AGE VALIDATION AND POPULATION STRUCTURE OF WARSAW GROUPER AND SNOWY GROUPER, AND AGE AND GROWTH RELATIONSHIPS OF WARSAW GROUPER IN THE GULF OF MEXICO

A Dissertation

by

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ABSTRACT

Warsaw grouper (*Hyporthodus nigritus*) and snowy grouper (*H. niveatus*) are deepwater grouper (F. Epinephelidae) found in the western Atlantic Ocean from the northern eastern seaboard of the United States of America to the central coast of Brazil, including in the Gulf of Mexico and Caribbean Sea. Considered periodic life history strategists, both species are long-lived, with slow growth and late maturation and therefore, are exceptionally vulnerable to overexploitation. Furthermore, both species are likely protogynous hermaphrodites (individuals first mature as females and after an environmentally dictated size or age transition to male) whose populations are sustained through episodic recruitment and infrequent year-class success when environmental conditions promote survival and settlement. However, little is known of their general life history due to the difficulties of common fisheries research methods for deepwater species due to barotrauma caused mortality associated preventing the use of techniques designed to directly estimate population demographics (e.g. abundance, density, age and growth, mortality, etc.), such as in-situ visual surveys or mark and recapture. Here, I attempt to clarify some population demographics using indirect estimation methods to assist in the development of effective fisheries policy. In order two accomplish these ends, the population structure of both species was assessed by analyzing and comparing the geochemical makeup of fish otoliths ("ear stones") collected from different geographical regions of the Gulf of Mexico (GOM). For both Warsaw grouper and snowy grouper, the chemical composition in otoliths resulted in region-specific signatures leading to the conclusion that individual movement between regions is minimal and therefore mixing limited, thereby suggesting the population is composed of distinct regional components and potential subpopulations. Before evaluating potential differences in population demographics from potentially distinct regions, a bomb radiocarbon age validation was conducted on both species. Typical of deepwater species, bomb radiocarbon age validation revealed an inherent underage bias for older fish in age determination methods, likely due to inability to distinguish annual growth increments in later ages as a result of depressed growth rates. These revelations led to a substantial increase in longevity estimates for both species, with both validated to have a minimum estimate of age-56. Using a newly validated age determination method and insights into population structure, age-length relationships were developed for Warsaw Grouper from multiple regions of the GoM. Growth relationships consistently resulted in a slower growth and lower natural mortality than current estimates, with subtle differences between the eastern GoM and western GoM. These results help to clarify population structure and demographics for two data-limited species subjected to commercial and recreational fishery exploitation that can be directly applied to fishery management and the development of effect conservation policy.

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TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

CHAPTER 1

INTRODUCTION

The Gulf of Mexico (GoM) commercial and recreational fisheries are important components of the fishing economy of the United States (U.S.). In 2016 the GoM was responsible for $912 million in commercial fisheries landings revenue from 1.75 billion pounds of landings, the 2nd and 3rd largest totals, respectively, of the 8 fisheries management regions in the country (NMFS 2017). Composed of five fishery management plans (FMPs), the Grouper-Tilefish Individual Fishing Quota (GT-IFQ) program is a major component of the commercial fisheries in the GoM. Of the five FMPs in the GT-IFQ (deep-water grouper, shallow-water grouper, gag grouper, red grouper, tilefish), the deep-water grouper (DWG) FMP accounts for the second-highest annual catch limits of the five. The DWG is composed of four species and has a combined sector (commercial and recreational) annual catch limit of 1.105 million lbs. Of these four species, the Warsaw grouper (*Hyporthodus nigritus*) and snowy grouper (*H. niveatus*) account for approximately 25% of the annual DWG catch by weight (GMFMC, 2018).

Warsaw grouper and snowy grouper are slow life history species that closely align with the periodic life history strategy of the Winemiller and Rose (1992) trilateral life history continuum, which makes them inherently vulnerable to exploitation pressure (Sadovy 1994, King & McFarlane 2005, Heyman 2014, Quetglas 2016). In particular, the selective removal of the largest and oldest individuals in the population, common in the recreational fishery, can lead to size and age truncation of the population thereby decreasing lifetime reproductive output of mature individuals (Longhurst 2002, Cooper et al. 2014, Secor et al. 2015). Furthermore, as populations that depend on episodic recruitment during years of favorable conditions, decreased survivorship to “match” years can greatly decrease the impact of successful year-classes (Cushing 1990, Berkeley et al. 2004, Secor 2007). As protogynous (sequential) hermaphrodites, selective removal of the largest individuals results in the disproportionate exploitation of males leading to highly skewed sex ratios further decreasing the spawning potential ratio (Heppell et al. 2006, Hixon et al. 2014).

Due to the increased vulnerability to exploitation pressures, accurate fishery parameters used in management policy are critical to ensure that catch limits do not lead to overexploitation and potential population collapse; however, the use of traditional fishery research techniques is limited for deepwater species such as Warsaw grouper and snowy grouper (Cailliet et al. 2001, Campana 2001). A lack of spatiotemporal variability of physicochemical conditions in deepwater environments decreases the direct influence of seasonal effects on water temperatures, decreasing the regularity of annual growth patterns (Beamish & McFarlane 1983). This in turn has led to inaccurate age determination for many deepwater species using traditional growth increment enumeration in otoliths, often resulting in age underestimation which has the potential to lead to unsustainable management policies (Caillet & Andrews 1998, Yule et al. 2008). Moreover, physical properties of deepwater environments, especially cold temperature, are more bioenergetically favorable for large individual size and increased longevity, and many deepwater reef-associated species have been validated to exhibit increased life-spans (Cailliet et al. 2001, Munk 2001). Natural mortality rates are typically low for long-lived slow-growth species, and consequently, consistent age underestimation for these populations has led to longevity and growth parameter estimates that overestimate sustainable yields (Francis et al. 2010). Within the DWG complex in the GoM, the interpretation of growth increments has been validated for two of the four species, speckled hind *Epinephelus drummondhayi* and yellowedge grouper *E.*

flavolimbatus, and for both age validation indicated fish were much older than previously considered (Cook et al. 2009, Andrews et al 2013). Therefore, it is critical that the interpretation of annual growth increments is validated for Warsaw grouper and snowy grouper populations to ensure the development of accurate age-length relationships and natural mortality estimates.

In addition to increased complexity in age determination, the application of conventional techniques to assess population structure are not effective for deepwater species due to barotrauma related release mortality and therefore require the analysis of natural tags to assess potential connectivity between regions. The decrease in ambient pressure between the surface and common depths Warsaw grouper and snowy grouper are caught (50-400 m) has led to a 100% mortality rate estimate for individuals released without descending devices (SEDAR 2013a, Schertzer et al. 2018). While these mortality rates and be decreased through the proper use of authorized descending devices, the survival rate is only 50% at best and only at the shallower depth range of the species (Runde & Buckel 2018, Runde et al. 2020). On the other hand, the analysis of natural tags in the otolith microstructure has the potential to elucidate population structure while avoiding the complications of release mortality on deepwater species (Kalish 1989, Campana 1999, Tanner et al. 2016). Though heterogeneity of physicochemical conditions in deep water masses minimizes the magnitude of spatial variability in ambient chemistry across regions relative to coastal waters, subtle differences in otolith chemistry among distinct population components is detectable (Edmunds et al. 1991, Sturrock et al 2012). Therefore, comparisons of otolith chemistry from fish collected from discrete regions of the GoM could provide insights into Warsaw grouper and snowy grouper population structure.

Estimates of population demographic parameters of Warsaw grouper (Manooch & Mason 1987) and snowy grouper (Matheson & Huntsman 1984, Wyanski et al. 2000, Costa et al. 2001) have produced mixed results and indicate that basic data on the age determination method, population structure, and life history of both species is critical to reduce their “unknown” stock status in the GoM. Listed as “near threatened” (Warsaw grouper) and “vulnerable” (snowy grouper) by the International Union for the Conservation of Nature Red List of threatened species (Aguilar-Perera et al. 2018, Bertoncini et al. 2018), additional insights are needed to provide the most accurate information to policy makers to ensure the development of sustainability fishery policy and the conservation of these species into the future.

Here, this dissertation attempts to fill in these data gaps through the investigation of the following research questions in the subsequent chapters.

Chapter II: How precise are current age determination methods (enumerating annuli) of Warsaw grouper and snowy grouper and are current longevity estimates (maximum age) accurate?

Chapter III: What is the population structure for Warsaw grouper in the GoM and does a mismatch of this population structure and the current single-stock management policy increase vulnerability of the population to exploitation?

Chapter IV: What is the population structure for snowy grouper in the GoM and western Atlantic Ocean, and are there any changes to the current management stock structure that could improve fishery and conservation policies?

Chapter V: What is the current age-length relationship for Warsaw grouper in the GoM and have dissimilar population demographics developed in different geographic regions? Specifically, are growth curves and growth parameters different from potentially discrete subpopulations as informed by chapter III. In addition, potential changes in the size and age composition of the fishery between the early 2000s and early 2010s is assessed to address changes in the fishery over time.

CHAPTER II

BOMB RADIOCARBON AGE VALIDATION OF WARSAW GROUPER AND SNOWY GROUPER*

Introduction

Age structure is an integral component in the development of fish stock assessments used to evaluate population status and inform management policy. Age data are commonly coupled with length or weight information to estimate growth rates, and age-specific data are used to determine the timing of sexual maturation, mortality rates, and catch limits (Ricker 1975, Gulland 1987, Pauly & Morgan 1987). Assessments depend on accurate population age structures; therefore, the validation of age determination techniques is critical (Beamish & McFarlane 1983, Campana 2001). The most common method of age determination for marine teleosts involves counting growth increments deposited in the otolith ("ear stone"); however, increments in the otolith microstructure may not be deposited annually, and enumerating the presence and location of annual growth increments (annuli) often requires subjective interpretation (Melvin & Campana 2010, Buckmeier 2011). Even in cases where estimated ages from multiple readers are similar, incorrect interpretation of annuli has led to incorrect age determination (Rivard & Foy 1987). This is particularly true for long-lived marine fishes that exhibit extremely slow growth at older ages, which can result in closely spaced, difficult-to-interpret growth increments (Cailliet & Andrews 2008). As a result, validating the accuracy of methods used for age determination is especially important in long-lived, slow-growth species (Campana 2001, Munk 2001).

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Global atmospheric atomic weapons tests conducted in the 1940s and 1950s led to a proliferation of the radiocarbon isotope (14C; hereafter, radiocarbon) in the atmosphere that spread through both atmospheric and oceanic circulation (Broecker et al. 1985, Druffel 1992). Increased environmental radiocarbon isotope concentrations resulted in increased radiocarbon deposition in biogenic carbonate structures (e.g., coral skeletons, otoliths, shells, etc.), functioning as a natural tag that can be used to accurately estimate the age of marine fish (Kalish 1993, Campana 2001). This "modern" radiocarbon chronology offers a method to validate ages for fish from cohorts with year–classes during and after this increase in oceanic radiocarbon concentrations. The hatch (birth) year of an individual is estimated by comparing radiocarbon concentrations in otolith cores (i.e., first year of life) with the concentrations in a biogenic carbonate reference series, such as the skeletons of hermatypic corals (Campana 2001).

In the Gulf of Mexico and Caribbean basin, radiocarbon concentrations rose dramatically from pre-bomb levels (Δ14C < −50‰) beginning in the late 1950s, peaked in the early to mid-1970s (Δ14C = 120–160‰; see review by Druffel 1992), and have since undergone a slow decline of approximately −27‰ Δ14C per decade (Moyer & Grottoli 2011). This radiocarbon chronology is consistent across multiple hermatypic coral reference series from the western Caribbean Sea and Gulf of Mexico: Belize (Druffel 1980), Flower Garden Banks (Wagner et al. 2009), Florida Keys (Druffel 1989), and Puerto Rico (Moyer & Grottoli 2011). In addition, more recent work on fish otoliths shows that the radiocarbon decline rate has remained constant into the early 2000s (Cook et al. 2009, Andrews et al. 2013, Barnett et al. 2018).

Large groupers (*F. Epinephelidae*) share life history strategies that make them vulnerable to overfishing; most are long-lived, slow growing, late to mature, and sequential hermaphrodites (Sadovy 1994, Coleman et al. 1999, Heyman 2014). Recent age validation studies have shown that some deepwater epinephelids are much older than previously estimated via counting annuli (Cook et al. 2009, Andrews et al. 2013), suggesting the potential for increased longevity in species with similar life histories. As such, there is a clear need to validate the ages of additional deepwater groupers, particularly those with a "vulnerable" conservation status.

The Warsaw Grouper *Hyporthodus nigritus* and Snowy Grouper *H. niveatus* are key components of the deepwater grouper fishery in the Gulf of Mexico (Runde & Buckel 2018, Schertzer et al. 2018). They are currently listed by the International Union for Conservation of Nature as "near threatened" and "vulnerable" species, respectively (Aguilar-Perera et al. 2018, Bertoncini et al. 2018). Given that age-specific life history traits influence stock assessments, an improved understanding of the age structure and longevity of both species is needed to develop conservation strategies based on accurate population demographics to ensure healthy, exploitable stocks in the future. Here, we apply the bomb radiocarbon approach to validate annual growth increment formation for Warsaw Grouper and Snowy Grouper, which will have broad implications for future population assessments and rebuilding plans for both species.

Methods

Sample Preparation and Bomb Radiocarbon Analysis

Archived Warsaw Grouper and Snowy Grouper sagittal otoliths were obtained from the Southeast Fisheries Science Center Panama City Laboratory (National Oceanic and Atmospheric Administration [NOAA] Fisheries). All archived samples from NOAA Fisheries were collected in the Gulf of Mexico and stored in paper envelopes. Additional otoliths of both species were also obtained from port sampling in Galveston, Texas, to expand sample sizes in the northwestern Gulf of Mexico. Otoliths were cleaned with double-deionized water (DDI-H2O;

ultrapure, 18-MΩ/cm water), allowed to air dry, weighed to the nearest 0.1 mg, and embedded in Struers epoxy resin following an established protocol (Rooker et al. 2008). Embedded otoliths were sectioned at 1.5-mm thickness on a transverse plane using a Buehler Isomet saw and were mounted onto a petrographic glass slide with Crystalbond 509 thermoplastic glue. Otolith thin sections were polished until the core was clearly visible without surpassing 1-mm thickness.

Otoliths were selected for bomb radiocarbon analysis based on an individual's back-calculated hatch year, with the intent of selecting fish from cohorts produced in the zone of rapid radiocarbon increase (1960 to early 1970s). Each otolith was aged by two independent readers counting annuli on the transverse cross section. The mean of the two reads was reported as the age, and the average percent error (APE) between reads was calculated to ensure that variability between readers was within acceptable limits. Measurements from the primordium to the edge of the age- 1 opaque zone (viewed with transmitted light) of young individuals (age-1 and age-2) delineated the area of the otolith corresponding to the age-0 period (i.e., first year of life; hereafter, "otolith core"; Supplemental Figure S1). Otolith cores of both Warsaw Grouper and Snowy Grouper were extracted for radiocarbon analysis to estimate deposition year and therefore the hatch year of each fish. In addition to isolating core material, transects outside otolith cores along specific growth increments were also sampled (Supplemental Figure S2). from Warsaw Grouper (n = 2) and Snowy Grouper (n = 1) with estimated hatch years during or before the period of radiocarbon rise. This approach allowed us to obtain otolith material that corresponded to additional years within the desired period of rapidly increasing radiocarbon and inspect changes in radiocarbon concentrations associated with increased fish age.

Otolith material was removed using a New Wave Research Micromill with a 300-µm-diameter drill bit (Figure 1). Drill depth per pass was 55 µm, and total depth sampled for each otolith was approximately 775 µm. Extracted otolith material was weighed to the nearest 0.1 mg and stored in 0.6-mL centrifuge vials packed in 2-mL, sealed Whirl-pak bags. Centrifuge vials were sterilized in a 10% HNO3 bath for a minimum of 24 h, triple rinsed with DDI-H2O, and air dried under a clean hood before core extraction. All radiocarbon analyses were performed at the National Ocean Sciences Accelerated Mass Spectrometry Lab (Woods Hole Oceanographic Institute). Results are reported in Δ14C values, representing the per mille deviation from the 14C activity in 19th-century wood corrected for isotopic fractionation.

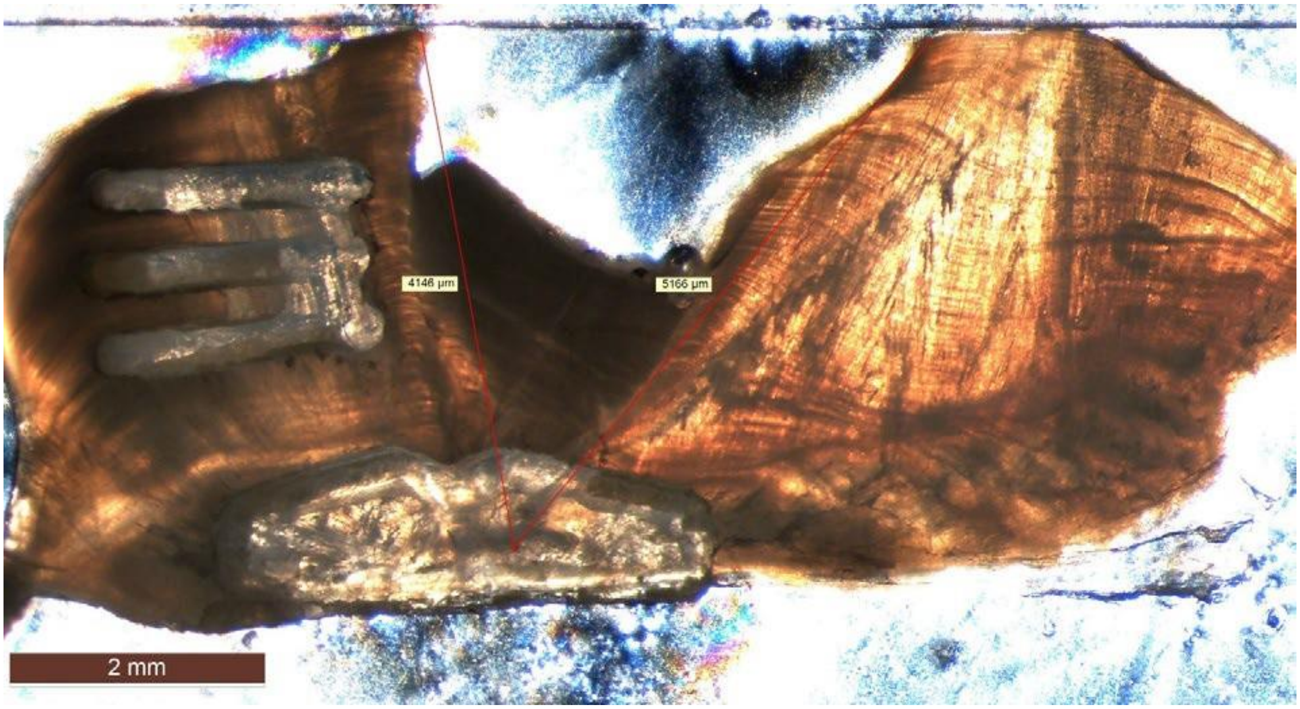

Figure 1. Otolith core and growth increment extraction location and mean sulcus height measurements. Growth increment samples were powdered, 300-µm-wide transects. For the core extraction, only the center portion was removed, and all material within 300 µm of the drill perimeter was powdered and discarded. Sulcus height was calculated as the average of the two measurements (4,146 and 5,166 µm) from the core to the dorsal and ventral sulcal groove processes. The otolith is from Warsaw Grouper sample WRG19.

Data Analysis

Warsaw Grouper and Snowy Grouper Δ14C values were visually compared to a spline model (RStudio, package "mgcv") developed from reference radiocarbon chronologies for hermatypic corals between 10- and 20-m depth from the Flower Garden Banks National Marine Sanctuary (Wagner et al. 2009) and the Florida Keys (Druffel 1989) and for two fish species, the Speckled Hind *Epinephelus drummondhayi* (Andrews et al. 2013) and Red Snapper *Lutjanus campechanus* (Barnett et al. 2018). The two coral radiocarbon chronologies were chosen based on their geographic proximity to our study area; the fish chronologies were selected to extend the reference series into the present. An age bias analysis was run on Snowy Grouper ages with hatch years during the radiocarbon rise through a quantitative comparison with the Flower Garden Banks reference radiocarbon chronology. Following the method described by Francis et al. (2010), a 95% confidence interval was constructed to calculate an age bias in Snowy Grouper age determination. An age bias analysis was not possible for Warsaw Grouper due to an insufficient number of samples with determined back-calculated hatch years during the radiocarbon rise and peak. Since the two coral reference radiocarbon chronologies do not extend far enough into the present to overlap temporally, otolith core Δ14C values for Warsaw Grouper with hatch years after 1978 (radiocarbon peak) were compared to the established post-peak radiocarbon chronologies reported for Speckled Hind and Red Snapper. An analysis of covariance (ANCOVA) was conducted to compare the slopes of the three linear regressions. Speckled Hind data were removed for a second ANCOVA, since the difference in their estimated deposition dates caused the continuous variable "year" to be confounded with the factor "species," preventing an intercept test. The second ANCOVA compared the slopes and intercepts between Warsaw Grouper and Red Snapper only (RStudio, package "nlme").

A mean sulcus height metric was calculated for both Warsaw Grouper and Snowy Grouper by taking the average of two measurements: (1) primordium to the dorsal process of the sulcal groove and (2) primordium to the ventral process of the sulcal groove (Figure 1). A mean of the two measurements acted to remove individual measurement variation due to the curve of the sulcus as a result of non-uniform growth and deviation in the angle of the otolith thin section cut. Linear regressions were developed to test the relation- ships of mean sulcus height to age and otolith mass to age to assess the value of these proxies for estimating ages of the two species.

Results

Warsaw Grouper

We selected 20 Warsaw Grouper (915–2,010 mm TL) collected in the years 2011–2016 for bomb radiocarbon age validation (Table 1). Age estimates from counting annuli on the otolith microstructure ranged from 9 to 59 years, with a total APE of 9.6% between the two reads. Otolith core Δ14C values of Warsaw Grouper as a function of hatch year (based on age determination from otolith microstructure analysis) were generally similar to the reference radiocarbon chronology for the Gulf of Mexico (Figure 2A), supporting the age estimates. Although overall patterns between the Gulf of Mexico reference radiocarbon chronology and Warsaw Grouper values were comparable, the otolith core Δ14C values were visibly lower than reference values, including the two fish with pre-bomb hatch years. The two individuals with the oldest determined ages (55 and 59 years) had pre-bomb Δ14C values of −70.6‰ and −68.6‰, which are lower than mean coral Δ14C values during the decade immediately preceding the postbomb rise (1949–1958) for both the Gulf of Mexico (−51.2‰) and Florida Keys (−57.6‰) reference chronologies. Otolith core and transect Δ14C values for Warsaw Grouper at the peak

Table 1. List of all otolith core samples in the study and their analysis values. Ages and year–classes with an asterisk are Snowy Grouper collected from 2011 to 2016, with age estimates derived from the following otolith mass–age equation ($R^2 = 0.74$): Age = −4.6 + (42.5 × Otolith Mass).

Fish ID	Catch year	TL (mm)	Sulcus height (μm)	Otolith mass (g)	Age estimate	Year-class estimate	$\Delta^{14}C$ (‰)	$\Delta^{14}C$ error
Warsaw Grouper								
WRG01	2015	1,064	2,650	0.9477	34	1981	130.42	2.1
WRG02	2014	1,287	2,095	0.5112	12	2002	57.49	2.4
WRG04	2016	1,275	2,283	0.6632	17	1999	62.86	2.4
WRG05	2014	1,252	1,862	0.6604	9	2005	47.44	3.2
WRG06	2015	1,219	1,925	0.5129	13	2002	51.46	2.5
WRG08	2016	1,283	1,855	0.4942	12	2004	58.99	2.4
WRG09	2014	1,341	2,033	0.6527	10	2004	50.98	3.3
WRG10	2016	1,222	2,108	0.6910	11	2005	59.37	2.3
WRG11	2012	2,010	1,954	0.5289	11	2001	67.59	2.7
WRG12	2016	1,525	2,339	0.7794	16	2000	66.06	2.1
WRG13	2012	1,755	3,326	NA	41	1971	108.88	2.1
WRG14	2014	1,702	2,597	0.9323	19	1995	76.46	2.5
WRG15	2011	1,810	4,214	NA	55	1956	−70.61	2.2
WRG16	2011	1,501	1,755	0.7115	13	1998	72.20	2.3
WRG17	2012	1,471	2,016	0.6939	16	1996	75.20	2.3
WRG18	2014	NA	3,524	1.2179	39	1975	115.22	2.3
WRG19	2016	1,790	4,656	1.5871	59	1957	−68.60	1.8
Snowy Grouper								
SNG02	1982	740	1,685	NA	11	1971	139.32	2.3
SNG03	1982	763	1,946	0.4401	14	1968	131.69	2.5
SNG04	1982	765	1,840	0.5179	15	1967	101.26	2.2
SNG05	1982	715	1,564	0.3837	11	1971	113.80	2.6
SNG06	1982	724	1,857	0.5216	16	1966	92.97	2.2
SNG07	1982	769	1,803	0.4548	17	1965	82.02	2.9
SNG08	1982	790	1,908	0.6044	21	1961	−31.99	1.9
SNG09	1982	788	1,824	0.4728	16	1966	143.43	2.3
SNG10	1982	769	1,942	0.5023	17	1965	42.26	2.3
SNG11	1982	747	2,020	0.5541	21	1961	−54.13	2.0
SNG12	2011	1,191	3,645	1.9541	78*	1933*	−63.87	1.9
SNG13	2015	1,121	3,558	1.8472	73*	1942*	−63.33	3.2
SNG14	2016	1,108	3,914	2.1191	85*	1931*	−65.48	1.8
SNG15	2013	1,218	3,798	1.4966	59*	1955*	−64.49	1.9
SNG16	2015	1,193	3,895	2.1025	84*	1933*	−63.45	2.1
SNG17	2015	1,132	2,877	1.2607	49*	1964*	114.52	2.3
SNG18	2016	1,162	4,192	1.9540	78*	1938*	−67.67	1.8

of the radiocarbon rise in the 1970s ranged from 101.2‰ to 130.4‰. Otolith core and transect Δ14C values near the end of the chronology in the 1990s and 2000s ranged between 76.5‰ and 39.8‰. The observed rate of decline from the peak in the 1970s corresponds to Δ14C values observed in the otolith cores of the postbomb chronologies developed for Red Snapper and Speckled Hind (ANCOVA slope test: F = 0.35, df = 2, P = 0.70; Figure 3). No difference in the rate of decline for Δ14C values between Warsaw Grouper and Red Snapper was detected (ANCOVA slope test: F = 0.54, df = 1, P = 0.47) but the magnitude of Warsaw Grouper Δ14C values was significantly lower (ANCOVA intercept test: F = 51.40, df = 1, P < 0.001).

Snowy Grouper

We selected 18 Snowy Grouper (330–1,218 mm TL) for bomb radiocarbon age validation, with 11 collected in 1982 and 7 collected from 2011 to 2016 (Table 1). Age estimates from counting annuli on the otolith microstructure ranged from 2 to 52 years, with a total APE of 6.0% between the two reads. Otolith core Δ14C values of Snowy Grouper as a function of hatch year were generally similar to the coral radiocarbon chronologies in the Gulf of Mexico for individuals with age estimates less than 25 years (Figure 2B). The nine Snowy Grouper collected in 1982 with back-calculated hatch years during the radiocarbon rise were selected for the age bias analysis. The 95% confidence interval for the age bias analysis (−10.5%, 1.7%) supported the conclusion that no significant age bias existed (Figure 4). Otolith core Δ14C values of the six largest Snowy Grouper, with ages derived from otolith microstructure analysis between 34 and 52 years, ranged from −63.33‰ to −67.67‰, confirming hatch years that predated the radiocarbon rise (pre-1960). Therefore, all six had validated ages of at least 51 years, with two at least 56 years (collected in 2016).

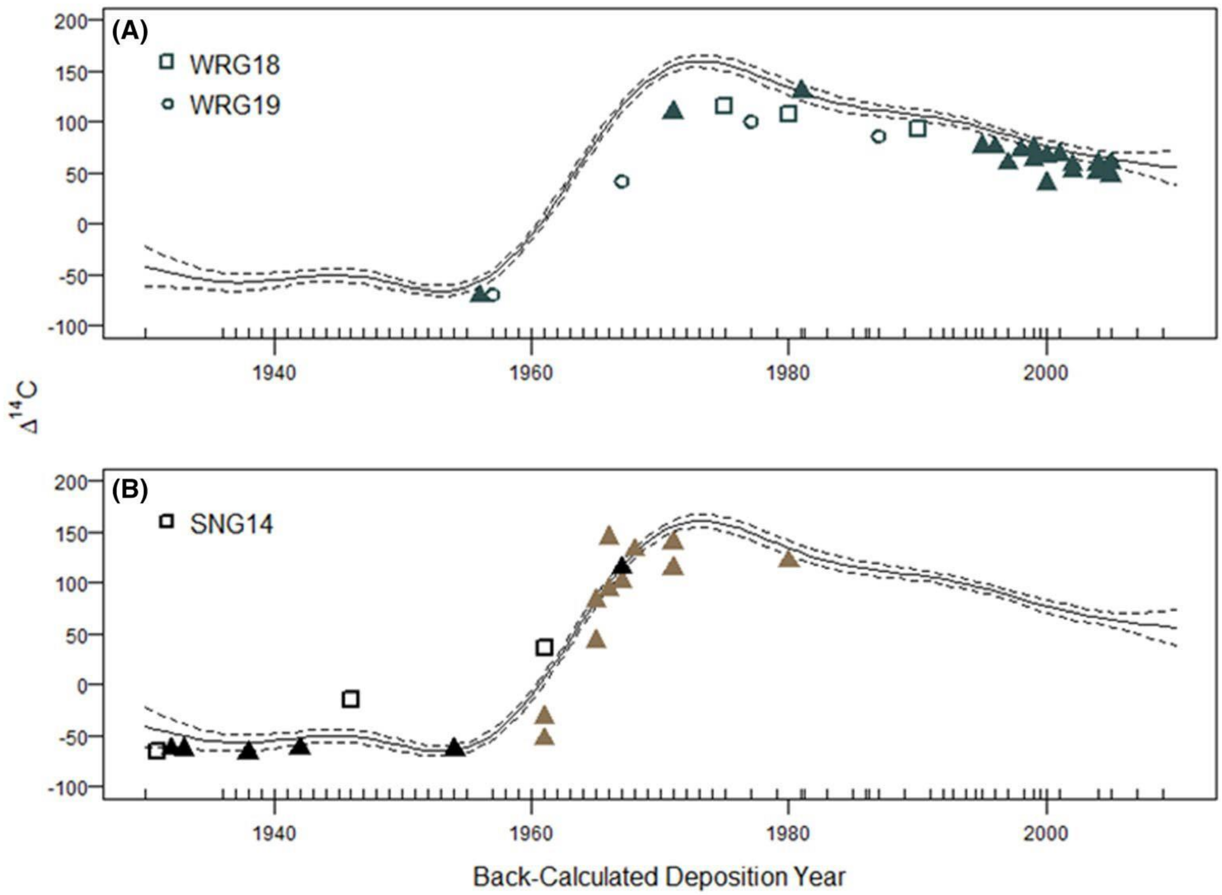

Figure 2. Radiocarbon values for core (solid triangles) and growth increment (hollow squares/circles) analyses of (A) Warsaw Grouper and (B) Snowy Grouper. Blue and gold symbol back-calculated deposition year is a function of conventional age determination. Black symbols denote Snowy Grouper with back-calculated deposition year as a function of otolith weight–age estimation. Unique hollow symbols refer to individual fish (Warsaw Grouper WRG18 and WRG19; Snowy Grouper SNG14). Smoothed reference line was developed from a combination of published radiocarbon chronologies from the Gulf of Mexico: Flower Gar- den Banks corals (Wagner et al. 2009), southern Florida corals (Druffel 1989), Speckled Hind (Andrews et al. 2013), and Red Snapper (Barnett et al. 2018).

Two individuals collected in 2015, with initial estimates of 34 and 37 years, had minimum validated ages of 55 years—much older than the microstructure analysis estimates. The seventh of the 2011–2016 Snowy Grouper was collected in 2015 and had an annular age

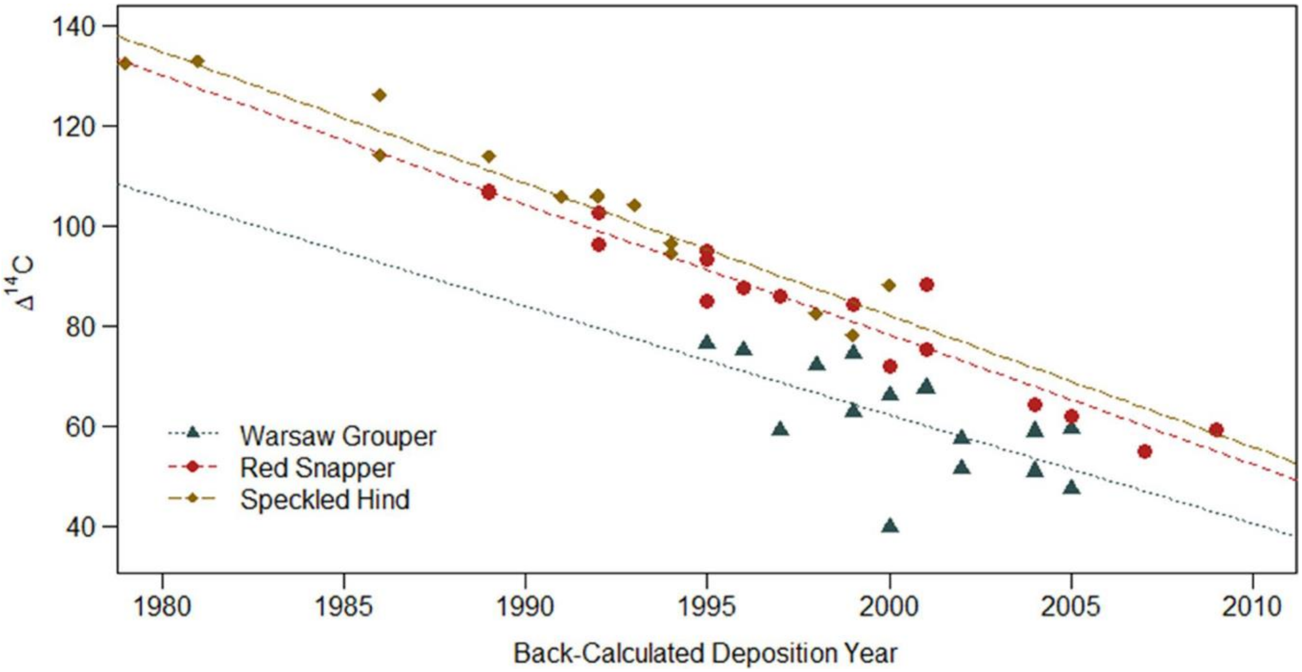

Figure 3. Post-peak radiocarbon decline trends for Warsaw Grouper, Speckled Hind (Andrews et al. 2013), and Red Snapper (Barnett et al. 2018) from 1978 through the early 2000s. Rate of decline was not significantly different among the three species (analysis of covariance [ANCOVA] slope test: *F* = 0.35, df = 2, *P* = 0.70); however, Warsaw Grouper had lower-magnitude values than Red Snapper (ANCOVA intercept test: *F* = 51.4, df = 1, *P* < 0.001).

estimate of 25 years; this individual had an otolith core Δ14C value (114.42‰) approaching the peak values for the reference series. Therefore, its hatch year could be assigned to either before or after the peak of the radiocarbon rise (years 1969 versus 1989), correlated with a radiocarbon age of either approximately 26 or 46 years, respectively. Although the 26-year radiocarbon age estimate is similar to the microstructure analysis estimate, the large otolith mass (1.26 g) and fish TL (1,131 mm) indicate that this Snowy Grouper was much older. Combined with the extreme age underestimation of the six largest Snowy Grouper, to which its otolith weight and TL were much closer, this individual was likely closer to 46 years old than to 26. Otolith core and transect Δ14C values for Snowy Grouper during the radiocarbon rise and peak from 1960 to 1980 ranged from −32.0‰ to 143.4‰.

Otolith Morphometrics

Bomb radiocarbon samples were composed of a large range of otolith masses for both Warsaw Grouper (0.49–1.59 g) and Snowy Grouper (0.44–2.11 g). Otolith mass was a good predictor of age for validated Warsaw Grouper (R2 = 0.88, df = 16, P < 0.001) and Snowy Grouper (R2 = 0.74, df = 7, P < 0.010; Figure 5A). The Snowy Grouper otolith mass–age equation (Age = −4.56 + [42.46 × Otolith Mass]) was used to estimate ages for the seven fish collected between 2011 and 2016. Using the otolith mass–age equation, these seven Snowy Grouper had predicted ages between 49 and 85 years (Table 2) and back-calculated hatch dates that correlated with their radiocarbon results (Figure 6). Age–mean sulcus height linear relationships for fish with validated ages were significant for both species and indicated that the metric is a useful proxy for approximating age of adult Warsaw Grouper (R2 = 0.93, df = 18, P < 0.001) and Snowy Grouper (R2 = 0.55, df = 9, P < 0.01; Figure 5B). For Snowy Grouper, this relationship was strengthened considerably (R2 = 0.96, df = 15, P < 0.001) when adding the six samples with pre-bomb hatch years and with ages derived from the otolith mass–age equation above. It is important to note that linear relationships described above were disproportionately influenced by the oldest individuals of each species, which extended the range of years included and increased the amount of natural variability explained. Otolith weight-derived ages should be considered estimates and not validated ages.

Discussion

Use of the postbomb radiocarbon chronology is a well-established tool to validate age (see review by Campana 2001). Where reference chronologies are available, the bomb radiocarbon age validation technique can be applied to any biogenic carbonate with an estimated deposition date. As a result, bomb radiocarbon age validations have been used for freshwater (Campana et al. 2008, Bruch et al. 2009, Davis- Foust et al. 2009), estuarine (Campana and Jones 1998), and marine megafauna, including toothed whales (Stewart et al. 2006), sharks (Kneebone et al. 2008, Hamady et al. 2014), and a myriad of bony fishes (Andrews et al. 2007, Treble et al. 2008). This method has proven especially useful for hard-to-age fishes that do not experience regular seasonal environmental variation, such as mesophotic species. The application of this

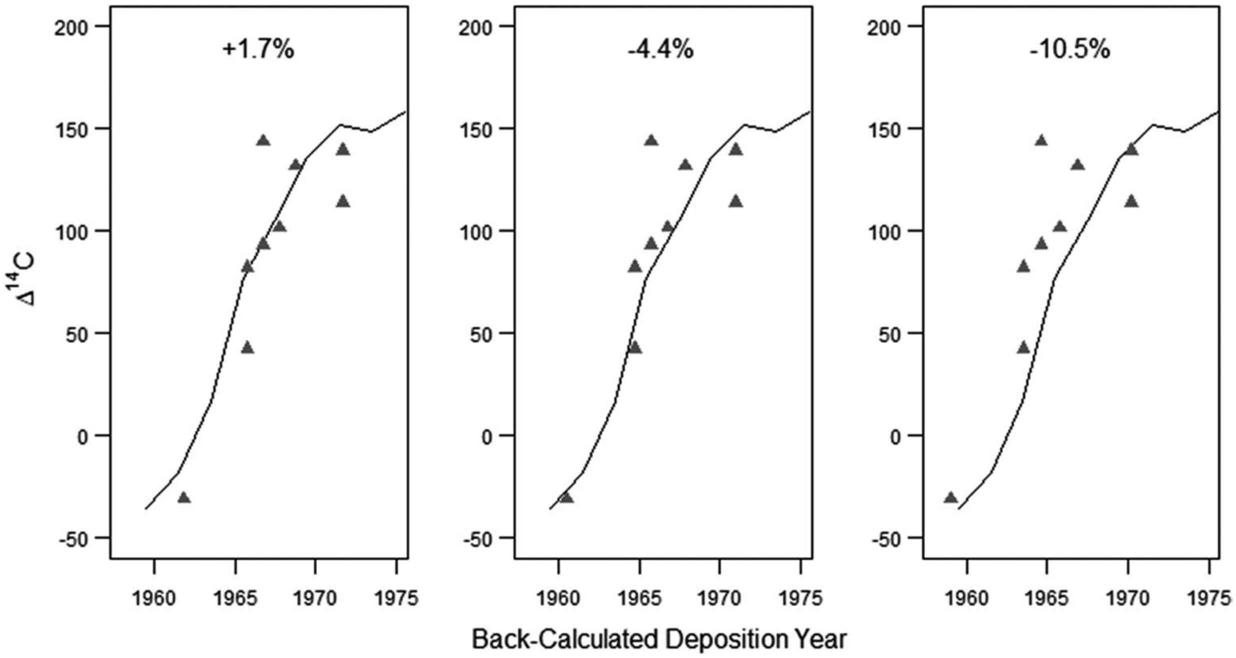

Figure 4. Age bias plots for Snowy Grouper with hatch years during the postbomb radiocarbon rise (1960–1975). Gold triangles are replotted ages assuming an age bias percent of + 1.7, −4.4, and −10.5%, which correspond to the mean percent bias and 95% confidence interval around the mean.

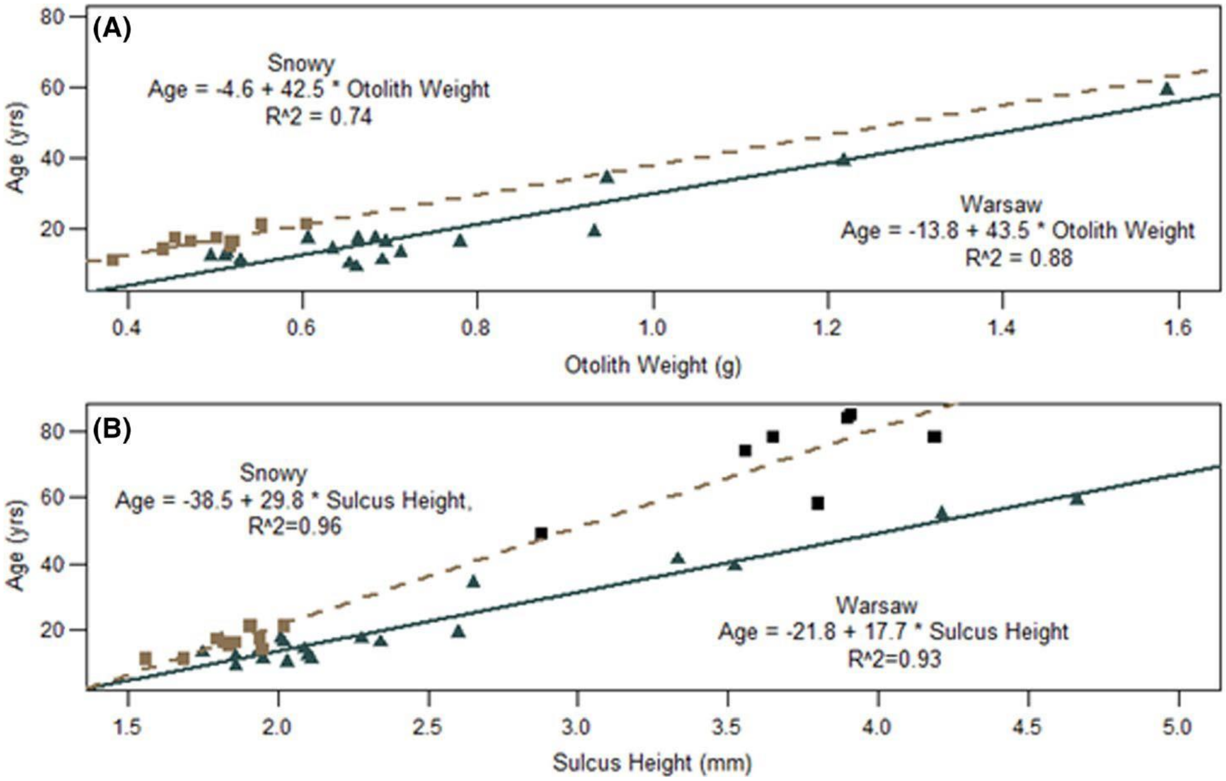

Figure 5. (A) Otolith weight–age linear regressions and (B) sulcus height–age linear regressions for Warsaw Grouper and Snowy Grouper. Gold squares are validated Snowy Grouper collected in 1982 with annulus count age estimates; black squares are Snowy Grouper collected in 2011–2016 with otolith-weight- derived age estimates.

promising validation technique often leads to greater longevity estimates (Cailliet and Andrews 2008), as was seen here for both Warsaw Grouper and Snowy Grouper.

Bomb radiocarbon age validation supports annulus formation in the otolith microstructure of all Warsaw Grouper and medium-sized Snowy Grouper (715–790 mm TL) but indicated that ages of larger Snowy Grouper (1,108–1,218 mm TL) were greatly underestimated. Bomb radiocarbon evidence supports an age estimate of 59 years for the largest Warsaw Grouper in this study, increasing the current longevity by at least 18 years (Manooch & Mason 1987). This increased longevity reflects recent bomb radiocarbon age validation results for other deepwater fish species (Cailliet et al. 2001, Horn et al. 2012). Radiocarbon values for medium-sized Snowy

Grouper closely matched the hermatypic coral radiocarbon chronology for the Gulf of Mexico (Wagner et al. 2009), with no bias in reader ages, suggesting that annuli are discernable up to at least 25 years. However, otolith radiocarbon values of larger Snowy Grouper indicated that the fish were considerably older than expected, which was due in part to difficulties in identifying annuli farther up the growth axis. More conspicuous annuli were present for Warsaw Grouper from the primordium to the margin of the otolith along the sulcal groove, and this appears to explain the difference in age estimate accuracy between the species. Initially, the low APE and reasonable maximum age from two readers led to confidence that age estimates for the largest Snowy Grouper were accurate; however, the youngest of the seven large individuals was given a validated age of 49 years, markedly higher than the annulus age estimate of 25 years. In fact, the six largest Snowy Grouper had minimum validated ages between 51 and 56 years based on collection years, with many exceeding the oldest age estimate determined by counting annuli in this study (52 years). Even with pre-bomb Δ14C values and therefore an inability to calculate a precise radiocarbon age, the minimum validated age of 56 years increases the current longevity

Table 2. Estimated ages for the seven Snowy Grouper that were collected from 2011 to 2016. Ages were estimated using the otolith mass–age equation developed in this study ($R^2 = 0.74$): Age = −4.6 + (42.5 × Otolith Mass). The 85-year age estimate is 29 years older than the 56-year mini- mum longevity validated in this study.

Sample ID	TL (mm)	Otolith mass (g)	Annulus age (years)	Minimum longevity (years)	Otolith mass-estimated age (years)
SNG12	1,191	1.9541	34	51	78
SNG13	1,121	1.8472	34	55	73
SNG14	1,108	2.1191	52	56	85
SNG15	1,218	1.4966	38	53	59
SNG16	1,193	2.1025	37	55	84
SNG17	1,132	1.2607	25	NA	49
SNG18	1,162	1.9540	45	56	78

estimate for Snowy Grouper (Costa et al. 2011) and greatly exceeds the maximum age used in the last stock assessment (SEDAR 2013a).

Otolith core radiocarbon values in both Warsaw Grouper and Snowy Grouper were observed to be lower than the radiocarbon values in the reference chronologies. The comparison of fishes with post-radiocarbon-peak hatch years suggested that age-0 Warsaw Grouper settle deeper than Speckled Hind and Red Snapper or migrate to deep water during their first year of life. In the Gulf of Mexico and western Atlantic Ocean, radiocarbon concentrations decrease with increasing depth (Broecker et al. 1985, Hansman et al. 2009), with measurable changes between surface waters and the mesopelagic zone (Stuiver & Ostlund 1980). Furthermore, radiocarbon analyses from otolith deposition farther up the growth axis did not show an additional decrease in radiocarbon values relative to the reference chronologies, which would be expected with an ontogenetic depth migration (Cook et al. 2009). Although there have been observations of newly settled individuals for both species on the northeastern Gulf of Mexico continental shelf, the sightings are rare (Heemstra & Randall 1993, Dance et al. 2011). Moreover, young juveniles are commonly caught at depths below 50 m (Wyanski et al. 2000, Schertzer et al. 2018). Reduced radiocarbon values for both Warsaw Grouper and Snowy Grouper relative to the reference chronologies could be indicative of age determination bias, but it is important to note that otolith radiocarbon values from pre-bomb fish were also consistently lower than pre-bomb radiocarbon values from the reference chronologies.

Strong linear relationships between age and both mean sulcus height and otolith weight measurements suggest that each represents a useful proxy for estimating adult Warsaw Grouper and Snowy Grouper ages. Sulcus height (Steward et al. 2009, Williams et al. 2015) and otolith weight (Pawson 1990, Pilling et al. 2003, Pino et al. 2004) have been previously reported to

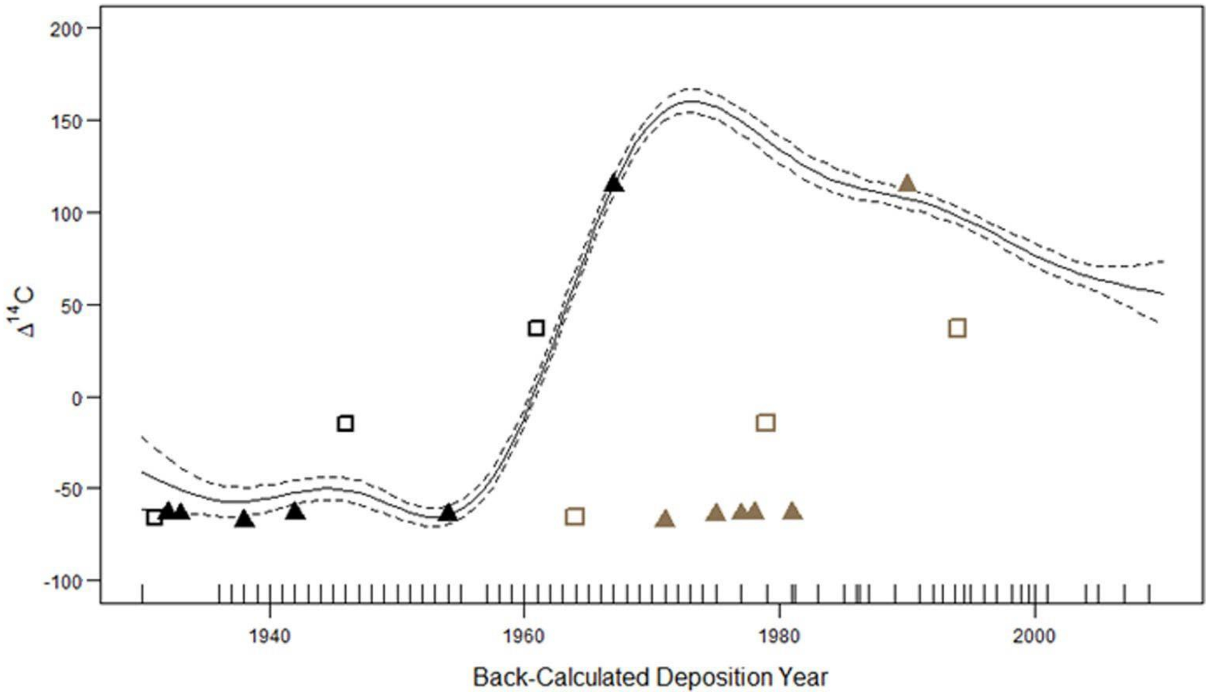

Figure 6. Plotted radiocarbon values for Snowy Grouper collected in 2011–2016, showing the shift from back-calculated hatch years based on ages derived from conventional age estimation techniques (gold triangles/squares) and ages derived from the otolith weight–age equation (black triangles/squares). Hollow squares represent the core, 15-year, and 30-year growth incre- ment samples for Snowy Grouper sample SNG14.

correlate with fish age in other species. Using the relationships developed here for Warsaw Grouper and Snowy Grouper with validated ages, we approximated the age of larger individuals with hatch years that predated the radiocarbon rise. Based on otolith masses, age estimates for the six largest Snowy Grouper ranged from 59 to 85 years, indicating that longevity may be considerably greater than previously estimated (Wyanski et al. 2000, Costa et al. 2011, SEDAR 2013a). While the predicted age of the largest Warsaw Grouper in this study was 59 years, larger individuals with greater otolith masses than any samples analyzed in our study have been collected. In fact, a 179-kg individual recently caught in Louisiana had a 2.56-g otolith mass that was 66% heavier than the otolith mass from the 59-year-old fish (1.59 g) included in our sample. This suggests that Warsaw Grouper longevity could approach the greater than 80-year longevity estimates that have been reported previously for other large, deepwater groupers (Cook et al. 2009, Andrews et al. 2013).

This bomb radiocarbon age validation extends the current documented longevities for both Warsaw Grouper and Snowy Grouper, bringing into question the current population models for both species in the Gulf of Mexico. Underestimations of longevity in aged based population models result in high estimates of natural mortality and low estimates of survivor- ship for the older age classes (Hoenig 1983, Yule et al. 2008). It can also lead to decreased estimates of the reproductive contribution for individuals that may live to spawn more years than previously expected (Secor 2000). Current stock assessments for both species indicate decreasing trends in abundances due to overfishing, with very little known about the conservation status of populations in the Gulf of Mexico (Aguilar-Perera et al. 2018, Bertoncini et al. 2018). Increased longevities for Warsaw Grouper and Snowy Grouper could act as a buffer against sustained fishery pressure if a segment of the population survives to older ages (Secor 2000), serving to increase the opportunities for successful recruitment in years when larvae or new settlers experience favorable environmental conditions (Cushing 1990). However, sustained fishery pressure targeting large individuals may lead to age truncation in a population, potentially offsetting the resilience associated with increased longevity for slow-growth species (Longhurst 2002, Secor et al. 2014). Here, we applied a holistic aging approach to advance our understanding of life history attributes shared by Warsaw Grouper and Snowy Grouper to theorize how exploitation may be affecting populations in the Gulf of Mexico. For long-lived, slow-growing species that likely experience episodic recruitment success, it is essential to consider conservation policies that stress the importance of older age-classes.

CHAPTER III

APPLICATION OF OTOLITH CHEMISTRY AT MULTIPLE LIFE HISTORY STAGES TO ASSESS POPULATION STRUCTURE OF WARSAW GROUPER IN THE GULF OF MEXICO*

Introduction

A variety of techniques are used to assess the movement and stock structure of marine fishes (see review by Cowen et al. 2007), including genetic markers (Ruzzante et al. 2000, Beltrán et al. 2017), conventional and electronic tagging (Merten et al. 2016, Rooker et al. 2019), and natural tracers in fish hard parts (e.g. otoliths, spines, vertebrae) (Rooker et al. 2010, Wells et al. 2010, Kitchens et al. 2018). Natural tracers in otoliths (ear stones) are increasingly applied to retrospectively determine the origin and movement of fishes as the relationship between otolith chemistry and water chemistry becomes clearer and analytical precision improves (Tanner et al. 2016). This approach has been effectively utilized to determine the natal origin of an individual (Zapp Sluis et al. 2012, Rooker et al. 2014) and the timing of ontogenetic habitat shifts (Thorrold et al. 1998, Gillanders 2005, Elsdon et al. 2008) by targeting distinct regions along the otolith growth axis. Moreover, quantifying chemical composition across the entire growth axis of the otolith (core to edge) serves as an integrated signature of all water masses occupied during the lifetime of an individual (Campana 1999, Thresher 1999), and therefore, has effectively been used to assess the connectivity, or lack thereof, of individuals from geographic regions with dissimilar water chemistries (Wells et al. 2015, Pita et al. 2016). While otolith chemistry has

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become a common tool for assessing the population connectivity of estuarine, nearshore, and oceanic fishes (Sturrock et al. 2012, Pita et al. 2016), comparable studies on species that occupy demersal habitats on the outer continental shelf or slope where physicochemical conditions of water masses are less pronounced are surprisingly limited (Elsdon et al. 2008), although a recent in- crease in studies around the Southern Ocean has proven the use of its application (Ashford et al. 2005, 2012, Zhu et al. 2018).

Warsaw grouper *Hyporthodus nigritus* (Epinephelidae) are a species of concern in the western Atlantic Ocean due to harvest pressure from recreational and commercial fisheries (Farmer & Karnauskas 2013, Aguilar-Perera et al. 2018, Shertzer et al. 2018). These fish are periodic strategists within the Winemiller & Rose (1992) trilateral continuum (high fecundity, late maturity, and long lived) that spawn in the spring and summer along the outer continental shelf (Smith 1971, Manooch & Mason 1987, Parker & Mays 1998, Sanchez et al. 2019). Therefore, Warsaw grouper are particularly susceptible to steady, long-term perturbations such as fishing exploitation, especially in age-truncated populations (Gerber & Kendall 2016, Quetglas et al. 2016). Warsaw grouper are economically important in the Gulf of Mexico (GoM) as the third largest component of the commercial deepwater grouper fishery and are targeted trophy fish by recreational anglers (GMFMC 2018). As the second largest grouper species in the western Atlantic Ocean (> 230 cm total length) and with an elevated trophic status (i.e. apex predator), Warsaw grouper exert top- down regulation and thus play an important ecological role in structuring deepwater communities (Shurin et al. 2002).

Throughout their range, Warsaw grouper are demersal and typically occur in deep, hard-bottom habitats below 50 m depth (Mejía-Ladino et al. 2003, Bender et al. 2013, Farmer & Karnauskas 2013). The patchy and limited distribution of these habitats across the GoM likely restricts the movement of individuals, potentially causing geographic isolation and the development of sub-populations with different demographic traits (Koenig & Coleman 2013). Nevertheless, Warsaw grouper in the GoM are currently managed as a single stock even though their population structure is presently unknown (Aguilar-Perera et al. 2018). This unknown status complicates their conservation because region- or sub-population- specific demographics are required for the management of species displaying a metapopulation structure (Policansky & Magnuson 1998, Begg et al. 1999). A single-stock management approach for marginally connected sub-populations can overestimate the population-wide maximum sustainable yield and therefore lead to unsustainable exploitation rates (Coleman et al. 2000, Longhurst 2002), highlighting the importance of an accurate population structure designation to develop effective conservation policy for threatened fish species (Thorrold et al. 2001).

Here, we applied natural chemical markers in otoliths of Warsaw grouper to investigate their population structure in the GoM, an important marginal sea in the center of their range. Two classes of chemical markers — trace elements (Li, Mg, Mn, Co, Cu, Zn, Sr, Ba) and stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) — were quantified from 4 regions of the GoM (Texas, Louisiana, Alabama−NW Florida, and SW Florida), and region-specific signatures were evaluated at 3 different life history stages of the otolith to assess early life, most recent growth, and lifetime variability in these markers. We predicted that limited movement of Warsaw grouper among regions and/or the presence of sub-populations with unique environmental histories would result in strong region-specific trends in otolith chemistry at all life history stages.

Methods

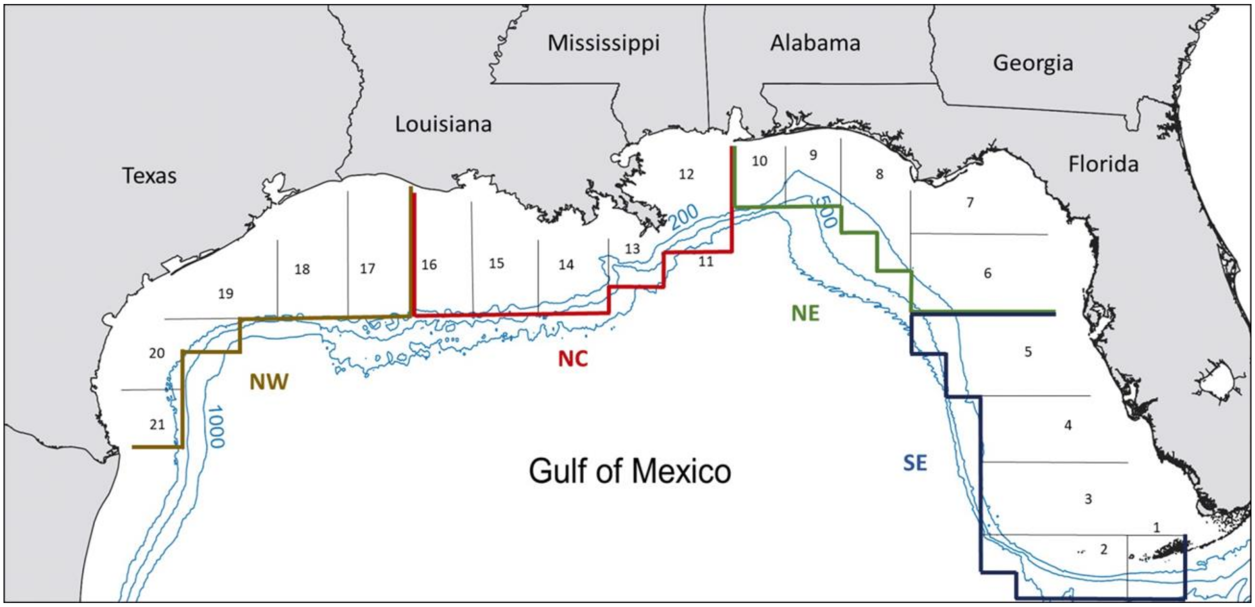


Figure 7. Gulf of Mexico commercial shrimp zones with the 4-region groups labeled; SE (Southeast, Zones 1−5), NE (Northeast, 6−10), NC (Northcentral, 11−16), NW (Northwest, 17−19). No samples were collected in shrimp Zones 1, 11, 12, 20, or 21. The 200, 500, and 1000 m depth contours are shown in blue.

Sample Collection and Age Determination

Archived otoliths from Warsaw grouper were acquired from the National Oceanic and Atmospheric Administration (NOAA) Fisheries' Southeast Regional Office in Panama City, Florida. Otoliths were collected by NOAA commercial fishery observers on benthic longline and handline fishing vessels. Analyzed specimens were restricted to a maximum total length (TL) of 100 cm and collection years 2011−2015 to minimize the confounding effects of ontogenetic and temporal variability on elemental composition of otoliths. Every available archived otolith that fit these criteria was selected and processed for this study. While depth data were not available for most gear sets, depth of set for the GoM deepwater grouper fishery typically ranges between 100 and 300 m. Samples were grouped into 4 regions determined by the reported commercial GoM shrimp zone catch location: Southeast (SE: Zones 1−5; Florida Keys to Tampa Bay, Florida), Northeast (NE: Zones 6−10; Tampa Bay, Florida, to Mobile Bay, Alabama), Northcentral (NC: Zones 11−16; Mobile Bay, Alabama, to central Louisiana), and Northwest (NW: Zones 17−21; western Louisiana and Texas) (Fig. 7). Our regional sampling design was based on expected differences in ambient water chemistry resulting from the geographic positions of Mississippi and Atchafalaya River System inflow and the Loop Current in the GoM.

Otoliths were cleaned of biological material with Teflon-coated forceps and double-deionized water (DDIH20; ultrapure, 18 MΩ cm−1 water), allowed to air dry, weighed to the nearest 0.1 mg, and embedded in Struers epoxy resin following Rooker et al. (2008). Embedded otoliths were then sectioned along the transverse plane at 1.5 mm thickness using a Buehler ISOMET saw to expose the primordium and growth increments. Otolith thin sections were mounted onto petrographic slides with Crystalbond 509 thermoplastic glue and polished on one side until the center of the primordium was visible while attempting not to reduce thickness below 1 mm. Two independent readers determined fish ages by counting annual growth increments on the otolith micro-structure, a method previously validated for Warsaw grouper (Sanchez et al. 2019).

Trace elements

Trace element composition of Warsaw grouper otoliths was assessed using an ultraviolet New Wave Research laser ablation (LA) system (NWR213) coupled with a quadrupole Thermo Scientific XSeries 2 inductively coupled plasma mass spectrometer (ICP-MS) located at Texas A&M University (Galveston Campus). Multiple otolith thin sections were mounted onto single petrographic slides, with slides presented randomly to the machine. A series of 70 μm diameter spots from the core to the margin were ablated on each otolith, with a spacing of 150 µm between centroids of successive spots (ca. 80 µm non-quantified area between edges of consecutive ablation spots). Organization of ablation spots targeted 3 distinct regions of the otolith thin section: (1) core (young-of-the year [YOY] period), (2) edge (recent growth period), and (3) lifetime (complete growth axis from core to edge) (Fig. 8A). The core consisted of 5 spots arranged in an M-shape contained within the opaque primordium with a central point at the base of sulcal groove. Two points, one on each side, followed the respective dorsal and ventral grooves up 150 µm, while 2 additional points were placed approximately 150 µm lateral (along the fast growth axis) of the central point. Lifetime samples included all points along the growth axis from the core to the edge of the otolith on the dorsal side of the sulcal groove. Edge

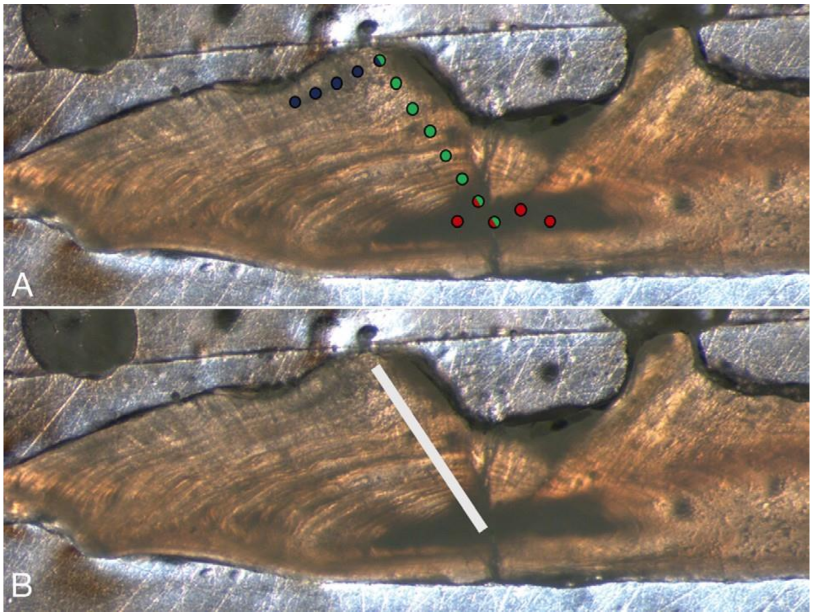


Figure 8. Example of (A) trace element sample groupings and (B) stable isotope sample transect on the Warsaw grouper otolith thin section. Red circles: core (first year) ablation points; green circles: lifetime transect ablation points; black circles: edge (recent growth period) ablation points. Points with 2 designations (half/half) were ablated once but included in both corresponding signatures. The stable isotopes transect was milled with a 300 µm drill bit, and all material was pooled into a single powdered sample per otolith.

samples (i.e. recently accreted material) consisted of 5 points positioned laterally along the distal edge of the otolith.

National Institute of Standards and Technology (NIST) 612 carbonate standards, with ablation set- tings identical to the otolith samples, were analyzed to calibrate trace element concentrations from counts per second. To assess potential drift within the ICP- MS, 1 NIST 612 standard was ablated after the 10th sample ablation spot for each individual otolith, and a cycle of 1 MACS and 1 NIST 612 standard was run after each otolith. All regions of otoliths and carbonate standards assayed were first pre-ablated at 60% laser output and 10 Hz intensity for a 1 s dwell time to remove any potential contamination from the surface of the otolith. Spots were then ablated for trace element analysis at 50% laser output, 10 Hz intensity, 30 s laser warmup (blank run), and 30 s dwell time (sample run). Trace element concentrations in parts per billion (ppb) were measured for 7Lithium (Li), ^{24}Magnesium (Mg), ^{43}Calcium (Ca), ^{55}Manganese (Mn), ^{59}Cobalt (Co), ^{65}Copper (Cu), ^{66}Zinc (Zn), ^{88}Strontium (Sr), and ^{137}Barium (Ba). Concentrations were then converted to element:Ca (μmol mmol–1) ratios using the formula:

(1) $X{:}Ca = (ppb_X \times 0.001)/Z_X)/(0.38/43)$

where X is the element analyzed, ppb_X its measured concentration, Z_X its atomic mass, and the denominator (0.38/43) is the standard parts per million concentration (0.38) and atomic mass (43) of ^{43}Calcium in aragonitic calcium carbonate.

Stable isotopes

A subset of the otoliths analyzed for trace elements was selected for δ18O and δ13C analysis. To develop a more balanced sample design for evaluating both classes of chemical markers, selection included all otoliths from the SE (n = 19), NE (n = 10), and NW (n = 15) regions not removed in the post-processing of life- time results, and 19 randomly selected otoliths from the NC. Selected otolith thin sections from LA-ICP- MS runs were lightly polished until ablation spots were no longer visible, removing the upper layer of material where trace element analysis was conducted. A precision micromill (New Wave Research Mi- croMill) was used to collect otolith material along a transect running from the core to the distal edge along the growth axis (Fig. 8B). The transect line was ca. 300 μm wide, and 14 passes at 55 μm depth were performed resulting in a sample depth of 770 μm. We assumed that otolith powder collected from the transect line for δ^{18}O and δ^{13}C analysis corresponded to the lifetime sample described above for trace elements. Otolith δ^{18}O and δ^{13}C analysis 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), NBS- 19 and NBS-18, with 6 standards run for every 40 samples; precision was ± 0.08 ‰ (SD) and ± 0.11 ‰ for δ18O and δ13C, respectively. Otolith δ^{18}O and δ^{13}C values are reported relative to the Vienna Pee Dee Belemnite (VPDB) scale after comparison to an in- house laboratory standard calibrated to VPDB.

Data analysis

Before analysis, data were post-processed to re move outliers. Element:Ca values were pooled by region, and all samples with values ± 5 SD of the mean for each individual marker were considered outliers and removed from any subsequent analysis. Post-processing was conducted for each life history stage independently and therefore resulted in different sample sizes for each due to differences in the number of otoliths considered outliers and removed. Multivariate analysis of variance (MANOVA) and ANOVA were used to determine whether chemical signatures (multiple markers) or individual markers, respectively, differed among geographic regions, with year incorporated as an interactive factor ($p = 0.05$) (RStudio, package 'nlme'). Tukey's highly significant difference (HSD) tests with a 'Shaffer' corrected alpha ($p = 0.05$) were run on interaction models (Region × Year) to analyze region-specific differences for individual markers with significant ANOVA results. Quadratic discriminant function analysis (QDFA) was used to assess regional classification of Warsaw grouper based on trace element signatures from the otolith core and edge using a standard set of elements identified as influential ($p = 0.10$) from ANOVA tests (SYSTAT 13). Jackknifed cross-validated classification success was determined for models with 4-region and 2-region groupings with prior probabilities based on region-specific sample size to account for unbalanced sample sizes across regions. The 2-region grouping was created by combining the NW, NC, and NE GoM regions due their close proximity to the Mississippi River inflow. MANOVA and ANOVA again were used to assess regional differences in chemical signatures (trace elements and stable isotopes) and individual markers. For lifetime signatures, classification success (QDFA) was determined using models based on combined marker classes (both trace elements and stable isotopes) and individual marker classes (trace elements only or stable isotopes only) to determine the value of each for classifying Warsaw grouper to the different geographic regions.

Results

Otoliths from 129 Warsaw grouper from the NOAA Fisheries' archives that fit selection criteria were available for geochemical analysis. For all samples, TL was 810 ± 40 mm (mean ±

Table 3. Sample size (n), mean age, and mean total length (TL), of all Warsaw grouper in the study (Total) and by Gulf of Mexico region. NW: northwest; NC: north central; SE: southeast; NE: northeast; APE: average percent error

Region	n	Mean age (yr)	SD age (yr)	APE (%)	Mean TL (mm)	SD TL
Total	129	4.4	1.2	6.9	810	68
NW	18	4.9	1.6	7.6	836	69
NC	79	4.5	1.2	5.4	820	67
SE	22	3.8	0.7	5.4	777	53
NE	10	3.6	0.4	7.6	783	55

SD), and the mean determined age was 4.4 ± 1.2 yr with an average percent error of 7.0% (0.6 yr) between 2 readers (Table 3). Five elements (Li, Mg, Mn, Sr, and Ba) in the otoliths were consistently above detection limits and were used for statistical testing; Co, Cu, and Zn were often below detection limits and removed from further analyses. After data post-processing, 3 and 8 otoliths were removed from the core ($n = 126$) and edge ($n = 121$) analyses, respectively.

Chemical signatures in the otolith$_{\text{core}}$ (YOY period) of Warsaw grouper varied significantly among the 4 regions (MANOVA, $p < 0.001$) (Table 4). Univariate contrasts indicated that otolith$_{\text{core}}$ Mn:Ca, Sr:Ca, and Ba:Ca ratios were significantly different (ANOVA, $p < 0.05$) between GoM regions. Otolith$_{\text{core}}$ Mn:Ca, Sr:Ca, and Ba:Ca values were higher in the NC region (Mn:Ca = 3.09 ± 1.32; Sr:Ca = 2715 ± 304; Ba:Ca = 5.03 ± 2.04) than the SE region (Mn:Ca =1.12 ± 0.38; Sr:Ca = 2527 ± 274; Ba:Ca = 3.45 ± 1.94) (Fig. 9A−C). Otolith$_{\text{core}}$ element:Ca ratios for individuals from the NW (Mn:Ca = 2.55 ± 0.91; Sr:Ca = 2451 ± 271; Ba:Ca = 4.19 ± 2.09) and NE regions (Mn:Ca = 2.26 ± 1.03; Sr:Ca = 2579 ± 390; Ba:Ca = 5.07 ± 3.60) were intermediate between the NC and SE, with the exception of Sr:Ca in the NW (Table A1 in the Appendix). No regional differences in otolith cores were observed for otolith$_{\text{core}}$ Mg:Ca or Li:Ca ratios (ANOVA, $p > 0.05$). Overall jackknifed classification success from

Table 4. Multivariate analysis of variance (MANOVA) degrees of freedom (numerator, denominator), F-values, and p-values (Pillai's trace) of 4-region (Northwest [NW], Northcentral [NC], Northeast [NE], and Southeast [SE]) and 2-region (NW/NC/NE and SE) groupings of Warsaw grouper otoliths in the Gulf of Mexico. The only interactive effect between Region and Year was for edge (recent growth period) samples in the 4-region analysis.

Otolith sample		Region			Year			Region × Year		
		df	F	p	df	F	p	df	F	p
4-Region	Core	15, 321	4.5	< 0.001	20, 432	2.7	< 0.001	55, 545	1.0	> 0.05
	Edge	15, 306	3.4	< 0.001	20, 412	2.3	< 0.01	55, 520	1.6	< 0.01
	Lifetime	21, 153	3.1	< 0.001	7, 49	2.9	< 0.05	21, 153	1.2	> 0.05
2-Region	Core	5, 114	10.8	< 0.001	20, 468	2.8	< 0.001	20, 468	0.78	> 0.05
	Edge	5,109	6.1	< 0.001	20, 448	2.4	< 0.01	20, 448	0.95	> 0.05
	Lifetime	7, 53	8.9	< 0.001	7, 53	3.4	< 0.01	7, 53	1.5	> 0.05

QDFA to the 4 regions using otolith$_{core}$ element ratios (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) was 72%, with a high level of variation in classification success among regions: NW (25%), NC (92%), NE (0%), and SE (67%). The 2-region grouping resulted in significant differences between regions and among years (MANOVA, $p < 0.05$) with no interaction, and increased overall jackknifed classification success to 94% (Table 5, Fig. 10A).

Chemical signatures in the otolith$_{edge}$ of Warsaw grouper (most recent period) also varied significantly among the 4 regions (MANOVA, $p < 0.001$) (Table 4). Univariate contrasts indicated that otolith$_{edge}$ Sr:Ca and Ba:Ca were different (ANOVA, $p < 0.05$) between regions. Otolith$_{edge}$ Sr:Ca and Ba:Ca were higher in the NC region (Sr:Ca = 3045 ± 373; Ba:Ca = 1.89 ±95) than the SE region (Sr:Ca = 2637 ± 345; Ba:Ca = 0.85 ± 0.18) (Fig. 9E,F). Otolith$_{edge}$ Sr:Ca and Ba:Ca ratios for individuals from the NW (Sr:Ca = 2944 ± 538; Ba:Ca = 1.49 ± 0.61) and NE (Sr:Ca = 2731 ± 409; Ba:Ca = 1.36 ± 0.48) regions were intermediate between the NC and SE. No regional differences were observed for otolith$_{edge}$ Mg:Ca, Mn:Ca, or Li:Ca values

(ANOVA, $p > 0.05$). Overall jackknifed classification success from QDFA to the 4 regions using otolith$_{\text{edge}}$ element ratios (Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca) was 67%, with a high level of variation in classification success among regions: NW (11%), NC (86%), NE (20%), and SE (71%).n The 2-region grouping resulted in significant differences between regions and among years (MANOVA, $p < 0.05$) with no interaction, and increased overall jackknifed classification success to 88% (Table 5, Fig. 10B).

Otolith$_{\text{lifetime}}$ signatures using combined marker classes (trace elements and stable isotopes) varied significantly among the 4 regions (MANOVA, $p < 0.001$) (Table 4). Univariate contrasts indicated that otolith$_{\text{lifetime}}$ Mn:Ca, Sr:Ca, Ba:Ca, and $\delta^{18}O$ were different (ANOVA, $p < 0.05$) between regions. Otolith$_{\text{lifetime}}$ Mn:Ca, Sr:Ca, and Ba:Ca ratios were higher in the NC region (Mn:Ca = 1.20 ± 0.38; Sr:Ca = 2692 ± 210; Ba:Ca = 3.53 ± 0.97) than the SE region (Mn:Ca = 0.46 ± 0.35; Sr:Ca = 2467 ± 200; Ba:Ca = 1.93 ± 0.72) (Fig. 11A−C). Otolith$_{\text{lifetime}}$ element:Ca ratios for individuals from the NW (Mn:Ca = 0.92 ± 0.52; Sr:Ca = 2618 ± 166; Ba:Ca = 2.45 ± 0.86) and NE (Mn:Ca = 0.68 ± 0.36; Sr:Ca = 2570 ± 189; Ba:Ca = 2.74 ± 1.18) regions were intermediate between the NC and SE. Otolith$_{\text{lifetime}}$ $\delta^{18}O$ values were higher in the SE (−0.01 ± 0.29) than all other regions (NW = −0.29 ± 0.27; NC = −0.41 ± 0.16; NE = −0.39 ± 0.21) (Fig. 11D). No regional differences were observed for Mg:Ca, Li:Ca, or $\delta^{13}C$ (ANOVA, $p > 0.05$). Overall classification success from QDFA using a combination of markers (Mg:Ca, Mn:Ca, Sr:Ca, Ba:Ca, and $\delta^{18}O$) to the 4-region grouping using otolith$_{\text{lifetime}}$ signatures was 60%, with a high level of variation in classification success among regions: NW (33%), NC (74%), NE

(30%), SE (84%). The 2-region grouping resulted in significant differences between regions and among years (MANOVA, $p < 0.05$) with no interaction, and increased overall jackknifed classification success to 84% (Table 5, Fig. 10C). Total classification success for the

2-region grouping (NC/NW/NE and SE) remained relatively consistent whether using trace elements only (83%), stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) only (84%), or a combination of trace elements and $\delta^{18}O$ (84%) (Table 6).

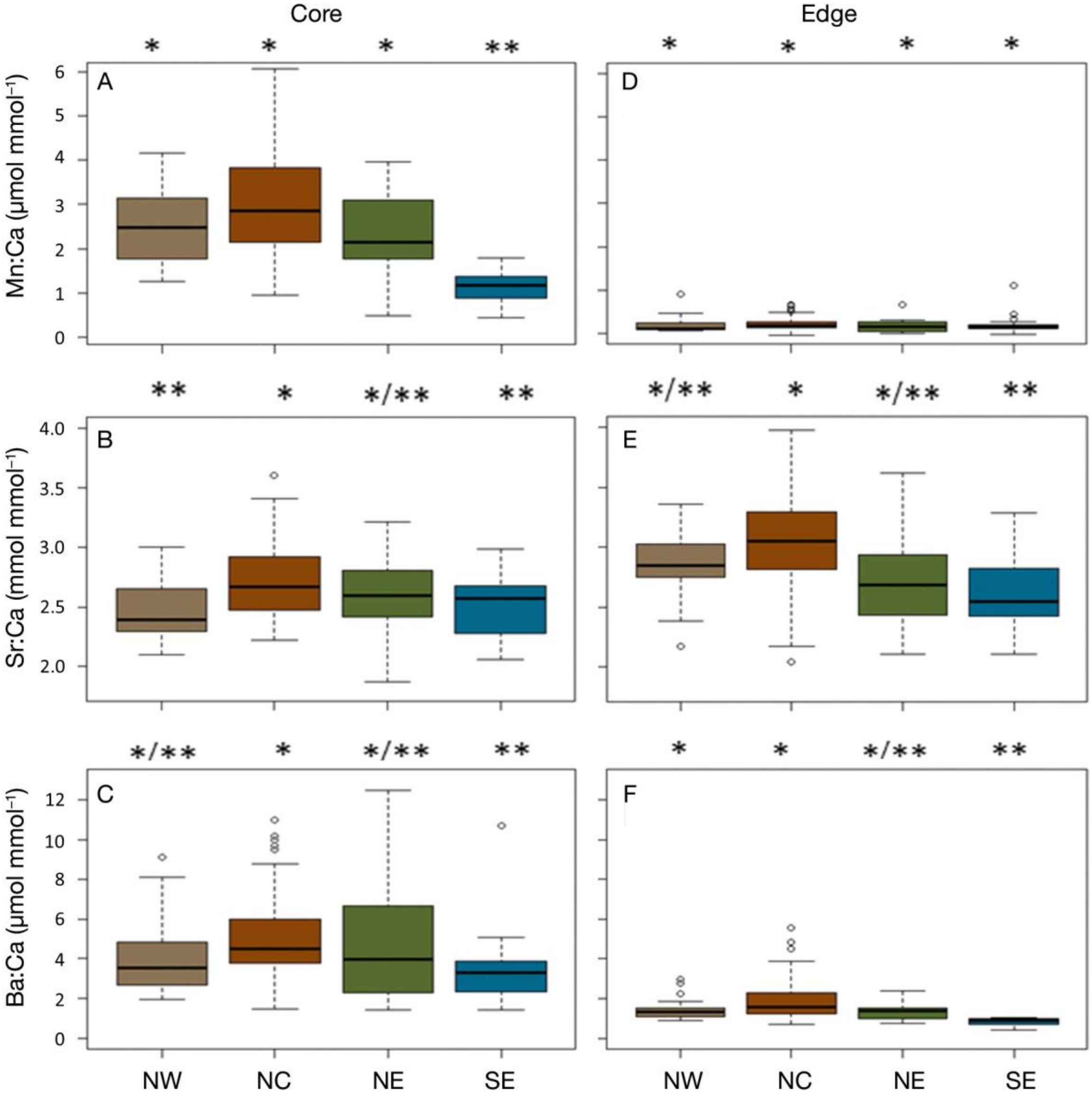


Figure 9. (A,D) Mn:Ca, (B,E) Sr:Ca, and (C,F) Ba:Ca ratios for core (A−C) and edge (D−F) values based on the Gulf of Mexico 4-region grouping for all Warsaw grouper otolith samples in the study (n = 126). Asterisks denote Tukey HSD groupings (p = 0.05) for the univariate (ANOVA) tests. Box range represents the IQR of the data with the mean indicated by the black line, circles are outliers. NW: Northwest; NC: Northcentral; NE: Northeast; SE: Southeast

Table 5. Jackknifed classification success (%) from quadratic discriminant function analysis (QDFA) for 4-region and 2-region groupings for Warsaw grouper otolith core (first year) and edge (recent growth period) samples using trace elements only (Mg, Mn, Sr, Ba), and lifetime samples using trace elements and $\delta^{18}O$ with sample sizes (n) per region. NW: Northwest; NC: North- central; NE: Northeast; SE: Southeast.

	n	Region	NW	NC	NE	SE	%	n	2-Region	%
Core	16	NW	4	9	1	2	25	105	NW/NC/NE	98
	79	NC	5	73	1	0	92	21	SE	76
	10	NE	0	7	1	2	0		**Overall**	**94**
	21	SE	1	4	2	14	67			
		Overall					**72**			
Edge	18	NW	2	11	1	4	11	104	NW/NC/NE	90
	76	NC	4	65	3	4	86	17	SE	71
	10	NE	1	4	2	3	20		**Overall**	**88**
	17	SE	0	5	0	12	71			
		Overall					**67**			
Lifetime	15	NW	5	5	1	4	33	44	NW/NC/NE	89
	19	NC	3	14	1	1	74	19	SE	74
	10	NE	3	2	3	2	30		**Overall**	**84**
	19	SE	3	0	0	16	84			
		Overall					**60**			

Discussion

Distinct chemical signatures (trace elements and stable isotopes) and high overall classification success (~85% or above) to specific geographic regions indicate that the Warsaw grouper population in the GoM likely consists of multiple stock components (Kalish 1989, Ashford et al. 2012, Tanner et al. 2016), with contingents or sub-populations emerging in the NW/NC/NE GoM and the SE GoM. Thus, the single-stock framework currently used to manage the population may not accurately represent the actual population structure of Warsaw grouper in the GoM, which compromises the management of the stock. For example, individual stocks (local populations) should be managed independently for a metapopulation based on stock-specific demographics and exploitation rates (Begg et al. 1999, Pita et al. 2016). Under the single stock framework, exploitation rates for more vulnerable stocks in a metapopulation may exceed sustainable levels under the false assumption of connectivity and replenishment by other segments of the population (Ying et al. 2011). For a species with a periodic life history strategy like the Warsaw grouper, overexploitation can lead to truncation of the population age structure, thereby decreasing the reproductive potential in years when environmental conditions would otherwise lead to a successful year-class, potentially resulting in local extirpation (Longhurst 2002, Winemiller 2005, Secor et al. 2015). For Warsaw grouper in the GoM, region-specific differences in otolith chemistry along multiple growth zones suggests that connectivity across

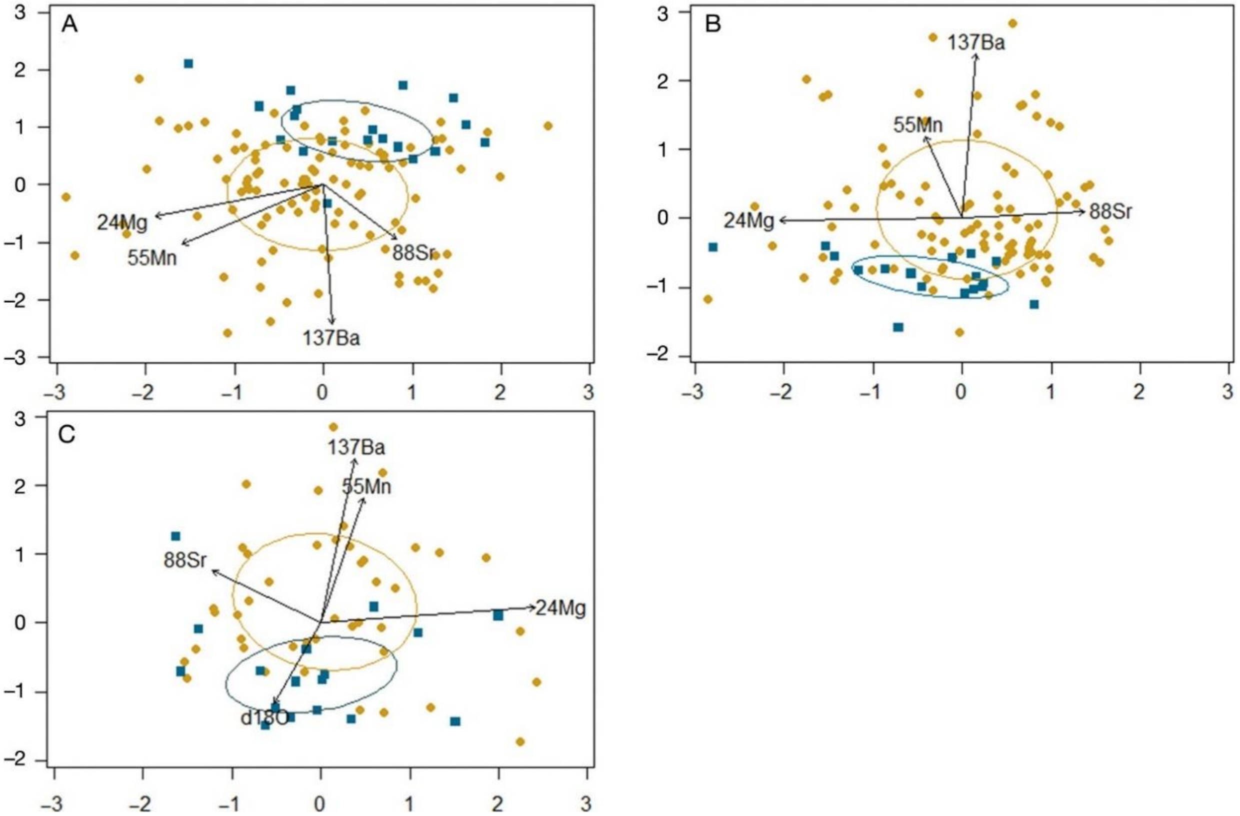


Figure 10. Canonical correspondence plots of chemical signatures with overlaid 2-region ordination ellipses for (A) core samples and (B) edge samples of trace elements only (Mg, Mn, Sr, Ba), and (C) lifetime samples with trace elements and δ^{18}O. Vector influence of each analytical parameter overlaid on CCA plots. North: Northwest, Northcentral, and Northeast regions (yellow), South: Southeast region (blue).

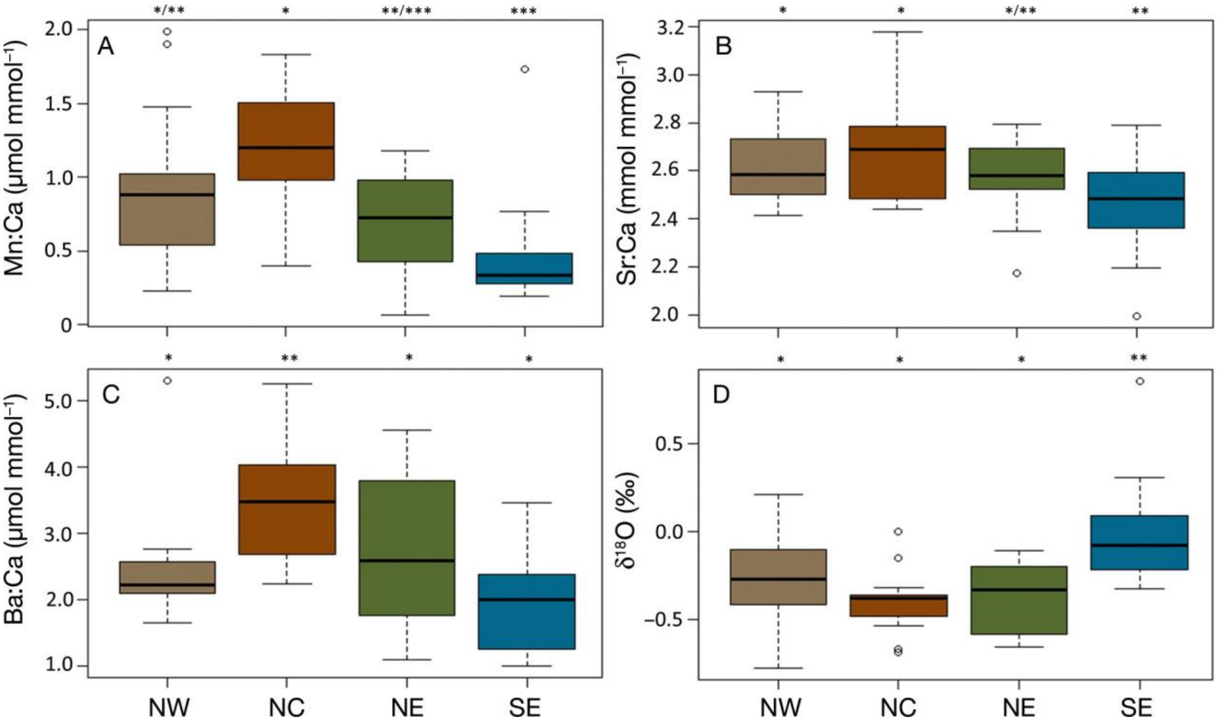


Figure 11. (A) Mn:Ca, (B) Sr:Ca, and (C) Ba:Ca ratios, and (D) δ^{18}O lifetime values by Gulf of Mexico 4-region grouping for the Warsaw grouper stable isotope sample subset (n = 61). Other details as in Fig. 9.

regions is lower than currently expected. Patterns in chemical signatures between the NW/NC/NE and SE regions for all 3 life history stages assessed (otolith$_{\text{core}}$, otolith$_{\text{edge}}$, and otolith$_{\text{lifetime}}$) were similar, suggesting that individuals likely remained within specific regions from early life to adult periods. In fact, back-calculated birth years for Warsaw grouper collected in the NW/NC/NE ranged from 2004−2012; nevertheless, region-specific classification success using otolith$_{\text{core}}$ signatures (=YOY period) was very high (98%). Therefore, our findings imply that regional differences in otolith chemistry, as influenced by water chemistry, at the geographic scale investigated remain distinct over time and lend credence to the premise that movement or mixing of individuals between these 2 regions is negligible for juvenile and adult stages of Warsaw grouper (Secor 2010). Moreover, region-specific differences were generally

Table 6. Jackknifed classification success (%) from quadratic discriminant function analysis (QDFA) for 2-region grouping of the stable isotope sample subset lifetime signature from Warsaw grouper otoliths using trace elements only (Mg, Mn, Sr, Ba), stable isotopes only ($\delta^{18}O$ and $\delta^{13}C$), and trace elements and $\delta^{18}O$. NW: Northwest; NC: Northcentral; NE: Northeast; SE: Southeast.

Region	n	Trace elements only	$\delta^{18}O$ and $\delta^{13}C$ only	Trace elements with $\delta^{18}O$
NW/NC/NE	44	89	95	89
SE	19	68	58	74
Overall		83	84	84

consistent between the 2 regions for both YOY/juvenile (otolith$_{core}$) and sub-adult/adult (otolith$_{edge}$) life history stages, supporting the assertion that regional contingents or sub-populations may exist for Warsaw grouper in the GoM (Ashford et al. 2005, Rooker & Secor 2019).

Classification success for Warsaw grouper from the NW and NE in the 4-region grouping using otolith$_{core}$, otolith$_{edge}$, and otolith$_{lifetime}$ signatures was distinctly lower than the NC and SE regions. While our inability to effectively classify individuals to these 2 regions using otolith$_{lifetime}$ signatures could be a result of otolith chemistries reflecting individual residency in a combination of chemically distinct regions (e.g. NC and SE) (Campana 1999), and therefore stock mixing, otolith$_{core}$ and otolith$_{edge}$ analyses suggest that low success in these 2 regions is a result of intermediate physicochemical conditions. Classification success for the NW and NC regions was low for both otolith$_{core}$ and otolith$_{edge}$ samples demonstrating that these signatures integrated both the juvenile (YOY) and adult periods, and consequently are not a result of movement through chemically distinct regions over a large geographic range. On the other hand, it seems reasonable to assume that if regional ambient water exhibited intermediate physicochemical characteristics, the integration of the signature in both long-term (lifetime) and short-term (core/edge) deposition periods would be expected, especially in regions with temporally stable water chemistries.

The 4 analytes (Mn, Sr, Ba, δ^{18}O) in the otoliths of Warsaw grouper that varied regionally showed a common spatial pattern across the GoM, with pronounced differences between the NC and SE regions, and values in the NE and NW regions generally intermediate to the NC and SE. Differences between the NC and SE region are likely a function of 2 mesoscale features that influence the ambient seawater chemistry in each region; the low salinity, nutrient rich Mississippi-Atchafalaya River System (MARS) in the NC and the relatively high salinity, oligotrophic Loop Current (LC) in the SE (Oey et al. 2005, Matli et al. 2018). In addition, the otolith chemistry of Warsaw grouper from the NW region was most similar to the NC region, which is not surprising because inflow from the MARS is known to affect the water chemistry of the NW GoM (Rabalais et al. 2002, Morey et al. 2003a,b). Although the MARS discharge predominantly flows westward, influencing physicochemical properties of the NW GoM (Rabalais et al. 1991, Oey et al. 2005, Matli et al. 2018), reverse flow occurs during the summer months (Morey et al. 2003a,b). Thus, while MARS influence is well known along the Louisiana-Texas coastal shelf (NW region), it also likely alters the seawater chemistry in the NE GoM (Walker et al. 2005) and subsequently influences chemical signatures in the otoliths of Warsaw grouper collected in this region.

The MARS influence is apparent when comparing individual chemical markers in the otoliths of Warsaw grouper, and regional patterns were typically retained in all 3 life history stages (core, edge, lifetime). While Mn:Ca and Sr:Ca ratios would be conservative in intermediate waters of the GoM if salinity was the predominant driver of variability (Sturrock et al. 2012), both elements were higher in the otoliths of Warsaw grouper from regions closest to the MARS inflow point (particularly the NC GoM). Element:Ca ratios for Mn, Sr, and Ba have been shown to increase in the otolith deposition of fish collected in nutrient-rich waters (Böhlke & Horan 2000, Martin et al. 2004, Martin & Thorrold 2005, Stanley et al. 2015), a characteristic of the MARS inflow, an inflow that has been shown to affect outer shelf waters in the NW region and is expected to influence those in the NE (Rabalais et al. 1991, Morey et al. 2003a,b). Furthermore, these relative patterns were exhibited for both core and edge life history stages even while absolute values changed, suggesting these changes resulted from either an ontogenetic depth shift to waters with less MARS influence or a shift in physiological regulation (Campana 1999, Ruttenberg et al. 2005), but not likely from migration to another region. No direct measurements of water chemistry were made, so it is not possible to reconcile causal mechanisms or the importance of additional factors (e.g. diet, depth shift).

Spatial variability of water chemistry in the Atlantic Ocean is well known, and its influence on otolith chemistry has been documented (Rooker et al. 2008, Wells et al. 2010, Tanner et al. 2016). Relatively few studies, however, have applied otolith chemistry to examine spatial dynamics and other aspects of the ecology of deepwater fishes (Edmonds et al. 1991, Thresher et al. 1994, Kalish et al. 1996), with the most recent applications on this group performed in the Southern Ocean (Ashford et al. 2005, 2012, Zhu et al. 2018). In our study, region-specific differences in $\text{otolith}_{\text{core}}$, $\text{otolith}_{\text{edge}}$, and $\text{otolith}_{\text{lifetime}}$ signatures of Warsaw grouper from the GoM were detected and indicated that natural markers could be used to correctly classify individuals to certain regions. The most striking difference in otolith chemistry was observed between Warsaw groupers from the NC and SE regions, with physicochemical conditions in the former region heavily influenced by the MARS (Oey et al. 2005, Matli et al. 2018). In contrast, the SE region was the farthest removed from MARS, and the otolith chemistry of Warsaw groupers from this region was distinct, indicating a lack of connectivity between groupers in the NC and SE regions. In addition, $\text{otolith}_{\text{core}}$ signatures (YOY or nursery period) were region-specific even though our samples comprised birth years that spanned nearly a decade. Therefore, the spatial variation in the otolith chemistry of Warsaw grouper is sufficient to overcome any temporal shifts in physicochemical conditions within regions. While ontogenetic shifts in migration are possible, little variation in chemical structure of $\text{otolith}_{\text{core}}$ and $\text{otolith}_{\text{edge}}$ regions suggest limited juvenile/sub-adult inter-regional movements. Our results indicate that assessing chemical signatures from multiple life history stages of an otolith has the potential to increase stock structure resolution for meta-populations whose contingents are spread between geographic areas with relatively homogeneous physicochemical conditions, a common characteristic in individual deepwater masses. Because juvenile/sub-adult Warsaw grouper are a major component of both the commercial and recreational deepwater fisheries and experience high release mortality rates, a multi-stock or metapopulation framework may be needed to improve future assessments and rebuilding plans for this species in the GoM.

CHAPTER IV

POPULATION STRUCTURE AND REGIONAL CONNECTIVITY OF SNOWY GROUPER, *HYPORTHODUS NIVEATUS*, IN THE GULF OF MEXICO AND WESTERN ATLANTIC OCEAN

Introduction

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 & Rose 1992, King &McFarlane 2003), with populations of the longest-lived species likely sustained through one successful year class every five to ten 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 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, large depth-related pressure gradients, and a lack of small-scale spatial variability in physicochemical conditions make it more difficult to assess population demographics (Campana 1999, Cailliet 2001, Campana 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). 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 & Wroblewski 2002, Kritzer & 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 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, *E. flavolimbatus*; snowy grouper, *Hyporthodus 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, *H. nigritus*) is unknown (Bertoncini et al. 2018). Bomb radiocarbon age validation analysis conducted on all four species concluded 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, *N. nigritus,* results indicated the presence of distinct population contingents within the Gulf of Mexico (herein GoM) (Sanchez et al. 2020), potentially increasing their vulnerability to fishing pressure.

Here, otolith chemistry analysis is applied to investigate the population structure and connectivity of snowy grouper along the continental shelf and slope in the Atlantic Ocean (Wyanski & White 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 (^{7}Li, ^{24}Mg, ^{55}Mn, ^{88}Sr, and ^{137}Ba) and stable isotopes (δ^{13}C and δ^{18}O), were measured for individuals collected from 4 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, it is anticipated 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, FL. All otoliths were collected by NOAA Fisheries' observers from 2012-2015 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 fishing shrimp 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) (Fig. 12). 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 (DDI$H_2$0; ultrapure, 18-MΩ/cm water), and allowed to air dry following Rooker et al. (2008).

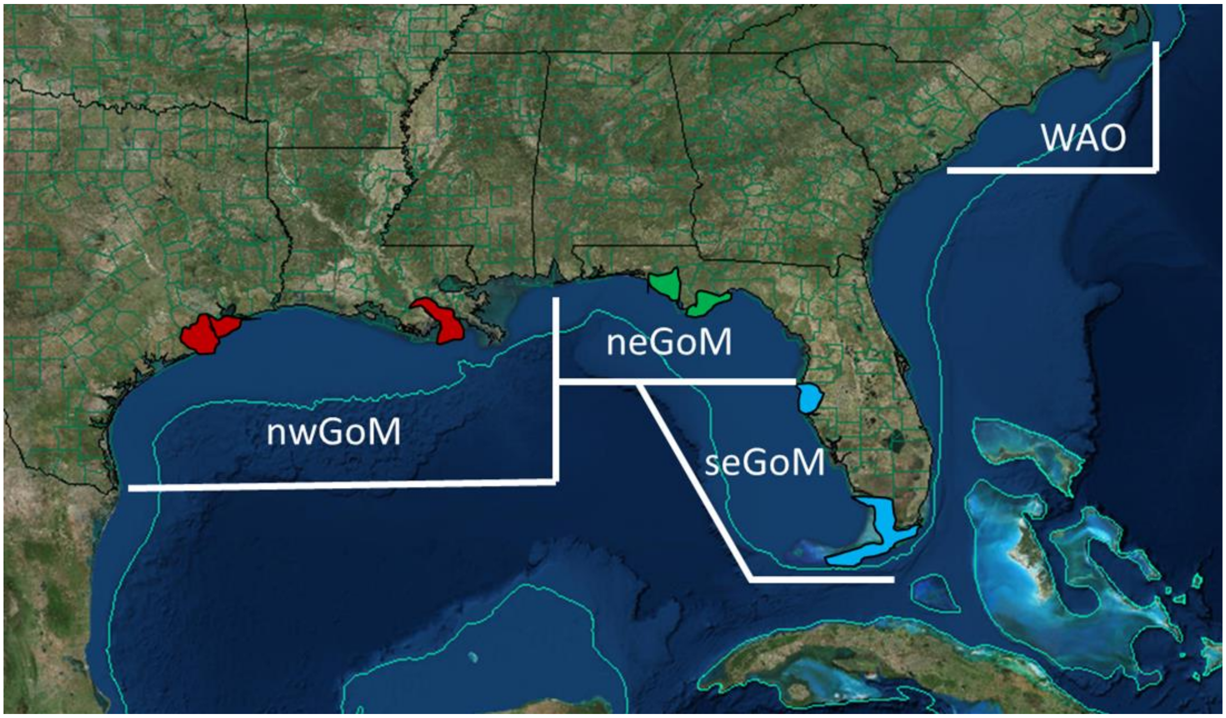


Figure 12. Map of the western Atlantic Ocean (WAO) and Gulf of Mexico (GoM) showing demarcations for regional groupings based on catch location (white lines). Colored polygons in the GoM represent sampled landing locations and light blue line denotes the 200-m depth contour. nw = northwest, ne = northeast, se = southeast.

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 where two reads were available, with the overall average percent error (APE) calculated.

Trace Elements

Trace element composition of snowy grouper otoliths was assessed using two different laser ablation inductively coupled plasma mass spectrometers (LA-ICP-MS); samples collected in 2012 and 2013 were assessed using an ultraviolet New Wave Research NWR213 LA system coupled with a quadrupole Thermo Scientific XSeries 2 ICP-MS located at Texas A&M University at Galveston (TAMUG); samples collected in 2014 and 2015 were assessed using an Elemental Scientific NWR193UC LA system coupled with an Agilent 7500ce ICP-MS at the University of Texas (UT) at Austin. On each instrument, multiple otolith thin sections were mounted onto single petrographic slides, with the exact number dependent on section width. Following a pre-ablation procedure to remove surface contamination (Sanchez et al. 2020), elemental concentrations were determined for a series of 70-µm diameter spots on each otolith, with 150-µm spacing between centroids (80-µm 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

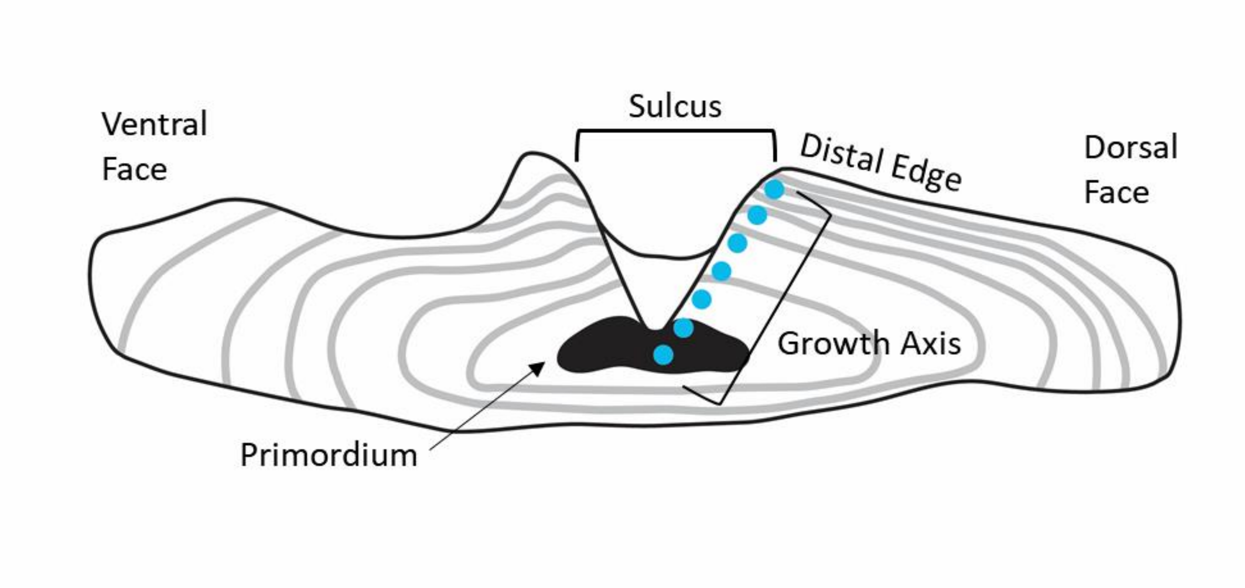


Figure 13. Sample design for A) trace element and B) stable isotope sampling on the otolith cross-section. Blue circles represent ablation spots, black section in center the primordium (earliest deposition), and gray rings growth increments. For trace elements, the otolith$_{core}$ includes the first three ablation spots moving up from primordium, otolith$_{edge}$ the last three ablation spots near distal edge, and otolith$_{life}$ all ablation spots up the growth axis. For stable isotopes, sample material was milled and pooled from the entire otolith$_{life}$ transect.

section (Fig 13). The total number of sampling spots per otolith was dependent on distance between otolith primordium and margin. Ablation spots were grouped into three life history stages: 1) otolith$_{core}$ (young-of-year [YOY] period), 2) otolith$_{edge}$ (recent growth period), and 3) otolith$_{life}$ (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$_{core}$ = first three spots from primordium, otolith$_{edge}$= last three spots from margin, otolith$_{life}$ = all spots along the transect. Replicates of National Institute of Standards and Technology (NIST) 612 standards were run at regular intervals to calibrate machine counts second^{-1} readings to element concentrations (ppm) and MACS-3 standards were used as the primary reference and to assess machine drift. Six elements in snowy grouper otoliths were quantified: ^{7}Li, ^{24}Mg, ^{43}Ca, ^{55}Mn, ^{88}Sr, ^{137}Ba. Element concentrations (ppm) were then converted to element:Ca ratios (µmol/mmol) using ^{44}Ca as the Ca standard at TAMUG and ^{43}Ca as the Ca standard at UT as described in Sanchez et al. (2020).

Stable Isotopes

For a subset of snowy grouper (n = 87), otolith δ^{18}O and δ^{13}C values were determined following analysis on the LA-ICP-MS. Otolith sections were initially prepared for stable isotope analysis by lightly polishing off laser ablation spots. Otolith material along the lifetime transect assayed on the LA-ICP-MS was then obtained using a high-precision micromill (New Wave Research MicroMill). Fourteen 55-µm deep passes of a 300-µm wide transect line from the otolith core to margin resulted in a sampling depth of 770 µm. Powdered otolith material from each sample was collected and pooled to obtain lifetime δ^{18}O and δ^{13}C values for each individual in the subset of snowy grouper otoliths. Stable isotope analysis (δ^{18}O and δ^{13}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), NBS-19 and NBS-18, with 6 standards ran for every 40 samples; precision was ± 0.08‰ (SD) and ± 0.11‰ (SD) for δ^{18}O and δ^{13}C, respectively. Otolith δ^{18}O and δ^{13}C values are reported relative to the Vienna Pee Dee Belemnite (VPDB) scale after comparison to an in-house laboratory standard calibrated to VPDB.

Statistical Analysis

Otolith element:Ca values of snowy grouper from 2012-13 (TAMUG) and 2014-15 (UT) were assessed independently. For each dataset, otolith$_{\text{life}}$, otolith$_{\text{core}}$, and otolith$_{\text{edge}}$ element:Ca values were organized by region and initially assessed for outliers which were defines as any element:Ca ratio outside 5 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. 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. MANOVA and ANOVA models incorporated an interaction term (region x 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 highly 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 (QDFA) 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 v.11). For otolith$_{\text{life}}$ values, a canonical correspondence analysis (CCA) 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 2012-13 dataset.

A subset of otolith $\delta^{18}O$ and $\delta^{13}C$ values for snowy grouper were analyzed with ANOVA to determine whether region-specific differences were present. Collection year was restricted to two years (2012, 2013) from the GoM and one year (2013) from the WAO; therefore, years were pooled and interaction between region and year was not assessed. QDFA 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: stable isotopes only, trace elements only, and combination of both markers.

Results

A total of 254 snowy grouper otoliths collected over four 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 length and age of snowy grouper in our final sample were 540 ± 35 mm and 7.3 ± 2.3 years, respectively, with no differences across the four regions (ANOVA, $p > 0.05$; Table 7).

Table 7. Sample size (n), length (mm), and age (years) data for fish from each region analyzed for trace elements. *Six otoliths not included in age comparison due to difficulties with age determination. WAO = western Atlantic Ocean, seGoM = southeast Gulf of Mexico, neGoM = northeast Gulf of Mexico, nwGoM = northwest Gulf of Mexico.

Region	n	Length Mean	Length SD	Age* Mean	Age* SD
WAO	65	540.2	23.9	6.8	1.7
seGoM	63	533.1	32.9	7.6	1.9
neGoM	44	535.8	51.8	7.7	3.1
nwGoM	63	548.6	31.5	7.3	3.1
Overall	235	539.7	35.1	7.3	2.3
ANOVA		F = 1.9, p > 0.05		F = 2.3, p > 0.05	

Trace Elements

Otolith$_{life}$ signatures of snowy grouper based on a combination of all five element:Ca ratios were significantly different among the four regions for both 2012-13 (MANOVA, Pillai's T = 0.87, $p < 0.001$) and 2014-15 (MANOVA, Pillai's T = 0.70, $p < 0.001$) collection periods. Otolith$_{life}$ Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca were each significantly different among the four regions for both 2012-13 and 2014-15 collection periods with the exception of Mg:Ca in 2014-15 (ANOVA, $p < 0.05$). Significant region x year interactions were observed for Sr:Ca and Ba:Ca in 2012-13, and Li:Ca and Mg:Ca in 2014-15; however, general inter-region patterns still emerged for most element:Ca ratios. Otolith$_{life}$ Li:Ca was consistently higher in the WAO (2012-13, 26.60 ± 21.04 µmol/mmol; 2014-15, 17.21 ± 20.77 µmol/mmol) relative to the nwGoM (2012-13, 3.22 ± 1.95 µmol/mmol; 2014-15, 5.11 ± 2.29 µmol/mmol), neGoM (2012-13, 4.08 ± 2.99; 2014-15 µmol/mmol, 4.20 ± 0.46 µmol/mmol), and seGoM (2012-13, 4.77 ± 6.59 µmol/mmol; 2014-15, 4.16 ± 0.53 µmol/mmol) (Table 8A). Similarly, mean otolith$_{life}$ Sr:Ca was consistently lowest in the nwGoM (2012-13, 2875 ± 177 µmol/mmol; 2014-15, 2213 ± 147 µmol/mmol) with a clear gradient towards higher values for this marker moving east into the neGoM (2012-13, 2924 ± 175 µmol/mmol; 2014-15, 2290 ± 132 µmol/mmol) and seGoM (2012-13, 2978 ± 179 µmol/mmol; 2014-15, 2350 ± 184 µmol/mmol); however; relative values fluctuated in the WAO, highest overall 2012-2013 (3226 ± 215 µmol/mmol) but lower than both the seGoM and neGoM in 2014-15 (2257 ± 90 µmol/mmol) (Table 8A). In contrast, mean otolith$_{life}$ Mn:Ca and Ba:Ca were highest in the nwGoM [Mn:Ca (2012-13, 0.97 ± 0.45 µmol/mmol; 2014-15, 0.98 ± 0.49 µmol/mmol), Ba:Ca (2012-13, 2.09 ± 0.75 µmol/mmol; 2014-15, 1.83 ± 0.43 µmol/mmol)] and decreased moving east into the neGoM [Mn:Ca (2012-13, 0.77

± 0.29 µmol/mmol; 2014-15, 0.87 ± 0.42 µmol/mmol), Ba:Ca (2012-13, 0.1.76 ± 0.59 µmol/mmol; 2014-15, 1.46 ± 0.31 µmol/mmol)], seGoM [Mn:Ca (2012-13, 0.55 ± 0.28

Table 8. Mean element:Ca ratios for each region, year, and life history stage. WAO = western Atlantic Ocean, seGoM = southeast Gulf of Mexico, neGoM = northeast Gulf of Mexico, nwGoM = northwest Gulf of Mexico.

A Life		Region	Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca
	2012	WAO	27.9	140.4	0.59	3152	1.98
		seGoM	6.5	105.1	0.55	2962	1.81
		neGoM	3.3	121.5	0.83	2999	1.91
		nwGoM	2.6	120.7	0.89	2917	2.17
	2013	WAO	18.7	142.1	0.51	3273	2.02
		seGoM	2.7	101.3	0.49	2959	1.39
		neGoM	3.8	123.3	0.82	2870	1.69
		nwGoM	2.7	115.2	0.90	2883	2.01
	2014	WAO	35.7	49.5	0.52	2299	1.59
		seGoM	4.0	49.8	0.85	2324	1.34
		neGoM	4.1	46.7	0.70	2290	1.40
		nwGoM	5.1	52.8	1.12	2295	1.74
	2015	WAO	3.7	53.8	0.43	2226	1.60
		seGoM	4.3	51.2	0.62	2381	1.69
		neGoM	4.3	67.1	1.02	2267	1.49
		nwGoM	5.0	51.3	0.87	2151	1.84
B Core			Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca
	2012	WAO	7.4	165.3	1.45	2972	3.20
		seGoM	4.8	145.8	1.50	2917	3.62
		neGoM	5.3	169.7	1.99	3023	3.45
		nwGoM	4.7	156.9	2.21	2892	3.45
	2013	WAO	7.8	174.7	1.26	3074	3.41
		seGoM	4.4	141.0	1.26	2831	2.50
		neGoM	5.0	160.3	1.98	2812	2.84
		nwGoM	4.6	161.6	2.16	2907	3.53
	2014	WAO	12.0	93.1	1.42	2265	2.90
		seGoM	6.1	94.4	2.23	2223	2.39
		neGoM	7.0	84.0	1.75	2219	2.58
		nwGoM	6.7	92.6	2.54	2324	3.31
	2015	WAO	5.4	81.9	1.00	2080	2.76
		seGoM	6.6	92.1	1.42	2288	2.98
		neGoM	6.7	107.9	2.34	2230	2.64
		nwGoM	6.0	87.1	1.81	2212	3.25

Table 8 Continued

C Edge	Region	Li:Ca	Mg:Ca	Mn:Ca	Sr:Ca	Ba:Ca
2012	WAO	50.5	146.9	0.07	3607	1.02
	seGoM	10.6	87.6	0.04	3367	0.77
	neGoM	2.0	98.6	0.09	3374	1.06
	nwGoM	3.8	113.8	0.12	3137	1.10
2013	WAO	34.8	132.0	0.06	3728	1.03
	seGoM	1.5	77.0	0.03	3367	0.72
	neGoM	2.9	116.5	0.08	3250	0.86
	nwGoM	1.9	95.8	0.10	3146	1.17
2014	WAO	68.4	29.5	0.03	2716	0.77
	seGoM	2.1	23.1	0.04	2733	0.62
	neGoM	2.5	24.6	0.06	2728	0.72
	nwGoM	4.2	32.5	0.05	2597	0.70
2015	WAO	2.4	34.8	0.03	2718	0.78
	seGoM	2.4	27.0	0.04	2807	0.75
	neGoM	2.5	53.3	0.05	2638	0.69
	nwGoM	5.2	27.8	0.12	2316	0.87

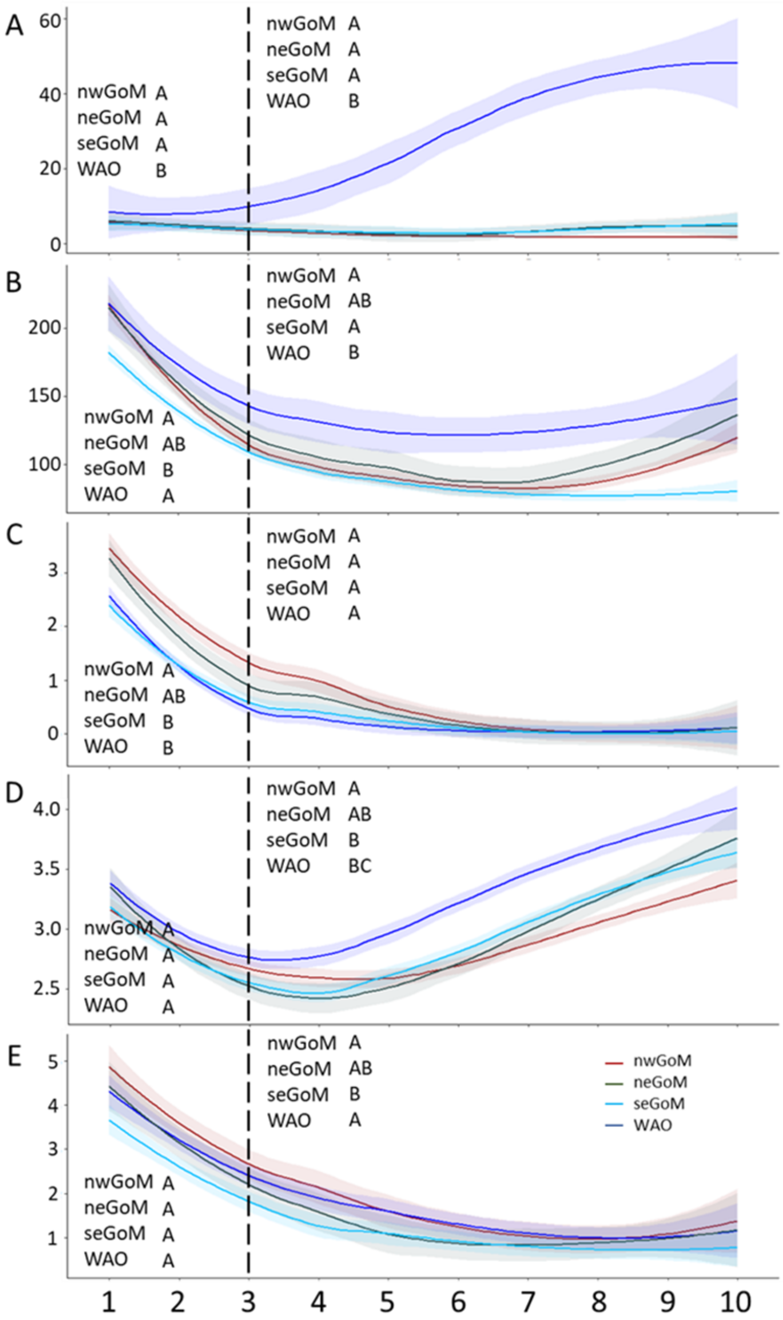
µmol/mmol; 2014-15, 0.73 ± 0.35 µmol/mmol), Ba:Ca (2012-13, 1.57 ± 0.60 µmol/mmol; 2014 15, 1.51 ± 0.48 µmol/mmol)] and WAO [Mn:Ca (2012-13, 0.55 ± 0.26 µmol/mmol; 2014-15, 0.47 ± 0.27 µmol/mmol), Ba:Ca (2012-13, 2.00 ± 0.59 µmol/mmol; 2014-15, 1.60 ± 0.46 µmol/mmol)] (Table 8A). Overall jackknifed classification success of snowy grouper to collection location based on otolith$_{\text{life}}$ signatures ranged from 56-70% among individual years (Table 9A). Average regional classification success was highest for snowy grouper from the WAO (80.5 ± 13.0%), with success rates lower for individuals from the nwGOM (64.8 ± 14.1%), the seGoM (58.5 ± 16.3%) and neGoM (41.8 ± 14.1%).

Otolith$_{\text{core}}$ (YOY) signatures of snowy grouper based on a combination of all five element:Ca ratios were significantly different among the four regions for both 2012-13 (MANOVA, Pillai's T = 0.51, $p < 0.001$) and 2014-15 (MANOVA, Pillai's T = 0.35, $p < 0.01$) collection periods. In 2012-13, otolith$_{\text{core}}$ Li:Ca, Mg:Ca, and Mn:Ca were significantly different among the four regions, while only otolith$_{core}$ Mn:Ca was significant in 2014-15 (ANOVA, $p < 0.05$). A significant region x 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$_{core}$ Li:Ca was higher in the WAO in 2012-13 (9.45 ± 9.21 umol/mmol) relative to the nwGoM (4.66 ± 1.52 umol/mmol), neGoM (5.15 ± 1.18 umol/mmol), and seGoM (4.67 ± 1.19 umol/mmol); no differences were observed for snowy grouper from the 2014-15 collection period (Table 8B). Conversely, otolith$_{core}$ Mn:Ca was consistently highest in the nwGoM (2012-13, 2.30 ± 1.13 umol/mmol; 2014-15, 2.13 ± 1.02 umol/mmol) and showed a clear gradient with values decreasing eastward through the neGoM (2012-13, 1.88 ± 0.76 umol/mmol; 2014-15, 2.08 ± 1.02 umol/mmol), seGoM (2012-13, 1.39 ± 0.71 umol/mmol; 2014-15, 1.80 ± 1.01 umol/mmol), and WAO (2012-13, 1.33 ± 0.60 umol/mmol; 2014-15, 1.18 ± 0.79 umol/mmol)

Table 9. Regional and overall classification success (%) for otolith$_{life}$, otolith$_{core}$, and otolith$_{edge}$ chemical signatures with mean values across years. WAO = western Atlantic Ocean, seGoM = southeast Gulf of Mexico, neGoM = northeast Gulf of Mexico, nwGoM = northwest Gulf of Mexico.

		Region	2012	2013	2014	2015	Mean ± 1SD
A	Life	WAO	74	75	100	73	80.5 ± 13.0
		seGoM	75	70	46	43	58.5 ± 16.3
		neGoM	50	33	27	57	41.8 ± 14.1
		nwGoM	56	80	73	50	64.8 ± 14.1
		Total	65	70	61	56	63.0 ± 5.9
B	Core	WAO	53	35	45	60	48.3 ± 10.8
		seGoM	63	55	69	43	57.5 ± 11.2
		neGoM	50	33	27	71	45.3 ± 19.7
		nwGoM	67	25	64	36	48.0 ± 20.7
		Total	59	38	52	53	50.5 ± 8.9
C	Edge	WAO	79	60	91	40	67.5 ± 22.3
		seGoM	50	80	69	36	58.8 ± 19.6
		neGoM	40	33	45	29	36.8 ± 7.1
		nwGoM	67	70	64	57	64.5 ± 5.6
		Total	62	65	67	57	58.5 ± 12.5

Figure 14. Element:Ca ratios (µmol/mmol) for A) Li:Ca (µmol/mmol), B) Mg:Ca (µmol/mmol), C) Mn:Ca (µmol/mmol), D) Sr:Ca (mmol/mmol), and E) Ba:Ca (µmol/mmol) for the first 10 successive ablation spots up the otolith growth axis for snowy grouper collected in 2012-13, grouped by collection region. Values for points 1-3 were averaged for the otolith$_{\text{core}}$ signature. Statistically significant regional groupings for otolith$_{\text{core}}$ and otolith$_{\text{edge}}$ are given on the left and right side of the dashed line, respectively. WAO = western Atlantic Ocean, neGoM = northeast Gulf of Mexico, seGoM = southeast Gulf of Mexico, nwGoM = northwest GoM.



(Table 8B). Otolith$_{core}$ Mg:Ca was lower in the seGoM (143.42 ± 20.92 umol/mmol) in 2012-13 when compared to the WAO (175.43 ± 44.61 umol/mmol), nwGoM (160.81 ± 21.32 umol/mmol), and neGoM (161.52 ± 19.51 umol/mmol) (Table 8B). Overall jackknifed classification success of snowy grouper to collection location based on otolith$_{core}$ signatures ranged from 38-59% for individual years (Table 9B). Average regional classification success of snowy grouper was low for all four regions, with only the seGoM over 50% (WAO, 48.3 ± 10.8%; neGOM, 45.3 ± 19.7%; seGoM, 57.5 ± 11.2%; nwGoM (48.0 ± 20.7%).

Otolith$_{edge}$ signatures of snowy grouper based on a combination of all five element:Ca ratios were significantly different among the four regions for both 2012-13 (MANOVA, Pillai's T = 0.85, $p < 0.001$) and 2014-15 (MANOVA, Pillai's T = 0.64, $p < 0.001$) collection periods. Otolith$_{edge}$ Li:Ca, Mg:Ca, Mn:Ca, Sr:Ca, and Ba:Ca were each significantly different among the four regions for both the 2012-13 and 2014-15 collection periods with the exception of Mn:Ca in 2012-13 and Ba:Ca in 2014-15 (ANOVA, $p < 0.05$). A significant region x year interaction was not observed for the 2012-13 collection period; however, in 2014-15 a significant interaction was observed for Li:Ca, Mg:Ca, and Sr:Ca, thereby indicating increased variability in regional differences between the years. Otolith$_{edge}$ Li:Ca was consistently higher in the WAO (2012-13, 46.94 ± 34.98 umol/mmol; 2014-15, 30.33 ± 39.68 umol/mmol) relative to the nwGoM (2012-13, 2.82 ± 3.84 umol/mmol; 2014-15, 4.87 ± 4.50 umol/mmol), neGoM (2012-13, 4.51 ± 8.76 umol/mmol; 2014-15, 2.38 ± 0.42 umol/mmol), and seGoM (2012-13, 6.27 ± 14.62 umol/mmol; 2014-15, 2.24 ± 0.38 umol/mmol) (Table 8C). Similarly, otolith$_{edge}$ Sr:Ca was consistently higher in the WAO (2012-13, 3674 ± 324 umol/mmol; 2014-15, 2717 ± 175 umol/mmol), neGoM (2012-13, 3301 ± 344 umol/mmol; 2014-15, 2678 ± 199 umol/mmol) and seGoM (2012-13, 3398 ± 241; 2014-15, 2771 ± 281 umol/mmol) than the nwGoM (2012-13, 3133 ± 267;

2014-15, 2433 ± 278 umol/mmol) (Table 8C). Otolith$_{edge}$ Mg:Ca was consistently lower from the seGoM (2012-13, 85.68 ± 16.68 umol/mmol; 2014-15, 24.34 ± 6.99 umol/mmol) when compared to the WAO (2012-13, 153.62 ± 140.58 umol/mmol; 2014-15, 32.54 ± 10.94 umol/mmol), nwGoM (2012-13, 99.43 ± 22.40 umol/mmol; 2014-15, 30.16 ± 13.39 umol/mmol), and neGoM (2012-13, 123.23 ± 76.62 umol/mmol; 2014-15, 40.04 ± 29.40 umol/mmol). Otolith$_{edge}$ Mn:Ca and Ba:Ca were low for all four regions for both 2012-13 and 2014-15. Overall jackknifed classification success of snowy grouper to collection location based on otolith$_{edge}$ signatures ranged from 57-67% for individual years (Table 9C). Average regional classification success of snowy grouper was generally similar in the WAO (67.5 ± 22.3%) nwGOM (64.5 ± 5.6%), and seGOM (58.8 ± 19.6%), with considerably lower success for classification of individuals to the neGoM (36.8 ± 7.1%).

Stable Isotopes

Otolith$_{life}$ $\delta^{13}C$ and $\delta^{18}O$ values of snowy grouper were significantly different across the four regions (ANOVA, $p < 0.05$; Table 7A). For otolith$_{life}$ $\delta^{13}C$, values of snowy grouper were significantly higher in the seGoM (-3.9 ± 0.5) and neGOM (-4.2 ± 0.4) relative to the nwGoM (-4.6 ± 0.3) (Tukey HSD, $p < 0.05$) with intermediate values for individuals from the WAO (-4.4 ± 0.2). For otolith$_{life}$ $\delta^{18}O$, values were higher in the seGoM (0.46 ± 0.13) and WAO (0.36 ± 0.04) relative to the nwGoM (-0.14 ± 0.09) and neGoM (0.11 ± 0.08) (Tukey HSD, $p < 0.05$). Overall jackknifed classification success of snowy grouper to collection location based on both otolith$_{life}$ element:Ca and stable isotope markers was 60%. Classification success based on each class of markers alone was 57% and 51% for otolith$_{life}$ element:Ca and stable isotopes, respectively.

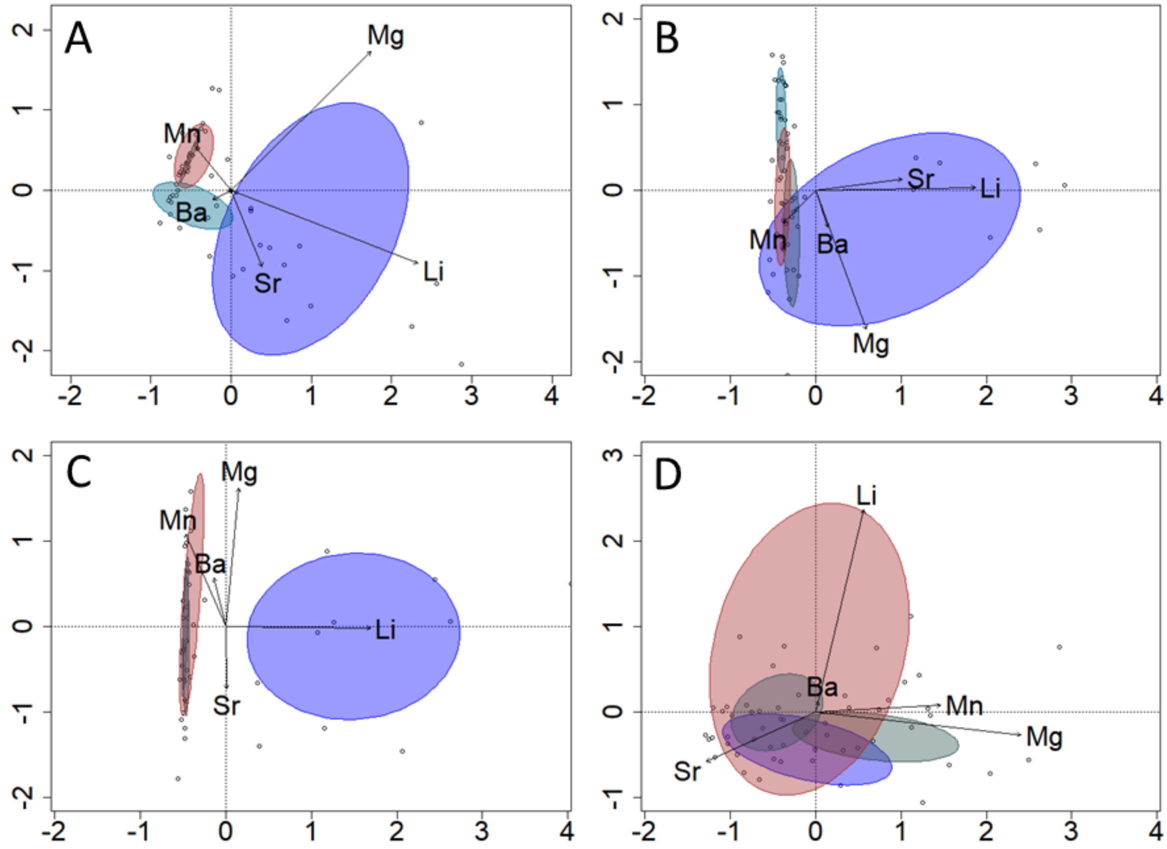


Figure 15. Constrained correspondence analysis plots of trace element signatures for the lifetime life history stage for A) 2012, B) 2013, C) 2014, D) 2015. Ellipses represent 1 SD for each region. Blue = west Atlantic Ocean (WAO), Light Blue = southeast Gulf of Mexico (seGoM), Green = northeast Gulf of Mexico (neGoM), Red = northwest Gulf of Mexico (nwGoM).

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 et al. 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 movement of snowy grouper across the regions investigated is limited.

Otolith$_{\text{life}}$ Mn:Ca and Ba:Ca in snowy grouper were elevated for individuals collected in the nwGoM, which is presumably the result of nutrient rich MARS inflow which 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 MARS, 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 MARS 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 et al. 2014). Similar to otolith Mn, Ba is a good indicator of nutrient rich waters and otolith Ba:Ca are often elevated in areas with high nutrient loading or upwelling (Bath et al. 2000, Walther & 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 GS, 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 Warsaw grouper (Sanchez et al. 2020) suggesting that regional differences are not conspecific but a result of large-scale geographic dynamics in physicochemical conditions of the GoM .

Regional variation otolith Sr:Ca and $\delta^{18}O$ 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 & Rooker 2000, Nelson & Powers 2019). Similarly, a positive relationship between otolith $\delta^{18}O$ 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 LC and GS and characterized by high salinity (Oey et al. 2005, Kemp et al. 2016), displayed elevated otolith Sr:Ca and $\delta^{18}O$ relative to the two other regions in the GoM that are heavily influenced by freshwater inflow from MARS. However, elevated Sr:Ca was 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. $\text{Otolith}_{\text{life}}$ Li:Ca for snowy grouper collected from 2012-2014 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 deposited in otoliths at equilibrium with environmental concentrations (Thresher 1999, Sturrock et al. 2012), thereby indicating ambient waters at these commercial fishing locations in the WAO appear to be enriched in Li. Of particular note, elevated otolith$_{life}$ values from 2012-2014 were driven by an increase in Li:Ca after the first year of life, with otolith$_{core}$ ratios (<12 µmol/mmol) markedly lower compared to otolith$_{edge}$ ratios (35-68 µmol/mmol), potentially indicative of an ontogenetic migration to waters enriched in Li. Potential point source(s) for Li in the South Atlantic Bight and Mid 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$_{life}$ element:Ca signatures (up to 70%) for snowy grouper suggests some degree of population structure (i.e., sub-populations) 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 & 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 MARS and LC in the region as the combination of a seasonal eastward flow of the MARS discharge and a seasonal northward intrusion of the LC causes two distinct water masses to intersect in the region (Morey et al. 2003a,b, Alvera-Azcárate et al. 2008). 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 grouper (*Mycteroperca microlepsis*), 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 grouper, 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 2001, Campana 2001).

Classification success of snowy grouper to collection region using otolith$_{\text{core}}$ element:Ca signatures was lower than expected (38-59%), suggesting that physicochemical conditions experienced by individuals during the YOY period are likely similar (Campana 1999, Tanner et al. 2016). While this may indicate regardless of collection region that during the YOY period, snowy grouper only inhabit a small geographic region as a result of only a 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 be an indication that effective juvenile habitats for snowy grouper are limited to locations with specific physicochemical conditions (Dahlgren et al. 2006). In addition, physiological mechanisms that regulate the chemical deposition of otoliths are less developed during early life and therefore, if regional variability exists in regulation rates, it may be less noticeable during this life stage (Campana 1999).

These findings indicate the possible existence of distinct population contingents of snowy grouper that appear to experience different environmental histories or exposures. 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. Physiochemical 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 potentially 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 from different geographic regions exhibited distinct otolith element:Ca signatures, suggesting the presence of unique population components within the region investigated. The potential for semi-isolated sub-populations of snowy grouper with limited connectivity and different demographic characteristics (i.e., metapopulation), challenges the single-stock management approach and may lead to increased likelihood of localized overexploitation or extirpation of vulnerable stock components.

CHAPTER V

AGE, LENGTH, AND MORTALITY OF WARSAW GROUPER, *HYPORTHODUS NIGRITUS*, IN THE GULF OF MEXICO

Introduction

Warsaw grouper, *Hyporthodus nigritus,* (F. Epinephelidae) are found throughout the western Atlantic Ocean, ranging from northern United States (U.S.) to south of Rio de Janeiro, Brazil, including throughout the Gulf of Mexico (GoM) and Caribbean Sea basins (Manooch & Mason 1987, Farmer & Karnauskas 2013, Aguilar-Perera et al. 2019). Because they exhibit periodic life history traits (e.g., slow growth, late maturity, episodic recruitment), Warsaw grouper are considered highly vulnerable to exploitation pressure due to their low naturality mortality and long generation interval (Winemiller & Rose 1992, Coleman et al. 2000). Perceived vulnerability combined with a population assessment indicating the population was overfished has led to strict regulations to avoid overexploitation of Warsaw grouper in all U.S. territorial waters (GMFMC 1999). Even with strict species-specific fishing regulations in place, Warsaw grouper habitat overlaps with commonly targeted congeners and release mortality is very high (Shertzer et al. 2018, Runde et al. 2020, Paxton et al. 2021), thereby highlighting the need for increased information on the demographics of this data-deficient species (Aguilar-Perera et al. 2019).

In U.S. territorial waters, Warsaw grouper are currently managed as two separate populations, one along the eastern seaboard where a year-round catch moratorium is in place and one in the GoM. However, the patchy and limited distribution of suitable habitat within these large geographic areas may support the development of spatially discrete populations (Hanski

1998, Koenig & Coleman 2013). A recent population structure assessment using chemical markers in otoliths suggests that individual movement of this species is limited across the GoM, suggesting that subpopulations may exist within this region (Sanchez et al. 2020). Under the assumption of a single well-mixed population in the GoM, previous stock assessments have not attempted to develop region-specific population demographics (e.g., growth, mortality, etc.). If population demographics differ among geographic regions, then a basin-wide total allowable catch may lead to extirpation of discrete subpopulations unable to sustain exploitation pressures (Kritzer & Sale 2004, Ying et al. 2011). This can be especially true if subpopulations exhibit limited connectivity (Holland & Herrera 2012). For a metapopulation sustained through episodic recruitment, extirpation of subpopulations can decrease the frequency of successful year-classes, further compounding deleterious effects of age truncation that often results from the overexploitation of a slow life history species (Longhurst 2002, Smedbol & Wroblewski 2002)

To address the potential for dissimilar population demographics among potentially discrete subpopulations in the GoM, regional age-length relationships and associated population parameters of Warsaw grouper were developed and compared for the northwest GoM (nwGoM), northcentral GoM (ncGoM), northeast GoM (neGoM), and the southeast GoM (seGoM). GoM-wide and regional age-length data were fitted to a von Bertalanffy growth function (VBGF) (von Bertalanffy 1938) to model fish growth and determine if regional differences exist in growth parameters (L_∞, K). In addition, declines in the log abundance on age were used to estimate total mortality (Z) of Warsaw grouper in the GoM. Using region-specific VBGF, age composition of the commercial catch of Warsaw grouper was assessed from 2001-2006 and 2011-2016 to expose any potential changes in the fishery between the two decades.

Methods

Archived otoliths from Warsaw grouper were provided by the Panama City Laboratory of the National Oceanic and Atmospheric Administration (NOAA) Southeast Fisheries Science Center. Otoliths were collected by NOAA Fisheries' observers from fishery dependent and fishery independent surveys in the GoM from 2011-2016. Collected otoliths were initially rinsed, dried, and archived in paper envelopes at the Panama City Lab, Florida. Additional otoliths were provided by the Louisiana Department of Wildlife and Fisheries, Texas A&M University-Corpus Christi, and Texas A&M University at Galveston (TAMUG). Otoliths were weighed (mg), embedded in Struers epoxy resin, and sectioned at 1.0 mm thickness with a Buehler ISOMET saw in the Fisheries Ecology Laboratory at TAMUG. Otolith cross-sections were mounted onto petrographic slides with Crystalbond 509 thermoplastic glue and polished using a series of 320-, 600-, and 800-grit sand paper to a thickness between approximately 0.5-0.8 mm until the core and growth increments were clearly visible. Age was determined for each otolith by enumerating growth increments using a previously validated method (Sanchez et al. 2019).

Age-length data for Warsaw grouper from the GoM were fitted to a VBGF (Eq.1) using the non-linear least squares method with 10,000 Monte Carlo simulations designed for data-limited stocks (Sparre & Venema 1998) to estimate growth parameters L_∞ (cm) and K with confidence intervals (RStudio, package "TropFishR"). Relationships between otolith mass and fish age were tested using a linear regression (RStudio, package "nlme").

Eq. 1. $$L_t = L_\infty(1 - e^{(-K(t-t_0))})$$

Age-length data were then organized into the four geographic regions to estimate region-specific growth rates: nwGoM (Texas continental shelf), ncGoM (Louisiana to Mobile Bay, AL), neGoM (Mobile Bay, AL to Tampa Bay, FL), and the seGoM (Tampa Bay, FL through the Florida Keys). Region-specific VBGFs were based on Warsaw grouper less than age-25 because fish with older than 25 years were rare or deficient in certain regions (RStudio, package “FSA”). Samples were also pooled into two larger regions—western GoM (nwGoM + ncGoM) and eastern GoM (neGoM + seGoM)—to further evaluate the influence of the entire age range on growth parameters.

Region-specific and overall GoM total instantaneous mortality rates (Z) were estimated with a traditional regression catch-curve analyses on age frequency data. For each catch-curve model, age-composition of the catch was binned by year (age) and the frequency (f) of each age was natural log transformed [ln(f)]. Natural log transformed frequency was then plotted by age and a linear regression was run through points beginning with the age with the highest catch frequency under the assumption that after this point gear selectivity and natural mortality are constant. Differences in regression slopes (Z) from the nwGoM, ncGoM, neGoM, and seGoM were analyzed using an ANCOVA (RStudio, package “stats”).

Length-frequency data of the 2001-2006 and 2011-2016 commercial fisheries were compared to assess changes in the fishery over the last two decades. Data were separated into catches from the wGoM and eGoM based on either the documented state that fish were landed (2001-2006) or shrimp zone of the catch (2011-2016) and converted to age by rearranging VBGFs; shrimp zone of catch was not available for a large proportion of the 2001-2006 catch data. Age-frequencies were developed for each region using region-specific (wGoM or eGoM) growth parameters. Age composition of the catch was compared between the two fishery periods using Kolmogorov-Smirnov tests, both for each region individually and with data for both regions pooled (RStudio, package “FSA”).

Results

Ages were determined for a total of 497 otoliths from Warsaw grouper collected between 2011 and 2018. Mean age was 9.2 ± 6.9 years with a range from age-1 to age-91 (mj 10). No difference in mean age was detected among the 4 regions (ANOVA, $F_{3,493} = 1.9$, $p < 0.05$); however, region-specific differences in length were detected with Warsaw grouper in our sample larger in the nwGoM and seGoM (101.8 cm and 104.8 cm TL) than the ncGoM and neGoM (94.5 cm and 90.6 cm TL) (ANOVA, $F_{3,493} = 9.1$, $p < 0.05$). The linear relationship between otolith mass and fish age was significant ($F_{1,301}= 1303$, $p < 0.001$) and resulted in linear model *Age (years) = 33.4 x Otolith Mass (g) - 3.2* (Figure 16).

Table 10. Summary statistics of age data for the entire basin (GoM) and individual regions, including estimated total mortality rates (Z) from age-based catch-curves. Group_L and Group_A show statistically significant groupings of regions for lengths and ages, respectively, (Tukey HSD, $p < 0.05$).

Region	n	Length (mm)	Group_L	Age	Group_A
GoM	497	970 ± 192	--	9.2 ± 6.9	--
nwGoM	102	1018 ± 233	A	10.2 ± 8.0	A
ncGoM	295	945 ± 149	B	8.8 ± 6.1	A
neGoM	38	906 ± 222	B	8.3 ± 8.9	A
seGoM	62	1048 ± 238	A	10.4 ± 6.9	A

Growth parameters for the VBGF for the whole GoM were $L_\infty = 188.8$ cm (CI: 168-217) and $K = 0.034$ (CI: 0.02-0.05) (Figure 17). When the sample was split into the eastern GoM and western GoM, L_∞ and K were not statistically different between the eastern GoM [$L_\infty = 176.3$ cm (149-230 cm), $K = 0.049$ (0.02-0.08)] and western GoM [$L_\infty = 192.3$ cm (167-229 cm), $K = 0.031$ (0.04-0.12)] (Figure 18). Regional-specific growth parameters from the VBGF for fish

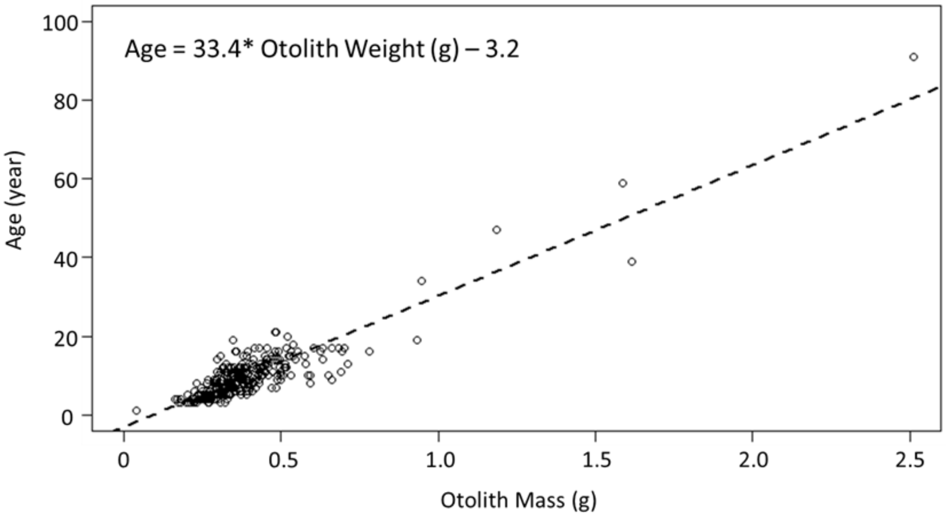


Figure 16. Linear regression between otolith mass (g) and age (years) for Warsaw grouper in the Gulf of Mexico. Linear model was significant (RSE = 3.2, df = 301, p < 0.001) and explained a large proportion of variability (R^2 = 0.81).

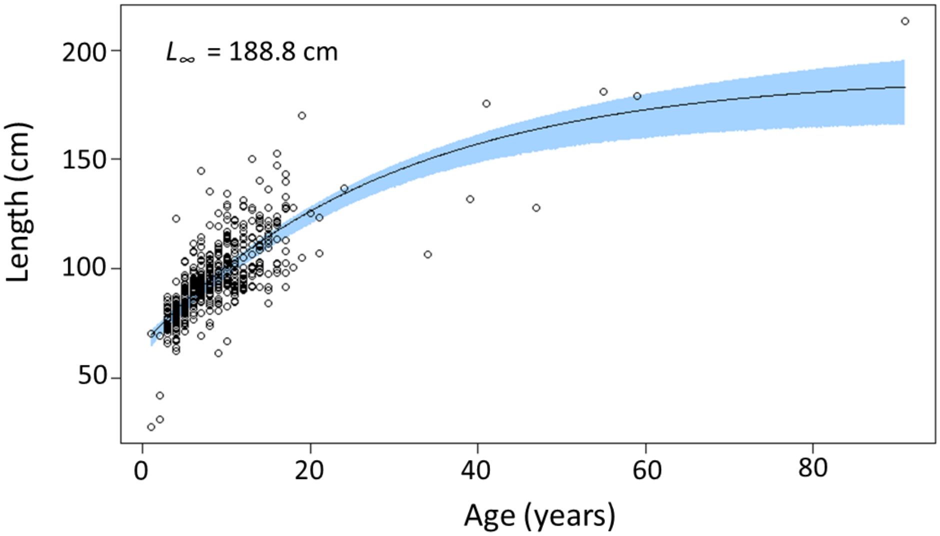


Figure 17. von Bertalanffy growth function and confidence interval (blue area) for the entire Gulf of Mexico population plotted using growth parameters developed with the non-linear least-squares method with 10,000 Monte Carlo simulations (L_∞ = 188.8 cm, K = 0.034).

less than age-25 varied among the four regions, with L_∞ highest and K lowest for Warsaw grouper from the seGoM (L_∞ = 153.3 cm, K = 0.077) (Figure 19), however statistical comparison with this region was not possible due to an inability to develop confidence intervals from the sample. Growth parameters were relatively similar for individuals collected from the other three regions in the northern GoM: nwGoM (L_∞ = 123.1 cm, K = 0.188), ncGoM (L_∞ = 117.5 cm, K = 0.121), neGoM (L_∞ = 113.2 cm, K = 0.156).

Total instantaneous mortality (Z) estimated from catch-curve analysis for Warsaw grouper from the GoM was 0.12 y^{-1} (Table 11), with Z in the western GoM (0.17 y^{-1}; Figure 18B) significantly higher than in the eastern GoM (0.08 y^{-1}; Figure 18C) (ANCOVA, $F_1 = 10.5$, $p < 0.01$). Regional-specific catch-curve slopes and therefore total instantaneous mortality rates were significantly different (ANCOVA, $F_3 = 6.7$, $p < 0.01$) with Z higher in the ncGoM (0.18 y^{-1}; Figure 20B), than the nwGoM (0.09 y^{-1}; Figure 20A), neGoM (0.12 y^{-1}; Figure 20C), and seGoM (0.06 y^{-1}; Figure 20D) (Tukey HSD, $p < 0.05$).

Table 11. Growth parameters from the von Bertalanffy Growth Function (L_∞, *K*) and associated natural mortality (*M*) rates calculated for the different regional groupings of age data in the Gulf of Mexico. The Hoenig$_{nls}$ *M* was estimated using a t_{max} = age-91. All *M* estimates from 2-region groups were calculated with the Pauly$_{nls}$ equation. For the 4-region grouping, samples only included data from fish estimated <age-25.

Grouping	Region	L_∞	K	Z	M
GoM	Hoenig$_{nls}$	188.8	0.034	0.12	0.079
	Pauly$_{nls}$				0.062
2-Region	wGoM	192.3	0.031	0.17	0.058
	eGoM	176.3	0.049	0.08	0.083
4-Region	nwGoM	123.1	0.188	0.09	
	ncGoM	117.5	0.121	0.18	
	neGoM	113.2	0.156	0.12	
	seGoM	153.3	0.077	0.06	

Age and mortality estimates derived for Warsaw grouper from the commercial fishery in the GoM indicated that the majority of fish were less than 10 years of age. For the 2001-2006 sample, mean age was 4.7 ± 8.3 years with a range from 0 to 100 years. For 2011-2016, mean fish age was 7.6 ± 6.4 years with a range from 0 to 58 years. Mean age of Warsaw grouper collected from the commercial fishery in 2011-2016 was higher across the entire GoM (KS Test, D = 0.33, $p < 0.001$) and for both the eastern GoM (KS Test, D = 0.31, $p < 0.001$) and western GoM (KS Test, D = 0.35, $p < 0.001$) independently. Age-based catch curves led to identical instantaneous total mortality rates ($Z = 0.23\ y^{-1}$) between the 2001-2006 collection period and the 2011-2016 collection period (Figure 21).

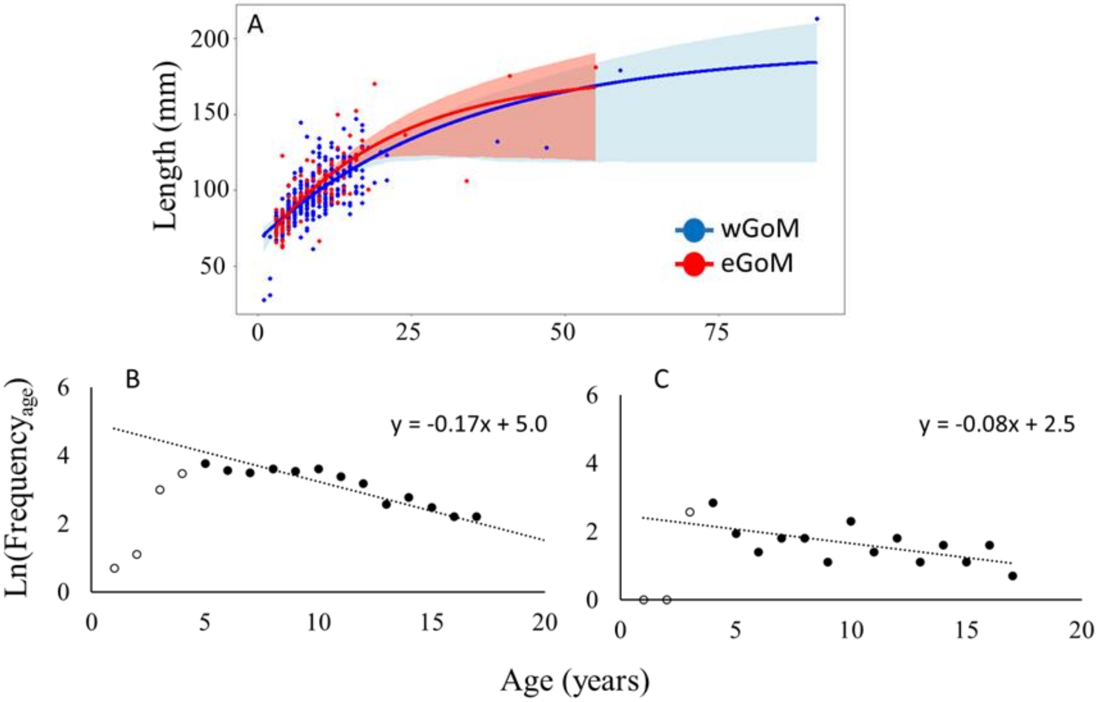


Figure 18. A) von Bertalanffy growth functions and age-based catch curves for age-length samples from the B) western Gulf of Mexico (wGoM) and (C) and eastern Gulf of Mexico (eGoM). Slope of the catch curve represents total mortality estimate (*Z*) for populations from the specific regions.

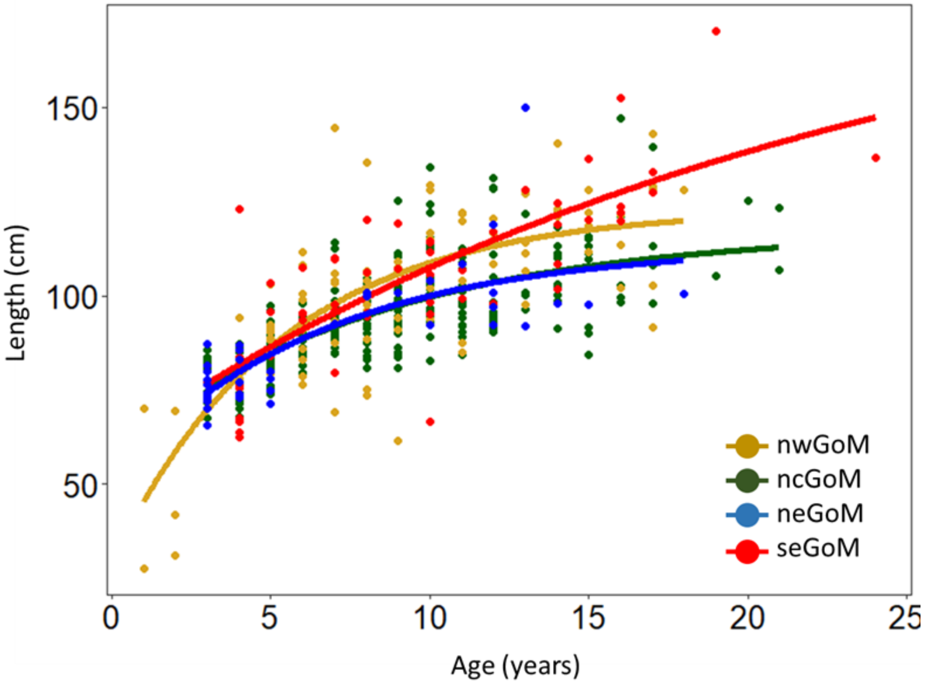


Figure 19. Region-specific von Bertalanffy growth functions and growth parameters A) northwest Gulf of Mexico (L_∞ = 123.1 cm, K = 0.188), B) northcentral Gulf of Mexico (L_∞ = 117.5 cm, K = 0.121), C) northeast Gulf of Mexico (L_∞ = 113.2, K = 0.156), and D) southeast Gulf of Mexico (L_∞ = 153.3, K = 0.077).

Discussion

The age-length relationship developed here for Warsaw grouper indicates that the population(s) in the GoM are long-lived, have low growth coefficients (K), and large asymptotic length (L_∞) relative to other exploited species in the western Atlantic Ocean (Wilson & Nieland 2001, Harris et al. 2011), including shallow water groupers (Hood & Schleider 1992, Crabtree & Bullock 1998, Lombardi-Carlson et al. 2008). The low K value for Warsaw grouper reveals that growth to asymptotic length is slow regardless of geographic location in the GoM, with estimates of L_∞ and K similar to Warsaw grouper in the western Atlantic Ocean along the U.S. eastern seaboard (Manooch & Mason 1987). These K and L_∞ estimates in the current study are considerably lower and higher, respectively, than a recent estimate for Warsaw grouper in the

GoM that estimated *K* and L_∞ to be 0.14 and 153.3 cm using a Bayesian parameter estimation method and simulated length-age data (Barnett et al. 2020). Large individuals were rare in both studies, a potential result of size truncation from overexploitation and preferential selection of large individuals, which can lead to correspondingly low L_∞ estimates when the parameter estimation method accounts for overexploitation (Taylor et al. 2005, Cooper et al. 2013). However, the common occurrence of large individuals in the recreational fishery (>200 cm TL) and the typical slow-growth of deepwater species suggest the lower *K* and larger L_∞ estimates are reasonable for the population in a more virgin state (Caillet et al. 2001, King & McFarlane 2003, Clark 2009).

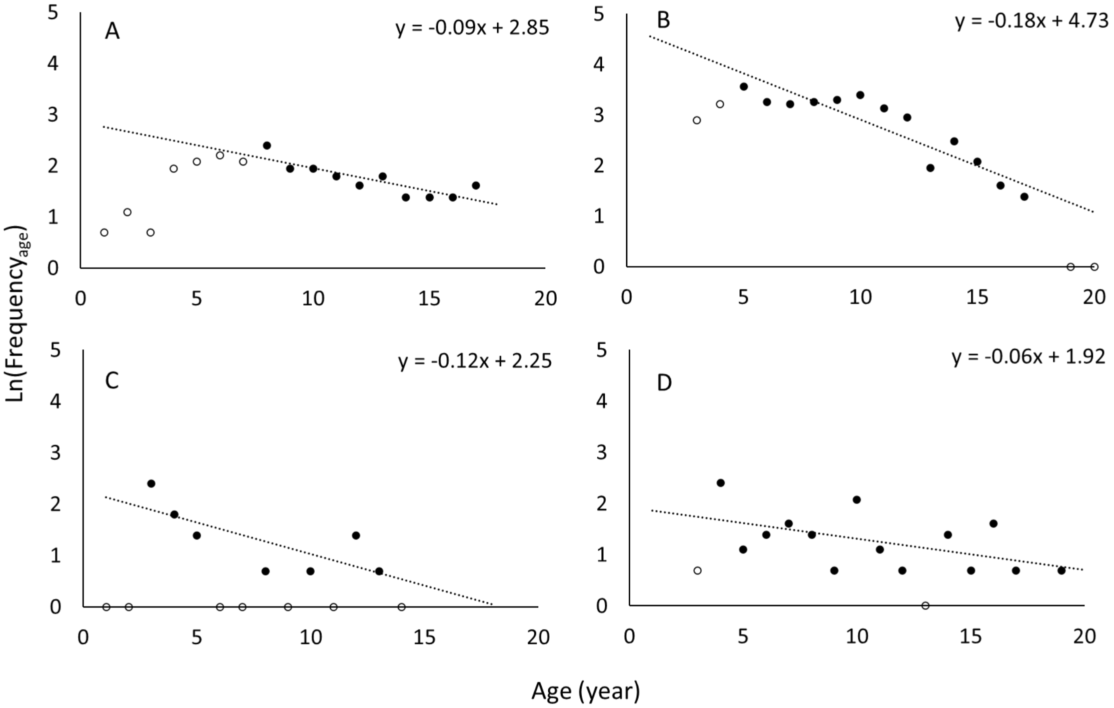


Figure 20. Age-based catch curves for age-length samples from the A) northwest Gulf of Mexico, B) northcentral Gulf of Mexico, C) northeast Gulf of Mexico, and D) southeast Gulf of Mexico. Slope of the catch curve represents total mortality estimate (*Z*) for populations from the regions.

A noteworthy finding from our study is the potential increase in longevity for Warsaw grouper, with the oldest fish in our sample (age-91) 30 years older than estimates in recent age validation studies (Sanchez et al. 2019, Barnett et al. 2020). While this marked increase in maximum age may seem surprising, this individual was 34.2 cm TL larger and had an otolith mass nearly 60% heavier than an age-59 fish previously validated with a bomb-radiocarbon analysis (Sanchez et al. 2019). Furthermore, longevity approaching 100 years is not uncommon for deepwater species (Caillet et al. 2001, Munk 2001, Horn et al. 2012) and longevity of 80 or more years has been reported for other species in the deepwater grouper complex using bomb

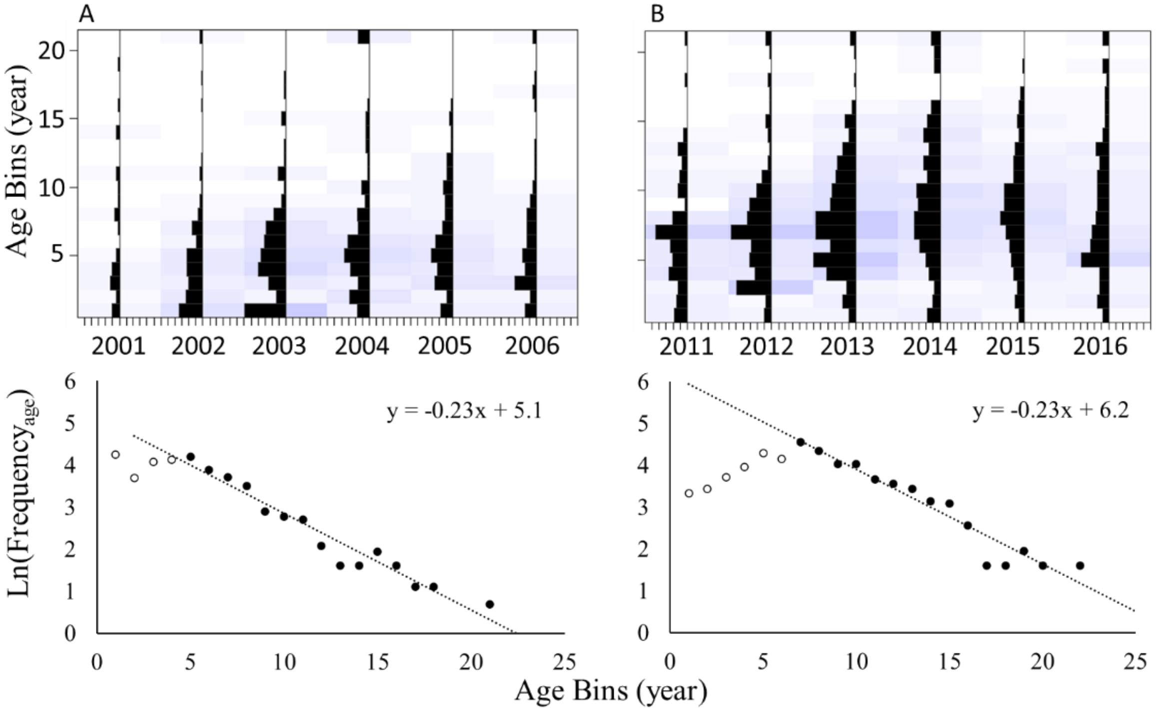


Figure 21. Annual age composition and catch curves from all years pooled from 2001-06 (A) and 2011-16 (B) in NOAA samples for the Warsaw grouper commercial fishery. Age was estimated from length data using rearranged von Bertalanffy Growth Functions developed for the eastern Gulf of Mexico and western Gulf of Mexico in this study. Slope of the catch curve represents total mortality estimate (*Z*) for populations from the timeframes.

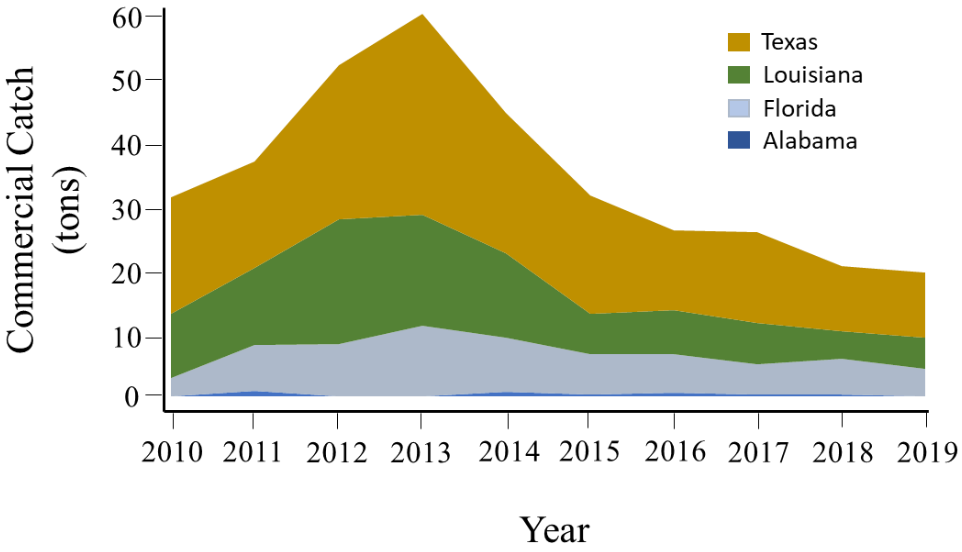


Figure 22. Commercial catch totals (tons) of Warsaw grouper by state landed since the Deepwater Grouper – Tilefish Individual Fishing Quota Fishery Management Program was implemented in 2010. Landing states Texas and Louisiana roughly correspond to sampling regions nwGoM and ncGoM, respectively, and combined comprise the western GoM.

radiocarbon validation techniques (Cook et al. 2008, Andrews et al. 2013, Sanchez et al. 2019). Such an increase in longevity would substantially decrease current SEDAR (Southeast, Data, and Assessment Review) natural mortality estimates for the species which use the Hoenig and Hewitt (1993) estimate, from 0.10 y^{-1} (SEDAR 2004) to 0.05 y^{-1} (our data).

Natural mortality estimates (M) using the modified $\text{Hoenig}_{\text{nls}}$ (0.079 y^{-1}) and modified $\text{Pauly}_{\text{nls}}$ (0.062 y^{-1}) equations (Then et al. 2014) indicate high vulnerability of Warsaw grouper to exploitation pressure and were well below the basin-wide instantaneous total mortality (0.12 y^{-1}). When grouped into larger eastern (neGoM, seGoM) and western (ncGoM, nwGoM) regions with VBGFs fitted to the entire age composition of Warsaw Grouper, the natural mortality rate was lower in the western GoM (0.058 y^{-1}) than eastern GoM (0.083 y^{-1}), potentially indicating decreased resilience to exploitation (Pauly 1980, Gislason et al. 2010). The lower M estimate derived from the western GoM using the VBGF growth parameters method corresponds to the region that catch curves indicated higher total mortality (Z) and that has contributed most to the commercial fishery since the deepwater grouper fishery was changed to an Individual Fishing Quota Fishery Management Program in 2010 (Figure 22; Courtesy: NOAA Fisheries). Of additional concern, catch totals from the western GoM have decreased substantially since peaking in 2013, even while the total allowable catch has remained constant. It should be noted, however, that this decrease in catch does not necessarily indicate a decrease in catch per unit effort which was not calculated.

Region-specific (4-regions) comparisons for Warsaw grouper <age-25 indicated that age-length relationships were potentially distinct in the seGoM while similar through the northern GoM regions (nwGoM, ncGoM, neGoM). While a statistical comparison was not possible with the seGoM using the least squares method, this only occurred because the relationship with the age-length relationship was too linear to develop confidence intervals, thereby suggesting growth rates remained high for individuals in the seGoM through age-25. Visual inspection of VBGFs from all four regions indicates that growth rates appear to slow down for the regions in the nwGoM, ncGoM, and neGoM well before age-25. Furthermore, fish from the seGoM experience unique chemical histories when compared to the other regions in the (Sanchez et al. 2020). This combination of dissimilar otolith chemistry and increased growth rates highlight the region as a potential subpopulation with discrete population demographics.

The predicted age-composition of the Warsaw grouper from the commercial fishery changed substantially over the last two decades based on length-based converted ages. From 2001-2006 the fishery was comprised primarily of Warsaw grouper less than age-5 (mean: 4.7 years) while fish were significantly older (mean: 7.8 year) in the 2011-2016 fishery. The observed increase in age between the assessment time frames may be due to a reduction in fishery pressure as a result of regulation changes in 1999 that limited the recreational catch of Warsaw grouper to one fish per boat per day (GFMFC 1999). The decrease in fishing mortality likely increased the presence of older fish in more recent years and is a more likely cause than exploitation induced shifts in growth rates between the two periods (Hilborn & Minte-Vera 2008). It is also possible that changes in fishing methods may contribute to a shift in age-distribution of the catch between the two time periods. Average depth of gear deployment increased from 100 m from 2001-2006 to 110 m from 2011-2016. The shift to deeper sets could result in the targeting of larger individuals since Warsaw grouper may undergo an ontogenetic shift to deeper depths with age (Barnett et al. 2020) or could represent a move to new fishing grounds with more virgin stocks. Regardless, the 2011-2016 fishery was mostly composed of fish less than age-10 (75%) and almost exclusively of fish less than age-20 (96%), an indication that past catch rates could have led to age-truncation of the population (Secor et al. 2015). For a species that relies on episodic recruitment success during years with favorable conditions, reestablishment of the full age-structure is critical and could take decades for such a long-lived species (Berkeley et al. 2004, Russ & Alcala 2004, van Gemert & Anderson 2018).

Here, I demonstrate that Warsaw grouper are longer-lived than previously considered but that the population in the GoM appears to be in a rebuilding state following changes in conservation policy (Manooch & Mason 1987, Hewitt & Hoenig 2005, Barnett et al. 2020). While average size of fish in the commercial fishery has increased significantly over the last two decades, past overexploitation has likely led to size and age truncation in the population and decreased resilience to stochastic events (Cooper et al. 2013, Rouyer et al. 2013, Secor et al. 2015). While increased size-composition of the commercial catch may be linked to changes in fish methods, it is just as likely that changes in management policy could have led to increased survivorship and an age-structure composed of relatively older fish. However, the small proportion of larger, older Warsaw grouper in the sample and commercial catch composition supports the need for continued strict management to allow the virgin age-structure to be reestablished.

CHAPTER VI

CONCLUSIONS

Effective management policy for targeted fishery species requires an understanding of fundamental population demographics for exploited stocks (age-length relationships, population structure, reproductive strategy, etc.). Unfortunately, for many some exploited species this information is currently unavailable due to logistical (access, costs, etc.) and methods limitations. This data-limitation is particularly troubling for slow life history species due to their increased vulnerability to exploitation pressure. Characterized by slow growth, long life spans, and late maturity, they are inherently vulnerable to fishery practices that target the largest and oldest individuals leading to age and size truncation of the populations, decreasing reproductive fitness and recruitment success, and thereby jeopardizing the replacement of exploited individuals in successive generations. The development of accurate population demographics for deepwater species has been particularly difficult using traditional assessment techniques due an increase in spatiotemporal stability in physicochemical conditions and high release mortality as a result of the effects of barotrauma.

In contrast to shallow-water and nearshore populations, a lack of seasonal temporality in water temperature of deepwater environments limits predictable variability in seasonal growth, and therefore decreased consistency of annual formation of growth increments. This has led to the development of highly inaccurate age-length relationships and longevity estimates for deepwater species that in turn have led to concerns in fishery parameters that influence management policy. In chapter II, these concerns with Warsaw grouper and snowy grouper were address via a bomb radiocarbon age validation which led to the development of new longevity estimates and a rethinking of their age-length relationships. For both species, longevity estimates are particularly important since the NOAA stock assessment group uses this parameter to estimate species-specific natural mortality rates and sustainable catch limits. Through the bomb radiocarbon age validation, each species was validated to have a longevity to at least age-56, with secondary evidence suggesting the maximum age for both likely to reach 80-90 years. These validated ages substantially increased the estimated longevities for Warsaw and snowy grouper, 41 and 35 years respectively, previously used in SEDAR stock assessments, and will increase the effectiveness of fishery parameters developed in future stock assessments used to calculate fishery catch quotas.

The effects of changes in ambient pressure between the surface and depths habited by Warsaw and snowy grouper prevent the use of any tagging related population structure analysis due to the very high release mortality that results from barotrauma in the gas bladder. Utilizing natural tags, the first attempts at defining the population structure in Warsaw grouper and snowy grouper were conducted in chapter III and IV, respectively, via comparisons of the otolith chemistry of fish collected from different geographic regions in the GoM. For both species, regional differences in otolith chemistry closely reflected expected spatial patterns in environmental chemistry, thereby indicating the chemical history of fish closely resembled local ambient conditions. Unsurprisingly, the influence of the MARS was substantial throughout the northern GoM, with the magnitude of its influence inversely related with distance from its inflow region. While classification success of individuals to collection region was only moderate for both species, the level of success suggests that population structure is more complex than previously considered, especially since spatial heterogeneity in deepwater physicochemical conditions is more limited than in surface waters. Nevertheless, nearshore processes were evident in the otolith chemistry of deepwater Warsaw grouper and snowy grouper (though with decreased magnitude of influence). The results of Chapters III and IV in this dissertation support the premise of multi-stock management framework for both Warsaw grouper and snowy grouper in the Gulf of Mexico.

Lastly, the development of region-specific age-length relationships for Warsaw grouper allowed for the estimation of growth parameters of potential subpopulations to determine if growth rates and natural mortality rates were comparable throughout the GoM. Region-specific von Bertalanffy growth functions and associated growth parameters indicated that growth rates were slower and natural mortality rates lower in regions in the western GoM when compared to the eastern GoM. In addition, significant differences in total mortality rates between the two regions indicate suggesting that exploitation rates are higher in the western GoM, matching the disparity in contribution of the two regions commercial fishery, of which a large majority of the catch occurs in the less resilient western GoM.

The objective of this dissertation was to address critical knowledge gaps in basic population demographics of Warsaw and snowy grouper to inform future stock assessments and fishery management policy. These results provide an accurate method of age determination for both species which highlighted shortcomings in traditional techniques and will improve age-length relationships in the GoM moving forward, including for potentially discrete populations. The analysis of geographic differences in growth rates for Warsaw grouper in the eastern and western GoM indicate that potential differences exist and that a reanalysis of management policy is warranted for both species in order to assist in the development of effective conservation measures that will ensure long-term stability of the populations for future generations.

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APPENDIX A

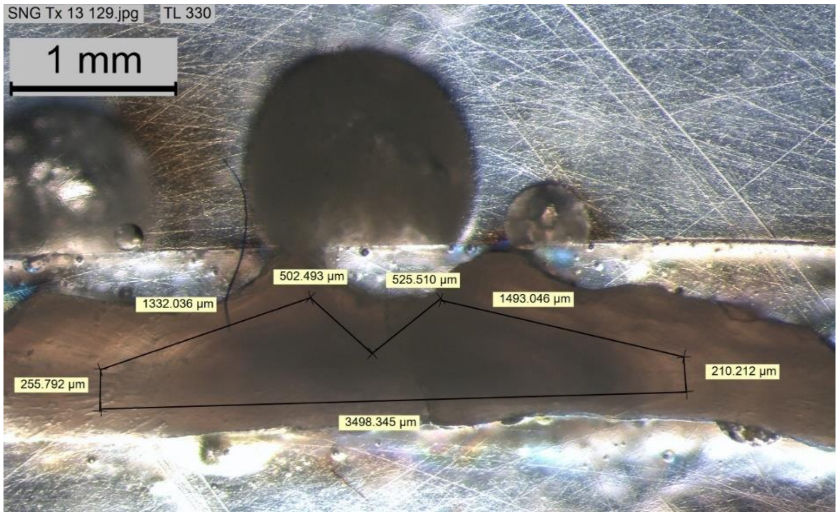

Figure A-1. An example of core template measurements on an age-1 Snowy Grouper *Hyporthodus niveatus*. Actual mill template extended 150 µm from measurements to account for the 150-µm radius drill bit.

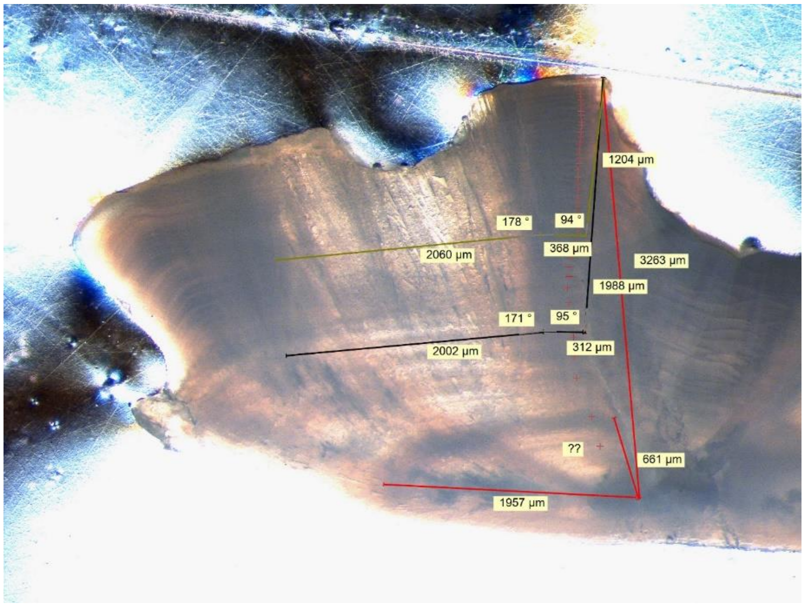

Figure A-2. An example of growth increment transect measurements on a validated age-38 Warsaw Grouper *Hyporthodus nigritus* WRG18 (1.22 g otolith). The two transects targeted the 5-year and 15-year growth increments. Measurements from the core assist in early growth increment interpretation. Annuli are visible up the slow growth axis and become compact around year 10.

APPENDIX B

Table B-1. Mean element:Ca and stable isotope ratios for $\text{otolith}_{\text{core}}$, $\text{otolith}_{\text{edge}}$, and $\text{otolith}_{\text{lifetime}}$ signatures from Warsaw grouper collected in the northwest Gulf of Mexico (NW), northcentral Gulf of Mexico (NC), northeast Gulf of Mexico (NE), and southeast Gulf of Mexico (SE).

		NW	NC	NE	SE
Core (‰)	^{7}Li:Ca	4.98 (0.80)	4.86 (1.43)	5.39 (0.88)	5.12 (1.16)
	^{24}Mg:Ca	140.8 (24.7)	150.9 (23.2)	144.2 (30.5)	136.4 (21.1)
	^{55}Mn:Ca	2.55 (0.91)	3.09 (1.32)	2.26 (1.03)	1.12 (0.38)
	^{59}Co:Ca	0.24 (0.24)	0.25 (0.17)	0.14 (0.14)	0.23 (0.19)
	^{65}Cu:Ca	1.06 (0.64)	0.68 (0.54)	0.92 (0.67)	0.60 (0.67)
	^{66}Zn:Ca	1.29 (0.61)	1.15 (0.55)	1.28 (1.06)	0.88 (0.41)
	^{88}Sr:Ca	2451 (271)	2715 (304)	2579 (390)	2527 (274)
	^{137}Ba:Ca	4.19 (2.09)	5.03 (2.04)	5.07 (3.60)	3.45 (1.94)
Edge (‰)	^{7}Li:Ca	1.91 (2.10)	2.13 (2.35)	1.60 (1.03)	2.68 (3.02)
	^{24}Mg:Ca	46.8 (10.1)	44.5 (10.4)	52.2 (12.3)	46.34 (8.49)
	^{55}Mn:Ca	0.11 (0.28)	0.21 (0.14)	0.19 (0.20)	0.20 (0.23)
	^{59}Co:Ca	0.18 (0.20)	0.25 (0.20)	0.09 (0.20)	0.16 (0.19)
	^{65}Cu:Ca	0.05 (0.33)	0.16 (0.35)	0.31 (0.39)	0.32 (0.38)
	^{66}Zn:Ca	0.52 (0.24)	0.51 (0.38)	0.43 (0.29)	0.44 (0.38)
	^{88}Sr:Ca	2944 (538)	3045 (373)	2731 (409)	2637 (345)
	^{137}Ba:Ca	1.49 (0.61)	1.89 (0.95)	1.36 (0.48)	0.85 (0.18)
Lifetime (‰)	^{7}Li:Ca	3.11 (1.41)	3.09 (1.51)	3.09 (0.31)	3.71 (1.36)
	^{24}Mg:Ca	83.5 (15.7)	81.5 (10.5)	91.8 (22.3)	79.2 (12.7)
	^{55}Mn:Ca	0.88 (0.49)	1.09 (0.49)	0.68 (0.36)	0.48 (0.35)
	^{59}Co:Ca	0.27 (0.19)	0.22 (0.16)	0.12 (0.14)	0.21 (0.15)
	^{65}Cu:Ca	0.50 (0.49)	0.35 (0.35)	0.33 (0.37)	0.39 (0.56)
	^{66}Zn:Ca	0.69 (0.55)	0.53 (0.35)	0.45 (0.33)	0.53 (0.29)
	^{88}Sr:Ca	2652 (220)	2773 (206)	2570 (189)	2459 (192)
	^{137}Ba:Ca	2.57 (0.95)	3.23 (0.93)	2.74 (1.18)	1.95 (0.71)
	δ^{13}C	-3.98 (0.33)	-4.15 (0.34)	-4.09 (0.30)	-4.05 (0.61)
	δ^{18}O	-0.29 (0.27)	-0.41 (0.16)	-0.39 (0.21)	-0.01 (0.28)

APPENDIX C

Table C-1. MANOVA results comparing chemical signatures of otoliths of snowy grouper between the 4-region grouping in the US western Atlantic in this study for otolith$_{life}$, otolith$_{edge}$, and otolith$_{core}$ chemical signatures. Degrees of freedom (DF) are equivalent for all MANOVA tests for each row.

	Otolith$_{life}$		Otolith$_{edge}$		Otolith$_{core}$		DF
Year	Pillai's T	P-value	Pillai's T	P-value	Pillai's T	P-value	(num, den)
2012-13	0.866	< 0.001	0.852	< 0.001	0.507	< 0.001	15, 381
2014-15	0.697	< 0.001	0.640	< 0.001	0.349	< 0.01	15, 297

Table C-2. ANOVA results for otolithl$_{ife}$, otolith$_{core}$, and otolith$_{edge}$ chemical signatures assessing an interaction between region and year (Region x Year) for snowy grouper 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^{13}C and Δ^{18}O for all years pooled.

			Region (df=3)		Year (df=3)		Region x Year (df=9)	
			F-value	p-value	F-value	p-value	F-value	p-value
A	Life	Li:Ca	34.16	**< 0.001**	4.43	**< 0.05**	2.49	> 0.05
		Mg:Ca	11.73	**< 0.001**	0.03	> 0.05	0.37	> 0.05
		Mn:Ca	13.91	**< 0.001**	6.97	**< 0.01**	0.95	> 0.05
		Sr:Ca	25.87	**< 0.001**	0.00	> 0.05	2.99	**< 0.05**
		Ba:Ca	5.47	**< 0.01**	7.69	**< 0.01**	4.29	**< 0.01**
		Δ^{13}C*	8.76	**< 0.001**	NA	NA	NA	NA
		Δ^{18}O*	8.31	**< 0.001**	NA	NA	NA	NA
B	Core	Li:Ca	7.16	**< 0.001**	1.74	> 0.05	0.88	> 0.05
		Mg:Ca	6.99	**< 0.001**	0.01	> 0.05	0.70	> 0.05
		Mn:Ca	10.91	**< 0.001**	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
C	Edge	Li:Ca	49.71	**< 0.001**	6.54	**< 0.05**	1.12	> 0.05
		Mg:Ca	5.20	**< 0.01**	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	**< 0.001**	0.01	> 0.05	1.08	> 0.05
		Ba:Ca	7.25	**< 0.001**	1.14	> 0.05	1.27	> 0.05

Table C-3. ANOVA results for otolith$_{\text{life}}$, otolith$_{\text{core}}$, and otolith$_{\text{edge}}$ chemical signatures assessing an interaction between region and year (Region x Year) for snowy grouper collected in 2014 and 2015.

			Region (df=3)		Year (df=3)		Region x Year (df=9)	
			F-value	p-value	F-value	p-value	F-value	p-value
A	Life	Li:Ca	22.65	**< 0.001**	33.85	**< 0.001**	35.25	**< 0.001**
		Mg:Ca	1.47	> 0.05	6.61	**< 0.05**	3.78	**< 0.05**
		Mn:Ca	8.33	**< 0.001**	0.89	> 0.05	2.57	> 0.05
		Sr:Ca	4.49	**< 0.01**	2.63	> 0.05	2.60	> 0.05
		Ba:Ca	3.62	**< 0.05**	3.01	> 0.05	0.94	> 0.05
B	Core	Li:Ca	2.18	> 0.05	9.11	**< 0.01**	7.17	**< 0.001**
		Mg:Ca	0.73	> 0.05	0.02	> 0.05	2.17	> 0.05
		Mn:Ca	5.67	**< 0.01**	4.16	**< 0.05**	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
C	Edge	Li:Ca	37.69	**< 0.001**	51.27	**< 0.001**	54.95	**< 0.001**
		Mg:Ca	4.20	**< 0.01**	6.29	**< 0.05**	3.79	**< 0.05**
		Mn:Ca	3.01	**< 0.05**	1.01	> 0.05	1.30	> 0.05
		Sr:Ca	10.77	**< 0.001**	2.38	> 0.05	3.05	**< 0.05**
		Ba:Ca	2.67	> 0.05	4.92	< 0.05	2.04	> 0.05