RAPID COMMUNICATION

Eye lens Δ^{14}C validates otolith-derived age estimates of Gulf of Mexico reef fishes

William F. Patterson III, Beverly K. Barnett, Thomas C. TinHan, and Susan K. Lowerre-Barbieri

Abstract: We tested whether Δ^{14}C values of eye lens protein (crystallin) formed in early life could be utilized to validate marine bony fish age estimates via the bomb radiocarbon chronometer. The slope of the relationship between red snapper (*Lutjanus campechanus*; $n = 8$; 0 to 27 years old), otolith and eye lens core Δ^{14}C values was not significantly different than 1, which was also true for seven additional reef fish species ($n = 21$; 0 to 24 years old). Results demonstrate eye lens core Δ^{14}C can be utilized to validate age estimates of marine fishes, which will be useful in numerous age validation applications.

Résumé : Nous avons vérifié si les valeurs de Δ^{14}C de la protéine du cristallin de l'óil (la cristalline) formée tôt durant la vie pouvaient être utilisées pour valider des estimations de l'âge de poissons osseux marins par chronométrie au radiocarbone dérivé de bombes atomiques. La pente de la relation entre les valeurs de Δ^{14}C de córs d'otolites et de cristallins de viva-neaux rouges (*Lutjanus campechanus*; $n = 8$, âgés de 0 à 27 ans) n'est pas significativement différente de 1, un résultat aussi observé pour sept autres espèces de poissons de récifs ($n = 21$, âgés de 0 à 24 ans). Ces résultats démontrent que le Δ^{14}C du noyau du cristallin peut être utilisé pour valider des estimations de l'âge de poissons marins, ce qui sera utile dans de nombreuses applications de validation d'âges. [Traduit par la Rédaction]

Introduction

The bomb radiocarbon (^{14}C) chronometer has been widely applied to validate age estimation in marine bony fishes. This approach relies on the approximate doubling of atmospheric ^{14}C in the 1950s and 1960s from nuclear weapons testing, which drove a concomitant ^{14}C increase in surface waters among the world's oceans when ^{14}C-enriched CO_2 dissolved into surface layers (Kalish 1993). The magnitude of oceanic ^{14}C increase differed among ocean basins due to factors such as initial delivery of ^{14}C and large-scale surface currents that drive water residence times and oceanic mixing rates (Kalish 1993; Druffel and Linick 1978). However, the general trend of a rapid increase in surface ocean ^{14}C in the 1950s and 1960s, a plateau in the 1970s, and then a decline thereafter has been fairly consistent across the globe (reviewed in Grottoli and Eakin 2007). The utility of this anthropogenic and oceanographic phenomenon for fish age validation owes to the fact that bomb ^{14}C has been incorporated into hermatypic coral skeletons and other biogenic carbonates around the planet. In turn, this provides a time series of ^{14}C, measured as Δ^{14}C, with which to compare otolith core Δ^{14}C values formed during the first year of life (Kalish 1993).

Employing the bomb ^{14}C chronometer for otolith age validation has become increasingly widespread in marine fisheries ecology, but several factors can affect the suitability of this approach. For example, otoliths vary widely in size among bony fish taxa (Paxton 2000), and accurately microsampling sufficient core material from small otoliths can be problematic. Typically, 100 µg of C is required for robust analysis of Δ^{14}C with accelerator mass spectrometry (AMS), which translates to 1 mg of otolith material given otoliths are ~12% C by mass. Low-mass analysis is possible (to ~10 µg), and recent advances in laser ablation-AMS provide a means to microsample ultralow C masses from otolith sections (Andrews et al. 2019). However, progressively greater measurement error typically occurs with decreasing sample mass.

Here, we tested the efficacy of deriving marine fish birth year Δ^{14}C signatures from eye lenses instead of otoliths for the purpose of age validation. The rationale is that eye lenses are composed of protein (crystallin) that is ~50% organic C by mass; thus, even fishes with relatively small lenses are likely to have lens cores with much greater than the 100 µg of C required for robust AMS analysis (Dahm et al. 2007). Eye lenses are similar to otoliths in that they grow throughout life and are metabolically inert once formed (Wallace et al. 2014). Chemical properties of eye lenses have been utilized for direct age estimation for other marine taxa, including marine mammals via analysis of amino acid racemization (George et al. 1999) and Greenland shark (*Somniosus microcephalus*) (Nielsen et al. 2016) via analysis of percent modern ^{14}C in eye lens cores. In the case of bony fishes, the juvenile portion (approximately first 3–6 months of life) of eye lenses can be easily extracted by drying and peeling lens layers down to the core (Wallace et al. 2014), the diameter of which can be estimated by measuring dried lenses of age-0 fish.

The objective here was to test whether Δ^{14}C derived from eye lens cores could be utilized to validate marine bony fish age

W.F. Patterson III. University of Florida, Fisheries and Aquatic Sciences, 7922 NW 71st Street, Gainesville, FL 32653, USA.

B.K. Barnett. University of Florida, Fisheries and Aquatic Sciences, 7922 NW 71st Street, Gainesville, FL 32653, USA; NOAA Fisheries, Southeast Fisheries Science Center, Panama City Laboratory, 3500 Delwood Beach Road, Panama City, FL 32408, USA.

T.C. TinHan. Texas A&M University at Galveston, Department of Marine Biology, 200 Seawolf Parkway, Galveston, TX 77554, USA.

S.K. Lowerre-Barbieri. University of Florida, Fisheries and Aquatic Sciences, 7922 NW 71st Street, Gainesville, FL 32653, USA; Florida Fish and Wildlife Research Institute, 100 8th Avenue SE, St. Petersburg, FL 33701, USA.

Corresponding author: William F. Patterson (email: will.patterson@ufl.edu).

14

Can. J. Fish. Aquat. Sci. Vol. 78, 2021

Fig. 1. Digital images of an adult red snapper (589 mm TL, age = 6 years) eye lens undergoing the process of (A) thawing, (B) drying, and (C) cleaving to produce (D) an extracted lens core.
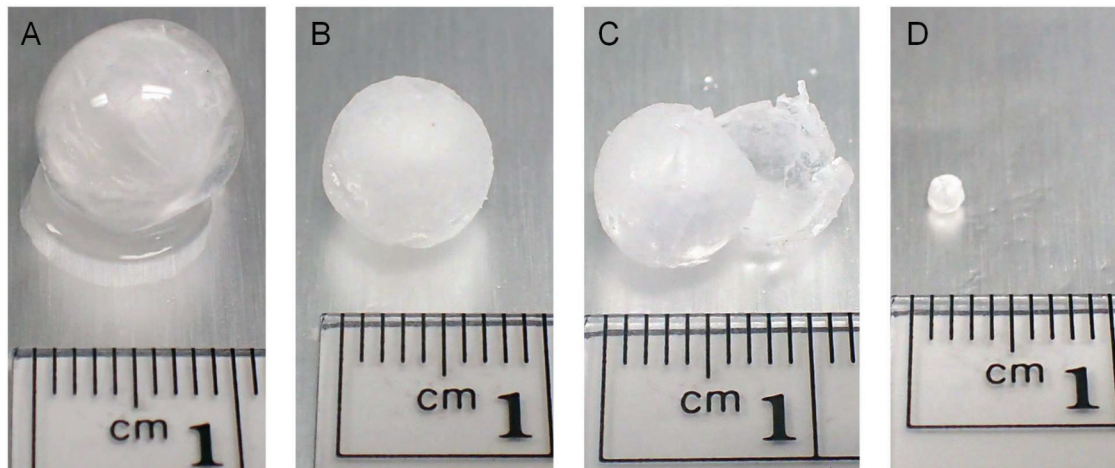
estimates via the bomb ^{14}C chronometer. This was accomplished through statistical comparisons of eye lens versus otolith core Δ^{14}C for northern Gulf of Mexico (nGOM) red snapper (*Lutjanus campechanus*) and a suite of additional nGOM reef fish species. Results are interpreted with respect to the efficacy of utilizing eye lens core Δ^{14}C signatures in applying the bomb ^{14}C chronometer to validate fish age estimation.

Methods

Red snapper (*n* = 8) and seven additional reef fish species (*n* = 3 individuals per species), including red grouper (*Epinephelus morio*), snowy grouper (*Hyporthodus niveatus*), yellowedge grouper (*Hyporthodus flavolimbatus*), scamp (*Mycteroperca phenax*), red porgy (*Pagrus pagrus*), short bigeye (*Pristigenys alta*), and vermilion snapper (*Rhomboplites aurorubens*), were captured on the nGOM continental shelf at depths between 24 and 200 m during fishery-independent trawl, longline, or hook and line sampling. Fish were measured to the nearest milli-metre total length (TL), and then both sagittal otoliths and eye lenses were extracted. Each sagitta was extracted by opening the otic cap-sule through the gill cavity with a chisel, removing the sagitta from the sacculus with tweezers, rinsing in distilled water, and storing dry in a labeled paper coin envelope. Lenses were extracted by mak-ing a small incision through the sclera, applying slight pressure, and then lightly pulling lenses free of suspensory ligaments. Lenses were stored in sterile plastic cell wells, then placed in a freezer at −20 °C onboard the sampling vessel. All samples were subsequently trans-ferred to the University of Florida, where otoliths were stored dry and lenses were stored in an ultralow freezer at −80 °C.

Transverse thin sections (0.5 mm) were prepared from left sag-ittae to estimate fish age, which was done by consensus between two experienced readers. Otoliths were first embedded in epoxy and cut with a double-bladed wafering saw, capturing the core, and then examined under a dissecting microscope with transmit-ted light to count the number of opaque zones present. Otolith processing for AMS analysis occurred in a class-100 clean room, and all metal tools, aluminum foil, or glass vials coming in con-tact with otolith or eye lens core samples were baked in a muffle furnace at 525 °C for 24 h. Right otoliths of age-1 and older fish were prepared for AMS analysis by preparing 1.5 mm thin sec-tions and then extracting otolith cores with a micromill follow-ing the method of Barnett and Patterson (2010). Otoliths of age-0 fish were analyzed whole. All otolith cores and whole age-0

otoliths were flooded with 1% ultrapure HNO_3 for 30 s and then repeatedly rinsed with double-deionized water to remove any surface contamination. Otolith cores and whole otoliths were allowed to dry overnight under a class-10 clean hood and then placed in acid-leached glass vials.

Preliminary analysis of age-0 red snapper (*n* = 8; mean total length ± 95% CIs = 117.1 ± 13.4 mm) eye lenses indicated they had a mean diameter of 2.1 ± 0.4 mm and a dry mass of 5.7 ± 1.2 mg; thus, eye lens cores of adult reef fishes were extracted to target those dimensions. Frozen lenses of age-1 and older fish were placed on prebaked aluminum foil for core extraction following the method of Wallace et al. (2014). Lenses were allowed to thaw naturally at room temperature. As a lens started to dry, fissures formed at its poles, which were utilized to peel lens layers, even-tually revealing the spherical lens core (Fig. 1). The entire process took ~0.5 h per sample. Extracted eye lens cores were placed in acid-leached glass vials.

Otolith and eye lens cores were analyzed for Δ^{14}C and δ^{13}C at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at Woods Hole Oceanographic Institution. The stable C isotope ^{13}C is reported as the delta value δ^{13}C, computed as the ratio ^{13}C/^{12}C relative to a standard (Pee Dee Belemnite). Radiocarbon (^{14}C) is reported as the delta value Δ^{14}C that repre-sents the activity of a sample relative to a standard corrected for age and δ^{13}C (Stuiver and Polach 1977). Linear regressions were computed in Microsoft Excel between otolith and eye lens core Δ^{14}C values separately for red snapper versus all other reef fishes. One-sample *t* tests were computed to test whether regression slopes were significantly different than 1, as well as whether regression *y* intercepts were significantly different than 0. Lastly, otolith and eye lens core Δ^{14}C values were overlain on linear func-tions of the linear descending limb (>1980) of a regional coral and known-age red snapper otolith Δ^{14}C reference time series (Barnett et al. 2018). Coral sample locations were the Florida Keys (Druffel 1989; Toggweiler et al. 2019) and the Flower Garden Banks off Texas and Louisiana in the nGOM (Wagner 2009). Known-age nGOM red snapper otolith data were reported by Barnett et al. (2018). However, one otolith edge sample from that dataset was omitted here due to it being a statistical outlier and of much larger mass than other otolith edge samples. The effect of this was to decrease the 95% prediction intervals of the linear regression of Δ^{14}C versus year of formation for the reference time series.

Table 1. Descriptive data, otolith core $\Delta^{14}C$ and $\delta^{13}C$, and eye lens core $\Delta^{14}C$ and $\delta^{13}C$ values for reef fish samples analyzed with accelerator mass spectrometry at the National Ocean Sciences Accelerator Mass Spectrometry (NOSAMS) facility at Woods Hole Oceanographic Institution.

Species	Sample date	Age (years)	Birth year	Total length (mm)	Otolith NOSAMS No.	Otolith core $\Delta^{14}C$ (‰)	Otolith core $\delta^{13}C$ (‰)	Eye lens NOSAMS No.	Lens core $\Delta^{14}C$ (‰)	Lens core $\delta^{13}C$ (‰)
Epinephelus morio	16 July 2017	1	2016	188	144513	36.75	−5.91	144495	38.41	−16.25
	17 July 2017	3	2014	337	144514	34.98	−7.41	144496	44.19	−17.65
	21 Mar. 2017	13	2004	830	144507	64.83	−5.95	144489	69.62	−16.97
Hyporthodus niveatus	11 Nov. 2010	2	2008	134	144512	59.92	−6.28	144494	61.23	−18.33
	8 July 2017	4	2013	508	144521	38.61	−5.51	144503	36.44	−16.98
	8 July 2017	16	2001	815	144520	68.26	−4.95	144502	73.10	−16.77
Hyporthodus flavolimbatus	18 Oct. 2007	1	2006	159	144510	64.41	−5.89	144492	64.94	−18.59
	30 Oct. 2013	1	2012	189	144511	48.60	−6.53	144493	54.58	−18.29
	9 July 2017	24	1993	895	144522	91.72	−5.21	144504	99.36	−17.86
Lutjanus campechanus	18 Oct. 2016	0	2016	331	140131	37.26	−4.59	142383	36.45	−17.73
	11 Sept. 2016	3	2013	331	142355	41.15	NA	142375	43.58	−18.75
	11 Sept. 2016	9	2007	832	142353	63.56	−4.54	142377	61.94	−16.82
	11 Sept. 2016	12	2004	805	142356	60.50	−3.47	124376	66.10	−17.39
	8 Sept. 2016	15	2001	749	142351	73.24	−3.95	142379	69.54	−17.78
	11 Sept. 2016	18	1998	843	142354	78.03	−4.07	142380	75.29	−16.33
	11 Sept. 2016	20	1996	870	142358	84.58	−4.08	142381	85.97	−17.01
	11 Sept. 2016	27	1989	882	142357	106.89	−4.79	142382	111.02	−17.50
Mycteroperca phenax	11 July 2012	0	2012	98	144515	56.57	−7.59	144497	48.79	−18.56
	21 Mar. 2017	12	2005	502	144506	57.82	−6.61	144488	62.09	−17.92
	21 Mar. 2017	24	1993	625	144517	89.17	−6.10	144499	88.98	−15.69
Pagrus pagrus	15 Nov. 2013	1	2012	237	144516	51.68	−5.54	144498	47.66	−17.99
	22 Apr. 2017	11	2006	530	144519	56.75	−5.53	144501	58.62	−17.36
	22 Apr. 2017	19	1998	554	144518	78.89	−3.85	144500	73.86	−16.68
Pristigenys alta	9 May 2016	4	2012	208	142359	48.75	−4.83	142392	46.48	−17.48
	9 May 2016	8	2008	284	142360	56.09	−4.71	142393	59.27	−18.21
	19 May 2016	18	1998	301	142362	78.21	−4.08	142395	73.03	−16.29
Rhomboplites aurorubens	14 Nov. 2016	0	2016	162	144508	42.51	−6.27	144490	42.73	−19.08
	14 Nov. 2016	6	2010	317	144509	45.76	−6.58	144491	57.57	−19.17
	16 Apr. 2017	13	2003	495	144523	65.37	−6.34	144505	66.94	−18.13

Results

Red snapper samples ranged in age from 0 to 27 years, while the age range among the additional reef fishes was 0 to 24 years (Table 1). The slope and y intercept ($\pm$CI) for the regressions of lens versus otolith core $\Delta^{14}C$ were 1.01 ($\pm$0.12) and −0.79 ($\pm$8.43) for the red snapper model and 1.02 ($\pm$0.16) and −0.57 ($\pm$8.60) for the model including the other species. Slopes were not significantly different than 1 for red snapper (t test, df = 7, t = 0.206, p = 0.842) or the additional species examined (t test, df = 20, t = 0.266, p = 0.902; Fig. 2). The y intercepts of the regressions also were not significantly different than 0 for red snapper (t test, df = 7, t = 0.415, p = 0.689) or the other reef fish species (t test, df = 20, t = 0.229, p = 0.826). All otolith and eye lens core $\Delta^{14}C$ values versus fish birth year (year of collection minus age estimate) were within the 95% prediction intervals of the regression fit to the regional coral and known-age red snapper otolith $\Delta^{14}C$ time series (Fig. 3).

Discussion

Our results clearly demonstrate the efficacy of utilizing eye lens cores to derive birth year $\Delta^{14}C$ values for marine fish age validation via the bomb ^{14}C chronometer. The method for extracting lens cores is relatively quick and straightforward, with no specialized equipment required. More importantly, data from nGOM reef fishes indicate there is a strong agreement between eye lens and otolith core $\Delta^{14}C$ values. The correspondence between otolith and eye lens core $\Delta^{14}C$ values is stronger for red snapper (R^2 = 0.98) than among samples from the other seven reef fishes examined (R^2 = 0.90), but in both cases linear regressions have slopes

that are not significantly different than 1 and y intercepts not significantly different than 0. However, it is unclear whether the greater amount of unexplained variance in the regression computed among the other reef fishes is due to process or measurement error. The potential for greater measurement error exists given lens core extraction methods were based on lens dimension and mass data from age-0 red snapper, and perhaps species-specific data for the other fishes would have decreased a potential source of measurement error. Some of the additional species examined also have smaller otoliths than red snapper, which can present a challenge when micromilling core material for AMS analysis. If otolith material from more recent years than the birth year was milled, it would have biased the estimate of otolith core $\Delta^{14}C$.

Eye lens as well as otolith core $\Delta^{14}C$ values matched expectation, meaning they were within the 95% prediction intervals of the linear regression fitted to the reference time series, although there was greater variance in the reef fish data beyond red snapper. These results are insufficient to be considered a definitive validation of age estimation procedures for any of the species examined. Nonetheless, they suggest birth years, hence age estimates, of our samples are accurate. This is clearly the most important component of any age validation procedure: that it produce accurate results. This is reinforced in the current study given age estimation via otolith opaque zone counts was previously validated with the bomb ^{14}C chronometer for nGOM red snapper (Barnett et al. 2018), yellowedge grouper (Cook et al. 2009), and snowy grouper (Sanchez et al. 2019).

Other important advantages of deriving birth year $\Delta^{14}C$ values from eye lens versus otolith cores for marine bony fishes are the greater proportion of C in lenses versus otoliths (~0.5 versus 0.1,

16

Can. J. Fish. Aquat. Sci. Vol. 78, 2021

Fig. 2. Eye lens core versus otolith core Δ^{14}C for (A) red snapper ($n = 8$) and (B) other reef fishes ($n = 21$) sampled in the northern Gulf of Mexico. Error bars are σ, which is the analytical standard error estimated from ^{14}C counts during accelerator mass spectrometry analysis. Dashed lines indicate line of 1:1 agreement between eye lens and otolith core Δ^{14}C values; solid lines are linear regression fits to the data, with equations indicated on panels.
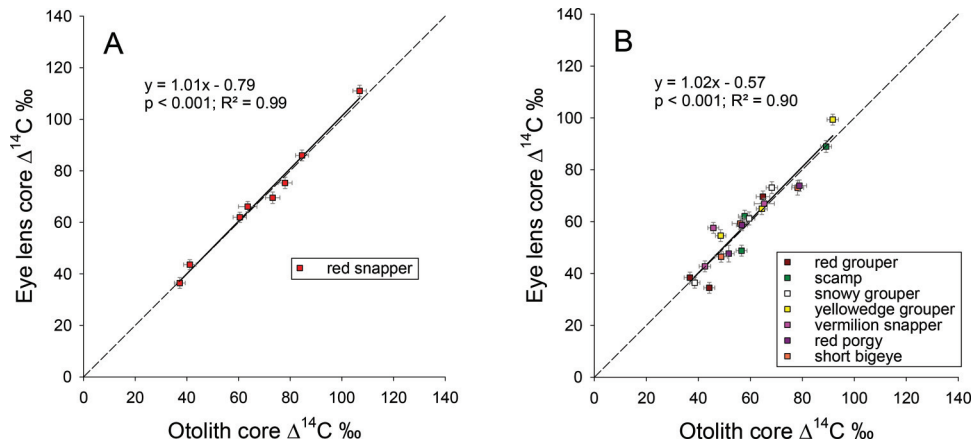

Fig. 3. Northern Gulf of Mexico (nGOM) red snapper (A) otolith core and (B) eye lens core Δ^{14}C versus year of formation (i.e., estimated birth year for otolith or eye lens core samples). Data in each panel are overlain on a linear regression fitted to a Δ^{14}C reference time series consisting of coral samples from the Florida Keys (Druffel 1989; Toggweiler et al. 2019) and the Flower Garden Banks off Texas and Louisiana (Wagner 2009), as well as known-age nGOM red snapper otolith samples (Barnett et al. 2018). Similar data for (C) otolith core and (D) eye lens core Δ^{14}C are also plotted for seven additional nGOM reef fishes. The solid line indicates the fitted regression ($y = 130.6 - 2.57x$ with 1980 as the initial year ($p < 0.001$, $R^2 = 0.98$), and dashed lines are 95% prediction intervals. Legend in panel A applies to panel B, and legend in panel C applies to panel D.
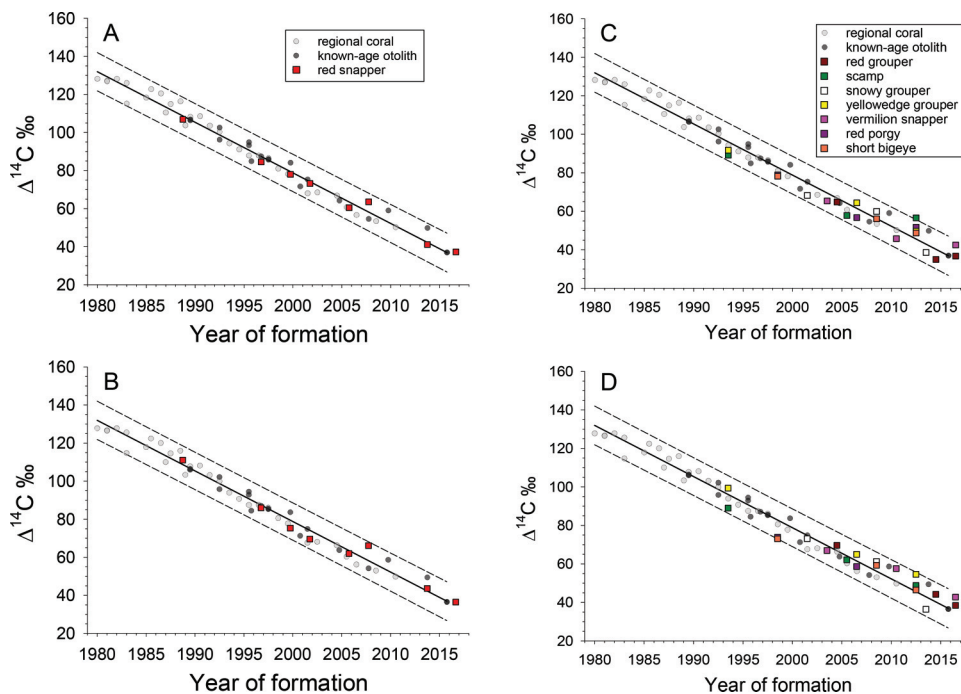
respectively) and the fact that eye lenses are derived 100% from metabolic carbon (Dahm et al. 2007), while otolith C is typically derived 60%–80% from dissolved inorganic carbon (Solomon et al. 2006). With respect to mass, approximately five times less mass of dried eye lens versus otolith core material is required to produce 100 μg of C for robust AMS analysis. Accurately milling enough otolith core material for AMS is not likely to be an issue

for fishes like red snapper that have relatively large otoliths (Barnett and Patterson 2010; Barnett et al. 2018), but can be problematic or impossible for fishes, such as demersal slope and mesopelagic or deeper fishes, that often have much smaller otoliths. However, application of the bomb ^{14}C chronometer for age validation for these deeper marine fishes has historically faced an even greater issue in that their otolith Δ^{14}C values do not

correspond to hermatypic coral time series from shelf habitats given that the influence of bomb ^{14}C diminishes rapidly with depth (Barnett et al. 2020). The fact that eye lens proteins are derived from metabolic sources (Dahm et al. 2007; Nielsen et al. 2016), and nearly all of the organic C in the deep ocean is derived from phytoplankton in the photic zone (Broecker and Peng 1982), means eye lens C of deepwater fishes should still have a surface-derived Δ^{14}C signature, while their otoliths will be depleted in ^{14}C relative to surface waters.

The one clear advantage of utilizing otolith cores, given they are sufficiently large, to derive birth year Δ^{14}C values for shelf or epipelagic marine bony fishes is the fact that otolith opaque zones provide clear landmarks for micromilling age-0 otolith material. However, if the dimensions of age-0 eye lenses can be measured or approximated for species of interest, that would provide target dimensions for extracting eye lens cores from adults. Dissecting and archiving eye lenses from sampled fishes is likely to become more routine given the life history and ecological information they store (Wallace et al. 2014), and deriving birth year Δ^{14}C values from them to apply the bomb ^{14}C chronometer for age validation would allow otoliths to be reserved for other analyses.

Acknowledgements

References

Andrews, A.H., Yeman, C., Welte, C., Hattendorf, B., Wacker, L., and Christl, M. 2019. Laser ablation AMS reveals complete bomb ^{14}C signal in an otolith with confirmation of 60-year longevity for red snapper (*Lutjanus campechanus*). Mar. Freshw. Res. **70**(12): 1768–1780. doi:10.1071/MF18265.

Barnett, B.K., and Patterson, W.F. 2010. The effect of coring and pulverizing juvenile red snapper, *Lutjanus campechanus*, otoliths on their chemical signatures. Environ Biol Fishes, **89**(3–4): 463–471. doi:10.1007/s10641-010-9697-7.

Barnett, B.K., Thornton, L., Allman, R., Chanton, J.P., and Patterson, W.F., III. 2018. Linear decline in red snapper (*Lutjanus campechanus*) otolith Δ^{14}C extends the utility of the bomb radiocarbon chronometer for fish age validation in the Northern Gulf of Mexico. ICES J. Mar. Sci. **75**(5): 1664–1671. doi:10.1093/ICESJMS/FSY043, doi:10.1093/icesjms/fsy043.

Barnett, B.K., Chanton, J.P., Ahrens, R., Thornton, L., and Patterson, W.F., III. 2020. Life history of northern Gulf of Mexico Warsaw grouper *Hyporthodus nigritus* inferred from otolith radiocarbon analysis. PLoS ONE, **15**(1): e0228254. doi:10.1371/journal.pone.0228254.

Broecker, W.S., and Peng, T.-H. 1982. Tracers in the sea. Lamont Doherty Geological Observatory, Palisades, New York.

Cook, M., Fitzhugh, G.R., and Franks, J.S. 2009. Validation of yellowedge grouper, *Epinephelus flavolimbatus*, age using nuclear bomb-produced radiocarbon. Environ. Biol. Fishes, **86**(4): 461–472. doi:10.1007/s10641-009-9536-x.

Dahm, R., Schonthaler, H.B., Soehn, A.S., van Marle, J., and Vrensen, G.F.J.M. 2007. Development and adult morphology of the eye lens in the zebrafish. Exp. Eye Res. **85**(1): 74–89. doi:10.1016/j.exer.2007.02.015.

Druffel, E.R.M. 1989. Decadal time scale variability of ventilation in the North Atlantic: high-precision measurements of bomb radiocarbon in banded corals. J. Geophys. Res. **94**(C3): 3271–3285. doi:10.1029/JC094iC03p03271.

Druffel, E.M., and Linick, T.W. 1978. Radiocarbon in annual coral rings of Florida. Geophys. Res. Lett. **5**(11): 913–916. .doi:10.1029/GL005i011p00913.

George, J.C., Bada, J., Zeh, J., Scott, L., Brown, S.E., O'Hara, T., and Suydam, R. 1999. Age and growth estimates of bowhead whales (*Balaena mysticetus*) via aspartic acid racemization. Can. J. Zool. **77**(4): 571–580. doi:10.1139/z99-015.

Grottoli, A.G., and Eakin, C.M. 2007. A review of modern coral δ^{18}O and Δ^{14}C proxy records. Earth-Sci. Rev. **81**(1-2): 67–91. doi:10.1016/j.earscirev.2006.10.001.

Kalish, J.M. 1993. Pre- and post-bomb radiocarbon in fish otoliths. Earth Planet. Sci. Lett. **114**(4): 549–554. doi:10.1016/0012-821X(93)90082-K.

Nielsen, J., Hedeholm, R.B., Heinemeier, J., Bushnell, P.G., Christiansen, F.S., Olsen, J., et al. 2016. Eye lens radiocarbon reveals centuries of longevity in the Greenland shark (*Somniosus microcephalus*). Science, **353**(6300): 702–704. doi:10.1126/science.aaf1703.

Paxton, J.R. 2000. Fish otoliths: do sizes correlate with taxonomic group, habitat and/or luminescence? Philos. Trans. R. Soc. Lond. Ser. B, **355**(1401): 1299–1303. doi:10.1098/rstb.2000.0688.

Sanchez, P.J., Pinsky, J.P., and Rooker, J.R. 2019. Bomb radiocarbon age validation of Warsaw grouper and snowy grouper. Fisheries, **44**(11): 524–533. doi:10.1002/fsh.10291.

Solomon, C.T., Weber, P.K., Cech, J.J., Jr., Ingram, B.L., Conrad, M.E., Machavaram, M.V., et al. 2006. Experimental determination of the sources of otolith carbon and associated isotopic fractionation. Can. J. Fish. Aquat. Sci. **63**(1): 79–89. doi:10.1139/f05-200.

Stuiver, M., and Polach, H.A. 1977. Discussion: reporting of ^{14}C data. Radiocarbon, **19**(3): 355–363. doi:10.1017/S0033822200003672.

Toggweiler, J.R., Druffel, E.R.M., Key, R.M., and Galbraith, E.D. 2019. Upwelling in the ocean basins north of the ACC: 1. On the upwelling exposed by the surface distribution of Δ^{14}C. JGR Oceans. **124**: 2591–2608. doi:10.1029/2018JC014794.

Wagner, A.J. 2009. Oxygen and carbon isotopes and coral growth in the Gulf of Mexico and Caribbean Sea as environmental and climate indicators. Ph.D. dissertation, Department of Oceanography, Texas A&M University, College Station, Texas.

Wallace, A.A., Hollander, D.J., and Peebles, E.B. 2014. Stable isotopes in fish eye lenses as potential recorders of trophic and geographic history. PLoS ONE, **9**(10): e108935. doi:10.1371/journal.pone.0108935.