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Interspecific variation in juvenile snapper otolith chemical signatures in the northern Gulf of Mexico

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ABSTRACT: The objective of this study was to evaluate whether age-0 lane snapper *Lutjanus synagris* otolith chemical signatures could serve as accurate proxies for those of its congener, red snapper *L. campechanus*, among northern Gulf of Mexico (GOM) nursery regions. Red (n = 90) and lane (n = 53) snappers were sampled from 3 regions of the northern GOM in fall 2005, and their otolith chemistry was analyzed with sector field-inductively coupled plasma-mass spectrometry (Ba:Ca, Mg:Ca, Mn:Ca, Sr:Ca, Li:Ca) or stable isotope ratio-mass spectrometry ($\delta^{13}C$ and $\delta^{18}O$). Chemical signatures were significantly different among regions (MANOVA, $p < 0.001$) and between species (MANOVA, $p = 0.029$), with the species effect being driven by significant differences in 4 of the 7 constituents analyzed (ANOVA, $p < 0.036$). The significant region effect persisted (MANOVA, $p < 0.001$), but the species effect was non-significant (MANOVA, $p = 0.964$) when constituent values were normalized to species-specific means. Mean regional classification accuracies from linear discriminant functions computed with otolith constituent data were 84% for lane snapper and 80% for red snapper whether data were normalized or not. Maximum likelihood models parameterized with normalized lane snapper otolith chemistry data estimated red snapper regional composition reasonably well among mixed-region samples (mean error = 9.7% among models). Therefore, it appears age-0 lane snapper otolith chemical signatures can serve as accurate proxies for those of red snapper in the northern GOM. These results have broader implications for deriving natural tags based on otolith chemistry for fishes that may have low abundance in parts of their range.

KEY WORDS: Otolith chemistry · Snapper · Nursery areas

INTRODUCTION

The global rise of ecosystem-based management, including coastal zone planning and the creation of marine protected areas, has resulted in an increased demand for understanding the population structure of marine organisms (Hall & Mainprize 2004, Crowder et al. 2006, Ciannelli et al. 2013). Several approaches have been developed to examine population structure and connectivity among invertebrate and vertebrate marine fauna, including molecular techniques, modeling egg and larval transport, and applying artificial and natural tags to examine population connectivity. In reef fishes, results

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from recent population genetics studies and oceanographic transport modeling suggest self-recruitment may be more widespread than previously thought (Cowen et al. 2000, Swearer et al. 2002, van der Meer et al. 2012, Ackiss et al. 2013). Furthermore, population connectivity in some species may be driven by post-settlement movement, rather than egg and larval transport (Bolden 2000, Lindberg et al. 2006, Patterson 2007). Unfortunately, artificial tagging is impractical for many reef fishes, and the ability to estimate site fidelity or movement can be limited in tagged fishes due to tag loss, uneven fishing effort, or low reporting rates. Therefore, the need to develop accurate, reliable natural tags is paramount to examine post-settlement movement and population connectivity in reef fishes.

Otolith chemical signatures have been shown to be effective natural tags in fishes, and examining population connectivity and estimating nursery sources with otolith chemical signatures has become widespread in fish ecology (reviewed in Campana & Thorrold 2001, Elsdon et al. 2008, Chang & Geffen 2013). Employing otolith chemical signatures has been particularly effective for examining recruitment dynamics and population connectivity in estuarine-dependent and diadromous fishes. This is due to typically high variability in water chemistry among estuaries driving differences in otolith chemical signatures, or a significant contrast existing between the water chemistry fish experience as juveniles versus adults. Among reef fishes, otolith chemical signatures have been demonstrated to distinguish nursery areas or systems for a variety of estuarine-dependent species (Gillanders & Kingsford 2003, Hanson et al. 2004). However, otolith chemical signatures have also proven effective in distinguishing nursery areas or regions in reef fishes that spend their entire life cycle on the shelf (H. M. Patterson et al. 2005, Ruttenberg et al. 2008). Among those is red snapper *Lutjanus campechanus* (Patterson et al. 2008, Zapp Sluis et al. 2012), a large (body mass to 25 kg and total length to 1 m) lutjanid that is one of the more ecologically and economically important reef fishes in the northern GOM.

Red snapper is the most targeted reef fish in US waters of the GOM despite being estimated to be severely overfished for over 30 yr (Porch 2007, SEDAR 2013). The stock has begun to recover in recent years, principally due to increasingly restrictive management measures mandated by the re-authorization of the Magnuson-Stevens Fishery Management and Conservation Act, which was passed by the US Congress in 2006. The GOM fishery began along the west Florida shelf in the 1800s (Stearns 1883, Collins 1885), but by the turn of the 19th century the red snapper population was commercially extinct in Florida waters (Porch et al. 2007). A clear sign of stock recovery in recent years has been increasing catch rates and abundance estimates in the eastern GOM (SEDAR 2013). Artificial tagging data indicate some young fish (<5 yr old) move from the north central GOM to the west Florida shelf post-settlement (Patterson 2007, Addis et al. 2013), but it is unknown what percentage of the growing red snapper population found there is locally produced versus recruits from other regions of the GOM.

Zapp Sluis et al. (2012) examined age-0 red snapper otolith chemical signatures among 6 regions of the GOM, including the west Florida shelf and 2 regions in Mexican waters, in an attempt to develop natural tags to examine sources of recruits to rebuilding red snapper populations in the northern GOM. Despite significant sampling efforts, they collected relatively few juvenile red snapper from the west Florida shelf, and had no samples from that region for 1 of the 3 yr classes they examined. Lane snapper *Lutjanus synagris*, however, are abundant on the west Florida shelf, thus the question arose as to whether lane snapper otolith chemical signatures could serve as a proxy for those of red snapper, given similarities in ecology, early life history, and GOM habitats between these 2 congeners (Workman et al. 2002, Mikulas & Rooker 2008, Wells et al. 2008). To test this question, age-0 red and lane snapper juveniles were sampled concurrently among 3 northern GOM regions, and differences in otolith chemical signatures between species were tested. Then, the ability of lane snapper otolith chemical signatures to accurately distinguish mixed-region red snapper samples was examined.

METHODS

Age-0 red and lane snappers were sampled in October and November 2005 from 3 regions in the northern GOM during the National Oceanic and Atmospheric Administration (NOAA) Fisheries Fall Groundfish Survey (FGS): north central Gulf (NCG), northwest Gulf (NWG), and southwest Gulf (SWG) (Fig. 1). Following Patterson et al. (2008), the boundary between NWG and NCG regions was longitude 89.0° W, and the boundary between NWG region and SWG regions was 94.5° W. Fish were placed in plastic bags, frozen onboard the research vessel, and then transferred to the laboratory for processing. Fish

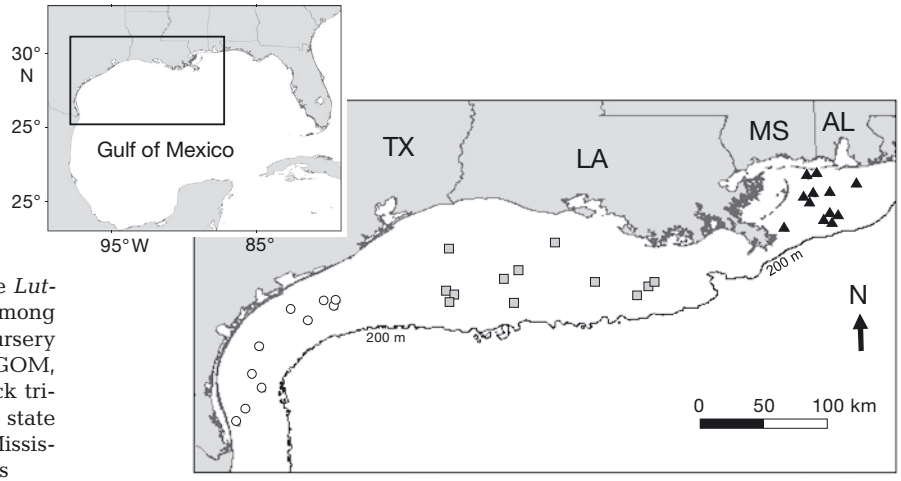

Fig. 1. Sampling locations for juvenile *Lutjanus campechanus* and *L. synagris* among northern Gulf of Mexico (GOM) nursery regions. Unfilled circles = southwest GOM, gray squares = northwest GOM, black triangles = north central GOM. US state abbreviations: AL = Alabama, MS = Mississippi, LA = Louisiana, TX = Texas

were thawed, measured to the nearest mm total length (TL) and wet mass recorded to the nearest 0.01 g. Right and left sagittae were extracted with acid-leached glass probes and Teflon forceps and rinsed with ultrapure (18.3 MΩ cm^{-1}) water. Each otolith was cleaned with 1% ultrapure HNO_3 for 30 s to remove any surface tissue, rinsed repeatedly with ultrapure water to remove acid, and then placed under a Class 10 laminar flow hood to air dry for at least 24 h.

Whole otoliths were analyzed for chemical constitutes such that otolith chemical signatures were integrated over the entire nursery period experienced by juveniles prior to sampling. Right otoliths were processed for elemental analysis by dissolving them in 1% ultrapure HNO_3 at a dilution factor of approximately 1000×; exact dilution factors were determined by mass. Aliquots (5 ml) of otolith solutions were analyzed with a ThermoFisher Element 2 sector field-inductively coupled plasma-mass spectrometer (SF-ICP-MS) in the Department of Marine Science at the University of Southern Mississippi. 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, ^{24}Mg, and ^{88}Sr, with concentrations of individual elements expressed as molar ratios to Ca. All elements were analyzed in medium resolution mode, except for Ba, Li, and Mn, which were analyzed in low resolution. Blanks of 1% ultrapure HNO_3 were processed through the same stages of preparation as sample solutions. Blanks were analyzed concurrently with sample solutions to estimate instrument limits of detection (LOD), which were estimated as 3 standard deviations of mean blank values. Instrument performance and matrix effects were checked by assaying elemental concentrations of an otolith standard reference material (SRM; FEBS-1) prepared from adult red snapper otoliths (Sturgeon et al. 2005). SRM solutions were prepared with the exact protocols as otolith samples.

Left otoliths were processed for stable isotope analysis by pulverizing samples to a fine, homogenized powder with acid-leached glass mortars and pestles and then transferred to microcentrifuge tubes. Subsamples (>1 mg) of pulverized otoliths were analyzed at the Stable Isotope Laboratory in the Department of Geology at the University of California at Davis with a Finnigan MAT 251 isotope ratio mass spectrometer (IR-MS). Instrument calibration was conducted against the International Atomic Energy Agency's carbonate standard, NBS-19. Analytical run accuracies were measured through routine analysis of an in-house check standard which had been stringently calibrated against NBS-19. Results of IR-MS analysis are reported in δ-notation $\{\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$, where $X = {}^{13}C$ or ^{18}O and $R = {}^{13}C / {}^{12}C$ or $^{18}O / {}^{16}O\}$, and are expressed as per mil (‰) relative to the international carbonate standard Vienna Peedee Belemnite (V-PDB).

Parametric assumptions of element:Ca and stable isotope data were tested prior to statistical analysis; all statistical tests were conducted with an experiment-wise error rate (α) of 0.05. Normality was tested with Ryan-Joiner tests and homogeneity of variances was tested with F_{max} tests. Constituents that violated parametric assumptions were either ln-transformed or reciprocal-transformed prior to statistical analysis. Species-specific relationships between TL and element:Ca or stable isotope values were tested with correlation analysis. Differences in otolith chemical signatures were tested with a 2-factor multivariate analysis of variance (MANOVA), with species and region main effects. These effects also were tested with ANOVA for individual constituents (i.e. element:Ca

or stable isotope ratios) (SAS Institute 2009). Following initial analysis of otolith chemical signatures, data were normalized for each constituent by dividing individual values by the species-specific mean among regions. Species and region effects then were retested with MANOVA and ANOVA as indicated above.

Linear discriminant function (LDF) analysis was conducted with non-normalized and normalized data for both red and lane snappers to evaluate whether juveniles could be distinguished among northern GOM regions with otolith chemical signatures (SAS Institute 2009). Lastly, maximum likelihood stock mixing models were parameterized with normalized lane snapper data to test whether the regional composition of unknown red snapper samples could be estimated accurately based on lane snapper otolith chemical signatures (Millar 1990). Regional composition of unknown red snapper samples included 100% from a single region (n = 30), 50% from 1 region and 25% randomly selected from each of the other 2 regions (n = 60), and 33% from each region (n = 90).

RESULTS

A total of 143 age-0 red (n = 90) and lane (n = 53) snapper juveniles was sampled among northern GOM regions (Fig. 1, Table 1). Samples were drawn from a broad geographic range within each region, although FGS stations produced fewer lane than red snapper samples. The range in TL was similar among regions within each species, but mean TL was between 17 and 32 mm greater for lane snapper than for red snapper sampled in the same region (Table 1). There was no significant difference in red snapper TL among regions (ANOVA, p = 0.589), but TL was significantly different among regions for lane snapper (ANOVA, p = 0.006).

Table 1. Descriptive statistics of *Lutjanus campechanus* and *L. synagris* sampled from the 3 regions in the northern Gulf of Mexico (Gulf) in fall 2005. NCG = north central Gulf, NWG = northwest Gulf, SWG = southwest Gulf

Species	Region	n	Stations	Mean ± SE total length (mm)
L. campechanus	NCG	30	12	110.0 ± 3.2
	NWG	30	13	112.5 ± 3.2
	SWG	30	10	110.3 ± 3.2
L. synagris	NCG	16	7	127.4 ± 4.4
	NWG	18	9	144.8 ± 4.2
	SWG	19	9	137.1 ± 4.1

Elemental concentrations were at least 2 orders of magnitude above LODs for each element analyzed with SF-ICP-MS, and analysis of the FEBS-1 SRM yielded concentration estimates within 5% of certified values. Values for $\delta^{13}C$ and $\delta^{18}O$ in the carbonate standard were within 1% of certified values during IR-MS analysis. All element:Ca ratios were ln-transformed and both stable isotope ratios were reciprocal-transformed to meet parametric assumptions.

The only constituent that was significantly correlated to TL for either species was Mn:Ca ($p < 0.04$, $r = -0.362$ for lane snapper and $r = -0.328$ for red snapper). However, the correlations were weak and removing the effect of TL from ln-transformed Mn:Ca had no effect on subsequent statistical analyses.

Otolith chemical signatures were significantly different among regions and between species (MANOVA, $p < 0.001$; Table 2, Fig. 2). The region effect was significant for all constituents except Ba:Ca and Sr:Ca (ANOVA, $p \geq 0.053$), and the species effect was significant for all constituents except Mg:Ca, Mn:Ca, and $\delta^{13}C$ (ANOVA, $p \geq 0.057$; Table 2, Fig. 2). Despite the significant species effect for most constituents, trends in mean constituent values were similar between species. The significant region effect persisted (MANOVA, $p < 0.001$; Table 3, Fig. 3) but the species effect was non-significant (MANOVA, $p = 0.964$) when constituent values were normalized to species-specific means. The removal of the species effect is also reflected in results from ANOVAs run on individual constituents (Table 3).

Mean region-specific jackknifed classification accuracies from LDFs computed with otolith constituent data were 84% for lane snapper and 80% for red snapper (Fig. 4). Identical results were produced with normalized data, thus indicating the variance-covariance structure of otolith chemical signatures was unaffected by normalizing the data. Maximum likelihood models parameterized with normalized lane snapper otolith chemistry data estimated red snapper regional composition reasonably well among mixed-region samples (Table 4). The mean error was 9.7% among models; however, results were least accurate for models in which red snapper samples were derived 100% from a single region.

DISCUSSION

Several criteria must be met for nursery-specific otolith chemical signatures of one fish species to be effectively employed as proxies for another species. Chemical signatures must be sufficiently different

Table 2. Results of MANOVA and ANOVA models computed to test for differences in otolith chemical signatures between age-0 *Lutjanus campechanus* and *L. synagris* among Gulf of Mexico regions. The statistic computed in the MANOVA model was Pillai's Trace and mean square error (from Type III sum of squares) in ANOVA models

Model	Statistic value	*F*-value	df	prob. > *F*
MANOVA				
Region	0.804	12.78	14,264	<0.001
Species	0.490	17.99	7,131	<0.001
Region × Species	0.283	3.11	14,264	<0.001
Ba:Ca ANOVA				
Region	0.003	0.03	2,142	0.971
Species	0.379	4.49	1,142	0.036
Region × Species	0.137	1.63	2,142	0.200
Li:Ca ANOVA				
Region	0.214	18.81	2,142	<0.001
Species	0.067	5.90	1,142	0.016
Region × Species	0.038	3.30	2,142	0.040
Mg:Ca ANOVA				
Region	0.363	21.50	2,142	<0.001
Species	0.062	3.69	1,142	0.057
Region × Species	0.009	0.51	2,142	0.600
Mn:Ca ANOVA				
Region	0.913	9.45	2,142	<0.001
Species	0.093	0.97	1,142	0.328
Region × Species	0.209	2.16	2,142	0.119
Sr:Ca ANOVA				
Region	0.014	3.00	2,142	0.053
Species	0.052	11.09	1,142	0.001
Region × Species	0.019	4.14	2,142	0.018
δ^{13}C ANOVA				
Region	0.026	36.63	2,142	<0.001
Species	0.001	0.57	1,142	0.453
Region × Species	0.005	6.54	2,142	0.002
δ^{18}O ANOVA				
Region	0.115	3.62	2,142	0.030
Species	2.996	77.23	1,142	<0.001
Region × Species	0.053	1.35	2,142	0.262

among nursery areas or regions for each species, high species-specific classification accuracy from statistical models computed with chemical signatures must exist, and nursery origin of a sample of juveniles from the second species must be accurately estimated based on a rule function derived from signatures of the first species. Based on these criteria, results of the current study suggest that region-specific otolith chemical signatures of lane snapper can be employed as accurate proxies for those of its congener, red snapper, in the northern GOM.

Otolith chemical signatures clearly were significantly different among regions for both red and lane snapper juveniles (see Patterson et al. 2008 and Zapp Sluis et al. 2012 for interpretation of regional differ-

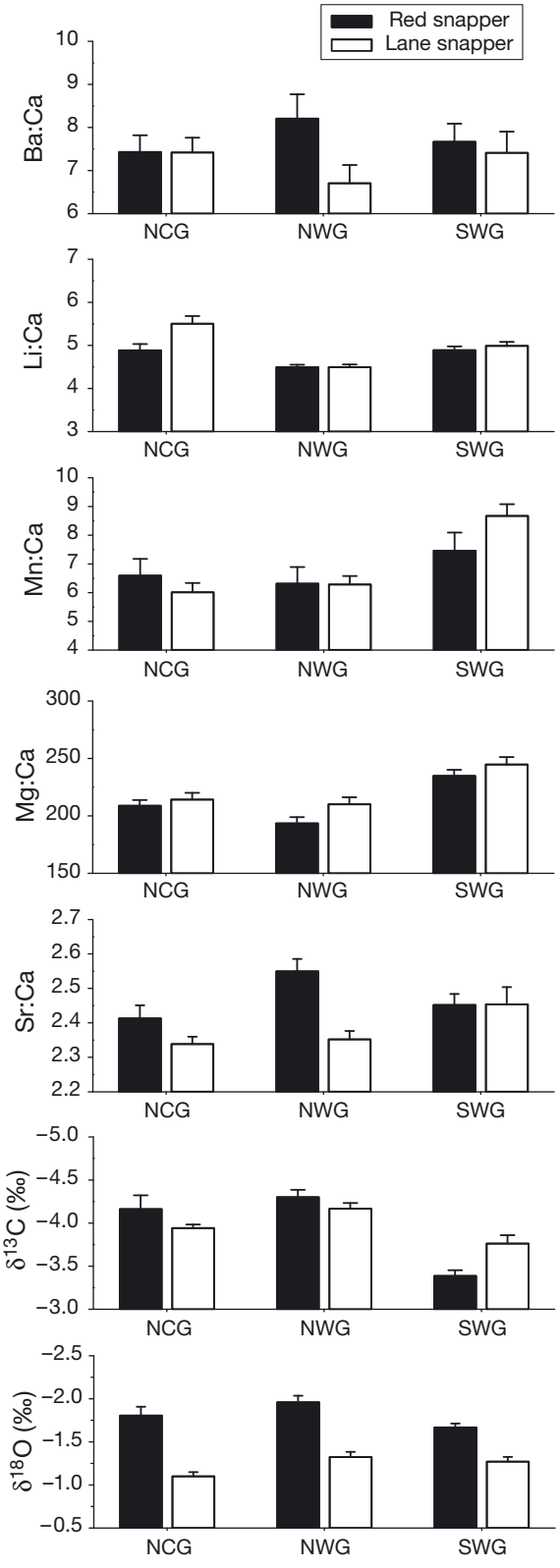

Fig. 2. Mean (+SE) values for otolith element:Ca ratios or carbonate stable isotopes for juvenile *Lutjanus campechanus* and *L. synagris* sampled from northern Gulf of Mexico nursery regions. See Table 1 for region abbreviations

Table 3. Results of MANOVA and ANOVA models computed to test for differences in normalized otolith chemical signatures between age-0 *Lutjanus campechanus* and *L. synagris* among Gulf of Mexico regions. The statistic computed in the MANOVA model was Pillai's Trace and mean square error (from Type III sum of squares) in ANOVA models

Model	Statistic value	*F*-value	df	prob. > *F*
MANOVA				
Region	0.808	12.78	14, 264	<0.001
Species	0.014	0.27	7,131	0.964
Region × Species	0.285	3.14	14, 264	<0.001
Ba:Ca ANOVA				
Region	0.003	0.03	2,142	0.971
Species	0.018	0.22	1,142	0.643
Region × Species	0.137	1.63	2,142	0.200
Li:Ca ANOVA				
Region	0.214	18.81	2,142	<0.001
Species	0.001	0.12	1,142	0.730
Region × Species	0.038	3.30	2,142	0.040
Mg:Ca ANOVA				
Region	0.363	21.50	2,142	<0.001
Species	0.001	0.01	1,142	0.932
Region × Species	0.009	0.51	2,142	0.600
Mn:Ca ANOVA				
Region	0.913	9.45	2,142	<0.001
Species	0.016	0.16	1,142	0.685
Region × Species	0.209	2.16	2,142	0.119
Sr:Ca ANOVA				
Region	0.014	3.00	2,142	0.053
Species	0.001	0.01	1,142	0.984
Region × Species	0.019	4.14	2,142	0.018
$\delta^{13}C$ ANOVA				
Region	0.398	36.42	2,142	<0.001
Species	0.001	0.07	1,142	0.787
Region × Species	0.070	6.40	2,142	0.002
$\delta^{18}O$ ANOVA				
Region	0.232	3.62	2,142	0.030
Species	0.056	0.88	1,142	0.351
Region × Species	0.093	1.46	2,142	0.237

ences). Chemical signatures also were significantly different between species when tested with non-normalized data, which was driven by significant differences in the individual constituents, Ba:Ca, Li:Ca, Sr:Ca, and $\delta^{18}O$. However, patterns among regions were similar between species even for constituents for which the species effect was significant. Normalizing the data to species-specific mean values effectively removed the species effect from MANOVA and ANOVA models while preserving the variance structure of the data with respect to the regional effect, as inferred from nearly identical results for the region effect for the models computed with normalized data. Normalizing the data also had no effect on

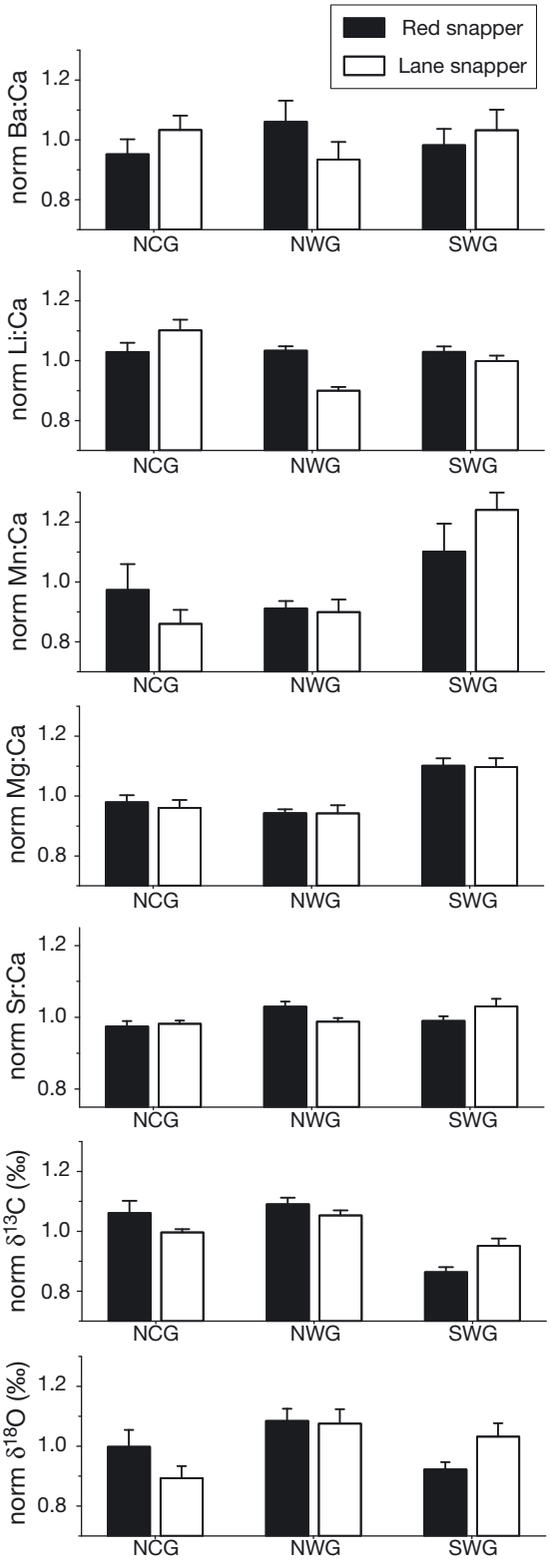

Fig. 3. Normalized mean (+SE) values for otolith element:Ca ratios or carbonate stable isotopes for juvenile *Lutjanus campechanus* and *L. synagris* sampled from northern Gulf of Mexico nursery regions. See Table 1 for region abbreviations

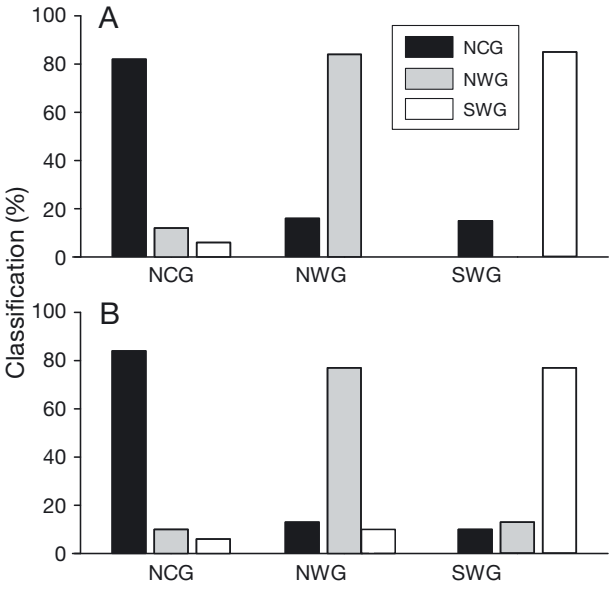

Fig. 4. Jackknifed classification accuracies from linear discriminant function analysis of (A) *Lutjanus synagris* and (B) *L. campechanus* samples from northern Gulf of Mexico nursery regions. See Table 1 for region abbreviations

high (≥80%) jackknifed nursery region classification accuracies from LDFs computed for either lane or red snapper. However, the ultimate test of lane snapper otolith chemical signatures serving as proxies is their ability to distinguish red snapper nursery sources, and maximum likelihood models parameterized with normalized lane snapper otolith chemical signatures accurately (mean error <10%) predicted nursery region of mixed-source red snapper samples.

Authors of other studies have reported significant differences in otolith chemical signatures among species sampled from the same nursery habitats or systems (Gillanders & Kingsford 2003, Hamer & Jenkins 2007, Reis-Santos et al. 2008, 2012). Typically, co-located species which are more closely related phylogenetically and ecologically tend to have more similar otolith chemical signatures (reviewed in Chang & Geffen 2013). For example, Brown (2006) reported otolith elemental composition was similar between juvenile flatfishes English sole *Pleuronectes vetulus* and speckled sanddab *Citharichthys stigmaeus* co-located among 3 regions along the coast of California, and that mean region-specific LDF classification accuracies differed little when species were modeled separately (78 and 79%, respectively) versus jointly (77%). Swearer et al. (2003) also reported that otolith signatures more similar among southern California estuaries for fishes that were more closely related phylogenetically and ecologically. Among species they examined, the flatfishes *Paralichthys californicus* and *Hypsopsetta guttulata* had otolith chemical signatures more similar to each other than to the other species sampled, as did the gobies *Clevelandia ios* and *Ilypnus gilberti*. Furthermore, chemical signatures were more similar between the flatfishes and gobies than between these benthic fishes and the mid-water topsmelt *Atherinops affinis*. It also should be noted that the flatfishes were juveniles of estuarine-dependent species that eventually emigrate out of estuaries to shelf environments, while the gobies were adults that typically have restricted (10s of m^2) home ranges (Swearer et al. 2003). Therefore, other factors such as life stage, growth rate, and feeding ecology also may have affected observed interspecific differences.

The 2 species examined in the current study belong to the subfamily Lutjaninae within the family Lutjanidae. Not only are they closely related phylogenetically (Gold et al. 2011), they also have very

Table 4. Maximum likelihood regional composition estimates of mixed-region age-0 *Lutjanus campechanus* samples from the northern Gulf of Mexico. Models were parameterized with region-specific otolith chemical signatures of *L. synagris*. Model percentage indicates the regional composition of *L. campechanus* mixed-region samples. See Table 1 for region abbreviations

Model percentage			Estimated percentage			Difference		
NCG	NWG	SWG	NCG	NWG	SWG	NCG	NWG	SWG
100	0	0	72.5	9.1	18.4	−27.5	9.1	18.4
0	100	0	0.0	77.1	22.9	0.0	−22.9	22.9
0	0	100	11.3	18.3	70.4	11.3	18.3	−29.6
33	33	33	26.3	36.1	37.6	−6.7	3.1	4.6
50	50	0	33.8	44.9	21.2	−16.2	−5.1	21.2
50	0	50	41.9	13.7	44.4	−8.1	13.7	−5.6
0	50	50	3.2	48.5	47.3	3.2	−1.5	−2.7
50	25	25	41.7	31.8	26.7	−8.3	6.8	1.7
25	50	25	22.0	50.9	28.8	−3.0	0.9	3.8
25	25	50	34.1	24.7	45.9	9.1	−0.3	−4.1

similar ecologies in the northern GOM, especially during early life. Both species spawn during spring and summer months and juveniles settle out in a variety of shelf habitats, although they are most concentrated in shell rubble habitat (Workman et al. 2002, Mikulas & Rooker 2008, Wells et al. 2008). Juveniles typically spend at least their first year of life associated with these habitats prior to recruiting to reefs later in their second year of life, and their feeding ecologies overlap as well, with juvenile diets consisting principally of zooplankton, benthic invertebrates, squids, and transitioning to greater piscivory with ontogeny (Franks & VanderKooy 2000, W. F. Patterson et al. 2005, McCawley & Cowan 2007, Mikulas & Rooker 2008). Therefore, few species are as similar phylogenetically and ecologically in the northern GOM as red and lane snappers, factors that Swearer et al. (2003) and Chang & Geffen (2013) concluded had great influence on the similarity of otolith chemical signatures between species.

A variety of factors, such as salinity, temperature, growth, or food, can affect the incorporation of constituents examined in red and lane snapper otoliths (Elsdon et al. 2008, Chang & Geffen 2013). For example, Sr and Ba concentrations in otoliths of several species, including gray snapper *Lutjanus griseus*, have been shown to reflect ambient concentrations in water, although their incorporation into otoliths may be affected to a lesser extent by water temperature and growth rate (Bath et al. 2000, Elsdon & Gillanders 2002, Martin & Wuenschel 2006, Walther & Thorrold 2006). Red and lane snappers have overlapping spawning seasons in the northern GOM, thus juveniles likely were present on the shelf and exposed to ambient conditions over the same time period. The large size for lane snapper juveniles may result from faster growth, as their mean growth rate tends to be on the upper end of the range observed for red snapper juveniles (Mikulas & Rooker 2008, Wells et al. 2008).

Differential growth rate could have implications for $\delta^{18}C$ but it is unlikely to cause differences observed in $\delta^{18}O$ (Thorrold et al. 1997, Høie et al. 2003). Regional patterns in $\delta^{18}O$ were similar between snapper species, with fish sampled in the NWG having the lowest and fish in the SWG having the highest $\delta^{18}O$ values, which likely resulted from lower $\delta^{18}O$ in Mississippi River water relative to GOM water and relatively little freshwater input onto the shelf from Texas rivers (Bowen & Wilkinson 2002, Dutton et al. 2005, Wagner & Slowey 2011). The inter-specific difference in $\delta^{18}O$, however, is more difficult to explain. Within a species, $\delta^{18}O$ has been shown to be incorporated into otoliths in close equilibrium with ambient water, with fractionation driven by water temperature and independent of growth or metabolic effects (Thorrold et al. 1997, Høie et al. 2003). However, differences in $\delta^{18}O$ fractionation have been reported among species such that a universal equation relating water temperature to $\delta^{18}O$ in otoliths has been elusive (Patterson et al. 1993, Thorrold et al. 1997, Høie et al. 2004). Differences in $\delta^{18}O$ observed between red and lane snappers ranged from 0.40 to 0.71‰ among GOM regions. This is within the range in otolith $\delta^{18}O$ reported among species for a given water temperature (Høie et al. 2004), although it is unclear if one should expect such a difference in $\delta^{18}O$ for 2 species as closely related as red and lane snappers without the fish having experienced different water temperatures.

The last otolith constituent for which there was a significant difference between snapper species was Li:Ca. Lithium is typically 2 orders of magnitude more concentrated in oceanic than riverine waters, and that is true of the Mississippi River, the predominant freshwater source in the northern GOM (Huh et al. 1998). The volume of Mississippi River water on the shelf in the NWG likely explains the lower otolith Li:Ca in that region. However, the incorporation of Li into otoliths is poorly understood so no definitive inference can be drawn either with respect to Li:Ca among regions or between snapper species. Furthermore, it should be noted that Li:Ca values were similar for red and lane snapper juveniles in the NWG and SWG, but the significant species effect was driven by Li:Ca being approximately 10% higher for red snapper in the NCG.

Overall, results from this study suggest that juvenile lane snapper otolith chemical signatures can serve as effective proxies for those of red snapper among northern GOM nursery regions. Significant differences were apparent between species as well as among regions when non-normalized data were analyzed, but normalizing the data effectively removed the species effect. Therefore, if juvenile red snapper samples were unavailable or lacking from GOM regions then lane snapper otolith chemical signatures could be utilized to produce proxies for red snapper. Such an approach could be employed on the west Florida shelf where juvenile red snapper currently are rarely encountered. This would be critical for parameterizing maximum likelihood or Bayesian assignment models computed to estimate source regions for adults because without west Florida juvenile signatures or proxies, models could never predict local self-recruitment. Beyond red snapper, our results may have implications for other

closely related species as well, especially if one species is relatively rare in some part of its range, but the proxy approach should be validated by controlled experiments to examine what factors affect constituents of interest in otoliths of these species.

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