, for the 2005, 2006 and 2007 year classes for A.) all regions sampled and B.) for only the AL, LA and TX regions. Asterisks denote nonsignificant correlations ($\alpha = 0.05$).

the test statistic because it is the most robust to violations of homogeneity of variance (Wilkinson et al. 1996). However, only the regions sampled each year of the study (AL, LA and TX) were examined in the MANOVA. Analysis of variance (ANOVA) was used to test element:Ca and stable isotope ratios individually to determine a source of variance among regions. ANOVAs were performed on just the northern regions (AL, LA, TX) to examine significant effects of independent variables (region, year class and their interaction) and were also used to assess significant levels of chemical signatures for each region within each year class separately.

To determine which elements were the most significant in discriminating among regions within year classes, a stepwise discriminant analysis (SDA) was computed. An SDA is used to find a set of the original quantitative variables that best discriminate samples among sites or groups. To distinguish regions with otolith chemical signatures, year class-specific discriminant function analyses were performed, as well as with all year classes combined. A quadratic discriminant function analysis (QDFA) was computed instead of a linear DFA because variance-covariance matrices of elemental and stable isotope variables were significantly different among regions. Jackknifed crossvalidation classification accuracies were analyzed to estimate classification success to respective regions by year class and with all year classes combined. A canonical discriminant analysis (CDA) was used to compare otolith chemical concentrations of each region by year class, and for all year classes combined. The CDA determines the best linear combination of quantitative variables where the means of the groups are most different and whether this difference varies by year class. All analyses were performed with the Statistical Analysis System (SAS Institute 2006) with a significance level of $\alpha = 0.05$.

Results

A total of 430 age-0 red snapper collected from 6 nursery regions across the Gulf was analyzed for otolith chemical signatures (Table 1.1). Concentrations of all 5 elements (^{137}Ba, ^{48}Ca, ^{7}Li, ^{55}Mn, ^{25}Mg, ^{86}Sr) were at least two orders of magnitude above detection limits for all elements analyzed in all samples. The SRM samples were within 5% of certified values for elements analyzed with SF-ICP-MS. Chemical signatures differed significantly among nursery regions (MANOVA, $F_{14, 512} = 12.70$, $p < 0.001$), year classes (MANOVA, $F_{14, 512} = 13.44$, $p < 0.001$), and in the interaction between regions and year classes (MANOVA, $F_{28, 1032} = 6.82$, $p < 0.001$). Most element:Ca and stable isotope ratios differed significantly (ANOVA, $p \leq 0.05$) among nursery regions, year classes and their interaction when testing the northern regions only. The exceptions were Mg:Ca for region and year class interaction effects (ANOVA, $p = 0.1167$), Sr:Ca for region effect (ANOVA, $p = 0.2898$), and δ^{13}C for year class effect (ANOVA, $p = 0.6253$).

Mean concentrations of element:Ca and stable isotope ratios varied across nursery regions and year classes (Figure 1.3; see also Appendix A). All element:Ca and stable isotope ratios differed significantly (ANOVA, $p \leq 0.05$) among regions within year classes, except Ba for the 2005 year class (ANOVA, $p = 0.4514$). When present, fish sampled from FL tended to have constituent values either lower or higher relative to the other regions. The same is true for fish sampled from MEX2. In fact, for the 2007 year class, samples from FL and MEX2 had similar element:Ca and stable isotope ratios with the only exception being Li. Fish collected from TX tended to have higher values for Mg:Ca and Mn:Ca across all year classes, and Sr:Ca steadily decreased over the years. Overall, AL and LA tended to have similar constituent values for each year class (Figure 1.3; see also Appendix A).

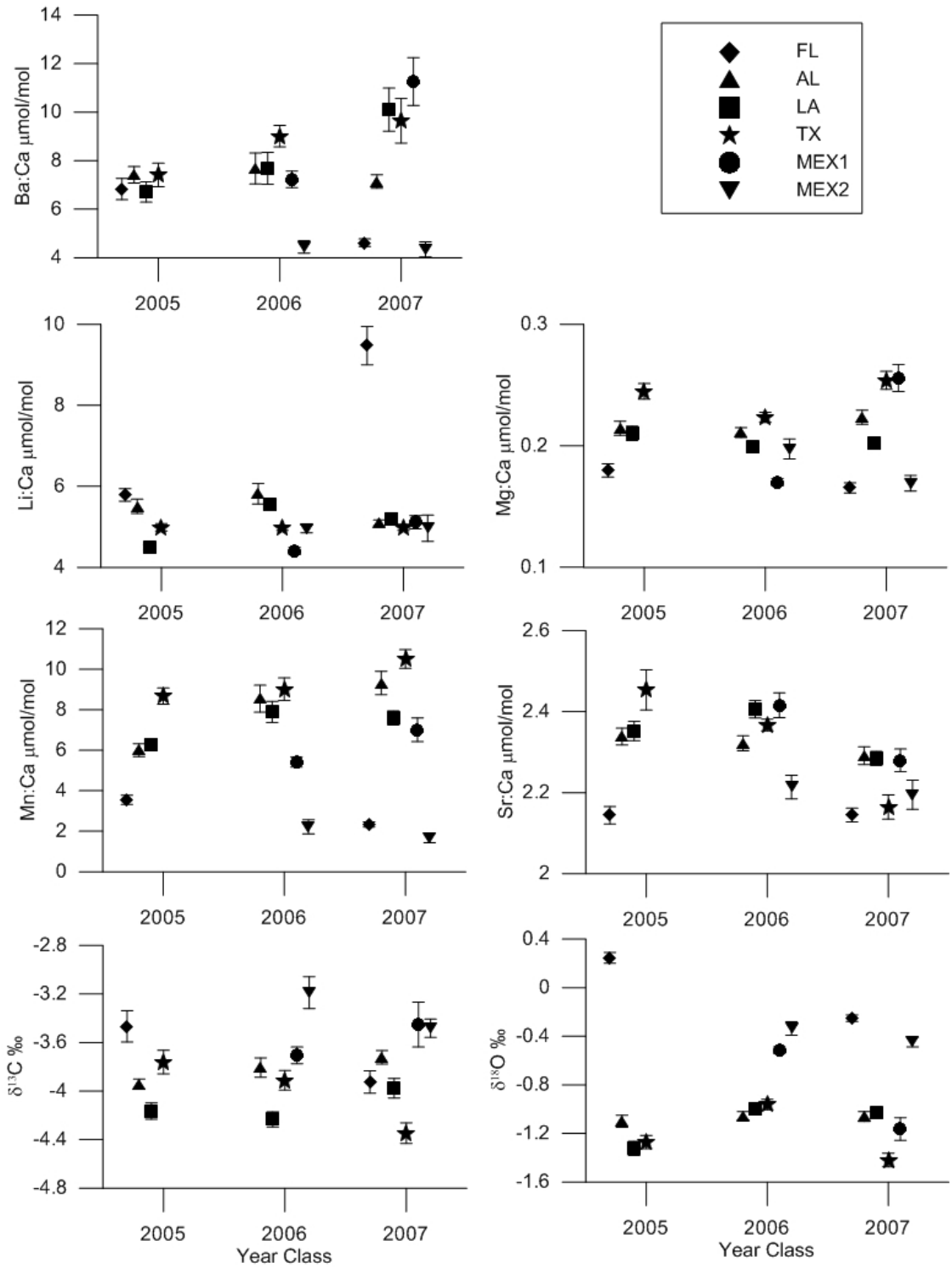

Figure 1.3. Mean (± SE) region- and year class-specific otolith element:Ca or stable isotope ratios for age-0 red snapper, *Lutjanus campechanus*, collected from six Gulf of Mexico nursery regions in 2005, 2006 and 2007.

The stepwise discriminant analysis retained all element:Ca and stable isotope ratios for each year class. Mean jackknifed classification accuracies of the QDFA models were 84.2% for 2005, 73.9% for 2006, 71.1% for 2007, and 72.4% for all year classes combined (Figure 1.4). Combining all year classes resulted in a slightly higher classification success compared to the classification success of the 2007 year class. The lowest classification success was for MEX1 samples from the 2007 year class (50%), and the highest classification success was for FL samples from the 2005 year class (100%). With the exception of the especially low classification success of 2007 MEX1 samples, red snapper collected from LA typically had the lowest classification success for each year class, with the majority of misclassifications resembling AL samples. Samples from AL had the next lowest classification success for each year class, with misclassifications resembling LA samples. For the 2007 year class, which contained all nursery regions, FL and MEX2 had the highest classification success and misclassification error from FL resembled MEX2 and vice versa (Figure 1.4). The CDA provided the best separation for the 2005 year class, and showed a general trend of overlapping between the northern nursery regions for all year classes (Figure 1.5; see also Appendix A). When all year classes are combined, the plot closely resembles the 2007 year class plot, the only year class to include all six nursery regions.

Discussion

The discriminant classifications of region-specific nursery signatures for the three consecutive year classes studied validate the utility of natural otolith tags to estimate the source of recruits to regions in the Gulf and to examine red snapper population connectivity. Patterson et al. (2008) also demonstrated the potential for using otolith chemical signatures to discriminate among red snapper nursery regions, but they reported an overall higher mean classification

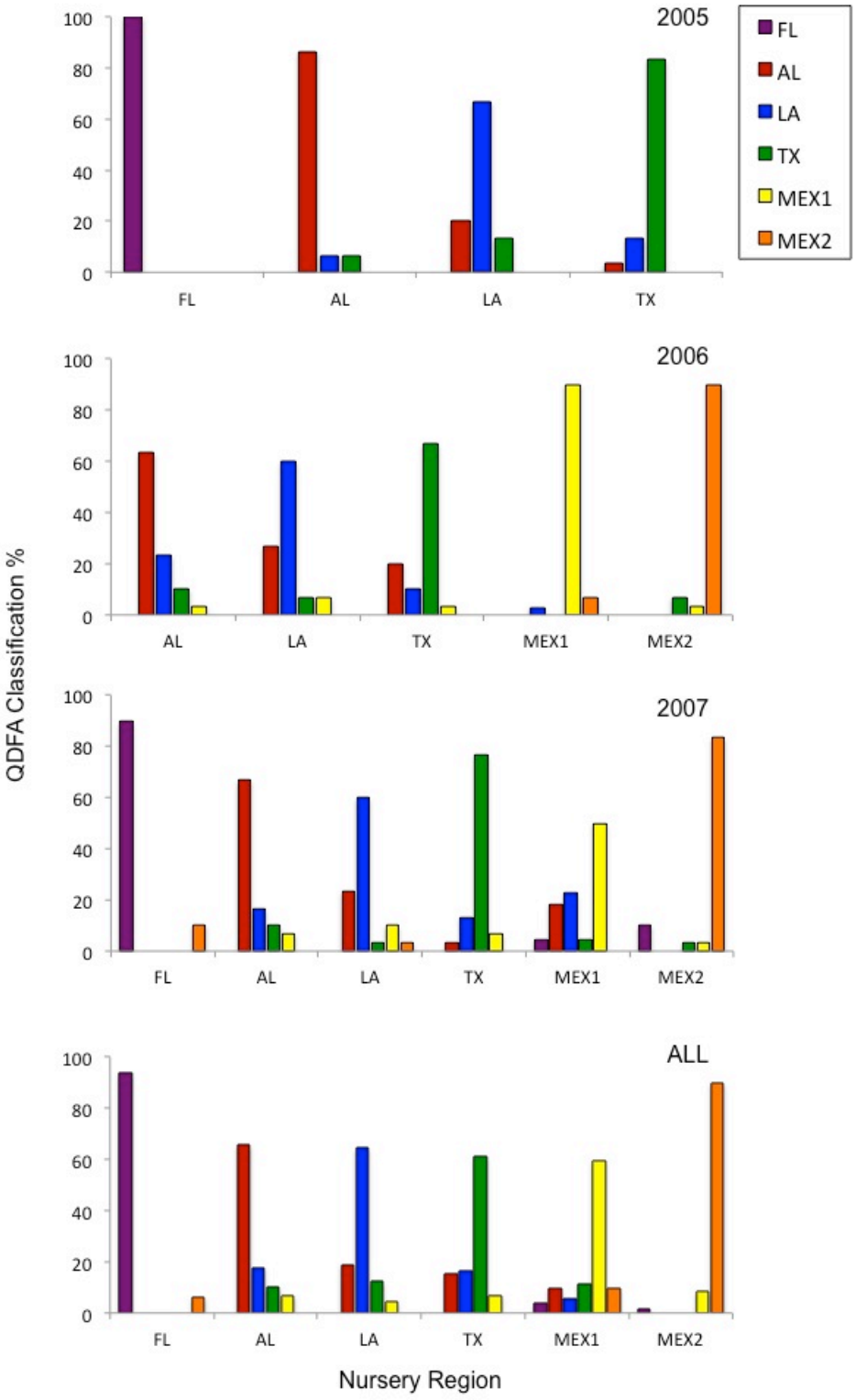

Figure 1.4. Jackknifed classification percentages estimated with quadratic discriminant function analysis (QDFA) of otolith chemical signatures of age-0 red snapper, *Lutjanus campechanus*, to six nursery regions in the Gulf of Mexico collected in 2005, 2006 and 2007. "ALL" indicates all three year classes combined.

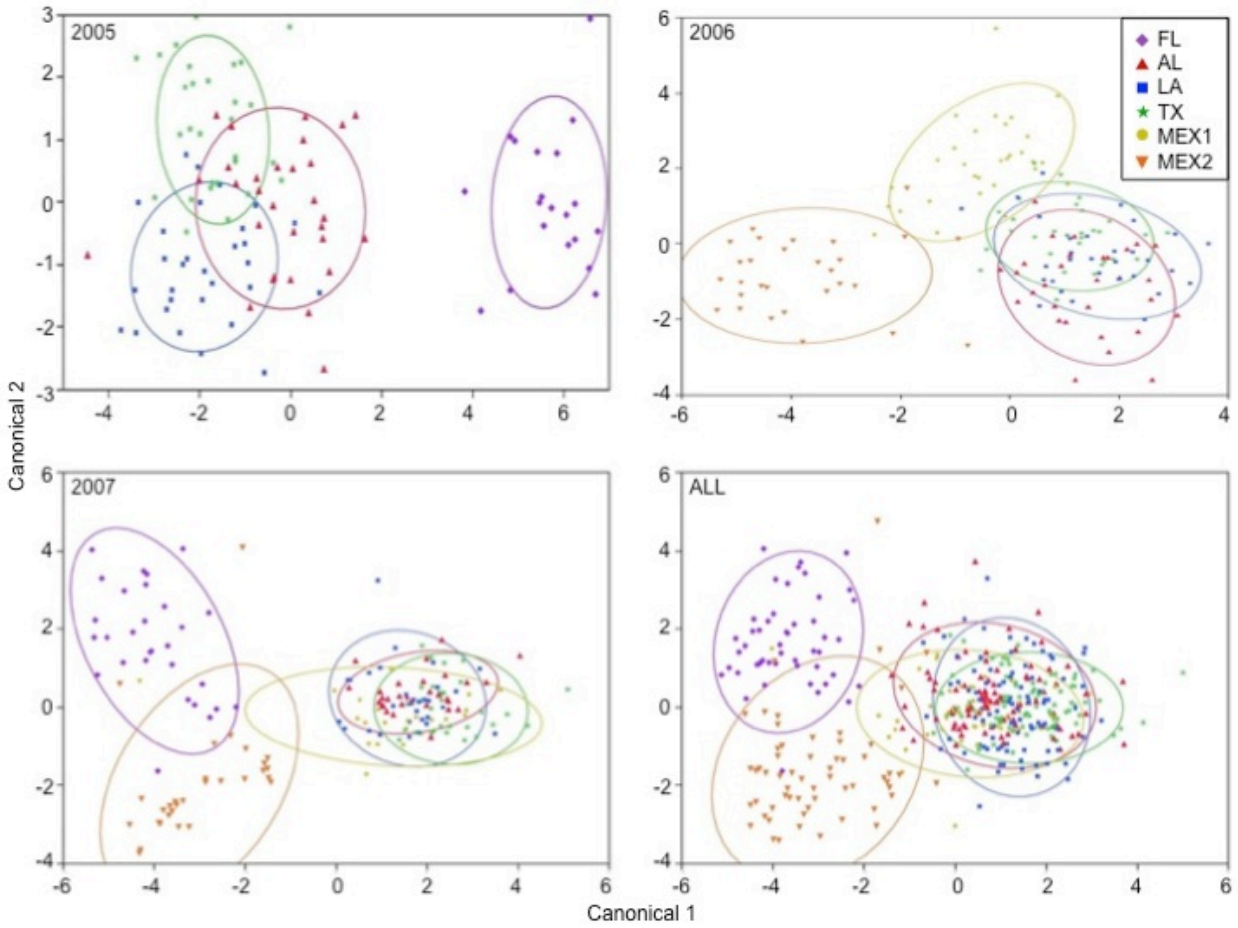

Figure 1.5. Canonical plot scores of otolith chemical signatures of age-0 red snapper, *Lutjanus campechanus*, from six nursery regions in the Gulf of Mexico for the 2005, 2006 and 2007 year classes. Ellipses indicate 95% confidence levels, and "ALL" indicates all three year classes combined.

success (80%) than was seen in this study. However, Patterson only examined the three northern nursery regions (AL, LA, TX), compared to the six analyzed in this study. The 2005 year class had the highest classification success with the lowest number of regions, whereas the 2007 year class had the lowest classification success with the highest number of regions. Thus, it would appear that the increase in regions caused a decrease in classification accuracy. Yet, misclassifications in the northern regions are mainly among those regions, and removal of FL, MEX1 and MEX2 does not significantly alter the classification errors.

Elements can be incorporated into the otolith by substituting for Ca in the calcium carbonate matrix, binding to proteins in the organic matrix, or inserted in to the interstitial spaces (Campana 1999). Elements that both directly substitute Ca within the carbonate matrix and reflect ambient water conditions are preferred when developing otolith chemical signatures to retain the signature within the otolith and use it to discriminate among fish groups. Before elements are incorporated into the carbonate matrix of the otolith they undergo branchial uptake, cellular transport through the blood plasma, and crystallization in the endolymph fluid surrounding the otolith. Hence, physiological regulations, along with environmental processes such as salinity and temperature, can also affect the assimilation of elements into otoliths (Kalish 1989; Campana 1999; Fowler et al. 1995). Strontium and Ba substitute for Ca, are deposited in proportion to ambient water conditions (Bath et al. 2000), and reflect salinity gradients. Higher concentrations of Sr appear in shelf waters and lower concentrations in riverine waters, whereas Ba follows a nutrient-type profile with higher concentrations in riverine and near-coastal waters (Thorrold et al. 1997a). While Li and Mn also reflect ambient water conditions and temperature, Mg demonstrates no trend with temperature but instead is at least partially regulated physiologically (Campana et al. 2000; Martin and Thorrold 2005).

Stable isotope ratios in otoliths have been used to reconstruct temperature history, differentiate among groups of fish, infer metabolic history, and reconstruct migratory patterns (Campana 1999). The oxygen isotope ratio of otoliths is deposited in equilibrium with ambient water and is inversely related to temperature (Radtke et al. 1996). Evaporation and freshwater input can also alter $\delta^{18}O$ values, resulting in heavier isotopes being associated with seawater and lighter isotopes being deposited into freshwater systems via precipitation (Lenanton et al. 2003). The carbon isotope ratio in otoliths is influenced by nutritional sources depending upon the

carbon-fixing pathway of the primary producers, the level of fractionation to higher trophic levels, and the metabolic rate of the consumer (Radtke et al. 1996). Approximately 17-40% of otolith carbon is derived from metabolic sources (see Solomon et al. 2006 for review), with the remainder coming from dissolved inorganic carbon (DIC). However, unlike oxygen isotopes, carbon isotopes are deposited in otoliths under non-equilibrium conditions as a result of metabolic affects. Studies have shown a positive correlation between somatic growth and $\delta^{13}C$, as well as otolith precipitation rates and $\delta^{13}C$ (Thorrold et al. 1997b; Gibson et al. 2010).

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 Rivers 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 (Justic 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, along-shore 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 concentrations were similar among the three northern regions, especially between AL and LA. Although interannual differences were present, AL and LA samples tended to have similar concentrations of Mg:Ca, Sr:Ca, and $\delta^{18}O$. Red snapper collected from TX differed more than the other two regions mostly owing to higher concentrations in Mg:Ca and Mn:Ca. Although not all of the results of this study supported the findings of Patterson et al. (2008), some similarities are evident. For instance, in both studies TX samples had higher concentrations of Mg:Ca compared to the other two regions, and LA samples

had lower concentrations of Mn:Ca while AL and TX Mn:Ca concentrations were similar. Patterson et al. (2008) concluded that red snapper otolith chemical signatures were a reflection of ambient water elemental concentrations. Therefore, differences between the studies would be expected resulting from changes in water elemental concentrations over time, with similarities possibly caused by persistent ambient concentrations of Mg and Mn within the northern regions.

Although red snapper samples were not collected from FL, MEX1 and MEX2 for all year classes, when present these regions consistently had otolith chemical concentrations that differed from the northern regions. The exception was MEX1 samples, which tended to have similar concentrations to the northern regions, primarily for the 2007 year class. The southern Gulf coastal waters are influenced by river runoff from the Grijalva-Usumacinta Rivers system, which produces the second-largest volume of freshwater discharge into the Gulf (Signoret et al. 2006). The river plume is displaced westward towards the Tamaulipas-Veracruz (TAVE) shelf caused by a westward coastal current. In the spring-summer, there is an up-coast current on the TAVE shelf that reaches the southern Texas continental shelf where it encounters a down-coast current favoring offshore transport. This current reverses in the fall and winter, and the now down-coast current extends to the southern Bay of Campeche where it meets an opposing along-shelf current, generating seasonal offshore transport. This current reversal allows water from the Mississippi-Atchafalaya Rivers to reach the TAVE shelf (Zavala-Hildalgo et al. 2003). Hence, the high freshwater inflow from the Grijalva-Usumacinta Rivers, along with seasonal inflow from the Mississippi-Atchafalaya Rivers system, likely contributes to the similarities between MEX1 and the northern Gulf regions.

Prevailing upwelling winds cause circulation on the western Campeche Bank to flow westward along the coast throughout the entire year (Zavala-Hildalgo et al. 2003). These

circulation patterns likely prevent mixing between MEX1 and MEX2 coastal waters, which is made evident by the differences in otolith chemical signatures between the two regions. The FL and MEX2 regions were also greatly enriched in $\delta^{18}O$ compared to the other nursery regions. Since lighter isotopes are associated with freshwater (Lenanton et al. 2003), this trend most likely reflects the river influence to the northern and MEX1 regions. Furthermore, FL and MEX2 samples had lower Ba:Ca values, which is associated with riverine waters and further confirms the dominant river influence to the northern and MEX1 regions. Another notable difference was FL and MEX2 had significantly lower Mn:Ca concentrations than the northern regions. Hanson et al. (2004) reported that otolith Mn concentration of gag, *Mycteroperca microlepis*, increased with latitude corresponding to the same trend in coastal sediment Mn concentration along the Florida Gulf coast. Thus, latitudinal differences, absence of heavy freshwater input and lack of water mixing as a result of circulation patterns likely contribute to the separation of FL and MEX2 from the other regions.

The efficiency of using otolith chemical concentrations as natural tags is partially dependent upon the temporal stability of the signature. Studies of temporal stability of otolith chemical signatures have shown either differences between two consecutive years (Patterson et al. 1999) or negligible differences over two year intervals with drastic changes occurring after 4-13 years (Campana et al. 2000). Yet, even though interannual differences are present, studies have shown that separation patterns among regions can still be similar and regional differences are the cause of variability in otolith chemical concentrations (Edmonds et al. 1992; Campana and Gagne 1995). Although thorough statistical testing of temporal stability was not possible because of the unbalanced design of this study, year class differences were significant for the three northern regions, which was 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 of the combined year classes was not much lower than the 2006-year class classification success and was a slight improvement from the 2007-year class classification success. Therefore, while developing cohort-specific otolith chemical signatures would be appropriate for Gulf red snapper, because of the unbalanced design of this study 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 to develop year class- and region-specific otolith chemical signatures to differentiate among nursery regions of the Gulf. The ultimate goal of this collaborative research is to utilize these natural tags to estimate the source of recruits to regions in the Gulf. Specifically, more work should be undertaken to estimate the source of recruits to the west Florida shelf, to examine the connectivity between populations of the western Gulf and northeast Mexico, and to 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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CHAPTER 2: APPLICATION OF OTOLITH NURSERY SIGNATURES TO ESTIMATE POPULATION CONNECTIVITY OF RED SNAPPER IN THE WESTERN GULF OF MEXICO.

Introduction

The Gulf of Mexico (Gulf) red snapper, *Lutjanus campechanus*, fishery began over 150 years ago off the coast of Pensacola, Florida, but due to severe overfishing the stock became depleted by the late 1800s (Camber 1955). This caused the fishery to shift to the western Gulf from the mouth of the Mississippi River to the southern coast of Texas, and even as far south as the Campeche Banks off the coast of Mexico. High red snapper landings continued until the early 1980s when the US fishing fleet was banned from Mexican waters, restricting the fleet to the western Gulf from Mississippi/Alabama to Texas (Gallaway et al. 1998). Catches continued to decline due to high levels of commercial and recreational exploitation, and bycatch mortality from the shrimp fishery, resulting in Gulf red snapper being currently overfished (GMFMC 2010).

Overexploitation of the red snapper fishery is also evident in Mexican Gulf waters. The Campeche Bank fishery was the national leader in red snapper production. However, due to adverse affects from Mexican and Cuban commercial fisheries, and bycatch mortality from the Mexican shrimp fishery, landings of red snapper from the Campeche Banks declined by 51.2% from the 1980s to the late 1990s (Monroy-García et al. 2002) and the Mexican stock was estimated to be severely overfished by 2005 (SAGARPA 2006 as cited in Brule et al. 2010). Mexico has established fishing regulations, including commercial finfish permits, hook size restrictions, and an annual catch quota for the Cuban fleet. Yet there is still a need for stricter regulations and Mexico plans to implement constraints similar to those applied to the red snapper in US waters (Monroy-García et al. 2002).

Management of the US Gulf red snapper stock was implemented in November 1984 by the Gulf of Mexico Fishery Management Council's (GMFMC) reef fish fishery management plan designed to rebuild declining fish stocks. Several amendments have been adapted to the plan to comply with regulations set by the Magnuson-Stevens Fishery Conservation and Management Act to end overfishing and rebuild the red snapper stock by 2032. Currently, constraints are placed on both directed fisheries (annual catch limits, bag and minimum size limits, seasonal closures, and reef fish permits) and on the Gulf shrimp fishery (reduction in effort, area closures and bycatch reduction devices (BRDs) on shrimp trawls; GMFMC 2010).

The red snapper population has been categorized into eastern and western substocks divided by the Mississippi River (SEDAR 7 2005) based on demographic differences in size at age, maturation rates, and genetic effective population size of red snapper that occur across the Gulf (Fischer et al. 2004; Jackson et al. 2007; Saillant and Gold 2006). However, plans to rebuild red snapper biomass are applied Gulf-wide and not on the individual management sub-units. Gold and Saillant (2007) determined that the genetic effective population size of red snapper off the coast of Louisiana was an order of magnitude larger than off the coasts of Alabama and Texas, alluding to spatial differences in viable adults able to produce surviving offspring. Reducing fishing mortality uniformly Gulf-wide with the higher stock biomass of the western substock, along with an estimated lower fishing mortality than the eastern substock, is expected to result in the western substock recovering faster and to a greater spawning stock biomass (SSB) level than the eastern substock (SEDAR 2009). Thus, without reconfiguring the management techniques, the western substock is projected to continue to be larger than the eastern substock.

Demographic differences also exist within the western substock alone. Studies have shown that red snapper collected off Texas are significantly smaller at age and reach smaller maximum size than red snapper collected off Louisiana (Fischer et al. 2004; Saari 2011). Saari (2011) also reported a higher proportion of older fish collected off north Texas and Louisiana than from all other Gulf regions, which was possibly attributed to the higher stock abundance of the western Gulf. Although differences in red snapper growth rates has been linked in past reports with increased primary production associated with the Mississippi River plume (Fischer et al. 2004), understanding population structure and connectivity could further explain demographic differences within the western Gulf. Furthermore, degree of connectivity that exists between the red snapper population off south Texas and northeast Mexico coasts is unknown. With the Mexican stock being severely overfished, if connectivity between Texas and Mexican red snapper populations is high, then the Mexican fishery could serve as a sink for Texas recruits (Crowder et al. 2000).

The use of otolith (earstone) microchemistry to develop natural tags has become an effective tool for fishery scientists to distinguish juveniles from distinct nursery areas and then estimate the contribution of different nursery areas to adult stocks (Thorrold et al. 1998, 2001; Rooker et al. 2001, 2008). The otolith precipitates as the fish grows and is metabolically inert, which means chemical signatures from surrounding seawater accreted onto the growing surface will be permanently retained (Campana 1999). This allows material that is deposited during the juvenile stage to act as a natural marker for the nursery of origin. As a result, chemical signatures contained within the core, or juvenile portion, of the otolith can then be used to identify the nursery of origin of the adult fish. Barnett and Patterson (2010) determined that the otolith core from an adult red snapper could be mechanically extracted and would yield effective

results for analyzing nursery chemical signatures. Furthermore, Patterson et al. (2008) and the results of Chapter 1 have demonstrated that it is possible to distinguish red snapper nursery regions within the Gulf using otolith chemical signatures. Employing these signatures to examine population connectivity and mixing dynamics is essential to the development of marine population dynamics and management of fishery stocks (Cowen et al. 2000).

The current study is part of a collaborative project to examine red snapper population connectivity and mixing across the Gulf. The purpose of this study was to apply the otolith chemical nursery signatures identified in Chapter 1 to estimate population structure and connectivity in the western Gulf. Specifically, natural tags derived from otolith element:Ca and stable isotope ratios of age-0 red snapper collected gulf-wide were compared to core element:Ca and stable isotope ratios of sub-adult and adult red snapper collected from the western Gulf and Mexican portions of the Gulf. The objective was to gain better knowledge as to the source of recruits to the Texas continental shelf, as well as examine any potential mixing dynamics between Texas and Mexico.

Methods

Sample Collection

Adult red snapper were sampled from the northwestern Gulf (LA), western Gulf (TX), and, when available, from the southwestern Gulf off Veracruz, Mexico (MEX1) and the Campeche Banks (MEX2; see Figure 1.1). To correspond to nursery signatures developed for the 2005-2007 year classes, age-1 red snapper were targeted during the summers (May through August) of 2006-2008, age-2 red snapper were targeted during the summers of 2007-2008, and age-3 red snapper were targeted during the summer of 2008. The objective was to collect fifty red snapper per year class (2005, 2006, and 2007) for each region over a three year period,

equaling 1200 samples total ((n=50 x 1 year class x 4 regions) + (n=50 x 2 year classes x 4 regions) + (n=50 x 3 year classes x 4 regions) = 1200). Sub-adult and adult red snapper were collected onboard NMFS scientific surveys, from recreational landings around Port Aransas, TX and Port Fourchon, LA, and from bycatch samples from the Mexican shrimp fishery. Collecting samples from MEX1 and MEX2 was difficult to achieve and resulted in sampling occurring later in the winter (December through March), with no samples obtained in 2008. Red snapper total length (TL) was measured to the nearest mm and both sagittae were extracted either in the field or laboratory, rinsed free of associated tissue with double deionized water (ultra-pure 18 MΩ cm^{-1} water; $DDIH_2O$) and stored in individual paper coin envelopes until further laboratory analysis.

Otolith Preparation and Analysis

Otoliths were cleaned with a synthetic bristle brush to remove any adhering tissue, rinsed with $DDIH_2O$, and placed in polyethylene vials to air-dry under a class-100 clean hood. The left sagitta was used to determine fish age for each sample. Transverse sections of the otolith were viewed under a dissecting microscope with transmitted light to count opaque zones and accurately determine age (following the protocol of Patterson et. al. 2001a and Fischer et. al. 2002). Once age was verified, stratified random sampling was used to select up to 50 fish per region per year class in each summer of sampling for otolith coring and chemical analysis.

Right otoliths selected for chemical analysis were embedded in epoxy resin and a transverse section containing the core was cut with a Beuhler Isomet low-speed saw fitted with twin diamond blades separated by a 1.5 mm nylon spacer. Empty sections of epoxy resin from the same block containing the otolith were also cut and affixed to an acid-leached microscope slide with Loctite Super Glue Control Gel. Anterior and posterior ends of the associated epoxy

of the embedded transverse otolith section were then affixed to the empty epoxy section with Loctite Super Glue Control Gel, such that the glue did not come into contact with the otolith section. Otolith cores were removed from the embedded transverse section with a New Wave MicroMill precision drilling instrument. The empty epoxy resin section was used to protect the drill bit from possibly hitting the slide, as well as to prevent the otolith core from cracking during the drilling process. A pre-determined path based on average age-0 otolith transverse section perimeters of 20 red snapper samples was programmed into the MicroMill system to extract the age-0 core section of sub-adult and adult samples (Figure 2.1 A,B). The drilling process required 24 passes at 75 μm depth per pass with a scan speed of 85 μm per second at 80% drill speed. Otolith cores were easily extracted from the transverse section with this process (Figure 2.1 C). Extracted cores were placed in clear micro-centrifuge tubes and sent to the University of West Florida (UWF) to be prepared for elemental and stable isotope analyses as part of the collaborative study.

Extracted cores were cleaned prior to elemental or stable isotope analysis under a class-100 clean hood. Dried cores were weighed before and after cleaning to the nearest 0.01 mg. Whole cores were immersed in 1% ultrapure nitric acid (HNO_3) for 30 seconds to oxidize any material adhering to the surface, and then flooded repeatedly with $DDIH_2O$ to remove the acid. Cores were dried under a class-100 clean hood for at least 24 hours. Once dried and reweighed, cores were pulverized with an acid-leached mortar and pestle, and the resulting homogenized powder was divided. Half of the core powder was weighed to the nearest 0.1 mg and then dissolved in an acid-leached high-density polyethylene (HDPE) vial by adding 1% ultrapure HNO_3 until a dilution factor of approximately 1,000x was achieved. Although dissolution typically was complete within 1 hour, samples were not manipulated for at least 24 hours once

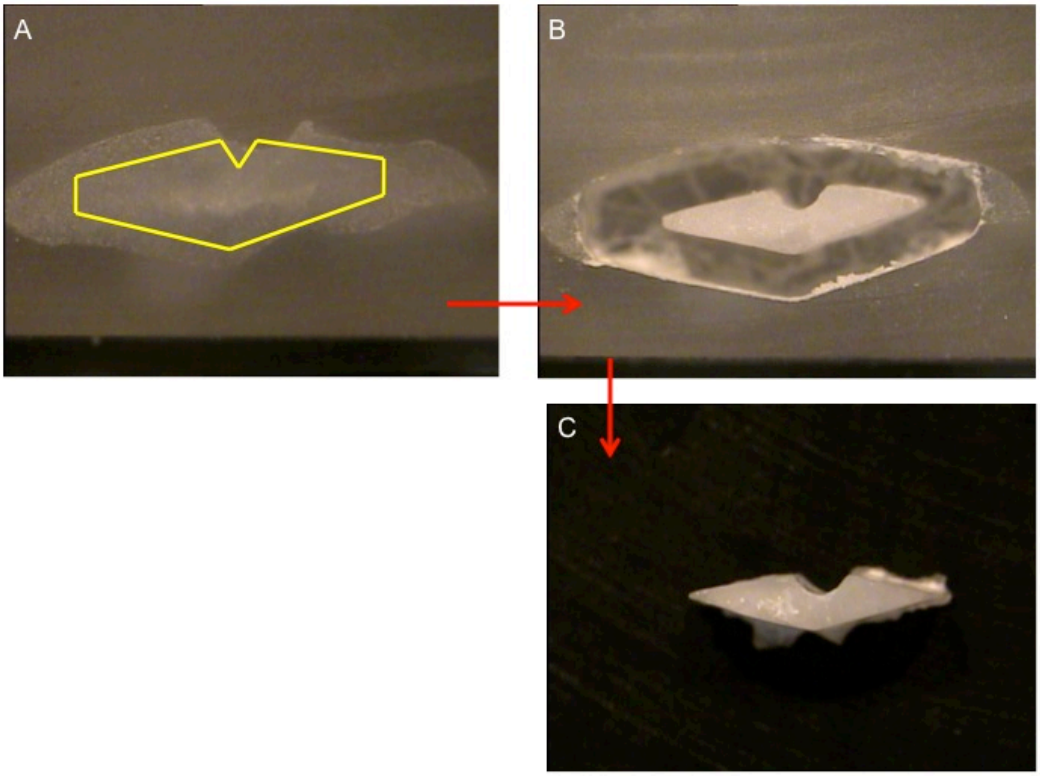

Figure 2.1. Transverse section of an adult red snapper, *Lutjanus campechanus*, sagittal otolith depicting a yellow outline of the template pattern (A) used to extract the age-0 core with a MicroMill precision drilling instrument (B). The resulting intact extracted core is represented in image C.

acid digestion began. Aliquots (5 ml) of the core solutions were sent to the Department of Marine Sciences at the University of Southern Mississippi for trace elemental analysis with a Finnigan MAT Element2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS). Core solutions were spiked with Indium at a concentration of 2.5 parts per billion (ppb) as an internal standard and then analyzed for ^{137}Ba, ^{48}Ca, ^{7}Li, ^{55}Mn, ^{25}Mg and ^{86}Sr. Blanks were prepared from 1% ultrapure HNO_3 and processed through the same stages of sample preparation as sample solutions. Blanks were analyzed concurrently with sample solutions to estimate instrument limits of detection (LOD), which were estimated as three standard deviations of mean blank values. Instrument performance and matrix effects were checked by assaying elemental

concentrations of an otolith standard reference material (SRM) prepared from adult red snapper otoliths (Sturgeon et al. 2005). Solutions of the SRM were prepared and analyzed similarly to red snapper otolith core samples.

The other half of the otolith core powder was placed into 2 ml microcentrifuge tubes and sent to the Stable Isotope Laboratory in the Department of Geology at the University of California at Davis for stable isotope ($\delta^{13}C$ and $\delta^{18}O$) analysis with a Finnigan MAT 251 isotope ratio mass spectrometer (IR-MS). The instrument was calibrated against the International Atomic Energy Agency's carbonate standard, NBS-19. The isotopic composition of otolith cores are reported in standard δ notation relative to standards (V-PDB reference standard for $\delta^{13}C$ and standard mean ocean water for $\delta^{18}O$) with the standard equation:

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

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

Statistical Analysis

Prior to statistical analysis, element:Ca ratios first were ln transformed to correspond to the constituents of the previously reported nursery signatures in Chapter 1. Then, cohort- and year-specific residual values were computed by subtracting mean element:Ca and stable isotope ratios from each respective sample ratio. This process was repeated for cohort-specific age-0 red snapper element:Ca and stable isotope ratios presented in Chapter 1. Residuals were computed for age-0 and core chemical signatures to remove extraneous sources of variance (i.e., ontogenetic effects of disproportionate primordium representation in cored otoliths versus original 3-dimensional structure of age-0 otoliths, instrument drift between sample analysis of age-0 and core samples, etc.) when estimating the source regions of sub-adult and adult samples (Thorrold et al. 2001, Barnett & Patterson 2010).

A maximum likelihood mixed-stock analysis 'HISEA' developed by Millar (1990) was used to estimate the source of recruits to a given region in a given sampling year. The baseline data set consisted of residual values of age-0 red snapper otolith nursery signatures. Sub-adult and adult core otolith signature residuals were classified as unknowns, or mixed data, against the age-0 baseline data to estimate their natal origins based on maximum likelihood estimates (MLE) of mixed-stock proportions. Mixed data for each region in each age group for each year sampled was classified individually into year class-specific and pooled year class baseline data. Direct MLE of nursery sources and standard deviations were computed in HISEA by bootstrapping with 1,000 resampled baselines.

Results

A total of 1,338 sub-adult and adult red snapper was collected from four Gulf regions and aged. Based on these age estimates, only 725 individuals corresponding to designated regions and cohorts were cored for otolith chemical analysis (Table 2.1; see also Appendix B). Unfortunately, when adequate MEX1 and MEX2 samples were obtained, they usually did not correspond to study year classes, resulting in low sample sizes for these regions. All 5 elements (Ba, Ca, Li, Mn, Mg, Sr) were present in concentrations at least two orders of magnitude above detection limits, and stable isotope delta values were within 1% of accepted values for IR-MS analysis.

Mean concentrations and natural variability of element:Ca and stable isotope ratios varied across regions and year classes, as would be expected based upon similar trends of age-0 baseline data (see Figure 1.3; see also Appendix A). Variations in element:Ca and stable isotope ratios also existed among age groups within a single cohort. For the 2005 cohort, age-2 red snapper otoliths collected from LA had higher Ba:Ca, Mg:Ca, and Mn:Ca concentrations

Table 2.1. Sample size and size range of sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four regions across the Gulf of Mexico during the summers of 2006, 2007 and 2008. LA = Louisiana; TX = Texas; MEX1 = Veracruz, Mexico; MEX2 = Campeche Banks, Mexico.

Sampling Year	Age	Region	Samples Cored and Analyzed	Size Range (mm TL)
2006	1	LA	51	153 - 241
		TX	52	151 - 226
		MEX1	18	250 - 280
		MEX2	3	240 - 250
2007	1	LA	56	151 - 235
		TX	44	153 - 258
		MEX1	31	230 - 380
		MEX2	3	240 - 280
	2	LA	55	186 - 443
		TX	60	232 - 348
		MEX1	50	240 - 320
		MEX2	1	480
2008	1	LA	50	152 - 209
		TX	50	151 - 237
		MEX1	-	-
		MEX2	-	-
	2	LA	50	220 - 410
		TX	50	165 - 422
		MEX1	-	-
		MEX2	-	-
	3	LA	50	335 - 470
		TX	50	301 - 457
		MEX1	-	-
		MEX2	-	-

compared to the other age groups, there was a steady decrease in Li:Ca concentrations as age increased, and only $\delta^{13}C$ remained constant and comparable to baseline nursery ratios (Figure 2.2; see also Appendix B). Texas red snapper otolith concentrations for the 2005 cohort remained constant across age groups, except for a similar increase in age-2 otolith Ba:Ca, Mg:Ca and Mn:Ca concentrations. The 2005 cohort MEX1 red snapper otolith concentrations remained constant across age groups except for Li:Ca, $\delta^{13}C$ and $\delta^{18}O$, and MEX2 otolith concentrations decreased between age groups for every element except $\delta^{18}O$, which increased. For the 2006 cohort, LA red snapper otolith concentrations remained fairly constant between age groups except for a decrease in Mg:Ca and Mn:Ca (Figure 2.3; see also Appendix B). Texas red snapper otolith concentrations for the 2006 cohort only remained constant between age groups for Li:Ca and $\delta^{13}C$, and also exhibited the same decrease patterns as LA samples. Minor fluctuations were observed between the 2006 cohort MEX1 and MEX2 red snapper otolith concentrations and baseline age-0 data. For the 2007 cohort, LA red snapper otolith concentrations were comparable to corresponding baseline age-0 samples except for being more enriched in $\delta^{18}O$ (Figure 2.4; see also Appendix B). The 2007 cohort TX red snapper otolith concentrations were lower in Ba:Ca and Mg:Ca, and also more enriched in $\delta^{18}O$ compared to baseline data. Interestingly, $\delta^{18}O$ ratios increased in LA and TX otolith concentrations for each age group within each cohort compared to baseline age-0 nursery data.

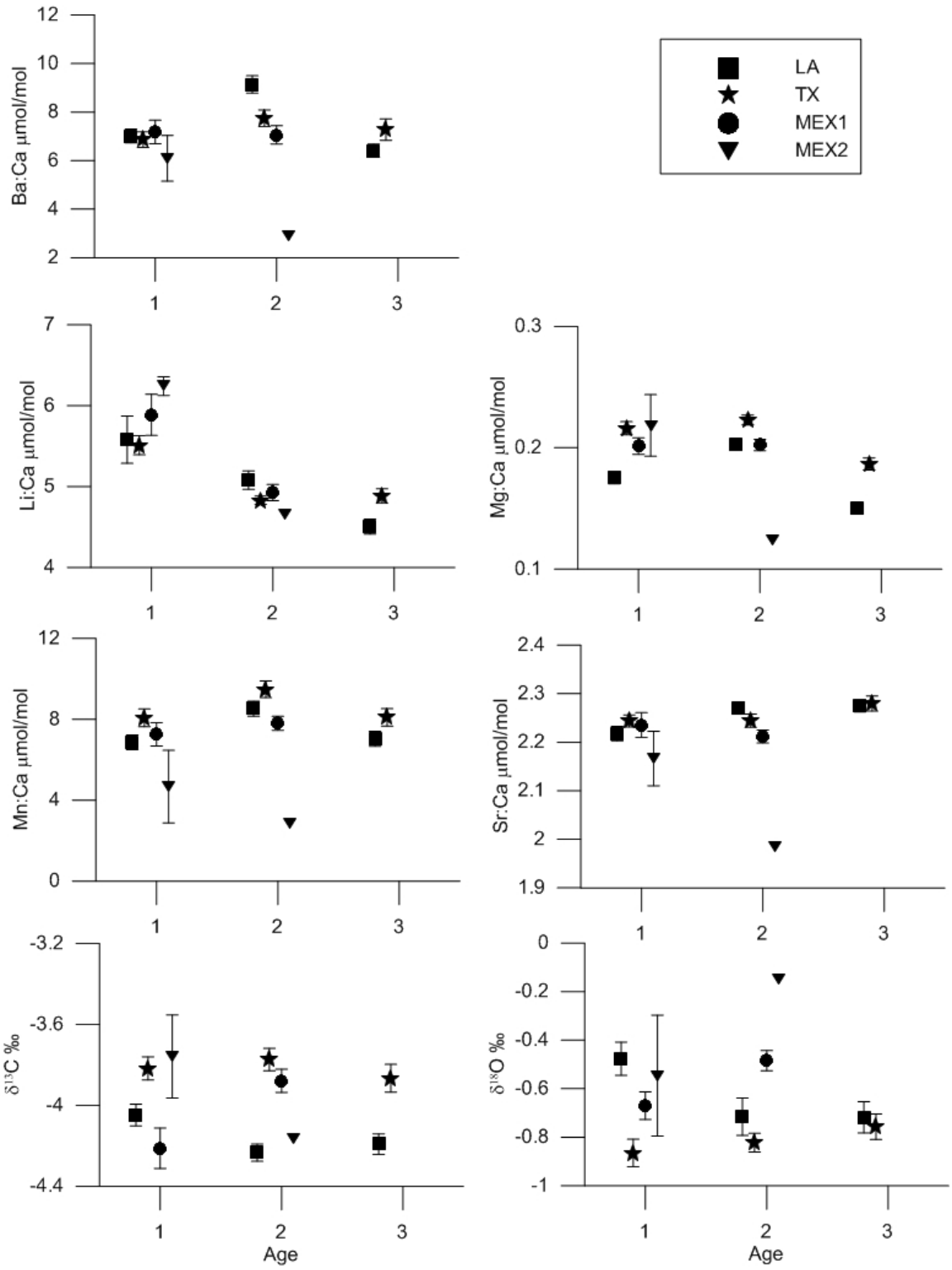

Figure 2.2. The 2005 cohort mean (± SE) region- and age-specific otolith core element:Ca or stable isotope delta ratios for sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico regions during the summers of 2006, 2007 and 2008.

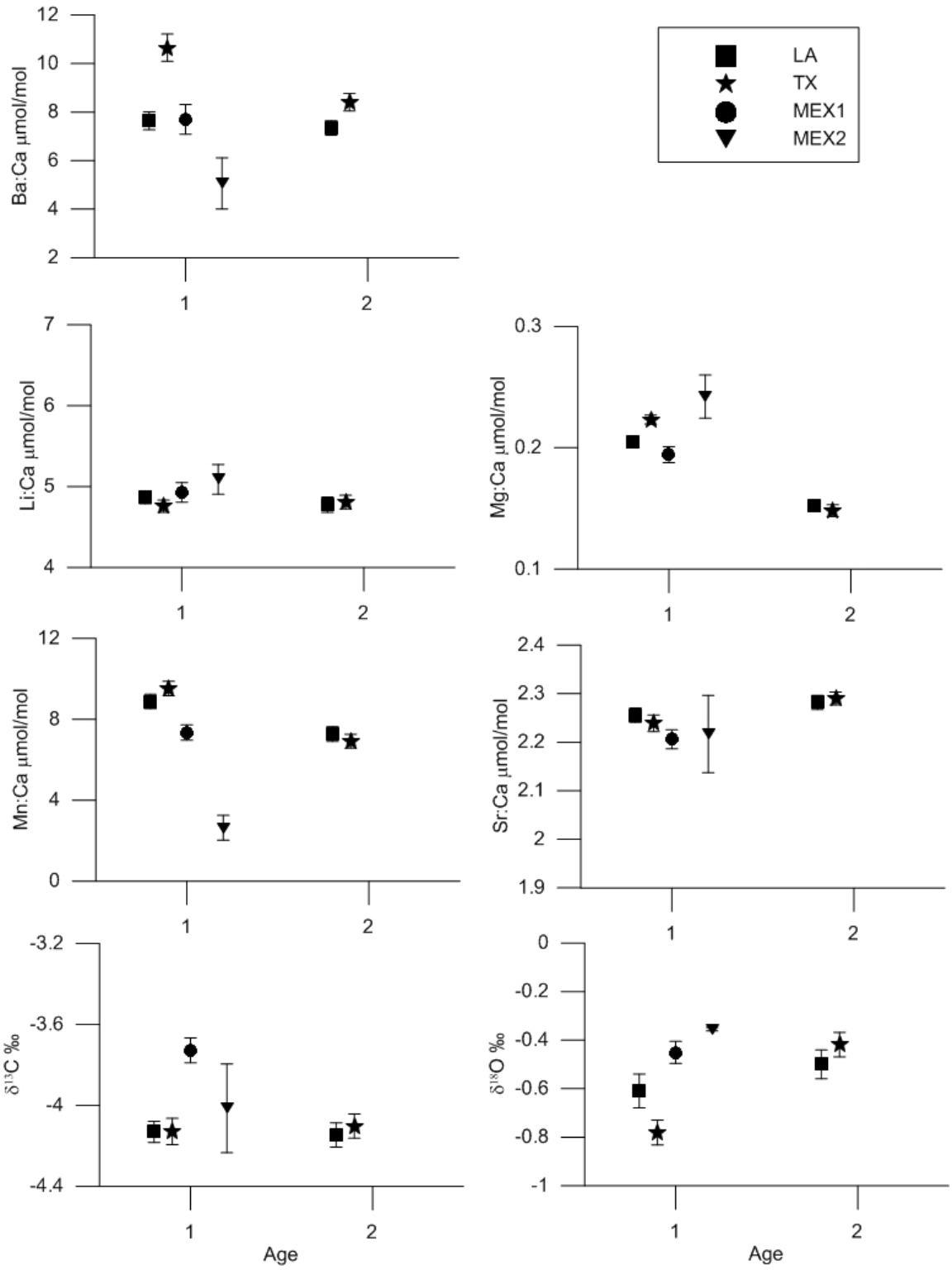

Figure 2.3. The 2006 cohort mean (± SE) region- and age-specific otolith core element:Ca or stable isotope delta ratios for sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico regions during the summers of 2007 and 2008.

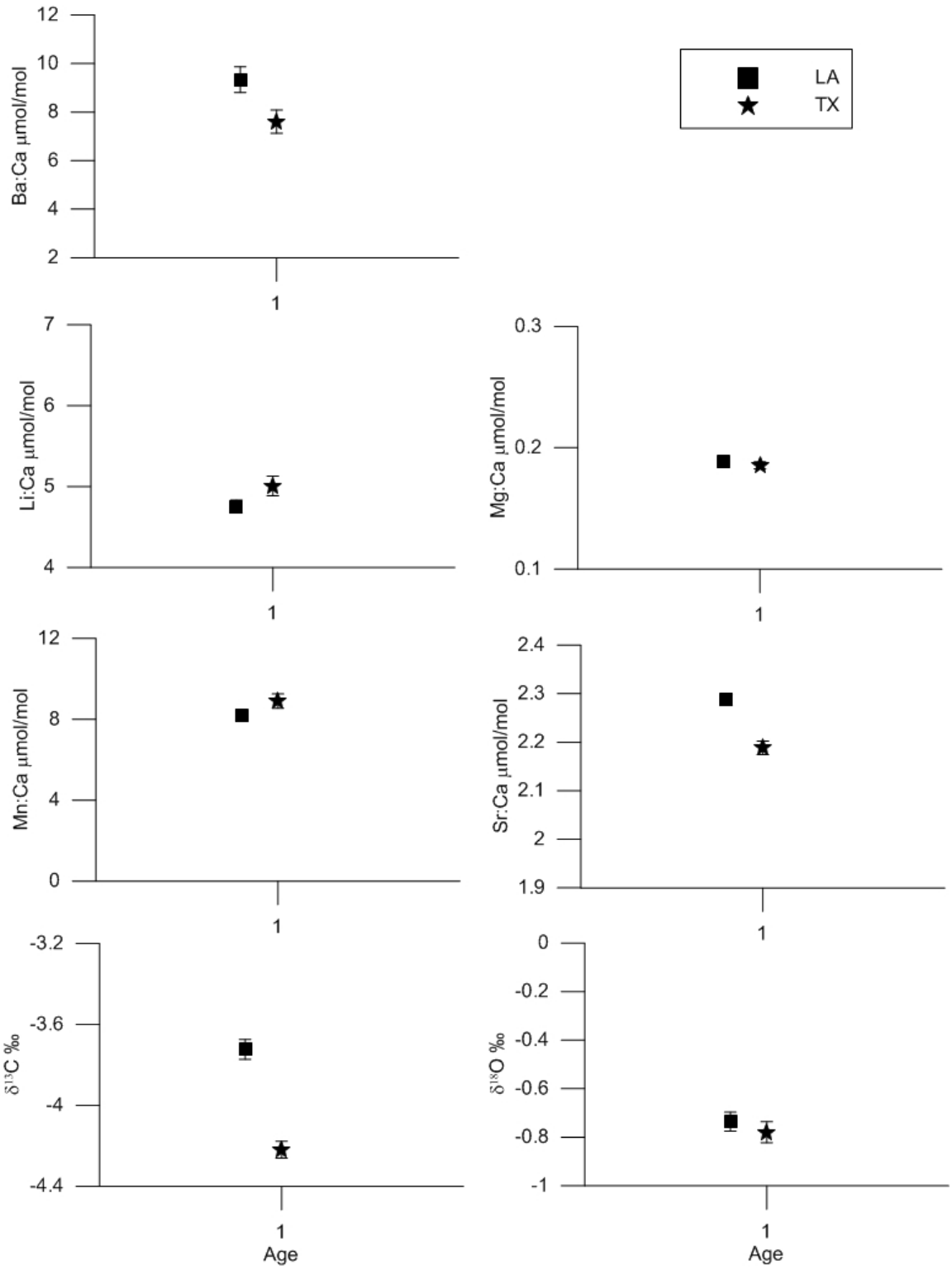

Figure 2.4. The 2007 cohort mean (± SE) region- and age-specific otolith core element:Ca or stable isotope delta ratios for sub-adult and adult red snapper, *Lutjanus campechanus*, collected from two Gulf of Mexico regions during the summer of 2008.

Maximum likelihood estimates indicate red snapper sampled from LA for the 2005 cohort exhibited an increase from 37.3% to 82.7% in locally derived fish as age increased. The secondary source of recruits to LA was estimated to be the AL nursery region, the contribution from which decreased from 37.3% to 13.1% as age increased (Figure 2.5). Estimates for the TX 2005 cohort fluctuated in locally derived fish from 52.4% to 88.4% to 54% for age-1, age-2 and age-3 fish, respectively. This sampling region also displayed a decrease in estimated AL derived fish (40 - 25%) and an increase in LA derived fish (7 - 20%) across ages 1-3. Although MEX1 and MEX2 age-1 and age-2 samples were collected for the 2005 cohort, baseline nursery data was not available for these regions and, thus, they were not included in the HISEA models for the 2005 cohort. For the 2006 cohort age-1 samples, LA fish were estimated to consist mainly of locally derived (32.2%) and MEX1 recruits (35%) with even contributions from AL and TX (16.5% and 15.8%, respectively). Texas fish were estimated to be largely locally derived (81.3%). Samples from MEX1 were estimated to be locally derived (52.4%) with a secondary source from TX recruits (26.8%), while MEX2 fish were estimated to be 100% locally derived. For the 2006 cohort age-2 samples, LA source estimates remained divided between locally produced (32.4%) and MEX1 recruits (33.9%), but with an increase in TX recruits (20.8%). Nursery source estimates for TX fish displayed a decrease in local recruits (30.5%) with the larger source now originating from MEX1 (45.9%) and an increase in LA recruits (17.8%). The 2007 cohort age-1 red snapper from LA were estimated to be primarily locally derived (79.78%) with a secondary source of recruits originating from MEX1 (17.6%). Texas samples were estimated to be sourced primarily from LA (71.8%) followed by local recruits (22%).

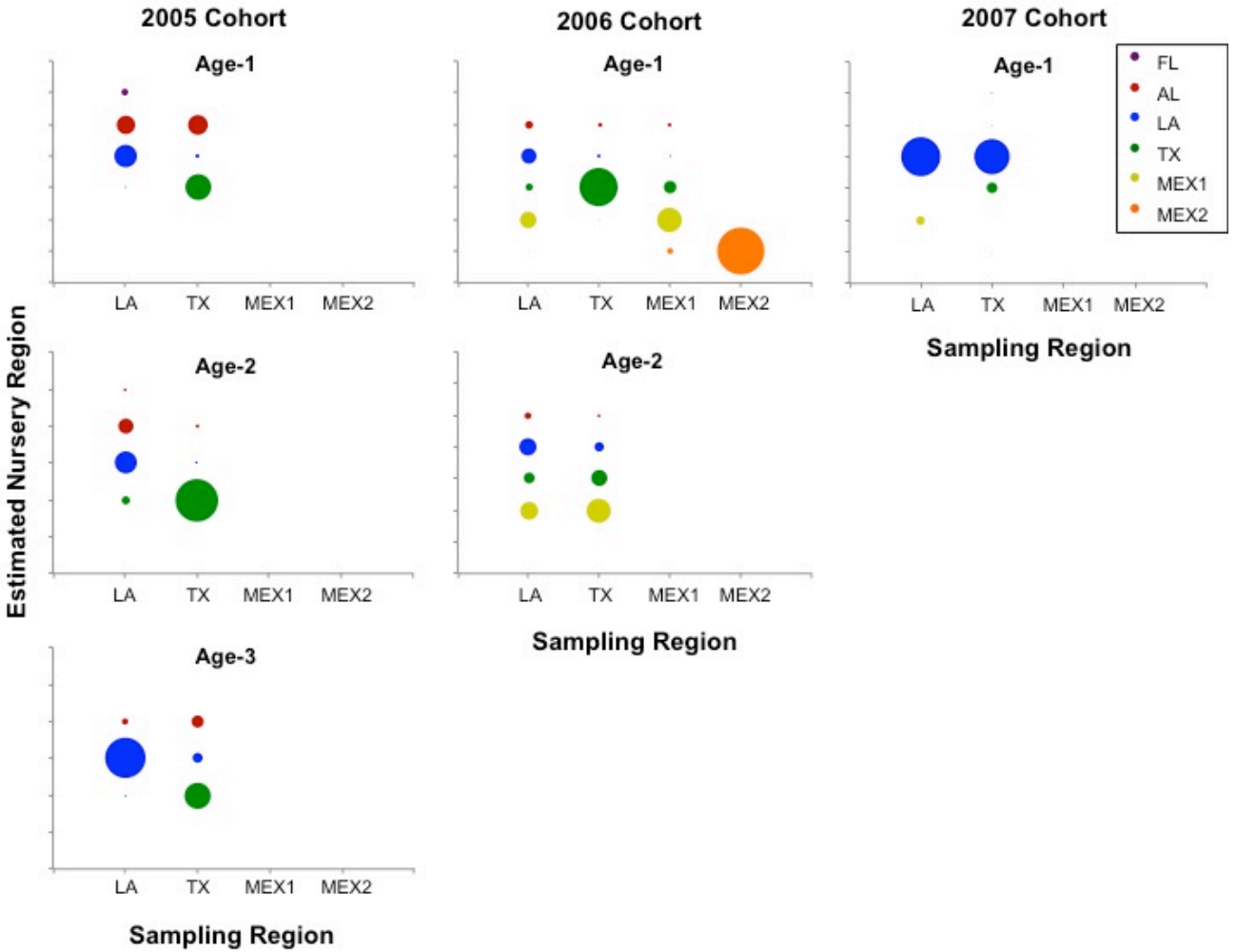

Figure 2.5. Bubbleplots of percent composition estimates derived from year class-specific otolith chemistry-based maximum likelihood estimate analysis indicate the nursery of origin of sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico sampling regions during the summers of 2006, 2007 and 2008. Columns are cohort-specific, panels indicate fish age, and bubbles are scaled by diameter.

When analyzing MLE of nursery sources with pooled year class baseline data, several of the same trends emerge with some minor differences (Figure 2.6). For the 2005 cohort, LA red snapper again exhibit an increase in estimates of locally derived fish as age increased (41.6 to 98.3%). However, the secondary source of recruits now originated from MEX1 for age-1 red snapper only (37.4%), with little to no influence from AL. The same fluctuating pattern was also observed for locally derived recruits to the TX sampling region (28.6 – 75.7 – 34.9%) with a decrease in AL recruits (59.4 to 36.8%) and an increase in LA recruits for age-3 samples (23.2%). The age-1 red snapper from MEX1 were estimated to be sourced largely from LA (49.3%), with a secondary source from AL (36.4%). The age-2 fish from MEX1 again were estimated to be primarily sourced from LA (31.7%), with even contribution between locally derived and AL recruits (29% each). The MEX2 age-1 red snapper were estimated to be primarily locally derived, however this is based on a low sample size (n = 3), and age-2 fish could not be analyzed due to a sample size of one. For the 2006 cohort red snapper, estimates of LA local recruits increased as age increased (45.3 – 74.4%), but MEX1 influence was estimated to remain high while AL influence decreased. Texas red snapper were no longer estimated to be locally derived, but instead dominated by LA and AL recruits (29.5% and 30.9%, respectively) for age-1 samples and by LA and MEX1 recruits (31% and 64.6%, respectively) for age-2 samples. The 2006 cohort age-1 MEX1 fish were estimated to be locally derived (81.5%), while MEX2 fish were estimated to be 100% locally derived. For the 2007 cohort age-1 red snapper, the LA nursery again dominated estimates of the sources of recruits to TX (98.4%), but LA fish were now divided between local (30.7%) and MEX1 (63.8%) recruits.

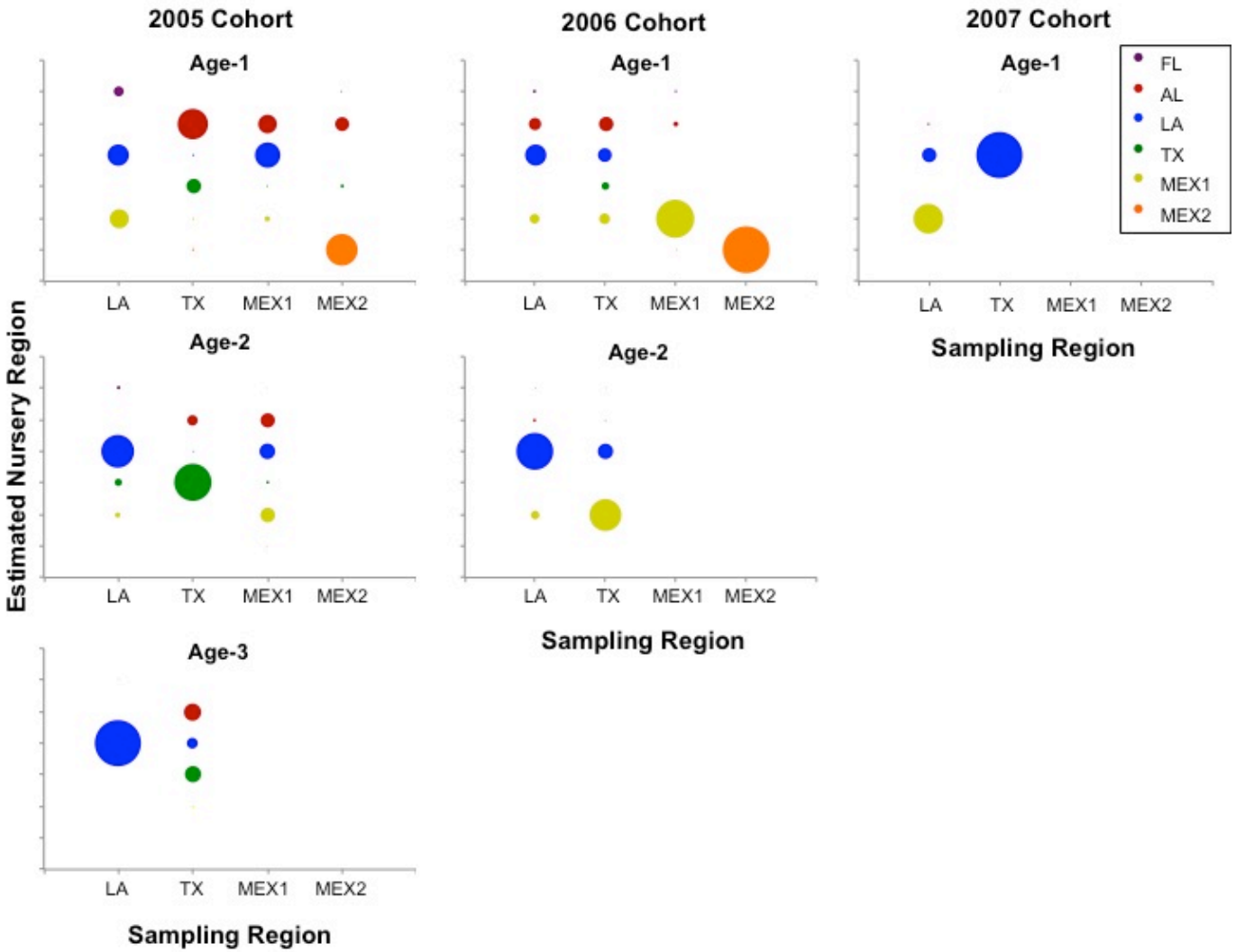

Figure 2.6. Bubbleplots of percent composition estimates derived from pooled year class otolith chemistry-based maximum likelihood estimate analysis indicate the nursery of origin of sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico sampling regions during the summers of 2006, 2007 and 2008. Columns are cohort-specific, panels indicate fish age, and bubbles are scaled by diameter.

Discussion

Otolith chemical signatures have proven to be effective for estimating the relative contribution of recruits from different nursery areas to adult populations (Gillanders & Kingsford 2000; Thorrold et al. 2001; Rooker et al. 2008). Using otolith chemical signatures as natural tags to evaluate population dynamics relies on the assumptions that otoliths are metabolically inert prohibiting the reabsorption of deposited elements and that elements incorporated onto the growing otolith surface are influenced by ambient water conditions (Campana 1999). The practicality of using otolith chemical concentrations as natural tags is also partially dependent upon the temporal stability of the signature. Inter-annual stability of chemical signatures is desired to avoid the need to develop yearly baseline data. Patterson et al. (2008) and Chapter 1 showed significant differences among year classes for age-0 red snapper otolith chemical signatures. However, when all year classes were combined in Chapter 1, the classification accuracy was relatively high (72.41%). Therefore, both cohort-specific and pooled year class chemical signatures were analyzed to test the effectiveness of the pooled chemical signatures and evaluate collection gaps within the data.

The overall objective of this study was to determine the source of recruits to the Texas continental shelf by using otolith chemical signatures from six nursery regions in the Gulf. Based on MLE results using cohort specific signatures, TX red snapper populations appear to be either locally derived or largely comprised of LA recruits, with only a couple of exceptions. First, age-1 red snapper collected in 2006, corresponding to the 2005 cohort, show a moderate influence of AL recruits. Larval transport estimates have shown that only a small portion of the western substock larvae would be able to cross the Mississippi River plume, but would most likely be transported away from the continental shelf with less chance of survival. Conversely,

during September and October, strong westerly winds would allow the eastern substock larvae to cross closer to the Mississippi River mouth with greater chance of survival and genetic mixing (Johnson et al. 2009). Similar migration patterns would need to continue for juvenile and sub-adult red snapper to be evident in otolith chemical signatures as the larval portion (initial 4 week planktonic period; Johnson et al. 2009) of the otolith would be too small to influence the signature. Further, the AL and LA red snapper otolith nursery signatures were shown to be similar in Chapter 1, which could cause discrepancies in discerning the source of recruits between these regions. However, estimates of an AL source of recruits diminish and LA recruits increase as age increased for red snapper collected off TX within the same cohort. Thus, similarities between AL and LA nursery chemical concentrations may not be an issue, and AL could in fact be a recruitment source to TX. Secondly, higher percentages of MEX1 recruits were evident among the 2008 TX red snapper, referring to the 2006 cohort age-2 samples. Interestingly, these recruits occur in similar proportions for LA red snapper as well. Although there was overlap between LA, TX and MEX1 nursery otolith signatures for the 2007 cohort, the 2006 cohort nursery signatures for MEX1 were distinctively separate from TX nursery signatures. Therefore, MEX1 may be another potential recruitment source of TX red snapper. It is also interesting to note that the fluctuating pattern of local recruits to TX samples for the 2005 cohort and the decrease of local recruits for the 2006 cohort is reflected in the Mg:Ca and Mn:Ca mean concentrations. Based on Chapter 1 results, these elements appeared in higher concentrations for TX nursery otolith signatures. Thus, these results further confirm Mg and Mn as TX nursery markers.

Previous red snapper otolith chemistry studies have indicated that significant post-settlement movement occurs between the northwest (LA) and southwest (TX) Gulf regions

(Cowan et al. 2003; Patterson 2007). In the present study, moderate to high percentages of LA recruits were observed among TX red snapper, but only a small percentage of TX recruits were observed among LA samples. Thus, it would appear that the LA region is an important source of recruits to the TX red snapper population based on the year classes examined. The current study also shows that LA red snapper populations may be predominantly composed of locally recruited fish. There was a high percentage of AL derived fish for the 2005 cohort age-1 samples, but again, a high degree of uncertainty exists in the connectivity between AL and LA due to the high misclassification rates in their age-0 otolith chemical signatures. However, the percentage of estimated AL recruits decreases as age of LA red snapper increases, possibly inferring some contribution to the younger age groups. The 2006 cohort samples from LA were almost evenly partitioned among all nursery areas except FL. The LA nursery area did have the lowest classification successes for age-0 otolith chemical signatures reported in Chapter 1. Thus, these results simply could be a reflection of low classification success. Gold and Saillant (2007) estimated that the genetic effective population size of LA red snapper is ten-fold higher than red snapper collected from AL and TX. Furthermore, the 2009 red snapper stock assessment indicated that age distribution in the eastern Gulf is truncated compared to the western Gulf, and the eastern substock is projected to have lower productivity than the western substock (SEDAR 2009). Therefore, despite uncertainties in nursery chemical signatures, observed MLE percentages indicate the importance of LA as a source of recruits to the western Gulf red snapper substock.

Due to the unfortunate unbalanced design of the MEX regional data, only the 2006 cohort age-1 samples could be analyzed unless nursery chemical signatures were pooled across all year classes. For the 2006 cohort, MEX2 red snapper were estimated to be locally derived, while

more than 70% of MEX1 fish were composed of local and TX recruits. When examining MLE results based on pooled year class data, MEX2 red snapper still were estimated to be primarily locally derived. Although the 2005 cohort age-1 samples from MEX2 appear to be influenced by AL recruits, this result is based on one out of three total samples for the region and thus not conclusive. The 2005 cohort age-1 red snapper from MEX1 were were estimated to be composed of AL and LA recruits, while the age-2 fish were mainly composed of LA recruits with similar contributions from AL and local recruits. When baseline age-0 nursery signatures were combined for all year classes, MEX1 signatures were similar to AL, LA and TX signatures (see Chapter 1). Thus, data were too inconclusive to determine the source of recruits to the Mexico red snapper populations.

Minor differences were observed when examining MLE derived from pooled year class signatures. For the 2005 cohort, the proportion of locally derived LA red snapper still were estimated to increase with age, however a secondary MEX1 influence replaced the AL influence based upon cohort specific nursery signatures. This may reflect the presence of MEX1 recruits among LA red snapper when analyzing MLE based on year class-specific signatures. The other notable change was TX red snapper for the 2006 cohort were estimated to be composed of more LA recruits than local recruits. This change was interesting and reflected the strong LA influence in the composition of the 2007 cohort. However, because significant differences were observed among year classes for age-0 otolith chemical signatures, evaluating MLE based on pooled year class signatures should be interpreted with caution.

Previous otolith chemistry studies indicated limited movement of red snapper in the first year of life (Cowan et al. 2003; Patterson 2007; Patterson et al. 2008), but results of the current study may indicate mixed movement patterns among cohorts. The 2005 cohort age-1 red

snapper exhibited moderate contribution from all nursery regions that decreased to more locally derived recruits as age increased. This may suggest that red snapper are capable of moving over longer distances during the juvenile stage than previously inferred. It could be speculated that the active 2005 hurricane season, which included hurricanes Katrina and Rita, may be responsible for the large movement of age-1 red snapper (Patterson et al. 2001b). Nonetheless, 2005 age-0 red snapper used in the development of nursery otolith chemical signatures were collected after the major hurricane impacts and exhibited the highest classification success, making a hurricane effect less likely. Conversely, the 2006 cohort age-1 red snapper exhibited more locally derived recruits with an increase in other nursery region contributions for the age-2 samples, suggesting increased movement with age. While the 2007 cohort showed strong movement in one direction from LA to TX. The 2005 and 2006 cohorts were strong year classes compared to the 2007 cohort (SEDAR 2009; Cowan 2011; Saari 2011), and may partially explain why higher mixing rates were evident for those cohorts. However, much of this is speculation as a sample size of n = 30 for each nursery region per year class may be too small to accurately discriminate sources of recruits for sub-adult and adult populations. Increasing the sample size and age groups examined may allow better resolution for understanding mixing dynamics of red snapper populations.

Despite collection flaws and lack of distinctiveness of otolith chemical signatures for age-0 red snapper collected from northern Gulf regions, one constant trend was evident regardless of the MLE model used. A moderate to strong contribution of LA recruits was apparent for red snapper sampled from TX. Unfortunately, connectivity between the western Gulf and MEX regions is inconclusive at this time and more data is required before inferences can be made. It has been estimated that most of the recent increase in Gulf red snapper SSB has occurred in the

western Gulf and this is projected to continue into the near future (SEDAR 2009). Based on the results of this study, the center of abundance off the coast of Louisiana may be expanding outward towards the TX continental shelf. Future work should also determine if the population recovery in the western Gulf is contributing to the relatively recent reappearance of red snapper in the far eastern Gulf as well. Determining population connectivity between eastern and western red snapper substocks would be beneficial to the development of efficient red snapper regional management.

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CHAPTER 3: SPATIAL DIFFERENCES IN RED SNAPPER OTOLITH MICROCHEMISTRY DERIVED FROM TRACE METALS ASSOCIATED WITH OIL AND GAS PLATFORMS

Introduction

In 1947, Kerr-McGee Oil Industries installed the first offshore oil well 70 km south of Morgan City, LA in 5.6 m of water (Kasprzak 1998). Today, according to estimates by the Bureau of Ocean Energy Management (BOEM), there are approximately 4,000 offshore oil and gas production platforms in the Gulf of Mexico (Gulf), with a majority located off the coast of Louisiana. The addition of production platforms to the northern Gulf has resulted in the largest unplanned artificial reef complex, perhaps in the world, increasing reef habitat by 4.1% (10.4% for LA alone; Stanley and Wilson 2003) to an ecosystem composed primarily of mud and sand substrate (Parker et al. 1983). While there has been support for artificial reef development, debates regarding their effectiveness still persist. Do artificial reefs produce new fish biomass or are fish simply attracted to them due to behavioral preferences? If artificial reefs are providing new habitat in a substrate-limited environment, they could potentially help increase fisheries production. However, if fish are aggregating to new, well-marked habitat (i.e. platforms) without producing new biomass, then unmanaged fishing could lead to a decline in reef-associated fish stocks. When overfishing becomes a problem, platforms are less likely to increase production, but instead make remaining fish populations more vulnerable to fishing pressures (Bohnsack 1989).

Red snapper, *Lutjanus campechanus*, is a commercially important reef-associated fish in the Gulf that is currently overfished due to high exploitation rates of directed and shrimp fisheries (GMFMC 2010). Typical of reef-associated fish, red snapper tend to aggregate near structured environments on the sea floor, but are not dependent on such habitat to complete their

life cycle. Newly settled juveniles are attracted to low-profile reefs, relic-shell habitats and adjacent mud/sand bottom habitats (Rooker et al. 2004: Szedlmayer and Howe 1997; Wells et al. 2008a). As red snapper mature, a natural ontogentetic shift in habitat occurs resulting in movement to more complex natural and artificial reef habitats, including oil and gas platforms. Although it has been observed that red snapper recruit to platforms as early as age-1, platform populations are primarily dominated by age-2 and age-3 fish (Nieland and Wilson 2003; Gitschlag et al. 2003). It is unclear as to whether this recruitment pattern is attributable to attraction or production. The decrease in individuals older than age-3 on platforms may be caused by emigration, low site fidelity or reduced recruitment of older fish. However, by age-2 and age-3 red snapper enter the directed fishery and high fishing pressure at platforms may result in fewer older individuals at these habitats (Nieland and Wilson 2003; Patterson 2007). Therefore, further research to examine recruitment and movement patterns associated with platforms could prove beneficial to red snapper management strategies.

The use of otolith (ear stone) microchemistry to develop natural tags has become an effective tool among fishery scientists to examine movement patterns of adult stocks (Gillanders and Kingsford 1996; Thorrold et al. 2001; Rooker et al. 2008). To achieve this, nursery signatures are developed by analyzing elemental and stable isotope concentrations accreted onto juvenile fish otoliths from surrounding waters. Since the otolith precipitates as the fish grows and is metabolically inert (Campana 1999), the juvenile portion, or core, of an otolith can be used to identify the nursery of origin of an adult fish and thus be used to examine movement patterns. However, nursery signatures in otoliths based on the usual suite of elements examined (Ba, Li, Mg, Mn, Sr) can differ among years due to temporal variability in temperature, salinity and water mass characteristics (Gillanders and Kingsford 2000; Rooker et al. 2001), requiring

cohort specific signatures to be identified. Spencer et al. (2000) determined that lead isotopes based on anthropogenic sources could be detected in otoliths and used to reconstruct the nursery of origin in Hawaiian estuaries. Thus, it may be possible to avoid chemical concentration variations of elements in otoliths by establishing signatures based upon a known anthropogenic source in a particular area.

Oil spills, drilling fluids and cuttings, produced water, protective antifouling paints and sacrificial anodes associated with oil and gas platforms all have the potential to release toxic chemicals into the surrounding water column and sediments. Several trace metals found in drilling fluids and produced waters (Ag, Ba, Be, Cd, Cr, Cu, Fe, Ni, Pb and Zn) have been detected at significantly higher levels than natural marine sediments and seawater (Neff et al. 1987). In a pilot study, Nowling et al. (2011) tested whether oil and gas platforms impart a detectable signature in the otoliths of adult red snapper. That study proved successful in identifying unique otolith chemical signatures for oil and gas platforms off the Louisiana coast, as well as unique signatures for artificial reefs east and west of the Mississippi River.

The primary purpose of this study was to determine if oil and gas platforms impart detectable chemical signatures in red snapper otoliths collected from a broader geographical range over multiple (2) years. Specifically, natural tags derived from otolith trace metal concentrations were used to examine temporal and geographical stability of platform signatures among three regions and two habitats on the continental shelf of the northern Gulf. Region- and habitat-specific chemical signatures were developed to determine if discriminant classifications were strong enough to validate the use of platform signatures to estimate the percent contribution of platform-reared recruits to regions devoid of platforms.

Methods

Sample Collection

Red snapper were collected during the summers 2007 and 2008 off the coasts of Port Aransas and Galveston, Texas (TX), Port Fourchon, Louisiana (LA) and Dauphin Island, Alabama (AL; Figure 3.1). Within LA, samples were collected in the Ship Shoal (SS), South Timbalier (ST) and Grand Isle (GI) federal (BOEM) mineral leasing areas. The objective was to collect 1000 red snapper each year with 300 coming from TX, 500 from LA and 200 from AL. Fish were collected from two habitat types within each region; oil and gas platforms (both standing and toppled) and non-platform habitats (natural bottom, artificial cement reefs, and wrecks). It is important to note that decommissioned oil and gas platforms that had been toppled to serve as artificial reefs were still categorized as platform due to the fact that potential contaminants would remain in the area. Fish collected within 50 m of oil and gas platforms were also categorized as being collected from platforms. Red snapper samples were collected from recreational landings, the Dauphin Island Deep Sea Fishing Rodeo, sampling trips aboard the *R/V Acadiana*, and the National Marine Fisheries Service's (NMFS) Vertical Longline Survey.

Due to large sample sizes, red snapper otolith extraction occurred in the field. Both sagittae were extracted, rinsed free of associated tissue with deionized (DI) water and stored in individual paper coin envelopes until further laboratory analysis. Fish total lengths (TL) were measured to the nearest mm; however measurements were not obtained for 451 individuals (23% of all individuals sampled). Estimated fish length was calculated based upon power relationships between TL and otolith weight (mg; Pawson 1990). Total length was strongly correlated with otolith weight in red snapper ($y = 16.487x^{0.530}$, $r^2 = 0.947$) and this relationship was used to

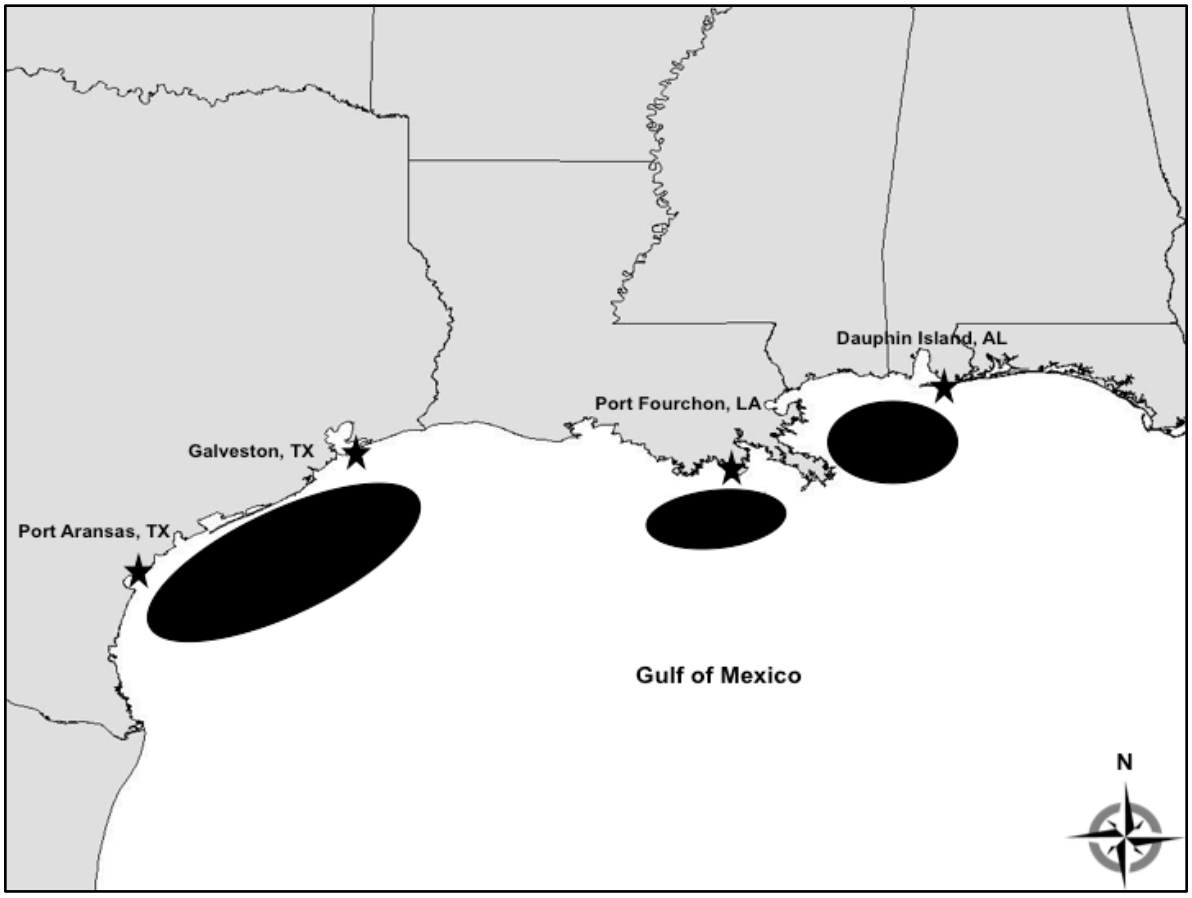

Figure 3.1. Sampling regions along the continental shelf of the northern Gulf of Mexico (Gulf) where adult red snapper, *Lutjanus campechanus*, were sampled on platform and non-platform habitats during the summers of 2007 and 2008.

approximate TL of the individuals that were not directly measured in the field. Red snapper with a TL between 250 – 650 mm were targeted to obtain a majority of fish between ages two through six years (Fischer et al. 2004; Saari 2011).

Otolith Preparation and Analysis

In the laboratory, sagittae were cleaned with a synthetic bristle brush to remove any adhering tissue, rinsed with DI water and placed in polyethylene vials to air-dry under a class-100 clean hood. The rest of the procedures occurred in a class-100 clean room under laminar flow using acid-washed supplies. Materials and solution blanks were tested before sample

preparation to insure there were no sources of contamination. Right sagittae were selected for trace elemental analysis for the following elements: ^{107}Ag, ^{109}Ag, ^{27}Al, ^{11}B, ^{138}Ba, ^{209}Bi, ^{111}Cd, ^{114}Cd, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{7}Li, ^{55}Mn, ^{98}Mo, ^{60}Ni, ^{206}Pb, ^{123}Sb, ^{120}Sn, ^{205}Tl, ^{238}U, ^{51}V, ^{64}Zn, ^{66}Zn. Dry otoliths were weighed before and after cleaning to the nearest 0.01 mg. Whole otoliths were immersed in 1% ultra-pure nitric acid (HNO_3) for 5 minutes to remove surface contamination. The otolith was then rinsed with double deionized water (ultra-pure 18 MΩ cm^{-1} water; $DDIH_2O$) to remove any remaining acid and dried under a class-100 clean hood for 24 hours. Otoliths remained in acid-leached polystyrene Falcon® tubes during the entire cleaning process. Once otoliths were dried and reweighed, the tubes were capped and placed in double Ziploc® bags. Otolith samples, along with blanks prepared from 1% ultra-pure HNO_3 and processed through the same stages of sample preparation, were sent to the Scandinavia ALS Laboratory Group in Luleå, Sweden for total digestion and trace elemental analysis.

Once samples arrived at the ALS laboratory, otoliths were transferred to individual acid washed Teflon vessels and 2 ml of concentrated ultrapure HNO_3 was added. When dissolution was completed (30-45 minutes), a second 2 ml aliquot of HNO_3 was added. After one hour, 6 ml of $DDIH_2O$ was added to the vessels and digested solutions were transferred to acid washed 15 ml polypropylene tubes. Samples were not manipulated for the next 24 hours, at which point digested solutions were further diluted using 1.4 M HNO_3 in $DDIH_2O$ to obtain a final dilution factor of 1,000 to 1,500-fold. All sample preparation was performed in a clean laboratory with a constant supply of HEPA-filtrated air. Diluted digests were analyzed with a Thermoscientific Element2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS) using an All-Teflon introduction system, self-aspiration and methane addition to plasma. Both low resolution (LR) and medium mass resolution (MR) acquisition modes were used. At least two preparation

blanks were analyzed concurrently with each batch of 56 otolith sample solutions to estimate instrument limits of detection (LOD), which were estimated as three standard deviations of mean blank values. The combination of external calibrations (synthetic blanks and standards prepared in 1.4 M HNO_3) and internal standardization (In and Lu added to all solutions at 200 ppt level) was employed for quantification. Detection and quantification capabilities were evaluated with results from preparation blanks.

Statistical Analysis

To meet parametric assumptions, data were ln transformed prior to statistical analysis. Due to a variety of ages being examined simultaneously, residual values were analyzed in order to compensate for mass differences and ontogenetic shifts within otoliths of fish of varying ages (see Barnett and Patterson 2010). Year-specific residual values were computed by subtracting mean elemental concentrations from each respective sample concentration. Multivariate analysis of variance (MANOVA) was used to test for differences in otolith elemental signatures among years, regions and habitats, with Pillai trace (V) as the test statistic because it is the most robust to violations of homogeneity of variance (Wilkinson et al. 1996). No significant year differences were detected (MANOVA, $F_{17, 1755} = 0.12$, $p < 1.00$); therefore subsequent models were blocked by year. An analysis of variance (ANOVA) was used to test elemental concentrations individually to determine a source of variance among regions and between habitats. ANOVA's were performed to examine significant effects of independent variables (region, habitat and their interaction), and were also used to assess significant levels of chemical signatures for each region and/or habitat. Reported values are based upon least square (LS) means.

To determine which elements are the most significant in discriminating between regions, habitats and habitats within regions (from now on referred to as location), a stepwise

discriminant analysis (SDA) was used. A SDA was used to find a set of the original quantitative variables that best discriminate among sites or groups. To distinguish regions, habitats and locations with otolith chemical signatures, discriminant function analyses were performed. As variance-covariance matrices of elemental and stable isotope variables were dissimilar between red snapper otolith samples from each region, habitat or location, a quadratic discriminant function analysis (QDFA) was used along with jackknifed crossvalidation classifications to quantify classification success to respective locations, regions and habitats. A canonical discriminant analysis (CDA) was used to compare otolith chemical concentrations of each region, habitat and location. The CDA determines the best linear combination of quantitative variables where the means of the groups are most different and whether this difference varies by year class. All analyses were performed with the Statistical Analysis System (SAS Institute 2006) with a significance level of $\alpha = 0.05$.

Results

A total of 1,964 red snapper otolith samples collected from three regions across the Gulf was processed for otolith chemical analysis. However, due to poor sample quality or inadequate detection limits only 1,778 samples were used to determine otolith chemical signatures (Table 3.1). Seven of the 24 elements (^{107}Ag, ^{109}Ag, ^{27}Al, ^{114}Cd, ^{60}Ni, ^{123}Sb, ^{238}U) were below LOD and were discarded. The remaining 17 elements (^{11}B, ^{138}Ba, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{7}Li, ^{55}Mn, ^{98}Mo, ^{206}Pb, ^{120}Sn, ^{205}Tl, ^{51}V, ^{64}Zn, ^{66}Zn) were present in red snapper otoliths above LODs. The chemical signatures were significantly different among regions (MANOVA, $F_{34, 3512} = 74.69$, $p < 0.001$), habitats (MANOVA, $F_{17, 1755} = 21.52$, $p < 0.001$), and locations (MANOVA, $F_{34, 3512} = 11.82$, $p < 0.001$).

Table 3.1. Sample size and size range of red snapper, *Lutjanus campechanus*, collected from three regions across the Gulf of Mexico during the summers of 2007 and 2008. AL = Alabama; LA = Louisiana; TX = Texas; P = platform habitat; NP = non-platform habitat.

Sample Year	Region	Habitat	Samples Collected	Samples Analyzed	Size Range (mm TL)
2007	AL	P	92	86	251 - 613
		NP	108	103	402 - 615
	LA	P	340	292	268 - 599
		NP	160	144	248 - 611
	TX	P	238	218	286 - 596
		NP	62	57	266 - 530
2008	AL	P	82	77	281 - 625
		NP	115	111	293 - 647
	LA	P	328	298	253 - 650
		NP	139	119	326 - 642
	TX	P	267	243	284 - 513
		NP	33	30	299 - 523

Mean concentrations of elements varied across regions and habitats (Table 3.2). Although all elemental concentrations differed significantly (ANOVA, $p \leq 0.05$) among locations and regions overall, some elemental concentrations were not significantly different between two regions. For instance, red snapper otoliths collected from AL and LA did not significantly differ in ^{59}Co ($p = 0.1834$), ^{206}Pb ($p = 0.2979$) and ^{205}Tl ($p = 0.4907$) concentrations, otoliths collected from AL and TX had non-significant differences in ^{63}Cu ($p = 0.9467$), ^{65}Cu ($p = 0.4985$) and ^{56}Fe ($p = 0.9806$) concentrations, and otoliths collected from LA and TX had non-significant differences in ^{59}Co ($p = 0.1531$), ^{120}Sn ($p = 0.9353$), ^{64}Zn ($p = 0.3905$) and ^{66}Zn ($p = 0.0838$) concentrations. Red snapper otoliths collected from AL had higher concentrations of ^{111}Cd, ^{7}Li, ^{98}Mo and ^{120}Sn, otoliths collected from LA had higher concentrations of ^{11}B and ^{138}Ba, and otoliths collected from TX had higher concentrations of ^{206}Pb and ^{205}Tl.

Table 3.2. Summary of raw data for region and habitat otolith elemental concentrations (ppb) for red snapper, *Lutjanus campechanus*, collected from the Gulf of Mexico during the summers of 2007 and 2008. Bolded values represent significantly higher values. AL = Alabama; LA = Louisiana; TX = Texas; P = platform habitat; NP = non-platform habitat.

Element	AL (n = 377)		LA (n = 853)		TX (n = 548)		P (n = 1214)		NP (n = 564)	
	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
^{11}B	772.97	13.28	**950.81**	17.34	629.67	8.52	743.34	8.46	**966.46**	23.54
^{138}Ba	6551.17	121.63	**6745.06**	59.55	6268.80	66.01	**6613.90**	53.36	6435.03	76.35
^{209}Bi	0.13	0.01	0.14	0.01	0.06	0.00	0.10	0.01	**0.13**	0.01
^{111}Cd	**0.17**	0.01	0.13	0.00	0.14	0.00	0.14	0.00	0.14	0.00
^{59}Co	0.14	0.01	0.14	0.01	0.13	0.00	**0.14**	0.01	0.12	0.00
^{63}Cu	45.11	0.47	43.36	0.29	45.23	0.40	**44.96**	0.27	42.90	0.32
^{65}Cu	42.24	0.45	41.51	0.29	43.50	0.40	**42.96**	0.27	40.80	0.32
^{56}Fe	78.43	6.71	61.94	3.61	74.62	3.98	**79.12**	3.45	48.30	2.98
^{7}Li	**615.74**	12.84	503.16	7.41	388.09	4.26	439.07	4.34	**604.56**	11.53
^{55}Mn	885.17	20.02	753.87	7.93	797.09	9.22	**819.22**	8.46	742.95	8.87
^{98}Mo	**3.07**	0.07	2.49	0.04	2.25	0.05	2.48	0.03	2.66	0.06
^{206}Pb	1.89	0.27	1.59	0.14	**2.77**	0.12	**2.30**	0.13	1.42	0.11
^{120}Sn	**3.23**	0.11	2.59	0.13	2.08	0.06	2.20	0.06	**3.34**	0.16
^{205}Tl	0.42	0.01	0.39	0.00	**0.58**	0.01	0.45	0.01	0.46	0.01
^{51}V	0.23	0.02	0.15	0.01	0.22	0.01	**0.20**	0.01	0.16	0.00
^{64}Zn	356.35	6.60	450.80	5.48	481.39	8.32	**464.31**	5.06	388.31	6.27
^{66}Zn	295.43	6.03	383.98	5.24	421.36	7.84	**400.78**	4.83	324.94	5.77

Almost all elemental concentrations differed significantly between habitats, with the exceptions being ^{111}Cd and ^{98}Mo (ANOVA, $p = 0.9059$ and $p = 0.1213$, respectively). Otoliths from red snapper collected at platform habitats had higher concentrations of ^{138}Ba, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{55}Mn, ^{206}Pb, ^{51}V, ^{64}Zn and ^{66}Zn, while samples collected at non-platform habitats had higher concentrations of ^{11}B, ^{209}Bi, ^{7}Li, ^{120}Sn and ^{205}Tl.

The stepwise discriminant analysis retained all otolith elements for the location model, retained all elements except ^{66}Zn for the region model and retained all elements except ^{63}Cu for the habitat model. However, removal of these elements resulted in insignificant changes to the QDFA models, thus all elements were retained in all of the models. Mean jackknifed classification accuracies of the QDFA models were 61.9% for location, 85.8% for region, and 76.4% for habitat (Figure 3.2). The low classification success among locations was primarily due to misclassifications within regions. In fact, the lowest classification success was for AL platform samples (58.3%), with 29.5% of those samples misclassified as being collected from AL non-platform habitats. Therefore, regions alone were analyzed and resulted in the highest classification successes. The largest misclassification among regions was 12.7% of LA red snapper were misclassified as being collected from TX. Habitats analyzed separately also had higher classification success than locations, with higher classification success occurring for red snapper collected from platforms (80.6%) than non-platform (72.2%) habitats.

The canonical variable plot for locations further displays significant separation of regions with major overlap of habitats within regions (Figure 3.3; see also Appendix C). Thus, locations again were disregarded, and a plot for regions alone was developed (Figure 3.4; see also Appendix C). Based on the analyses of the elemental variables, AL red snapper otolith signatures appear to be correlated with ^{111}Cd, ^{7}Li, ^{98}Mo and ^{120}Sn, LA otolith signatures

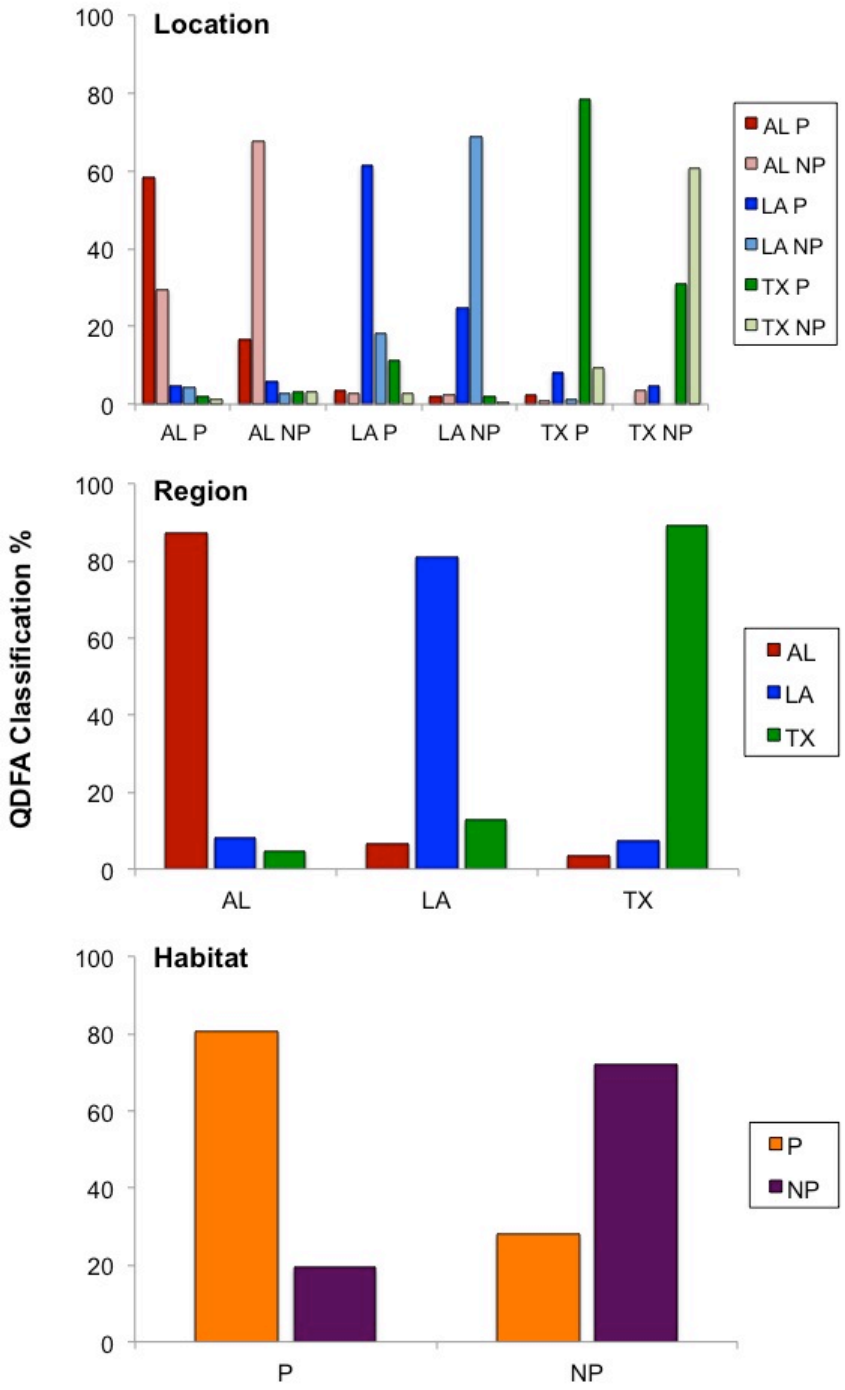

Figure 3.2. Jackknifed classification percentages of red snapper, *Lutjanus campechanus*, to six locations, three regions and two habitats in the Gulf of Mexico collected during the summers of 2007 and 2008. Percentages were estimated with quadratic discriminant function analyses (QDFA) of otolith chemical signatures. AL = Alabama; LA = Louisiana; TX = Texas; P = platform habitat; NP = non-platform habitat.

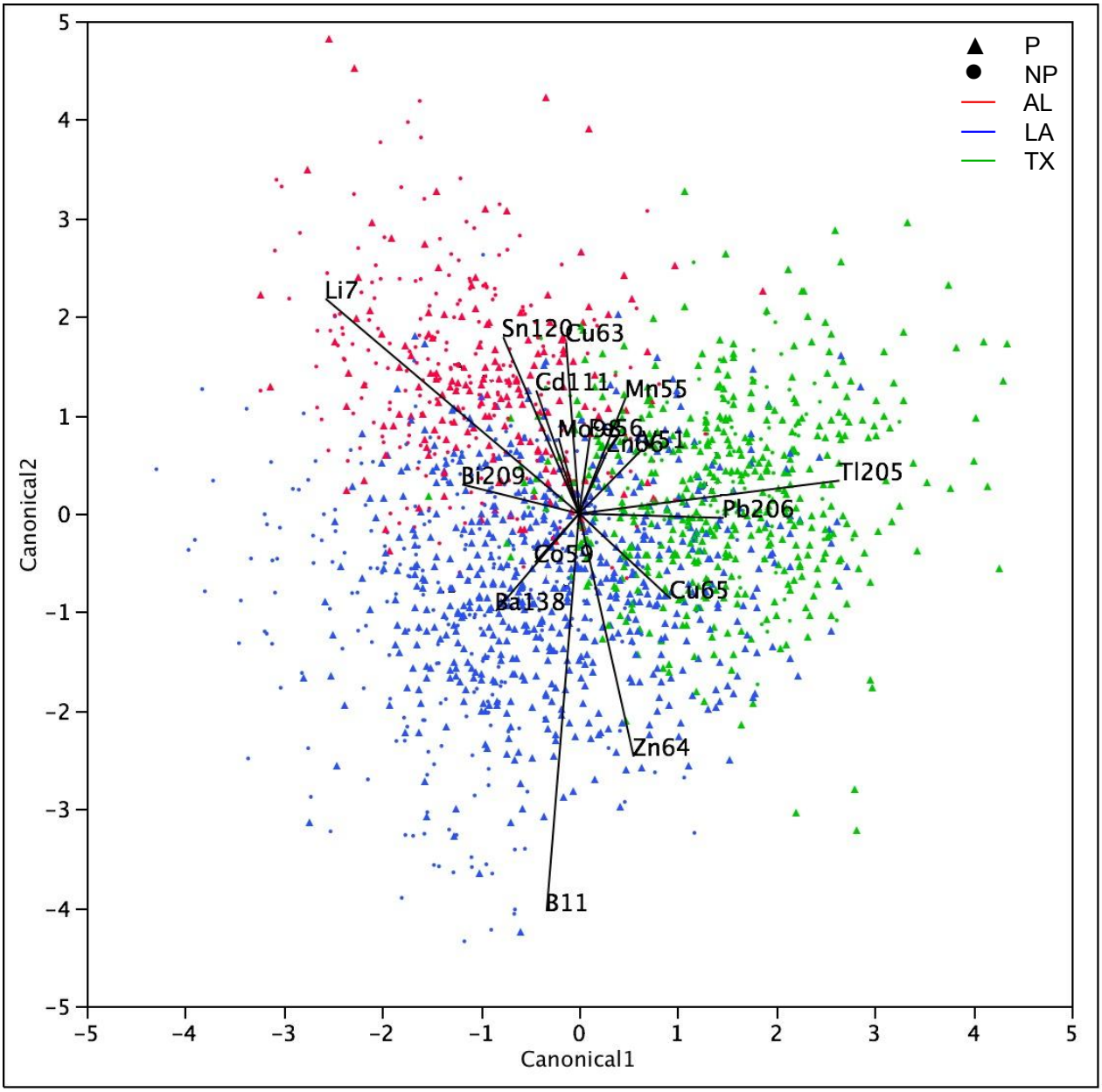

Figure 3.3. Canonical plot scores derived from otolith chemical signatures of red snapper, *Lutjanus campechanus*, collected from six locations in the Gulf of Mexico during the summers of 2007 and 2008. AL = Alabama; LA = Louisiana; TX = Texas; P = platform habitat; NP = non-platform habitat.

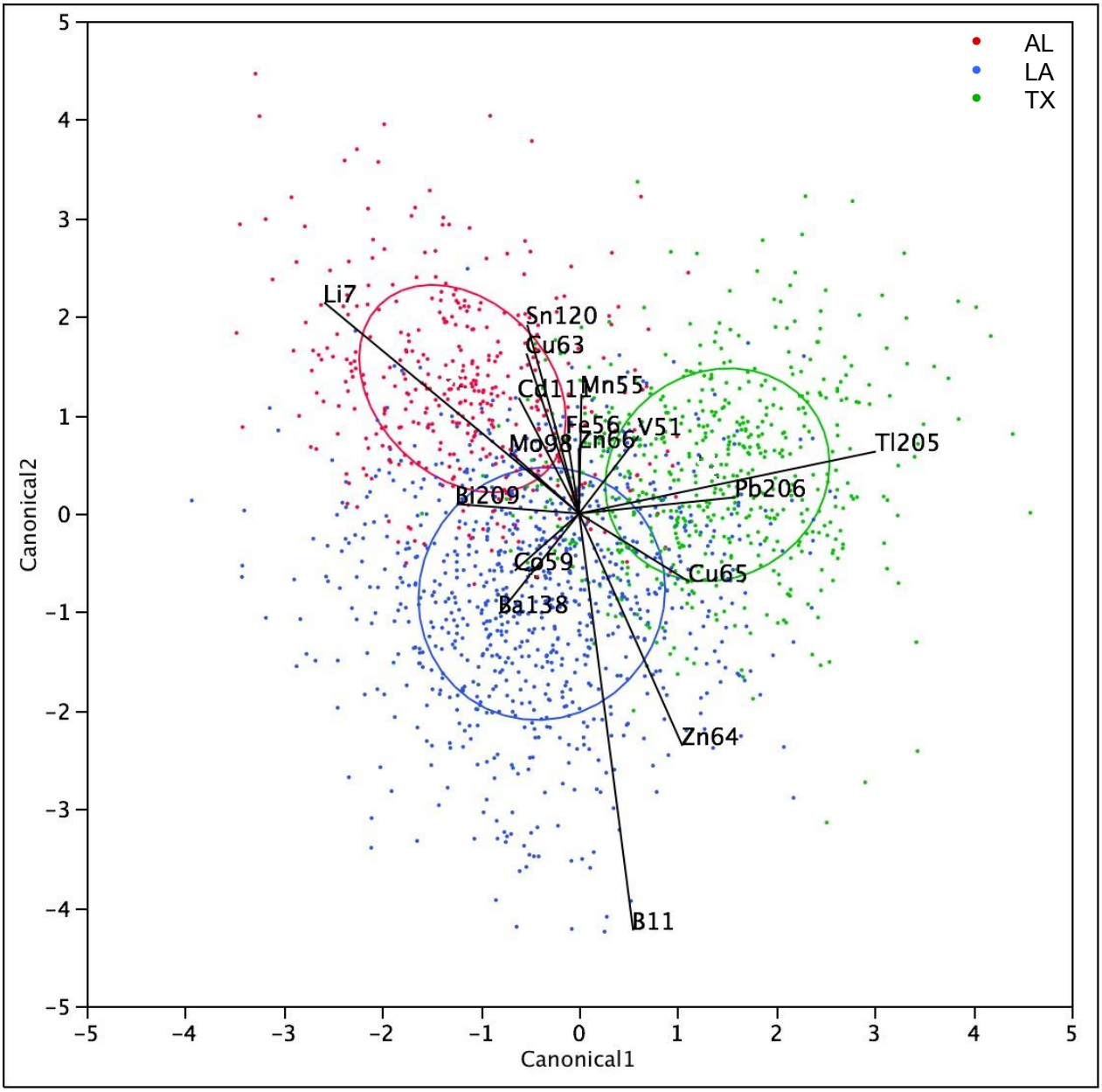

Figure 3.4. Canonical plot scores derived from otolith chemical signatures of red snapper, *Lutjanus campechanus*, collected from three regions in the Gulf of Mexico during the summers of 2007 and 2008. Ellipses indicate 95% confidence levels. AL = Alabama; LA = Louisiana; TX = Texas.

appear to be correlated with ^{11}B and ^{138}Ba, and TX otolith signatures appear to be correlated with ^{206}Pb and ^{205}Tl. These results coincide with mean elemental concentrations for each region (Table 3.2). Although the plot shows that LA red snapper otolith signatures may also be correlated with ^{59}Co, these concentrations did not differ significantly from the other two regions. Thus, ^{59}Co was not considered a substantial element to the development of LA otolith signatures. A canonical variable plot for habitats further confirms platform otolith signatures to be correlated with ^{138}Ba, ^{59}Co, ^{56}Fe, ^{55}Mn, ^{206}Pb, and ^{51}V, and non-platform signatures to be correlated with ^{11}B, ^{209}Bi, ^{7}Li, ^{120}Sn and ^{205}Tl (Figure 3.5; see also Appendix C). Although ^{63}Cu, ^{65}Cu, ^{64}Zn and ^{66}Zn mean concentrations were higher for platform samples, these elements appear divided among habitat types in Figure 3.5. As expressed by the QDFA classification accuracies, more overlap occurs between habitats than among regions. In addition, several platform samples that were misclassified as being non-platform samples were collected at fishing rodeos or from GI platforms (Figure 3.6). Removal of these samples improved the QDFA model for habitat by 5% (81.5% classification accuracy).

Discussion

Otolith trace metal concentrations were temporally stable for red snapper collected in the northern Gulf over the two-year study period. When developing natural tags, inter-annual stability of chemical signatures is desired to avoid the need to produce annual baseline data and further validate the effectiveness of the tag. The temporal stability of otolith chemical signatures can vary within months, between two consecutive years, or show negligible differences over a two-year period with drastic changes occurring after 4-13 years (Patterson et al. 1999; Campana et al. 2000; Gillanders 2002). Thus, while the temporal stability of otolith trace metal

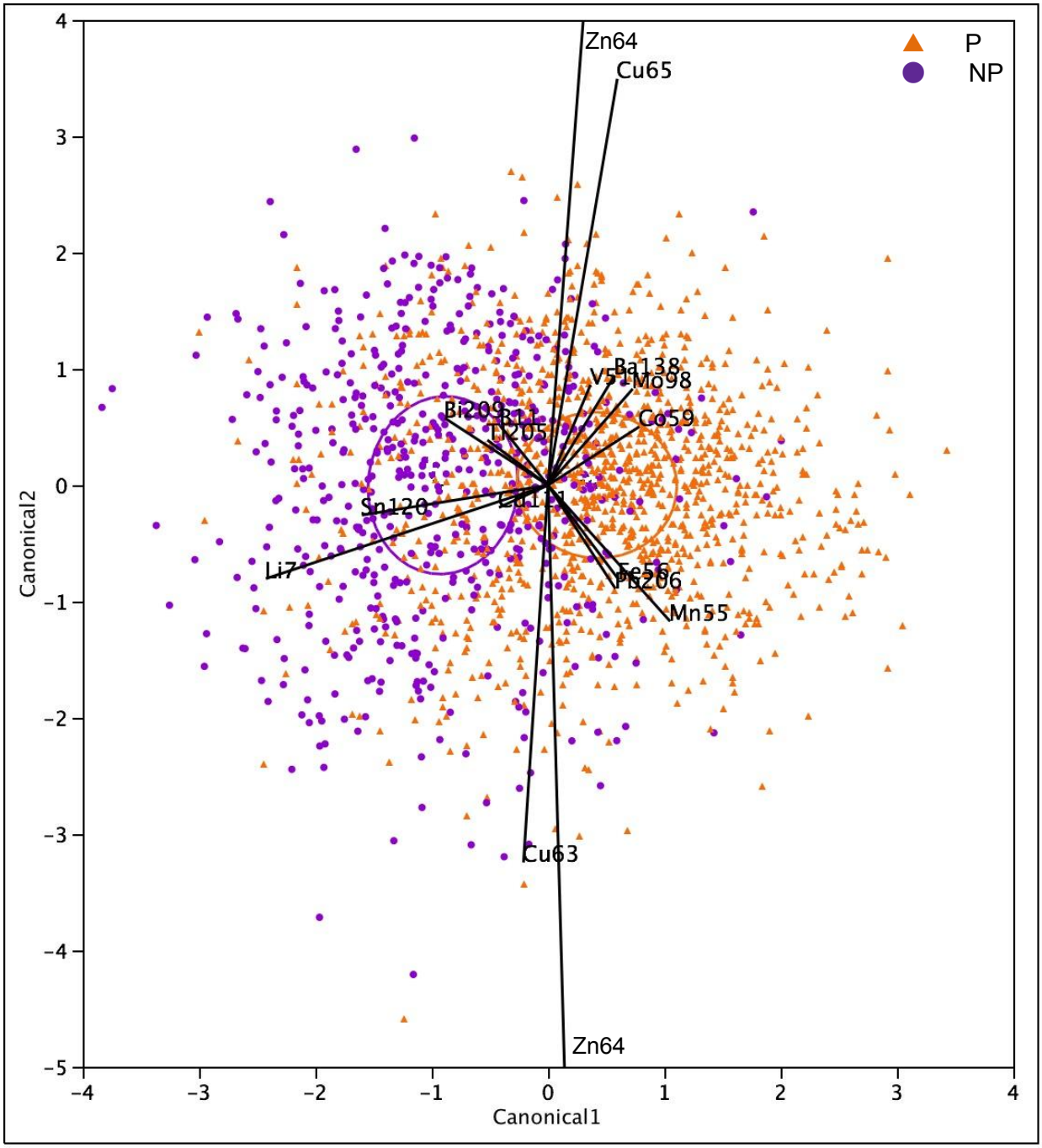

Figure 3.5. Canonical plot scores derived from otolith chemical signatures of red snapper, *Lutjanus campechanus*, collected from two habitats in the Gulf of Mexico during the summers of 2007 and 2008. Ellipses indicate 95% confidence levels. P = platform habitat; NP = non-platform habitat.

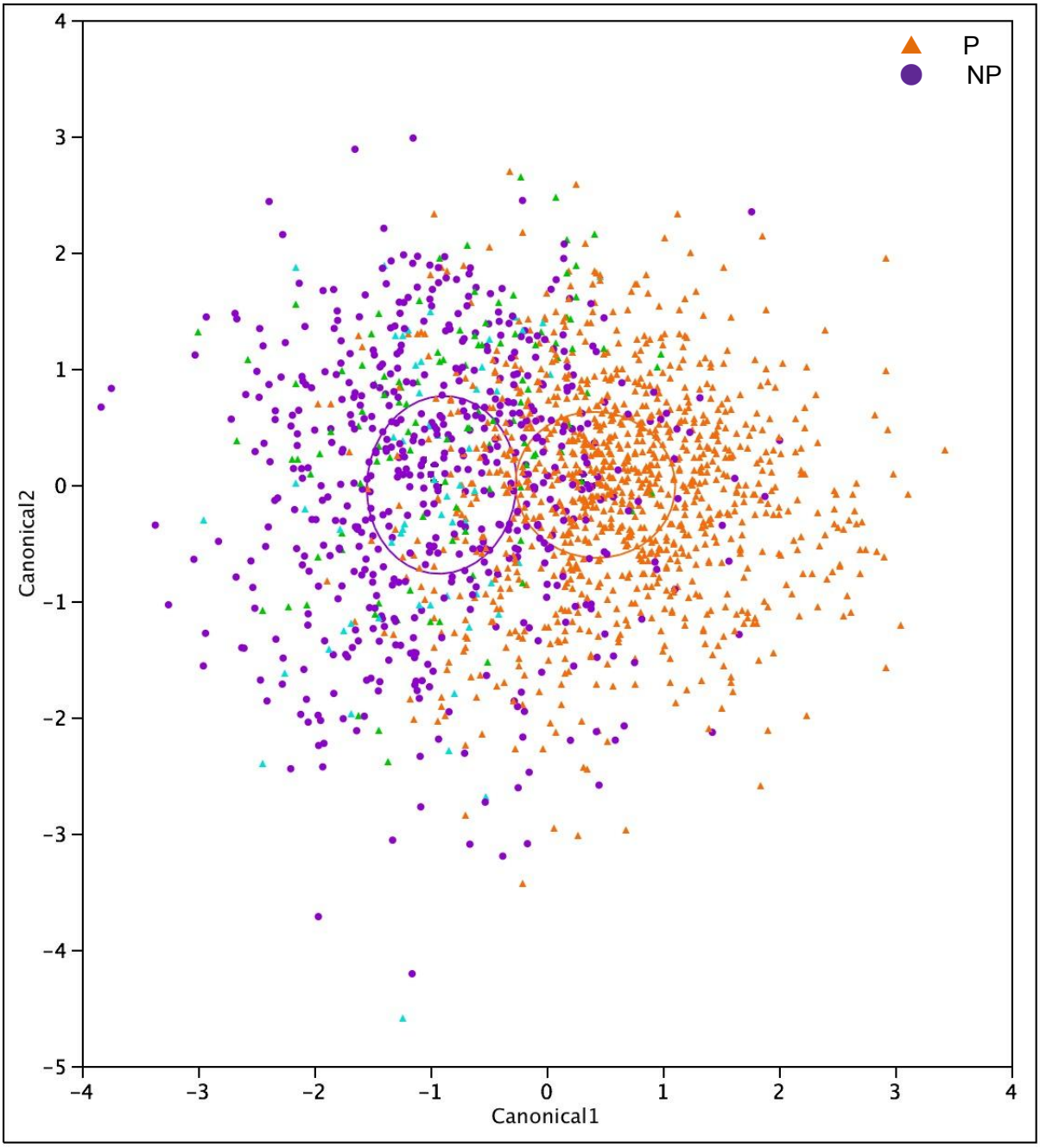

Figure 3.6. Canonical plot scores derived from otolith chemical signatures of red snapper, *Lutjanus campechanus*, collected from two habitats in the Gulf of Mexico during the summers of 2007 and 2008. Blue triangles represent red snapper collected from fishing rodeos that may have been mislabeled as platform fish and green triangles represent fish collected from Grand Isle platforms. Ellipses indicate 95% confidence levels. P = platform habitat; NP = non-platform habitat.

concentrations in this study is significant, it should be noted that there is potential for concentrations to change in the future.

Natural tags derived from red snapper otolith trace metal concentrations demonstrated significant spatial differences. Classification success was high for regions and habitats when analyzed separately, whereas locations had a much lower classification success. The high classification error of locations is attributed to misclassification between habitats within the same region. Although red snapper aggregate near platforms, they tend to periodically move away from platforms possibly for foraging purposes (Bortone et al. 1998; McDonough 2009). Platforms are occasionally placed only a few hundred meters apart from other platforms, artificial reefs or natural habitats, allowing red snapper to encounter new habitat while foraging away from these structures. In fact, acoustic studies have revealed that red snapper do move between closely spaced platforms and surrounding habitats (Westmeyer et al. 2007). Patterson and Cowan (2003) reported that red snapper site fidelity to artificial reefs was low, with substantial dispersion. However, a consistent pattern of red snapper tagging studies is that most fish only move short distances and slowly diffuse away from tagging sites (<10 km; Patterson 2007), with larger fish more likely to travel greater distances than smaller fish (Patterson et al. 2001). Therefore, localized movement within regions, along with different habitat types in close proximity to one another, may explain the low classification success of locations and high regional classification success in this study.

Otoliths of red snapper collected from AL had significantly higher concentrations of ^{111}Cd, ^{7}Li, ^{98}Mo and ^{120}Sn. These results differed from Nowling et al. (2011) in which higher concentrations of ^{59}Co and ^{62}Ni were found in otoliths of red snapper collected east of the Mississippi River, which they attributed to discharge from Mobile Bay. Although temporal

variations in ambient water elemental concentrations could be a factor, differences between these studies may be caused by variations in analytical procedures and sample sizes (see Nowling 2005). Cadmium, Li, Mo and Sn can all be associated with anthropogenic materials used to construct artificial reefs. While few platforms exist east of the Mississippi River, approximately 20,000 artificial reefs have been deployed in a 3,100 km^2 designated area beginning in the early 1950s (Minton & Heath 1998; Patterson et al. 2001). These artificial reefs are constructed from a variety of objects, including car bodies, liberty ships, shrimp boats, barges, concrete, military tanks and small planes. During the 1950s, Cd was primarily used as a protective coating on iron and steel parts associated with tanks, automobiles, ships and aircrafts (Lansche 1958), but the use of Cd coating has gradually decreased over time due to environmental concerns (Tolcin 2011). Molybdenum has principally been used as an alloying agent in iron and steel products to enhance durability and protect against corrosion (Polyak 2011). Before the introduction of the Li battery, Li compounds were mainly used in ceramics, glass and aluminum (Jaskula 2011). Tributyltin (TBT)-based antifouling paints were used on ship hauls because of the durable (5 years) protection it provided until it was banned in 2003 over environmental concerns (Hayman et al. 2000). Tributyltin degrades slowly in marine environments until it becomes inorganic Sn (MacLeod et al. 2004) and gradually less toxic. While the ban resulted in lower water column levels, TBT and associated degradation products are still retained in marine sediments of affected areas (Antizar-Ladislao 2008). The various objects used to construct artificial reefs are properly cleaned until they are deemed environmentally safe before being disposed into the ocean. However, over time these items will corrode due to natural processes. Thus, the higher concentrations of ^{111}Cd, ^{7}Li, ^{98}Mo and ^{120}Sn in red snapper otoliths collected from AL may reflect the unique materials used to create the region's artificial reef system. Future research

should determine if water and sediment samples around these artificial reefs also exhibit high levels of these metals.

Before 2002, red snapper collected from Louisiana waters accounted for more than 50% of commercial landings in the Gulf (http://www.st.nmfs.noaa.gov/st1/commercial/). The majority of red snapper are harvested around artificial structure, including the large number of oil and gas platforms in the area, because natural hard substrate is limited on the LA continental shelf. In fact, a survey of recreational fishers determined that 70% preferred to fish on LA platforms (Stanley & Scarborough-Bull 2003). In this study, red snapper otoliths collected from LA had higher concentrations of ^{11}B and ^{138}Ba, both of which are associated with platform production processes. Elevated concentrations of B have been associated with oilfield brines (Collins 1975) and Ba as barite is a main component in drill muds (Kennicutt et al. 1996). Although higher concentrations of ^{138}Ba were associated with platform habitats in this study, the opposite is true for ^{11}B, which was associated with non-platform habitats. The main source of B input into the oceans seems to originate from continental discharge (Lemarchand et al. 2002). Weathering of natural rocks and mineral deposits as a result of riverine processes has been a significant source of metals to estuaries (Summers et al. 1996). The Mississippi River system drains 41% of the conterminous United States (Turner & Rabalais 1991) and its plume has been known to extend well offshore. Furthermore, Ba is deposited into the otolith in proportion to ambient water conditions (Bath et al. 2000) and follows a nutrient-type profile with higher concentrations in riverine and near-coastal waters (Thorrold et al., 1997). Thus, higher concentrations of ^{11}B and ^{138}Ba in LA red snapper otoliths may actually be attributed to influences of the Mississippi River discharge, rather than platform production processes. Again, these results differed from Nowling et al. (2011) in which higher concentrations of ^{114}Cd, ^{65}Cu,

^{238}U, ^{107}Ag and ^{109}Ag were found in otoliths of red snapper collected west of the Mississippi River. Several of these elements were below detection limits in the current study.

Concentrations of ^{206}Pb and ^{205}Tl were higher in red snapper otoliths collected from Texas. Lead has been detected in drilling muds resulting from trace impurities in barite and in produced waters from drilling operations (Neff et al. 1987). Studies have shown that Pb and Ba are not highly correlated in sediments collected around Gulf platforms (Kennicutt et al. 1996), and at times Pb in marine sediments can be equal to or higher than levels in drilling muds (Neff et al. 1987). Therefore, other sources are likely responsible for high Pb levels, including produced water, welding operations, lubricants and corrosion of galvanized structures associated with offshore oil development. Lead concentrations in otoliths reflect ambient water conditions and can serve as an environmental monitor (Geffen et al. 1998, Ranaldi & Gagnon 2010). In the current study, platform samples had higher concentrations of ^{206}Pb and, with the majority of TX red snapper having been collected at platforms, these associations may explain the correlation of ^{206}Pb with TX otolith signatures. Conversely, high Tl concentrations were not associated with platform samples. Thallium occurs naturally in trace concentrations in the earth's crust within sulfide ores of Zn, Cu and Pb (Peter and Viraraghavan 2005). Higher concentrations of Tl can be found in sulfide deposits (i.e. pyrite) and released into the water column through weathering of Tl-rich sulfides (Xiao et al. 2003). Interestingly, recent work based on a subset of samples collected during this study (Zapp Sluis et al., in review) showed that sulfur isotopes were more enriched in red snapper tissue samples collected from TX compared to the other two regions. Further research is needed to determine if high Tl levels are correlated with high levels of sulfur in sediment samples from this region.

Otolith concentrations of ^{11}B, ^{209}Bi, ^{7}Li, ^{120}Sn and ^{205}Tl were significantly higher in red snapper otoliths collected from non-platform habitats versus platform habitats. Each of these elements, except Bi, were presented above as being linked to a region in a way not associated with platforms. It may be possible that each of these elements were correlated to non-platform habitats in their respective regions and combining regions has grouped them together to form the non-platform signature, which may also explain the lower classification success of habitats compared to regions. The dominant source of Bi to the ocean is via aeolian inputs originating from volcano processes and European-Asian arid land regions (Lee et al. 1986, Bertine et al. 1996). As such, it would be assumed that ^{209}Bi concentrations should be uniform across the Gulf, or at least between habitats within regions. In the United States, Bi is used primarily by the chemical and pharmaceutical industries, as well as for additives used in casting and galvanizing (Carlin 2010). Red snapper otoliths from AL and LA had higher concentrations of ^{209}Bi compared to TX samples, thus it could be possible that Bi enriched chemicals and pharmaceuticals were leaked into the ocean through riverine input. However, again both habitat types should be affected equally. While it is unknown at this time as to why ^{209}Bi is higher in non-platform versus platform otolith samples, it most likely is not a strong contributing factor to non-platform otolith signatures.

An important concern facing future offshore oil and gas platform development is the long-term biological and environmental effects they might create. Drilling fluids and produced water associated with oil production processes, and corrosion of the rig structure, antifouling paints and sacrificial anodes can all be responsible for leaching metals into the water column and sediments around platforms. In the current study, red snapper otoliths collected from platform habitats had higher concentrations of ^{138}Ba, ^{59}Co, ^{56}Fe, ^{55}Mn, ^{206}Pb, and ^{51}V. Each of these

metals has been detected in drilling fluids, produced water and crude oil (Neff et al. 1987, Bezerra et al. 2007, Kennicutt et al. 1996). Tillery et al. (1981, as cited in Neff 1987) analyzed sediments, invertebrates and fish for common metals (Ba, Cd, Cr, Cu, Fe, Ni, Pb, V, and Zn) associated with oil and gas platforms. Concentrations of Ba, Cr, Cu, Pb, and Zn were elevated in sediments within 100 m of the platform, but there was no indication of metal bioaccumulation in tissues of marine fauna associated with platforms. Fast turnover rates may prevent high levels of metal from accumulating in tissues of marine fauna, but the inert property of otoliths will allow metal concentrations to continuously increase the longer the fish resides on a platform. Accordingly, analyzing whole otoliths may display stronger platform signatures because they represent the metal accumulation for the entire duration of time spent on a platform, which could be multiple years. Furthermore, Kennicutt et al. (1996) confirmed that metal contamination levels due to drilling and discharge effects at deeper water sites (>80 m) remain stable in sediments for several years, possibly decades (except Pb which increased over time). Thus, it may be possible for otolith platform signatures to remain temporally stable for longer than the two-year period of this study. Nowling et al. (2011) also observed higher concentrations of ^{206}Pb and ^{51}V in otoliths collected from platform habitats, however they did not test for ^{138}Ba, ^{56}Fe, and ^{55}Mn.

Concentrations of ^{63}Cu, ^{65}Cu, ^{64}Zn and ^{66}Zn were significantly higher in otoliths of red snapper collected from platforms. Both of these metals are associated with oil production processes and have been found in higher concentrations in sediments near platforms (Tillery et al. 1981, as cited in Neff 1987). However, concentrations of Cu and Zn in otoliths may not be proxies of ambient water conditions as they are both influenced by physiological regulations (Campana 1999). Zinc is primarily absorbed through the intestines and dietary exposure is

responsible for most of the Zn assimilation in teleosts. Since otolith formation requires a large amount of Zn, it is unlikely that Zn concentrations in otoliths accurately represent ambient water concentrations (Miller et al. 2006). In fact, it is not uncommon for high concentrations of Zn to be present in otoliths, and these increased levels most likely represent diet or metabolism influences (Friedrich & Halden 2010). For the same reasons, Cu concentrations in otoliths will not reflect ambient waters unless extreme conditions occur in which Cu levels are high enough to stress the fish and the liver can no longer remove Cu adequately (Milton & Chenery 2001). However, because whole otoliths were analyzed, higher concentrations of Cu and Zn in red snapper otoliths may reflect accumulation of these elements over several years without harmful health affects being detected in fish. Furthermore, red snapper feed mostly on the benthos (McCawley and Cowan 2007; Wells et al. 2008b), possibly causing increased Cu and Zn concentrations in platform sediments to be assimilated through the food web. Significantly higher concentrations of Zn were observed for red snapper collected off LA and TX compared to fish collected off AL. Thus, increased levels of these elements can be useful for distinguishing among red snapper populations as demographic differences in physiological regulation and metabolic influences may exist between regions.

While analyzing the habitat data, I observed that a majority of the misclassified platform samples were red snapper otolith samples collected from AL fishing rodeos or GI platforms off the LA coast. Sampling at fishing rodeos occurred dockside and habitat was assigned based upon the recreational fisher's word. However, it was not possible to verify the true location of these catches and AL recreational fishers prefer to fish for red snapper on the abundant artificial reefs deployed in the area. Thus, red snapper samples collected from AL fishing rodeos that were labeled platform may need to be removed or relabeled as non-platform samples.

Conversely, habitat was accurately defined for samples collected from GI platforms. The GI mineral leasing area is closest to the Mississippi River mouth compared to the other two leasing areas in which LA red snapper samples were collected. While it may be possible for large inputs of river water to muddle otolith platform signatures, not all GI platform samples were misclassified. A more likely scenario may be that misclassified samples represent red snapper that recently inhabited the platform prior to capture and a strong platform signature had not yet developed. As this may be the case for all misclassified habitat samples, and no conclusive rationale for the misclassification of GI platform samples can be defined, these samples should remain as originally labeled.

Numerous studies have shown that oil and gas platforms are utilized by red snapper, but whether these unique habitats are beneficial is still debatable. The results of this study indicate that trace metals associated with platforms can be used to develop otolith chemical signatures to differentiate among regions, and to a lesser extent habitats, in the Gulf. Although the overall goal was to develop an oil and gas platform otolith signatures based upon a suite of trace metals, this combinations of trace metals proved to work best for discriminating among regions because of unique features, e.g. the Mississippi River, that differentially affect each area. The next step will be to utilize these natural tags to estimate the source of adults to regions devoid of platforms in the Gulf. Specifically, region-specific chemical signatures can be used to further explore mixing dynamics between red snapper populations east and west of the Mississippi River, and habitat-specific signatures can be used to estimate the percent contribution of platform-reared recruits to regions devoid of platforms, i.e. Florida.

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CHAPTER 4: UTILIZING REGIONAL AND HABITAT OTOLITH MICROCHEMICAL SIGNATURES TO EXAMINE STOCK STRUCTURE OF RED SNAPPER IN THE NORTHERN GULF OF MEXICO.

Introduction

Beginning in the 1950s, thousands of *de facto* artificial reefs have been deployed in the northern Gulf of Mexico (Gulf) as a result of oil and gas platform development, primarily centered off the Louisiana coast (Kasprzak 1998). The development of Alabama's Artificial Reef Program began at the same time, which has led to the deliberate construction of thousands of reefs using opportunistic materials for the purpose of enhancing reef fish habitat (Minton and Heath 1998). By the end of the decade, landings of Gulf red snapper, *Lutjanus campechanus*, greatly increased with a majority of the landings occurring west of the Mississippi River. This has been attributed to the end of World War II, technology innovations and an increase of the commercial fishing fleet (see Porch et al. 2007 for review). The development of artificial reefs around this time may have also contributed to the increased landings, but the question remains to what extent do artificial reefs benefit the red snapper stock.

Gulf red snapper are currently overfished, although the western substock is estimated to be recovering from overfishing (SEDAR 2009). It has been widely debated as to whether artificial reefs, including oil and gas platforms, benefit red snapper production by increasing habitat or if individuals simply aggregating to these structures become vulnerable to the associated intense fishing pressure (Shipp 1999; Cowan et al. 1999; Shipp and Bortone 2009; Patterson and Cowan 2003; Gallaway et al. 2009; Cowan et al. 2010). According to Bohnsack (1989) the debate is not that simple, but instead involves a continuum of factors including site fidelity, habitat availability, recruitment limitation, and fishing pressure. Artificial reefs are

likely to increase production if fish are habitat-limited versus recruitment-limited, not experiencing overfishing, and exhibit high site fidelity.

Red snapper production in the Gulf does not appear to be limited by habitat, especially when considering that natural habitats supported the population before it was heavily exploited (Cowan et al. 1999; Lindberg 1997). The association of red snapper with artificial reefs may actually make them more vulnerable to fishing pressure by concentrating them to well-marked areas more accessible to commercial and recreational fishers (Bohnsack 1989; Cowan et al. 2010). Furthermore, red snapper annual site fidelity is estimated to range between 25 – 60% for fish associated with artificial reefs off the Alabama coast (Patterson and Cowan 2003; Schroepfer and Szedlmayer 2006; Strelcheck et al. 2007). Nonetheless, as red snapper recover from overfishing, data suggest that red snapper populations are also showing signs of recovery on the west Florida and south Texas continental shelf (SEDAR 2009). It is unknown if population expansions off west Florida and south Texas are caused by self recruitment in response to stricter management strategies, or if regions with higher abundance are supplying recruitment subsidies, as conventional tagging studies suggest (Patterson et al. 2001a; Addis et al. 2008). If other regions are supplying recruits, it is unknown if artificial reefs contributed production.

Problems associated with conventional tagging (see Patterson 2007 for review) can cause red snapper movement to be underestimated. For this reason fishery scientists have turned to otoliths (ear stones) as natural tags for a more efficient way to study population connectivity (Thorrold et al. 2001; Gillanders 2002; Rooker et al. 2008). The inert quality of the otolith allows elemental and stable isotope concentrations accreted onto the otolith from ambient water to be permanently retained as the fish grows (Campana 1999). This makes it possible for

material that is deposited during the juvenile stage to act as a natural marker to determine the nursery of origin of adult fish (Chapter 1 and 2). In Chapter 3, a unique suite of trace metals associated with artificial habitat was used to identify region and habitat of origin of adult red snapper based on otolith chemical analysis. Employing these signatures can help examine population connectivity of older red snapper in the northern Gulf. Furthermore, if a platform signature is evident in otoliths of fish collected in areas devoid of platforms, such as Florida, it may indicate some contribution of platform-reared recruits.

Although the initial goal was to develop a platform signature, it was determined in Chapter 3 that a suite of elements associated with artificial habitat was more useful for distinguishing region of origin than habitat of origin for Gulf red snapper. This result may be caused by the relatively low site fidelity of red snapper (Patterson and Cowan 2003), along with close proximity of other habitat types (McDonough 2009; Westmeyer et al. 2007), which can muddle the habitat signature. Gray triggerfish (*Balistes capriscus*) is another reef-associated fish that is known to display high site fidelity with limited dispersion from reefs (63 - 87% per year for Alabama artificial reefs; Ingram 2001). Based on these traits, Ingram and Patterson (2001) concluded that gray triggerfish would benefit more from artificial habitat established within marine protected areas than would red snapper. Additionally, higher site fidelity of gray triggerfish would make it a better candidate for testing the accuracy of the platform otolith signature.

The primary purpose of this study was to apply otolith chemical signatures described in Chapter 3 to estimate region and habitat of origin for adult red snapper collected from areas devoid of platforms. Specifically, natural tags derived from otolith trace metal concentrations of red snapper collected from platform and non-platform habitats from three regions in the Gulf

were compared to otolith concentrations of adult red snapper collected from natural habitats in the western Gulf and from areas devoid of oil and gas development in the eastern Gulf. Otolith trace metal concentrations of gray triggerfish collected from platform and non-platform habitats were also analyzed to test the accuracy of the platform otolith signature described in Chapter 3. The objective was to use regional signatures to further examine red snapper population connectivity among Gulf regions, and use platform signatures to estimate the contribution of platform-reared recruits to regions devoid of platforms.

Methods

Sample Collection

Adult red snapper were collected during the summer of 2009 off the coasts of South Padre Island, Texas (TX), Port Fourchon, Louisiana (LA), Destin, Florida (DFL) and Tampa, Florida (TFL; Figure 4.1). The objective was to collect 500 red snapper total with 100 coming from TX, 100 from LA and 300 combined from the two Florida (FL) regions. Red snapper were collected from recreational landings and sampling trips aboard a research vessel. To test the effectiveness of regional and platform signatures developed from red snapper otolith samples collected in 2007 and 2008, adult red snapper were targeted on natural habitat or other habitats in areas devoid of oil and gas platforms. Red snapper in TX were collected on natural rock outcrops, LA samples were collected on shelf edge banks (Alderdice Bank, Bouma Bank and Jakkula Bank), DFL samples were collected on natural habitat, artificial reefs and wrecks, and TFL samples were collected on the FL middle grounds. Both red snapper sagittae were extracted in the field, rinsed free of associated tissue with deionized (DI) water and stored in individual plastic coin envelopes until further laboratory analysis. Fish total lengths (TL) were measured to the nearest mm. In attempt to collect older individuals that are more likely to have migrated

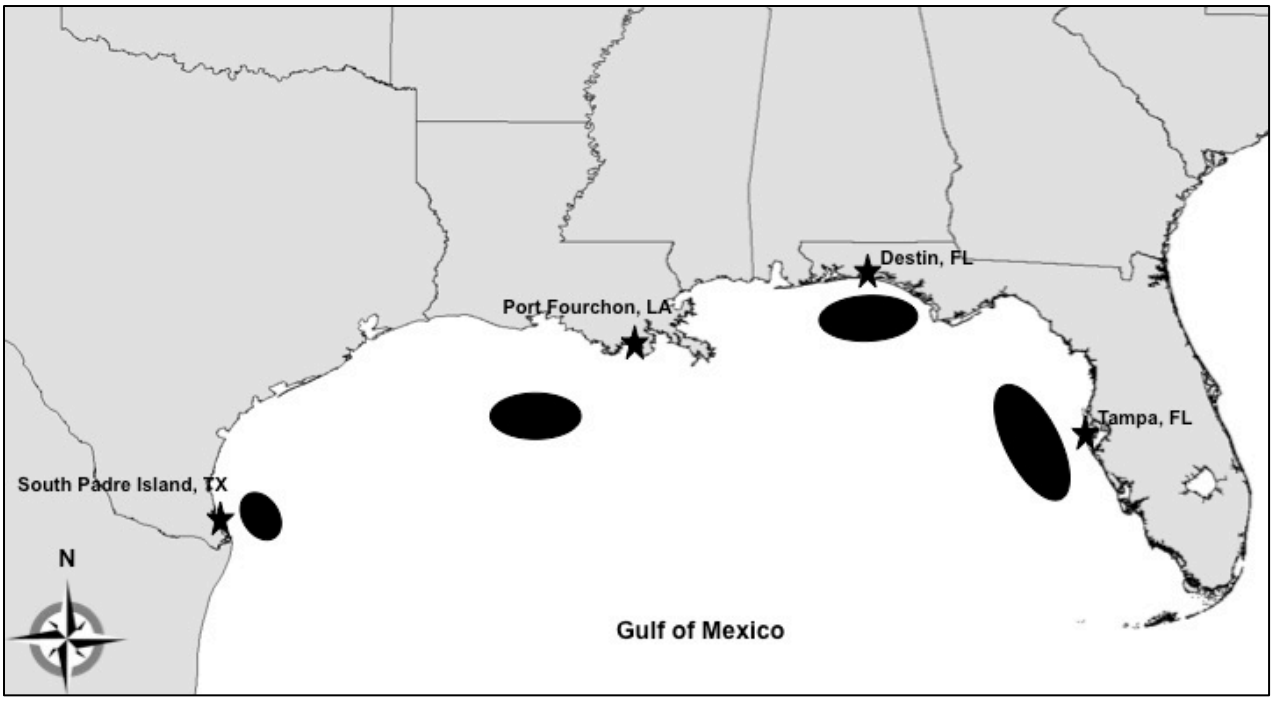

Figure 4.1. Sampling regions along the continental shelf of the northern Gulf of Mexico (Gulf) where adult red snapper, *Lutjanus campechanus*, were sampled on natural habitat during the summer of 2009.

away from platforms, red snapper with a TL greater than 500 mm were targeted to obtain a majority of fish that were older than age-5 (Fischer et al. 2004; Saari 2011).

Gray triggerfish also were collected during the summer of 2009 off the coasts of Port Fourchon, LA and DFL. To determine if the platform signature from Chapter 3 was valid for other species, gray triggerfish were targeted on platforms (Eugene Island mineral leasing area; LA) and natural habitat (DFL). Gray triggerfish were collected from recreational landings and sampling trips aboard a research vessel. Fish TLs were measured in the field to the nearest mm. Heads were removed in the field and frozen until further laboratory processing. In the laboratory, both sagittae were extracted, rinsed free of associated tissue with deionized (DI) water and stored in cell trays until further analysis.

Otolith Preparation and Analysis

In the laboratory, red snapper and gray triggerfish sagittae were cleaned with a synthetic bristle brush to remove any adhering tissue, rinsed with DI water and placed in polyethylene cell trays to air-dry under a class-100 clean hood. The rostrum and postrostrum ends of otoliths extracted from red snapper larger than 700 mm (n=17) were removed with the precision grinder on a Hillquist model 800 thin-sectioning machine until the otolith was 22 mm in length, which was the average length of otolith samples in this study. This was done to homogenize otolith sizes and to remove additional material that may dilute the regional and platform signal. The remaining procedures were carried out in a class-100 clean room under laminar flow using acid-washed supplies. Materials and solution blanks were tested before sample preparation to ensure there were no sources of contamination. Right sagittae were selected for trace elemental analysis for the following elements: ^{11}B, ^{138}Ba, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{7}Li, ^{55}Mn, ^{98}Mo, ^{206}Pb, ^{120}Sn, ^{205}Tl, ^{51}V, ^{64}Zn, and ^{66}Zn. Dry otoliths were weighed before and after cleaning to the nearest 0.01 mg. If the right sagitta of triggerfish weighed less than 5 mg, both sagittae were combined to ensure an adequate amount of sample material. Whole otoliths were immersed in 1% ultra-pure nitric acid (HNO_3) for 5 minutes to remove surface contamination. Each otolith was then rinsed with double deionized water (ultra-pure 18 MΩ cm^{-1} water; $DDIH_2O$) to remove any remaining acid and dried under a class-100 clean hood for 24 hours. Otoliths remained in acid-leached polystyrene Falcon® tubes during the entire cleaning process. Once otoliths were dried and reweighed, the tubes were capped and placed in double Ziploc® bags. Otolith samples, along with blanks prepared from 1% ultra-pure HNO_3 and processed through the same stages of sample preparation, were sent to the Scandinavia ALS Laboratory Group in Luleå, Sweden for total digestion and trace elemental analysis.

Once samples arrived at the ALS laboratory, otoliths were transferred to individual acid washed Teflon vessels and 2 ml of concentrated ultrapure HNO_3 was added. When dissolution was completed (30-45 minutes), a second 2 ml aliquot of HNO_3 was added. After one hour, 6 ml of $DDIH_2O$ was added to the vessels and digested solutions were transferred to acid washed 15 ml polypropylene tubes. Samples were not manipulated for the next 24 hours, at which point digested solutions were further diluted with 1.4 M HNO_3 in $DDIH_2O$ to obtain a final dilution factor of 1,000 to 1,500-fold. All sample preparation was performed in a clean laboratory with a constant supply of HEPA-filtrated air. Diluted digests were analyzed with a Thermoscientific Element2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS) using an All-Teflon introduction system, self-aspiration and methane addition to plasma. Both low resolution (LR) and medium mass resolution (MR) acquisition modes were used. At least two preparation blanks were analyzed concurrently with each batch of 56 otolith sample solutions to estimate instrument limits of detection (LOD), which were estimated as three standard deviations of mean blank values. The combination of external calibrations (synthetic blanks and standards prepared in 1.4 M HNO_3) and internal standardization (In and Lu added to all solutions at 200 ppt level) was employed for quantification. Detection and quantification capabilities were evaluated using results from preparation blanks.

Statistical Analysis

Prior to statistical analysis, data were ln transformed to correspond to the constituents of the regional and platform signatures that are described in Chapter 3. Due to the variety of ages being examined simultaneously, and again to comply with the constituents of the developed signatures, residual values were analyzed in order to compensate for mass differences and ontogenetic shifts within otoliths of fish from varying ages (see Barnett and Patterson 2010).

Residual values were computed by subtracting mean elemental concentrations from each respective sample concentration. Multivariate analysis of variance (MANOVA) was used to determine if differences existed in red snapper otolith elemental signatures between DFL and TFL, with Pillai trace (V) as the test statistic because it is the most robust to violations of homogeneity of variance (Wilkinson et al. 1996). This was done to determine if the regions needed to be analyzed separately or as a combined FL region. An analysis of variance (ANOVA) was used to test elemental concentrations individually to determine the source of variance among regions. Reported values are based upon least square (LS) means. All analyses were performed using the Statistical Analysis System (SAS Institute 2006) with a significance level of $\alpha = 0.05$.

A maximum likelihood mixed-stock analysis 'HISEA' developed by Millar (1990) was used to estimate either the region or habitat of origin of adult red snapper in areas devoid of platforms. The baseline data set consisted of residual values of red snapper otolith region and habitat signatures that are reported in Chapter 3. It should be noted that all red snapper collected from fishing rodeos that were believed to be collected from platforms (n = 58) were relabeled as being collected from non-platform habitats based on the argument presented in Chapter 3. This improved the classification accuracy for habitat to 79.4% and location to 72.7% (Figure 4.2). The 2009 adult red snapper otolith data were classified as unknowns, or mixed data, against the baseline data to determine their presumed origin based upon maximum likelihood estimates (MLE) of mixed-stock proportions. Mixed data for each region was classified individually into region- and habitat-specific baseline data. Direct MLE and standard deviations were developed in HISEA by bootstrapping with 1000 resampled baselines.

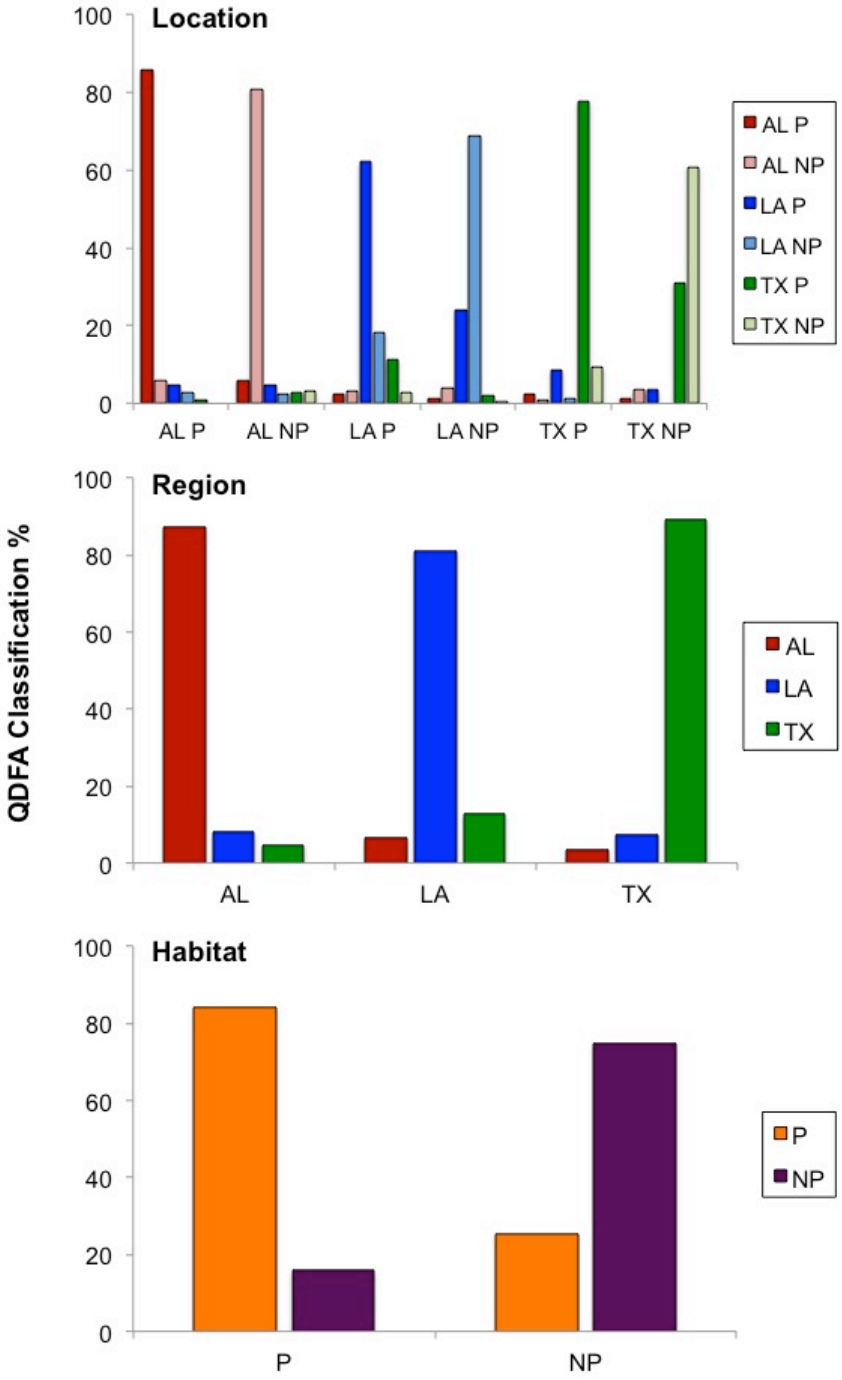

Figure 4.2. Jackknifed classification percentages of red snapper, *Lutjanus campechanus*, to six locations, three regions and two habitats in the Gulf of Mexico collected during the summers of 2007 and 2008 after relabeling all Alabama fishing rodeo samples as being collected from non-platform habitat. Percentages were estimated with quadratic discriminant function analyses (QDFA) of otolith chemical signatures. AL = Alabama; LA = Louisiana; TX = Texas; P = platform habitat; NP = non-platform habitat.

Multivariate analysis of variance (MANOVA) was used to determine if differences existed in gray triggerfish otolith elemental signatures between habitats, again using Pillai trace (V) as the test statistic. An analysis of variance (ANOVA) was used to test elemental concentrations individually to determine the source of variance between habitats. Reported values are based upon least square (LS) means. The HISEA model was used to determine if gray triggerfish otolith samples could be accurately classified to the habitat from which they were collected. The gray triggerfish otolith data were classified as mixed data against the red snapper otolith habitat signature baseline data to determine their presumed origin based on MLE of mixed-stock proportions. Mixed data for each habitat was classified individually into the habitat-specific baseline data in HISEA by bootstrapping with 1000 resampled baselines.

Results

A total of 500 adult red snapper otolith samples collected from four regions across the Gulf was processed for otolith chemical analysis. However, due to either poor sample quality or inadequate detection limits, only 487 otolith samples were compared to otolith signatures described in Chapter 3 to determine region and habitat origin (Table 4.1). All 17 elements (^{11}B, ^{138}Ba, ^{209}Bi, ^{111}Cd, ^{59}Co, ^{63}Cu, ^{65}Cu, ^{56}Fe, ^{7}Li, ^{55}Mn, ^{98}Mo, ^{206}Pb, ^{120}Sn, ^{205}Tl, ^{51}V, ^{64}Zn, ^{66}Zn) were present in adult red snapper otoliths above LODs. Trace metal concentrations were significantly different between DFL and TFL (MANOVA, $F_{17, 275} = 40.71$, $p < 0.001$); therefore these two regions were analyzed separately.

Mean concentrations of elements differed among regions (Table 4.2), and all elemental concentrations differed significantly (ANOVA, $p \leq 0.05$) among regions overall. As reported in Chapter 3, red snapper otoliths collected from LA and TX continued to not differ significantly in ^{59}Co ($p = 0.8496$), ^{120}Sn ($p = 0.1692$), ^{64}Zn ($p = 0.9569$) and ^{66}Zn ($p = 0.9976$) concentrations.

Table 4.1. Sample size and size range of red snapper, *Lutjanus campechanus*, and gray triggerfish, *Balistes capriscus*, collected from the Gulf of Mexico during the summer of 2009. DFL = Destin, Florida; TFL = Tampa, Florida; LA = Louisiana; TX = Texas.

Species	Region	Samples Collected	Samples Analyzed	Size Range (mm TL)
Red Snapper	DFL	155	153	447 - 747
	TFL	145	140	452 - 764
	LA	100	96	458 - 735
	TX	100	98	517 - 708
Gray Triggerfish	DFL	15	15	409 - 548
	LA	39	15	254 - 587

Additionally, these samples also had similar ^{11}B ($p = 2534$), ^{111}Cd ($p = 0.9448$), ^{98}Mo ($p = 0.9974$), and ^{51}V ($p = 1.000$). The only elements not significantly different between red snapper otoliths collected from DFL and TFL were ^{209}Bi ($p = 0.0785$), ^{98}Mo ($p = 0.2798$), ^{120}Sn ($p = 0.7193$) and ^{64}Zn ($p = 0.8965$). Red snapper otoliths collected from LA and DFL did not differ significantly in ^{111}Cd ($p = 0.2022$) and ^{206}Pb ($p = 0.1253$) concentrations, whereas LA and TFL had non-significant differences in ^{11}B ($p = 0.0638$), ^{111}Cd ($p = 0.6956$), ^{56}Fe ($p = 0.9715$), and ^{205}Tl ($p = 0.8604$) concentrations. More similarities existed in elemental concentrations of red snapper otoliths collected from TX and the FL regions. For instance, TX and DFL red snapper otoliths had non-significant differences in ^{111}Cd ($p = 0.5242$), ^{63}Cu ($p = 0.5166$), ^{65}Cu ($p = 0.1063$), ^{56}Fe ($p = 0.7014$), and ^{55}Mn ($p = 0.1730$) concentrations, while TX and TFL otoliths had non-significant differences in ^{11}B ($p = 0.9621$), ^{209}Bi ($p = 0.3210$), ^{111}Cd ($p = 0.3229$), ^{63}Cu ($p = 0.880$), ^{65}Cu ($p = 0.7428$), and ^{7}Li ($p = 0.9232$) concentrations. Red snapper otoliths collected from DFL had significantly higher concentrations of ^{11}B, ^{138}Ba, ^{59}Co, ^{7}Li, ^{205}Tl, and ^{51}V compared to the other regions (Table 4.2). As was also observed in Chapter 3, TX red snapper otoliths continued to have the highest concentrations of ^{206}Pb.

Table 4.2. Summary of raw data of otolith elemental concentrations (ppb) for red snapper, *Lutjanus campechanus*, and gray triggerfish, *Balistes capriscus*, collected from the Gulf of Mexico during the summer of 2009. Bold number represent significantly higher concentrations in regions. DFL = Destin, Florida; TFL = Tampa, Florida; LA = Louisiana; TX = Texas.

Element	Red Snapper								Gray Triggerfish			
	DFL (n = 153)		TFL (n = 140)		LA (n = 96)		TX (n = 98)		DFL (n = 15)		LA (n = 15)	
	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
^{11}B	**1177.82**	43.51	723.07	18.53	673.68	33.86	705.32	20.37	**2405.73**	145.06	1852.13	176.69
^{138}Ba	**6911.20**	180.85	4234.40	194.24	4420.33	105.61	5704.91	103.01	20306.53	2633.96	**40439.73**	5628.88
^{209}Bi	0.08	0.01	0.07	0.01	0.03	0.00	0.04	0.00	0.14	0.04	0.11	0.02
^{111}Cd	0.11	0.00	0.09	0.00	0.10	0.00	0.10	0.00	0.87	0.21	0.95	0.18
^{59}Co	**0.26**	0.01	0.12	0.01	0.17	0.01	0.18	0.01	1.06	0.15	1.50	0.26
^{63}Cu	50.78	0.59	47.49	0.59	40.92	0.57	49.51	0.71	74.93	6.28	83.59	9.81
^{65}Cu	49.16	0.56	46.33	0.57	38.70	0.59	47.22	0.72	73.03	6.10	81.68	9.99
^{56}Fe	70.23	4.24	52.88	4.09	55.62	6.03	65.21	4.14	1180.33	252.52	1099.47	286.33
^{7}Li	**498.56**	8.22	385.49	6.38	415.66	8.00	376.79	5.42	502.53	30.13	653.53	40.34
^{55}Mn	553.76	11.32	404.41	10.77	469.57	12.19	598.26	17.12	1409.47	114.15	1610.20	223.77
^{98}Mo	2.27	0.05	2.20	0.07	1.66	0.04	1.79	0.08	3.87	0.23	3.81	0.46
^{206}Pb	1.43	0.11	0.94	0.09	1.46	0.08	**1.82**	0.08	6.32	1.42	9.00	1.33
^{120}Sn	1.37	0.04	1.45	0.13	0.99	0.10	0.95	0.05	**10.45**	0.89	7.45	0.58
^{205}Tl	**0.72**	0.01	0.53	0.01	0.51	0.02	0.67	0.02	1.00	0.07	1.16	0.06
^{51}V	**0.22**	0.01	0.14	0.01	0.16	0.01	0.16	0.01	1.28	0.19	**6.41**	3.31
^{64}Zn	396.33	16.92	404.27	25.18	442.83	10.92	465.89	16.48	11761.13	1846.41	17656.40	3073.33
^{66}Zn	366.71	16.63	335.34	23.83	412.45	10.68	428.33	16.28	11782.27	1845.26	17698.20	3089.39

Direct MLE based on region-specific baseline data indicate that red snapper collected from DFL were estimated to predominately originate from LA (82.1%) and secondarily from AL (16.1%; Figure 4.3). Red snapper collected from TFL were also estimated as originating from LA (53.6%) with more influence from AL (37.8%). However, the original objective of this study was to test the validity of platform signatures collected from regions where platforms were evident; hence baseline DFL and TFL data were not collected. Surprisingly, the suite of elements believed to be associated with platforms and other artificial habitats performed better for discriminating among Gulf regions than between habitats, and were applied here to further examine population connectivity among red snapper Gulf regions. Thus, evaluating MLE based on regional signatures for DFL and TFL should be interpreted with caution as baseline samples for these regions were not collected causing biased results. Louisiana red snapper mainly consisted of locally derived recruits (66.2%) with small contributions from AL and TX (16.2% and 17.6%, respectively). Texas red snapper were largely locally derived (85.7%). Direct MLE based on habitat-specific baseline data indicated that red snapper collected from DFL, LA and TX were derived from platform habitats (79.3%, 98%, and 100%, respectively). Only red snapper collected from TFL were classified as originated from non-platform habitats (97.6%). Although classification success was the lowest for location-specific baseline data, direct MLE based on location-specific baseline data mimics the overall trends displayed in region- and habitat-specific MLE results (Figure 4.3).

A total of 54 adult gray triggerfish otoliths was collected from DFL and LA to test the validity of the platform signature described in Chapter 3 on other reef associated species. Only 30 samples were processed for otolith chemical analysis to allow even numbers to be processed from both regions/habitats (Table 4.1). Each LA gray triggerfish was collected from platform

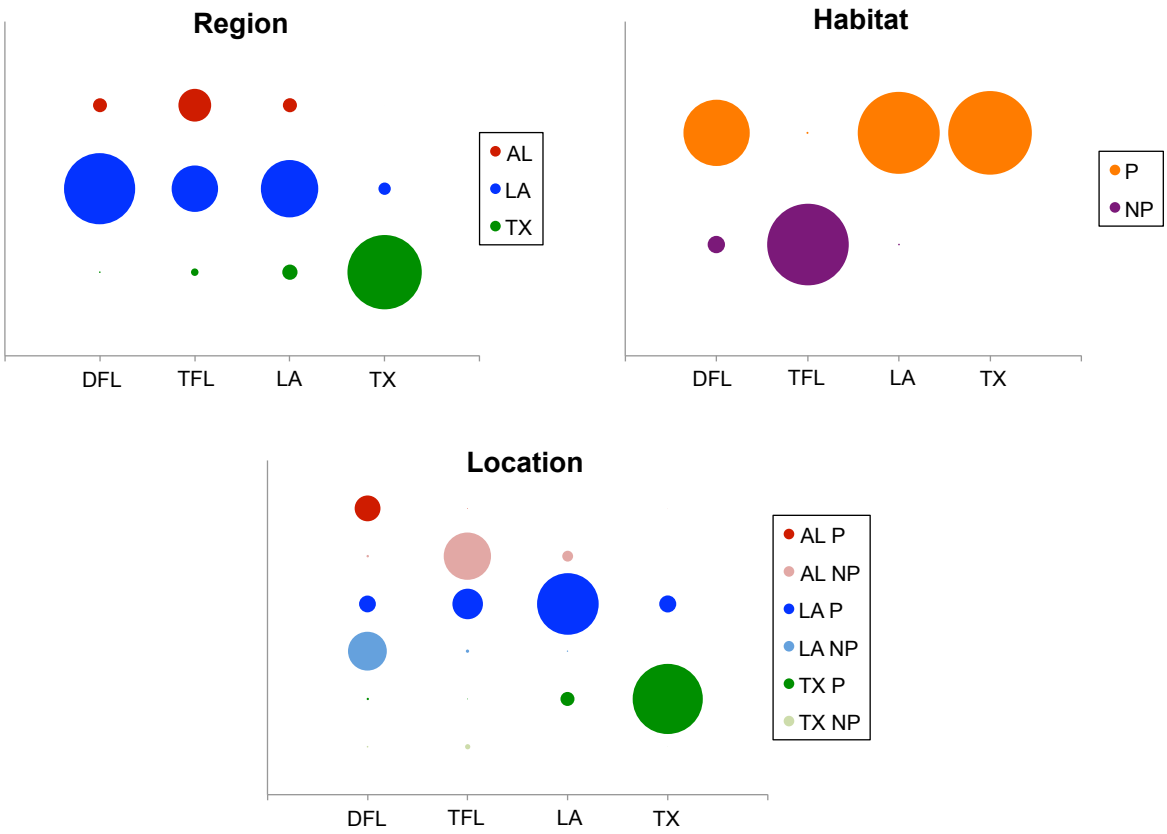

Figure 4.3. Bubbleplots of percent composition estimates derived from region-, habitat- and location-specific otolith chemistry-based discriminant function analysis indicate the origin of adult red snapper, *Lutjanus campechanus*, collected from four regions and two habitat types within the Gulf of Mexico during the summer of 2009. Bubbles are scaled by diameter; DFL = Destin, Florida; TFL = Tampa, Florida; LA = Louisiana; TX = Texas; AL = Alabama; P = platform habitat; NP = non-platform habitat.

habitats and each DFL gray triggerfish was collected from non-platform habitats. However, trace metal concentrations were not significantly different among regions/habitats (MANOVA, $F_{17, 12} = 2,48$, $p < 0.0576$). Only ^{11}B ($p = 0.0145$), ^{138}Ba ($p = 0.0055$), ^{120}Sn ($p = 0.0068$) and ^{51}V ($p = 0.0005$) concentrations were significantly different in gray triggerfish otoliths collected from these two regions. Direct MLE based on habitat-specific baseline data using all 17 elements indicate that 60.8% of DFL gray triggerfish were collected at non-platform habitats and 78.4% of

LA gray triggerfish were collected from platform habitats (Figure 4.4A). Yet, direct MLE based on habitat-specific baseline data using only the 4 significant elements better reflects actual collection sites with 99.9% of DFL gray triggerfish classified as being collected from non-platform habitats and 98.8% of LA gray triggerfish classified as being collected from platform habitats (Figure 4.4B).

Discussion

The overall objective of this study was to establish if microchemical signatures in red snapper otoliths collected from oil and gas platform described in Chapter 3 were evident in red snapper collected from regions devoid of platforms, including the west FL shelf. Based on MLE results using habitat-specific signatures, the platform marker was apparent in red snapper otoliths collected from FL, but primarily for fish collected from the DFL region. Additionally, all red snapper collected from natural habitats in LA and TX exhibited the platform marker. A natural ontogenetic shift in habitat is known to occur in red snapper as juveniles move from low-profile reefs, relic-shell and mud habitats to more complex habitats with increasing vertical dimension (see Patterson 2007 for review). As red snapper continue to mature, larger individuals are less dependent upon structure, including platforms, and can be found on outer shelf-edge reefs (Render 1995; Mitchell et al. 2004). The dominance of age-2 and age-3 red snapper on platforms has been attributed to this ontogenetic shift in habitat, as well as intense fishing pressure associated with platforms (Nieland and Wilson 2003; Patterson 2007). While this study cannot rule out fishing pressure as a cause for the reduction of older individuals on platforms, the fact that red snapper collected from natural habitat and shelf-edge banks exhibited the platform marker implies an ontogenetic shift from platform habitats to natural, lower relief habitats.

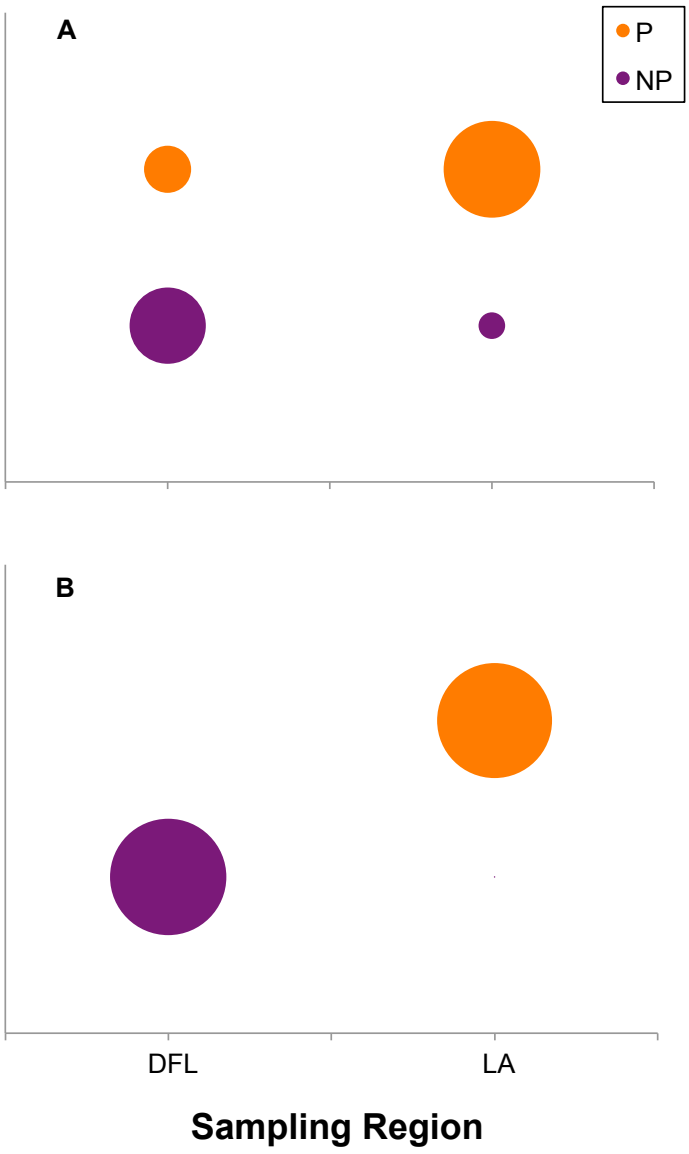

Figure 4.4. Bubbleplots of percent composition estimates derived from habitat-specific otolith chemistry-based discriminant function analysis using A.) all 17 elements or B.) only ^{11}B, ^{138}Ba, ^{120}Sn, and ^{51}V to indicate the habitat of origin of adult gray triggerfish, *Balistes capriscus*, collected from two regions representing two habitat types within the Gulf of Mexico during the summer of 2009. All DFL gray triggerfish were collected on non-platform habitats and all LA gray triggerfish were collected on platform habitats. Bubbles are scaled by diameter; DFL = Destin, Florida; LA = Louisiana; P = platform habitat; NP = non-platform habitat.

Low site fidelity of red snapper, along with localized movement between habitat types within regions, likely contributed to the lower classification success of habitats compared to regions as discussed in Chapter 3. To minimize the effect of movement between habitats and the accumulation of additional platform elements, red snapper sampled from LA and TX in this study were collected on shelf-edge banks and rock formations away from platforms. This proved more difficult for the LA region due to the abundance of platforms in the area (see Figure 4.5), which may have caused the platform signature to be continually evident in red snapper otoliths from this region as a result of the close proximity of habitats. However, mean otolith elemental concentrations for LA red snapper in this study were lower than the mean concentrations for the LA region and platform habitat reported in Chapter 3. Red snapper on the outer shelf-edge banks are exposed to fewer platforms and are farther removed from the influence of the Mississippi River plume. Therefore, new material that is incorporated onto the otolith while the fish resides on the shelf-edge bank will likely have lower elemental concentrations than fish collected further inshore and on platform habitats. Because whole otoliths were analyzed, if a red snapper resided on a platform at one time during it's life, the platform signature would still be present within the otolith due to the inert property of the otolith. However, the elemental concentration of the signature may be diluted by additional material accumulated on the otolith after the fish migrated away from the platform.

Gray triggerfish otoliths were analyzed to further examine the effectiveness of the platform signature in a reef-associated fish with higher site fidelity. The MLE revealed the platform signature was able to accurately predict the habitat of origin for gray triggerfish. However, for the signature to be highly accurate, elements had to be removed based on significance levels between regions examined. Patterson et al. (2010) discovered differences in

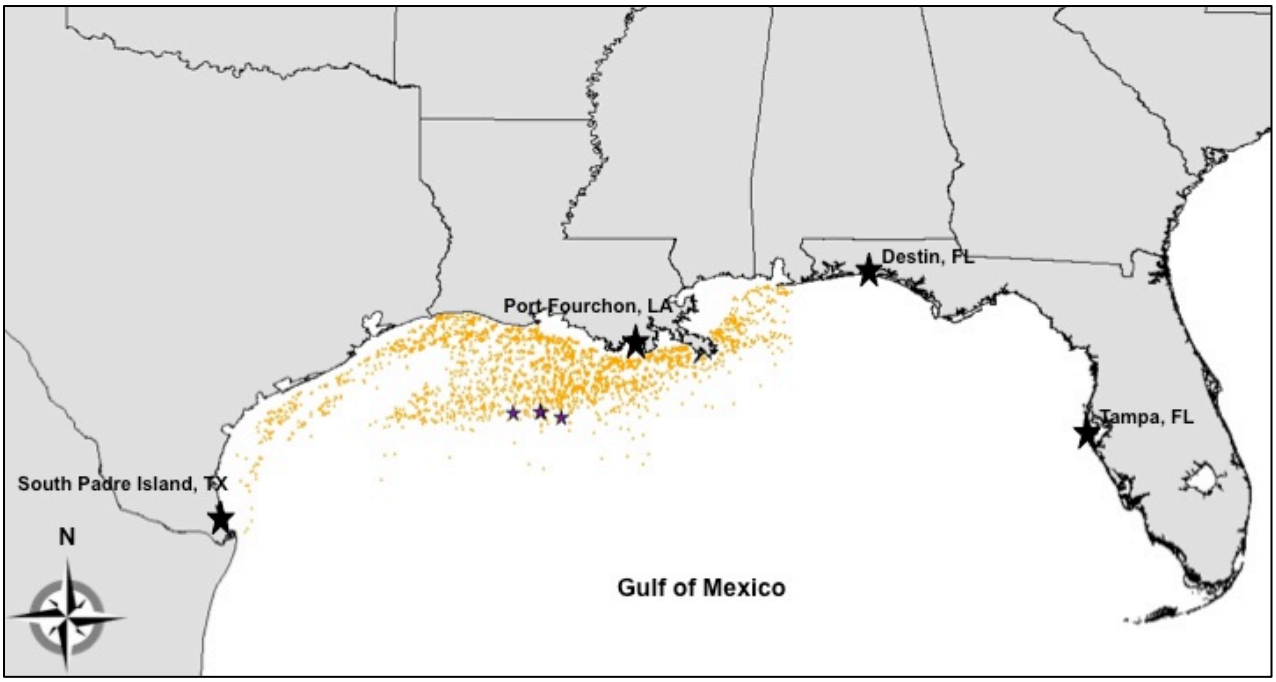

Figure 4.5. Distribution of oil and gas platforms (orange dots) along the continental shelf of the northern Gulf of Mexico (Gulf) in relation to the 2009 sampling regions. The purple stars represent three natural shelf-edge banks where adult red snapper were collected off the coast of Louisiana (LA).

otolith chemical signatures between age-0 lane snapper (*Lutjanus synagris*) and red snapper of similar sizes. Unlike red snapper, lane snapper may recruit to estuaries before migrating offshore, which may contribute to the observed differences. Furthermore, some of the elements (i.e., Cu and Zn) contained in the platform signature are physiologically regulated (see Chapter 3 for review). Thus, differences in life history parameters and physiological regulation among fish species may cause variability in the levels of metal incorporation into otoliths, making it necessary to alter signatures based on species and regions being analyzed.

It may also be speculated that the estimates of habitat origin is confounded by region since all gray triggerfish platform samples were collected off LA and all non-platform samples were collected off DFL. Otoliths from gray triggerfish collected at platforms had significantly

higher concentrations of Ba and V. While high concentrations of Ba likely can be attributed to the Mississippi River discharge (see Chapter 3 for review), Ba as barite is also the main component in drill muds associated with oil production processes (Kennicutt et al. 1996). Further, average Ba concentrations in gray triggerfish otoliths were six times greater than average Ba concentrations of red snapper collected from Louisiana or platforms. Average V concentrations were also greater in gray triggerfish otoliths compared to concentrations in red snapper otoliths collected from Louisiana or platforms. Vanadium is present at significant levels in crude oil (Kennicutt et al. 1996). Thus, the higher site fidelity of gray triggerfish may result in elevated otolith concentrations of Ba and V for samples collected at platforms. Additionally, gray triggerfish collected from non-platform habitats had significantly higher concentrations of Sn, a metal associated with anti-fouling paints (see Chapter 3 for review). Several of the gray triggerfish collected off DFL were from shipwrecks. Again, average Sn otolith concentrations of gray triggerfish were greater than average Sn otolith concentrations of red snapper collected from non-platform habitats. Therefore, the estimates of gray triggerfish origins may in fact be the result of habitat differences between regions.

Since the beginning of the Alabama Artificial Reef Program in 1953, approximately 20,000 artificial reefs have been deployed in a 3,100 km^2 designated area (Minton and Heath 1998; Patterson et al. 2001a). Despite AL's small coastline, red snapper caught there represent nearly 40% of the total recreational landings in the US Gulf. Fishery scientists have debated whether the artificial reef system off AL has increased production of red snapper or if it merely serves as a sink for stock-specific production (Szedlmayer and Shipp 1994; Shipp 1999; Cowan et al. 1999; Patterson et al. 2001b; Shipp and Bortone 2009; Cowan et al. 2010). Red snapper collected off AL and LA have similar growth rates and size distributions (Patterson et al. 2001b;

Fischer et al. 2004; Saari 2011). Although both regions have unique artificial habitat, age distribution in the eastern Gulf is truncated compared to the western Gulf and the eastern substock is projected to have lower productivity than the western substock (SEDAR 2009). Region-specific MLE results showed little contribution from AL red snapper to the 2009 sampling regions. The largest estimated contribution of AL red snapper was to the TFL region. This result is biased because no FL baseline samples were collected. However, it does confirm conventional tagging data in which fish tagged off AL and the FL panhandle were shown to move east and southeast, with red snapper recaptured as far south as TFL, but only one fish tagged off AL has been recaptured west of the Mississippi River (Patterson et al. 2001a; Addis et al. 2008). Additionally, the low contribution of AL red snapper to neighboring regions could imply that the AL artificial reef system is not highly productive and high fishing morality in the area may actually cause the artificial reef system to serve as a net sink for the Gulf-wide population.

By the late 1960s, the majority of commercial landings for red snapper were being obtained in the western Gulf. In fact, a significant portion of red snapper landed at ports in the eastern Gulf was obtained off the coast of LA (Goodyear 1995). The genetic effective population size of LA red snapper is estimated to be ten-fold greater than red snapper originating from AL and TX (Gold and Saillant 2007). Furthermore, the recent increase in red snapper spawning potential ratio (SPR) has been attributed to the western Gulf (SEDAR 2009). Thus, it is not surprising that MLE results of this study estimate a large contribution of LA red snapper to the FL regions. Again, these results are biased as FL baseline samples were not collected for comparison. However, the platform marker was evident in red snapper collected from DFL, and with LA red snapper being highly correlated with the platform signal, this may imply a western

substock contribution to the area. The results of Chapter 2 indicate that LA was an important source of recruits for the western red snapper substock and results from a collaborative project revealed that LA may potentially be a source of recruits to the west FL shelf (Patterson et al. 2010). While data were insufficient to determine the stock structure of FL red snapper, observed MLE based upon regional and habitat otolith signatures, along with the results of Chapter 2 and Patterson et al. (2010), suggest LA may be a potentially important source of red snapper recruits for the entire northern Gulf.

Based upon MLE results using cohort specific signatures, TX red snapper appear to be locally derived with a relatively small contribution from LA. Further, TX red snapper appear to contribute little to the other Gulf regions sampled. These results are supported by results described in Chapter 2 in which TX juvenile red snapper were primarily locally derived with LA being a secondary important source of recruits depending on the year class examined. Previous red snapper otolith chemistry studies indicated limited movement in the first year of life (Cowan et al. 2003; Patterson 2007; Patterson et al. 2008). However, the large proportion of LA and TX red snapper assigned to their respective regions in the current study, combined with the results of Chapter 2 where more movement and mixing occurred for age-1 fish, suggest that red snapper move more in the juvenile stage and settle down as they get older. Diamond et al. (2007) reported small-scale movement of tagged red snapper along the TX coast, supporting an isolated stock theory. They concluded that an isolated stock could explain the smaller sizes of TX red snapper compared to LA and AL, and supported the notion of a separate demographic TX substock. Kritzer and Sale (2004) define a metapopulation as 'a system of discrete local populations, each of which determines its own internal dynamics to a large extent, but with a degree of identifiable and nontrivial demographic influence from other local populations through

dispersal of individuals.' The idea of managing red snapper as a metapopulation is not a new concept (Pruett et al. 2005; Gold and Saillant 2007; Patterson 2007; Saillant et al. 2010). The microchemical otolith results of the western Gulf red snapper in this study demonstrated discrete regional populations with some dispersal from neighboring regions, further supporting the notion of a metapopulation.

Previous studies have determined that trace metals based on known anthropogenic sources could be detected in otoliths and used to discriminate the nursery or location of origin (Dove and Kingsford 1998; Spencer et al. 2000). While otolith trace metal analysis is not a novel idea, this study is novel in terms of the large suite of trace metals analyzed and the intent of trying to use these metals to distinguish between habitat types that co-occur in the open ocean. Otolith chemical signatures based on trace metals associated with oil and gas production platforms were able to discriminate among three red snapper collection regions, and to a lesser extent between habitats, in the Gulf. Furthermore, this study can provide baseline data for future projects examining the effects of oil and gas production in the Gulf. On April 20, 2009 the Deepwater Horizon oil well blowout near the Mississippi River delta resulted in the largest oil spill in U.S. history. Morales-Nin et al. (2007) found that some elements associated with the oil of a sunken oil tanker were incorporated into turbot (*Scophthalmus maximus*) otoliths through a diet laboratory study. Thus, three years of "pre-spill" otolith trace metal concentrations are present in this study that could be compared to otolith concentrations of red snapper collected "post-spill" to see if a spike in elements associated with crude oil (i.e., Cu, Ni, V) occurred. If so, it may infer the assimilation of crude oil into the diet of red snapper collected from areas affected by the oil spill.

Whole otolith analysis has proven useful for distinguishing among fish stocks or among fish inhabiting different niches for some period of time (Campana et al. 1994; Patterson et al. 1999; Elsdon and Gillanders 2003). However, whole otolith analysis incorporates the entire life of the fish, and it is not possible to determine when geographic separation occurred. Future work should utilize laser ablation inductively coupled plasma-mass spectrometry (LA-ICP-MS) to analyze specific loci along a transverse section of the otolith to determine an approximate age at which red snapper migrate away from platforms. If this age coincides with the disappearance of older red snapper from platforms, it would further demonstrate that a natural ontogenetic shift in habitat does occur, which may be more prominent to the disappearance of older fish from platforms than fishing pressure. Moreover, evidence of the platform signature in regions devoid of platforms may not indicate that platforms enhance the production of red snapper, but instead reflect a possible western contribution to the eastern Gulf. Additional analysis, including the collection of FL baseline samples, is needed to determine mixing dynamics and stock separation in the eastern Gulf. Further study may show that Gulf red snapper populations should be divided into four separate substocks (TX, LA, AL/MS and FL), instead of the two-stock management approach currently established.

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GENERAL SUMMARY AND CONCLUSIONS

The two main objectives of this study were to estimate red snapper, *Lutjanus campechanus*, mixing dynamics in the western Gulf of Mexico (Gulf), and to evaluate potential linkages via movement between western Gulf and eastern Gulf portions of the red snapper stock. The use of otolith chemical signatures developed from both nursery regions and from metals associated with oil and gas platforms proved successful in differentiating red snapper collected from different regions. Although the sample design was incomplete for some aspects of the project, this study proves that such signatures can serve as an effective tool to examine postsettlement movement and population connectivity among Gulf red snapper.

In Chapter 1, nursery signatures were developed through the analysis of age-0 red snapper otoliths collected from six different regions within the Gulf waters of US and Mexico. The discriminant classifications of region-specific nursery signatures for the three consecutive year classes studied validate the utility of natural otolith tags to estimate the source of recruits to regions in the Gulf and to examine red snapper population connectivity. The largest misclassifications occurred in northern Gulf regions, which is likely attributable to the heavy fluvial influence to these area. Although red snapper were not collected from southern Gulf regions for all year classes studied, when present these regions consistently had otolith chemical concentrations that greatly differed from the northern regions.

In Chapter 2, red snapper otolith nursery signatures defined in Chapter 1 were used to estimate the source of recruits to the Texas continental shelf, as well as examine any potential mixing dynamics between Texas and Mexico, based on otolith core concentrations of sub-adult and adult red snapper. Moderate to high percentages of LA recruits were observed among TX red snapper, but only a small percentage of TX recruits were detected among LA samples. Thus,

it would appear that the LA region is an important source of recruits to the TX red snapper population based on the year classes examined. Louisiana red snapper populations appeared to be predominantly comprised of locally recruited fish, further indicating the importance of LA as a source of recruits to the western Gulf red snapper substock. Unfortunately, data were not sufficient to determine the source of recruits to the Mexico red snapper populations, or to examine any potential connectivity between Mexican and western Gulf red snapper.

In Chapter 3, natural tags derived from otolith trace metal concentrations were used to examine temporal and geographical stability of platform signatures among three regions of the northern Gulf. Otolith trace metal concentrations were temporally stable for red snapper collected in the northern Gulf over the two-year study period. Natural signatures derived from trace metal concentrations demonstrated significant spatial differences, with classification success being higher for regions as compared to habitats. Localized movement within regions, along with different habitat types in close proximity to each other, most likely contributed to the higher regional classification success of this study.

When examining regional differences in otolith concentrations, each region had distinct metal levels that could be used to discriminate among them. Higher concentrations of ^{111}Cd, ^{7}Li, ^{98}Mo and ^{120}Sn in red snapper collected off Alabama may reflect the unique materials used to create the region's artificial reefs, many of which were created from materials of opportunity. Higher concentrations of ^{11}B and ^{138}Ba in Louisiana samples may result from combined influences of oil and gas platform production process and Mississippi River discharge. Red snapper collected off Texas had higher concentrations of ^{206}Pb and ^{205}Tl, which may reflect platforms and higher sulphur concentrations in the region. Thus, while the results of this study indicate that trace metals associated with platforms can be used to develop otolith chemical

signatures, trace metals ultimately worked best for discriminating among regions due to unique features and habitats in each region.

In Chapter 4, otolith signatures described in Chapter 3 were used to estimate region and habitat of origin for adult red snapper collected from natural habitats in the western Gulf and from areas in the eastern Gulf that are free of platforms. The platform marker was apparent in red snapper otoliths collected off Destin, Florida and for all red snapper collected from natural habitats in Louisiana and Texas, possibly reflecting an ontogenetic shift from platform habitats to natural, less complex habitats. Otolith trace metal concentrations of gray triggerfish collected from platform and non-platform habitats were also analyzed to test the accuracy of the platform otolith signature developed in Chapter 3. The platform signature was highly accurate for classifying habitat of origin for gray triggerfish once elements were removed based on significance levels between regions examined. Results of this study are insufficient to determine if the platform marker in regions devoid of platforms indicates new biomass production. However, the large contribution of Louisiana red snapper to the Florida region, with low contribution from the neighboring Alabama region, and evidence of the platform marker in Florida red sapper otoliths may reflect a western contribution of red snapper to the eastern Gulf.

The elements used to develop the platform signature in this study are unique and not the typical suite of elements used in otolith chemical analysis. Because this unique suite of elements worked better for discriminating among regions than habitats, it would be interesting to apply these elements to the group of elements and stable isotopes used to develop the nursery signature. The combination of these elements may result in higher discriminant classification results of the nursery regions and possibly minimize the need to develop cohort-specific signatures.

The otolith chemical results of western Gulf red snapper in this study demonstrated discrete regional populations with some dispersal from neighboring regions, supporting the notion of a metapopulation structure. Further analysis is needed to verify the results of this study and to investigate the population structure of the eastern Gulf. If this trend continues for the western Gulf, and if it is also evident in the eastern Gulf, it may be appropriate to amend the current two-stock management approach and instead divide the Gulf red snapper population into four separate substocks. Overall, the results of this study demonstrate the potential of using natural tags to examine postsettlement movement and population connectivity to benefit the management and recovery of Gulf red snapper stocks.

APPENDIX A: CHAPTER 1 SUPPLEMENTARY DATA

Table A.1. Summary of raw data for the 2005 year class region-specific otolith element:Ca (μmol/mol) or stable isotope (ppb) ratios for age-0 red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico nursery regions. FL = Florida; AL = Alabama; LA = Louisiana; TX = Texas.

Element	FL (n = 20)		AL (n = 30)		LA (n = 30)		TX (n = 30)	
	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Ba:Ca	6.83	0.44	7.42	0.34	6.71	0.42	7.41	0.48
Li:Ca	5.79	0.15	5.51	0.17	4.50	0.06	4.99	0.09
Mg:Ca	0.18	0.01	0.21	0.01	0.21	0.01	0.24	0.01
Mn:Ca	3.55	0.23	6.01	0.32	6.28	0.29	8.67	0.40
Sr:Ca	2.14	0.02	2.34	0.02	2.35	0.02	2.45	0.05
$\delta^{13}C$	-3.47	0.13	-3.94	0.04	-4.17	0.07	-3.76	0.10
$\delta^{18}O$	0.25	0.04	-1.10	0.05	-1.32	0.06	-1.27	0.05

Table A.2. Summary of raw data for the 2006 year class region-specific otolith element:Ca (μmol/mol) or stable isotope (ppb) ratios for age-0 red snapper, *Lutjanus campechanus*, collected from five Gulf of Mexico nursery regions. AL = Alabama; LA = Louisiana; TX = Texas, MEX1 = Veracruz, Mexico; MEX2 = Campeche, Mexico.

Element	AL (n = 30)		LA (n = 30)		TX (n = 30)		MEX1 (n = 30)		MEX2 (n = 29)	
	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Ba:Ca	7.68	0.64	7.68	0.66	9.01	0.44	7.23	0.34	4.45	0.26
Li:Ca	5.82	0.25	5.54	0.14	4.97	0.06	4.40	0.09	4.95	0.10
Mg:Ca	0.21	0.00	0.20	0.00	0.22	0.00	0.17	0.00	0.20	0.01
Mn:Ca	8.54	0.66	7.89	0.52	9.01	0.56	5.41	0.25	2.22	0.35
Sr:Ca	2.32	0.02	2.41	0.02	2.37	0.01	2.42	0.03	2.21	0.03
$\delta^{13}C$	-3.81	0.08	-4.23	0.06	-3.91	0.08	-3.71	0.07	-3.19	0.13
$\delta^{18}O$	-1.05	0.03	-0.99	0.05	-0.96	0.04	-0.52	0.03	-0.34	0.06

Table A.3. Summary of raw data for the 2007 year class region-specific otolith element:Ca (µmol/mol) or stable isotope (ppb) ratios for age-0 red snapper, *Lutjanus campechanus*, collected from six Gulf of Mexico nursery regions. FL = Florida; AL = Alabama; LA = Louisiana; TX = Texas, MEX1 = Veracruz, Mexico; MEX2 = Campeche, Mexico.

Element	FL (n = 29)		AL (n = 30)		LA (n = 30)		TX (n = 30)		MEX1 (n = 22)		MEX2 (n = 30)	
	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
Ba:Ca	4.62	0.15	7.14	0.28	10.11	0.89	9.65	0.92	11.26	0.99	4.34	0.31
Li:Ca	9.48	0.47	5.10	0.07	5.20	0.15	4.99	0.08	5.11	0.16	4.96	0.33
Mg:Ca	0.17	0.00	0.22	0.01	0.20	0.00	0.25	0.01	0.26	0.01	0.17	0.01
Mn:Ca	2.34	0.13	9.32	0.58	7.59	0.35	10.51	0.46	7.01	0.58	1.66	0.23
Sr:Ca	2.14	0.02	2.29	0.02	2.28	0.02	2.16	0.03	2.28	0.03	2.19	0.04
$\delta^{13}C$	-3.93	0.09	-3.72	0.06	-3.98	0.08	-4.35	0.08	-3.45	0.18	-3.48	0.08
$\delta^{18}O$	-0.25	0.03	-1.06	0.04	-1.03	0.04	-1.42	0.06	-1.16	0.09	-0.45	0.04

Table A.4. Raw canonical coefficients for canonical discriminant analysis comparing region and year class-specific otolith chemical signatures of age-0 red snapper, *Lutjanus campechanus*, from six nursery regions in the Gulf of Mexico for the 2005, 2006 and 2007 year classes. "All" indicates all three year classes combined.

Element	2005 Year Class		2006 Year Class		2007 Year Class		All Year Classes	
	Can 1	Can 2	Can 1	Can 2	Can 1	Can 2	Can 1	Can 2
Ba:Ca	1.987	-0.131	0.240	0.043	0.495	-0.178	-0.073	0.812
Li:Ca	2.374	2.660	0.779	-6.462	-2.894	4.682	-2.018	4.128
Mg:Ca	-0.143	2.528	-3.070	-1.907	-1.329	-0.819	-1.284	-1.728
Mn:Ca	-1.337	2.510	1.640	1.478	2.116	1.226	1.738	1.247
Sr:Ca	-5.039	1.295	1.047	8.363	2.272	3.702	3.216	-0.494
$\delta^{13}C$	0.581	1.615	-0.711	-0.264	-0.031	0.077	-0.445	0.036
$\delta^{18}O$	3.352	0.770	-3.081	2.855	-1.498	0.237	-2.110	1.020

APPENDIX B: CHAPTER 2 SUPPLEMENTARY DATA

Table B.1. Sample size and size range of sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four regions across the Gulf of Mexico during the summers of 2006, 2007 and 2008. LA = Louisiana; TX = Texas; MEX1 = Veracruz, Mexico; MEX2 = Campeche Banks, Mexico.

Sampling Year	Region	Samples Aged	Samples Cored and Analyzed	Total Size Range (mm TL)
2006	LA	167	52	151 - 325
	TX	142	52	151 - 293
	MEX1	31	18	250 - 280
	MEX2	27	3	230 - 260
2007	LA	147	111	151 - 443
	TX	128	104	152 - 366
	MEX1	110	81	240 - 380
	MEX2	132	4	240 - 480
2008	LA	248	150	151 - 699
	TX	206	150	151 - 682
	MEX1	-	-	-
	MEX2	-	-	-

Table B.2. Summary of raw data for the 2005 year class region and age-specific otolith element:Ca (μmol/mol) or stable isotope (ppb) ratios for sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico regions during the summers of 2006, 2007 and 2008. LA = Louisiana; TX = Texas; MEX1 = Veracruz, Mexico; MEX2 = Campeche, Mexico.

Age	Region	Total	Ba:Ca		Li:Ca		Mg:Ca		Mn:Ca		Sr:Ca		$\delta^{13}C$		$\delta^{18}O$	
			Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
1	LA	51	7.02	0.29	5.58	0.29	0.18	0.01	6.86	0.36	2.22	0.01	-4.05	0.05	-0.48	0.07
	TX	52	6.87	0.32	5.51	0.12	0.22	0.01	8.07	0.44	2.24	0.01	-3.82	0.06	-0.86	0.06
	MEX1	18	7.18	0.49	5.89	0.25	0.20	0.01	7.25	0.58	2.24	0.03	-4.21	0.10	-0.67	0.06
	MEX2	3	6.10	0.94	6.24	0.12	0.22	0.03	4.67	1.79	2.17	0.06	-3.76	0.21	-0.55	0.25
2	LA	55	9.14	0.36	5.08	0.11	0.20	0.00	8.52	0.37	2.27	0.01	-4.23	0.04	-0.72	0.08
	TX	60	7.75	0.34	4.83	0.05	0.22	0.00	9.49	0.41	2.24	0.01	-3.77	0.06	-0.82	0.04
	MEX1	50	7.06	0.37	4.92	0.10	0.20	0.00	7.80	0.34	2.21	0.01	-3.88	0.06	-0.48	0.04
	MEX2	1	2.88	.	4.65	.	0.12	.	2.84	.	1.98	.	-4.17	.	-0.15	.
3	LA	50	6.41	0.27	4.50	0.09	0.15	0.00	7.03	0.37	2.05	0.02	-4.19	0.05	-0.72	0.06
	TX	50	7.28	0.44	4.88	0.09	0.19	0.00	8.10	0.43	2.21	0.02	-3.87	0.07	-0.76	0.05
	MEX1	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	MEX2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table B.3. Summary of raw data for the 2006 year class region- and age-specific otolith element:Ca (µmol/mol) or stable isotope (ppb) ratios for sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico regions during the summers of 2007 and 2008. LA = Louisiana; TX = Texas; MEX1 = Veracruz, Mexico; MEX2 = Campeche, Mexico.

Age	Region	Total	Ba:Ca		Li:Ca		Mg:Ca		Mn:Ca		Sr:Ca		$\delta^{13}C$		$\delta^{18}O$	
			Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
1	LA	56	7.64	0.37	4.87	0.08	0.20	0.00	8.88	0.36	2.26	0.01	-4.13	0.05	-0.61	0.07
	TX	44	10.65	0.56	4.76	0.07	0.22	0.00	9.52	0.35	2.24	0.02	-4.13	0.06	-0.78	0.05
	MEX1	31	7.70	0.61	4.93	0.12	0.19	0.01	7.35	0.38	2.21	0.02	-3.73	0.06	-0.45	0.05
	MEX2	3	5.06	1.06	5.09	0.19	0.24	0.02	2.64	0.62	2.22	0.08	-4.01	0.22	-0.36	0.01
2	LA	50	7.35	0.30	4.77	0.10	0.15	0.00	7.27	0.37	2.28	0.01	-4.15	0.06	-0.50	0.06
	TX	50	8.40	0.36	4.81	0.08	0.15	0.00	6.92	0.35	2.29	0.01	-4.10	0.06	-0.42	0.05
	MEX1	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	MEX2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table B.4. Summary of raw data for the 2007 year class region- and age-specific otolith element:Ca (µmol/mol) or stable isotope (ppb) ratios for sub-adult and adult red snapper, *Lutjanus campechanus*, collected from four Gulf of Mexico regions during the summer 2008. LA = Louisiana; TX = Texas; MEX1 = Veracruz, Mexico; MEX2 = Campeche, Mexico.

Age	Region	Total	Ba:Ca		Li:Ca		Mg:Ca		Mn:Ca		Sr:Ca		$\delta^{13}C$		$\delta^{18}O$	
			Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err	Mean	Std Err
1	LA	50	9.34	0.53	4.75	0.08	0.19	0.00	8.21	0.26	2.29	0.01	-3.72	0.05	-0.74	0.04
	TX	50	7.61	0.48	5.01	0.12	0.19	0.00	8.90	0.35	2.19	0.01	-4.22	0.04	-0.78	0.04
	MEX1	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	MEX2	0	.	.	.	.	.	.	.	.	.	.	.	.	.	.

APPENDIX C: CHAPTER 3 SUPPLEMENTARY DATA

Table C.1. Raw canonical coefficients for canonical discriminant analysis comparing location, region and habitat otolith chemical signatures of red snapper, *Lutjanus campechanus*, from three regions and two habitats in the Gulf of Mexico during the summers of 2007 – 2008.

Element	Location		Region		Habitat	
	Can 1	Can 2	Can 1	Can 2	Can 1	Can 2
^{11}B	-0.251	-3.079	0.391	-3.016	-0.275	0.343
^{138}Ba	-0.797	-0.934	-0.755	-0.946	0.530	0.872
^{209}Bi	-0.302	0.075	-0.313	0.024	-0.216	0.140
^{111}Cd	-0.195	0.561	-0.274	0.525	-0.184	-0.084
^{59}Co	-0.182	-0.202	-0.261	-0.232	0.316	0.202
^{63}Cu	-0.188	2.471	-0.749	2.276	-0.296	-4.546
^{65}Cu	1.273	-1.184	1.518	-0.941	0.809	4.745
^{56}Fe	0.029	0.207	-0.034	0.213	0.158	-0.211
^{7}Li	-2.093	1.774	-1.981	1.641	-1.805	-0.598
^{55}Mn	0.418	1.039	0.020	1.034	0.889	-0.991
^{98}Mo	-0.105	0.386	-0.352	0.312	0.356	0.405
^{206}Pb	0.514	-0.016	0.544	0.056	0.188	-0.289
^{120}Sn	-0.240	0.557	-0.159	0.578	-0.481	-0.077
^{205}Tl	1.931	0.244	2.162	0.452	-0.344	0.256
^{51}V	0.216	0.220	0.201	0.262	0.120	0.283
^{64}Zn	0.443	-1.975	0.826	-1.858	0.256	-9.053
^{66}Zn	0.203	0.446	-0.002	0.468	0.613	8.086

VITA

Michelle Zapp Sluis was born on May 25th, 1982 in Houston, Texas. She grew up in Sugar Land, Texas and graduated from Stafford High School in 2000. At the end of her high school senior year, Michelle participated in the Science and Engineering Fair of Houston and was awarded a full scholarship for the Texas A&M University at Galveston Summer School at Sea program for the summer of 2000. After completing her freshman year at the University of Texas, Michelle decided her interests would be better met at Texas A&M University at Galveston and transferred in August 2001. There she earned her Bachelor of Science in marine biology with a concentration in marine fisheries in 2004. While a student at Texas A&M, Michelle worked in Dr. Jay Rooker's Fisheries Ecology and Ecosystem Research Lab. After graduation, she continued on as a research technician working cooperatively for both the research lab and the National Marine Fisheries Service Galveston Branch. It was there that she was introduced to the world of otoliths. In August 2005, Michelle entered the Department of Oceanography and Coastal Sciences as a graduate student under the supervision of Dr. James H. Cowan, Jr. She earned a Doctor of Philosophy degree from the Louisiana State University in December 2011.