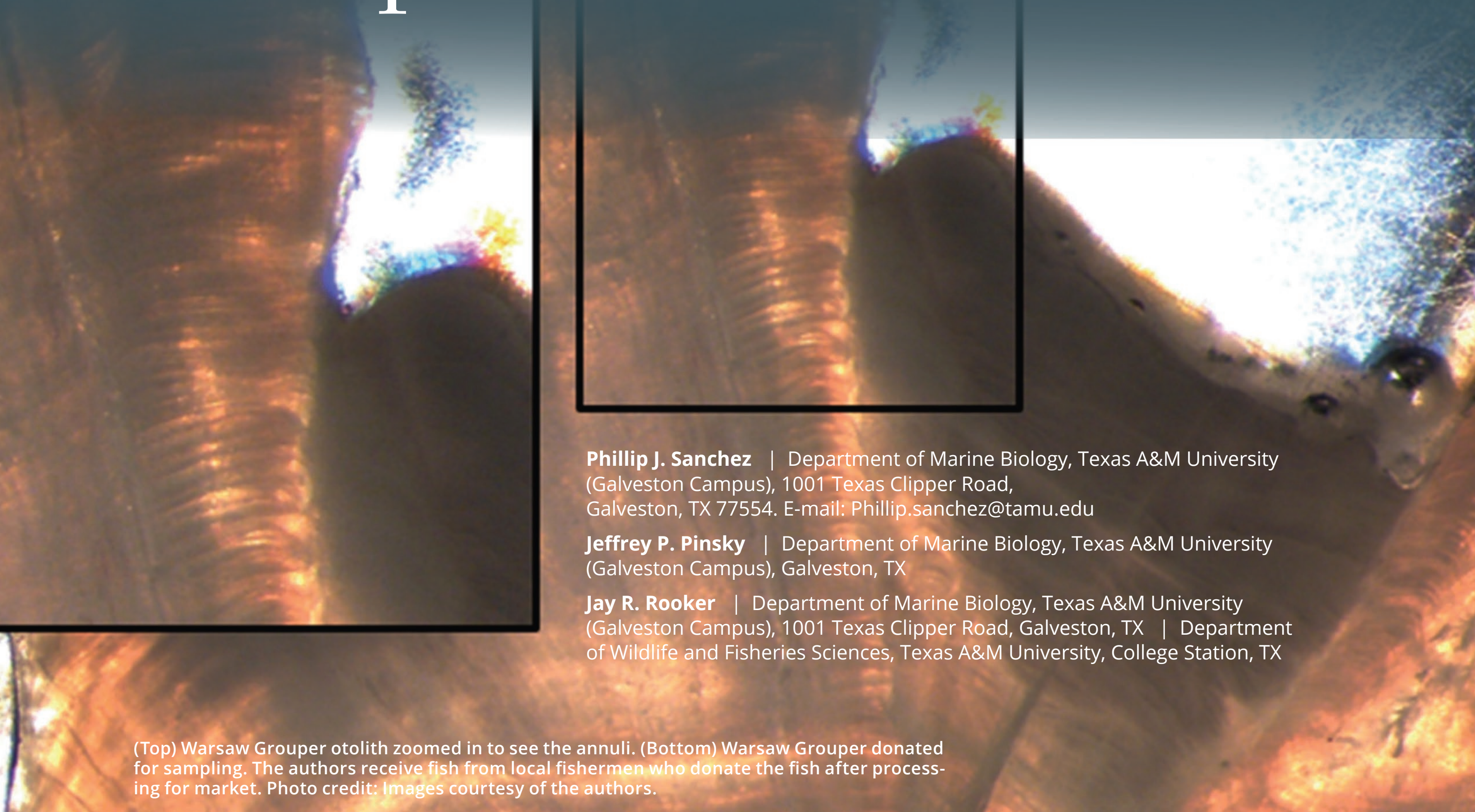
FEATURE

Bomb Radiocarbon Age Validation of Warsaw Grouper and Snowy Grouper



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(Top) Warsaw Grouper otolith zoomed in to see the annuli. (Bottom) Warsaw Grouper donated for sampling. The authors receive fish from local fishermen who donate the fish after processing for market. Photo credit: Images courtesy of the authors.

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Current stock assessments for both the Warsaw Grouper *Hyporthodus nigritus* and the Snowy Grouper *H. niveatus* are based on age-structured population models determined using traditional otolith-based aging techniques. However, recent studies using bomb radiocarbon validation have shown that many deepwater fishes live much longer than previously estimated when relying on conventional age determination methods. In this study, we conducted bomb radiocarbon age validations of Warsaw Grouper and Snowy Grouper from the Gulf of Mexico. Radiocarbon age validation supported annual growth increment formation for all Warsaw Grouper size classes and medium-sized Snowy Grouper. Conversely, ages of larger, older Snowy Grouper were greatly underestimated due to difficulty in discriminating annuli. This bomb radiocarbon analysis validates a minimum 56-year longevity for both Warsaw Grouper and Snowy Grouper, increasing the currently published longevities of 41 and 54 years, respectively.

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 and Morgan 1987). Assessments depend on accurate population age structures; therefore, the validation of age determination techniques is critical (Beamish and 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 and 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 and 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 and 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).

Global atmospheric atomic weapons tests conducted in the 1940s and 1950s led to a proliferation of the radiocarbon isotope (^{14}C; 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 ($\Delta^{14}C < -50‰$) beginning in the late 1950s, peaked in the early to mid-1970s ($\Delta^{14}C = 120–160‰$; see review by Druffel 1992), and have since undergone a slow decline of approximately $-27‰$ $\Delta^{14}C$ per decade (Moyer and 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 and 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 and 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-H_2O; 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% HNO_3 bath for a minimum of 24 h, triple rinsed with DDI-H_2O, 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 $\Delta^{14}C$ values, representing the per mille deviation from the ^{14}C activity in 19th-century wood corrected for isotopic fractionation.

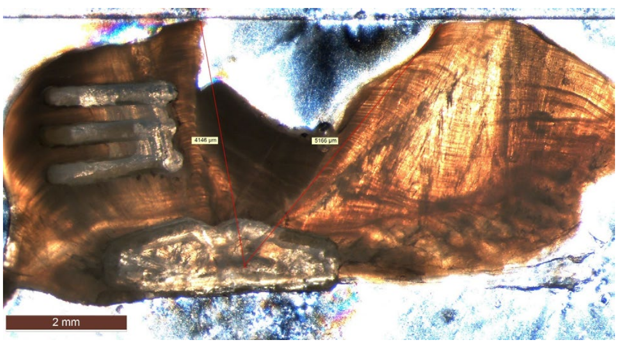

Figure 1. Otolith core and growth increment extraction location and mean sulcus height measurements. Growth increment samples were powdered, 300-µm-wide transects. For the core extraction, only the center portion was removed, and all material within 300 µm of the drill perimeter was powdered and discarded. 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 relationships 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ values were visibly lower than reference values, including the two fish with pre-bomb

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 (years)	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
WRG20	2014	1,405	2,092	0.6340	14	2000	39.80	2.7
WRG21	2016	915	2,008	0.6051	17	1997	59.10	2.0
WRG22	2016	1,430	1,807	0.6825	17	2009	74.40	2.4
Snowy Grouper								
SNG01	1982	330	NA	NA	2	1980	120.78	2.3
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

hatch years. The two individuals with the oldest determined ages (55 and 59 years) had pre-bomb $\Delta^{14}C$ values of −70.6‰ and −68.6‰, which are lower than mean coral $\Delta^{14}C$ 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 $\Delta^{14}C$ values for Warsaw Grouper at the peak of the radiocarbon rise in the 1970s ranged from 101.2‰ to 130.4‰. Otolith core and transect $\Delta^{14}C$ 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 $\Delta^{14}C$ values observed in the otolith cores of the postbomb chronologies developed for Red Snapper and Speckled Hind (ANCOVA slope test: F = 0.35,

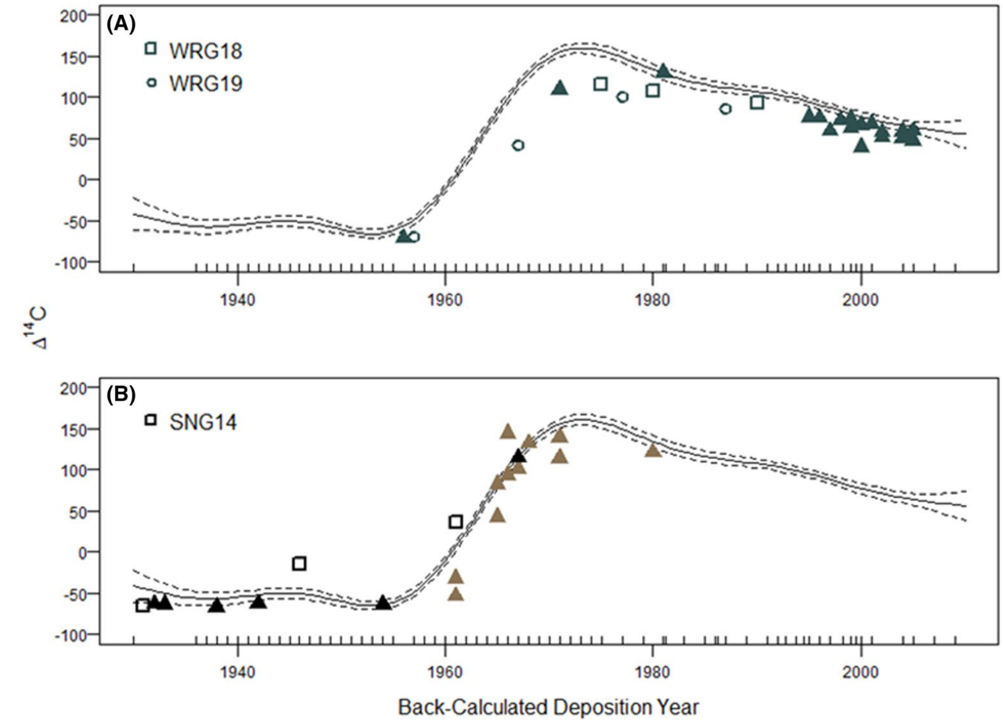

Figure 2. Radiocarbon values for core (solid triangles) and growth increment (hollow squares/circles) analyses of **(A)** Warsaw Grouper and **(B)** Snowy Grouper. Blue and gold symbol back-calculated deposition year is a function of conventional age determination. Black symbols denote Snowy Grouper 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 Garden Banks corals (Wagner et al. 2009), southern Florida corals (Druffel 1989), Speckled Hind (Andrews et al. 2013), and Red Snapper (Barnett et al. 2018).

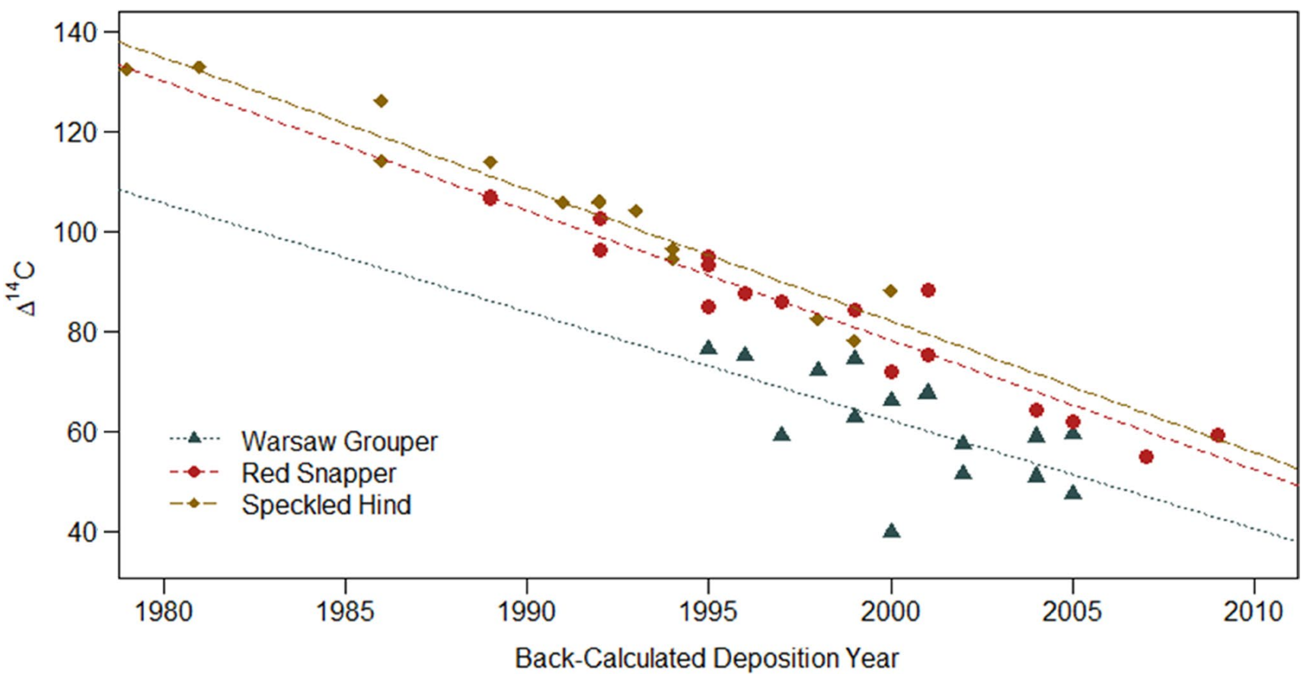

Figure 3. Post-peak radiocarbon decline trends for Warsaw Grouper, Speckled Hind (Andrews et al. 2013), and Red Snapper (Barnett et al. 2018) from 1978 through the early 2000s. Rate of decline was not significantly different among the three species (analysis of covariance [ANCOVA] slope test: $F = 0.35$,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

df = 2, P = 0.70; Figure 3). No difference in the rate of decline for $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 $\Delta^{14}C$ 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). 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 estimate of 25 years; this individual had an otolith core $\Delta^{14}C$ 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 $\Delta^{14}C$ 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 (R^2 = 0.88, df = 16, P < 0.001) and Snowy Grouper (R^2 = 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 (R^2 = 0.93, df = 18, P < 0.001) and Snowy Grouper (R^2 = 0.55, df = 9, P < 0.01; Figure 5B). For Snowy Grouper, this relationship was strengthened considerably (R^2 = 0.96, df = 15, P < 0.001) when adding the six samples with prebomb 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

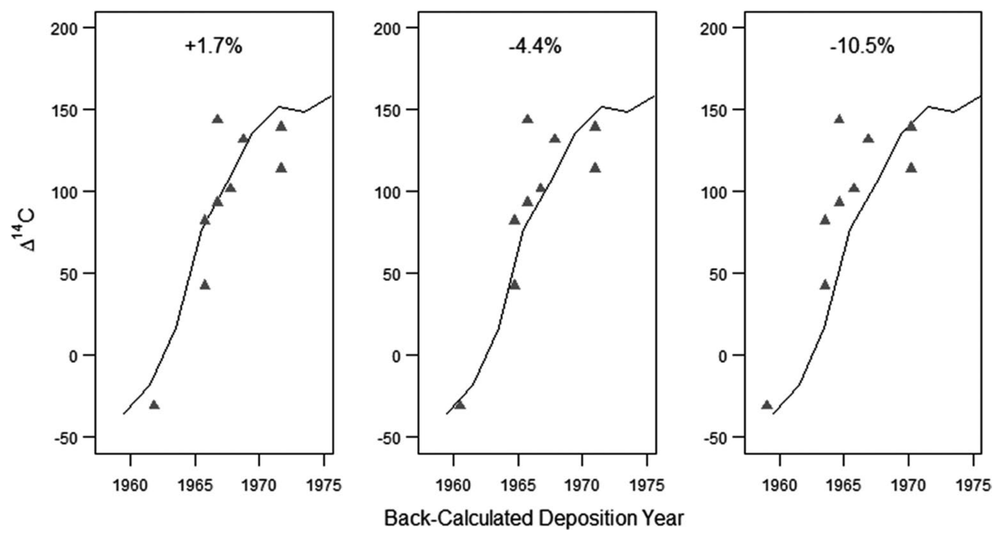

Figure 4. Age bias plots for Snowy Grouper with hatch years during the postbomb radiocarbon rise (1960–1975). Gold triangles are replotted ages assuming an age bias percent of + 1.7, −4.4, and −10.5%, which correspond to the mean percent bias and 95% confidence interval around the mean.

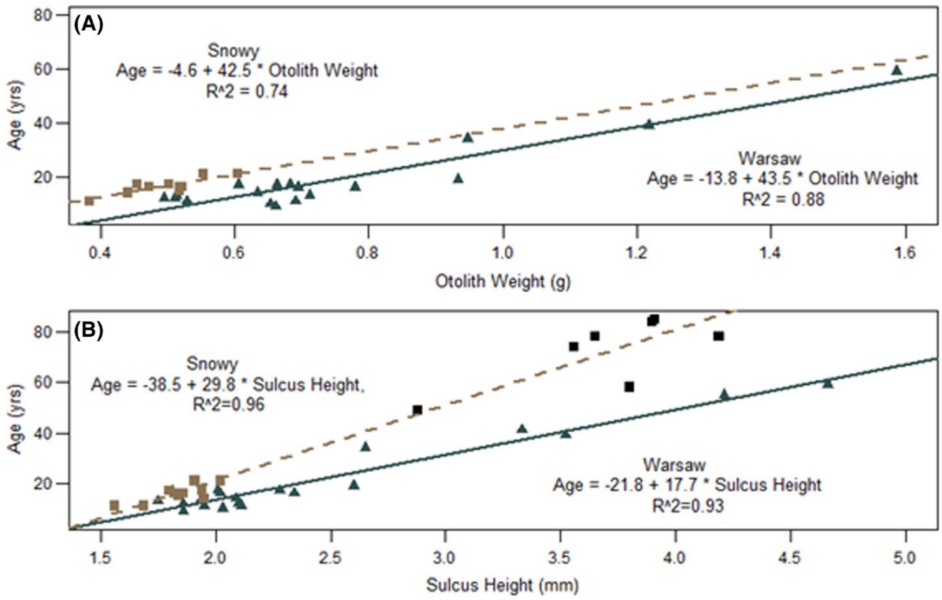

Figure 5. **(A)** Otolith weight–age linear regressions and **(B)** sulcus height–age linear regressions for Warsaw Grouper (triangles) and Snowy Grouper (squares), with regression formulas and R^2 values given. Gold squares are validated Snowy Grouper collected in 1982 with annulus count age estimates; black squares are Snowy Grouper collected in 2011–2016 with otolith-weight-derived age estimates.

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 minimum 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

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 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 and 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

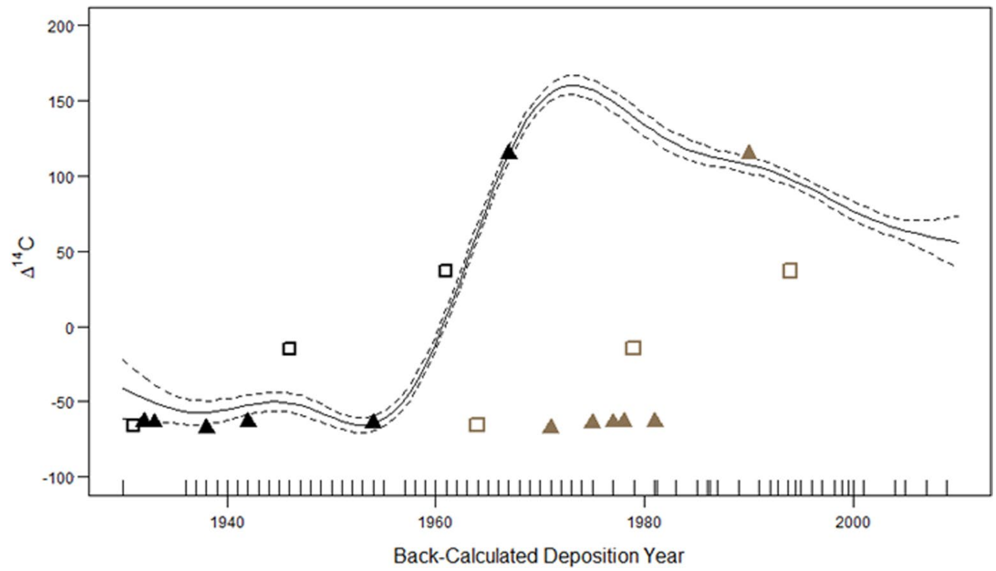

Figure 6. Plotted radiocarbon values for Snowy Grouper collected in 2011–2016, showing the shift from back-calculated hatch years based on ages derived from conventional age estimation techniques (gold triangles/squares) and ages derived from the otolith weight–age equation (black triangles/squares).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

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 $\Delta^{14}C$ values and therefore an inability to calculate a precise radiocarbon age, the minimum validated age of 56 years increases the current longevity estimate for Snowy Grouper (Costa et al. 2011) and greatly exceeds the maximum age used in the last stock assessment (SEDAR 2013).

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 and 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 and 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 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 2013). 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 survivorship 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.

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