1-1-2015

Application of Otolith Chemical Signatures to Estimate Population Connectivity of Red Snapper In the Western Gulf of Mexico

Michelle Zapp Sluis
Louisiana State University

Beverly K. Barnett
NOAA Fisheries Service

William F. Patterson
University of South Alabama

James H. Cowan
Louisiana State University

Alan M. Shiller
University of Southern Mississippi

Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 7:483–496, 2015
Published with license by the American Fisheries Society
ISSN: 1942-5120 online
DOI: 10.1080/19425120.2015.1088492

ARTICLE

Application of Otolith Chemical Signatures to Estimate Population Connectivity of Red Snapper in the Western Gulf of Mexico

Michelle Zapp Sluis*[1]
Department of Oceanography and Coastal Sciences, Louisiana State University, Energy, Coast, and Environment Building, Baton Rouge, Louisiana 70803, USA

Beverly K. Barnett
National Marine Fisheries Service, Southeast Fisheries Science Center, Panama City Laboratory, 3500 Delwood Beach Road, Panama City Beach, Florida 32408, USA

William F. Patterson III
Department of Marine Sciences, University of South Alabama, and Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, Alabama 36528, USA

James H. Cowan Jr.
Department of Oceanography and Coastal Sciences, Louisiana State University, Energy, Coast, and Environment Building, Baton Rouge, Louisiana 70803, USA

Alan M. Shiller
Department of Marine Science, University of Southern Mississippi, 1020 Balch Boulevard, Stennis Space Center, Mississippi 39529, USA

Abstract

Otolith chemical signatures of Red Snapper *Lutjanus campechanus* from six nursery regions were used to estimate the sources of recruits to four sampling regions in the western Gulf of Mexico (Gulf) and to estimate whether postsettlement mixing of Red Snapper occurs between the U.S. and Mexican portions of the western Gulf. In a previous study, region-specific otolith signatures (element : Ca ratios: Ba:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Li:Ca; stable isotope delta values: $\delta^{13}C$ and $\delta^{18}O$) were developed based on age-0 Red Snapper (2005–2007 year-classes) sampled from the six nursery areas. In the present study, subadult and adult Red Snapper (ages 1–3) belonging to those same year-classes were collected from four sampling regions within the western Gulf (two regions in U.S. waters; two regions along the Mexican continental shelf) during summer in 2006–2008. Left sagittal otoliths were used to age subadults and adults to the corresponding nursery year-classes, and right sagittal otoliths were cored for chemical analysis. Off the southwestern U.S. coast, the sampled age-1–3 Red Snapper included locally derived recruits as well as recruits from the northwestern Gulf nursery region. However, analytical results were inconclusive with respect to estimating the connectivity between Red Snapper populations in U.S. and Mexican waters of the western Gulf.

Subject editor: Carl Walters, University of British Columbia, Canada

*Corresponding author: sluism@tamug.edu
[1]Present address: Department of Marine Biology, Texas A&M University at Galveston, 1001 Texas Clipper Road, Galveston, Texas 77554, USA.

Received May 4, 2015; accepted August 25, 2015

The Gulf of Mexico (hereafter, Gulf) fishery for Red Snapper *Lutjanus campechanus* began over 150 years ago off the coast of Pensacola, Florida; however, due to severe overfishing, the stock became depleted by the late 1800s (Camber 1955). The fishery then shifted 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 landings of Red Snapper continued until the early 1980s, when the U.S. fishing fleet was banned from Mexican waters, thereby 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 Banks fishery was initially the national leader in Red Snapper production. However, due to adverse effects 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% between the 1980s and the late 1990s (Monroy-García et al. 2002), and the Mexican stock was estimated to be severely overfished by 2005 (SAGARPA 2006, as cited by 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, but there is still a need for stricter regulations.

Management of the U.S. Gulf Red Snapper stock was implemented in November 1984 via the Gulf of Mexico Fishery Management Council's fishery management plan for reef fishes, which was designed to rebuild declining fish stocks. In compliance with regulations set by the Magnuson–Stevens Fishery Conservation and Management Act, several amendments have been adopted 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 on shrimp trawls; GMFMC 2010).

The Red Snapper population has been categorized into eastern and western substocks (divided by the Mississippi River; SEDAR 2005) based on demographic differences in size at age, maturation rates, and genetic effective population size (N_e) of Red Snapper that occur across the Gulf (Fischer et al. 2004; Saillant and Gold 2006; Jackson et al. 2007). However, plans to rebuild Red Snapper biomass are applied Gulf-wide rather than at the level of individual management subunits. Gold and Saillant (2007) determined that the N_e of Red Snapper off the Louisiana coast was an order of magnitude larger than the N_e off the Alabama and Texas coasts, alluding to spatial differences in the number of viable adults that were able to produce surviving offspring. Uniform reduction of fishing mortality Gulf-wide is expected to result in the western substock recovering faster and to a greater spawning stock biomass level than the eastern substock, since the western substock has a higher biomass and an estimated lower fishing mortality relative to the eastern substock (SEDAR 2013). Thus, without a reconfiguration of the current management approach, the greater size of the western substock is projected to continue.

Demographic differences also exist within the western substock. Studies have shown that Red Snapper collected off Texas are significantly smaller at age and reach a smaller maximum size than those collected off Louisiana (Fischer et al. 2004; Saari 2014). Saari (2014) also reported a higher proportion of older fish collected off north Texas and Louisiana than from all other Gulf regions, which was possibly attributable to the higher stock abundance of the western Gulf. Although differences in Red Snapper growth rates have been linked to increased primary production associated with the Mississippi River plume (Fischer et al. 2004), an understanding of population structure and connectivity could further explain the demographic differences within the western Gulf. Furthermore, the degree of connectivity that exists between the Red Snapper population off south Texas and the population along the northeastern coast of Mexico is unknown. Given that the Mexican stock is severely overfished, high connectivity between Texas and Mexican Red Snapper populations could mean that the Mexican fishery serves as a sink for Texas recruits (sensu Crowder et al. 2000).

The use of otolith chemistry 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 once formed; thus, the chemical signatures from surrounding seawater accreted onto the growing surface will be permanently retained (as reviewed by Campana 1999). This allows material that is deposited during the juvenile stage to act as a natural marker indicating the nursery of origin. Chemical signatures contained within the core (or juvenile portion) of the otolith can then be used to identify the nursery of origin of 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 Zapp Sluis et al. (2012) demonstrated that Red Snapper from various nursery regions within the Gulf can be distinguished based on otolith chemical signatures. Employing otolith signatures to examine population connectivity and mixing dynamics is essential to the development of marine population dynamics and the management of fishery stocks (Cowen et al. 2000).

The purpose of this study was to apply the otolith chemical nursery signatures identified by Zapp Sluis et al. (2012) to estimate the population structure and connectivity of Red Snapper

in the western Gulf. Specifically, natural tags derived from element : Ca and stable isotope ratios in otoliths of age-0 Red Snapper sampled from six regions throughout the Gulf were compared to element : Ca and stable isotope ratios for the otolith cores in subadult and adult Red Snapper sampled from four western Gulf regions. The objectives were to estimate the sources of Red Snapper recruits to these regions and to examine Red Snapper mixing dynamics between U.S. and Mexican regions of the western Gulf.

METHODS

Sample collection.—Subadult and adult Red Snapper were sampled from the northwestern Gulf (NWG); southwestern Gulf (SWG); southern Gulf shelf between Tampico and Veracruz, Mexico (MEX1); and Campeche Banks, Mexico (MEX2; Figure 1). To correspond to nursery signatures developed for the 2005–2007 year-classes (see Zapp Sluis et al. 2012), Red Snapper of the following ages were targeted during the summer (May–August): age-1 fish were targeted in 2006–2008, age-2 fish were targeted in 2007–2008, and age-3 fish were targeted in 2008. The objective was to sample 50 Red Snapper per year-class in each region over a 3-year period, equaling 1,200 samples total ([50 fish × 1 year-class × 4 regions] + [50 fish × 2 year-classes × 4 regions] + [50 fish × 3 year-classes × 4 regions] = 1,200). Subadult and adult Red Snapper were sampled onboard National Marine Fisheries Service vessels during scientific bottom trawl surveys; from recreational landings around Port Aransas, Texas, and Port Fourchon, Louisiana; and from bycatch in the Mexican shrimp fishery. Due to difficulty in collecting samples from MEX1 and MEX2, sampling in those regions occurred later in the winter (December–March), and no samples were obtained in 2008. Red Snapper TL was measured to the nearest millimeter; both sagittal otoliths were extracted (either in the field or in the laboratory), rinsed free of associated tissue by using double-deionized water ($DDIH_2O$; ultra-pure 18-MΩ/cm water), 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 via the protocols of Patterson et al. (2001a) and Fischer et al. (2002). Once age was verified, stratified random sampling was used to select the otoliths of up to 50 fish per region per year-class from each summer's sample, and those otoliths were used for 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 Buhler 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 with the embedded transverse otolith section were then affixed to the empty epoxy section with Loctite gel such that the glue did not come into contact with the otolith section. Using the method of Barnett and Patterson (2010), otolith cores were removed from the embedded transverse section with a New Wave MicroMill precision drilling instrument. The empty section of epoxy resin was used to protect the drill bit from possibly hitting the slide and to prevent the otolith core from cracking during the drilling process. A pre-determined path based on the average transverse section perimeters for otoliths from 20 age-0 Red Snapper was programmed into the MicroMill system to extract the age-0 core section from each subadult/adult otolith sample (Figure 2A, B). The drilling process required 24 passes at 75-μm depth per pass with a scan speed of 85 μm/s and at 80% drill speed. Otolith cores were easily extracted from the transverse section with this process (Figure 2C). Extracted cores were placed in clear microcentrifuge tubes for storage until analysis of chemical signatures.

Prior to elemental analysis or stable isotope analysis, the extracted otolith cores were cleaned under a class-100 clean hood. Dried cores were weighed to the nearest 0.01 mg before

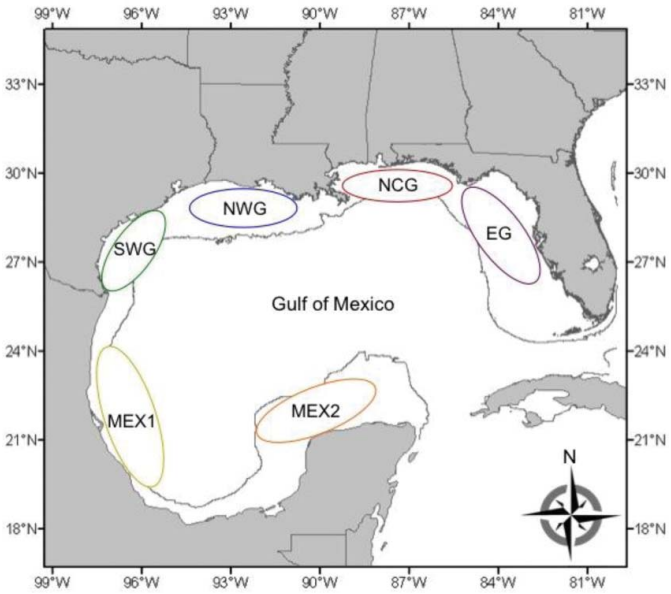

FIGURE 1. Six nursery regions along the continental shelf in the Gulf of Mexico (Gulf), where age-0 Red Snapper were sampled during 2005–2007 (Zapp Sluis et al. 2012). Subadult and adult Red Snapper of those same cohorts were sampled from four of the regions during 2006–2008: the northwestern Gulf (NWG), southwestern Gulf (SWG), Mexico region 1 (MEX1), and Mexico region 2 (MEX2). Additional nursery regions include the eastern Gulf (EG) and north-central Gulf (NCG). The 200-m depth contour line indicates the continental shelf edge.

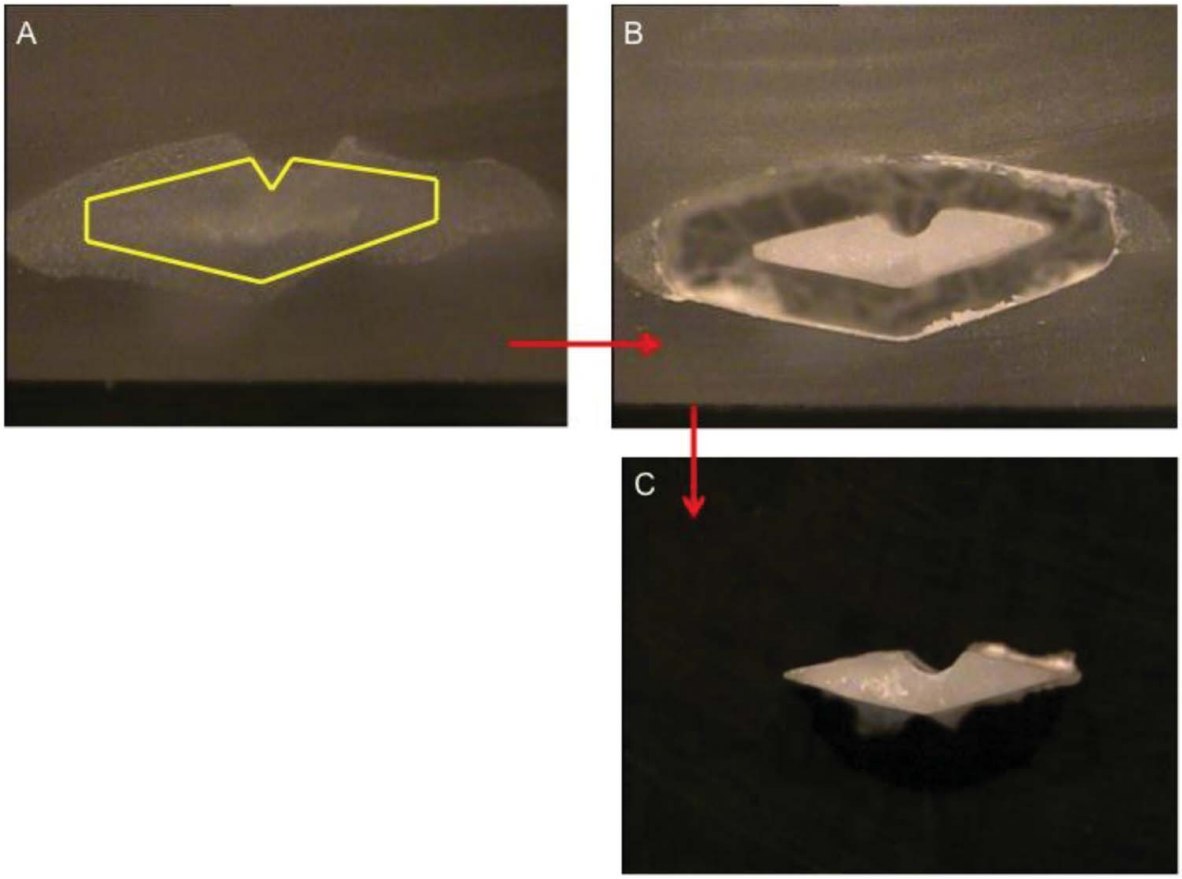

FIGURE 2. Transverse section of a sagittal otolith from an adult Red Snapper, depicting **(A)** a yellow outline of the template pattern, which was used to extract **(B)** the age-0 core with a MicroMill precision drilling instrument. **(C)** The resulting intact extracted core is also shown.

and after cleaning. Whole cores were immersed in 1% ultrapure nitric acid (HNO_3) for 30 s to clean the surface and then were flooded repeatedly with $DDIH_2O$ to remove the acid. Cores were dried under a class-100 clean hood for at least 24 h. Once dried and reweighed, the otolith cores were pulverized with an acid-leached mortar and pestle, and the resulting homogenized powder was divided into two approximately equal proportions. Half of the otolith core powder was weighed to the nearest 0.1 mg and then dissolved in an acid-leached, high-density polyethylene vial by adding 1% ultrapure HNO_3 until a dilution factor of approximately 1,000-fold was achieved. Although total dissolution typically occurred within 1 h, samples were not manipulated for at least 24 h after acid digestion began. Aliquots (5 mL) of the core solutions were sent to the University of Southern Mississippi for trace elemental analysis with a Thermo Fisher Element2 sector field (SF) inductively coupled plasma (ICP) mass spectrometer. Core solutions were spiked with indium at a concentration of 2 ng/mL as an internal standard and then were analyzed for ^{137}Ba, ^{48}Ca, ^{7}Li, ^{55}Mn, ^{25}Mg, and ^{86}Sr. Calibration was achieved using (1) external standards that were made to include approximately the same Ca concentration as the samples and (2) standards that were made without added Ca. Blanks were prepared from 1% ultrapure HNO_3 and were processed through the same sample preparation stages as the sample solutions. Blanks were analyzed concurrently with sample solutions to estimate instrument limits of detection as three SDs of the blank values. Instrument performance and matrix effects were checked by assaying elemental concentrations of an otolith standard reference material that was prepared from adult Red Snapper otoliths (Sturgeon et al. 2005). Solutions of the standard reference material were prepared and analyzed similarly to the otolith core samples. Measured precision (% relative SD; $n = 22$) of the method was 2% for Ba:Ca, 5% for Li:Ca, 6% for Mn:Ca, 16% for Mg:Ca, and 1% for Sr:Ca. Recovery estimates were 102% for Ba:Ca, 79% for Li:Ca, 102% for Mn:Ca, 116% for Mg:Ca, and 94% for Sr:Ca based on comparison with the certified values from Sturgeon et al. (2005). Note that Sturgeon et al.'s (2005) certified values have uncertainties ranging from 2% (Mn:Ca) to 13% (Li:Ca), and thus our results are indicative of satisfactory agreement.

The other half of the powder from each otolith core sample was placed into a 2-mL microcentrifuge tube and was sent to the Stable Isotope Laboratory at the Department of Geology,

University of California–Davis, for stable isotope ($\delta^{13}C$, $\delta^{18}O$) analysis with a Finnigan MAT 251 isotope ratio (IR) mass spectrometer. 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 that had been stringently calibrated against NBS-19. Method precision based on long-term monitoring of the NBS-19 standard was ±0.02‰ for $\delta^{13}C$ and ±0.06% for $\delta^{18}O$. The isotopic composition of otolith cores are reported in standard delta (δ) notation relative to the Vienna Pee Dee belemnite reference standard:

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

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

Statistical analysis.—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 the cohort-specific age-0 Red Snapper element : Ca and stable isotope ratios presented in Zapp Sluis et al. (2012). Residuals were computed for otolith chemical signatures from age-0 fish and from subadult/adult fish (core samples) to remove extraneous sources of variance (i.e., ontogenetic effects of disproportionate primordium representation in cored otoliths versus the original three-dimensional structure of age-0 otoliths; instrument drift between sample analysis of age-0 samples and otolith core samples; etc.) when estimating the source regions for subadult and adult samples (Thorrold et al. 2001; Barnett and Patterson 2010).

A Bayesian model was used to estimate the source of recruits to a given region in a given sampling year. Full methodological details on the Bayesian model and accompanying R package (R Development Core Team 2007) used in this study are provided by Smith and Campana (2010). The baseline data set consisted of the residual values of otolith signatures from age-0 Red Snapper that were sampled in nursery regions. Residuals of otolith core signatures from adults and subadults were classified as unknowns (or mixed data) against the age-0 baseline data to estimate their nursery source(s). Even though significant differences among year-classes were evident for the age-0 otolith chemical signatures (Zapp Sluis et al. 2012), they were also combined to determine whether nursery signatures pooled across year-classes could help fill data gaps. Thus, the mixed data for each subadult/adult age-group in each region and each year sampled were classified individually to the year-class-specific baseline data as well as to the baseline data pooled across year-classes. Posterior distributions were used to calculate 95% credible intervals (CIs) for the proportion of mixed samples assigned to each baseline group (i.e., nursery region). An overlap in CIs indicated an ambiguous assignment to the baseline groups.

RESULTS

In total, 1,338 subadult and adult Red Snapper were collected from the four western Gulf sampling regions. Based on the ages estimated for those fish, only 725 individuals corresponded to the designated regions and cohorts of interest, and their otoliths were cored for chemical analysis (Table 1). Few of the samples obtained from MEX1 and MEX2 corresponded to the targeted year-classes, resulting in low sample sizes for those regions. All six elements (Ba, Ca, Li, Mn, Mg, and Sr) were present in concentrations at least two orders of magnitude above detection limits in all samples.

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 in the age-0 baseline data (see Zapp Sluis et al. 2012). The element : Ca and stable isotope ratios also varied among age-groups within a given cohort. For the 2005 cohort, otoliths of age-2 Red Snapper collected from NWG had higher Ba:Ca, Mg:Ca, and Mn:Ca values than the other age-groups. There was also a steady decrease in Li:Ca values with increasing age, and only $\delta^{13}C$ remained constant and within the same range as baseline nursery values (Figure 3). In the 2005-cohort samples from SWG, otolith element : Ca and stable isotope ratios remained constant across age-groups except for a similar increase in otolith Ba:Ca, Mg:Ca, and Mn:Ca values for age-2 fish. In MEX1, otolith ratios for the 2005 cohort remained constant across age-groups except for Li:Ca, $\delta^{13}C$, and $\delta^{18}O$. For MEX2 samples of the 2005 cohort, otolith ratios decreased between age-groups for every element except $\delta^{18}O$, which increased.

For the 2006 cohort, element : Ca and stable isotope ratios in the otoliths of NWG adult/subadult Red Snapper remained fairly constant between age-groups except for a decrease in Mg:Ca and Mn:Ca (Figure 4). Otolith ratios for the 2006 cohort sampled from SWG only remained constant between age-groups for Li:Ca and $\delta^{13}C$ and also exhibited the same decrease in values as the 2006-cohort samples from NWG. For the 2007 cohort, otolith element : Ca and stable isotope ratios in NWG Red Snapper were similar to the corresponding baseline age-0 samples (see Zapp Sluis et al. 2012) except for being more enriched in $\delta^{18}O$ (Figure 5). Otolith ratios for the 2007-cohort samples from SWG were lower in Ba:Ca and Mg:Ca and more enriched in $\delta^{18}O$ relative to the baseline data. Interestingly, in the NWG and SWG samples, $\delta^{18}O$ ratios increased for each age-group within each cohort relative to the baseline age-0 nursery data (see Zapp Sluis et al. 2012).

Classification of subadults and adults (mixed nursery origin) from the 2005 cohort sampled in NWG indicated that the proportion of locally derived fish (i.e., NWG nursery region) increased as age increased (Figure 6). Furthermore, the proportion of NWG-sampled adults assigned to the NWG nursery region differed significantly from the proportions assigned to the other nursery regions, as indicated by the non-overlapping 95% CIs (Figure 6). The secondary source of recruits to NWG was estimated to be the NCG nursery region, with the

TABLE 1. Sample size and TL range of subadult and adult Red Snapper collected from four regions across the Gulf of Mexico (Gulf) during summer in 2006–2008 (NWG = northwestern Gulf; SWG = southwestern Gulf; MEX1 = Mexico region 1; MEX2 = Mexico region 2).

Sampling year	Age	Year-class	Region	Otolith samples (cored and analyzed)	TL (mm) range
2006	1	2005	NWG	51	153–241
			SWG	52	151–226
			MEX1	18	250–280
			MEX2	3	240–250
2007	1	2006	NWG	56	151–235
			SWG	44	153–258
			MEX1	31	230–380
			MEX2	3	240–280
	2	2005	NWG	55	186–443
			SWG	60	232–348
			MEX1	50	240–320
			MEX2	1	480
2008	1	2007	NWG	50	152–209
			SWG	50	151–237
			MEX1		
			MEX2		
	2	2006	NWG	50	220–410
			SWG	50	165–422
			MEX1		
			MEX2		
	3	2005	NWG	50	335–470
			SWG	50	301–457
			MEX1		
			MEX2		

contribution from NCG decreasing as age increased; however, the CIs overlapped with those for the other two nursery regions. Classification of adults from the 2005 cohort sampled in SWG appeared to be locally derived, yet only the age-2 samples differed significantly in the proportion assigned to SWG versus the other nursery regions (Figure 6). Although age-1 and age-2 samples for the 2005 cohort were collected from MEX1 and MEX2, baseline nursery data were not available for these regions; therefore, the samples were not included in Bayesian models for the 2005 cohort. For the 2006 cohort, adult samples from NWG were primarily assigned to the NWG and SWG nursery regions. Among the 2006-cohort adults sampled in SWG, age-1 fish were classified to MEX1 and MEX2 nursery regions, and age-2 fish were primarily assigned to the SWG nursery region. However, the only instance in which CIs did not overlap for the 2006 cohort was for the proportion of MEX2 adult samples that were assigned to the MEX2 nursery region. Although not significantly different at the 95% level, age-1 fish of the 2007 cohort sampled in NWG were classified to both the NWG and SWG nursery regions, while age-1 fish from SWG were typically assigned to the NWG nursery region (Figure 6).

When nursery sources were analyzed by using baseline data that were pooled across year-classes, similar trends emerged. For the 2005 cohort of Red Snapper, adults sampled from NWG were still classified as being primarily locally derived; however, the only case in which CIs did not overlap was for age-3 individuals (Figure 7). Adult samples from SWG were mostly classified to the NWG nursery region rather than being locally derived. Age-1 samples from MEX1 were primarily classified to the MEX1 nursery region, and a large proportion of age-2 samples from MEX1 were assigned to the MEX2 nursery region; however, all of the 95% CIs overlapped. For age-1 fish sampled from MEX2, assignments were mainly to the EG nursery region (although not significantly so); however, this estimate was based on a low sample size ($n = 3$). The age-2 sample from MEX2 could not be analyzed because the sample size was one fish. For the 2006 cohort, age-1 individuals sampled from NWG were assigned to both the NWG and SWG nursery regions, whereas age-2 fish were primarily classified as originating from the SWG nursery region. Adult samples from SWG were predominantly locally derived; the 95% CIs for age-2 fish were nonoverlapping. Adult samples obtained in MEX1 were also assigned to the local MEX1 nursery baseline signature, although there were some instances of CI overlap. The MEX2 adult samples were primarily assigned to the NWG nursery region, but again the CIs greatly overlapped. For the 2007 cohort of Red Snapper, age-1 fish

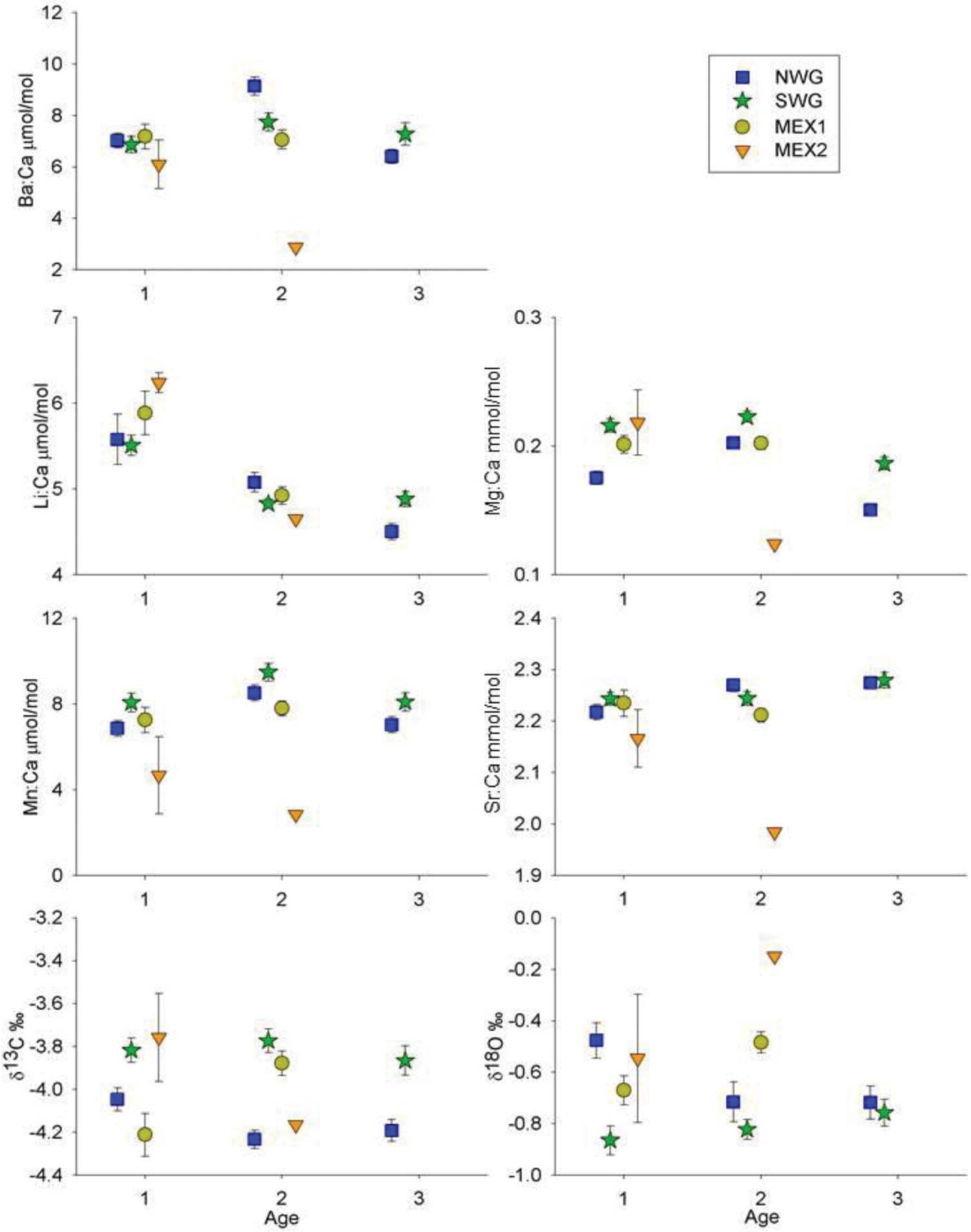

FIGURE 3. Mean (±SE) element : Ca or stable isotope delta ratios for otolith cores from 2005-cohort Red Snapper (subadults and adults, ages 1–3) sampled in four regions of the western Gulf of Mexico (Gulf) during summer in 2006–2008 (NWG = northwestern Gulf; SWG = southwestern Gulf; MEX1 = Mexico region 1; MEX2 = Mexico region 2).

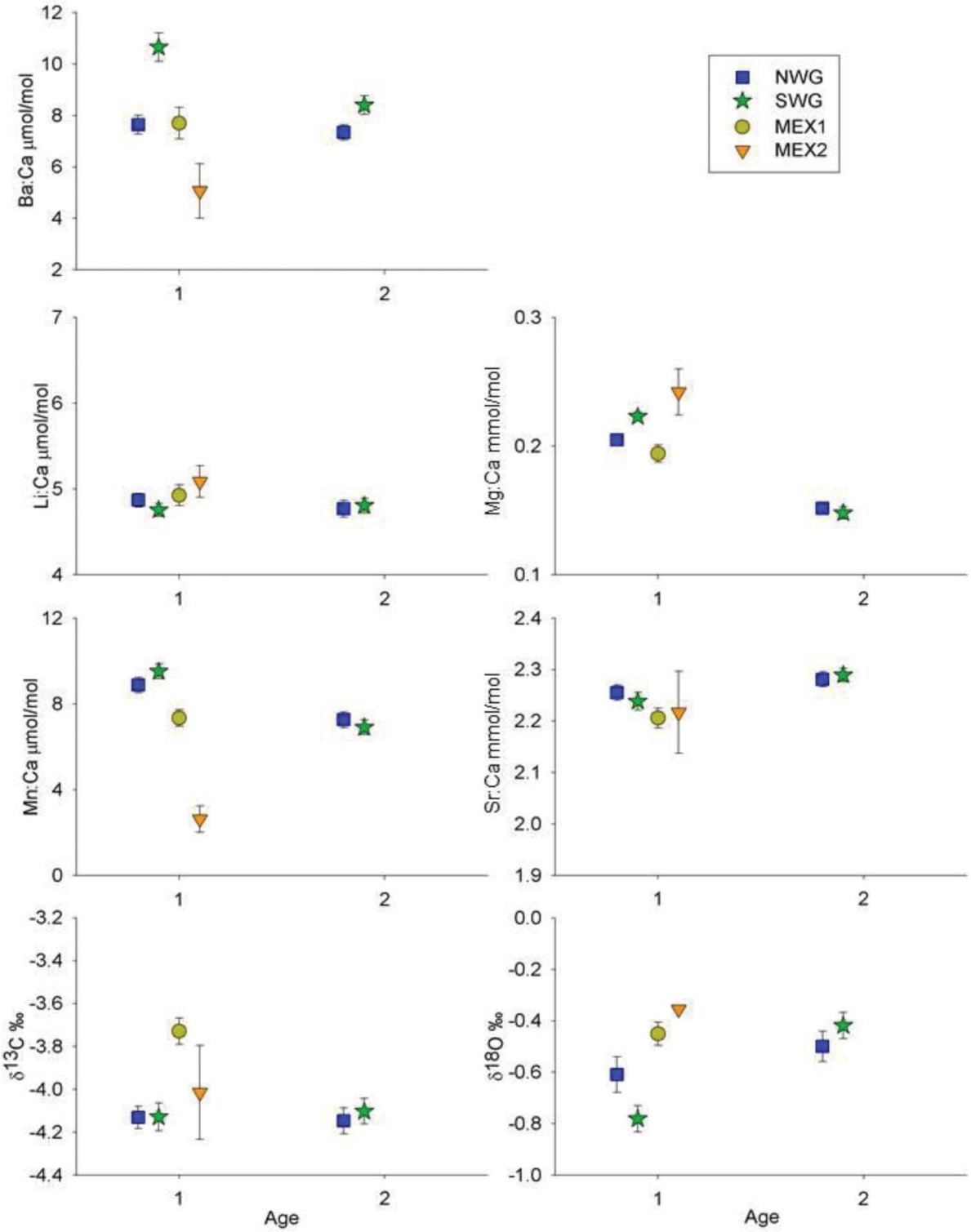

FIGURE 4. Mean (±SE) element : Ca or stable isotope delta ratios for otolith cores from 2006-cohort Red Snapper (subadults and adults, ages 1–2) sampled in four regions of the western Gulf of Mexico during summer in 2007 and 2008 (region codes are defined in Figure 3).

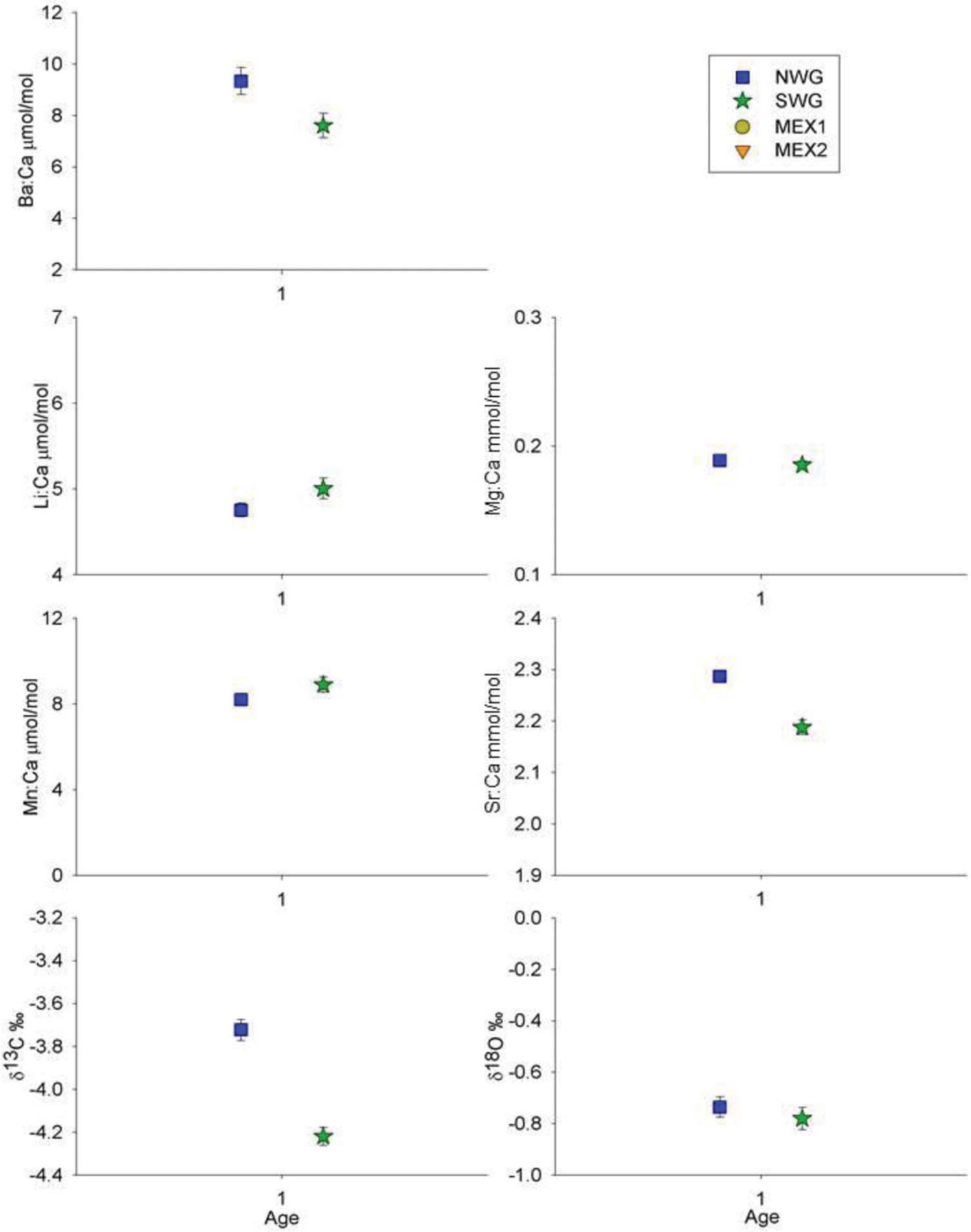

FIGURE 5. Mean (±SE) element : Ca or stable isotope delta ratios for otolith cores from 2007-cohort Red Snapper (subadults and adults, age 1) sampled in two regions of the western Gulf of Mexico during summer 2008 (region codes are defined in Figure 3).

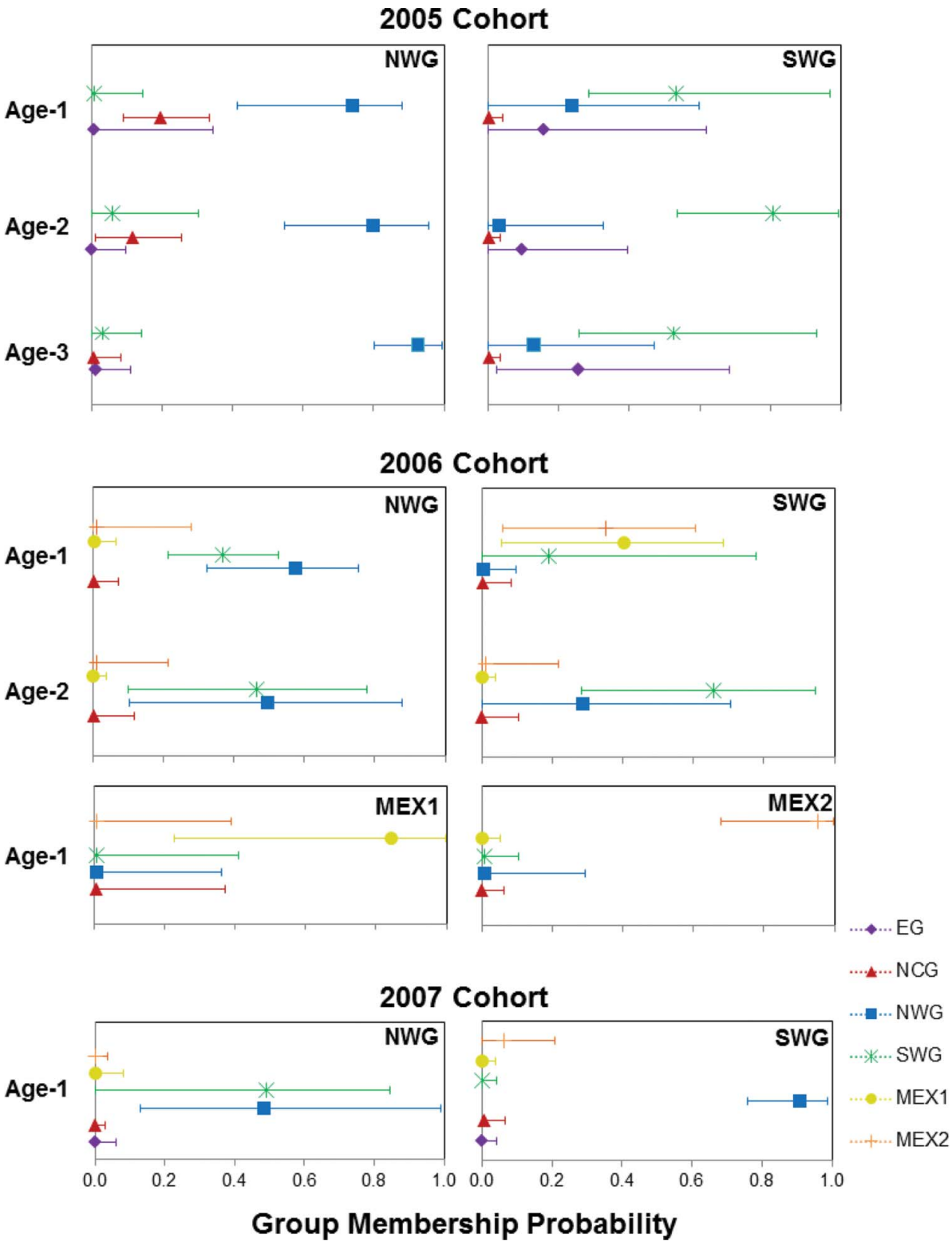

FIGURE 6. Estimated proportions (±95% credible interval) of subadult and adult (ages 1–3) Red Snapper sampled from the western Gulf of Mexico (Gulf) that were assigned to nursery regions based on year-class-specific otolith element : Ca and stable isotope ratio data for age-0 fish from the corresponding (2005–2007) cohorts. The subadult/adult sampling region is specified in the upper-right corner of each panel; symbols represent the assigned nursery regions (EG = eastern Gulf; NCG = north-central Gulf; NWG = northwestern Gulf; SWG = southwestern Gulf; MEX1 = Mexico region 1; MEX2 = Mexico region 2).

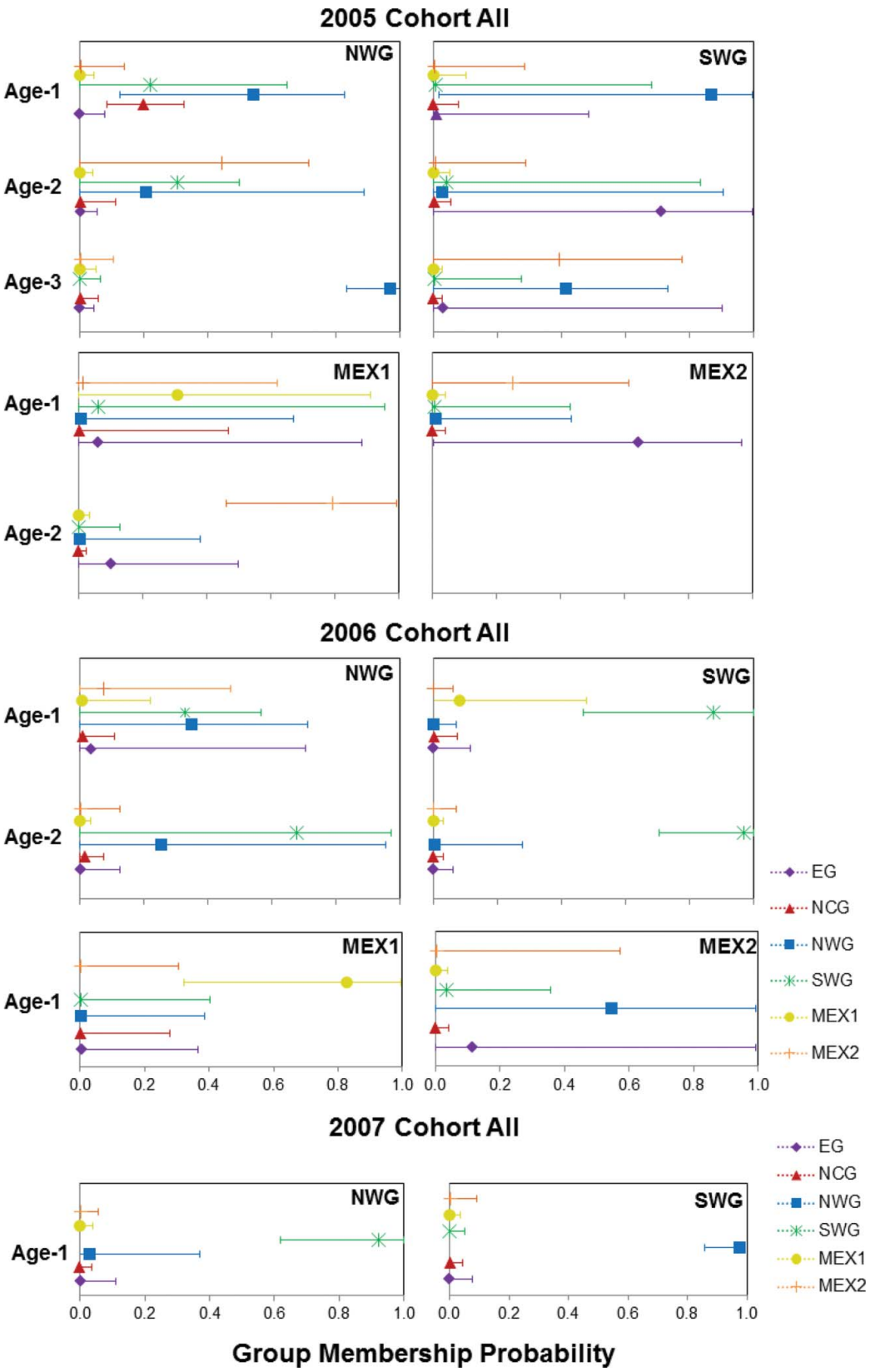

FIGURE 7. Estimated proportions (±95% credible interval) of subadult and adult (ages 1–3) Red Snapper sampled from the western Gulf of Mexico (Gulf) that were assigned to nursery regions based on otolith element : Ca and stable isotope ratio data for age-0 fish pooled across cohorts (2005–2007 year-classes). The subadult/adult sampling region is specified in the upper-right corner of each panel; symbols represent the assigned nursery regions (region codes are defined in Figure 6).

sampled from NWG were classified to the SWG nursery region; likewise, age-1 samples from the SWG were classified to the NWG nursery region.

DISCUSSION

Previous studies of Red Snapper otolith chemistry have indicated that significant postsettlement movement occurs between NWG and SWG (Cowan et al. 2003; Patterson 2007). In the present study, moderate to high percentages of recruits from the NWG nursery were observed among Red Snapper subadults and adults sampled from SWG; in some cases, almost equal proportions of recruits from the NWG and SWG nursery regions were observed among the NWG samples. Therefore, results of this study provide evidence that substantial postsettlement mixing of subadult/adult Red Snapper occurs between U.S. regions within the western Gulf. However, little evidence was detected of Red Snapper mixing between the eastern and western Gulf or between the U.S. and Mexican portions of the Gulf.

Gold and Saillant (2007) estimated that the N_e of Red Snapper was 10-fold higher in NWG than in NCG and SWG. Furthermore, the 2009 Red Snapper stock assessment (SEDAR 2009) indicated that the age distribution in the eastern Gulf was truncated relative to that in the western Gulf; the eastern Gulf population was also projected to have lower productivity than the western substock. The current study demonstrates that (1) the 2005 cohort of Red Snapper in NWG was predominantly composed of locally derived fish; and (2) although some cohorts in SWG were locally derived, the NWG nursery region was an important source of 2007-cohort recruits to SWG. Interestingly, Kulaw (2012) discovered that female Red Snapper in SWG reached 100% maturity faster than females in NWG. This was attributed to signs of juvenescence in the SWG population as it rebuilds from overfishing, whereas the NWG population may have moderate to low fecundity and later maturation due to its higher population size. Therefore, it is possible for the SWG population to be locally derived during strong year-classes (e.g., 2005 cohort; Cowan 2011; SEDAR 2013; Saari 2014) and to receive recruitment from other regions when year-classes are not as strong (e.g., 2007 cohort). Thus, in combination with past research on Red Snapper, the observed classification proportions in this study indicate the NWG's importance as a source of recruits to Red Snapper populations in the western U.S. Gulf.

Previous otolith chemistry studies have indicated that postsettlement movement of Red Snapper does occur but that their movement is limited during the first year of life (Cowan et al. 2003; Patterson 2007; Patterson et al. 2008). However, results of the current study may indicate otherwise. For the 2005 cohort of Red Snapper in NWG, the estimated proportion of locally derived recruits increased as the age of sampled fish increased. If limited movement should occur in the first year of life, with a potential increase in movement as the fish ages, then the trend displayed for locally derived recruits for the NWG 2005 cohort should be reversed. This suggests 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 estimated large-scale movement of age-1 Red Snapper (Patterson et al. 2001b). Nonetheless, the 2005-cohort age-0 Red Snapper used in the development of nursery signatures were collected after the major hurricane impacts and exhibited the highest classification success, making a hurricane effect less likely. The 2007 cohort showed strong movement in one direction—from NWG to SWG. The 2005 and 2006 cohorts were stronger year-classes than the 2007 cohort (Cowan 2011; SEDAR 2013; Saari 2014), and this may partially explain why higher mixing rates were evident for the 2005 and 2006 cohorts, whereas the 2007 cohort in SWG consisted primarily of recruits from the NWG nursery region. However, much of this is speculation, as a sample size of 30 fish·year-class^{-1}·nursery region^{-1} may be too small to permit accurate discrimination of subadult/adult recruitment sources. Increasing the sample size and the number of age-groups examined may allow for better resolution in understanding the mixing dynamics of Red Snapper populations.

The majority of subadults and adults sampled in SWG were classified as originating from the SWG or NWG nursery region, with only one exception. The 2006-cohort age-1 samples from SWG were proportionately assigned to the MEX1 and MEX2 nursery regions, although the CIs overlapped, indicating a lack of significance at the 95% level. It is perplexing that SWG samples were assigned to and overlapped with the MEX1 and MEX2 nursery regions because (1) the MEX1 and SWG baseline otolith signatures did not overlap for the 2006 cohort; and (2) the MEX2 baseline signatures remained separate from the SWG signatures for both the 2005 and 2006 cohorts (see Zapp Sluis et al. 2012). Although the MEX1 nursery region could be another potential source of Red Snapper recruits for SWG, the transfer of Red Snapper from MEX2 to SWG seems highly unlikely. Prevailing upwelling winds cause circulation on the western Campeche Banks to flow westward along the coast, and during the fall and winter this is met with a down-coast current that extends to the southern Bay of Campeche and generates seasonal offshore transport (Zavala-Hidalgo et al. 2003). These circulation patterns, along with the separation of distance, likely impede the mixing of Red Snapper between SWG and MEX2.

Due to the unbalanced design of the Mexican 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, subadult/adult Red Snapper in MEX2 were estimated to be locally derived, and MEX1 fish were classified to MEX1 baseline samples, although some overlap between 95% CIs was apparent. When examining classification results based on age-0 data

pooled across year-classes, the MEX2 Red Snapper were no longer estimated to be locally derived; instead, MEX2 samples from the 2005 and 2006 cohorts were primarily classified to the EG and NWG nursery regions, respectively. For the 2006-cohort samples from MEX1, the classification results based on age-0 data from single year-classes appeared to be the same as the results obtained based on the pooled year-classes. For the 2005 cohort, the pooled classification results indicated that age-1 Red Snapper sampled in MEX1 consisted mainly of recruits from MEX1, although with significant overlap of CIs; the age-2 samples from MEX1 were mainly composed of fish from the MEX2 nursery region. Even though combining the baseline signatures across year-classes resulted in relatively high classification accuracy (70.3%), significant differences in otolith chemical signatures were evident among year-classes for age-0 Red Snapper (Zapp Sluis et al. 2012). Consequently, analyses of classification estimates based on cohort-specific data are more accurate, as evidenced by the notable changes in classification to the NWG and SWG samples when pooled year-class signatures were used. Thus, analytical results were inconclusive regarding the source of recruits to the Mexican Red Snapper populations.

Based on the Red Snapper sampled in this study, a moderate to strong contribution of recruits from the NWG nursery was apparent among adults sampled from NWG and SWG. Unfortunately, connectivity between the western Gulf and Mexican regions is inconclusive at this time, and more data would be required before inferences can be made. Most of the recent increase in spawning stock biomass of Gulf Red Snapper is estimated to have occurred in the western Gulf, and this is projected to continue into the near future (SEDAR 2013). Based on the results of the current study, the center of abundance off the coast in NWG may be expanding outward toward the SWG continental shelf. Furthermore, it appears that some self-recruitment is occurring in SWG. Future work should also determine whether population recovery in the western Gulf is contributing to the relatively recent reappearance of Red Snapper in the far eastern portion of the Gulf. Determining connectivity between eastern and western populations of Red Snapper would be beneficial to the development of efficient regional management for this species.

ACKNOWLEDGMENTS

The present research was supported by the Marine Fisheries Initiative under Grant Number NA05NMF4331072 awarded to W. Patterson and J. Cowan. We thank Steve Tomeny Charters, Fisherman's Wharf Deep Sea Fishing, the National Marine Fisheries Service (fall groundfish surveys and vertical longline surveys), and numerous recreational anglers for allowing us to sample their catch. We are especially grateful to K. Simonsen, M. Hedges Monk, and K. Boswell for assistance in the field; G. Martınez for sampling fish in the MEX1 and MEX2 regions; X. Wang for assistance in running the SF-ICP mass spectrometry samples; D. Winter for running the IR mass spectrometry samples; S. Smith for troubleshooting the program; and two anonymous reviewers for helpful feedback that improved the manuscript. We also thank B. Kline and A. Fischer for their assistance in the laboratory.

REFERENCES

Barnett, B., and W. F. Patterson III. 2010. The effect of coring and pulverizing juvenile Red Snapper, *Lutjanus campechanus*, otoliths on their chemical signatures. Environmental Biology of Fishes 89:463–471.

Brule, T., T. Colas-Marrufo, E. Perez-Diaz, and J. C. Samano-Zapata. 2010. Red Snapper reproductive biology in the southern Gulf of Mexico. Transactions of the American Fisheries Society 139:957–968.

Camber, C. I. 1955. A survey of the Red Snapper fishery of the Gulf of Mexico, with special reference to the Campeche Banks. Florida Board of Conservation Marine Research Laboratory Technical Series 12.

Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Marine Ecology Progress Series 188:263–297.

Cowan, J. H. Jr. 2011. Red Snapper in the Gulf of Mexico and U.S. South Atlantic: data, choices and conclusions. Fisheries 36:319–331.

Cowan, J. H. Jr., M. Woods, W. Patterson III, and D. Nieland. 2003. Otolith microchemistry (and reproductive biology) portion–stock structure of Red Snapper in the northern Gulf of Mexico: is their management as a single stock justified based on spatial and temporal patterns of genetic variation, otolith microchemistry, and growth rates? Marine Fisheries Initiative, Southeast Regional Office, Final Report NA87FF0425, St. Petersburg, Florida.

Cowen, R. K., K. M. M. Lwiza, S. Sponaugle, C. B. Paris, and D. B. Olson. 2000. Connectivity of marine populations: open or closed? Science 287:857–859.

Crowder, L. B., S. J. Lyman, W. F. Figueira, and J. Priddy. 2000. Source-sink population dynamics and the problem of sitting marine reserves. Bulletin of Marine Science 66:799–820.

Fischer, A. J., M. S. Baker Jr., and C. A. Wilson. 2004. Red Snapper (*Lutjanus campechanus*) demographic structure in the northern Gulf of Mexico based on spatial patterns in growth rates and morphometrics. U.S. National Marine Fisheries Service Fishery Bulletin 102:593–603.

Fischer, A. J., C. A. Wilson, and D. L. Nieland. 2002. Age and growth of Red Snapper, *Lutjanus campechanus*, in the northwestern Gulf of Mexico: implications to the unit stock hypothesis. Proceedings of the Gulf and Caribbean Fisheries Institute 53:496–506.

Gallaway, B. J., M. Longnecker, J. G. Cole, and R. M. Meyer. 1998. Estimates of shrimp-trawl bycatch of Red Snapper (*Lutjanus campechanus*) in the Gulf of Mexico. Pages 817–839 *in* F. Funk, T. J. Quinn, J. Heifetz, J. N. Ianelli; J. E. Powers, J. F. Schweigert, P. J. Sullivan, and C. I. Zhang, editors. 15th Lowell Wakefield Fisheries Symposium: fishery stock assessment models for the 21st century. Alaska Sea Grant College Program, AK-SG-98-01, Anchorage.

GMFMC (Gulf of Mexico Fishery Management Council). 2010. Final regulatory amendment to the reef fish fishery management plan to set total allowable catch for Red Snapper. GMFMC, Tampa, Florida.

Gold, J. R., and E. Saillant. 2007. Population structure of Red Snapper in the northern Gulf of Mexico. Pages 201–216 *in* W. F. Patterson III, J. H. Cowan Jr., D. A. Nieland, and G. R. Gitzhugh, editors. Red Snapper ecology and fisheries in the U.S. Gulf of Mexico. American Fisheries Society, Symposium 60, Bethesda, Maryland.

Jackson, M. W., J. H. Cowan Jr., and D. A. Nieland. 2007. Demographic differences in northern Gulf of Mexico Red Snapper reproductive maturation: implication for the unit stock hypothesis. Pages 217–227 *in* W. F. Patterson III, J. H. Cowan Jr., G. R. Fitzhugh, and D. L. Nieland, editors. Red Snapper

ecology and fisheries in the U.S. Gulf of Mexico. American Fisheries Society, Symposium 60, Bethesda, Maryland.

Kulaw, D. 2012. Habitat- and region-specific reproductive biology of female Red Snapper (*Lutjanus campechanus*) in the Gulf of Mexico. Master's thesis. Louisiana State University, Baton Rouge.

Monroy-García, C., M. G. Andrade, and J. C. Espinoza. 2002. Análisis de la pesqueria de huachinango (*Lutjanus campechanus*) en el Banco de Campeche. [Analysis of the Red Snapper fishery (*Lutjanus campechanus*) on the Campeche Bank.] Proceedings of the Gulf and Caribbean Fisheries Institute 53:507–515.

Patterson, W. F. III. 2007. A review of Gulf of Mexico Red Snapper movement studies: implications for population structure. Pages 221–236 *in* W. F. Patterson III, J. H. Cowan Jr., G. R. Fitzhugh, and D. L. Nieland, editors. Red Snapper ecology and fisheries in the U.S. Gulf of Mexico. American Fisheries Society, Symposium 60, Bethesda, Maryland.

Patterson, W. F. III, J. H. Cowan Jr., C. A. Wilson, and Z. Chen. 2008. Temporal and spatial variability in juvenile Red Snapper otolith elemental signatures in the northern Gulf of Mexico. Transactions of the American Fisheries Society 137:521–532.

Patterson, W. F. III, J. H. Cowan Jr., C. A. Wilson, and R. L. Shipp. 2001a. Age and growth of Red Snapper, *Lutjanus campechanus*, from an artificial reef off Alabama in the northern Gulf of Mexico. U.S. National Marine Fisheries Service Fishery Bulletin 99:617–627.

Patterson W. F. III, J. C. Watterson, R. L. Shipp, and J. H. Cowan Jr. 2001b. Movement of tagged Red Snapper in the northern Gulf of Mexico. Transactions of the American Fisheries Society 130:533–545.

R Development Core Team. 2007. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available: www.R-project.org. (October 2015).

Rooker, J. R., D. H. Secor, G. DeMetrio, A. J. Kaufman, A. B. Rios, and A. Ticina. 2008. Evidence of trans-Atlantic movement and natal homing of Bluefin Tuna from stable isotopes in otoliths. Marine Ecology Progress Series 368:231–239.

Rooker, J. R., D. H. Secor, V. S. Zdanowicz, and T. Itoh. 2001. Discrimination of northern Bluefin Tuna from nursery areas in the Pacific Ocean using otolith chemistry. Marine Ecology Progress Series 218:275–282.

Saari, C. R. 2014. Regional differences in the age and growth of Red Snapper (*Lutjanus campechanus*) in the U.S. Gulf of Mexico. U.S. National Marine Fisheries Service Fishery Bulletin 112:261–273.

SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pescay Alimentación). 2006. Carta nacional pesquera. [National fishery chart.] Pages 1–128 *in* A. López-González, editor. Diario official de la Federación, tomo DCXXXV 19, segunda sección). [Official diary of the Federation, volume DCXXXV 19, second section.] Interior Ministry, Mexico City.

Saillant, E., and J. R. Gold. 2006. Population structure and variance effective size of Red Snapper (*Lutjanus campechanus*) in the northern Gulf of Mexico. U.S. National Marine Fisheries Service Fishery Bulletin 104:136–148.

SEDAR (Southeast Data, Assessment, and Review). 2005. Stock assessment report, Gulf of Mexico Red Snapper (SEDAR 7). SEDAR, North Charleston, South Carolina.

SEDAR (Southeast Data, Assessment, and Review). 2009. Update assessment of Red Snapper in the Gulf of Mexico. SEDAR, North Charleston, South Carolina.

SEDAR (Southeast Data, Assessment, and Review). 2013. Stock assessment report, Gulf of Mexico Red Snapper (SEDAR 31). SEDAR, North Charleston, South Carolina.

Smith, S. J., and S. E. Campana. 2010. Integrated stock mixture analysis for continuous and categorical data, with application to genetic-otolith combinations. Canadian Journal of Fisheries and Aquatic Sciences 67:1533–1548.

Sturgeon, R. E., S. N. Willie, L. Yang, R. Greenberg, R. O. Spatz, Z. Chen, C. Scriver, V. Clancy, J. W. Lam, and S. Thorrold. 2005. Certification of a fish otolith reference material in support of quality assurance for trace element analysis. Journal of Analytical Atomic Spectrometry 20:1067–1071.

Thorrold, S. R., C. M. Jones, P. K. Swart, and T. E. Targett. 1998. Accurate classification of juvenile Weakfish *Cynoscion regalis* to estuarine nursery areas based on chemical signatures in otoliths. Marine Ecology Progress Series 173:253–265.

Thorrold, S. R., C. Latkoczy, P. K. Swart, and C. M. Jones. 2001. Natal homing in a marine fish metapopulation. Science 291:297–299.

Zapp Sluis, M., B. K. Barnett, W. F. Patterson III, J. H. Cowan Jr., and A. M. Shiller. 2012. Discrimination of juvenile Red Snapper otolith chemical signatures from Gulf of Mexico nursery regions. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science [online serial] 4:587–598.

Zavala-Hidalgo, J., S. L. Morey, and J. J. O'Brien. 2003. Seasonal circulation on the western shelf of the Gulf of Mexico using a high-resolution numerical model. Journal of Geophysical Research [online serial] 108(C12):3389.