Environ Biol Fish (2009) 86:461–472
DOI 10.1007/s10641-009-9536-x

Validation of yellowedge grouper, *Epinephelus flavolimbatus*, age using nuclear bomb-produced radiocarbon

Melissa Cook · Gary R. Fitzhugh · James S. Franks

Received: 19 June 2008 / Accepted: 28 September 2009 / Published online: 31 October 2009

Abstract Age validation and estimates of longevity of yellowedge grouper (*Epinephelus flavolimbatus*) from the Gulf of Mexico (GOM) are needed to inform fishery management decisions. Yellowedge grouper sagittal otoliths (n=100) were collected, aged using conventional means, and cores were submitted for radiocarbon (^{14}C) measurement. Radiocarbon values of yellowedge grouper otoliths were compared to established radiocarbon chronologies in the region to validate the age and ageing methodology of this species. The yellowedge grouper chronology displayed a similar sigmoidal trend as previously published chronologies. In addition to the core analysis, multiple areas on otolith sections from eight specimens were analyzed for $\Delta^{14}C$ to validate age estimates for fish born prior to the ^{14}C increase. Our results indicate that yellowedge grouper live longer than previously reported (minimum of 40 years based on radiocarbon measurements). The validated ageing methodology supported an estimated maximum longevity of 85 years and established that yellowedge grouper have the longest lifespan currently known for any species of grouper in the GOM. Results also indicate a depth-age interaction in that material extracted from adult otolith sections assigned to post-bomb dates exhibited lower $\Delta^{14}C$ values than cores (juvenile material) assigned to the same post-bomb dates. This finding is likely explained by lower ^{14}C levels reported from water masses at deeper depths (>100 m) which are inhabited by adults.

Keywords Age determination · Age validation · Radiocarbon analysis · Yellowedge grouper · Gulf of Mexico · Longevity

M. Cook (✉) · G. R. Fitzhugh
National Marine Fisheries Service,
NOAA Fisheries Service,
3500 Delwood Beach Road,
Panama City, FL 32444, USA
e-mail: Melissa.Cook@noaa.gov

J. S. Franks
Gulf Coast Research Laboratory,
Center for Fisheries Research and Development,
University of Southern Mississippi,
703 Beach Drive,
Ocean Springs, MS 39564, USA

Introduction

The yellowedge grouper (*Epinephelus flavolimbatus,* Poey 1865) is found in the western Atlantic from North Carolina (Huntsman 1976) to southern Florida, the Gulf of Mexico (GOM), Cuba (Smith 1971), the West Indies, Central America, and the northern coast of South America to Brazil (Smith 1971; Fischer 1978). The introduction of large-scale commercial longlining beginning in the early 1980s greatly increased commercial harvest of grouper in the GOM (Bullock and Smith 1991). Yellowedge grouper are managed in the Reef Fish Resources of the Gulf of

Mexico Fisheries Management Plan as part of a five species deepwater grouper complex (GMFMC 2001) where they comprise the majority of the deepwater catch. Since 1992, yellowedge grouper has been the third most abundant grouper harvested in the GOM with an average of 436 metric tons, valued at over $2.2 million dollars, landed annually (National Marine Fisheries Service 2007). The first stock assessment on yellowedge grouper in the northern GOM was conducted in 2002 (Cass-Calay and Bahnick 2002). However, due to a lack of available life history information and the short catch-per-unit-effort and landings time series (1985–2001) the assessment was inconclusive and the population dynamics remains unknown (Cass-Calay and Bahnick 2002).

Grouper are thought to be sensitive to exploitation since many are relatively large in size, often slow growing and long-lived (Huntsman et al. 1999; Musick 1999), and yellowedge grouper may be no exception. Currently, there is a lack of available information on yellowedge grouper growth and longevity due to ageing difficulties experienced by previous researchers. Keener (1984) used sagittal otoliths, viewed with reflected light, to estimate the age range of yellowedge grouper in the South Carolina commercial fishery as 2–15 years. Keener (1984) reported only 27% of otoliths used in the study were readable and, due to the uncertainty of assigning ages to larger fish, estimated that ages could exceed 20 years. Bullock et al. (1996) used polarized light to age yellowedge grouper from western Florida but reported most sagittal otoliths were unreadable and ageing attempts were unsuccessful. Bullock et al. (1996) and Keener (1984) independently concluded that yellowedge grouper otoliths were difficult to read because opaque growth increments were not easily distinguishable. Manickchand-Heileman and Phillip (2000), however, reported observable annuli for 89% of otoliths sampled from Trinidad and Tobago and reported ages up to 35 years. Due to ageing difficulties, an age verification and validation technique is needed for yellowedge grouper from subtropical to temperate waters.

Kalish (1993) reported that atmospheric nuclear bomb testing between 1952 and 1963 left a dated mark on otoliths which could provide a method to validate annuli and to determine accurate, absolute ages of long-lived fish. Prior to the industrial revolution, radiocarbon (^{14}C) levels in the ocean and atmosphere were fairly constant (Kalish 1995). Nuclear bomb testing increased atmospheric ^{14}C levels by ~100% and oceanic ^{14}C levels by ~20% (Druffel and Linick 1978; Nydal and Lövseth 1983). Radiocarbon was incorporated into carbonate structures such as hermatypic corals (Druffel and Linick 1978; Druffel 1980), otoliths (Kalish 1993; Campana 1997; Campana and Jones 1998) and mollusks (Weidman and Jones 1993) in concentrations proportional to ambient levels in the water column. The oceanic ^{14}C increase was observed in temperate and tropical waters in ~1957 (Druffel and Linick 1978; Druffel 1980) but may vary by location as new chronologies are developed.

The bomb-related increase in oceanic radiocarbon can be used for age validation because the dramatic rise of radiocarbon levels was observed over a brief period of time (Kalish 1995). Radiocarbon validation studies typically isolate the otolith core, the portion of the otolith that was formed during the first year of growth. Core analysis provides the level of ^{14}C at the time of fish birth and the earliest period of development. Fish with presumed birth years that occurred during the 1960–1970 increase in oceanic ^{14}C are preferred candidates; however, given that levels of radiocarbon are gradually declining with time, it is possible to use otoliths from fish born after 1970 (Kalish 1995). Since surface water ^{14}C levels were relatively constant prior to the bomb-produced increase (Druffel 1980; Druffel and Suess 1983), it is not possible to use an otolith core to determine an absolute birth date prior to the rise in ^{14}C. Radiocarbon analysis can only determine whether or not a fish was born prior to the observable bomb ^{14}C increase indicated by negative ^{14}C results. A fish collected today but born prior to the nuclear bomb testing would have an otolith containing both positive and negative ^{14}C results. Initial otolith growth increments contain negative ^{14}C values and increments deposited after the bomb-produced ^{14}C increase contain positive ^{14}C values and thus provides the basis for validation.

Analysis of bomb-produced ^{14}C provided successful age validation for GOM red snapper, *Lutjanus campechanus*, (Baker and Wilson 2001) and other commercially important species around the world (Campana 1997; Kalish et al. 1997; Kerr et al. 2005; Piner et al. 2005). Our objective was to compare the amount of ^{14}C found in the cores of yellowedge grouper otoliths to previously determined ^{14}C levels

from otoliths of red snapper and from corals to confirm the presumed birth dates determined from conventional otolith ageing.

Materials and methods

Yellowedge grouper sagittal otoliths used in this study were selected from the National Marine Fisheries Service, Panama City, Florida Laboratory otolith archive. Fish were collected in the northern GOM from the commercial fishing industry and scientific research studies from 1979–2001. All sagittal otoliths were weighed (mg), processed and analyzed as follows.

Age estimation

In order to validate ages throughout the life history, yellowedge grouper (n=100) ranging in size from 177–1,160 mm total length (TL) were selected for empirical age analysis. Yellowedge grouper were selected based on fish size or availability of both sagittal otoliths. Otoliths were embedded whole in araldite epoxy resin and sectioned (0.6 mm) transversely through the core using an IsoMet® Low Speed saw with a diamond blade. Sections were polished with 1500-grit fine grade silicon carbide paper and mounted to a glass slide with Crystalbond™ mounting adhesive. Final polishing was completed using a Foredom® bench polisher and Buehler® Micropolish Alumina II (0.3 micron) polishing compound. Otolith sections were examined using a dissecting microscope with transmitted light at a magnification of 10x to 50x. The number of opaque growth increments per otolith was counted three to five times independently by two readers who lacked knowledge of specimen length or date of capture, and the median count was recorded. Due to difficulty experienced by previous investigators otoliths were also assigned a readability code (good, readable, difficult or unreadable) based on Kuo and Tanaka (1984). Indices of reader precision were determined using the coefficient of variation (CV) (Chang 1982).

Radiocarbon core sample preparation

Otoliths were not randomly selected for ^{14}C analysis, only otoliths assigned a readability code of good or readable were considered. Right otoliths were primarily used for ^{14}C core analysis. Otoliths were embedded and two to three cross-sections (0.7 mm each) were removed, as described above, from selected otoliths, which provided adequate sample material for the removal of the core region. In order to obtain enough material for otolith core analysis (minimum of 3.0 mg for a standard sample), core sections and often as much as the first 2 years of growth were extracted from otoliths. Desired sections were first identified and a Staedtler® pigment liner pen was used to mark the outer boundary (which was not included in the sample). Initially, core samples (n=9) were removed by a dentist who used a standard dental drill (330 bur) to isolate the core. Subsequent samples were removed using a Dremel® Multipro™ (n=33) and digital microsampler (n=5) (see below) rotary tools. A Dremel® Multipro™ rotary tool was fitted with a 1.4 mm diamond needle bit which was used to pulverize the otolith into a powder. The distal portion of the otolith located below the core was removed first to avoid contamination by other growth increments. Each drill bit was replaced with a clean bit for use with each otolith. Cores were stored in sterile vials which were first rinsed three times each with 1 N HCl then distilled water and dried in an oven. Replicate core samples from nine otoliths were submitted for ^{14}C analysis to test the precision of the accelerator mass spectrometry (AMS) instrument used to produce the radiocarbon measurements. Replicate samples were collected using several methods: four taken with the dental tool, four taken with rotary tools and a whole otolith from an estimated age one fish.

Isolated cross-section sample preparation

In order to validate ages of fish born prior to the bomb increase and thus prior to atmospheric increase in ^{14}C, multiple areas on a single otolith cross-section were isolated using a digital microsampler (predecessor of the MicroMill) rotary tool. The objective was to isolate areas on the otolith, determined by counting growth increments, which corresponded to a time period either prior to the nuclear bomb testing (pre-bomb) or after the ^{14}C increase (post-bomb). If the ^{14}C results from the isolated cross-section corresponded to the conventional age-estimated time period, the result would support our hypothesis that growth increments were deposited annually. A sub-

sample of fish (n=8) were selected from a range of sizes. Otoliths were embedded in araldite epoxy resin as before, but thicker cross-sections (1.1 mm) were made through the core. A photograph of the otolith section was taken using a Nikon® SMZ-1500 Stereomicroscope fitted with a Nikon® DMX-1200 digital camera and used to create digital points which the microsampler used as a reference. The otolith section was adhered to the base plate of the microsampler using Crystalbond™. Areas on the cross-section to be isolated and removed (1–3 per otolith) were digitized by the microsampler, moving from the sulcus toward the ventral side of the otolith. Since the amount of calcium carbonate deposited decreases with age (Campana and Thorrold 2001), isolated otolith sections contained numerous years of growth to obtain enough material for ^{14}C analysis. Following a digitized path, the microsampler was then used to shave the isolated sections which were nearly as long as the ventral half of the otolith and approximately 0.7 mm deep. Shavings were collected and stored in sterile glass vials and submitted for AMS analysis.

Radiocarbon analysis

All otolith samples (core and isolated cross-section) were analyzed by the National Ocean Sciences Accelerator Mass Spectrometry Facility at the Woods Hole Oceanographic Institute, Woods Hole, MA. Otolith samples were analyzed for radiocarbon using the procedures described by Baker and Wilson (2001). Samples were also analyzed for ^{13}C which was used to correct for natural and machine fractionation effects. Radiocarbon values were subsequently reported as $\Delta^{14}C$, which is the per mil (‰) deviation of the sample from the radiocarbon activity of 19th-century wood, corrected for isotopic fractionation and sample age decay prior to 1950 according to methods by Stuiver and Polach (1977).

Radiocarbon core results were compared to published $\Delta^{14}C$ values from GOM red snapper (Baker and Wilson 2001) and corals collected in Belize, Bermuda and Florida (Druffel 1980; Druffel 1989; Druffel personal communication) to determine if yellowedge grouper $\Delta^{14}C$ values followed the same trend as other fish and coral from a similar region. The timing of the onset of the ^{14}C increase above pre-bomb levels was calculated for all chronologies using a deterministic coupled-functions model described by Hamel et al. (2008). The method models a bomb-produced pulse of radiocarbon as a Gaussian curve over time combined with a continuous exponential decay function (Hamel et al. 2008).

Results

Yellowedge grouper otoliths were successfully aged by conventional means although difficulties were encountered. Of the 100 aged otoliths originally sampled from the archive, 51 otoliths were retained for ^{14}C analysis. Otoliths were selected based on fish age and clarity of growth increments; only otoliths with discernable increments (Fig. 1) were included. Selected yellowedge grouper had a wide range of presumed birth dates (1915–1999) and ranged in age from 1–85 years old (median age=24 years). The CV for yellowedge grouper selected for ^{14}C analysis was 10.90%. As estimated ages increased, reader precision decreased. The CV was 9.78% for yellowedge grouper≤24 years old and 12.17% for fish 25 years old and older. With increasing age, the distance between otolith growth increments narrowed which made it difficult to interpret individual increments. The edge was the most difficult section of the otolith to interpret since it often appeared dark in color.

Otolith core analysis indicated a rapid $\Delta^{14}C$ accumulation by yellowedge grouper during the 1960s (Fig. 2). Fish with presumed birth dates prior to the nuclear bomb testing had negative $\Delta^{14}C$ levels that ranged from −85.9 to −22.1, whereas fish with estimated birthdates after the bomb increase had elevated $\Delta^{14}C$ levels of 11.3 to 149.4 (Table 1). Fish born after 1978 (n=10) had gradually decreasing $\Delta^{14}C$ levels that ranged from 126.5 to 62.5. Yellowedge grouper $\Delta^{14}C$ values declined at a rate almost three times slower than their increase and have yet to reach pre-bomb levels. Current $\Delta^{14}C$ levels in the GOM range from 62.5 to 80, declining from peak level in 1978 but still considerably higher than pre-bomb levels. Replicate core samples were similar in $\Delta^{14}C$ value, differing by 1.8 to 28.4, which indicated acceptable precision in AMS processing relative to the range from pre- to post-bomb values (Table 1).

We noted some possible effects due to methods used to extract otolith core material. Use of the dental drill may have caused deviations because those core samples were composed of several small pieces of

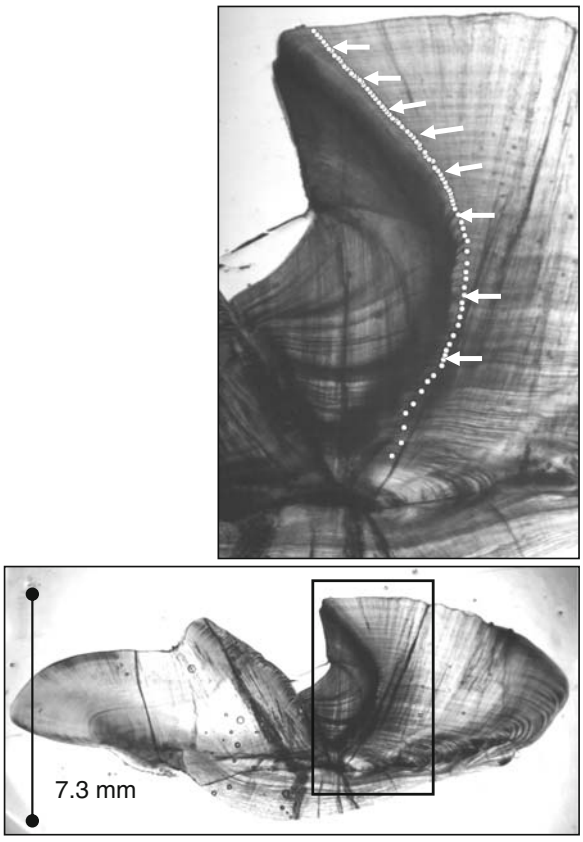

Fig. 1 Transverse section of sagittal otolith from an estimated 85 year old (1,148 mm TL) yellowedge grouper (*Epinephelus flavolimbatus*) collected in 2000. Inset shows the sulcal groove along the ventral side of the otolith which was used for age determination. Growth increments are annotated and arrows are placed every ten years beginning in 1925

core possibly resulting in heterogeneous samples. Samples may have contained additional otolith growth increments from the distal area below the core which could have influenced $\Delta^{14}C$ levels. Among the replicate samples, those removed using the dental drill differed by $\Delta^{14}C$ levels of 12.7 to 28.4. Replicate core samples removed using the rotary tools were more homogenous and results were more precise; $\Delta^{14}C$ values differed by 1.8 to 11.8.

The yellowedge grouper otolith core $\Delta^{14}C$ chronology followed a pattern similar to GOM red snapper and corals (Fig. 3). The year of initial increase for yellowedge grouper was 1959 which was similar to that of red snapper and the corals (Table 2). While displaying a similar sigmoidal trend as the previously published chronologies, the yellowedge grouper chronology did have the lowest peak $\Delta^{14}C$ value.

The area cross-section analysis conducted on 8 fish (ranging from 755 to 1148 mm TL and 21–85 years old), resulted in a total of 13 areas isolated into pre- and post-bomb sections (Table 3). In general, samples from the isolated cross-section analysis incorporated a greater age range (about 3–15 years) in contrast to age ranges represented in core samples (about 1–3 years). We inferred that 11 of the isolated otolith sections were correctly assigned to the expected time period, i.e., either pre-bomb sections (with negative $\Delta^{14}C$ values) or post-bomb sections (with positive $\Delta^{14}C$ values). The incorrectly assigned isolated sections both contained lower than expected radiocarbon values for samples assigned as post-bomb. Figure 4 illustrates a yellowedge grouper otolith cross-section from a fish believed to be 85 years old (born in 1915) based on growth increment counts. Radiocarbon $\Delta^{14}C$ values for the first three isolated sections (−80.5, −71.1, −94.7, respectively) were consistent with expected negative pre-bomb $\Delta^{14}C$ levels formed before the bomb increase. The most recently formed isolated section had a positive $\Delta^{14}C$ value of 38.9, which was consistent with formation after the bomb increase.

The results of both the core and the isolated cross-section radiocarbon analyses validated our hypothesis that increments are formed annually and are interpretable using traditional ageing methodologies. In addition, we observed a positive linear relationship with a high coefficient of determination for age and otolith weight ($F_{1,47}$=310, P<0.001, R^2=0.87;

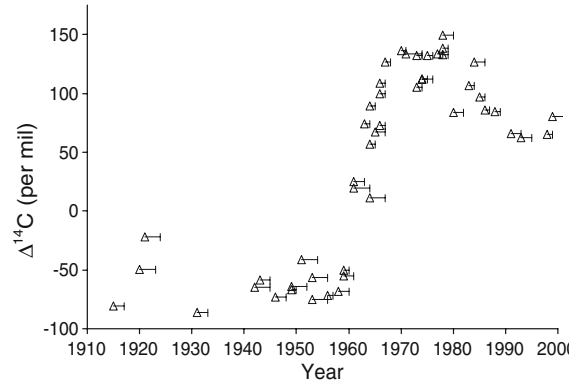

Fig. 2 Yellowedge grouper otolith core radiocarbon ($\Delta^{14}C$) verses birth year determined by counting annual growth increments on sagittal otolith sections. The horizontal error bar represents the number of years estimated to be contained in the core sample

Table 1 Yellowedge grouper data and otolith core radiocarbon results. Age and birth year were determined by counting otolith annual growth increments. Year(s) sampled identifies core sample year and additional consecutive years, if any, submitted for ^{14}C analysis. $\delta^{13}C$ ($^{13}C/^{12}C$) was used to correct for isotopic fractionation to calculate $\Delta^{14}C$. SD refers to standard deviation of the $\Delta^{14}C$ result. RC refers to replicate core samples

Sample ID	Total length (mm)	Year collected	Age (years)	Birth year	Otolith weight (mg)	Sample weight (mg)	Year(s) sampled	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	±1 SD (‰)	RC $\Delta^{14}C$ (‰) (±1 SD)
753	1148	2000	85	1915	6991	6.70	1915–1916	−3.90	−80.5	3.4	−78.7 (5.2)
[a]206	1160	1991	71	1920	4663	21.61	1920–1922	−2.98	−49.7	5.9	
[a]333	1085	1991	70	1921	4566	24.07	1921–1923	−3.96	−22.1	5.4	
1424	930	2001	70	1931	3261	3.40	1931–1932	−4.60	−85.9	4.9	
415	1100	1992	50	1942	3731	5.20	1942–1944	−3.60	−65.1	3.4	
283	1080	1991	48	1943	2969	4.20	1943–1945	−4.39	−58.6	5.1	
1097	967	2001	55	1946	2713	3.70	1946–1947	−1.29	−73.2	10.5	−82.5 (3.1)
[a]271	840	1991	42	1949	1594	22.11	1949–1951	−3.59	−63.8	6.0	
1457	910	1979	30	1949	2377	7.40	1949	−4.44	−67.1	4.2	
[a]329	1010	1991	40	1951	2652	16.72	1951–1953	−4.11	−41.6	5.2	−64.8 (5.2)
[a]253	965	1991	38	1953	2895	26.80	1953–1955	−3.56	−56.6	4.1	
[a]325	1080	1991	38	1953	2703	21.82	1953–1955	−3.74	−75.1	3.9	−59.6 (3.4)
1577	945	2001	45	1956	2497	8.50	1956	−4.37	−71.9	3.4	
1486	603	1983	25	1958	937	4.10	1958–1959	−5.24	−68.0	3.4	
1487	630	1983	24	1959	910	3.10	1959	−5.62	−50.2	10.2	
1578	999	2001	42	1959	2553	6.10	1959–1960	−4.83	−55.3	5.6	−53.2 (5.0)
197	930	1991	30	1961	2359	19.76	1961–1963	−4.43	19.3	7.3	
1466	765	1984	23	1961	1332	4.50	1961–1962	−4.28	25.0	4.2	
1473	740	1984	21	1963	1068	4.90	1963	−4.34	74.0	4.7	
[a]372	1100	1992	28	1964	2320	18.05	1964–1966	−3.72	11.3	7.3	
1138	1015	2001	37	1964	3049	3.20	1964	−4.78	57.1	4.5	45.3 (3.7)
1521	670	1984	20	1964	1016	6.20	1964	−4.77	89.0	3.1	
1502	662	1983	18	1965	938	5.90	1965–1966	−5.31	67.5	5.7	
1465	795	1982	16	1966	1282	6.00	1966	−3.70	72.6	3.6	
1507	524	1983	17	1966	831	5.40	1966	−5.00	108.8	3.2	
1520	705	1984	18	1966	949	6.40	1966	−4.64	99.8	18.8	
1483	656	1984	17	1967	859	6.00	1967	−5.01	126.7	3.2	
[a]1425	991	2001	31	1970	2507	6.30	1970	−4.51	136.3	8.1	107.9 (3.9)
[a]649	1050	2000	29	1971	2460	28.90	1971–1973	−4.20	133.5	6.3	146.2 (4.4)
1442	803	2001	28	1973	1722	5.40	1973	−5.00	105.2	3.2	
1482	620	1984	11	1973	637	3.80	1973	−5.41	131.9	5.5	
1434	854	2001	27	1974	1819	9.80	1974–1975	−5.06	112.3	6.4	
1471	659	1984	10	1974	710	6.40	1974	−4.52	112.3	3.4	
516	1005	1999	24	1975	2388	9.20	1975	−4.50	132.1	4.6	
1469	585	1984	7	1977	607	5.70	1977	−5.54	133.3	6.4	
631	785	2000	22	1978	1429	10.15	1978–1979	−4.95	149.4	3.2	
640	815	2000	22	1978	1650	6.20	1978	−5.05	138.4	11.3	
1504	488	1983	5	1978	432	5.70	1978	−4.77	132.8	5.4	
1423	873	2001	21	1980	1725	10.00	1980–1981	−3.67	83.8	7.3	
648	802	2000	17	1983	1411	8.69	1983	−5.00	106.9	4.0	

Table 1 (continued)

Sample ID	Total length (mm)	Year collected	Age (years)	Birth year	Otolith weight (mg)	Sample weight (mg)	Year(s) sampled	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	±1 SD (‰)	RC $\Delta^{14}C$ (‰) (±1 SD)
634	745	2000	16	1984	1103	11.00	1984–1985	−5.00	126.6	3.5	
650	706	2000	15	1985	1140	7.40	1985	−5.29	96.9	6.2	
1441	772	2001	15	1986	1178	4.90	1986	−4.67	86.2	3.8	
639	740	2000	12	1988	1098	6.50	1988	−4.75	84.8	5.6	
636	590	2000	9	1991	694	5.48	1991–1992	−5.33	65.5	3.4	
1437	568	2001	8	1993	571	8.00	1993–1994	−5.63	62.5	3.2	
674	262	2000	2	1998	114	2.36	1998	−5.60	65.4	12.0	
825	177	2000	1	1999	52	29.29	1999–2000	−6.21	80.2	3.5	82.9 (3.3)

[a] Samples removed using a dentist drill; remaining samples removed using rotary tools

Fig. 5). The intercept was near the origin (0.063) which suggested that early increments were being correctly interpreted (see Morison et al. 1998; Allman and Fitzhugh 2007). By not rejecting the hypothesis that the yellowedge bomb chronology was reasonably precise and increments were formed annually, the ^{14}C results indicated that yellowedge grouper reach an age of at least 40 years based on the core analysis and supported the maximum age estimate of 85 years based on empirical ageing.

However, there were notable deviations between the expected $\Delta^{14}C$ results of the core analysis and the isolated area analysis (Fig. 6). A comparison indicated that $\Delta^{14}C$ values from the isolated cross-section results were lower than values from the core analysis for the same post-bomb years. While we understand there is an age difference in the comparison (core values reflect the juvenile stage and isolated cross-section values reflect older ages) there may also be a habitat or depth effect as older fish inhabit deeper depths. The yellowedge grouper used in this study were collected between 85–298 m (although depth information was not available for all fish). To further examine a possible depth effect we obtained data from Stuvier and Öslund (1980) for depth and $\Delta^{14}C$ in ambient seawater. In general, an increase in depth from 100 to 300 m is associated with a $\Delta^{14}C$ decrease of≥100 (Fig. 7). The number of years incorporated into each sample may have also contributed to the deviations between the cores and isolated areas. Although some cores contained up to 3 years of growth, all isolated areas contained multiple years of growth. This could have resulted in a weighted average effect on the radiocarbon measurements.

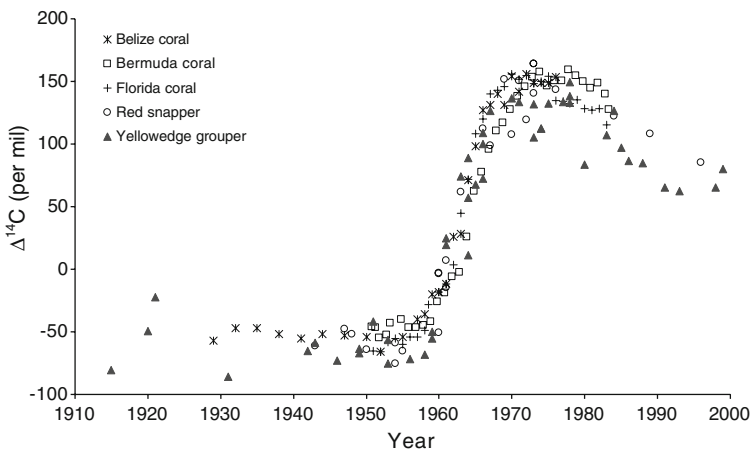

Fig. 3 Time series of radiocarbon ($\Delta^{14}C$) values for yellowedge grouper (this study), corals from Belize (Druffel 1980), Bermuda (Druffel 1989), and south Florida (Druffel 1989) and Gulf of Mexico red snapper (Baker and Wilson 2001). Yellowedge grouper and red snapper data points represent estimated year of birth. Coral points represent year of growth increment formation

Table 2 Predicted parameters and derived quantities from deterministic coupled-functions model fitted to otolith and coral radiocarbon series. Parameters are the total inputted radiocarbon (k), mean year of increase (μ), exponential decay rate (r), standard deviation of the cumulative normal (σ), estimated timing of initial radiocarbon increase (μ — σ), minimum radiocarbon level observed (y_{min}), maximum radiocarbon level that would occur in the absence of r (y_{asym}). The standard deviation (SD) is in parentheses, y_{asym} and k have the same SD, no error term is associated with y_{min} since it is an observed point. Radiocarbon data taken from yellowedge grouper (this study), Gulf of Mexico red snapper (Baker and Wilson 2001) and corals from Belize (Druffel 1980), Bermuda (Druffel 1989), and south Florida (Druffel 1989)

Source	k (‰)	μ (year)	r	σ	μ — σ (year)	y_{min} (‰)	y_{asym} (‰)
Yellowedge grouper	240.32 (9.84)	1962.94 (0.29)	0.015 (0.002)	4.14 (0.38)	1958.80 (0.55)	−75.1	165.22
Red snapper	255.63 (12.84)	1963.25 (0.41)	0.014 (0.003)	4.61 (0.51)	1958.60 (0.93)	−75.2	180.43
Belize	237.55 (16.85)	1963.10 (0.31)	0.008 (0.007)	3.93 (0.28)	1959.17 (0.54)	−66.0	171.55
Bermuda	232.25 (7.84)	1965.33 (0.19)	0.010 (0.003)	4.38 (0.19)	1960.95 (0.36)	−54.8	177.45
Florida	254.19 (5.10)	1963.36 (0.11)	0.016 (0.002)	3.59 (0.13)	1959.77 (0.21)	−66.0	188.20

Since we do not know the radiocarbon levels for the individual years in the sample it is not possible to further examine this idea.

Discussion

We generally found yellowedge grouper otolith sections to be interpretable with lower reader precision (9.8% CV for ages≤24 years and 12.2% CV for ages>24) than observed for other species (7.6% CV for 117 species; Campana 2001). In routine production-style ageing we would expect precision for yellowedge grouper to be even lower. While this may be a more difficult species to age than most, both the core analysis and the isolated cross-section analysis supported the hypothesis that yellowedge grouper otoliths form one annulus per year. Our inference about age interpretation was further supported by a linear relationship accounting for 87% of the variability between otolith weight and fish age. Longevity is at least 40 years based on the radiocarbon core results and at least 85 years of age based on empirical ageing which suggests they have the longest lifespan currently known for any grouper found in the GOM and U.S. South Atlantic (cf., Collins et al. 1987; Manooch and Mason 1987; Bullock et al. 1992; Crabtree and Bullock 1998; Wyanski et al. 2000).

Table 3 Radiocarbon ($\Delta^{14}C$) results of yellowedge grouper isolated section analysis. Pre-bomb refers to otolith growth increments formed prior to the 1960s increase of ^{14}C. Post-bomb refers to otolith growth increments formed after the 1960s increase of ^{14}C. Each isolated section contained approximately 3–15 years of growth increments

Sample ID	Sample Description	Years Sampled	Fish Age (Years)	Year Born	Sample Weight (mg)	$\Delta^{14}C$ (‰)	±1 SD (‰)
101 A	Post-bomb	1960–1972	70	1921	4.1	−37.1	3.8
283 B	Post-bomb	1965–1976	48	1943	2.8	−39.9	3.6
415 B	Pre-bomb	1952–1958	50	1942	4.0	−51.9	3.5
753 B	Pre-bomb	1920–1929	85	1915	3.3	−71.1	7.5
753 C	Pre-bomb	1937–1943	85	1915	2.9	−94.7	4.5
753 D	Post-bomb	1972–1987	85	1915	3.5	38.9	4.3
922 A	Pre-bomb	1939–1948	70	1931	3.3	−51.5	3.9
922 B	Post-bomb	1963–1969	70	1931	4.4	45.2	3.8
1097 B	Pre & Post-bomb	1954–1964	55	1946	4.3	−74.7	3.9
1424 B	Pre-bomb	1938–1944	70	1931	2.9	−101.3	3.3
1424 E	Post-bomb	1999–2001	70	1931	1.6	85.9	8.4
1470 A	Post-bomb	1967–1969	21	1963	3.4	30.9	4.0
1470 B	Post-bomb	1973–1976	21	1963	3.2	23.8	3.7

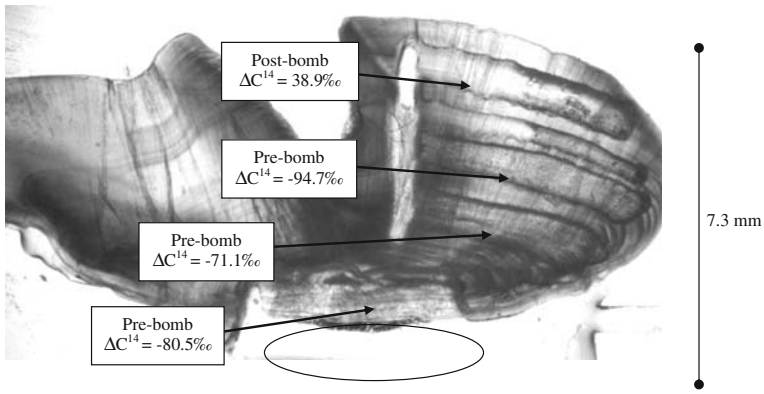

Fig. 4 An estimated 85 year old yellowedge grouper otolith (the second of a pair; the first viewed in Fig. 1) with isolated areas of multiple growth increments removed (identified by arrows). The reported ΔC^{14} values represent a combination of years of growth instead of a single point estimate. Pre-bomb= prior to 1960s increase, and post-bomb=during or after 1960s increase. The distal area below the core (circled) was removed prior to sampling to avoid contamination by other growth increments

Previous yellowedge grouper longevity estimates were considerably less than those found in our study, most likely due to unsuccessful ageing or biased-low age estimates because readers had difficulty determining individual growth increments. The longevity of yellowedge grouper compared to many other grouper species may be due to a preference for deepwater habitat and physiological processes related to low levels of light, oxygen, temperature and prey availability. Deepwater species may live considerably longer than shallow-water species and several deepwater marine fishes have validated age estimates indicative of long life spans including rockfishes which reach ages of 60–205 years (Cailliet et al. 2001).

The yellowedge grouper otolith core $\Delta^{14}C$ chronology was the primary evidence supporting the hypothesis that growth increments are annual. The chronology followed a pattern very similar to GOM red snapper and corals. The year of initial ^{14}C increase occurred in 1959 for yellowedge grouper, red snapper (Baker and Wilson 2001) and the coral from Belize (Druffel 1980), in 1960 for South Florida coral and in 1961 in Bermuda coral (Druffel 1989). The timing of initial ^{14}C increase was similar to other published chronologies from the Northern hemisphere (Hamel et al. 2008).

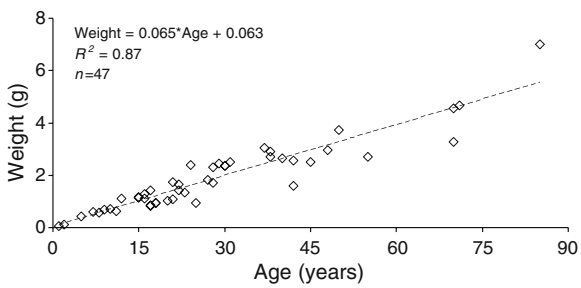

Fig. 5 Relationship between yellowedge grouper sagittal otolith weight and empirical fish age. Linear equation represented by dashed line

Our study was the first to isolate multiple areas on a single otolith cross-section for radiocarbon analysis. The extractions from the isolated cross-sections mapped the radiocarbon that was deposited within the otolith as age increased. For instance, the multiple isolated areas with negative $\Delta^{14}C$ values on a single otolith were consistent with the timing of otolith deposition expected before the bomb increase in ^{14}C. However, the material extracted from adult otolith sections assigned to post-bomb dates exhibited lower $\Delta^{14}C$ values than cores (juvenile material) assigned to the same post-bomb dates. This would suggest that the fish were considerably under-aged, unlike the evidence from the core chronology (which perhaps explains a one to 3 year age bias) and unlike the otolith weight-age relationship. Another more likely possibility is that the differing $\Delta^{14}C$ levels reflect age/habitat differences mapped within the otolith during the post-bomb period. It is known that adult yellowedge grouper are found at deeper depths (typically 125–300 m, but as deep as 390 m) than juveniles (between 35–125 m) (Cook 2007). As levels of ^{14}C in otoliths are derived (about 70%) from dissolved inorganic carbon (DIC) in the surrounding

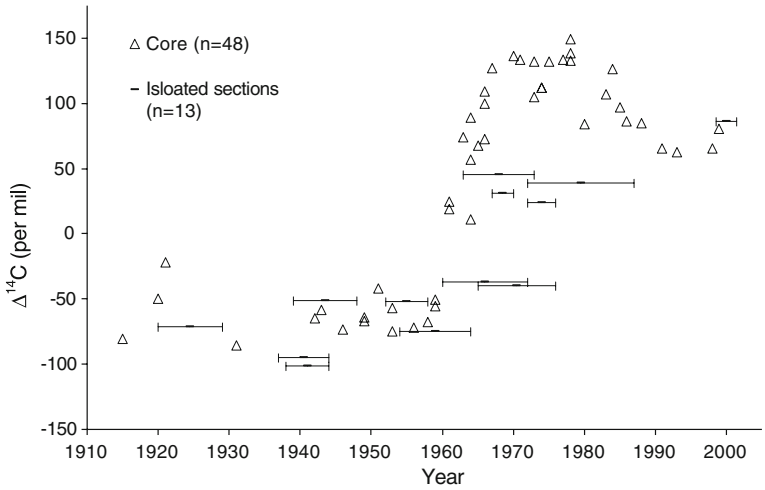

Fig. 6 Yellowedge grouper isolated otolith cross-sections (bar) and core (triangle) radiocarbon ($\Delta^{14}C$) verses birth year determined by counting annual growth increments on sagittal otolith sections. The horizontal bar represents the number of years (approximately 3-15 growth increments) contained in the isolated sample

water (Degens et al. 1969; Kalish 1991a, b), any discrepancy between the core analysis, reflecting the juvenile stage, and isolated cross-section analysis, reflecting older ages, may be the result of an age/depth interaction.

Seawater in the GOM originates from several sources. Surface waters extending to depths of ~100 m originate as North and South Atlantic Ocean surface waters and are transported into the GOM via the Caribbean, Guiana and North Equatorial Currents (Gore 1992). Deeper waters, extending to ~500 m are composed of cooler Subtropical Underwater originating from the equatorial Atlantic Ocean and Caribbean Sea (Gore 1992). Stuvier and Östlund (1980) reported that DIC in seawater collected from the tropical Atlantic Ocean between 100–300 m depths had $\Delta^{14}C$ levels 30–154% less than surface waters

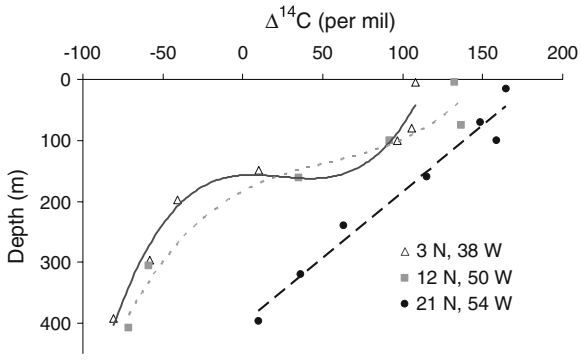

Fig. 7 Comparison of $\Delta^{14}C$ measurements of dissolved inorganic carbon in ambient seawater by depth from three locations in the tropical Atlantic Ocean [data taken from Stuvier and Östlund 1980]

(Fig. 7). Others have also reported that the appearance of the radiocarbon signal is delayed below about 100 m in depth (Williams et al. 1987; Campana 2002). So, it appears that different water masses with notably different $\Delta^{14}C$ levels characterize the depth boundary separating juvenile and adult yellowedge grouper habitats. Thus it is very likely that radiocarbon levels in adult portions of yellowedge grouper otoliths were affected by lower levels of $\Delta^{14}C$ at deeper depths.

A depth effect may also explain the lower peak ^{14}C value as red snapper juveniles are found in shallower depths (19–37 m) than juvenile yellowedge grouper (Gallaway et al. 1999; Cook 2007). It is also possible that there may be latitudinal or spatial patterns in ^{14}C deposition. Druffel (1996) also observed that $\Delta^{14}C$ values from corals from the tropical Atlantic Ocean, associated with lower water mass ^{14}C levels, peaked at lower levels than corals from Florida and Bermuda.

Although we took care in our methods, such as using sterile vials and refraining from using carbon-based polishing materials in the final surface preparations, we cannot discount some possible contamination or preparation effects. There were limits in the age-precision of the otolith material extracted given the minimum amount needed for analysis. Even with the use of a microsampler the isolated cross-section analysis included several years (possibly to 15) in samples, in contrast to core samples (1–3 years estimated) which were removed with dental and rotary tools. Thus the isolated cross-section approach was less precise in providing $\Delta^{14}C$ values at age. Two (of 13) isolated cross-section samples were classified as post-bomb sections based on estimated age but exhibited pre-bomb $\Delta^{14}C$ values.

It is possible that additional pre-bomb growth increments were also included in the samples and contributed to lower than expected $\Delta^{14}C$ values. We found that using a rotary tool to extract core samples increased our precision somewhat over our initial use of a dental tool. But as we treated all preparations similarly and had reasonable precision in blind duplicate samples submitted for AMS, we feel we minimized or accounted for processing effects to the degree possible with the tools available to us. With continued innovations and standardized applications, the ^{14}C age-environment interactions within otoliths are likely to become better quantified.

Long-lived fishes are more vulnerable to overfishing, are slow to recover once exploited (Huntsman et al. 1999; Musick 1999; Coleman et al. 2000), and it has been questioned whether they can be sustainably managed (Devine et al. 2006). Little information is available regarding maximum age estimates for most deepwater groupers in the GOM. Additional ageing studies should be conducted to determine if other deepwater grouper species follow the same life history pattern as yellowedge grouper and reach comparable longevities. If radiocarbon methods are used to validate ages for outer continental shelf species, possible spatial and water-depth radiocarbon effects and their interaction with age needs to be carefully considered. For species occupying habitats deeper than the ^{14}C mixing zone (approximately 100 m) in the juvenile stage, other validation approaches may be more appropriate.

Acknowledgments We would like to thank the National Ocean Sciences Accelerator Mass Spectrometry facility staff, especially Ann McNichol, Kathy Elder, Kari Paro and Chris Weidman, for scientific advice and use of their digital microsampler. We would also like to thank Jan Welker, James Warren and David Bonderer for otolith preparation and sample removal. Ellen Druffel and Scott Baker, Jr. provided scientific advice and raw data for corals and red snapper, respectively, which were vital for the comparison of ^{14}C curves. Owen Hamel provided executable files and details necessary to run the deterministic models. We would also like to thank Bruce Comyns and three anonymous reviewers for comments which resulted in significant improvements to this manuscript. This study was supported in part by funds from the National Marine Fisheries Service Marine Fisheries Initiative (MARFIN) project # 02MFIH09. The statements, findings, conclusions and recommendations are those of the authors and do not necessarily reflect the views of the Department of Commerce or NOAA or any of its sub-agencies. Use of brand names within this study should not be considered an endorsement of the product. Brand names are included solely for informational purposes, and their inclusion does not imply an agency endorsement.

References

Allman RJ, Fitzhugh GR (2007) Temporal age progressions and relative year-class strength of Gulf of Mexico red snapper. In: Patterson WF III, Cowan JH Jr, Fitzhugh GR, Nieland DL (eds) Red snapper ecology and fisheries in the US Gulf of Mexico. Am Fish Soc Symp 60, Bethesda, Maryland, pp 311–328

Baker MS Jr, Wilson CA (2001) Use of bomb radiocarbon to validate otolith section ages of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico. Limnol Oceanogr 46:1819–1824

Bullock LH, Godcharles MF, Crabtree RE (1996) Reproduction of yellowedge grouper, *Epinephelus flavolimbatus*, from the eastern Gulf of Mexico. Bull Mar Sci 59:216–224

Bullock LH, Murphy MD, Godcharles MF, Mitchell ME (1992) Age, growth, and reproduction of jewfish, *Epinephelus itajara*, in the eastern Gulf of Mexico. Fish Bull 90:243–249

Bullock LH, Smith GB (1991) Seabasses (Pisces: Serranidae). Mem Hourglass Cruises 8(2):1–243

Cailliet GM, Andrews AH, Burton EJ, Watters DL, Kline DE, Ferry-Graham LA, Grant CS (2001) Age determination and validation studies of marine fishes: do deep-dwellers live longer? Exp Gerontol 36:739–764

Campana SE (1997) Use of radiocarbon from nuclear fallout as a dated marker in the otoliths of haddock, *Melanogrammus aeglefinus*. Mar Ecol Prog Ser 150:49–56

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Bio 59:197–242

Campana SE (2002) Bomb dating and age determination of large pelagic sharks. Can J Fish Aq Sci 59:450–455

Campana SE, Jones CM (1998) Radiocarbon from nuclear testing applied to age validation of black drum, Pogonias cromis. Fish Bull 96:185–192

Campana SE, Thorrold SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can J Fish Aquat Sci 58:30–38

Cass-Calay SL, Bahnick M (2002) Status of the yellowedge grouper fishery in the Gulf of Mexico. Sustainable Fisheries Division Contribution No SFD-02/03-172. National Marine Fisheries Service, Southeast Fisheries Science Center, Miami, FL.

Chang WYB (1982) A statistical method for evaluating the reproducibility of age determination. Can J Fish Aquat Sci 39:1208–1210

Coleman FC, Koenig CC, Huntsman GR, Musick JA, Eklund AM, McGovern JC, Chapman RW, Sedberry GR, Grimes CB (2000) Long-lived reef fishes: the grouper-snapper complex. Fish 25(3):14–21

Collins MR, Waltz CW, Roumillat WA, Stubbs DL (1987) Contribution to the life history and reproductive biology of gag, *Mycteroperca microlepis* (Serranidae), in the South Atlantic Bight. Fish Bull 85:648–653

Cook M (2007) Population dynamics, structure and per-recruit analysis of yellowedge grouper, *Epinephelus flavolimbatus*, from the northern Gulf of Mexico. Doctoral dissertation, University of Southern Mississippi, Hattiesburg, Mississippi 172p

Crabtree RE, Bullock LH (1998) Age, growth, and reproduction of black grouper, *Mycteroperca bonaci*, in Florida waters. Fish Bull 96:735–753

Degens ET, Deuser WG, Haedrich RL (1969) Molecular structure and composition of fish otoliths. Mar Biol 2:105–113

Devine JA, Baker KD, Haedrich RL (2006) Deep-sea fishes qualify as endangered. Nature 439(5):29

Druffel EM (1980) Radiocarbon in annual coral rings of Florida and Belize. Radiocarbon 22:363–371

Druffel EM (1996) Post-bomb radiocarbon records of surface corals from the tropical Atlantic Ocean. Radiocarbon 38:563–572

Druffel EM, Suess HE (1983) On the radiocarbon recorded in banded corals: exchange parameters and net transport of $^{14}CO_2$ between atmosphere and surface ocean. J Geophys Res 88:1271–1280

Druffel EM (1989) Decadal time scale variability of ventilation in the North Atlantic: High-precision measurements of bomb radiocarbon in banded corals. J Geophys Res 94:3271–3285

Druffel EM, Linick TW (1978) Radiocarbon in annual coral rings of Florida. Geophys Res Lett 5:913–916

Fischer W (ed) (1978) Rome, FAO. FAO species identification sheets for fishery purposes: Western Central Atlantic (fishing area 31). Vol 4

Gallaway BJ, Cole JG, Meyer R, Roscigno P (1999) Delineation of essential habitat for juvenile red snapper in the northwestern Gulf of Mexico. Trans Am Fish Soc 128:713–726

Gore RH (1992) The Gulf of Mexico: a treasury of resources in the American Mediterranean. Pineapple Press, Sarasota, Florida

GMFMC (Gulf of Mexico Fisheries Management Council) (2001) Public hearing draft amendment 18 to the reef fish fishery management plan for the reef fish resources of the Gulf of Mexico. Gulf of Mexico Fisheries Management Council, Tampa, FL

Hamel OS, Piner KR, Wallace JR (2008) A robust deterministic model describing the bomb radiocarbon signal for use in fish age validation. Trans Am Fish Soc 137:852–859

Huntsman GR (1976) Offshore bottom-fisheries of the United States south Atlantic coast. In: Bullis HR Jr, Jones AC (eds) Proceedings: Colloquium on snapper-grouper fishery resources of the western central Atlantic Ocean. FL Sea Grant Rep 17, pp 192–221

Huntsman GR, Potts J, Mays RW, Vaughan D (1999) Groupers (Serranidae, Epinephelinae): Endangered apex predators of reef communities. In: Musick JA (ed) Life in the slow lane, ecology and conservation of long-lived marine animals. Am Fish Soc Symp 23, Bethesda, Maryland, pp 217–231

Kalish JM (1991a) Oxygen and carbon stable isotopes in the otoliths of wild and laboratory-reared Australian salmon (*Arripis trutta*). Mar Biol 110:37–47

Kalish JM (1991b) ^{13}C and ^{18}O isotopic disequilibria in fish otoliths: Metabolic and kinetic effects. Mar Ecol Prog Ser 75:191–203

Kalish JM (1993) Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet Sci Lett 114:549–554

Kalish JM (1995) Radiocarbon in fish biology. In: Secor DH, Dean JM, Campana SE (eds) Recent development in fish otolith research. University of South Carolina Press, Columbia, South Carolina, pp 637–653

Kalish JM, Johnston JM, Smith DC, Morison AK, Robertson SG (1997) Use of the bomb radiocarbon chronometer for age validation in blue grenadier, *Macruronus novaezelandiae*. Mar Biol 128:557–563

Keener P (1984) Age, growth, and reproductive biology of the yellowedge grouper, *Epinephelus flavolimbatus*, off the coast of South Carolina. Masters thesis, College of Charleston, Charleston, South Carolina, 65 p

Kerr LA, Andrews AH, Munk K, Coale KH, Frantz BR, Cailliet GM, Brown TA (2005) Age validation of quillback rockfish (*Sebastes maliger*) using bomb radiocarbon. Fish Bull 103:97–107

Kuo CL, Tanaka S (1984) Otolith features and reliability for age-determination of hoki, *Macruronus novaezelandidae* (Hector), in waters around New Zealand. Bull Jpn Soc Sci Fish 50:1349–1355

Manickchand-Heileman SC, Phillip DAT (2000) Age and growth of the yellowedge grouper, *Epinephelus flavolimbatus*, and the yellowmouth grouper, *Mycteroperca interstitialis*, off Trinidad and Tobago. Fish Bull 98:290–298

Manooch CS III, Mason DL (1987) Age and growth of the Warsaw grouper and black grouper from the Southeast region of the United States. Northeast Gulf Sci 9:65–75

Morison AK, Coutin PC, Robertson SG (1998) Age determination of black bream, *Acanthopagrus butcheri* (Sparidae), from the Gippsland Lakes of south-eastern Australia indicates slow growth and episodic recruitment. Mar Fresh Res 49:491–498

Musick JA (1999) Ecology and conservation of long-lived marine animals. In: Musick JA (ed) Life in the slow lane, ecology and conservation of long-lived marine animals. Am Fish Soc Symp 23, Bethesda, Maryland, pp 1–10

National Marine Fisheries Service (2007) Commercial fishery landings data. February 26, 2007 from http://www.st.nmfs.noaa.gov/st1/commercial/index.html

Nydal R, Lövseth K (1983) Tracing bomb 14C in the atmosphere 1962–1980. J Geophys Res 88:3621–3642

Piner KR, Hamel OS, Menkel JL, Wallace JR, Hutchinson CE (2005) Age validation of canary rockfish (*Sebastes pinniger*) from off the Oregon coast (USA) using the bomb radiocarbon method. Can J Fish Aquat Sci 62:1060–1066

Smith CL (1971) A revision of the American groupers: *Epinephelus* and allied genera. Bull Am Mus Nat Hist 146(2):67–242

Stuiver M, Polach HA (1977) Discussion: Reporting of ^{14}C data. Radiocarbon 19:355–363

Stuiver M, Öslund HG (1980) GEOSECS Atlantic radiocarbon. Radiocarbon 22:1–24

Weidman CR, Jones GA (1993) A shell-derived time history of bomb ^{14}C on Georges Bank and its Labrador Sea implications. J Geophys Res 98:14577–14588

Williams PM, Druffel ERM, Smith KL Jr (1987) Dietary carbon sources for deep-sea organisms as inferred from their organic radiocarbon activities. Deep-Sea Res 34:253–266

Wyanski DM, White DB, Barans CA (2000) Growth, population age structure, and aspects of the reproductive biology of snowy grouper, *Epinephelus niveatus*, off North Carolina and South Carolina. Fish Bull 98:199–218