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Bomb Radiocarbon Age Validation of Warsaw Grouper and Snowy Grouper

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Abstract

Current stock assessments for both Warsaw Grouper *Hyporthodus nigritus* and 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 deep-water 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 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, and therefore, validating 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 fishes (Kalish 1993; Campana 2001). This "modern" radiocarbon chronology offers a method to validate ages for fishes from cohorts with year classes during and after this increase in oceanic radiocarbon concentrations. Hatch (birth) year of an individual is estimated by comparing radiocarbon concentrations in otolith cores (i.e., first year of life) with 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 prebomb levels ($< -50\ \Delta^{14}C$) beginning in the late 1950s, peaked in the early to mid-1970s (120–160 $\Delta^{14}C$) (See Review, 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 2009), Florida Keys (Druffel 1989), and Puerto Rico (Moyer and Grottolli 2011). In addition, more recent work on fish otoliths shows the radiocarbon decline rate has remained consistent into the early 2000s (Cook et al. 2009; Andrews et al. 2013; Barnett et al. 2018).

Large groupers (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 deep-water epinephelids are much older than previously estimated via counting annuli (Cook et al. 2009; Andrews et al. 2013), suggesting potential for increased longevity in species with similar life histories. Thus, there is a clear need to validate the ages of additional deep-water groupers, particularly those with a "vulnerable" conservation status. Warsaw Grouper *Hyporthodus nigritus* and Snowy Grouper *H. niveatus* are key components of the deep-water grouper fishery in the Gulf of Mexico (GMFMC 2001–2016; Runde and Buckel 2018; Schertzer et al. 2018). They are currently listed by the International Union for the Conservation of Nature (IUCN) 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 National Oceanic and Atmospheric Administration (NOAA) Fisheries' Panama City Laboratory, Southeast Fisheries Science Center. 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 (DDIH$_2$O; 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 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 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 and hereafter "otolith core;" Supplemental File 1). Otolith cores of both Warsaw Grouper and Snowy Grouper were extracted for radiocarbon analysis as a secondary estimate of 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 File 2) 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 Whirlpacks. Centrifuge vials were sterilized in a 10% HNO_3 bath for a minimum of 24 hours, triple rinsed with $DDIH_2O$, 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 were reported in $\Delta^{14}C$ values, the per mil deviation from the ^{14}C activity in 19th century wood corrected for isotopic fractionation.

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 2009) and the Florida Keys (Druffel 1989), and two fish species, 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 and the fish chronologies 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 in 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 ages 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 confounding 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 weight to age to assess the value of these proxies for estimating ages of the two species.

Results

Warsaw Grouper

We selected 20 Warsaw Grouper (915–2010 mm TL) collected in 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. While overall patterns between the Gulf of Mexico reference radiocarbon chronology and Warsaw Grouper values are comparable, the otolith core $\Delta^{14}C$ values are visibly lower than reference values, including the two fish with prebomb hatch years. The two individuals with the oldest determined ages (55 and 59 years) had prebomb $\Delta^{14}C$ 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 range 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, $p = 0.700$, $F = 0.35$, $df = 2$; 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, $p=0.470$ $F=0.54$, $df=1$) but the magnitude of Warsaw Grouper $\Delta^{14}C$ values are significantly lower (ANCOVA intercept test, $p<0.001$, $F=51.40$, $df=1$).

Snowy Grouper

We selected 18 Snowy Grouper (330–1218 mm TL) for bomb radiocarbon age validation, with 11 collected in 1982 and 7 additional 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 Δ^{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%) concluded no significant age bias existed (Figure 4). Otolith core Δ^{14}C values of the six largest Snowy Grouper, with ages derived from otolith microstructure analysis between 34 to 52 years, were between -63.33 and -67.67, confirming hatch years that predate the radiocarbon rise (pre-1960). Therefore, all six have 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, have minimum validated ages of 55 years, much older than the microstructure analysis estimates. The seventh of the 2011–2016 Snowy Grouper, collected in 2015 and with an annular age estimate of 25 years, had an otolith core Δ^{14}C value (114.42) approaching the peak values for the reference series. Therefore, its hatch year could be attributable to either before or after the peak of the radiocarbon rise (years 1969 vs 1989), correlated with a radiocarbon age of either approximately 26 or 46 years, respectively. While the 26-year radiocarbon age estimate is similar to the microstructure analysis estimate, the large otolith mass (1.26 g) and fish total length (1131 mm) indicate it was much older. Combined with the extreme age underestimation of the six largest Snowy Grouper, of which its otolith weight and total length were much closer in size, it is likely closer to 46 than 26 years old. Otolith core and transect Δ^{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 to 1.59 g) and Snowy Grouper (0.44 to 2.11 g). Otolith mass was a good predictor of age for validated Warsaw Grouper (df = 16, $p < 0.001$, $R^2 = 0.88$) and Snowy Grouper (df = 7, $p < 0.010$, $R^2 = 0.74$) (Figure 5a). The Snowy Grouper otolith mass – age equation (Age = -4.56 + 42.46 * Otolith Weight) 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 correlate with their radiocarbon results (Figure 6). Age and mean sulcus height linear relationships for fish with validated ages was significant for both species and indicate that the metric is a useful proxy for approximating age: Warsaw Grouper (df = 18, $p < 0.001$, $R^2 = 0.93$) and Snowy Grouper (df = 9, $p < 0.010$, $R^2 = 0.55$; Figure 5b). For Snowy Grouper, this relationship is strengthened considerably (df = 15, $p < 0.001$, $R^2 = 0.96$) when adding the six samples with prebomb hatch years 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, 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 et al. 1998), and marine megafauna, including tooth 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 seen for both Warsaw Grouper and Snowy Grouper here.

Bomb radiocarbon age validation supports annuli 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 (1108–1218 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 deep-water fish species (Cailliet et al. 2001; Horn et al. 2012). Medium-sized Snowy Grouper radiocarbon values closely matched the hermatypic coral radiocarbon chronology for the Gulf of Mexico (Wagner 2009) with no bias in reader ages, suggesting that annuli appear to be discernable up to at least 25 years. However, otolith radiocarbon values of larger Snowy Grouper indicated fish were considerably older than expected, which was due in part to difficulties 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 annuli age estimate of 25 years. In fact, the six largest Snowy Grouper have minimum validated ages between 51 to 56 years based on collection years, with many exceeding the oldest age estimate determined by counting annuli (52 years). Even with prebomb Δ^{14}C values, and therefore an inability to calculate a precise radiocarbon age, the minimum validated ages 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 36).

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 suggests 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). While there have been observations of newly settled individuals for both species on the northeastern Gulf of Mexico continental shelf, the sightings are rare (Hardy 1978; 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). Although, reduced radiocarbon values for both Warsaw Grouper and Snowy Grouper relative to the reference chronologies could be indicative of age determination bias, it is important to note that otolith radiocarbon values from prebomb fish were also consistently lower than prebomb 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 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 relationships developed here for Warsaw Grouper and Snowy Grouper with validated ages, we approximated the age of larger individuals with hatch years that predate the radiocarbon rise. Based on otolith masses, age estimates for the six largest Snowy Grouper range from 59 to 85 years, indicating that longevity may be considerably greater than previously estimated (Wyanski et al. 2000; Costa et al. 2011; SEDAR 36). 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, deep-water grouper (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 2005). 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 2005), 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. 2015). 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. As 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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Supplementary Material

Two supplementary figures provide examples of measurements used to develop the otolith core milling template to determine growth increment transect locations and annuli interpretation in age determination. One figure includes an example of the annuli counts on sample WRG18.

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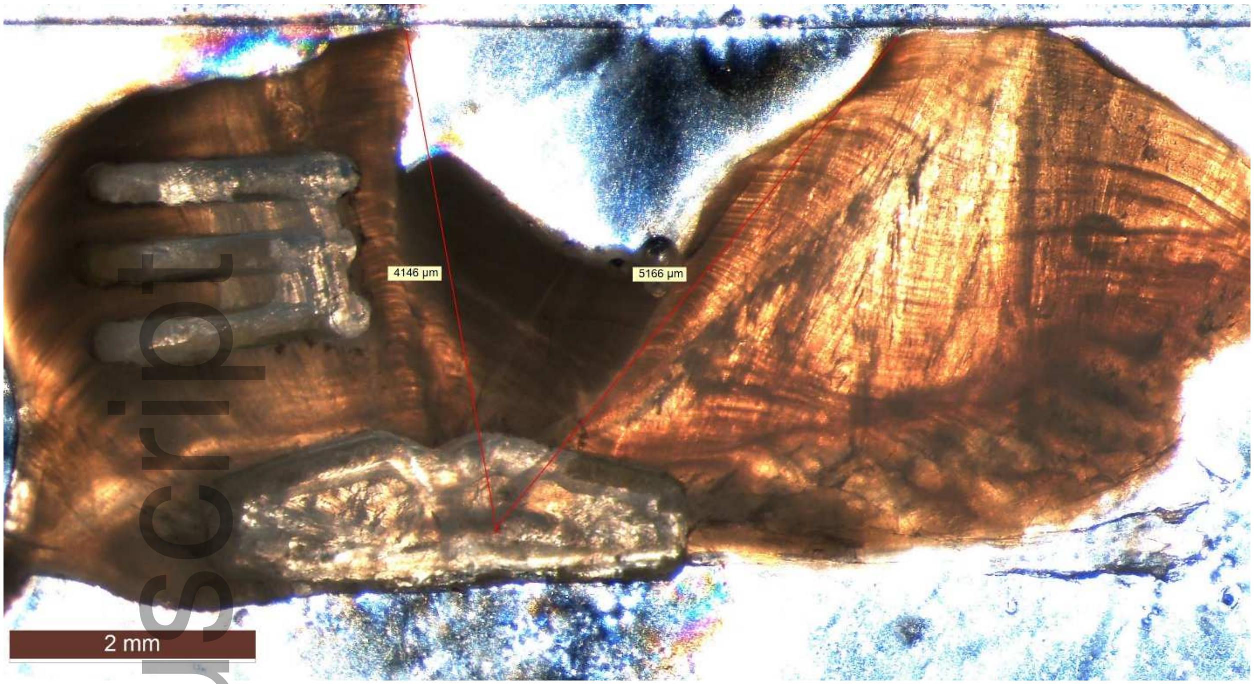
Table 1. List of all otolith core samples in the study and their analysis values. Ages and year classes with a * are Snowy Grouper, Hyporthodus niveatus, collected from 2011-2016 with age estimates derived from the otolith weight – age equation: Age = -4.6 + 42.5 * Otolith Weight, R² = 0.74.

Fish ID	Species	Catch year	Total length (mm)	Sulcus height	Otolith weight (g)	Age Estimate	Year class estimate	$\Delta^{14}C$	$\Delta^{14}C$ Error
WRG01	Warsaw	2015	1064	2650	0.9477	34	1981	130.42	2.1
WRG02	Warsaw	2014	1287	2095	0.5112	12	2002	57.49	2.4
WRG04	Warsaw	2016	1275	2283	0.6632	17	1999	62.86	2.4
WRG05	Warsaw	2014	1252	1862	0.6604	9	2005	47.44	3.2
WRG06	Warsaw	2015	1219	1925	0.5129	13	2002	51.46	2.5
WRG08	Warsaw	2016	1283	1855	0.4942	12	2004	58.99	2.4
WRG09	Warsaw	2014	1341	2033	0.6527	10	2004	50.98	3.3
WRG10	Warsaw	2016	1222	2108	0.6910	11	2005	59.37	2.3
WRG11	Warsaw	2012	2010	1954	0.5289	11	2001	67.59	2.7
WRG12	Warsaw	2016	1525	2339	0.7794	16	2000	66.06	2.1
WRG13	Warsaw	2012	1755	3326	NA	41	1971	108.88	2.1
WRG14	Warsaw	2014	1702	2597	0.9323	19	1995	76.46	2.5
WRG15	Warsaw	2011	1810	4214	NA	55	1956	-70.61	2.2
WRG16	Warsaw	2011	1501	1755	0.7115	13	1998	72.2	2.3
WRG17	Warsaw	2012	1471	2016	0.6939	16	1996	75.2	2.3
WRG18	Warsaw	2014	NA	3524	1.2179	39	1975	115.22	2.3
WRG19	Warsaw	2016	1790	4146	1.5871	59	1957	-68.6	1.8
WRG20	Warsaw	2014	1405	2092	0.6340	14	2000	39.8	2.7
WRG21	Warsaw	2016	915	2008	0.6051	17	1997	59.1	2.0
WRG22	Warsaw	2016	1430	1807	0.6825	17	2009	74.4	2.4
SNG01	Snowy	1982	330	NA	NA	2	1980	120.78	2.3
SNG02	Snowy	1982	740	1685	NA	11	1971	139.32	2.3
SNG03	Snowy	1982	763	1946	0.4401	14	1968	131.69	2.5
SNG04	Snowy	1982	765	1840	0.5179	15	1967	101.26	2.2
SNG05	Snowy	1982	715	1564	0.3837	11	1971	113.8	2.6
SNG06	Snowy	1982	724	1857	0.5216	16	1966	92.97	2.2
SNG07	Snowy	1982	769	1803	0.4548	17	1965	82.02	2.9
SNG08	Snowy	1982	790	1908	0.6044	21	1961	-31.99	1.9
SNG09	Snowy	1982	788	1824	0.4728	16	1966	143.43	2.3
SNG10	Snowy	1982	769	1942	0.5023	17	1965	42.26	2.3
SNG11	Snowy	1982	747	2020	0.5541	21	1961	-54.13	2.0
SNG12	Snowy	2011	1191	3645	1.9541	78*	1933*	-63.87	1.9

SNG13	Snowy	2015	1121	3558	1.8472	73*	1942*	-63.33	3.2
SNG14	Snowy	2016	1108	3914	2.1191	85*	1931*	-65.48	1.8
SNG15	Snowy	2013	1218	3798	1.4966	59*	1955*	-64.49	1.9
SNG16	Snowy	2015	1193	3895	2.1025	84*	1933*	-63.45	2.1
SNG17	Snowy	2015	1132	2877	1.2607	49*	1964*	114.52	2.3
SNG18	Snowy	2016	1162	4192	1.9540	78*	1938*	-67.67	1.8

Table 2. Estimated ages for the seven Snowy Grouper, Hyporthodus niveatus, collected from 2011-2016 using the otolith weight – age linear equation developed in this study: Age = -4.6 + 42.5 * Otolith Weight, $R^2 = 0.74$. The 85-year estimate is 29 years older than the 56-year minimum longevity validated in this study.

Sample ID	Total length (mm)	Otolith weight (g)	Annuli age	Minimum longevity	Otolith weight estimated age
SNG_12	1191	1.9541	34 years	51 years	78 years
SNG_13	1121	1.8472	34 years	55 years	73 years
SNG_14	1108	2.1191	52 years	56 years	85 years
SNG_15	1218	1.4966	38 years	53 years	59 years
SNG_16	1193	2.1025	37 years	55 years	84 years
SNG_17	1132	1.2607	25 years	NA	49 years
SNG_18	1162	1.9540	45 years	56 years	78 years



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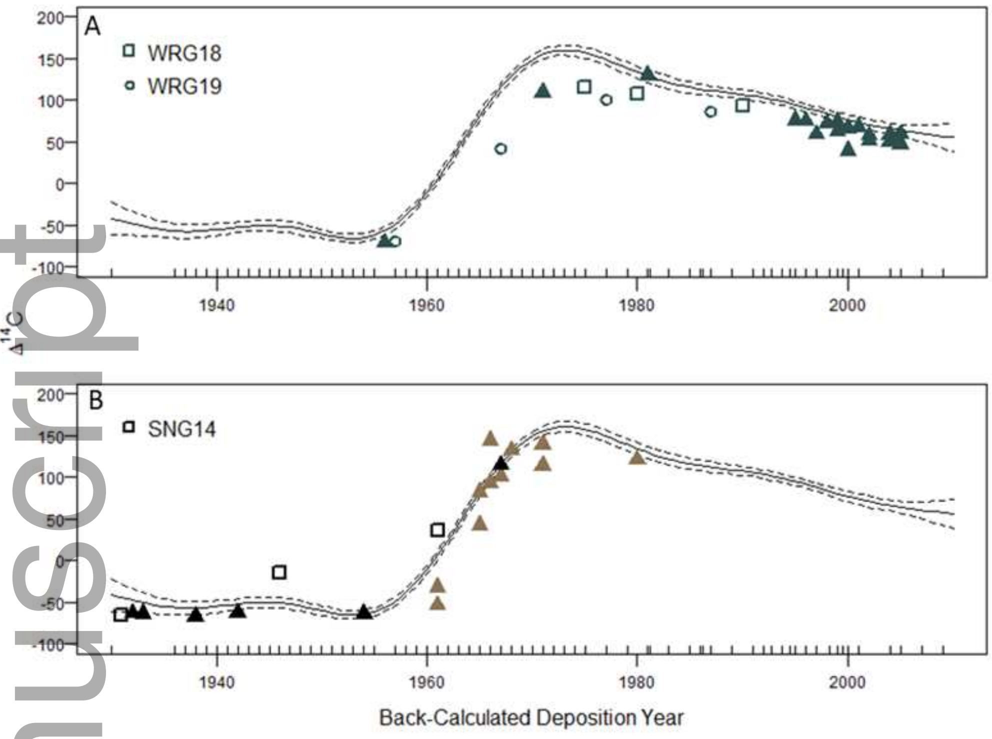

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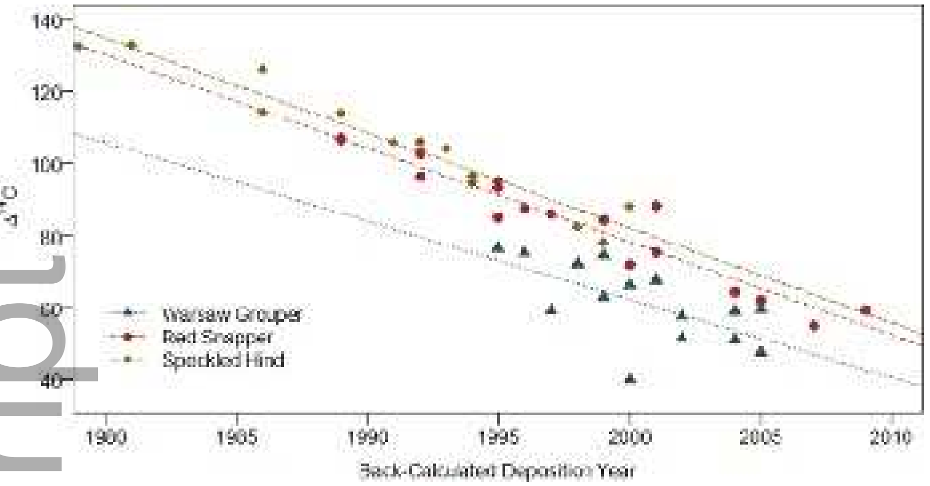

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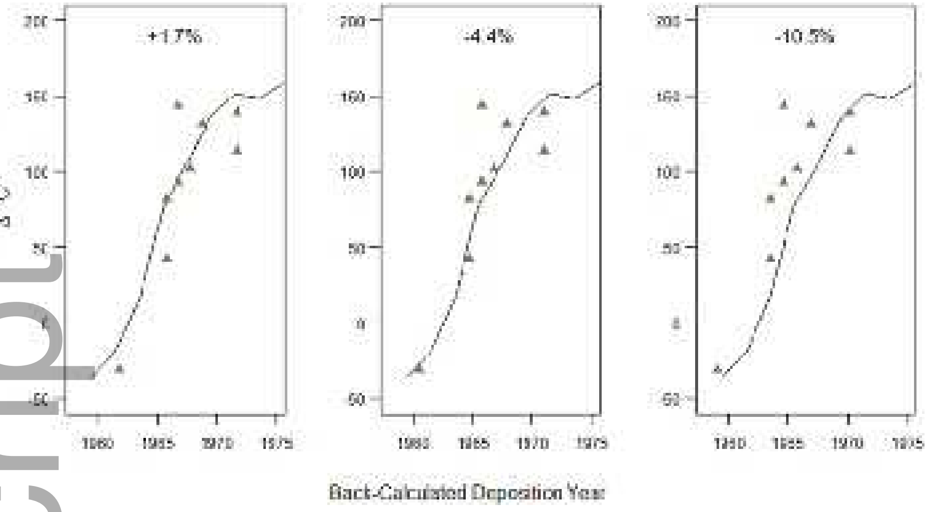

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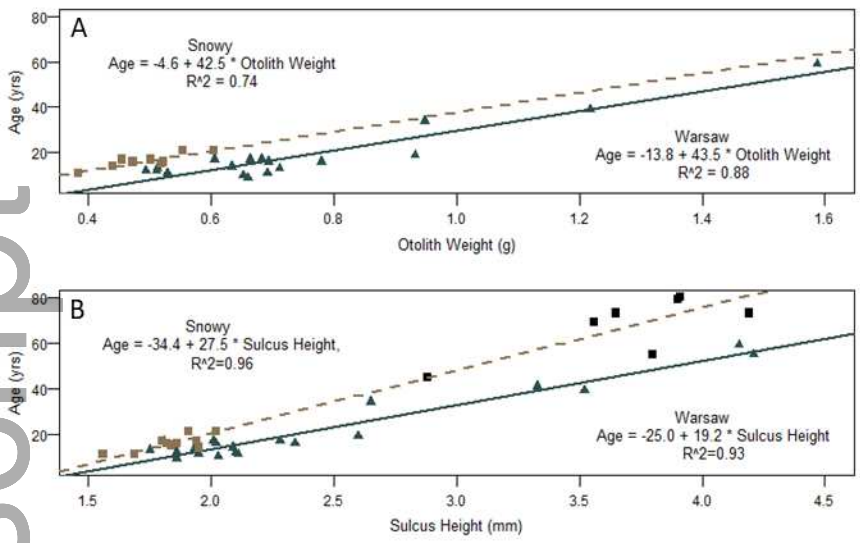

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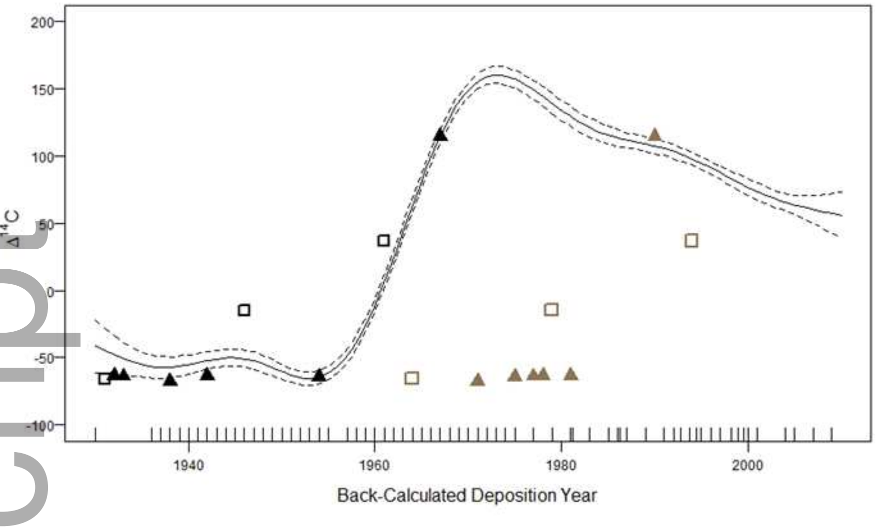

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