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Linear decline in red snapper (*Lutjanus campechanus*) otolith Δ^{14}C extends the utility of the bomb radiocarbon chronometer for fish age validation in the Northern Gulf of Mexico

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Radiocarbon (Δ^{14}C) was analyzed in northern Gulf of Mexico (nGOM) red snapper (*Lutjanus campechanus*) otolith cores ($n = 23$), otolith edge samples ($n = 12$), and whole age-0 otoliths ($n = 9$), with edge samples and whole age-0 otoliths constituting known-age samples. There was no significant difference in the linear relationship of Δ^{14}C versus year of formation between regional corals and known-age otolith samples, and a linear regression fit to the combined data from 1980 to 2015 extends the utility of the bomb radiocarbon chronometer for age validation. The entire regional coral and known-age otolith data set (1940 to 2015) was then utilized as a reference series to validate otolith-derived red snapper age estimates for cored otolith samples. A loess regression was fit to the reference data and then the sum of squared residuals (SSR) was computed from predicted versus observed birth years for cored adult otolith samples. This process was then repeated for ages biased $\pm$1–4 years. Ages with no bias applied had the lowest SSR, thus validating red snapper age estimates and demonstrating the utility of the combined regional coral and known-age red snapper otolith Δ^{14}C time series for age validation of nGOM marine fishes.

Keywords: age validation, otoliths, radiocarbon, red snapper

Introduction

Accurately determining fish age is critical to estimating life history parameters, such as growth, mortality, maturity functions, and longevity, but is also fundamental to age-structured stock assessment models (Ricker 1969; Beamish and McFarlane 1983; Reeves 2003). Ageing errors are more often associated with underestimating rather than overestimating age, which leads to biased growth and mortality rates (Campana 2001). In turn, age underestimation can then lead to unsustainably high fishing mortality threshold estimates, thus overfishing (Beamish and McFarlane 1983; Campana *et al.*,1990; Smith *et al.*, 1995). Therefore, validation or verification of age estimates is critically important when examining fish population ecology or estimating stock dynamics (Beamish and McFarlane 1983; Campana 2001).

Common methods of age validation or verification include chemically marking otoliths of fish that are later recaptured to examine periodicity of opaque zone formation, mark-recapture studies in which tagged fish growth is compared to growth estimated from otolith-derived ages, or marginal increment analysis. However, these methods require either large sample sizes to ensure sufficient recaptures or monthly sampling of multiple age classes to examine the margins of otolith thin sections. Another age validation method that is applicable to long-lived species for which age estimations are considerably different is radiochemical dating, such as ^{210}Pb:^{226}Ra, that relies on radioactive decay of radioisotopes into their radioactive daughter products (Campana 2001). However, low radioisotope concentrations typically result in low precision in radiochemical dating estimates (Campana 2001). An alternative approach to age validation in marine fishes is the application of the bomb radiocarbon (^{14}C) chronometer (Kalish 1993; Campana 2001). This approach is based on the rapid increase in oceanic ^{14}C during the 1950s and 1960s due to atmospheric testing of thermonuclear weapons (Broecker *et al.* 1982), with the ^{14}C subsequently being incorporated into marine carbonates such as hermatypic corals (Druffel 1980, 1989; Wagner 2009; Moyer and Grottoli 2011), bivalve shells (Weidman and Jones 1993), and fish otoliths (Kalish 1993). Although ^{14}C measurements from these calcified structures are assumed to represent ^{14}C of ocean surface dissolved inorganic carbon levels in surrounding waters, these values are temporally and spatially dependent on water residence times and oceanic mixing rates (Druffel and Linick 1978; Druffel 1989; Kalish 1993; Weidman and Jones 1993). Data from Δ^{14}C analysis of otolith cores, along with estimated fish birth year from otolith opaque zone counts and year of collection, are plotted against coral Δ^{14}C vs. year of formation to validate fish age estimates, hence ageing methods. Critically important to this method is the fact that the coral and fish must live in and experience a similar water mass (Kalish 1995; Campana and Jones 1998; Kastelle *et al.*, 2016).

The bomb ^{14}C chronometer has been successfully applied as an age validation tool in numerous teleost ageing studies employing otoliths (Ewing *et al.*, 2007; Neilson and Campana 2008; Andrews *et al.*, 2012), some of which were performed on fishes from the GOM (Baker and Wilson 2001; Fischer *et al.*, 2005; Andrews *et al.*, 2013). Historically, the application has been used for fish with birth years in the informative period of rising Δ^{14}C values (approximately 1958–1968, depending on the region), but it is becoming increasingly difficult to locate samples with birth years in this range. Authors of early northern Gulf of Mexico (nGOM) studies utilizing the bomb ^{14}C radiocarbon chronometer to validate ageing for red snapper *Lutjanus campechanus* (Baker and Wilson 2001), yellowedge grouper *Hyporthodus flavolimbatus* (Cook *et al.*, 2009), and gray snapper *Lutjanus griseus* (Fischer *et al.*, 2005), respectively, noted that coral and otolith samples from after the mid-1970s coral Δ^{14}C peak suggested a post-peak linear decline existed in Δ^{14}C values. However, the reference coral Δ^{14}C chronology available in these early studies did not extend beyond 1983. Recently, a Δ^{14}C coral chronology from Puerto Rico in the Caribbean Sea through 2004 was reported by Moyer and Grottoli (2011), which Andrews *et al.* (2013) then applied to validate post-1975 ageing in speckled hind *Epinephelus drummondhayi* that were sampled principally from the southeastern GOM. We hypothesized that a similar relationship holds true for nGOM marine fishes, with red snapper serving as our model species.

Red snapper is a long-lived (to 60 years), demersal species that occurs over the shelf from North Carolina to Florida and throughout the GOM, where it has supported an economically important fishery since the mid-1850s (Collins 1885). Age estimates derived from otolith opaque zone counts have been verified or validated for this species with multiple methods, including marginal increment analysis (Patterson III *et al.*, 2001; Wilson and Nieland 2001; Fischer *et al.*, 2004), via growth estimate comparisons to tagged fish (Patterson III *et al.*, 2001), and radiochemical dating (Baker *et al.*, 2001). Perhaps the most definitive work validating otolith-based red snapper ageing was conducted by Baker and Wilson (2001), who analysed Δ^{14}C in otolith cores (estimated birth years 1943–1996) and then utilized the bomb ^{14}C chronometer to validate ages of fish as old as 38 years. Given this work, as well as the comprehensive treatment of age validation for this species in the literature, red snapper was judged to be an ideal model to examine whether the post-1970s decline phase in otolith Δ^{14}C could be employed to extend the utility of the bomb ^{14}C chronometer to more recent decades for nGOM marine fishes. Specific objectives were to (i) analyse Δ^{14}C of known-age reference otolith aragonite from the 1980s to 2010s and test whether the linear relationship between Δ^{14}C and year of formation was significantly different between known-age red snapper otoliths and regional coral, and (ii) analyse Δ^{14}C in otolith cores for red snapper with birth years in the 1950s–2010s and apply a temporally expanded bomb ^{14}C chronometer to validate age estimates for those fish.

Methods

Sample collection

Red snapper otolith samples were collected from offshore waters off Florida, Alabama, Louisiana, and Texas during fishery-dependent and fishery-independent sampling programs conducted in the U.S. GOM. Fishery-dependent sampling was conducted by National Marine Fisheries Service (NMFS) port agents. Collections of adult and sub-adult otolith samples were archived at the NMFS Panama City Laboratory. Otolith pairs were stored dry in paper coin envelopes; left otoliths were subsequently sectioned and aged following the protocols of Patterson III *et al.* (2001) (Figure 1).

Age-0 red snapper were sampled from offshore waters of Alabama and Louisiana during fall Southeast Area Monitoring and Assessment Program (SEAMAP) trawl sampling from 1995 to 2015, with otoliths from those fish being archived at the NMFS Panama City Laboratory or the University of Florida. Age-0 otoliths were rinsed with double deionized water and then allowed to air dry. Samples were then stored dry in plastic vials.

Sample processing

Adult red snapper otolith edge samples and whole age-0 otoliths were sampled to provide known-age otolith material. Otoliths from which edge samples were extracted were first cleaned with a succession of 70% ethanol, mild detergent, and deionized water, with sonication between each cleaning step following the method of Andrews *et al.* (2013). Edge samples were then extracted by applying pressure to the rostrum of whole adult or subadult otoliths such that an ~5 mg piece of otolith was removed. Age-0 otolith samples were cleaned as indicated above and left whole.

Otoliths selected for coring were first cleaned as above following the method of Andrews *et al.* (2013). All samples were air-dried overnight under a class-10 clean hood. Samples then were affixed sulcus side down to glass slides with a toluene-based

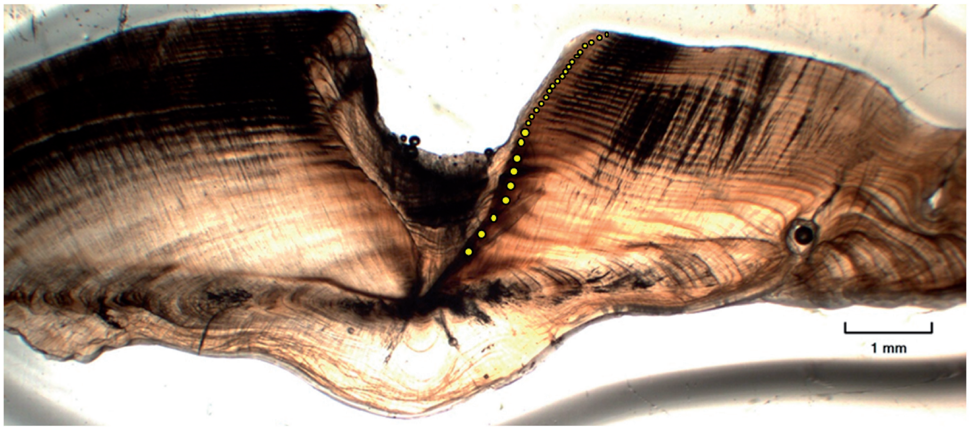

Figure 1. Image of a left sagittal otolith section, viewed with transmitted light, from an 882-mm total length female red snapper (sample 122-39 in Table 1) sampled in August 2016 and estimated to be 27 years old. The core of the right otolith from this fish (sample 122-39 in Table 1) was extracted and analysed for $\Delta^{14}C$.

acrylic resin that was allowed to cure for a week prior to further preparation. Wet hand grinding using 320- to 1000-grit carbide wet–dry sandpaper was performed on the distal side of the whole otolith until the otolith core was exposed. Once the otolith core was exposed, extraction of core aragonite utilized the computer automated capabilities of a New Wave Research® (ESI–NWR Division; Fremont, CA, USA) micromilling instrument. A 0.5-mm diameter Brasseler® (Savannah, GA, USA) bit was used to drill an overlapping surface scan conforming to the uneven surface structure of each whole otolith. Otolith material was extracted from the distal side of the whole otolith to the mean thickness of age-0 otoliths collected in fall, as this is when the first opaque zone begins to form (Patterson III *et al.*, 2001; Barnett and Patterson III 2010). Once extracted, core material was stored dry in acid-leached glass vials.

Accelerator mass spectrometry analysis

Otolith samples were analysed for $\Delta^{14}C$ with accelerator mass spectrometry (AMS) at either the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at the Woods Hole Oceanographic Institution, or at the University of Georgia's Center for Applied Isotope Studies (CAIS). Processing and analysis of otolith samples for $\Delta^{14}C$ proceeded similarly at NOSAMS and CAIS following standard methods. Prior to AMS analysis, all samples were prepared using acid hydrolysis procedures (additional information can be found online: www.whoi.edu/nosams/radiocarbon-data-calculations).

The stable C isotope ^{13}C is reported as the delta value $\delta^{13}C$ (‰), which is computed as the ratio of $^{13}C/^{12}C$ relative to a standard (Pee Dee Belemnite). Radiocarbon (^{14}C) is also reported as a delta value ($\Delta^{14}C$) that represents the activity of a sample relative to a standard (Stuiver and Polach 1977) and corrected for age and $\delta^{13}C$. Values of $\Delta^{14}C$ are reported with ± 1 *SD*, which incorporates statistical and analytical sources of error.

Data analysis and interpretation

The time series of $\Delta^{14}C$ compiled by Andrews *et al.* (2013) for Caribbean and GOM corals was used as the coral reference series in this study. Coral sample sites include Belize (Druffel 1980), the Florida Keys (Druffel 1989), Vera Cruz, Mexico, the Flower Garden Banks off Texas (Wagner 2009), and Puerto Rico (Moyer and Grottoli 2011). In each of these studies, coral cores were aged and then analysed with AMS to provide a time series of coralline aragonite $\Delta^{14}C$. A general linear model (GLM) was used to investigate the relationship between coral and known-age red snapper otolith sample $\Delta^{14}C$ values vs. year of aragonite formation for overlapping years (1989–2004) of the two time series. A linear regression was then fit to the combined coral and known-age otolith samples for years 1980–2015 to determine if the known-age otolith samples, particularly those with birth years >2004, would fall within the linear regression model 95% prediction intervals.

A loess regression was fit to the combined coral and known-age red snapper otolith (coral-otolith) reference $\Delta^{14}C$ data. Bonferroni-corrected confidence intervals were estimated around the loess regression following the method of Kastelle *et al.* (2008), with the α-value set by the number of adult red snapper otolith core validation sample comparisons to be made and calculated as $\alpha^* = \alpha/n^*$, where n^* represents the number of validation samples with unique years of aragonite formation. Model assumptions for normality and homogeneity of variance were tested with Shapiro–Wilk and Levene tests, respectively. Significance levels for the GLM were set at $\alpha = 0.05$.

Age estimates of cored samples were investigated for ageing bias by purposely shifting the estimated ages by ± 1–4 years and superimposing otolith core $\Delta^{14}C$ values on the coral-otolith reference $\Delta^{14}C$ time series to examine the fit of otolith core $\Delta^{14}C$ values to reference sample $\Delta^{14}C$ values. An age bias of 0 represented the original age estimates, while an age bias of +1 and +2 shifted the age estimate to the left (i.e. older) and an age bias of −1 and −2 shifted the age estimate to the right (i.e. younger). The sum of squared residuals (SSR) was then computed from predicted vs. observed (based on opaque zone counts) birth years for cored otolith samples, and repeated for the purposely biased ages (Kastelle *et al.*, 2008).

Results

Accelerator mass spectrometry was employed to analyse $\Delta^{14}C$ and $\delta^{13}C$ in nGOM red snapper otolith edge samples ($n = 12$), whole

Table 1. Red snapper otolith samples analysed for $\Delta^{14}C$ with AMS.

Sample number	Analysis number	TL mm	Otolith Mass (mg)	Sample date	Sample type	Year of formation	Age (years)	AMS Mass mg	$\delta^{13}C$ ‰	$\Delta^{14}C$ ‰
RS-07	OS-120900	830	3811.1	9/12/04	core	1951	53	4.3	−2.69	−59.8±2.0
RS-04	OS-120897	853	3767.5	8/6/02	core	1958	44	4.9	−3.59	−55.8±2.2
RS-01B	OS-127112	857	–	8/2/01	core	1959	42	1.2	−4.00	−12.8±2.3
RS-08C	OS-127217	870	3150.8	9/10/09	core	1965	44	0.8	−4.16	124.8±3.3
RS-16C	OS-127226	970	–	6/3/13	core	1968	45	0.7	−3.20	125.1±3.9
RS-12	OS-121097	905	2942.5	7/14/11	core	1980	31	6.6	−0.96	128.2±2.2
RS-14C	OS-127207	900	3349.3	9/10/11	core	1983	28	0.8	−4.47	127.5±3.0
RS-13	OS-121098	810	2753.4	7/20/11	core	1984	27	4.6	−3.36	116.8±2.3
RS-05C	OS-127095	740	1884.3	9/25/03	core	1987	16	5.0	−5.11	117.8±3.1
RS-15B	OS-127200	875	3518.4	9/10/12	core	1987	25	1.0	−4.97	113.1±3.2
RS-02	OS-120895	795	1731.2	3/29/02	core	1989	13	4.8	−2.97	101.7±2.9
RS-09	OS-121094	828	2629.4	9/19/09	core	1989	20	4.8	−2.72	111.3±2.3
122-39	OS-129782	882	3427.3	9/11/16	core	1989	27	8.75	−4.79	106.9±2.6
RS-11	OS-121096	682	1758.0	5/13/11	core	1992	19	4.9	−4.42	104.8±2.4
RS-06	OS-120899	864	2025.7	9/10/04	core	1993	11	5.2	−3.29	104.1±3.1
RS-03	OS-120896	730	1491.4	3/29/02	core	1994	8	4.6	−2.66	95.7±2.2
122-74	OS-129783	870	3115.2	9/11/16	core	1996	20	8.1	−4.08	84.6±2.5
121-59	OS-129779	843	2233.0	9/11/16	core	1998	18	9.35	−4.07	78.0±2.7
106-62	OS-129776	749	1486.2	9/8/16	core	2001	15	10.8	−3.95	73.2±2.8
121-41	OS-129778	832	1844.1	9/11/16	core	2004	12	10.8	−3.91	59.0±2.5
122-08	OS-129781	805	2037.4	9/11/16	core	2004	12	8.9	−4.54	60.5±2.6
117-34	OS-129777	670	1267.1	9/10/16	core	2008	8	10.2	−3.47	63.6±3.6
122-06	OS-129780	331	302.3	9/11/16	core	2013	3	11.2	−3.58	41.2±2.4
RS-R01	OS-115083	250	196.7	5/29/89	edge	1989	–	6.6	−3.78	106.6±2.5
RS-R02	OS-115084	312	–	10/30/89	edge	1989	–	7.8	−3.23	107.0±2.5
RS-R05	OS-117426	330	292.1	2/1/92	edge	1992	–	3.2	−3.56	102.7±1.8
RS-R06	OS-117427	406	395.7	10/10/92	edge	1992	–	1.9	−2.30	96.4±2.0
RS-R09	OS-115184	285	–	8/16/95	edge	1995	–	13.4	−3.10	93.5±2.6
RS-R10	OS-115185	385	–	3/6/95	edge	1995	–	7.7	−3.67	95.1±2.8
RS-R11	OS-117428	313	250.1	4/9/97	edge	1997	–	3.3	−2.19	85.9±1.9
RS-R12	OS-117580	380	382.7	10/30/97	edge	1997	–	2.7	−3.17	86.2±2.5
RS-R15	OS-115186	398	–	5/2/01	edge	2001	–	11.9	−4.72	88.4±2.5
RS-R16	OS-115187	282	–	12/1/01	edge	2001	–	8.2	−3.27	75.5±2.5
RS-R19	OS-117581	88	15.9	10/20/05	edge	2005	–	4.0	−3.67	61.9±2.2
RS-R20	OS-117582	65	9.2	10/26/05	edge	2005	–	5.1	−4.64	62.2±2.1
R595	OS-128463	134	37.0	10/15/00	whole	2000	0	33.7	−3.92	71.9±2.7
R612	OS-128464	140	31.6	10/15/99	whole	1999	0	27.0	−4.13	84.3±2.2
A15R	OS-128465	138	20.9	10/15/95	whole	1995	0	15.0	−3.62	85.1±2.7
1D17	OS-128466	125	39.0	10/15/96	whole	1996	0	34.0	−3.83	87.7±2.4
S121	OS-128467	104	–	10/15/04	whole	2004	0	28.3	−4.25	64.4±2.2
2821	OS-128468	114	36.3	10/28/07	whole	2007	0	33.2	−3.52	54.9±2.2
D-6368	UGA-19997	89	–	10/20/09	whole	2009	0	11.1	−3.43	59.3±2.8
12920	UGA-19998	121	35.2	11/3/13	whole	2013	0	32.0	−2.78	49.4±2.9
14200	OS-128479	122	40.8	11/20/15	whole	2015	0	36.9	−4.59	37.3±2.5

Sample type includes adult core, edge sample of adult otolith, or whole age-0 otoliths. Otoliths for which no otolith mass or age estimate was recorded are shown as –. Analysis number prefix: OS = NOSAMS, UGA = University of Georgia. Year of formation equals sample year for adult otolith edge or whole age-0 otoliths, and sample year minus opaque zone count for adult otolith cores.

age-0 otoliths ($n = 9$), and otolith cores ($n = 23$) (Table 1). Edge samples and age-0 whole otoliths constitute known-age samples, with dates of aragonite formation between 1989 and 2015 (Table 1). There was no significant difference (GLM, $F_{1;\,34} = 0.278$, $p = 0.783$) in the linear relationship of $\Delta^{14}C$ vs. year of aragonite formation between regional corals and known-age otolith samples for overlapping years (1989–2004) of the two time series. The linear regression fit to the combined >1980 coral and known-age otolith material time series was significant (GLM, $F_{1;\,63} = 1805$, $p < 0.001$; Figure 2), and nearly all coral and known-age otolith samples fell within the 95% prediction intervals of the function. The rate of decline of ^{14}C was 2.52‰ y^{-1} for the period 1980–2015.

The core samples extended the temporal range of the measurements, and fit with the documented changes in radiocarbon over the last 70 years. The loess (degree = 2, alpha = 0.2) fit to the regional coral-otolith reference chronology $\Delta^{14}C$ data plotted vs. estimated year of formation demonstrated a clear pattern of rapidly increasing values due to atmospheric testing of nuclear weapons in the 1950s and 1960s, then a declining trend after the 1970s (Figure 3).

Among cored red snapper otolith samples, fish TL ranged from 331 to 970 mm and estimated birth year ranged from 1951 to 2013 (Table 1). All but one cored red snapper otolith sample fell within the Bonferroni-corrected 95% confidence intervals around the loess regression fit to the coral-otolith reference series

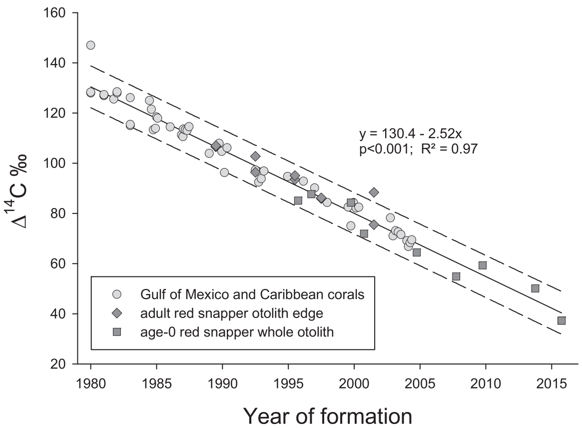

Figure 2. Scatterplot of aragonite $\Delta^{14}C$ vs. year of formation for regional corals ($n = 52$), adult red snapper otolith edge ($n = 12$), and whole age-0 otolith samples ($n = 9$). Solid line indicates a linear regression fit to the combined coral and red snapper otolith data, with year zero set to 1980. Dashed lines are 95% prediction intervals.

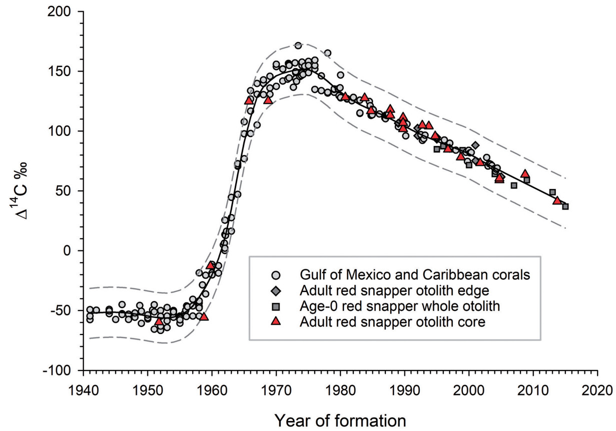

Figure 3. Scatterplot of regional coral plus known-age red snapper (adult edge and whole age-0) otolith $\Delta^{14}C$ values vs. year of formation. The solid line is a loess regression (degree = 2; $\alpha = 0.20$) fit to the combined coral and known-age red snapper data; dashed lines are Bonferroni-corrected 95% confidence intervals based on the sum of squared residuals (SSR) calculated following the method of Kastelle *et al.* (2008). Also plotted but not included in the loess fit are adult red snapper otolith core $\Delta^{14}C$ vs. estimated birth year derived from sample year minus otolith opaque zone counts.

(Figure 3). Bias plots of red snapper otolith core $\Delta^{14}C$ values relative to the loess regression fit to regional coral data indicate red snapper birth year estimates derived from otolith thin section opaque zone counts are accurate, given unmanipulated age estimates had the lowest SSR (2365) as calculated with the method of Kastelle *et al.* (2008), while purposely biased estimates had SSR ranging from 2904 (+1 year) to 19 801 (−4 years) (Figure 4).

Discussion

Results from this study clearly demonstrate a linear decline in regional coral $\Delta^{14}C$ and red snapper otolith $\Delta^{14}C$ from 1980 to 2015 that extends the utility of the bomb ^{14}C chronometer as a method to validate ageing in red snapper and other nGOM marine fishes to more recent decades. Clearly, we are not the first to suggest a linear trend in the descending limb of coral or otolith $\Delta^{14}C$ time series. Kalish *et al.* (1996) first noted this phenomenon with southern bluefin tuna *Thunnus maccoyii*, and the descending limb of coral and otolith time series has since been examined for speckled hind in the southeastern GOM (Andrews *et al.*, 2013), bluespine unicornfish *Naso unicornis* from Hawaii (Andrews *et al.*, 2016), Pacific bluefin tuna *Thunnus orientalis* from the north Pacific Ocean (Ishihara *et al.*, 2017), and three Hawaiian parrotfishes (DeMartini *et al.*, 2018). Here, we extend the utility of the approach geographically in the western Atlantic, as well as temporally by extending the time series. The extension of this approach into the nGOM is notable given that the regional coral $\Delta^{14}C$ record utilized for comparing the post-1980 decline phase was principally from Puerto Rico in the Caribbean Sea (Moyer and Grottoli 2011). That location is greater than 2000 km from the nGOM red snapper sample region, and coral $\Delta^{14}C$ time series have been reported to vary considerably among ocean basins (reviewed in Grottoli and Eakin 2007). However, the Caribbean Sea and GOM are connected by the Caribbean Current that flows into the GOM through the Yucatan Current (Candela *et al.*, 2002). Once this Current enters the GOM, it becomes known as the Loop Current which dominates GOM circulation patterns (Candela *et al.*, 2002), thus explaining the correspondence among overlapping western Atlantic coral $\Delta^{14}C$ time series (Druffel 1980, 1989; Wagner 2009; Moyer and Grottoli 2011).

The linear decline we report in regional corals and nGOM red snapper otolith $\Delta^{14}C$ is consistent with values of dissolved inorganic carbon (DIC) reported for the region, as well as declines observed in $\Delta^{14}C$ measured for DIC in other regions of the globe. This is especially important given dissolved inorganic carbon from seawater is the dominant (70–90%) source of the carbon deposited in otolith aragonite (Degens *et al.*, 1969; Weidman and Millner, 2000; Høie *et al.*, 2003; Solomon et al., 2006; Tohse and Mugiya, 2008; Nelson *et al.*, 2011). The record we report of $\Delta^{14}C$ declining at a rate of 2.52‰ year^{-1} reflects the declining $\Delta^{14}C$ of surface water DIC as the radiocarbon bomb spike mixes into the biosphere. Chanton *et al.* (2012) measured a $\Delta^{14}C$ value of $+41 \pm 14$ ‰ for DIC in surface waters of the nGOM which fits well with the otolith data at that time. The declining value of 2.52‰ year^{-1} is also similar but slightly less than the decrease of 3.5‰ year^{-1} observed in the surface waters of the northeast Pacific Ocean from 1995 to 2004 (Bertrand *et al.*, 2013), but roughly half the value for the decline of tropospheric CO_2 determined by Graven *et al.* (2012) of 5.5‰ year^{-1} for the period 1992–2007. The larger oceanic DIC reservoir buffers the decrease of marine DIC ^{14}C content relative to the atmospheric reservoir. Variations in the rate of ^{14}C depletion between water bodies are due to local mixing and advection effects (Mahadevan 2001). Interestingly, Bertrand *et al.* (2013) observed a similar rate of decline of ^{14}C in particulate organic carbon in the northeast Pacific (3.2‰year^{-1}) indicating that both the inorganic carbon source, and the fixed carbon derived from it are decreasing at similar rates. Both organic tissue, fixed in surface waters, and inorganic components follow the same trends providing multiple opportunities for age determination from records such as we report here.

Historically, utilizing the bomb ^{14}C chronometer required having samples with birth years in the 1950s and 1960s to validate ageing based on otolith $\Delta^{14}C$ values. However, the linear relationship described in this study provides a reference chronology to validate ages of young or short-lived nGOM marine fishes for

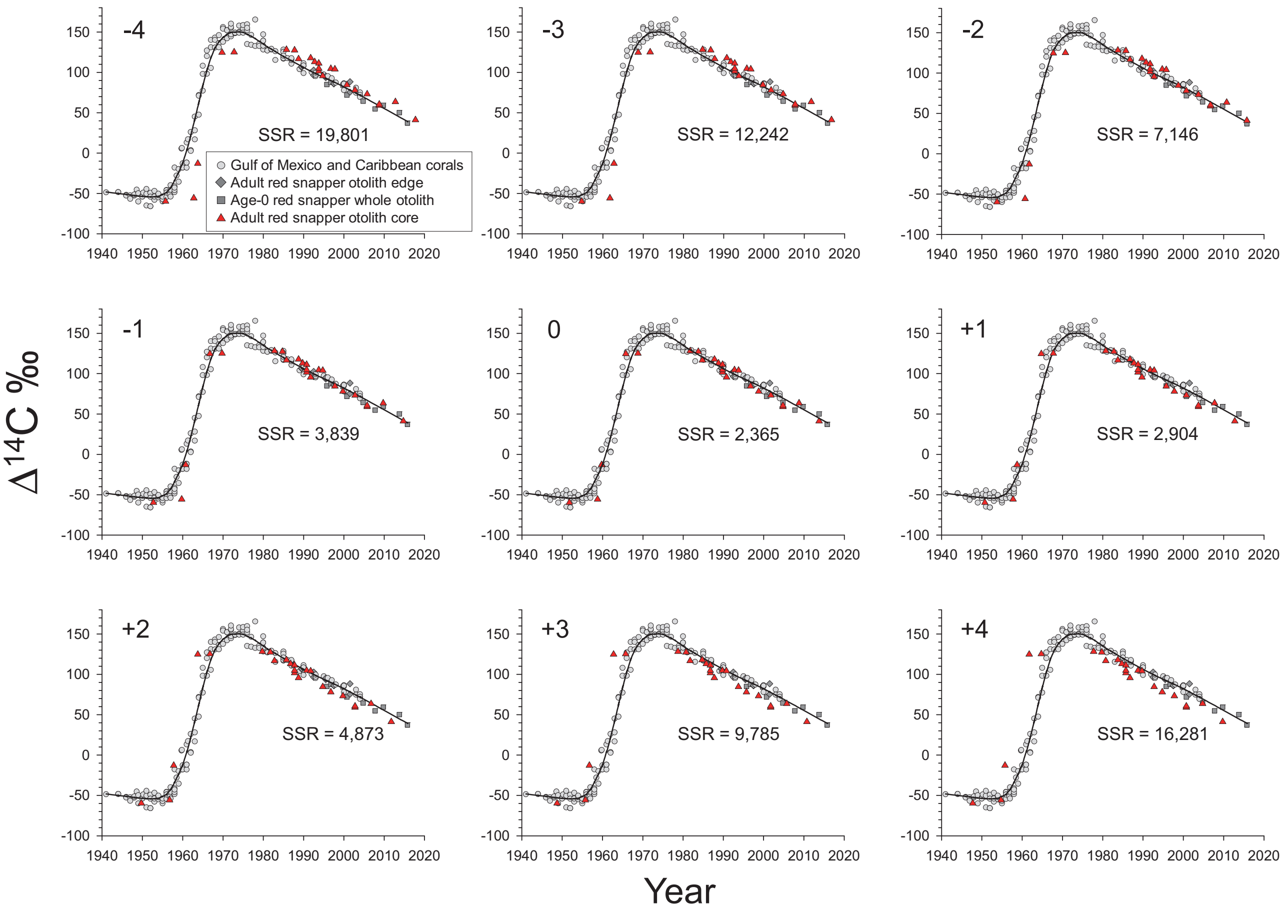

Figure 4. Bias plots of red snapper otolith core Δ^{14}C values relative to the loess regression fit to regional coral data (Figure 3). Panels are labeled with adjustments (0 to $\pm$ 4 years) made to estimated birth years derived from otolith thin section opaque zone counts and year of collection. The sum of squared residuals (SSR) calculated following the method of Kastelle *et al.* (2008) is given on each panel. Projected coral values for years 2005–2008 were estimated based on the linear regression reported in Figure 2.

which archived otoliths with 1950s and 1960s birth years do not exist. This is particularly important for reef fishes that exhibit high site fidelity and limited home ranges, given that localized conditions can drive high variability in size at age, therefore uncertainty in age estimates. Many nGOM reef fishes have otoliths with diffuse growth increment patterns making it difficult to interpret age estimates (Black *et al.*, 2011). Some of this variability has been attributed to latitudinal gradients where fish residing in semi-tropical waters (i.e. lower latitudes) have more diffuse banding patterns that are most likely due to a decrease in seasonal variability (Caldow and Wellington 2003). Uncertainty in age estimates can also be compounded by growth differences that occur on small spatial scales, which have been observed for several nGOM reef fishes that exhibit high site fidelity. For example, Allman (2007) reported differences in vermilion snapper *Rhomboplites aurorubens* size at age among reef sites separated by 10 s of km. Small-scale differences in growth have also been reported for gray triggerfish *Balistes capriscus* (Ingram 2001), white grunt *Haemulon plumieri* (Murie and Parkyn 2005), and red porgy *Pagrus pagrus* (DeVries 2006). Estimated longevity of these species, as well as many other nGOM fishery species, is <30 years, and often <20 years. This precludes the traditional usage of the bomb ^{14}C chronometer to validate ageing, at least with contemporary samples. However, the combined coral-red snapper otolith linear function reported here provides a prediction against which otolith Δ^{14}C vs. year of formation can be evaluated to validate age estimates of nGOM reef fishes with birth years between 1980 and 2015. An underlying assumption for using the decline period when validating age estimates is that the reference chronology (e.g. coral, otoliths from known-age fish) and fish must experience similar water masses (Kalish 1995; Campana and Jones 1998; Kastelle *et al.*, 2016).

Results of Δ^{14}C analysis of cored red snapper otoliths validate ageing protocols and age estimates for fish between 3 and 45 years of age. Three additional fish could not be validated since their estimated birth years are prior to nuclear testing (∼1960); however, there is no evidence of ageing bias in the maximum age estimate of 53 years. Results from this study were not surprising given results of studies in which marginal increment analysis (Patterson III *et al.*, 2001; Wilson and Nieland 2001; Fischer *et al.*, 2005), tagging data (Patterson III *et al.*, 2001), radiometric ageing (Baker *et al.*, 2001), and an earlier application of the bomb ^{14}C chronometer (Baker and Wilson 2001) have been employed to verify or validate otolith-based ageing in red snapper. In contrast to previous marginal increment studies that found opaque zone formation occurred in late winter to spring (Patterson III *et al.*, 2001; Wilson and Nieland 2001; Fischer *et al.*, 2004), Szedlmayer and Beyer (2011) found opaque zones formed in late summer to winter leading them to suggest that perhaps two opaque zones are formed annually in red snapper. However, the preponderance of data for this well-studied species clearly indicates a single opaque zone is laid down each year. Furthermore, the fact that otolith core $\Delta^{14}C$ values of red snapper matched expectation based on the regional coral and known-age otolith time series provides compelling evidence that opaque zone counts can be employed to accurately determine red snapper age for fish at least into their mid-1940s and probably longer.

Overall, results of this study could have broad implications for red snapper assessment and fishery management, as well as for estimating population dynamics parameters for other nGOM fishes that are not as long-lived as red snapper. Accurate age determination is fundamental to estimating fish growth and mortality rates, maturity functions, and longevity, but it also has important implications for estimating sustainable harvest rates for exploited species such as red snapper. Integrated stock assessment models rely on the accuracy of catch at age matrices, as well as life history parameter estimates, and quantitative stock assessment is an important component of informed management for many species. This is particularly true for red snapper given it supports the most economically important commercial and recreational finfish fisheries in the U.S. GOM, despite being estimated to be overfished since the 1970s (Cowan *et al*., 2010; SEDAR, 2016). Red snapper's overfished status and the stock rebuilding plan mandated by the Reauthorized Magnuson-Stevens Fishery Conservation and Management Act of 2006 have caused much consternation among fishery managers and constituencies alike (Cowan *et al*., 2010). Therefore, it is critical that the best scientific information available is utilized to assess and manage this marquee species. Results of this study not only provide overwhelming evidence that otolith-based ageing of red snapper produces accurate age estimates, but also the post-1980s decline in $\Delta^{14}C$ values establishes a method for validating ageing protocols that could be used for other, shorter-lived nGOM marine fishes.

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