Great longevity of speckled hind (*Epinephelus drummondhayi*), a deep-water grouper, with novel use of postbomb radiocarbon dating in the Gulf of Mexico

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Great longevity of speckled hind (*Epinephelus drummondhayi*), a deep-water grouper, with novel use of postbomb radiocarbon dating in the Gulf of Mexico

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Abstract: Growth characteristics are poorly understood for speckled hind (*Epinephelus drummondhayi*), a tropical deep-water grouper of economic importance that is considered overfished. Age has been validated for early growth, but the validity of adult age estimates is unknown. A few studies of growth zones in otoliths have revealed maximum age estimates of 15–35 years, which have been uncritically assumed as longevity. To answer questions about adult age, bomb radiocarbon dating was used to provide validated age estimates. A novel aspect of this study was use of the postbomb radiocarbon decline period (ca. 1980–2004) to age younger fish, an approach that was validated with known-age otoliths. Bomb radiocarbon dating provided valid length-at-age estimates ranging from ~5 years to more than 45 years. Age was unexpectedly greater than previous estimates for more than half the fish used in this study, and longevity may approach 60–80 years. This study extends the utility of bomb radiocarbon dating by more than 20 years and adds to the growing perspective that deep-water tropical fishes can be long-lived.

Résumé : Les caractéristiques de croissance ne sont pas bien comprises pour le mérou grivelé (*Epinephelus drummondhayi*), un poisson tropical d'eau profonde d'importance économique et considéré comme étant surexploité. Des estimations de l'âge ont été validées pour le début de la croissance, mais la validité des estimations de l'âge d'adultes n'est pas connue. Quelques études des zones de croissance d'otolites ont donné des estimations de l'âge maximum allant de 15 à 35 ans, dont il a été présumé sans évaluation critique qu'ils représentaient la longévité. Pour répondre aux questions touchant à l'âge des adultes, la datation au radiocarbone post-bombe a été utilisée pour obtenir des estimations validées de l'âge. Un aspect novateur de cette étude consiste en l'utilisation de la période de diminution du radiocarbone post-bombe (ca. 1980–2004) pour déterminer l'âge de poissons relativement jeunes, cette approche ayant été validée à l'aide d'otolites d'âges connus. La datation au radiocarbone post-bombe a fourni des estimations valides de la longueur selon l'âge allant de ~5 ans à plus de 45 ans. Contrairement aux attentes, les âges se sont avérés plus grands que les estimations antérieures pour plus de la moitié des poissons utilisés dans l'étude, et la longévité pourrait être de l'ordre de 60 à 80 ans. Cette étude élargit de plus de 20 ans la plage de temps pour laquelle la datation au radiocarbone post-bombe est utile et appuie la notion de plus en plus fréquemment invoquée selon laquelle les poissons tropicaux d'eau profonde peuvent vivre très longtemps. [Traduit par la Rédaction]

Introduction

The speckled hind (*Epinephelus drummondhayi*), a tropical deep-water grouper (Family Epinephelidae) of economic importance in the western North Atlantic and Gulf of Mexico, was identified as a "species of concern" in 1997 by the National Marine Fisheries Service owing to threats from fishing and incidental bycatch mortality (NMFS 2009). This species shows high site fidelity and may exhibit slow growth and late maturity, which make it susceptible to overexploitation (Matheson and Huntsman 1984; Manooch 1987; Parker and Mays 1998). As a result of these observations, the International Union for Conservation of Nature and American Fisheries Society has classified *E. drummondhayi* as endangered (Salamone 2012).

Age information provides the basis for describing life history parameters such as growth, mortality, recruitment, and productivity in fishes (Campana 2001). Deep-water grouper can be especially difficult to age because otoliths have provided challenging growth zone counting scenarios (Fig. 1). Studies using otoliths from *E. drummondhayi* collected along the southeastern United States have estimated maximum age as 35 years (Ziskin 2008), and no information exists for the Gulf of Mexico population (Table 1). Ziskin (2008) reported use of broad translucent bands consisting of multiple narrower zones and counted as annual increment groups. An assumption was made that the narrow increments were subannual with some validation using marginal increment analysis. However, in most cases the method can only be used to validate early age and growth and should not be used to validate long-lived species where increment width diminishes (Campana 2001), leaving accurate adult age in question for *E. drummondhayi*.

Bomb radiocarbon dating is a unique application in the age validation of fishes (Campana 2001). The approach relies on a conserved record of the rapid increase in radiocarbon that occurred in the oceans as a result of atmospheric testing of thermonuclear devices in the 1950s and 1960s (Broecker and Peng 1982). The uptake of bomb-produced radiocarbon by the marine environment is reported as delta carbon-14 ($\Delta^{14}C$) in reference to a prenuclear standard (Stuiver and Polach 1977). This signal was virtually synchronous in the mixed layer of mid-latitude oceans and was first measured in marine carbonates from hermatypic

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Fig. 1. Two images of the same transverse otolith section from specimen SPH-13 (*E. drummondhayi*) viewed with (A) reflected light on a black background and (B) off-axis transmitted light (Leica TL4000 Rotterman Contrast). Precise core extraction is visible as the grooved notch on the topside (distal margin) of the otolith section images. The $\Delta^{14}C$ value measured for this sample indicated that the age of this fish was at least 44 years. Note the complexity of the growth zone structure that can lead to a wide range of age estimate interpretations. The right side of the sulcus (ventral) reveals broad zone groupings quantifiable to approximately 25 years (A, B). The left side of the sulcus (dorsal) reveals a finer structure that can be quantified to more than 50 years and is more apparent with Rotterman Contrast transmitted light (B), which is consistent with bomb radiocarbon dating. Scale bar = 1 mm.

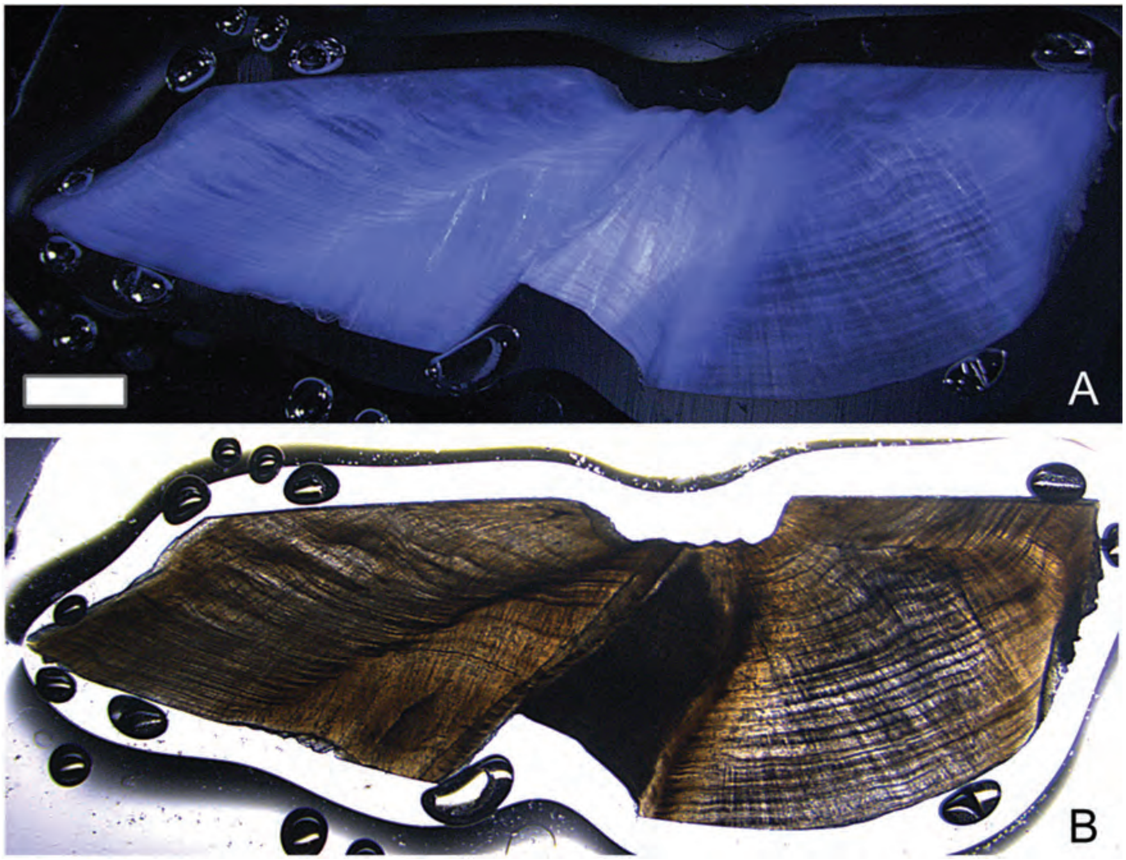


Table 1. Synopsis of von Bertalanffy growth function parameters for *Epinephelus drummondhayi* from studies performed in the United States.

					von Bertalanffy parameters		
Study	Region	Length (TL mm)	Estimated age (years)	Valid age (years)	L_∞ (mm FL)	K (years^{-1})	t_0 (years)
Ziskin et al. 2011	North Carolina to Florida	164–973	1–35	≤4	888 (1977–2007)*	0.12	–1.80
					945 (2004–2007)*	0.09	–2.76
Matheson and Huntsman 1984	North and South Carolina	185–861†	1–15	≤8	967	0.13	–1.01

*Parameters from all collection years and more recent only.
†Back-calculated mean lengths (length range of actual fish not reported).

corals (Knutson and Buddemeier 1973; Druffel and Linick 1978; Nozaki et al. 1978). Since that time, numerous bomb radiocarbon records have been recovered from shallow-water corals in all major tropical ocean basins (Grottoli and Eakin 2007). This time-specific $\Delta^{14}C$ rise and decline provides a reference period that can be used to determine age. Application to fishes began with an innovative comparison of $\Delta^{14}C$ values from otolith carbonate to $\Delta^{14}C$ records from hermatypic corals (Kalish 1993). In this and other studies, measured $\Delta^{14}C$ levels have provided validated age estimates that are independent of growth zone counting (Campana 1997; Kalish et al. 2001).

Bomb radiocarbon dating has since been applied successfully as an age validation tool in numerous teleost studies using otoliths (e.g., Ewing et al. 2007; Kastelle et al. 2008; Neilson and Campana 2008) and has expanded to other marine organisms ranging from calcareous algae and invertebrates (e.g., Frantz et al. 2005; Roark et al. 2006; Kilada et al. 2007) to sharks and toothed whales (Campana et al. 2002; Stewart et al. 2006; Andrews et al. 2011*b*). In the tropical and subtropical regions there have been successes that continue to expand the utility of the age validation tool (e.g., Andrews et al. 2011*a*, 2012), some of which were performed on fishes of the Gulf of Mexico (Baker and Wilson 2001; Fischer et al. 2005; Cook et al. 2009). Bomb radiocarbon dating is typically used to validate fish age with birth years during the rapid rise of $\Delta^{14}C$ between approximately 1955 and 1970 (depending on the region) and is considered the most sensitive period for $\Delta^{14}C$-based age

Fig. 2. Map of regional coral core locations that can be used as a temporal reference for the marine $\Delta^{14}C$ signal (star symbols). Some of these coral records were compiled to create the reference record used for age validation of *Epinephelus drummondhayi* and a broader regional $\Delta^{14}C$ reference that included a $\Delta^{14}C$ decline series (black star symbols). Other coral records were not used because of observed differences in the $\Delta^{14}C$ signal timing, which was attributed to different oceanographic circulation patterns and radiocarbon sources (white star symbols).

determination (Campana 2001). But recent information on the postbomb $\Delta^{14}C$ decline in some regions has the potential for providing validated age estimates for younger and more recently collected fish. This approach was initially investigated in the Gulf of Mexico for the gray snapper (*Lutjanus griseus*) but was limited to the use of red snapper (*Lutjanus campechanus*) otoliths as a reference for the $\Delta^{14}C$ decline period (Baker and Wilson 2001). Because the $\Delta^{14}C$ decline reference was from three red snappers with estimated ages and one juvenile, an assumption of age estimate accuracy was necessary. This assumption was supported by the alignment of older adult age estimates with the $\Delta^{14}C$ rise, but a truly independent $\Delta^{14}C$ decline time series was lacking for birth years more recent than the early 1980s.

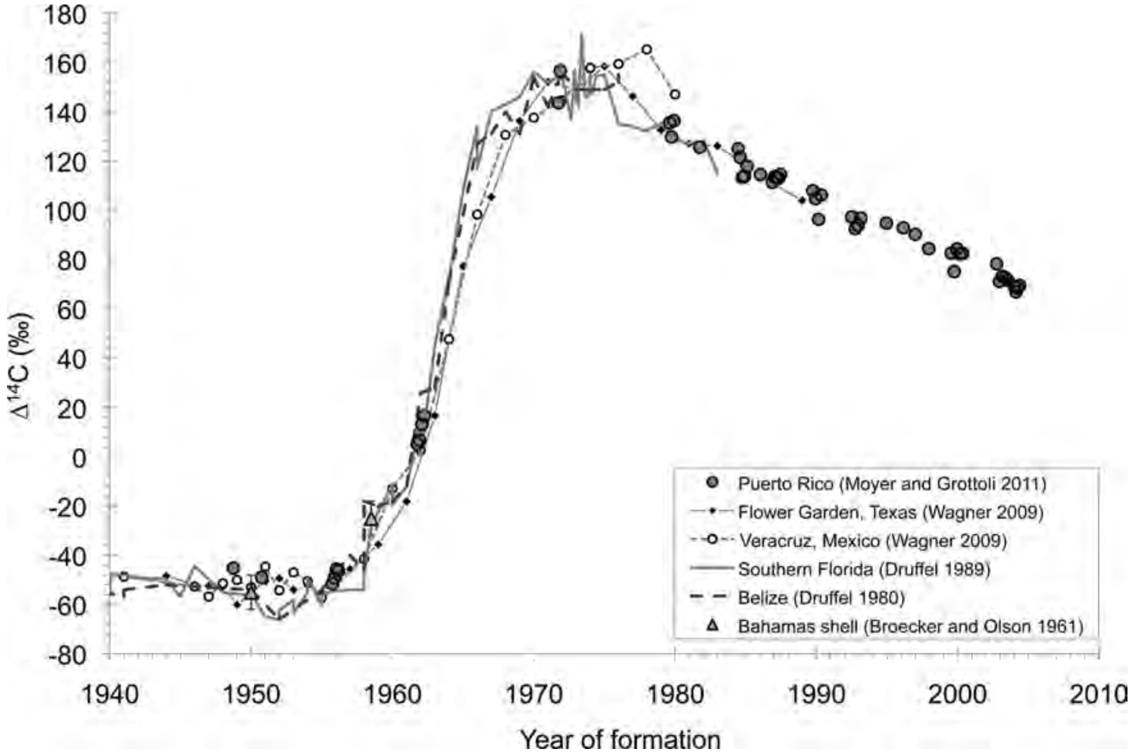
The objective of this study was to use bomb radiocarbon dating to provide valid age estimates for *E. drummondhayi*, thereby creating a validated basis for age-reading procedures for sectioned sagittal otoliths. The specific goals of this study were to (*i*) compile a more comprehensive regional $\Delta^{14}C$ reference record from hermatypic coral cores throughout the Gulf of Mexico; (*ii*) investigate a temporal extension of bomb radiocarbon dating to the period of postbomb $\Delta^{14}C$ decline; (*iii*) use known-age *E. drummondhayi* otoliths to validate the $\Delta^{14}C$ decline reference series; (*iv*) measure $\Delta^{14}C$ in the otolith cores of adult *E. drummondhayi*; (*v*) calibrate the age of each fish using otolith $\Delta^{14}C$ data relative to the regional $\Delta^{14}C$ reference record; and (*vi*) use validated age to make an estimate of longevity.

Materials and methods

Bomb radiocarbon dating reference

Reference $\Delta^{14}C$ records from previous studies were assembled to prepare for bomb radiocarbon dating of fishes in the Gulf of Mexico. These reference records were to provide a temporal basis for comparing measured $\Delta^{14}C$ levels from otoliths of *E. drummondhayi* specimens. The applicable bomb radiocarbon records in the Gulf of Mexico were from hermatypic coral cores collected at Belize (Druffel 1980), Veracruz, Mexico (Wagner 2009), Flower Garden Banks, Texas (Wagner 2009), several locations off the coast of southern Florida (Druffel 1989), and northeastern Puerto Rico (Moyer and Grottoli 2011; Fig. 2). The individual coral $\Delta^{14}C$ records selected for use in bomb radiocarbon dating provided variable degrees of temporal continuity and resolution for the critical time period, typically between ca. 1955 and ca. 1970 (Fig. 3). Collectively, the coral $\Delta^{14}C$ records continuously cover a period ranging from well before the bomb radiocarbon pulse to well after the peak through the 1970s and ending in 2004 during the postbomb decline period. Hence, it was deemed feasible that fish age could be validated using the combined $\Delta^{14}C$ records with an extended utility through the decline period (ca. 1980–2004). The individual $\Delta^{14}C$ records from the hermatypic corals were combined to form a generic regional reference curve used to estimate the age of fish. The temporal trend of the combined coral $\Delta^{14}C$ records was described with a Loess curve fit (spline interpolation smoothing parameter = 0.3, two-parameter polynomial; SigmaPlot 11.2). The

Fig. 3. Bomb radiocarbon references from the coral cores used in compiling a general Δ14C reference record for use in validating the age of fishes from the Gulf of Mexico. Original records from Glover Reef, Belize, and The Rocks off Southern Florida were coupled with three more recent studies from Flower Garden, Texas, Santiaguillo Reef, Mexico, and Cayo Ahogado, Puerto Rico. The Δ14C records from Flower Garden and Cayo Ahogado extend the reference series well into the Δ14C decline period for the region. Included are shell records from the Bahamas as part of the argument for extending the range of the regional reference series to the western North Atlantic.



most diagnostic portions of the curve were the rise and decline periods, as evidenced by the strong slopes. *Epinephelus drummondhayi* with known collection dates were used to validate the applicability of the Δ14C decline period to younger adult otolith cores.

Sample selection

To provide a range of validated age estimates for adult *E. drummondhayi*, an experimental design blind to estimated age (growth zone counting in otoliths) was devised for selection of the samples for radiocarbon dating. Otoliths were selected that differed considerably across the available range of fish-size and otolith-mass classes (i.e., low and high otolith mass samples were selected for a given fish length range). Samples that were considered in this manner were from collections made along the coast of Florida in the Gulf of Mexico in the years 2000–2005 from fishery-dependent sampling. Samples available for consideration ranged from fish with lengths of 500–550 mm FL (fork length) and otolith masses near 0.1 g to fish near maximum size (up to 1075 mm FL) and an otolith mass exceeding 1 g. In general, a range of otolith mass and fish length scenarios was chosen to cover birth year possibilities that may range through the informative rise and decline periods.

Radiocarbon analysis protocol

Core material from the selected otoliths was extracted using a micromilling machine. Individual otoliths were cleaned using alternating steps with deionized water and mild detergent, with sonication for several minutes. All otoliths appeared to be satisfactorily cleaned and were air-dried overnight prior to mounting for milling. Whole otoliths were mounted on glass slides with the sulcus side down, making the distal surface accessible for core extraction by micromilling. Cytoseal was used as an adhesive and was allowed to cure for a week prior to further preparation. Because the adult otoliths accrete a small amount of otolith material on the distal side of the otolith, wet hand grinding using 320- to 1000-grit carbide wet–dry sandpaper was performed to expose the earliest otolith growth. The first few years of growth were usually visible as grinding proceeded, and the concentric growth zone structure, as well as radial lines running outward from the nucleus, was used as a guide in exposing the core. Milling proceeded as an extraction of the smallest core structure visible. Guidance was provided using the dimensions of a 2-year-old juvenile otolith (6.2 mm long × 3.6 mm wide × 0.7–0.8 mm thick). Focus was aimed at material closest to the time of birth, and dimensions of 3.9 mm long × 2.1 mm wide were measured from what appeared to be the first annulus in a digital image of the whole otolith.

Extraction of the otolith core utilized the computer automated capabilities of a New Wave Research micromilling machine (ESI - NWR Division, Fremont, California, USA). A 0.5 mm diameter bit (Brasseler USA, Savannah, Georgia, USA) was used to drill an overlapping surface scan within the oval dimensions of 4 mm long × 2 mm wide. The surface scan was a guided extraction that conformed to the uneven surface structure of each otolith. Otolith material was extracted to a depth of 400 μm with two passes of the scan at 200 μm each (conservative relative to the reference otolith material). These dimensions appeared to match or reside within the 1-year-old otolith dimensions and were estimated to liberate a sample mass near 3–5 mg. The extracted samples were submitted to the National Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at Woods Hole Oceanographic Institution in Woods Hole, Massachusetts, for routine radiocarbon analyses using accelerator mass spectrometry (AMS).

Radiocarbon measurements were reported from NOSAMS as the fraction modern (Fm), which was used to calculate Δ14C with a

Table 2. Reference bomb radiocarbon series from known collection date speckled hind (*Epinephelus drummondhayi*).

Sample No. (WHOI No.)	Length (mm TL)	Collection date	Sample (mg)	Fm	$\Delta^{14}C$ (‰) ($\delta^{13}C$)
SPH-R1 (OS-93078)	449	18 Jan. 2000	3.2	1.0949	88.4±3.8 (–3.85)
SPH-R2 (OS-94584)	423	20 May 1999	5.3	1.0847	78.3±4.1 (–4.41)
SPH-R3 (OS-94641)	500	10 Mar. 1994	10.6	1.1024	96.6±4.0 (–4.82)
SPH-R4 (OS-93076)	337	9 Dec. 1994	4.3	1.1008	94.6±3.6 (–4.71)
SPH-R5 (OS-95754)	360	17 Feb. 1993	2.4	1.1100	104.3±4.4 (–3.95)
SPH-R6 (OS-93007)	347	16 June 1992	10.1	1.1115	105.9±4.0 (–5.59)
SPH-R7 (OS-94586)	357	16 June 1992	9.4	1.1117	106.1±3.5 (–4.66)
SPH-R8 (OS-93049)	282	10 Aug. 1991	8.5	1.1115	106.0±3.6 (–5.14)
SPH-R9 (OS-93070)	262	10 Aug. 1989	6.2	1.1194	114.1±4.2 (–5.28)
SPH-R10 (OS-95755)	310	19 July 1986	Lost	—	—
SPH-R11 (OS-94587)	376	4 Oct. 1986	4.9	1.1193	114.4±3.4 (–4.06)
SPH-R12 (OS-93077)	292	14 Aug. 1986	4.4	1.1312	126.3±3.9 (–5.11)
SPH-R13 (OS-93073)	339	28 Sept. 1981	5.3	1.1373	133.0±3.7 (–4.32)
SPH-R14 (OS-95756)	512	22 Oct. 1998	1.5	1.0890	82.7±4.4 (–4.29)

Note: Each sample analyzed was either part of a whole otolith or edge material from larger fish otoliths. All were collected along the Florida coast in the Gulf of Mexico. Fm, fraction modern.

correction for natural isotopic fractionation (Stuiver and Polach 1977). Fraction modern is the measured deviation of the $^{14}C/^{12}C$ ratio from a "modern" sample. This internationally agreed upon reference is defined as 95% of the radiocarbon concentration of the NBS Oxalic Acid I standard (SRM 4990B) normalized to $\delta^{13}C_{VPDB}$ (–19‰) in 1950 CE (VPDB, Vienna Pee Dee Belemnite geological standard; Coplen 1996). Sample Fm values were corrected for fractionation using a $\delta^{13}C$ value measured online (during the AMS analysis). In addition, a measured value from a partitioned sample provided a robust, sample-specific $\delta^{13}C$ value.

The calculated $\Delta^{14}C$ values reported in this study were corrected for age, or time of formation, based on an approximate birth year. Because age was not known or estimated prior to the radiocarbon analysis, a retrospective estimate was generated based on the initial $\Delta^{14}C$ value and its known proximity in time relative to the reference $\Delta^{14}C$ records. This kind of adjustment provides a $\Delta^{14}C$ value that is more comparable to the $\Delta^{14}C$ reference time series. The year used in the corrections was based on a $\Delta^{14}C$ criterion as follows:

Birth year approximation from adjustment $\Delta^{14}C$ criteria:
Prebomb period = 1950 for < –35‰
Rise period = 1962 for –35‰ to 125‰ (large otolith-fish)
Near-peak period = 1972 for >125‰
Decline period = 1992 for <125‰ (small otolith-fish)

The reason for such corrections is to provide a $\Delta^{14}C$ value that takes into account the decay that occurred between the approximate year of birth and the time of measurement, as is the case with all regional $\Delta^{14}C$ reference records (see review by Grottoli and Eakin 2007). An additional consideration was making a choice between rise and decline periods for $\Delta^{14}C$ values shared between scenarios, specified above as small fish with small otoliths for the decline period and large fish with large otoliths for the rise period (minor assumption based on empirical ontogenetic changes in fish otolith size with time); however, both age estimation scenarios are listed for consideration.

Bomb radiocarbon dating

Estimates of age were determined by projecting the measured $\Delta^{14}C$ values back in time from the measurement date to the regional $\Delta^{14}C$ reference series. For levels attributed to the rise period (most diagnostic), age was estimated based on a fit to the Loess curve (SigmaPlot 11.2 iteration routine) with an uncertainty from estimated prediction intervals. Smaller fish with $\Delta^{14}C$ values attributed to the decline period were aged using a linear regression and 99% prediction intervals. Levels measured near the regional peak in $\Delta^{14}C$ would be assigned an age range that could be attributed to the time the region held those $\Delta^{14}C$ levels. For prebomb levels, a minimum birth year and age was estimated based on the fit of the value to the Loess curve of the coral $\Delta^{14}C$ data. Values below the Loess curve minimum were given a year based on the last reference $\Delta^{14}C$ value near the measured $\Delta^{14}C$ value. Age estimated from these values was a minimum age, with a few years earlier or younger as an uncertainty for the distribution at the beginning of the rise in $\Delta^{14}C$. An upper limit to the age estimates for these fish was estimated loosely on an otolith mass-to-age relationship. Radiocarbon samples from *E. drummondhayi* with diagnostic age estimates were used to create a linear relationship that was used to estimate maximum age from otolith mass.

Results

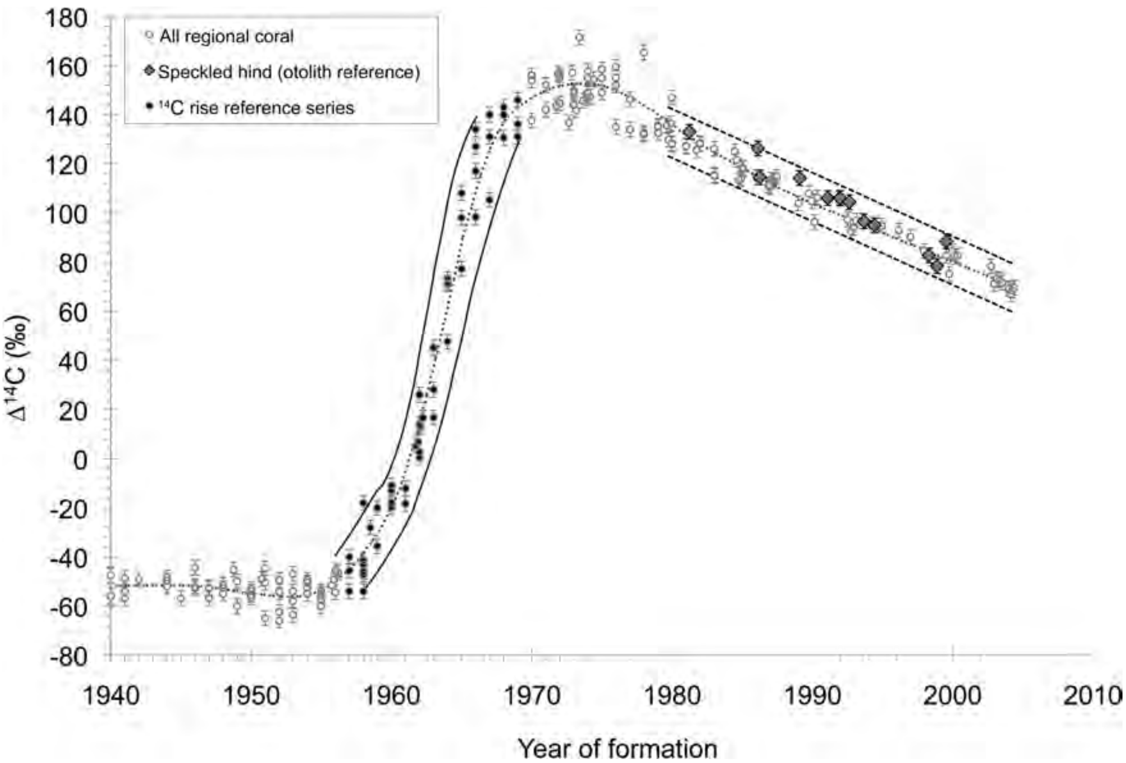
Bomb radiocarbon dating reference

Because use of the $\Delta^{14}C$ decline period has not been tested, a series of young *E. drummondhayi* otolith specimens with a known collection date were analyzed for $\Delta^{14}C$ to validate age calibration of adults. These fish were resourced from archived collections and ranged in collection year from 1981 to 2000 (Table 2). Fish selected for this analysis were 262–512 mm TL (total length, n = 14); the largest fish were sampled at the edge or rostrum tip for most recently deposited material. Other samples were small otolith fragments, some of which were a vestige of original envelope contents (e.g., broken otolith edge pieces). Measured $\Delta^{14}C$ levels from this otolith series were compared with the coral reference series, minus one lost sample (SPH-R10). Each $\Delta^{14}C$ value was assumed to be from approximately 1 year of material; hence, each $\Delta^{14}C$ value was plotted 0.5 years prior to collection date. The *E. drummondhayi* reference data were consistent with the coral $\Delta^{14}C$ reference series, ranging from a high of 133‰ in 1981 and declining to a low of 78.3‰ in 1999. These data provide support for the use of otolith cores from adults to age fish using the $\Delta^{14}C$ decline period.

In addition to the 13 *E. drummondhayi* of known collection date, three juvenile otolith $\Delta^{14}C$ records from prior studies of other species in the northern Gulf of Mexico were compared with the $\Delta^{14}C$ decline reference series (Fig. 4). One 1-year-old yellowedge grouper (*Epinephelus flavolimbatus*) in 2000 (replicated 80.2‰ and 82.9‰ plotted at 1999.5), and one 2-year-old red snapper (*Lutjanus campechanus*) cored to first year (85.3‰ at 1996.5), were also considered as practical reference material (Baker and Wilson 2001; Cook et al. 2009).

All *E. drummondhayi* otolith references were in close agreement with the coral $\Delta^{14}C$ reference series (Fig. 4). For the range of sample dates shared by coral and the otoliths of known-age juveniles,

Fig. 4. Compiled bomb radiocarbon reference series for all regional coral core $\Delta^{14}C$ data. Temporal correlation of otolith cores was most time-specific on the $\Delta^{14}C$ rise (filled circles noted as ^{14}C rise reference series) and the $\Delta^{14}C$ decline. The $\Delta^{14}C$ decline is emphasized and supported with data from otolith material of known collection date from *E. drummondhayi* (grey diamonds). Given a measured $\Delta^{14}C$ value from a fish otolith, age estimates were derived from a fitted Loess curve (rise) and linear regression (decline), with limits from 99% prediction intervals.



slopes were indistinguishable (ANCOVA, $P = 0.26$). Therefore, the two sample sets were pooled into a single regression used to estimate age prediction intervals. As a result, all coral and otolith data between 1980 and 2004 were used to establish a linear relationship for use in age determination (birth year = 2031 – $\Delta^{14}C \times 0.386$; $R^2 = 0.964$) with 99% prediction intervals (±4.1 years). These intervals were used to provide limits to the calibration of age from $\Delta^{14}C$ measured in core material of the otoliths of younger adults.

To provide similar limits for age estimation on the rise of $\Delta^{14}C$ in the 1950s and 1960s, data associated with this region of the curve were selected (Fig. 4). Because the slope is curvilinear, with more reference data variability toward the ends of the range, the residuals associated with the Loess curve fit were used to describe a limit that follows the observable variation. Low and high Loess residuals were separated into two data sets and a second Loess curve was applied to those data (0.6, two-parameter), plus and minus 1 year to shift the reference limit to just outside the observed $\Delta^{14}C$ reference distribution (age uncertainty ±1.3 years to ±1.9 years depending on position along the curve; Fig. 4).

Bomb radiocarbon dating

A total of 16 otolith samples were selected for radiocarbon analysis from adult *E. drummondhayi* collected in 2001–2005. Based on the experimental design previously described, fish length and otolith mass were used to cover the extremes of possibilities within various size ranges (Table 3). In addition, a loose estimate of potential age range was made from published length-at-age data from Ziskin et al. (2011) to roughly hypothesize the utility of the bomb radiocarbon reference series. The smallest fish were near 600 mm FL with otoliths near 0.2 g. It was hypothesized that these fish would be aged using the $\Delta^{14}C$ decline reference series at less than 10 years. Fish approaching 700 mm FL were selected based on fish with similar length (660–690 mm FL) and differences in otolith mass by up to a factor of 4 (0.14–0.55 g), but all with a hypothesized age of less than 15 years. Upper-medium to large-sized fish between 700 and 900 mm FL were considered as possibly ranging from more than 10 years of age to approaching or exceeding 30 years of age. Otolith masses crisscrossed the range, with the greatest contrast between a 710 mm FL fish having the highest otolith mass near 0.68 g, to a fish at 880 mm FL with an otolith nearly two times lighter. Selection of the largest fish otoliths came from fish ranging from 995 to 1075 mm FL. We hypothesized that these fish would exceed 30 years because Ziskin et al. (2011) provided an estimate of 35 years for a fish 930 mm TL (~882 mm FL; TL = 1.05FL + 3.52; Ziskin (2008)), but it was uncertain what fish would reach prebomb levels at nearly 45 years from the collection dates. Hence, the four specimens chosen ranged in otolith mass from near 0.89 to 1.16 g, a difference of 30% for fish that were similar in length (1000 and 1005 mm FL).

Radiocarbon was measured for all samples except one that was lost during the coring process (SPH-2). Calculated $\Delta^{14}C$ values ranged from a low value of –59.5‰ (prebomb) to a high value of 126.1‰ (near peak), a range similar to the regional coral $\Delta^{14}C$ reference series (Fig. 5). Three fish provided $\Delta^{14}C$ levels that could be attributed to either rise-age or decline-age scenarios. Two were from small fish with small otoliths, and an age from the decline scenario was selected over unreasonable ages near 30 years. The third fish provided the highest measured $\Delta^{14}C$ value, and both scenarios were applicable at approximately 20 and 35 years (SPH-11); neither otolith mass nor fish length (large fish with a small otolith) provided support for one age scenario over the other. Two larger fish provided diagnostic $\Delta^{14}C$ values for rise-age estimates near 40 years (SPH-8 and SPH-9). The remaining samples were unexpectedly greater than approximately 43–45 years based on prebomb $\Delta^{14}C$ levels and likely older by a significant margin for the largest fish with the most massive otoliths.

Table 3. Fish mass and length, otolith mass, and radiocarbon and age data for adult speckled hind (*Epinephelus drummondhayi*) used in this study.

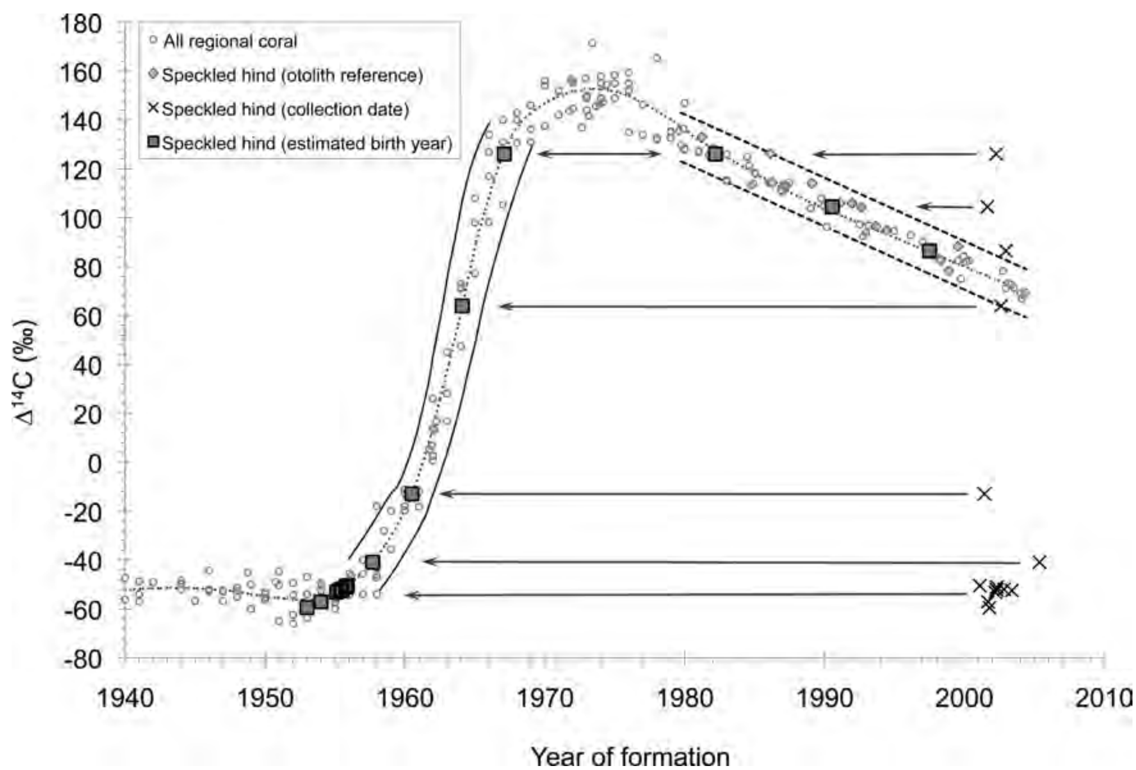
Sample No. (WHOI No.)	Length (mm FL)	Otolith mass (g)	Collection date	Mass (mg)	Fm	$\Delta^{14}C$ (‰)	$\delta^{13}C$ (‰)	Radiocarbon age (years)	Age scenario
SPH-1 (OS-94595)	610	0.2135	20 Sept. 2001	7.3	1.1101	104.5±3.6	–5.62	11.1±4.1 (7–15)	Decline
SPH-2 (—)	630	0.1483	13 Dec. 2002	Lost	—	—	—	—	
SPH-3 (OS-96332)	660	0.1416	13 Dec. 2002	2.2	1.0920	86.5±2.8	–5.59	5.4±4.1 (1–9)	Decline
SPH-4 (OS-95757)	670	0.5455	7 Sept. 2001	4.6	0.9429	–57.1±3.2	–5.89	47.7 (>44 min.)	Prebomb†
SPH-5 (OS-95752)	690	0.4969	5 Feb. 2001	5.7	0.9495	–50.5±2.9	–5.13	45.2 (>43 min.)	Prebomb†
SPH-6 (OS-94702)	710	0.6780	12 Mar. 2002	4.4	0.9468	–53.2±4.3	–5.23	47.1 (>44 min.)	Prebomb†
SPH-7 (OS-95758)	745	0.6092	30 Oct. 2002	5.4	0.9480	–52.0±3.8	–5.57	47.3 (>45 min.)	Prebomb†
SPH-8 (OS-95759)	850	0.6691	6 June 2001	5.4	0.9885	–12.9±3.1	–5.49	40.9±1.5 (40–44)	Rise
SPH-9 (OS-95760)	855	0.4744	8 Aug. 2002	5.6	1.0655	64.0±5.6	–5.33*	38.5±1.4 (37–41)	Rise
SPH-10 (OS-95761)	860	0.5726	9 Apr. 2002	5.4	0.9479	–52.1±3.1	–5.12	46.8 (>44 min.)	Prebomb†
SPH-11 (OS-94673)	880	0.3775	9 Apr. 2002	5.5	1.1291	126.1±4.1	–5.65	20.1±4.1 (33–37)	Rise and decline
SPH-12 (OS-95762)	995	0.9770	17 Oct. 2001	4.9	0.9405	–59.5±4.0	–5.16	48.8 (>44 min.)	Extrap. 69 years
SPH-13 (OS-94696)	1000	1.1586	26 Apr. 2002	5.7	0.9490	–51.0±3.5	–5.26	46.6 (>44 min.)	Extrap. 83 years
SPH-14 (OS-95753)	1005	0.8902	13 May 2005	5.5	0.9591	–40.9±3.1	–4.57	47.7±1.9 (>43 min.)	Extrap. 63 years
SPH-15 (OS-94697)	1075	0.9286	26 Apr. 2002	5.2	0.9478	–52.2±4.7	–5.09	46.9 (>44 min.)	Extrap. 65 years
SPH-16 (OS-94672)	1020	1.0553	22 May 2003	5.3	0.9475	–52.5±3.2	–5.23	48.0 (>45 min.)	Extrap. 75 years

Note: Radiocarbon age was calculated based on the difference in time from collection to either the decline regression or rise Loess curve. Uncertainty for decline age was from 99% prediction intervals and rise age was from Loess of residuals as described in the Materials and methods section. Resolved age is in parentheses as either an age range for well-defined estimates or greater than an age calculated from the last year where prebomb reference values were considered (1958). Extrapolated estimates of age were made to explore potential maximum age for fish with the most massive otoliths (estimate uncertainty unknown). Fm, fraction modern.

*Partitioned sample lost and mean of all measured samples (n = 14) was used.

†These fish may have ages near the minimum estimated age based on a possible otolith mass-to-age relationship used to extrapolate age for the largest fish.

Fig. 5. Alignment of the measured $\Delta^{14}C$ values from otolith cores of *E. drummondhayi* with estimates of age (range = ~5 to >45 years) determined from the compiled Gulf of Mexico bomb radiocarbon reference curve. Measured $\Delta^{14}C$ values were initially plotted at the date of collection ("×" symbol) and then projected to the appropriate location in time on the bomb radiocarbon reference series (grey squares). Age was determined from the time difference between collection date and alignment with the reference series. Each age was estimated on a case by case basis because of the variability of the reference series and because some samples had two potential age estimate scenarios.



To explore the possibilities of maximum age for the largest fish, an otolith mass-to-age relationship was loosely determined from the well-defined age estimates (age = 74.3 × otolith mass – 3.6; n = 4). The scarcity of validated age estimates makes this relationship far less than diagnostic, but can function as a first indication of what ages are possible for the largest fish (SPH-12 through SPH-16). All these fish had large otoliths that were 33%–73% more massive than those of the fish aged diagnostically at 41 years (SPH-8). Given the linear relationship determined here, these fish could have been older by approximately 20–40 years, for a roughly estimated maximum age near 60–80 years (SPH-13; Fig. 1).

Discussion

The combined coral core records from a broadly defined Gulf of Mexico region supports a long and fairly well-constrained Δ^{14}C reference record for use in age validation of regional fishes. Older coral core studies (Druffel 1980, 1989), combined with more recent studies (Wagner 2009; Moyer and Grottoli 2011), indicate that there is a high degree of consistency in the timing and amplitude of the Δ^{14}C rise across a broad region. Because of differences in the timing and amplitude of other Δ^{14}C records from the eastern Caribbean (Kilbourne et al. 2007; Wagner 2009) and Bermuda (Druffel 1989), those data were not considered for development of the bomb radiocarbon reference in the Gulf of Mexico. Use of the combined record for ageing fish from otolith core material provided age estimates with relatively high accuracy (±1.5 to 2 years) for the period of rising Δ^{14}C (ca. 1958–1967). Two *E. drummondhayi* were aged to approximately 39 and 42 years, exceeding the previous maximum age estimated for this species by approximately 4–7 years; these two fish were 75–80 mm smaller than the 35-year-old fish (930 mm TL, ~882 mm FL) reported by Ziskin et al. (2011). In addition, the validated ages reported here are two to three times older than the maximum age of 15 years reported for fish of similar size by Matheson and Huntsman (1984).

Moyer and Grottoli (2011) presented a Δ^{14}C reference record from northeastern Puerto Rico that provides an additional reference in support of the Δ^{14}C decline period for validating the age of younger fish. While Moyer and Grottoli (2011) were primarily interested in the decline period for other purposes, a series of samples was also measured from several periods within the prebomb and rise portions of their record. These data were consistent with the synthesis of other Δ^{14}C data sources throughout the Gulf of Mexico. Because of its easterly position relative to the Gulf of Mexico, there was some question as to why the record was not in better agreement with other eastern Caribbean Δ^{14}C reference records. After a detailed examination of the regional ocean current systems, it was apparent that the northeastern coast of Puerto Rico is strongly influenced by the northern Atlantic and the North Equatorial Current. This ocean system differs from the eastern Caribbean because of the dominant influx of ocean waters from the South Atlantic Equatorial Current, transported north and west into the Caribbean via the North Brazil Current system (Bischof et al. 2003; Bonhoure et al. 2004; Wagner 2009). Hence, these other Caribbean records are apparently attenuated and phase-lagged to an extent by a more equatorial and southern hemisphere Δ^{14}C signal (Broecker and Peng 1982; Druffel 1996). In addition, measurements of dissolved inorganic carbon Δ^{14}C within waters of the Antilles Current in the mid-1950s and from mollusk shells in the Bahamas (Broecker and Olson 1961) were consistent with the Moyer and Grottoli (2011) coral measurements in northeastern Puerto Rico and divergent from the Kilbourne et al. (2007) record from southwestern Puerto Rico. Hence, there was evidence for the utility of the Moyer and Grottoli (2011) record, adding 20 years of reference Δ^{14}C data, as a means to determine the age of younger and more recently collected fish from the Gulf of Mexico. The hypothesis was successfully tested with the series of young fish having known collection dates.

Compilation of the coral Δ^{14}C records, coupled with known-age otolith material, provided a validated basis for the use of the regional postbomb Δ^{14}C decline as a measure of age for Gulf of Mexico fishes. In numerous oceanic regions, the Δ^{14}C records indicate that this kind of analysis is not possible because of complications in the rise (due to conditions like upwelling of Δ^{14}C-depleted waters) or lack of a postbomb decline period (Δ^{14}C remains elevated long term) or both (e.g., Grottoli and Eakin 2007). In the waters of the coastal northeastern Pacific, strong seasonal upwelling has led to a very high degree of both temporal and spatial variation in the rise of bomb Δ^{14}C, which is reflected in a collection of numerous records from the region (Allen and Andrews 2012; Haltuch et al. 2013). Observed Δ^{14}C differences in seawater from Half Moon Bay, California, were as great as 100‰ within a single year (Robinson 1981). Conversely, Δ^{14}C levels past the peak in the tropical Indo-Pacific region can remain elevated for many decades and carry a lesser degree of seasonal variation owing to the stability of the mixed layer. Because reference records from this region exhibit a Δ^{14}C decline that is slow and has a low slope (Andrews et al. 2011*a*), fish with otolith core Δ^{14}C values at near-peak levels could not be age validated with any certainty. In addition, owing to the difficulty in obtaining coral cores from remote places and the relatively high cost of Δ^{14}C analysis, many reference records are simply incomplete. Coral Δ^{14}C reference records are also limited by the collection year, often to only a short time after peak bomb radiocarbon levels were reached (Druffel 2002). Despite these limitations and to properly evaluate the temporal relationship of Δ^{14}C, regionally specific records are necessary and should be tested with material of known age and be species specific if possible. However, it is not too great an assumption that coral Δ^{14}C records will reflect the same marine Δ^{14}C signal (Druffel 1997) that is also sequestered by fishes of the region.

There was confidence in the age estimates for the two youngest fish because of the agreement between juvenile *E. drummondhayi* otoliths and the coral Δ^{14}C reference record. The finding was consistent with the hypothesis that otolith mass is a good proxy for age, as opposed to fish length; the 5-year-old fish was 50 mm longer than the 11-year-old fish, yet otolith mass was positively correlated with age for these samples. Additionally, if the Δ^{14}C decline period were not well defined, then the selection of young versus old age scenarios would have been ambiguous for the relatively elevated Δ^{14}C value (64.0‰ ± 5.6‰; SPH-9). This fish was not one of the longest and otolith mass was moderate, but because the decline period is well defined, the older age scenario of approximately 39 years was the only plausible choice.

Otolith mass was used to resolve the age of one fish having complicated bomb radiocarbon results. The most elevated Δ^{14}C value (126.1‰ ± 4.1‰) was near peak for a large fish with low otolith mass (SPH-11), the combination of which precludes a clear age classification. Given that otolith mass may be a better proxy for age, the rise-age scenario is in better agreement with the age from otolith mass relationship established by larger fish (discussed below). Use of the younger age (~20 years) leads to ages derived from otolith mass that were unrealistically less than the minimum age established by prebomb levels by up to 12 years. Hence, it is more likely that this fish was ~35 years at a length of 880 mm FL. These observations require further investigation, and it is an issue worth considering in a future study of age estimation from otolith sections.

The otolith mass-to-age relationship was used to provide an estimate of *E. drummondhayi* longevity, as well as age estimates for the largest fish with prebomb Δ^{14}C levels. Otolith mass-to-age relationships for other fishes have been described as roughly linear, and estimates were made beyond the validated maximum age of 42 years. Based on this relationship, it is possible that five of the larger fish (SPH-4, 5, 6, 7, and 10) were at an age that would provide a birth year close to the beginning of the rise of Δ^{14}C. Further, the five largest fish may have been considerably older because of their more massive otoliths and age estimates ranging from near 60 to 80 years. It is recommended that these guidelines be used to search for an age estimation protocol that can account for not only the validated age estimates to 42 years, but beyond this age to approaching 60–80 years (Fig. 1). This approach is similar to what was performed for red snapper and yellowedge grouper in the Gulf of Mexico under similar bomb radiocarbon circumstances (Baker and Wilson 2001; Cook et al. 2009). Relative to these age estimates, the next available technique that can assay fish of this age is lead–radium dating. This approach requires an entirely different experimental design and is currently the most viable method available for fish age approaching 100 years (Campana 2001; Hutchinson et al. 2007; Andrews et al. 2009).

The additional three $\Delta^{14}C$ records for juvenile fish from two other studies in the northern Gulf of Mexico seem to support the applicability of the decline reference series to other species. The measurements from one red snapper (age 2 and cored to age 1) and one yellowedge grouper (age 1 and replicated) provided $\Delta^{14}C$ values that were remarkably consistent with the reference $\Delta^{14}C$ series (Baker and Wilson 2001; Cook et al. 2009). Application of the age estimates for other young red snapper (ages 7, 9, and 15 years; Baker and Wilson 2001) also provided a well-centered fit to the $\Delta^{14}C$ reference series and is apparently a good follow-up validation of age for these red snapper samples. In contrast, a reevaluation of the $\Delta^{14}C$ values and estimated ages of yellowedge grouper relative to the decline reference series revealed mostly differences and ages greater than expected. This kind of offset can be explained by (*i*) age underestimation or overestimation, (*ii*) the $\Delta^{14}C$ decline reference as not valid for this species, and (*iii*) an artifact of sample preparation or some combination of these factors. Because of the agreement of $\Delta^{14}C$ measurements from two 1-year-old juveniles with the reference record, it is possible that age estimation was a problem for this sample set. However, this interpretation is problematic because one set of otoliths has collection years prior to the appropriate $\Delta^{14}C$ decline years; hence, the fish would have been captured before being born. Based on this observation, and as previously discussed by Cook et al. (2009), the possibility of a deep-water effect on the $\Delta^{14}C$ levels in juvenile otoliths (recruitment to deep water) needs to be considered. This factor is especially relevant if the otolith core material used to measure $\Delta^{14}C$ consisted of several years of growth. Because the youngest yellowedge grouper was in sync with the $\Delta^{14}C$ decline series, it is possible that this fish represents settlement in shallower water and that the otolith sampled was strictly limited to the first year of growth, whereas the others were cores from slightly older fish and may have included a deeper water $\Delta^{14}C$ signal.

In addition to the work performed on yellowedge grouper, a study from the same region as the red snapper $\Delta^{14}C$ research was performed on gray snapper (Fischer et al. 2005). A reassessment of the gray snapper $\Delta^{14}C$ results relative to the $\Delta^{14}C$ decline reference series indicated that the estimated birth years were more consistent than yellowedge grouper but offset by 2–3 years more than was expected for five of the six samples. Deep-water $\Delta^{14}C$ complications do not appear to exist for gray snapper, suggesting a low degree of age overestimation. However, age estimation was not considered difficult for this species (Fischer et al. 2005), suggesting the need for calibration using known-age otolith material. Furthermore, some near-peak $\Delta^{14}C$ values were lower than expected for red snapper (Baker and Wilson 2001), also suggesting that proper calibration is necessary for this species to verify reference curve alignment and coring precision.

Precision and accuracy has increased in otolith radiocarbon analyses both in terms of sample extraction and AMS analysis. Extraction of *E. drummondhayi* otolith cores in this study employed techniques and equipment that allow much greater confidence in removal of target material (Fig. 1). Because previous studies have expressed concern about the precision of core or sample extraction, it is possible that the observed variability of otolith $\Delta^{14}C$ values can be reassessed for these species using an experimental design with greater precision. Physical extraction of samples on the order of 3–5 mg of otolith carbonate can be routine with a micromilling machine. Instead of removing all external material to reveal the target region, the target region itself is removed from an intact otolith, leaving a majority of the whole available for further analysis, such as verifying core extraction and age estimation from growth zone counting. For the previous studies, the core extractions regularly approached or exceeded 10 mg, with highly variable levels of precision (some ±1 SD > 10‰). At present, AMS precision of ±1 SD = 3‰–5‰ is attainable for a 1–2 mg sample, and in this study a mean sample mass of 5.2 mg produced a mean precision of ±1 SD = 3.7‰. This increase in both coring accuracy and AMS precision greatly increases the opportunity to sample otolith material from much earlier in the life history of the fish with confidence. Hence, the uptake of $\Delta^{14}C$ from waters complicated by environmental changes (i.e., movement into deeper water) decreases as the sample specificity approaches the otolith mass of larval and early juvenile stages.

Based on the findings presented in this study, the following recommendations can be made for future use of the compiled coral and otolith $\Delta^{14}C$ reference records. Research can continue with *E. drummondhayi* by selecting more samples with potential birth years in the late 1950s through to the 2000s to create a wide range of validated length-at-age estimates using the Gulf of Mexico bomb radiocarbon record. The approach can be similar to work performed on Hawaiian pink snapper (*Pristipomoides filamentosus*), where a series of previously unknown length-at-age classes were filled in by careful specimen selection (Andrews et al. 2012). Because *E. drummondhayi* longevity exceeds the utility of bomb radiocarbon dating (currently limited to ~55 years) the only method likely to address the potentially great age of this species (~80 years) is lead–radium dating (e.g., Andrews et al. 2009). As a baseline for lead–radium dating, studies on several fishes of the Gulf of Mexico found that radium-226 levels are relatively high in otoliths from the region (see Chapter 5 of Andrews 2009), indicating that a study would be practical (Andrews et al. 2001; Baker et al. 2001*a*, 2001*b*). In addition to *E. drummondhayi*, red snapper and gray snapper can be reinvestigated by taking advantage of increased core extraction accuracy and AMS precision. As with *E. drummondhayi*, it is recommended that known-age and species-specific otolith material be used to verify temporal comparisons to the Gulf of Mexico $\Delta^{14}C$ reference series for these and other species in the region. In general, this study provides a benchmark in bomb radiocarbon dating for the Gulf of Mexico, both as an innovative future reference for regional age and growth studies and as a possible approach for fishes in other regions across the globe where the $\Delta^{14}C$ decline is not known as a potential tool in age validation.

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