

## FEATURED PAPER

# Population Structure and Regional Connectivity of Young Snowy Grouper in the Gulf of Mexico and Western Atlantic Ocean

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## Abstract

Snowy Grouper *Hyporthodus niveatus* are long-lived, slow-growing demersal fish that occur throughout most of the western Atlantic Ocean. Currently, Snowy Grouper in U.S. territorial waters are managed as two stocks, one along the eastern Atlantic coast and one in the Gulf of Mexico (GoM), though population connectivity throughout the region is unknown. The population structure of juvenile Snowy Grouper in U.S. territorial waters was assessed with otolith chemistry analysis, both trace element ( $^7\text{Li}$ ,  $^{24}\text{Mg}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$ , and  $^{137}\text{Ba}$ ) and stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ), from three life stages (core [first-year deposition], edge [most recent deposition], and life [mean of lifetime deposition]) across four geographic regions (northwest GoM, northeast GoM, southeast GoM, and U.S. East Coast). Dissimilar element:Ca signatures were evident across regions for all three life history stages, with regional patterns in individual markers following expected differences in environmental chemistry based on the geographic location of dominant mesoscale features (Mississippi and Atchafalaya River system, Loop Current, and Gulf Stream) and associated physicochemical conditions. Moderate classification success of Snowy Grouper to collection region with lifetime signatures indicated that the population along the U.S. East Coast experienced different physicochemical conditions than individuals inhabiting the GoM. The close adherence in regional patterns of otolith chemistry with expected environmental chemistry indicates that movement of individuals is limited and population connectivity constrained in U.S. territorial waters.

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Slow life history species are of notable conservation concern due to their intrinsic vulnerability to sustained exploitation pressure (Winemiller 2005; Quetglas et al. 2016). These species are characterized by slow growth, late maturity, long life spans, and episodic recruitment (Winemiller and Rose 1992; King and McFarlane 2003), with

populations of the longest-lived species likely sustained through one successful year-class every 5 to 10 years (Secor 2007). When these life history traits are combined with exploitation pressures targeting the largest, oldest individuals, the eventual result is size and age truncation of the population (Longhurst 2002; Secor et al. 2015). This

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shift in demographics can lead to a decrease in spawning potential from either the removal of the most fecund females in a population (e.g., gonochoristic species) or the preferential removal of a single sex leading to highly skewed sex ratios within mature individuals (e.g., sexual dimorphic, sequential hermaphroditic species) (Heppell et al. 2006; Hixon et al. 2014).

Conservation of slow life history species is considerably more complex for deepwater fishes, where inaccessibility, increased pressure at depth, and a lack of small-scale spatial variability in physicochemical conditions make it more difficult to assess population demographics (Campana 1999, 2001; Cailliet et al. 2001). In addition, the application of conventional approaches used to determine movement, population size, and connectivity of fishes (e.g., tagging) are typically not suitable for these deepwater taxa due to high release mortality (Koslow et al. 2000; Newman et al. 2017). However, otolith chemistry of individual fish functions as a natural tag and when analyzed in conjunction with age information can provide approximate estimations of population connectivity and stock structure (Campana 2001; Tanner et al. 2016). Since the advent of Levins' (1969) metapopulation concept, defining the structure of exploited stocks has become a critical element for the development of fisheries stock assessments and management policy (Begg et al. 1999; Berkeley et al. 2004). For uncoupled local populations (i.e., subpopulations), differences in demographics can lead to divergences between sustainable exploitation rates and result in extirpation of the most vulnerable contingents, decreasing resiliency of the population as a whole (Smedbol and Wroblewski 2002; Kritzer and Sale 2004). When managing an exploited population, detrimental effects on the most vulnerable components within the population can be masked when assessed under a single-stock concept (Ying et al. 2011). This is especially true for slow life history species, where an improperly defined (or undefined) stock structure increases vulnerability for a species exposed to overexploitation.

The U.S. deepwater grouper (family Epinephelidae) fishery in the Atlantic Ocean is comprised of four slow life history species that are vulnerable to overexploitation (GMFMC 2018). Three of the four species that compose this fishery (Speckled Hind *Epinephelus drummondhayi*, Yellowedge Grouper *Hyporhodus flavolimbatus*, Snowy Grouper *Hyporhodus niveatus*) are experiencing decreasing population trends with a vulnerable or data-deficient stock status (Aguilar-Perera et al. 2018; Padovani-Ferreira et al. 2018; Sosa-Cordero and Russell 2018), while the population status of the fourth species (Warsaw Grouper *Hyporhodus nigrurus*) is unknown (Bertoncini et al. 2018). Bomb radiocarbon age validation analysis conducted on all four species concluded that the longevity for each is likely double that currently applied to natural mortality

estimations used to determine fishery catch limits revealing increased vulnerabilities to overexploitation (Cook et al. 2009; Andrews et al. 2013; Sanchez et al. 2019). For the only species in this deepwater grouper complex on which a population structure analysis was conducted, Warsaw Grouper, results indicated the presence of distinct population contingents within the Gulf of Mexico (hereafter, "GoM"; Sanchez et al. 2020), potentially increasing their vulnerability to fishing pressure.

Here, we apply otolith chemistry to investigate the population structure and connectivity of Snowy Grouper along the continental shelf and slope in the Atlantic Ocean (Wyanski et al. 2000; Costa et al. 2012). To date, the population structure of this species in the GoM and western Atlantic Ocean (WAO) is unknown and requires immediate attention because current estimates of natural mortality rates may be substantially higher than previously considered (SEDAR 2020). Chemical markers in the otoliths of Snowy Grouper, both trace elements (Li, Mg, Mn, Sr, and Ba) and stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ), were measured for individuals collected from four geographic regions in the GoM and WAO to determine whether region-specific differences in otolith chemistry were present during three life history stages: early life (first-year deposition), adult (most recent deposition), and lifetime (mean value of whole otolith). Due to Snowy Grouper dependence on benthic structure and the patchy nature of deepwater habitat in the regions investigated, we anticipate that movement and mixing across regions will likely be limited and lead to distinct chemical signatures for Snowy Grouper from each region.

## METHODS

*Sample collection and preparation.*—Archived otoliths from Snowy Grouper were provided by the National Oceanic and Atmospheric Administration (NOAA) Fisheries' Southeast Regional Office in Panama City, Florida. All otoliths were collected by NOAA Fisheries' observers from 2012 to 2015 in U.S. waters of the GoM and WAO. Otoliths from Snowy Grouper less than 60 cm total length (TL) were selected for analysis to control for confounding ontogenetic effects on otolith chemistry in older fish. Samples were organized into four regions determined by the Gulf of Mexico Fishery Management Council shrimp fishery statistical zone: northwest GoM (nwGoM; zones 13–21), northeast GoM (neGoM; zones 6–12), southeast GoM (seGoM; zones 1–6), and WAO (zones 701, 707, 708, 713, 714) (Figure 1). All fish in our sample from the WAO were landed in North Carolina and South Carolina. Initially dried and stored in paper envelopes in the Southeast Regional Office, Snowy Grouper otoliths were cleaned by scraping off all organic tissue with Teflon-coated forceps, rinsed with double deionized water

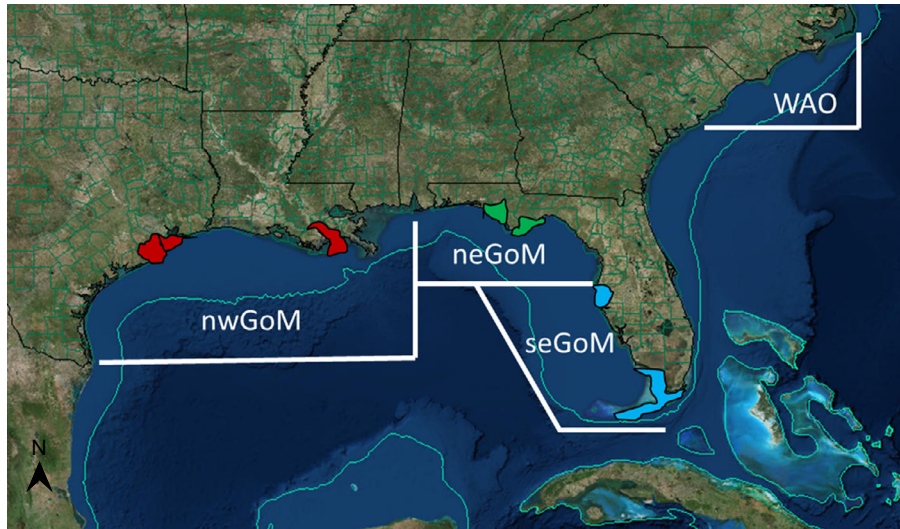


FIGURE 1. Map of the western Atlantic Ocean (WAO) and Gulf of Mexico (GoM; nw = northwest, ne = northeast, se = southeast), showing demarcations for regional groupings based on catch location (white lines). Colored polygons in the GoM represent sampled landing locations, and the light blue line denotes the 200-m depth contour.

(ultrapure, 18-M $\Omega$ /cm water), and allowed to air dry following Rooker et al. (2008). Cleaned and dried otoliths were then set in Struers epoxy resin, sectioned along a transverse plane, mounted on petrographic slides, and polished using a series of 240-, 600-, and 800-grit sandpaper until the otolith primordium (core) was exposed and growth increments were visible. Ages for each otolith were determined by enumerating growth increments, a method previously validated for Snowy Grouper less than 25 years old (Sanchez et al. 2019). A second reader assessed ages of 40% of the otoliths to verify readings. Fish age is reported as the mean age of the two reads when two reads were available, with the overall average percent error calculated.

**Trace elements.**—Trace element composition of Snowy Grouper otoliths was assessed using two different laser ablation inductively coupled plasma mass spectrometers (ICP-MS). Samples collected in 2012 and 2013 were assessed using an ultraviolet New Wave Research NWR213 laser ablation system coupled with a quadrupole Thermo Scientific XSeries 2 ICP-MS located at Texas A&M University at Galveston set to  $^{44}\text{Ca}$  as the Ca standard; samples collected in 2014 and 2015 were assessed using an Elemental Scientific NWR193UC laser ablation system coupled with an Agilent 7500ce ICP-MS at the University of Texas at Austin set to  $^{43}\text{Ca}$  as the Ca standard. On each instrument, multiple otolith thin sections were mounted onto single petrographic slides, with the exact number dependent on section width.

Following a preablation procedure to remove surface contamination (Sanchez et al. 2020), elemental concentrations were determined for a series of 70- $\mu\text{m}$ -diameter spots

on each otolith, with 150- $\mu\text{m}$  spacing between centroids (80- $\mu\text{m}$  distance between consecutive spot edges). The series of ablation spots ran from the otolith primordium to the otolith margin (most recent deposition) up the growth transect along the sulcal groove on the dorsal face of the otolith section (Figure 2). The total number of sampling spots per otolith was dependent on the distance between the otolith primordium and margin. Ablation spots were grouped into three life history stages: (1) otolith<sub>core</sub> (young-of-year period [age 0]), (2) otolith<sub>edge</sub> (most recent 2–3 years), and (3) otolith<sub>life</sub> (lifetime period). Elemental values for each life history stage were calculated as the mean of a series of successive spots up the growth axis transect: otolith<sub>core</sub> = the first three spots from the primordium, otolith<sub>edge</sub> = the last three spots from the margin, and otolith<sub>life</sub> = all spots along the transect. Inclusion of three spots was selected for otolith<sub>core</sub> and otolith<sub>edge</sub> signature development because preliminary analyses suggested that decreasing the number of spots (two or one) did not change analysis results though it did increase the influence of potential irregularities in values for an individual. Replicates of National Institute of Standards and Technology 612 standards were run at regular intervals to calibrate machine counts per second readings to element concentrations (ppm), and U.S. Geological Survey MACS-3 standards were used as the primary reference and to assess machine drift. Six elements in Snowy Grouper otoliths were quantified:  $^7\text{Li}$ ,  $^{24}\text{Mg}$ ,  $^{43}\text{Ca}$ ,  $^{55}\text{Mn}$ ,  $^{88}\text{Sr}$ , and  $^{137}\text{Ba}$ . National Institute of Standards and Technology 612 analyte recoveries ( $N=72$ ) were typically within 6% of GeoREM-preferred values. Element concentrations (ppm) were then converted to element:Ca ratios ( $\mu\text{mol/}$

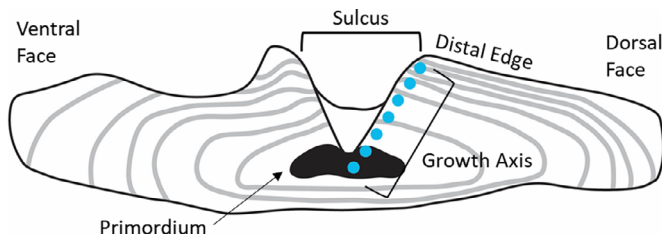


FIGURE 2. Collection locations on the otolith cross section for material sampled for trace elements and stable isotopes. The blue circles represent ablation spots, the black section in the center represents the primordium (earliest deposition), and the gray rings represent growth increments. For trace elements, otolith<sub>core</sub> includes the first three ablation spots moving up from the primordium, otolith<sub>edge</sub> includes the last three ablation spots near the distal edge, and otolith<sub>life</sub> includes all the ablation spots up the growth axis. For stable isotopes, sample material was milled and pooled across the entire otolith<sub>life</sub> transect.

mmol) using  $^{44}\text{Ca}$  as the Ca standard at Texas A&M University at Galveston and  $^{43}\text{Ca}$  as the Ca standard at University of Texas at Austin as described in Sanchez et al. (2020).

**Stable isotopes.**—For a subset of Snowy Grouper ( $n = 87$ ), otolith  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values were determined following laser ablation ICP-MS analysis. Otolith sections were initially prepared for stable isotope analysis by lightly polishing off laser ablation spots. Otolith material along the lifetime transect assayed of the laser ablation ICP-MS was then obtained using a high-precision micromill (New Wave Research MicroMill). Fourteen 55- $\mu\text{m}$ -deep passes of a 300- $\mu\text{m}$ -wide transect line from the otolith core to margin resulted in a sampling depth of 770  $\mu\text{m}$  (Figure 2). Powdered otolith material from each sample was collected and pooled to obtain lifetime  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values for each individual in the subset of Snowy Grouper otoliths. Stable isotope analysis ( $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ ) was performed at the University of Arizona using an automated carbonate preparation device (KIEL-III, Thermo Fisher Scientific) coupled to a Thermo Fisher Scientific MAT 252 isotope ratio mass spectrometer. Powdered otolith samples were reacted with dehydrated phosphoric acid under vacuum at 70°C. The isotope ratio measurement was calibrated based on repeated measurements of National Bureau of Standards (NBS-19 and NBS-18), with six standards run for every 40 samples; analytical precision was  $\pm 0.08\text{‰}$  (SD) and  $\pm 0.11\text{‰}$  (SD) for  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$ , respectively. Otolith  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values are reported relative to the Vienna Pee Dee Belemnite scale after comparison to an in-house laboratory standard calibrated to Vienna Pee Dee Belemnite.

**Statistical analysis.**—Otolith element:Ca values of Snowy Grouper during 2012–2013 (Texas A&M University at Galveston) and 2014–2015 (University of Texas at Austin) were assessed independently. For each data set, otolith<sub>life</sub>, otolith<sub>core</sub>, and otolith<sub>edge</sub> element:Ca values

were organized by region and initially assessed for outliers, which were defined as any element:Ca ratio outside five standard deviations of the region-specific mean value for that element. Any otolith with a single outlier for any element in any life history stage was removed from all analyses.

Multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) tests were run for each life history stage to determine whether otolith element:Ca signatures and individual elements for Snowy Grouper differed by region. The MANOVA and ANOVA models incorporated an interaction term (region  $\times$  year) to evaluate if regional differences changed among years (R Studio, package “nlme”). In cases where the interaction effect was not significant, a single-factor (region) ANOVA was run to assess regional variability for that individual element. A Tukey’s honestly significant difference (HSD) test ( $\alpha = 0.05$ ) was applied to significant univariate tests to determine factor levels (regions) that were significantly different from each other. Quadratic discriminate function analysis was run on samples for each life history stage with data from individual years to assess classification success of Snowy Grouper into collection region (SYSTAT version 11). For otolith<sub>life</sub> values, a canonical correspondence analysis plot for each year was developed to project signature dissimilarities in two-dimensional space overlaid with the influence of individual markers (R Studio, package “vegan”). To visualize changes in otolith element:Ca with ontogeny, mean element:Ca values per region were plotted for the first 10 ablation spots up the growth axis for all samples in the 2012–2013 data set.

A subset of otolith  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  values for Snowy Grouper were analyzed with ANOVA to determine whether region-specific differences were present. Collection year was restricted to 2 years (2012, 2013) from the GoM and 1 year (2013) from the WAO; therefore, years were pooled and interaction between region and year was not assessed. Quadratic discriminate function analysis was applied to the subset of Snowy Grouper otoliths that were assayed for both trace elements and stable isotopes. Classification success to region was evaluated using three different models for the subset—stable isotopes only, trace elements only, and a combination of both markers—and results were compared to assess success rates between the different signature types.

## RESULTS

A total of 254 Snowy Grouper otoliths collected over 4 years (2012, 2013, 2014, 2015) were analyzed for Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca. After the removal of otoliths with element:Ca outliers, 235 individuals were retained for further analysis. Mean  $\pm$  SD length and age of Snowy Grouper in our final sample were  $540 \pm 35$  mm

and  $7.3 \pm 2.3$  years (average percent error = 11%) for fish aged (Table 1), respectively, with no differences across the four regions (length ANOVA:  $F=1.9$ ,  $P<0.05$ ; age ANOVA:  $F=2.3$ ,  $P>0.05$ ).

### Trace Elements

Otolith<sub>life</sub> signatures of Snowy Grouper based on a combination of all five element:Ca ratios were significantly different among the four regions for both 2012–2013 (MANOVA, Pillai's  $T=0.87$ ,  $P<0.001$ ) and 2014–2015 (MANOVA, Pillai's  $T=0.70$ ,  $P<0.001$ ) collection periods. Otolith<sub>life</sub> Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca were each significantly different (ANOVA:  $P<0.05$ ) among the four regions for both 2012–2013 (Table 2) and 2014–2015 (Table 3) collection periods, with the exception of Mg:Ca in 2014–2015. Significant region  $\times$  year interactions were observed for Sr:Ca and Ba:Ca in 2012–2013 and for Li:Ca and Mg:Ca in 2014–2015; however, general interregion patterns still emerged for most element:Ca ratios (Appendix Table A.1). For 2012–2013 and 2014–2015, otolith<sub>life</sub> Li:Ca was higher in the WAO than in the nwGoM, neGoM, and seGoM (Figure 3A). Similarly, mean otolith<sub>life</sub> Sr:Ca was lowest in the nwGoM for 2012–2013 and 2014–2015, with a clear gradient towards higher values for this marker moving east into the neGoM and seGoM; however, relative values fluctuated in the WAO, with the highest overall in 2012–2013 but lower than both the seGoM and neGoM in 2014–2015 (Figure 3D). In contrast, mean otolith<sub>life</sub> Mn:Ca (Figure 3C) and Ba:Ca (Figure 3E) were higher in the nwGoM than in the neGoM, seGoM, and WAO. Overall jackknifed classification success of Snowy Grouper to collection location based on otolith<sub>life</sub> signatures ranged from 56% to 70% among individual years (Table 4). Average regional classification success was highest for Snowy Grouper from the WAO (mean  $\pm$  SD =  $80.5 \pm 13.0\%$ ), with success rates lower for individuals from the nwGoM ( $64.8 \pm 14.1\%$ ),

the seGoM ( $58.5 \pm 16.3\%$ ), and the neGoM ( $41.8 \pm 14.1\%$ ) (Figure 4).

Otolith<sub>core</sub> (age-0) signatures of Snowy Grouper based on a combination of all five element:Ca ratios were significantly different among the four regions for both 2012–2013 (MANOVA, Pillai's  $T=0.51$ ,  $P<0.001$ ) and 2014–2015 (MANOVA, Pillai's  $T=0.35$ ,  $P<0.01$ ) collection periods. In 2012–2013, otolith<sub>core</sub> Li:Ca, Mg:Ca, and Mn:Ca were significantly different among the four regions (Table 2), while only otolith<sub>core</sub> Mn:Ca was significant in 2014–2015 (Table 3) (ANOVA:  $P<0.05$ ). A significant region  $\times$  year interaction was not detected for any of the element:Ca ratios where region was significant, indicating that general regional patterns persisted across years. Otolith<sub>core</sub> Li:Ca was higher in the WAO in 2012–2013 than in the nwGoM, neGoM, and seGoM; no differences were observed for Snowy Grouper from the 2014–2015 collection period (Figure 5A). Conversely, otolith<sub>core</sub> Mn:Ca was highest in the nwGoM for 2012–2013 and 2014–2015 and showed a clear gradient with values decreasing eastward through the neGoM, seGoM, and WAO (Figure 5C). Otolith<sub>core</sub> Mg:Ca was lower in the seGoM in 2012–2013 than in the nwGoM and neGoM; however, like Li:Ca no differences were observed from the 2014–2015 collection period (Figure 5B). Overall jackknifed classification success of Snowy Grouper to collection location based on otolith<sub>core</sub> signatures ranged from 38% to 59% for individual years (Table 4). Average regional classification success of Snowy Grouper was low for all four regions, with only the seGoM over 50% (WAO =  $48.3 \pm 10.8\%$  [mean  $\pm$  SD]; neGoM =  $45.3 \pm 19.7\%$ ; seGoM =  $57.5 \pm 11.2\%$ ; nwGoM =  $48.0 \pm 20.7\%$ ).

Otolith<sub>edge</sub> signatures of Snowy Grouper based on a combination of all five element:Ca ratios were significantly different among the four regions for both the 2012–2013 (MANOVA, Pillai's  $T=0.85$ ,  $P<0.001$ ) and 2014–2015 (MANOVA, Pillai's  $T=0.64$ ,  $P<0.001$ ) collection periods. Otolith<sub>edge</sub> Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca were each significantly different among the four regions for both the 2012–2013 (Table 2) and 2014–2015 (Table 3) collection periods, with the exception of Mn:Ca in 2012–2013 and Ba:Ca in 2014–2015 (ANOVA:  $P<0.05$ ). A significant region  $\times$  year interaction was not observed for the 2012–2013 collection period; however, in 2014–2015 a significant interaction was observed for Li:Ca, Mg:Ca, and Sr:Ca, thereby indicating increased variability in regional differences between the years. Otolith<sub>edge</sub> Li:Ca was higher in the WAO for both 2012–2013 and 2014–2015 than in the nwGoM, neGoM, and seGoM (Figure 5A). Similarly, otolith<sub>edge</sub> Sr:Ca was highest in the WAO and seGoM and lowest in the nwGoM (Figure 5D). Otolith<sub>edge</sub> Mg:Ca was lower in the seGoM than the WAO and nwGoM for both 2012–2013 and 2014–2015 (Figure 5B). Otolith<sub>edge</sub> Mn:Ca (Figure 5C) and Ba:Ca

TABLE 1. Sample size ( $n$ ), length (mm), and age (years) data for Snowy Grouper from each region analyzed for trace elements. The asterisk indicates that six otoliths were not included in the age comparison due to difficulties with age determination. Abbreviations are as follows: WAO = western Atlantic Ocean, seGoM = southeast Gulf of Mexico, neGoM = northeast Gulf of Mexico, and nwGoM = northwest Gulf of Mexico.

Region	$n$	Length		Age*	
		Mean	SD	Mean	SD
WAO	65	540	24	6.8	1.7
seGoM	63	533	33	7.6	1.9
neGoM	44	536	52	7.7	3.1
nwGoM	63	549	32	7.3	3.1
Overall	235	540	35	7.3	2.3

TABLE 2. Results from ANOVA tests for otolith<sub>life</sub>, otolith<sub>core</sub>, and otolith<sub>edge</sub> chemical signatures assessing an interaction between region and year (region × year) for samples collected in 2012 and 2013. Stable isotope samples were not collected for multiple years for every region, so only a single-factor ANOVA was used to assess regional differences for δ<sup>13</sup>C and δ<sup>18</sup>O for all years pooled (indicated with an asterisk). The four regions are as follows = western Atlantic Ocean, northeast Gulf of Mexico, southeast Gulf of Mexico, and northwest Gulf of Mexico. Significant values are shown in bold italics.

Life stage	Natural marker	Region (df = 3)		Year (df = 3)		Region × year (df = 9)	
		<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Life	Li:Ca	34.16	<b>&lt;0.001</b>	4.43	<b>&lt;0.05</b>	2.49	>0.05
	Mg:Ca	11.73	<b>&lt;0.001</b>	0.03	>0.05	0.37	>0.05
	Mn:Ca	13.91	<b>&lt;0.001</b>	6.97	<b>&lt;0.01</b>	0.95	>0.05
	Sr:Ca	25.87	<b>&lt;0.001</b>	0.00	>0.05	2.99	<b>&lt;0.05</b>
	Ba:Ca	5.47	<b>&lt;0.01</b>	7.69	<b>&lt;0.01</b>	4.29	<b>&lt;0.01</b>
	δ <sup>13</sup> C*	8.76	<b>&lt;0.001</b>				
	δ <sup>18</sup> O*	8.31	<b>&lt;0.001</b>				
Core	Li:Ca	7.16	<b>&lt;0.001</b>	1.74	>0.05	0.88	>0.05
	Mg:Ca	6.99	<b>&lt;0.001</b>	0.01	>0.05	0.70	>0.05
	Mn:Ca	10.91	<b>&lt;0.001</b>	1.03	>0.05	0.17	>0.05
	Sr:Ca	1.77	>0.05	0.07	>0.05	1.51	>0.05
	Ba:Ca	1.21	>0.05	1.16	>0.05	0.68	>0.05
Edge	Li:Ca	49.71	<b>&lt;0.001</b>	6.54	<b>&lt;0.05</b>	1.12	>0.05
	Mg:Ca	5.20	<b>&lt;0.01</b>	0.09	>0.05	1.69	>0.05
	Mn:Ca	0.37	>0.05	3.60	>0.05	1.19	>0.05
	Sr:Ca	24.15	<b>&lt;0.001</b>	0.01	>0.05	1.08	>0.05
	Ba:Ca	7.25	<b>&lt;0.001</b>	1.14	>0.05	1.27	>0.05

TABLE 3. Results of ANOVA tests for otolith<sub>life</sub>, otolith<sub>core</sub>, and otolith<sub>edge</sub> life history stages assessing an interaction between region and year (region × year) for samples collected in 2014 and 2015. The four regions are as follows = western Atlantic Ocean, northeast Gulf of Mexico, southeast Gulf of Mexico, and northwest Gulf of Mexico. Significant values are shown in bold italics.

Life stage	Natural marker	Region (df = 3)		Year (df = 3)		Region × year (df = 9)	
		<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value	<i>F</i> -value	<i>P</i> -value
Life	Li:Ca	22.65	<b>&lt;0.001</b>	33.85	<b>&lt;0.001</b>	35.25	<b>&lt;0.001</b>
	Mg:Ca	1.47	>0.05	6.61	<b>&lt;0.05</b>	3.78	<b>&lt;0.05</b>
	Mn:Ca	8.33	<b>&lt;0.001</b>	0.89	>0.05	2.57	>0.05
	Sr:Ca	4.49	<b>&lt;0.01</b>	2.63	>0.05	2.60	>0.05
	Ba:Ca	3.62	<b>&lt;0.05</b>	3.01	>0.05	0.94	>0.05
Core	Li:Ca	2.18	>0.05	9.11	<b>&lt;0.01</b>	7.17	<b>&lt;0.001</b>
	Mg:Ca	0.73	>0.05	0.02	>0.05	2.17	>0.05
	Mn:Ca	5.67	<b>&lt;0.01</b>	4.16	<b>&lt;0.05</b>	2.64	>0.05
	Sr:Ca	1.28	>0.05	1.49	>0.05	1.82	>0.05
	Ba:Ca	1.75	>0.05	0.35	>0.05	0.57	>0.05
Edge	Li:Ca	37.69	<b>&lt;0.001</b>	51.27	<b>&lt;0.001</b>	54.95	<b>&lt;0.001</b>
	Mg:Ca	4.20	<b>&lt;0.01</b>	6.29	<b>&lt;0.05</b>	3.79	<b>&lt;0.05</b>
	Mn:Ca	3.01	<b>&lt;0.05</b>	1.01	>0.05	1.30	>0.05
	Sr:Ca	10.77	<b>&lt;0.001</b>	2.38	>0.05	3.05	<b>&lt;0.05</b>
	Ba:Ca	2.67	>0.05	4.92	<b>&lt;0.05</b>	2.04	>0.05

(Figure 5E) were low for all four regions for both 2012–2013 and 2014–2015. Overall jackknifed classification success of Snowy Grouper to collection location based on

otolith<sub>edge</sub> signatures ranged from 57% to 67% for individual years (Table 4). Average regional classification success of Snowy Grouper was generally similar in the WAO

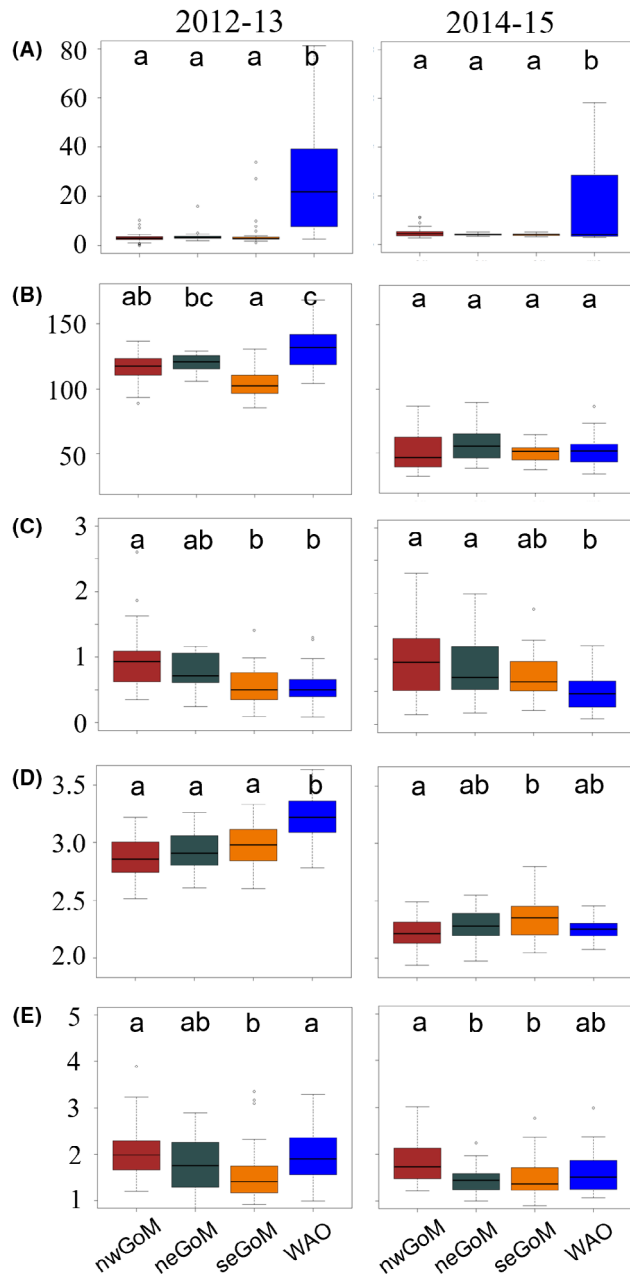


FIGURE 3. Boxplot of element :Ca ratios in otolith<sub>life</sub> signatures for (A) Li:Ca ( $\mu\text{mol}/\text{mmol}$ ), (B) Mg:Ca ( $\mu\text{mol}/\text{mmol}$ ), (C) Mn:Ca ( $\mu\text{mol}/\text{mmol}$ ), (D) Sr:Ca ( $\text{mmol}/\text{mmol}$ ), and (E) Ba:Ca ( $\mu\text{mol}/\text{mmol}$ ) for both the 2012–2013 (left column) and 2014–2015 (right column) collection years. The horizontal line in each box indicates the median, the box dimensions represent the 25th to 75th percentile ranges, the whiskers show the 10th to 90th percentile ranges, and dots are outliers. Statistically significant groupings (Tukey's HSD:  $P < 0.05$ ) are given in lowercase letters at the top of each plot; within year and element, regions that share a letter are not significantly different.

(mean  $\pm$  SD =  $67.5 \pm 22.3\%$ ), nwGOM ( $64.5 \pm 5.6\%$ ), and seGOM ( $58.8 \pm 19.6\%$ ), with considerably lower success for classification of individuals to the neGoM ( $36.8 \pm 7.1\%$ ).

TABLE 4. Regional and overall classification success (%) for otolith<sub>life</sub> (with  $\delta^{18}\text{O}$ ), otolith<sub>core</sub>, and otolith<sub>edge</sub> chemical signatures with mean values across years. Abbreviations are as follows: WAO = western Atlantic Ocean, seGoM = southeast Gulf of Mexico, neGoM = northeast Gulf of Mexico, and nwGoM = northwest Gulf of Mexico.

Life stage	Region and total	2012	2013	2014	2015	Mean $\pm$ SD
Life	WAO	74	75	100	73	$80.5 \pm 13.0$
	seGoM	75	70	46	43	$58.5 \pm 16.3$
	neGoM	50	33	27	57	$41.8 \pm 14.1$
	nwGoM	56	80	73	50	$64.8 \pm 14.1$
	Total	65	70	61	56	$63.0 \pm 5.9$
Core	WAO	53	35	45	60	$48.3 \pm 10.8$
	seGoM	63	55	69	43	$57.5 \pm 11.2$
	neGoM	50	33	27	71	$45.3 \pm 19.7$
	nwGoM	67	25	64	36	$48.0 \pm 20.7$
Edge	Total	59	38	52	53	$50.5 \pm 8.9$
	WAO	79	60	91	40	$67.5 \pm 22.3$
	seGoM	50	80	69	36	$58.8 \pm 19.6$
	neGoM	40	33	45	29	$36.8 \pm 7.1$
	nwGoM	67	70	64	57	$64.5 \pm 5.6$
Total	62	65	67	40	$58.5 \pm 12.5$	

### Stable Isotopes

Otolith<sub>life</sub>  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of Snowy Grouper were significantly different across the four regions (ANOVA:  $P < 0.05$ ; Table 2). For otolith<sub>life</sub>  $\delta^{13}\text{C}$ , values of Snowy Grouper were significantly higher in the seGoM and neGoM than in the nwGoM (Tukey HSD:  $P < 0.05$ ), with intermediate values for individuals from the WAO. For otolith<sub>life</sub>  $\delta^{18}\text{O}$ , values were higher in the seGoM and WAO than in the nwGoM and neGoM (Tukey HSD:  $P < 0.05$ ). Overall jackknifed classification success of Snowy Grouper to collection location based on both otolith<sub>life</sub> element :Ca and stable isotope markers was 60%. Classification success of samples in the subset based on element :Ca only and stable isotopes only was 57% and 51%, respectively.

### DISCUSSION

Trace element and stable isotope signatures in the otoliths of Snowy Grouper for all life history stages assessed (core, edge, and lifetime) showed distinct region-specific patterns among collection areas in the GoM and WAO. Consistent discrimination in otolith chemical signatures over multiple years indicates that differences in otolith chemistry are likely a result of distinct environmental and/or physicochemical conditions Snowy Grouper experience in these regions (Kalish 1989; Elsdon et al. 2008). Moreover, our finding of consistent regional differences in otolith chemistry of Snowy Grouper that reflect expected differences in ambient water chemistry imply that

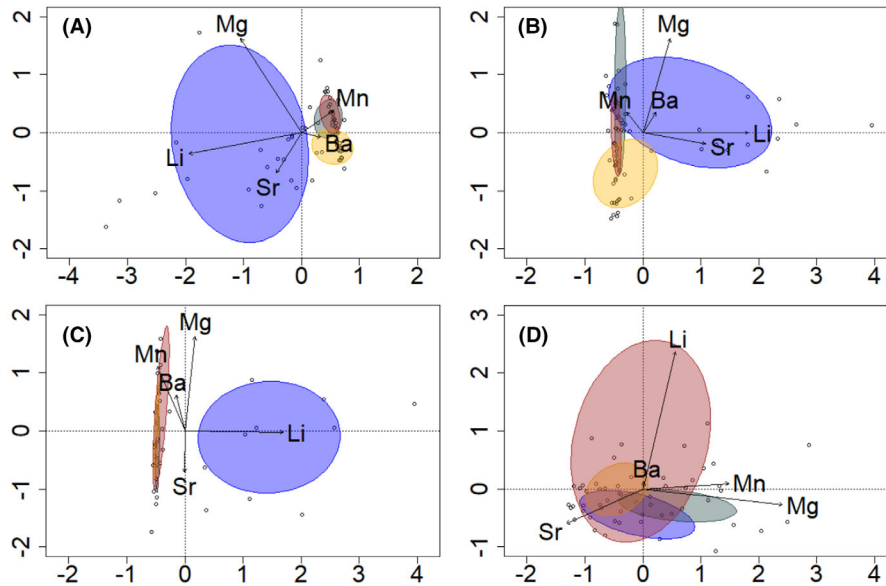


FIGURE 4. Constrained correspondence analysis plots of trace element signatures for the lifetime life history stage for (A) 2012, (B) 2013, (C) 2014, and (D) 2015. Ellipses represent one standard deviation for each region. Colors are coded as follows: purple = western Atlantic Ocean, orange = southeast Gulf of Mexico, green = northeast Gulf of Mexico, and red = northwest Gulf of Mexico.

movement of postsettlement juvenile Snowy Grouper across the regions investigated is limited.

Otolith<sub>life</sub> Mn:Ca and Ba:Ca in Snowy Grouper were elevated for individuals collected in the nwGoM, which is presumably the result of nutrient-rich Mississippi and Atchafalaya River system inflow that likely elevates concentrations of Mn and Ba in this region. Considered a scavenged element, Mn concentrations are often higher in environments near areas of high freshwater inflow such as the Mississippi and Atchafalaya River system, with concentrations of Mn typically decreasing as distance from the river or freshwater source increases (Sturrock et al. 2012). Moreover, dissolved Mn remains in solution longer in hypoxic waters, increasing its availability for uptake through the gills and incorporation into biomineralized structures such as otoliths (Limburg et al. 2015). In addition to the strong Mississippi and Atchafalaya River system influence in the nwGoM, hypoxic conditions are common along the Louisiana–Texas continental shelf, where a seasonal dead zone develops every year and where the benthos of the continental shelf is characterized by a thick nepheloid layer composed of resuspended anoxic sediment (Rabalais et al. 2002; Matli et al. 2018). Therefore, Mn may be more available for uptake for demersal species (Ashford et al. 2005; Limburg et al. 2011), though an actual increase in otolith Mn:Ca has not always been found in hypoxic waters (Mohan and Walther 2014). Similar to otolith Mn, Ba is a good indicator of nutrient-rich waters and otolith Ba:Ca is often elevated in areas with high nutrient loading or

upwelling (Bath et al. 2000; Walther and Thorrold 2006; Miller 2009). While the nwGoM is expected to have the most elevated levels of Ba in the GoM regions, ambient chemistry along the outer shelf of the WAO is likely influenced by nutrient-rich upwelling along the frontal eddies of the Gulf Stream, thereby elevating environmental concentrations of Ba in this region as well (Lee et al. 1981; Gula et al. 2016). Our finding of elevated otolith Mn:Ca and Ba:Ca in the nwGoM is consistent with levels found in Warsaw Grouper (Sanchez et al. 2020), suggesting that regional differences are not species-specific but a result of large-scale geographic dynamics in physicochemical conditions of the GoM.

Regional variation in otolith Sr:Ca and  $\delta^{18}\text{O}$  of Snowy Grouper again appear associated with the geographic position of mesoscale features and ambient seawater chemistry. In the present study, both of these markers were highest for Snowy Grouper collected from the seGoM and WAO. While Sr concentration is assumed to be conservative across the normal range of ocean salinity (33–37‰) found on the outer shelf in the GoM and WAO, a positive relationship between otolith Sr and salinity is often observed when moving between freshwater and marine systems (Secor and Rooker 2000; Nelson and Powers 2019). Similarly, a positive relationship between otolith  $\delta^{18}\text{O}$  and salinity has also been shown (Kalish 1991; Bastow et al. 2002; Kerr et al. 2007). Thus, it is not surprising that Snowy Grouper collected in seGoM and WAO, regions influenced by the Loop Current and Gulf Stream



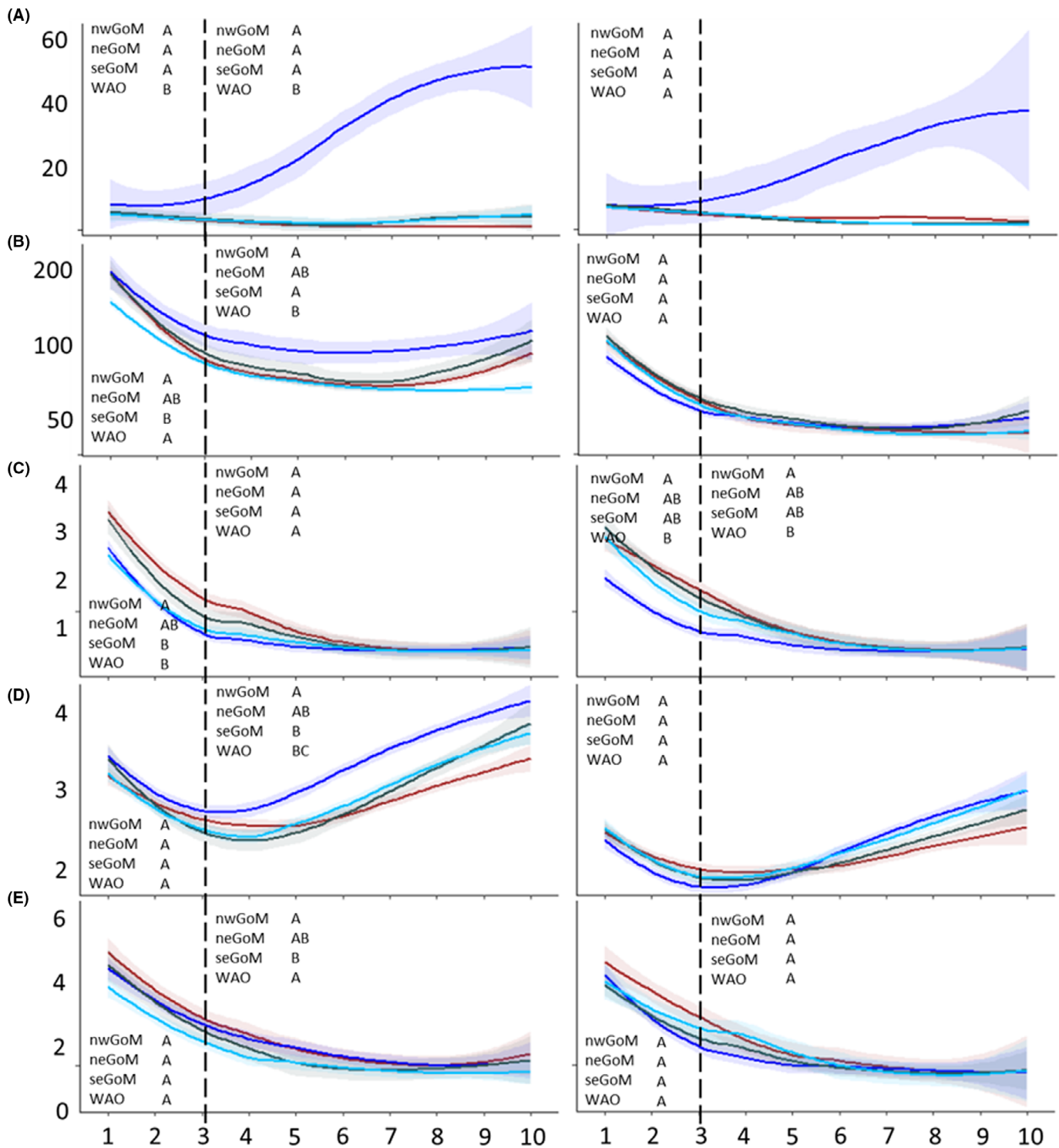


FIGURE 5. Mean element:Ca ratios (solid line) with two standard deviations (shaded region) for (A) Li:Ca ( $\mu\text{mol}/\text{mmol}$ ), (B) Mg:Ca ( $\mu\text{mol}/\text{mmol}$ ), (C) Mn:Ca ( $\mu\text{mol}/\text{mmol}$ ), (D) Sr:Ca ( $\text{mmol}/\text{mmol}$ ), and (E) Ba:Ca ( $\mu\text{mol}/\text{mmol}$ ) for the first 10 (approximate age 9) successive ablation spots up the otolith growth axis for Snowy Grouper collected in 2012–2013 (left column) and 2014–2015 (right column), grouped by collection region. The dashed line indicates the transition to deposition after age 0. Statistically significant regional groupings (Tukey's HSD:  $P < 0.05$ ) for otolith<sub>core</sub> and otolith<sub>edge</sub> are given on the left and right side of the dashed line, respectively, with edge groupings for Li:Ca, Mg:Ca, and Sr:Ca omitted as a result of a significant interaction term. Region abbreviations are as follows: WAO = western Atlantic Ocean, neGoM = northeast Gulf of Mexico, seGoM = southeast Gulf of Mexico, and nwGoM = northwest Gulf of Mexico.

and characterized by high salinity (Oey et al. 2005; Kemp et al. 2016), displayed elevated otolith Sr:Ca and  $\delta^{18}\text{O}$  relative to the two other regions in the GoM that are heavily influenced by freshwater inflow from the Mississippi and Atchafalaya River system. However, elevated Sr:Ca was also detected in Warsaw Grouper in the nwGoM (Sanchez et al. 2020), thereby suggesting factors other than water chemistry (e.g., diet, temperature, membrane transport regulation) are influencing Sr:Ca deposition rates (Campana 1999; Sturrock et al. 2012).

One noteworthy and unanticipated finding from this study that appears unrelated to factors described above was the pronounced increase in otolith Li:Ca observed for Snowy Grouper from the WAO. Otolith<sub>life</sub> Li:Ca for Snowy Grouper collected from 2012 to 2014 in the WAO displayed ratios that were nearly an order of magnitude higher than those collected from the three regions in the GoM. While Li typically follows a conservative profile in seawater, it is assumed to deposit in otoliths at equilibrium with environmental concentrations (Thresher 1999; Sturrock et al. 2012), thereby indicating that ambient waters at these commercial fishing locations in the WAO appear to be enriched in Li. Of particular note, elevated otolith<sub>life</sub> values from 2012 to 2014 were driven by an increase in Li:Ca after the first year of life, with otolith<sub>core</sub> ratios (<12  $\mu\text{mol}/\text{mmol}$ ) markedly lower compared with otolith<sub>edge</sub> ratios (35–68  $\mu\text{mol}/\text{mmol}$ ), potentially indicative of an ontogenetic migration to waters enriched in Li. Potential point source(s) for Li in the South Atlantic Bight and Middle Atlantic Bight are unclear, though the combination of detailed catch location and otolith chemical analysis could be used to highlight specific locations for further investigation.

Modest classification success to collection region using otolith<sub>life</sub> element:Ca signatures (up to 70%) for Snowy Grouper suggests that some degree of population structure (i.e., subpopulations) for juvenile fish occurs among the regions investigated. Given that classification success was highest for Snowy Grouper from the WAO, the most noticeable stock discontinuity (boundary) occurs around the Florida peninsula, separating what are possibly unique subpopulations in the GoM and WAO. Within the GoM, there may even be additional population structure as differences in otolith chemistry of Snowy Grouper among the three regions (nwGoM, neGoM, and seGoM) are consistent with differences in ambient physicochemical conditions (Ohlmann and Niiler 2005; Matli et al. 2018). Regional variation in otolith chemistry within the GoM was also reported for Warsaw Grouper, for which otolith element:Ca ratios from individuals collected in the neGoM were intermediate to the fish collected in the nwGoM and seGoM, similar to what was found for Snowy Grouper (Sanchez et al. 2020). This “intermediate signature” is likely a result of seasonal influences of both the Mississippi and Atchafalaya River

system and Loop Current in the region as the combination of a seasonal eastward flow of the Mississippi and Atchafalaya River system discharge and a seasonal northward intrusion of the Loop Current causes the two distinct water masses to mix in the region (Morey et al. 2003a, 2003b; Alvera-Azcárate et al. 2009). Furthermore, relative differences for otolith Mn:Ca and Sr:Ca in Snowy Grouper in the neGoM and seGoM is in accord with differences found in Gag *Mycteroperca microlepis*, with otolith Mn:Ca higher in the neGoM and otolith Sr:Ca higher in the seGoM (Hanson et al. 2004). Although classification success of Snowy Grouper from the neGoM and seGoM is relatively low, this is likely due to increased homogeneity of ambient chemistry in deep, offshore waters. Even while drawing a nearly identical geographic demarcation between the neGoM and seGoM for Snowy Grouper and Gag, spatial variability in ambient conditions in offshore deep water is much lower than nearshore coastal zones and therefore may be more difficult to detect (Cailliet et al. 2001; Campana 2001).

Classification success of Snowy Grouper to collection region using otolith<sub>core</sub> element:Ca signatures was lower than expected (38–59%), suggesting that physicochemical conditions experienced by individuals during the age-0 period are comparable (Campana 1999; Tanner et al. 2016). This may indicate that during the age-0 period, regardless of collection region, Snowy Grouper only inhabit a small geographic region as a result of few large transient spawning aggregations (Coleman et al. 2000; Brule et al. 2018). However, this similarity in environmental history during the early life stage may also indicate that effective juvenile habitats for Snowy Grouper are limited to locations with specific physicochemical conditions (Dahlgren et al. 2006). If variability in exposures to physicochemical conditions occurred for age-0 Snowy Grouper, it should be more noticeable during this life stage because physiological mechanisms that regulate the chemical deposition of otoliths are less developed during early life (Campana 1999).

These findings indicate the possible existence of distinct population contingents of young Snowy Grouper that experience different environmental exposures though the juvenile life stage. While classification success of Snowy Grouper to collection region using otolith element:Ca values was only modest for early life and recent growth periods, classification success using lifetime signatures was considerably higher indicating that long-term exposure to a specific region imparts a more reliable chemical imprint on the otolith of an individual’s geographic origin and/or environmental history. Physicochemical conditions in deepwater environments typically exhibit smaller fluctuations relative to coastal waters, potentially minimizing the effectiveness of otolith chemistry as a tool for assessing stock structure and population connectivity. However, long-term temporal stability of deepwater conditions (relative to shallow-water

counterparts) likely allows for the detection of more subtle differences in these chemical markers (Edmonds et al. 1991; Ashford et al. 2005; Sturrock et al. 2012). Regardless of the physicochemical drivers influencing the otolith chemistry of Snowy Grouper, individuals (mostly age 5 to age 10) from different geographic regions in this study exhibited distinct otolith chemical signatures, suggesting the presence of discrete juvenile/immature populations within the region investigated. While the study did not include older fish (approximately >age 10), subpopulations of Snowy Grouper with limited postsettlement connectivity of immature and recently mature fish supports the distinction between GoM and WAO management stocks. In addition, the potential low connectivity in the GoM combined with a fishery largely composed of small Snowy Grouper (~660 mm mean TL) could lead to an increased likelihood of overexploitation of vulnerable stock components.

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## REFERENCES

- Aguilar-Perera, A., B. Padovani-Ferreira, and A. A. Bertocchini. 2018. *Hyporthodus nigritus*. The International Union for Conservation of Nature's Red List of Threatened Species: e.T7860A46909320.
- Alvera-Azcárate, A., A. Barth, and R. H. Weisberg. 2009. The surface circulation of the Caribbean Sea and the Gulf of Mexico as inferred from satellite altimetry. *Journal of Physical Oceanography* 39:640–657.
- Andrews, A. H., B. K. Barnett, R. J. Allman, R. P. Moyer, and H. D. Trowbridge. 2013. Great longevity of Speckled Hind (*Epinephelus drummondhayi*), a deep-water grouper, with novel use of postbomb radiocarbon dating in the Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Sciences* 70:1131–1140.
- Ashford, J. R., C. M. Jones, E. Hofmann, I. Everson, C. Moreno, G. Duhamel, and R. Williams. 2005. Can otolith elemental signatures record capture site of Patagonian Toothfish (*Dissostichus eleginoides*), a fully marine fish in the Southern Ocean? *Canadian Journal of Fisheries and Aquatic Sciences* 62:2832–2840.
- Bastow, T. P., G. Jackson, and J. S. Edmonds. 2002. Elevated salinity and isotopic composition of fish otolith carbonate: stock delineation of Pink Snapper, *Pagrus auratus*, in Shark Bay, western Australia. *Marine Biology* 141:801–806.
- Bath, G. E., S. F. Thorrold, C. M. Jones, S. E. Campana, J. W. McLaren, and J. W. Lam. 2000. Strontium and barium uptake in aragonitic otoliths of marine fish. *Geochimica et Cosmochimica Acta* 64(10):1705–1714.
- Begg, G. A., K. D. Friedland, and J. B. Pearce. 1999. Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research* 43:1–8.
- Berkeley, S. A., M. A. Hixon, R. J. Larson, and M. S. Love. 2004. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. *Fisheries Management* 29(8):23–32.
- Bertoncini, A. A., B. Ferreira, and A. Aguilar-Perera. 2018. *Hyporthodus niveatus*. The International Union for Conservation of Nature's Red List of Threatened Species: e.T7861A46909546.
- Brule, T., T. Colas-Marrufo, E. Perez-Diaz, and C. Deniel. 2018. Biology, exploitation and management of groupers (Serranidae, Epinephelidae, Epinephelini) and snappers (Lutjanidae, Lutjaninae, *Lutjanus*) in the Gulf of Mexico. Pages 137–179 in K. Withers and M. Nipper, editors. *Environmental analysis of the Gulf of Mexico*. Harte Research Institute for Gulf of Mexico Studies, Special Publication Series Number 1, Corpus Christi, Texas.
- Cailliet, G. M., A. H. Andrews, E. J. Burton, D. L. Watters, D. E. Kline, and L. A. Ferry-Graham. 2001. Age determination and validation studies of marine fishes: do deep-dwellers live longer? *Experimental Gerontology* 36:739–764.
- Campana, S. E. 1999. Chemistry and composition of fish otoliths: pathways, mechanisms, and applications. *Marine Ecology Progress Series* 188:263–297.
- Campana, S. E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* 59:197–242.
- Coleman, F. C., C. C. Koenig, G. R. Huntsman, J. A. Musick, A. M. Eklund, J. C. McGovern, G. R. Sedberry, R. W. Chapman, and C. B. Grimes. 2000. Long-lived reef fishes: the grouper-snapper complex. *Fisheries* 25(3):14–21.
- Cook, M., G. R. Fitzhugh, and J. S. Franks. 2009. Validation of Yellowedge Grouper, *Epinephelus flavolimbatus*, age using nuclear bomb-produced radiocarbon. *Environmental Biology of Fishes* 86:461–472.
- Costa, P. A. S., A. C. Braga, J. P. Rubinich, A. O. Avila-Da-Silva, and C. M. Neto. 2012. Age and growth of the Snowy Grouper, *Epinephelus niveatus*, off the Brazilian coast. *Journal of the Marine Biological Association of the United Kingdom* 92:633–641.
- Dahlgren, C. P., G. T. Kellison, A. J. Adams, B. M. Gillanders, M. S. Kendall, C. S. Layman, J. A. Ley, I. Nagelkerken, and J. E. Serafy. 2006. Marine nurseries and effective juvenile habitats: concepts and applications. *Marine Ecology Progress Series* 312:291–295.
- Edmonds, J. S., N. Caputi, and M. Morita. 1991. Stock discrimination by trace-element analysis of otoliths of Orange Roughy (*Hoplostethus atlanticus*), a deep-water marine teleost. *Australian Journal of Marine and Freshwater Research* 42:383–389.
- Elsdon, R. S., B. K. Wells, S. E. Campana, B. M. Gillanders, C. M. Jones, K. E. Limburg, D. H. Secor, S. R. Thorrold, and B. D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: hypotheses, assumptions, limitations and inferences. *Oceanography and Marine Biology: An Annual Review* 46:297–330.
- GMFMC (Gulf of Mexico Fishery Management Council). 2018. Grouper-tilefish individual fishing quota program 5-year review. Available: <http://gulfcouncil.org/wp-content/uploads/B-9a-Grouper-Tilefish-Review-March-2018.pdf>. (February 2022.)

- Gula, J., M. J. Molemaker, and J. C. McWilliams. 2016. Submesoscale dynamics of a Gulf Stream frontal eddy in the South Atlantic Bight. *Journal of Physical Oceanography* 46:305–325.
- Hanson, P. J., C. C. Koenig, and V. S. Zdanowicz. 2004. Elemental composition of otoliths used to trace estuarine habitats of juvenile Gag *Mycteroperca microlepis* along the west coast of Florida. *Marine Ecology Progress Series* 267:253–265.
- Heppell, S. S., S. A. Heppell, F. C. Coleman, and C. C. Koenig. 2006. Models to compare management options for a protogynous fish. *Ecological Applications* 16:138–249.
- Hixon, M. A., D. W. Johnson, and S. M. Sogard. 2014. BOFFFF: on the importance of conserving old-growth age structure in fishery populations. ICES (International Council for the Exploration of the Sea) *Journal of Marine Science* 71:2171–2185.
- Kalish, J. M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *Journal of Experimental Marine Biology and Ecology* 132:151–178.
- Kalish, J. M. 1991.  $^{13}\text{C}$  and  $^{18}\text{O}$  isotopic disequilibria in fish otoliths: metabolic and kinetic effects. *Marine Ecology Progress Series* 75:191–203.
- Kemp, P. K., J. W. Day, A. Yañez-Arancibia, and N. S. Peyronnin. 2016. Can continental shelf river plumes in the northern and southern Gulf of Mexico promote ecological resilience in a time of climate change? *Water* 8:83.
- Kerr, L. A., D. H. Secor, and R. T. Kraus. 2007. Stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$ ) and Sr/Ca composition of otoliths as proxies for environmental salinity experienced by an estuarine fish. *Marine Ecology Progress Series* 349:245–253.
- King, J. R., and G. A. McFarlane. 2003. Marine fish life history strategies: applications to fishery management. *Fisheries Management Ecology* 10:249–264.
- Koslow, J. A., G. W. Boehlert, J. D. M. Gordon, R. L. Haedrich, P. Lorange, and N. Parin. 2000. Continental slope and deep-sea fisheries: implications for a fragile ecosystem. ICES (International Council for the Exploration of the Sea) *Journal of Marine Sciences* 57:548–557.
- Kritzer, J. P., and P. F. Sale. 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. *Fish and Fisheries* 5:131–140.
- Lee, T. N., L. P. Atkinson, and R. Legeckis. 1981. Observations of a Gulf Stream frontal eddy on the Georgia continental shelf, April 1977. *Deep Sea Research Part A. Oceanographic Research Papers* 28(4):347–378.
- Levins, R. 1969. Some demographic and genetic consequences of environmental heterogeneity for biological control. *Bulletin for the Entomological Society of America* 15:237–140.
- Limburg, K. E., C. Olson, Y. Walther, D. Dale, C. P. Slomp, and H. Hoie. 2011. Tracking Baltic hypoxia and cod migration over millennia with natural tags. *Proceedings of the National Academy of Sciences* 108:E177–E182.
- Limburg, K. E., B. D. Walther, Z. Lu, G. Jackman, J. A. Mohan, Y. Walther, A. Nissling, P. K. Weber, and A. K. Schmitt. 2015. In search of the dead zone: use of the otoliths for tracking fish exposure to hypoxia. *Journal of Marine Systems* 141:167–178.
- Longhurst, A. 2002. Murphy's law revisited: longevity as a factor in recruitment to fish populations. *Fisheries Research* 56:125–131.
- Matli, V. R. R., S. Fang, J. Guinness, N. N. Rabalais, J. K. Craig, and D. R. Obenour. 2018. Space-time geostatistical assessment of hypoxia in the northern Gulf of Mexico. *American Science and Technology* 52:12484–12493.
- Miller, J. A. 2009. The effects of temperature and water concentration on the otolith incorporation of barium and manganese in Black Rockfish *Sebastes melanops*. *Journal of Fish Biology* 75:39–60.
- Mohan, J. A., and B. D. Walther. 2014. Influence of constant and periodic experimental hypoxic stress on Atlantic Croaker otolith chemistry. *Aquatic Biology* 20:1–11.
- Morey, S. L., P. J. Martin, J. J. O'Brien, A. A. Wallcraft, and J. Zavala-Hidalgo. 2003a. Export pathways for river discharged fresh water in the northern Gulf of Mexico. *Journal of Geophysical Research* 108 (C10):3303.
- Morey, S. L., W. W. Schroeder, J. J. O'Brien, and J. Zavala-Hidalgo. 2003b. The annual cycle of riverine influence in the eastern Gulf of Mexico. *Geophysical Research Letters* 30(16):1867.
- Nelson, T. R., and S. P. Powers. 2019. Validation of species specific otolith chemistry and salinity relationships. *Environmental Biology of Fishes* 102:801–815.
- Newman, S. J., C. B. Wakefield, A. J. Williams, J. M. O'Malley, B. M. Taylor, S. J. Nicol, R. S. Nichols, S. A. Hesp, N. G. Hall, N. Hill, J. J. L. Ong, A. H. Andrews, C. M. Wellington, E. S. Harvey, P. Mous, Z. S. Oyafuso, C. Pardee, M. Bunce, J. D. DiBattista, and B. R. Moore. 2017. International workshop on advancing methods to overcome challenges associated with life history and stock assessments of data-poor deep-water snappers and groupers. *Marine Policy* 79:78–83.
- Oey, L. Y., T. Ezer, and H. C. Lee. 2005. Loop current, rings and related circulation in the Gulf of Mexico: a review of numerical models and future challenges. Pages 31–56 in W. Sturges and A. Lugo-Fernandez, editors. *Circulation in the Gulf of Mexico: observations and models*. American Geophysical Union, Volume 161, Washington, D.C.
- Ohlmann, J. C., and P. P. Niiler. 2005. Circulation over the continental shelf in the northern Gulf of Mexico. *Progress in Oceanography* 64:45–81.
- Padovani-Ferreira, B., A. A. Bertoni, and A. Aguilar-Perera. 2018. *Hyporhodus flavolimbatus*. The International Union for Conservation of Nature's Red List Threatened Species: e.T64400A46915591.
- Quetglas, A., L. Rueda, D. Alvarez-Berastegui, B. Guijarro, and E. Masuti. 2016. Contrasting responses to harvesting and environmental drivers of fast and slow life history species. *PLOS (Public Library of Science) ONE [online serial]* 11(2):e0148770.
- Rabalais, N. N., R. E. Turner, and W. J. Wiseman. 2002. Gulf of Mexico hypoxia, a.k.a. "The Dead Zone". *Annual Review of Ecological Systems* 33:235–263.
- Rooker, J. R., D. H. Secor, G. De Metro, R. Schloesser, B. A. Block, and J. D. Neilson. 2008. Natal homing and connectivity in Atlantic Bluefin Tuna populations. *Science* 322:742–744.
- Sanchez, P. J., J. Pinsky, and J. R. Rooker. 2019. Bomb radiocarbon age validation of Warsaw Grouper and Snowy Grouper. *Fisheries* 44:524–533.
- Sanchez, P. J., J. R. Rooker, M. Zapp Sluis, J. Pinsky, M. A. Dance, B. Falterman, R., and R. J. Allman. 2020. Application of otolith chemistry at multiple life history stages to assess population structure of Warsaw Grouper in the Gulf of Mexico. *Marine Ecology Progress Series* 651:111–123.
- Secor, D. H. 2007. The year-class phenomenon and the storage effect in marine fishes. *Journal of Sea Research* 57:91–103.
- Secor, D. H., and J. R. Rooker. 2000. Is otolith strontium a useful scalar of life cycles in estuarine fishes? *Fisheries Research* 46:359–371.
- Secor, D. H., J. R. Rooker, B. I. Gahagan, M. R. Siskey, and R. W. Wingate. 2015. Depressed resilience of Bluefin Tuna in the western Atlantic and age truncation. *Conservation Biology* 29:400–408.
- SEDAR (Southeast Data, Assessment, and Review). 2020. SEDAR 36 update. South Atlantic Snowy Grouper stock assessment report. SEDAR, North Charleston, South Carolina. Available: [https://safmc.net/download/BB%20Jan%20SSC%20Meeting/A01\\_SEDAR36U\\_SAR\\_November2020.pdf](https://safmc.net/download/BB%20Jan%20SSC%20Meeting/A01_SEDAR36U_SAR_November2020.pdf). (February 2022.)
- Smedbol, R. K., and J. S. Wroblewski. 2002. Metapopulation theory and northern cod population structure: interdependency of subpopulations in recovery of a groundfish population. *Fisheries Research* 55:161–174.
- Sosa-Cordero, E., and B. Russell. 2018. *Epinephelus drummondhayi*. The International Union for Conservation of Nature's Red List of Threatened Species: e.T7854A46909143.
- Sturrock, A. M., C. N. Trueman, A. M. Darnaude, and E. Hunter. 2012. Can otolith elemental chemistry retrospectively track migrations in fully marine fishes? *Journal of Fish Biology* 81:766–795.

- Tanner, S. E., P. Reis-Santos, and H. N. Cabral. 2016. Otolith chemistry in stock delineation: a brief overview, current challenges and future prospects. *Fisheries Research* 173:206–213.
- Thresher, R. E. 1999. Elemental composition of otoliths as a stock delin-  
eator in fishes. *Fisheries Research* 43:165–204.
- Walther, B. D., and S. R. Thorrold. 2006. Water, not food, contributes  
the majority of strontium and barium deposited in the otoliths of a  
marine fish. *Marine Ecology Progress Series* 311:125–130.
- Winemiller, K. O. 2005. Life history strategies, population regulation,  
and implications for fisheries management. *Canadian Journal of Fish-  
eries and Aquatic Sciences* 62:872–885.
- Winemiller, K. O., and K. A. Rose. 1992. Patterns of life-history diversi-  
fication in North American fishes: implications for population regula-  
tion. *Canadian Journal of Fisheries and Aquatic Sciences* 49:2196–  
2218.
- Wyanski, D. F., D. B. White, and C. A. Barans. 2000. Growth, population  
age structure, and aspects of the reproductive biology of Snowy Group-  
per, *Epinephelus niveatus*, off North Carolina and South Carolina. U.S.  
National Marine Fisheries Service Fishery Bulletin 98:199–218.
- Ying, Y., Y. Chen, L. Longshan, and G. Tianzian. 2011. Risks of ignor-  
ing fish population structure in fisheries management. *Canadian Jour-  
nal of Fisheries and Aquatic Sciences* 68:2101–2120.

## APPENDIX

TABLE A.1. Mean element : Ca ratios for each region, year, and life history stage. Abbreviations are as follows: WAO = western Atlantic Ocean, seGoM = southeast Gulf of Mexico, neGoM = northeast Gulf of Mexico, and nwGoM = northwest Gulf of Mexico.

Life stage	Year	Region	Element ratios				
			Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca
Life	2012	WAO	27.9	140.4	0.59	3,152	1.98
	2012	seGoM	6.5	105.1	0.55	2,962	1.81
	2012	neGoM	3.3	121.5	0.83	2,999	1.91
	2012	nwGoM	2.6	120.7	0.89	2,917	2.17
	2013	WAO	18.7	142.1	0.51	3,273	2.02
	2013	seGoM	2.7	101.3	0.49	2,959	1.39
	2013	neGoM	3.8	123.3	0.82	2,870	1.69
	2013	nwGoM	2.7	115.2	0.90	2,883	2.01
	2014	WAO	35.7	49.5	0.52	2,299	1.59
	2014	seGoM	4.0	49.8	0.85	2,324	1.34
	2014	neGoM	4.1	46.7	0.70	2,290	1.40
	2014	nwGoM	5.1	52.8	1.12	2,295	1.74
	2015	WAO	3.7	53.8	0.43	2,226	1.60
	2015	seGoM	4.3	51.2	0.62	2,381	1.69
	2015	neGoM	4.3	67.1	1.02	2,267	1.49
	2015	nwGoM	5.0	51.3	0.87	2,151	1.84
	Core	2012	WAO	7.4	165.3	1.45	2,972
2012		seGoM	4.8	145.8	1.50	2,917	3.62
2012		neGoM	5.3	169.7	1.99	3,023	3.45
2012		nwGoM	4.7	156.9	2.21	2,892	3.45
2013		WAO	7.8	174.7	1.26	3,074	3.41
2013		seGoM	4.4	141.0	1.26	2,831	2.50
2013		neGoM	5.0	160.3	1.98	2,812	2.84
2013		nwGoM	4.6	161.6	2.16	2,907	3.53
2014		WAO	12.0	93.1	1.42	2,265	2.90
2014		seGoM	6.1	94.4	2.23	2,223	2.39
2014		neGoM	7.0	84.0	1.75	2,219	2.58
2014		nwGoM	6.7	92.6	2.54	2,324	3.31
2015		WAO	5.4	81.9	1.00	2,080	2.76
2015		seGoM	6.6	92.1	1.42	2,288	2.98
2015		neGoM	6.7	107.9	2.34	2,230	2.64
2015		nwGoM	6.0	87.1	1.81	2,212	3.25

TABLE A.1. Continued.

Life stage	Year	Region	Element ratios				
			Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca
Edge	2012	WAO	50.5	146.9	0.07	3,607	1.02
	2012	seGoM	10.6	87.6	0.04	3,367	0.77
	2012	neGoM	2.0	98.6	0.09	3,374	1.06
	2012	nwGoM	3.8	113.8	0.12	3,137	1.10
	2013	WAO	34.8	132.0	0.06	3,728	1.03
	2013	seGoM	1.5	77.0	0.03	3,367	0.72
	2013	neGoM	2.9	116.5	0.08	3,250	0.86
	2013	nwGoM	1.9	95.8	0.10	3,146	1.17
	2014	WAO	68.4	29.5	0.03	2,716	0.77
	2014	seGoM	2.1	23.1	0.04	2,733	0.62
	2014	neGoM	2.5	24.6	0.06	2,728	0.72
	2014	nwGoM	4.2	32.5	0.05	2,597	0.70
	2015	WAO	2.4	34.8	0.03	2,718	0.78
	2015	seGoM	2.4	27.0	0.04	2,807	0.75
	2015	neGoM	2.5	53.3	0.05	2,638	0.69
	2015	nwGoM	5.2	27.8	0.12	2,316	0.87