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SEDAR100-RD-03

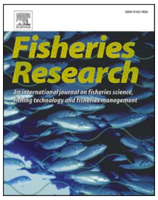
August 2025

Fisheries Research 279 (2024) 107123

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres



Bomb ^{14}C validates Gray Triggerfish (*Balistes capriscus*) dorsal spine and otolith ageing protocols

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ARTICLE INFO

Keywords:
Gray triggerfish
Age validation
Bomb radiocarbon
Dorsal spines
Otoliths

ABSTRACT

Gray triggerfish (*Balistes capriscus*) historically have been aged by counting translucent zones in thin sections prepared from their first dorsal spine because their small, fragile sagittal otoliths are difficult to extract and process for ageing. However, recent research suggests dorsal spine translucent zone counts produce biased age estimates, thus the historical dorsal spine-based ageing protocol results in a systematic underestimation of true age. Here, we employed the bomb radiocarbon chronometer to test the accuracy of age estimates ($n = 3$ readers) derived from opaque zone counts in whole otoliths, as well as dorsal spine section translucent zone counts produced with the historical ageing protocol and a new method that requires higher magnification to count translucent zones on the margin of dorsal spine sections. Results indicate historical dorsal spine-derived age estimates underestimate age, with the extent of bias increasing with age. There was no evidence of ageing bias for both whole-otolith opaque zone counts and new protocol dorsal spine translucent zone counts. New dorsal spine protocol ageing was slightly more precise among readers (iAPE = 9.4 %) than otolith ageing (iAPE = 10.1 %) and read times were 2–3x faster for dorsal spine sections than whole otoliths. Validation of the new dorsal spine ageing protocol is a critical step in effective production ageing of gray triggerfish. Archived dorsal spine sections can be re-aged with the new protocol to update historical age composition data, and future ageing will not have to rely on the logistically challenging extraction and processing of otoliths.

1. Introduction

Accurate age data are critical for estimating population dynamics, such as growth and mortality, and as inputs to assessment models computed to estimate stock status (Maunder and Punt, 2013; Reeves, 2003). Therefore, extensive efforts are often taken to estimate and minimize ageing error to ensure accurate and precise age estimates. Ageing bias most frequently appears as an underestimate rather than an overestimate of age, which results in biased estimates of growth and mortality and the overestimation of the fishing mortality rate that produces maximum sustainable yield for a given stock (Beamish and McFarlane, 1983; Campana, 2001; Lin Lai and Gunderson, 1987; Reeves, 2003). Without accurate age data, the ability of managers to effectively monitor and manage fish stocks becomes compromised, increasing the likelihood of overfishing. Consequently, accurate age data are fundamental to the sustainable management of fisheries stocks (Lin Lai and Gunderson, 1987; Reeves, 2003).

Age estimates for bony fishes are typically derived from growth zone counts in ageing structures, such as otoliths, scales, spines, or bones. Sagittal otoliths are the most common ageing structure but extracting them is an invasive, lethal method. Scales, spines, or fin rays are often used for non-lethal age estimation (Carroll et al., 2023; Murie et al., 2009), but scales can be lost and regenerated and spines and fin rays can be prone to metabolic reworking near the nucleus which can make growth zone interpretation difficult. For some fishes, these other structures are used for age estimation due to difficulty in extracting or

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https://doi.org/10.1016/j.fishres.2024.107123
Received 24 October 2023; Received in revised form 22 July 2024; Accepted 25 July 2024

processing otoliths for ageing. For example, gray triggerfish, *Balistes capriscus*, age has historically been estimated by counting translucent zones in dorsal spine thin sections (Allman et al., 2016; Ingram, 2001; Moore, 2001). This is because gray triggerfish sagittal otoliths are difficult to extract and prepare for ageing due to their exceedingly small size (<3.0 mm) and mass (<6.0 mg), and their ornate and brittle structure (Fig. 1 and S1). However, dorsal spine-based ageing suffers from poor reader agreement (iAPE >10 %; Allman et al., 2018, 2016; Burton et al., 2014; Jefferson et al., 2019; Kelly-Stormer et al., 2017), and inconclusive marginal increment analysis results (Allman et al., 2016) cast doubt on the accuracy of dorsal spine-derived age estimates.

Recent results suggest historical dorsal spine ageing produces biased gray triggerfish age estimates, while age estimates derived from counts of opaque zones in whole or sectioned otoliths provide accurate age estimates (Patterson et al., 2019; Potts et al., 2023). Patterson et al. (2019) applied the bomb radiocarbon (^{14}C) chronometer (Kalish, 1993) to test the accuracy of Gulf of Mexico (GOM) gray triggerfish age estimates derived from counts of translucent zones in dorsal spine sections, as well as opaque zone counts in otoliths. They employed an extensive regional coral and known-age fish otolith Δ^{14}C reference series (Andrews et al., 2013; Barnett et al., 2018) to gray triggerfish eye lens core (birth year) Δ^{14}C, sensu Patterson et al. (2021), to test if dorsal spine- or otolith-derived age estimates were accurate. Age estimates produced with the historical dorsal spine ageing method were shown to underestimate age for older fish, while otolith-derived age estimates were estimated to be accurate. Shervette and Rivera Hernández (2022a) later applied this same approach to validate age estimates derived from whole otoliths in Caribbean Sea queen triggerfish, *Balistes vetula*, while Shervette and Rivera Hernández (2022b) indicated only otoliths should be utilized to age gray triggerfish despite the difficulty in extracting and processing them for ageing.

Most recently, Potts et al. (2023) reported results of an Atlantic gray triggerfish (n = 101) rearing experiment in which ageing structures were chemically marked with calcein and then fish were held for up to 527 days to test the periodicity of growth zone formation in otoliths and dorsal spines, both of which were sectioned for ageing. They reported otolith opaque zones appeared to be formed once per year but there was a divergence between otolith opaque zone counts and dorsal spine translucent zone counts where otolith opaque zone counts for older age classes (age 5+) were higher than the corresponding dorsal spine translucent zone count, as had been reported earlier by others (Patterson et al., 2019). However, broad translucent zones were noted in some dorsal spine thin sections and increased magnification revealed the presence of compacted growth zones near the margin of the dorsal spine sections of some older (>age-5) specimens. Based on this observation, a new ageing protocol was developed that includes using increased magnification to examine broad translucent zones for additional growth zones (Potts et al., 2023). If this protocol produces accurate age estimates, then it would alleviate major problems associated with inaccurate underestimates of age produced with the historical dorsal spine ageing protocol. Therefore, there is a critical need to test the accuracy and precision of dorsal spine-based gray triggerfish age estimates based on this new ageing protocol.

The bomb radiocarbon (^{14}C) chronometer is an effective age validation tool and has been applied successfully to a suite of Gulf of Mexico (GOM) reef fishes (Baker and Wilson, 2001; Barnett et al., 2020, 2018; Chamberlin et al., 2023a, 2023b; Fischer et al., 2005) and provides an ideal approach to age validation for gray triggerfish from this region. The bomb ^{14}C chronometer is based on the rapid increase in atmospheric and oceanic ^{14}C in the 1950s and 1960s due to atmospheric testing of nuclear weapons (Kalish, 1993; Nydal and Lövseth, 1983). Regional chronometers have been developed which can then be compared to otolith core Δ^{14}C values to validate age estimates (Kalish, 1993). Typically, ≥100 µg of C is required for robust analysis of Δ^{14}C with accelerator mass spectrometry (AMS). Otoliths are approximately 12 % C by mass, meaning >1 mg of otolith core material is necessary for precise AMS analysis of Δ^{14}C. This is problematic for fishes with small otoliths, such as gray triggerfish, as the otolith core is unlikely to contain sufficient aragonite (biogenic CaCO3) to be precisely analyzed for Δ^{14}C, thus the bomb radiocarbon chronometer has not been applied as an age validation tool for gray triggerfish using otolith core birth year material. However, eye lenses have emerged as a useful source of birthyear ^{14}C (Patterson et al., 2021; Patterson and Chamberlin, 2023). Eye lenses are composed of approximately 50 % organic carbon (Andley, 2007; Dahm et al., 2007; Tzadik et al., 2017), which allows for a smaller amount of eye lens material to be analyzed for Δ^{14}C while maintaining analytical

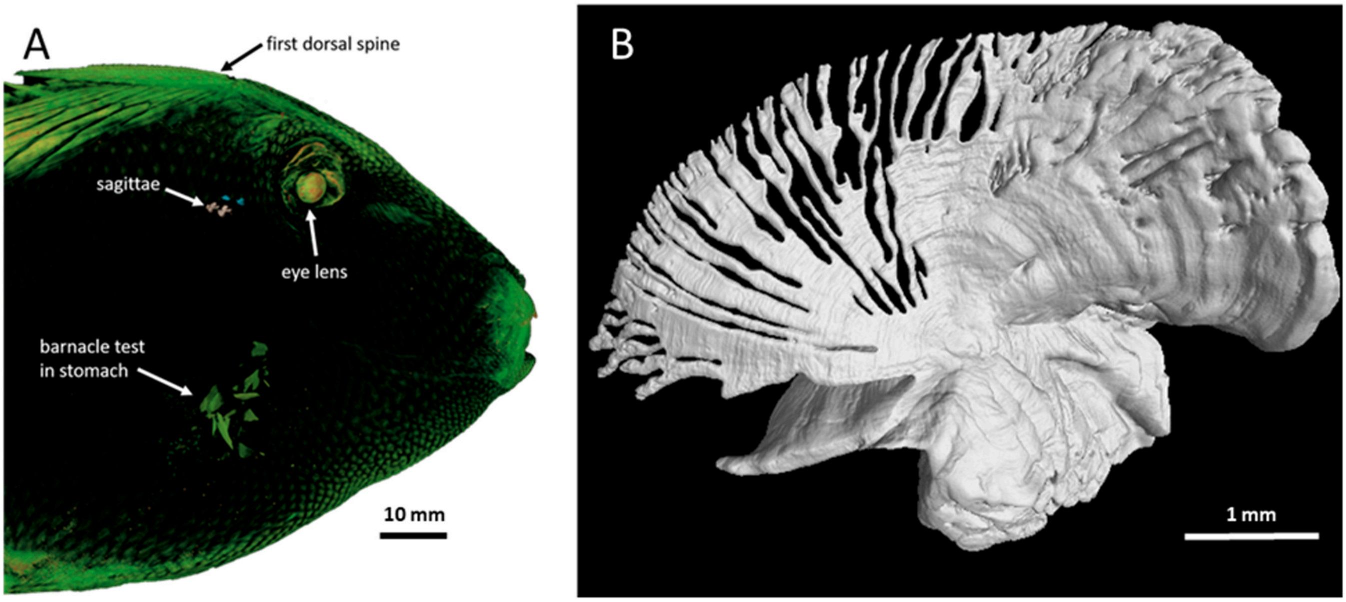

Fig. 1. A) Nano-computerized tomography (CT) scan image of a gray triggerfish with sagittal otoliths highlighted in white, and B) a nano-CT scan of a gray triggerfish sagittal otolith made while the extracted otolith remained within the sacculus. The lattice-like otolith projections in the top left, radiating from the core, are often destroyed upon removal from the sacculus. For an animated 360° video of the sagittal otolith in panel B the reader is referred to Fig. S1 in the online version of the supplementary materials.

accuracy and precision (Patterson et al., 2021) and eye lenses have recently been successfully used as a source of birth year ^{14}C to validate age estimates of several GOM reef fishes (Chamberlin et al., 2023a, 2023b).

The objectives of this study were to 1) test the accuracy of gray triggerfish age estimates derived from whole otoliths, as well as from translucent zone counts in dorsal spine sections following the historical ageing protocol (Kolmos et al., 2013) and the new dorsal spine ageing protocol (Potts et al., 2023); 2) estimate the precision of age estimates produced with each ageing protocol; and, 3) produce recommendations for the production ageing of gray triggerfish. We accomplished the age validation component of the study through the application of the bomb radiocarbon chronometer to gray triggerfish eye lens core Δ^{14}C values (Patterson et al., 2021, 2019), while ageing precision estimates were made among three independent readers of each structure. Study results have important implications for the assessment and management of gray triggerfish stocks throughout the species' range and are discussed in the context of correcting historical age composition data and producing accurate age composition data in the future.

2. Methods

2.1. Sample collection

Fishery-independent gray triggerfish samples were collected in the northern GOM (nGOM) in 2022 with hook and line. Fish were targeted on natural and artificial reefs across the nGOM (28.40 N to 30.20 N and 84.20 W to 86.94 W). Fish were labeled with a zip tie fastened around the caudal peduncle and placed on ice. Fishery-dependent gray triggerfish were collected in 2022 and 2023 from charter fishing vessels from natural and artificial reefs in the nGOM with hook and line and by commercial fishing vessels from natural and artificial reefs in the eastern GOM (eGOM) with benthic longlines and deck reels. Right and left vestibular systems were extracted whole after the top of the cranium was removed with a hand saw and stored dry in 0.5-ml microcentrifuge tubes. The first dorsal spine, including the condyle, was removed by cutting anterior and posterior to the spine. Both eyes were removed from the cranium, placed in plastic bags, and frozen in a −20 °C freezer.

2.2. Age estimation

Sagittal otoliths were removed from the vestibular tissue with forceps in the laboratory following rehydration of the tissue, and then stored dry in microcentrifuge vials. Given the otolith opaque zones were enumerated from whole, unsectioned otoliths, sample preparation beyond removal from the cranium and vestibular tissue was not necessary. Dorsal spines were prepared for ageing by boiling in water to remove soft tissue and subsequently scraping any remaining tissue (Allman et al., 2016). Each dorsal spine was then affixed to a glass microscope slide and a 0.5-mm thick transverse sections was cut approximately 5–10 mm above the condyle with a diamond-bladed, low-speed saw. The dorsal spine section was subsequently affixed to a glass microscope slide and the surface was covered with a toluene-based mounting medium to fill any cracks or abrasions from the sectioning process.

Dorsal spine translucent zones were enumerated from the focus of the dorsal spine to posterior lobes by reader 1 (R1; DWC) using the historical protocol of Kolmos et al. (2013), hereafter referred to as the "historical dorsal spine protocol," where zoom is kept constant. Given reader 2 (R2; JCP) and reader 3 (R3; WDR) had previously developed a new dorsal spine ageing protocol (Potts et al., 2023), only R1 read the dorsal spines with the historical dorsal spine protocol as reverting to the historical dorsal spine protocol after training with the new dorsal spine protocol would likely have produced biased results.

Ageing comparisons were made among three experienced readers for the other protocols examined. Whole otolith opaque zones were enumerated on the distal side of the otolith, with the sulcal groove facing downward, under a dissecting microscope with transmitted light at 25–50x magnification. Dorsal spine translucent zones were also enumerated by all three readers using the protocol of Potts et al. (2023), hereafter referred to as the "new dorsal spine protocol," where a dorsal spine section was initially examined under a dissecting microscope with transmitted light at 15–20x magnification and reexamined for additional growth zones at the margin or within earlier broad translucent zones at 40x if 5 or more translucent zones were initially counted or if a broad translucent zone was readily apparent. Both whole otolith and new dorsal spine protocol translucent zone counts were performed double-blind.

The index of average percent error (iAPE) was calculated to assess the precision of growth zone counts between readers for each ageing structure (Beamish and Fournier, 1981). Bias plots were also constructed to compare growth zone counts between readers for each ageing structure (Campana et al., 1995), as well as between ageing structures or dorsal spine protocols for each reader. All analyses were carried out in R (R Core Team, 2020).

2.3. Bomb ^{14}C age validation

A systematic random subsample of gray triggerfish was selected for age validation analysis. Two fish were randomly selected from ages 2–9 years and all fish in age classes ≥10 were selected from R1's otolith-derived opaque zone counts. The right eye of each sample was thawed, and its lens extracted through an incision made in the cornea. All materials used in the processing and storage of eye lenses were baked in a muffle furnace at 500°C for a 24-hr period to remove any residual petrocarbon from the manufacturing process. Extracted lenses were then wrapped individually in aluminum foil and freeze dried for 24 hours. This caused the outer layers of the lens to split and begin flaking, while leaving the core of the lens intact. Outer layers of the lens were peeled away with forceps, ultimately revealing the core, which was stored in a glass vial. Target lens core mass was 3.0 mg, which is the predicted dry eye lens mass from age-0 gray triggerfish in December, (mean fork length = 130 mm) estimated from a non-linear regression fit to FL and dry lens mass of age-0 gray triggerfish (n = 27; Figure S2) sampled October to December with trawls in the nGOM.

Once extracted, eye lens cores were analyzed for ^{14}C with accelerated mass spectrometry (AMS) at the National Ocean Sciences AMS (NOSAMS) facility at Woods Hole Oceanographic Institution. The resulting data are reported as Δ^{14}C, which is the ^{14}C activity relative to the absolute international standard (base year 1950) corrected for δ^{13}C fractionation and ^{14}C age (Stuiver and Polach, 1977). Eye lens core Δ^{14}C signatures were tested against a time series of coral (Chamberlin et al., 2023a; Druffel, 1980, 1989; Moyer and Grottoli, 2011; Toggweiler et al., 2019; Wagner, 2009), and known-age red snapper otolith Δ^{14}C (Barnett et al., 2018; Chamberlin et al., 2023a) values that constituted the regional Δ^{14}C reference series for this study (Figure S3). Gray triggerfish eye lens core Δ^{14}C signatures and birth year estimates were superimposed on the regional Δ^{14}C time series for each reader and age estimation protocol, with birth year = year of collection – growth zone count + 0.75 y. Adding the fractional year accounts for the fact that GOM gray triggerfish peak spawning occurs in June and July (Ingram, 2001), therefore the midpoint of the birth year time period is half-way through the second half of the year, or 0.75 y.

Ageing bias was estimated for dorsal spine and otolith-derived age estimates using a Bayesian spline model (Chamberlin et al., 2023b) fit to the compiled regional reference series. The model was fit with the cmdstanr package (Gabry and Češnovar, 2022) using 8 chains, 1000 warmup iterations per chain, and 125 sampling iterations per chain. The Gelman-Rubin statistic was used to assess chain convergence with values less than 1.1 assumed to indicate convergence (Gelman and Rubin, 1992). The 95 % credible interval was computed for the birth year adjustment posterior distribution, and the probability of the maximum a posteriori (MAP) value was zero was assessed, with an inference of no bias if $p(\delta_{\mathrm{MAP}} = 0) > 0.05$.

3. Results

3.1. Sample collection

In total 573 gray triggerfish were sampled in the GOM. This consisted of 153 fisheries-independent and 420 fisheries-dependent samples. Of these 573 gray triggerfish, 108 were collected in the eGOM and 465 in the nGOM (Figure S4). Sample sizes for otoliths ($n = 555$) and dorsal spines ($n = 564$) differed slightly due to missing structures for some samples.

3.2. Age estimation

Between-reader growth zone counts from whole otoliths and the new dorsal spine protocol showed strong agreement, as did intra-reader whole otolith and new dorsal spine protocol growth zone counts (Figs. 2 and 3). Intra-reader whole otolith and new dorsal spine protocol had an iAPE of 8.6 % for R1, 5.8 % for R2, and 12.2 % for R3 (Fig. 3A-C). Opaque zone counts derived from whole otoliths had an overall iAPE of 10.1 % among all three readers, an iAPE of 6.2 % between R1 and R2, 9.1 % between R1 and R3, and 9.7 % between R2 and R3 (Fig. 3D-F). Translucent zone counts using the new dorsal spine protocol had an overall iAPE of 9.4 % among all three readers, an APE of 7.3 % between R1 and R2, 9.1 % between R1 and R3, and 7.2 % between R2 and R3 (Fig. 3G-I). Age estimates across age classes were more consistent between readers using the new dorsal spine protocol, largely falling on the line of 1:1 agreement (Fig. 3G-I). Conversely, R1 and R2 had consistent otolith opaque zone counts across age classes but R3's counts were slightly elevated for age-2 and 3 (Fig. 3D-F).

Translucent zone counts for R1 using the historical dorsal spine protocol corresponded with whole otolith opaque zone counts and new dorsal spine protocol translucent zone counts for growth zone counts 2 through 4 but diverged at historical dorsal spine protocol growth zone counts ≥5 (Fig. 4A-4B). This matched the trend reported by Potts et al. (2023) (Fig. 4C). Notably, the degree of divergence in growth zone counts increased as otolith opaque zone or new dorsal spine protocol translucent zone counts increased, with the historical dorsal spine protocol consistently producing lower growth zone counts. The iAPE for R1 between historical dorsal spine protocol and otolith growth zone counts was 11.1 % and was 10.9 % between historical dorsal spine protocol and new dorsal spine protocol counts.

3.3. Bomb ^{14}C age validation

Thirty eye lens cores were submitted to NOSAMS for ^{14}C analysis. The eye lenses had a mean ± 95 % CI mass of 2.98 ± 0.36 mg. Estimated birth years ranged from 2008.75 to 2020.75 for the otolith samples and 2006.75–2020.75 for the new dorsal spine protocol, among all readers, and 2010.75–2020.75 for the historical protocol, for R1 (Table 1). Application of the Bayesian Δ^{14}C model demonstrated that there was no evidence of bias in otolith opaque zone counts and the new dorsal spine protocol age estimates ($p(\delta_{\mathrm{MAP}} = 0) > 0.05$) for all readers (Table 2, Fig. 5A-B). Trace plots and Gelman-Rubin convergence diagnostic (<1.10) indicated all models converged (Table 2, Figures S5-S10). Conversely, dorsal spines aged with the historical protocol produced age estimates that had a median negative bias of −1.72 years with a 95 %

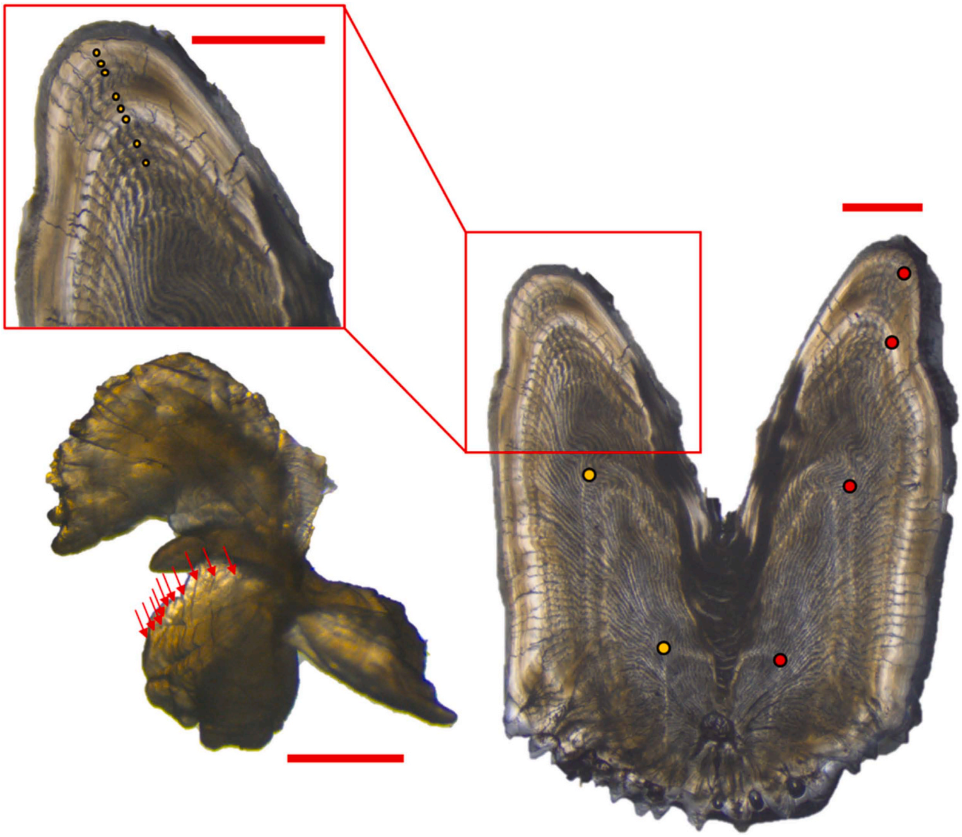

Fig. 2. Digital images viewed under a dissecting microscope with transmitted light of a whole sagittal otolith and a first dorsal spine thin section from a 479 mm gray triggerfish where the historical spine protocol translucent zone count is 6 lower than the counts derived from the otolith and new dorsal spine protocol. Inset is the magnified area of the spine with the broad translucent zone showing the compacted growth zones. Scale bars (1 mm) are included on each image. Red points denote the historical dorsal spine protocol count, orange points new dorsal spine protocol translucent zones, and red arrows denote otolith opaque zones.

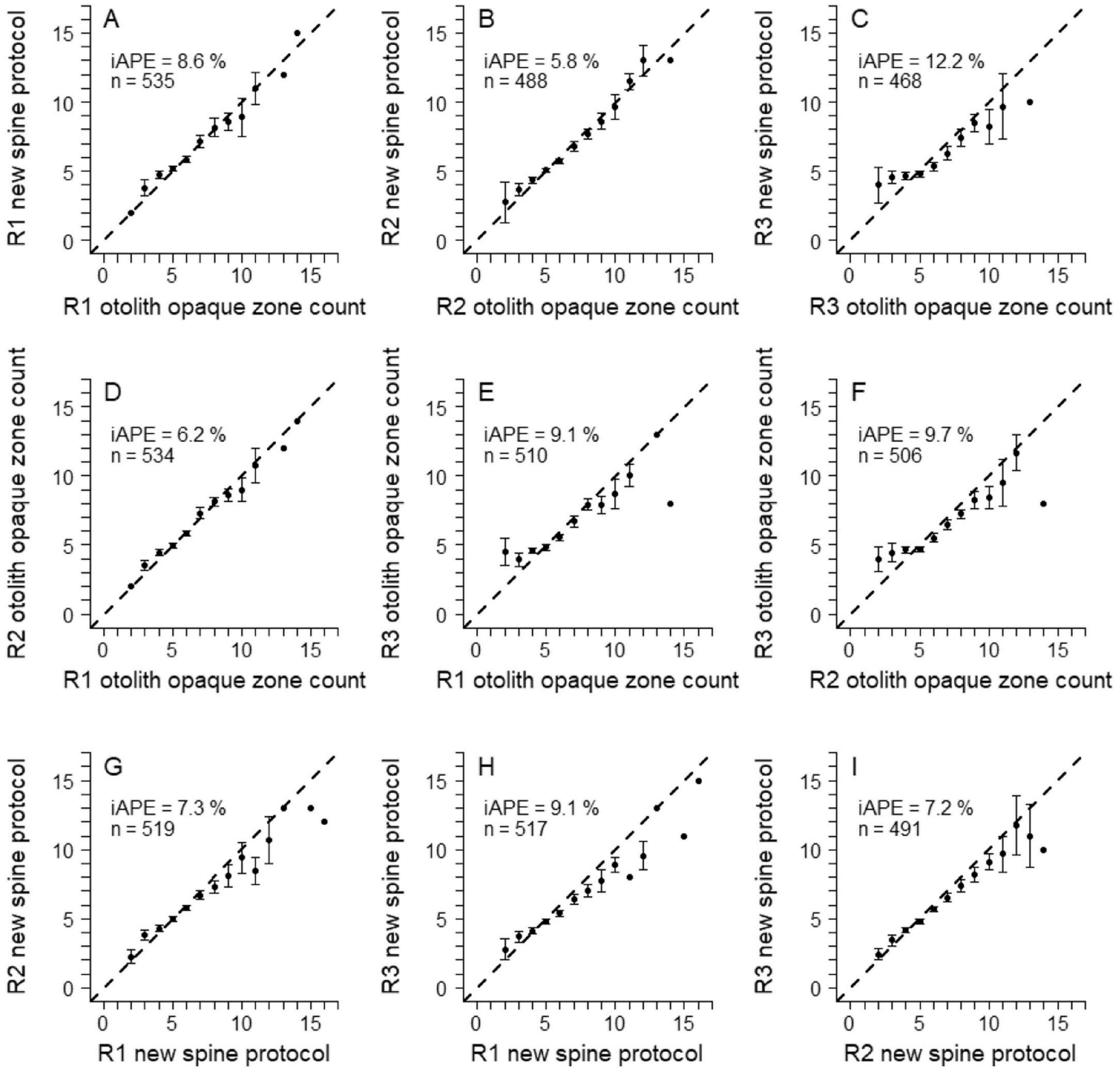

Fig. 3. Bias plots of A) R1 new dorsal spine protocol translucent zone counts versus R1 otolith opaque zone counts; B) R2 new dorsal spine protocol translucent zone counts versus R2 otolith opaque zone counts; C) R3 new dorsal spine protocol translucent zone counts versus R3 otolith opaque zone counts; D) R1 versus R2 otolith opaque zone counts; E) R1 versus R3 otolith opaque zone counts; F) R2 versus R3 otolith opaque zone counts; G) R1 versus R2 new dorsal spine protocol translucent zone counts; H) R1 versus R3 new dorsal spine protocol translucent zone counts; I) R2 versus R3 new dorsal spine protocol translucent zone counts. Error bars are 95 % confidence intervals. Dashed lines indicate the line of 1:1 agreement.

credible interval of −2.20 - −1.28, with this level of bias being significantly different from 0 ($p(\delta_{\mathrm{MAP}} = 0) < 0.0001$) (Table 2, Fig. 5C). As with the otolith-derived birth year model, the trace plots and Gelman-Rubin convergence diagnostic (<1.10) indicated the model had converged (Table 2, Figure S11).

4. Discussion

Results of this study demonstrate no evidence of bias in age estimates derived from gray triggerfish whole otolith opaque zone counts and new dorsal spine protocol translucent zone counts, based on eye lens radiocarbon data. Conversely, the historical dorsal spine protocol produces translucent zone counts that underestimate age, which could result in biased growth estimates and overestimating stock productivity (Lin Lai and Gunderson, 1987; Reeves, 2003). While there was no evidence of bias in age estimates derived from both whole otoliths and the new dorsal spine protocol, the new dorsal spine protocol also resulted in slightly more precise age estimates (iAPE = 9.4 %) compared to the whole otolith age estimates (iAPE = 10.1 %), among three readers.

The negative bias in historical dorsal spine protocol age estimates is of particular concern to production ageing scientists. Shervette and Rivera Hernández (2022b) indicated gray triggerfish should only be aged via otoliths moving forward, but their manuscript was published prior to the new dorsal spine-based ageing protocol proposed by Potts

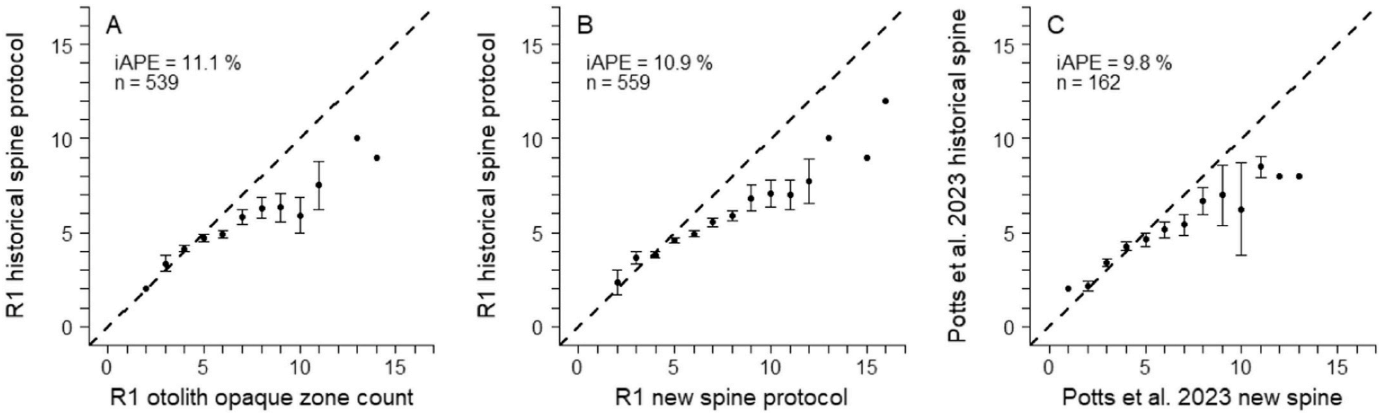

Fig. 4. Bias plot of A) R1 historical dorsal spine protocol translucent zone counts versus R1 otolith opaque zone counts, B) R1 historical dorsal spine protocol translucent zone counts versus R1 new dorsal spine protocol translucent zone counts, and C) Potts et al. (2023) historical dorsal spine protocol translucent zone counts versus new dorsal spine protocol translucent zone counts (Reproduced with permission of the author's). Error bars are 95 % confidence intervals. Dashed lines indicate the line of 1:1 agreement.

Table 1
Gray triggerfish eye lens core samples (n = 30) analyzed for Δ^{14}C with accelerator mass spectrometry, along with age estimates derived from otoliths and dorsal spines. R1 = Reader 1 (DWC), R2 = Reader 2 (JCP), and R3 = Reader 3 (WDR). Otolith = otolith opaque zone counts, new spine = new dorsal spine protocol translucent zone counts, and old spine = historical dorsal spine protocol translucent zone counts. NA = no age estimate. * = not measured.

	Eye lens core data			Age estimate years						
Sample year	Δ^{14}C ‰	σ	δ^{13}C ‰	R1 otolith	R1 new spine	R1 old spine	R2 otolith	R2 new spine	R3 otolith	R3 new spine
2022	34.51	2.0	−16.73	7	6	7	7	9	6	7
2022	47.45	2.2	−17.75	9	13	10	9	13	7	13
2022	59.19	2.2	−16.52	13	12	10	12	12	13	10
2022	36.52	2.1	−17.61	10	7	5	6	5	7	4
2022	31.33	2.7	−18.87	3	4	3	NA	10	NA	9
2022	47.88	2.4	−17.29	11	10	7	9	10	9	10
2022	35.69	2.2	−18.37	6	5	4	5	7	5	7
2022	31.08	2.6	−18.55	5	7	5	4	7	3	7
2022	46.7	2.2	−17.27	10	10	7	10	10	9	9
2022	48.42	2.1	−16.99	11	10	6	12	14	11	10
2023	30.46	2.0	*	5	4	4	5	5	5	4
2022	41.8	2.1	−16.33	10	12	7	10	10	10	11
2022	32.4	2.0	−16.64	10	6	5	10	NA	5	4
2022	48.64	2.1	−17.62	10	10	4	10	10	9	9
2022	46.18	2.2	−17.45	8	7	5	8	8	7	12
2022	24.82	2.0	−19.04	2	2	2	2	2	4	2
2022	34.96	2.1	−18.34	4	5	5	4	3	5	6
2022	27.65	2.1	−18.50	2	2	2	2	2	5	2
2022	31.37	2.0	−18.34	9	8	6	8	9	8	9
2022	31.28	2.0	−17.94	4	4	4	4	4	6	5
2022	33.96	2.1	*	3	3	4	3	4	3	4
2022	52.79	2.1	−17.69	10	10	6	10	9	9	7
2022	41.17	2.1	−18.60	10	10	8	8	9	8	8
2022	47.86	2.1	−17.31	11	12	9	11	11	10	9
2022	53.83	2.1	−17.56	14	15	9	14	13	8	11
2022	48.27	2.7	−17.15	8	16	12	11	12	11	15
2022	43.23	2.1	−17.98	10	11	8	8	10	9	8
2022	39.19	1.9	*	7	12	7	7	7	7	7
2023	45.63	1.9	*	11	12	8	11	12	10	11
2023	23.66	2.2	*	6	5	4	5	5	5	5

et al. (2023) and validated in the current study. Utilizing the new dorsal spine-based ageing protocol offers some clear advantages over otolith-based ageing. Collecting dorsal spines is a minimally invasive method that requires cutting an incision posterior and anterior to the first dorsal spine, leaving the fish largely intact and marketable as a whole product. By contrast, removing the otoliths requires removing the top of a fish's cranium, leaving the fish disfigured. Fishers, both commercial and recreational, are likely to be reluctant to allow samplers to disfigure their fish, particularly in the commercial fishery where removing the otoliths would likely result in decreased market value for fish sold whole. This would likely hinder the ability of samplers to collect sufficient fishery-dependent age composition data for stock assessment with otoliths.

Beyond the hurdles presented in collecting sufficient otoliths for robust age composition data, all three readers noted distinct limitations to otolith processing and reading compared to dorsal spines. The readability of the otolith can change dramatically based on the orientation of the otolith on the microscope stage. If not examined at a precise orientation all opaque zones may not be apparent, thus leading to an underestimate of age (Fig. 6). In addition to elevated iAPE, age-specific otolith opaque zone counts were less consistent between readers. This is indicative of the challenges in enumerating otolith opaque zones, particularly for younger fish. While we did not attempt to section the gray triggerfish otoliths in this study, Potts et al. (2023) stated they were

Table 2
Summary of Bayesian spline models fitted to the regional reference series to estimate ageing bias by reader and ageing method. Reported values include the median posterior values, 95% credible intervals, effective sample size (bulk and tail), Gelman-Rubin convergence diagnostic ($\widehat{R}$), *p*-value using the maximum *a posteriori* probability estimate ($p(\delta_{MAP} = 0) > 0.05$) for the estimated bias (BY_{adj}), variance in the reference series (σ_{ref}), and variance in the validation samples (σ_{obs}).

Reader	Ageing method	Parameter	Median estimate	95 % CI	Bulk ESS	Tail ESS	$\widehat{R}$	p-value
Reader 1	Otolith opaque zone counts	BY_{adj}	−0.09	−0.90 - 0.84	951	742	1.01	0.94
		σ_{ref}	6.04	5.98 - 6.09	1980	794	1.00	
		σ_{obs}	5.27	4.50 - 6.20	1138	643	1.02	
Reader 1	New dorsal spine protocol	BY_{adj}	0.05	−0.70 - 0.90	1832	703	1.02	0.97
		σ_{ref}	6.03	5.98 - 6.09	1886	585	1.01	
		σ_{obs}	5.27	4.55 - 6.15	1934	850	1.01	
Reader 1	Historical dorsal spine protocol	BY_{adj}	−1.72	−2.20 - −1.28	1298	790	1.02	<0.001
		σ_{ref}	6.03	5.98 - 6.09	2146	753	1.00	
		σ_{obs}	4.74	4.02 - 5.63	1593	772	1.01	
Reader 2	Otolith opaque zone counts	BY_{adj}	−0.19	−0.81 - 0.43	1467	704	1.00	0.87
		σ_{ref}	6.03	5.98 - 6.09	2375	733	1.01	
		σ_{obs}	4.89	4.23 - 5.79	1677	905	1.01	
Reader 2	New dorsal spine protocol	BY_{adj}	0.32	−0.58 - 1.26	1491	647	1.00	0.76
		σ_{ref}	6.03	5.98 - 6.09	2035	876	1.02	
		σ_{obs}	5.51	4.78 - 6.41	1621	773	1.01	
Reader 3	Otolith opaque zone counts	BY_{adj}	−0.47	−0.96 - 0.00	1420	627	1.01	0.13
		σ_{ref}	6.03	5.98 - 6.09	1622	627	1.02	
		σ_{obs}	4.38	3.71 - 5.28	2368	798	1.01	
Reader 3	New dorsal spine protocol	BY_{adj}	−0.14	−0.80 - 0.58	1595	815	1.00	0.93
		σ_{ref}	6.03	5.98 - 6.09	2127	859	1.01	
		σ_{obs}	5.34	4.58 - 6.28	2049	713	1.01	

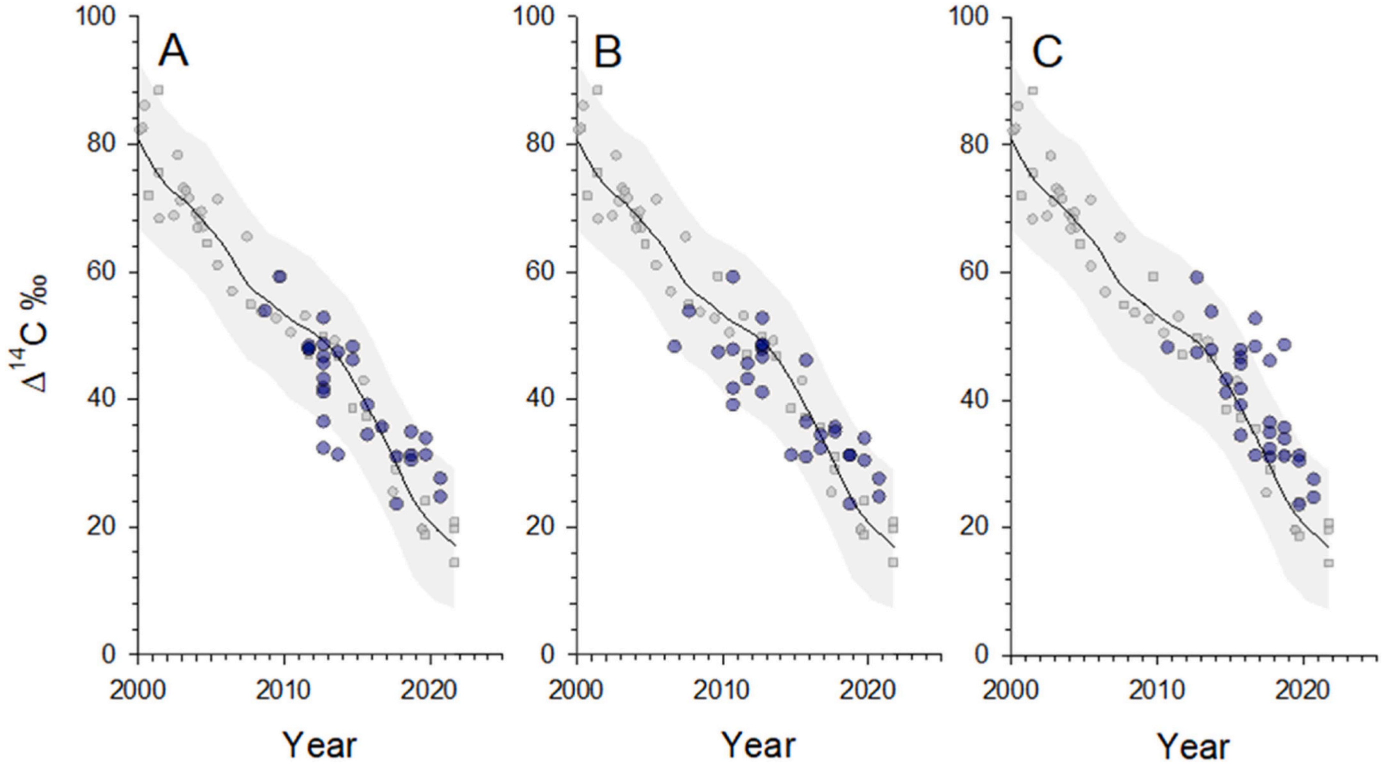

Fig. 5. Gray triggerfish eye lens (n = 30, blue circles) $\Delta^{14}C$ versus year of formation (birth year) estimates, derived from reader 1's A) otolith opaque zone counts, B) new dorsal spine protocol translucent zone counts, and C) historical dorsal spine protocol translucent zone counts, overlayed on a regional coral and known-age otolith $\Delta^{14}C$ reference series. The reference series has been truncated to 2000–2022, the reader is referred to Fig. S3 in the supplementary materials for the full reference series used in ^{14}C analysis. Gray polygons represent the 95 % credible intervals.

only able to successfully section ~50 % of their otoliths owing to unique shape and small size of gray triggerfish sagittae. This makes consistently aligning the otolith for sectioning difficult and missing the desired sectioning plane causes the section to be unreadable. Comparatively, dorsal spine sectioning is relatively straightforward, with sectioning issues only arising in a small portion of samples when the dorsal spine is misshapen or if section preparation was poor. There was also a notable difference in the rate samples could be read with dorsal spine sections versus whole otoliths. Approximately 15–20 whole otoliths could be read per hour compared to 40–70 dorsal spine sections per hour, and all readers noted substantially less eye strain with the dorsal spine sections. Translucent zone interpretation from gray triggerfish dorsal spine sections is still difficult, indicated by the somewhat elevated iAPE of 9.4 %. However, imprecision can easily be estimated by conducting paired

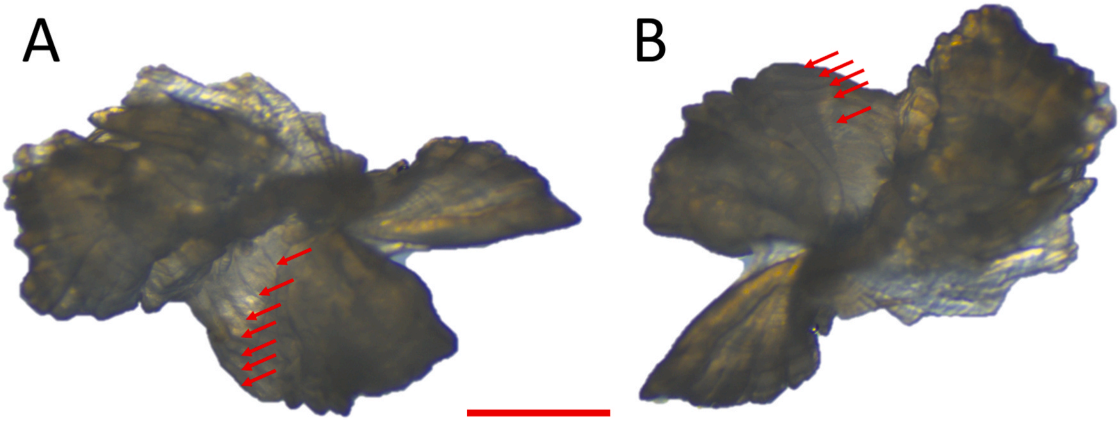

Fig. 6. Digital images viewed under a dissecting microscope with transmitted light the same whole sagittal otolith, from a 540 mm gray triggerfish, where A) opaque zones are apparent in a different orientation increasing the opaque zone count to 7 and B) opaque zones are obscured due to the otolith orientation resulting in only 5 opaque zones being observed. Scale bar (1 mm) included. Red arrows denote the observed opaque zones.

readings and subsequently estimating ageing error matrices for use in stock assessment, while accuracy is generally more difficult to quantify (Campana, 2001; Punt et al., 2008). The lack of bias in new dorsal spine protocol age estimates and the relative ease of dorsal spine collections strongly suggest dorsal spines should remain the preferred ageing structure for gray triggerfish, albeit with the new protocol proposed by Potts et al. (2023).

Validation of the new dorsal spine protocol allows for the continued use of dorsal spines as an ageing structure for gray triggerfish and eliminates the hurdles in sample acquisition and preparation that would arise if otoliths were the preferred ageing structure. Additionally, archived dorsal spine sections can be re-examined to produce updated historical age composition data, which would not be possible with otoliths as they have not been routinely sampled, thus management agencies do not have archives of gray triggerfish otoliths. Given the tight relationship between translucent zone counts using the historical dorsal spine protocol and the new dorsal spine protocol for fish aged <5 with the historical dorsal spine protocol (Fig. 4), production ageing labs could target their efforts to apply the new dorsal spine ageing protocol to re-age fish that were estimated to be ≥ 5 with the historical dorsal spine protocol. This would greatly reduce the effort required to update the historical age composition data.

The deleterious impacts of ageing error on stock assessments have been observed for numerous stocks (e.g., walleye pollock, orange roughy; Francis, 1992; Lin Lai and Gunderson, 1987; Mace et al., 1990; Reeves, 2003), thus the importance of estimating, accounting for, and correcting ageing error in gray triggerfish. The GOM gray triggerfish stock is estimated to have experienced overfishing for over two decades, which led to an overfished status in the early 1990s (Southeast Data, Assessment, and Review SEDAR, 2015b). Despite significant management actions, including size and daily bag limits and seasonal closures, being enacted since the late 1990s to reduce landings and eliminate overfishing, the GOM gray triggerfish stock has not recovered at projected rates (Southeast Data, Assessment, and Review SEDAR, 2015a). Natural mortality (M) is often estimated based on longevity, and there is a close relationship between M and F_{MSY} (Hamel and Cope, 2022; Then et al., 2015; Zhou et al., 2012). While our samples did not extend the longevity estimate of gray triggerfish (Southeast Data, Assessment, and Review SEDAR, 2015a), the overall trend of the historical dorsal spine protocol age estimates being lower than otolith and new dorsal spine protocol estimates suggests the longevity of gray triggerfish may be higher that previously reported. Additionally, the systematic underestimation of age would lead to the overestimation of the growth rate which combined with the potential overestimation of natural mortality could cause the overestimation of stock productivity. Therefore, uncertainty and bias in the ageing process may have caused overestimation of stock productivity leading to slower than predicted stock recovery (Lin Lai and Gunderson, 1987; Tyler et al., 1989).

While we did not attempt to estimate the effect of historical ageing error on the GOM gray triggerfish stock assessment in the current study, we did successfully implement a novel application of the bomb ^{14}C chronometer to conduct gray triggerfish age validation based on eye lens Δ^{14}C. Our results indicate otolith opaque zone counts are not biased for this species, but the new dorsal spine ageing protocol proposed by Potts et al. (2023) also lacked evidence of any bias and was slightly more precise than otolith-based age estimates. This study addresses key issues with historical gray triggerfish ageing, which has been uncertain and problematic (Allman et al., 2016; Potts et al., 2023; Shervette and Rivera Hernández, 2022b). The research presented here, following on the work of Potts et al. (2023), clearly demonstrates the efficacy and accuracy of the new dorsal spine ageing protocol and that compacted growth zones should be enumerated individually under increased magnification. The impracticality of collecting and processing large numbers of gray triggerfish otoliths combined with a lack of archived samples further support dorsal spine thin sections as the preferred ageing structure for gray triggerfish.

Funding

Funding for this study was provided by the Gulf of Mexico Fishery Management Council (NA20NMF4410007) and NOAA Marine Fisheries Initiative (NA21NMF4330501) to WFP. Additional funding was provided by the University of Florida.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors would like to thank the many researchers and fishers who were essential to collecting and processing the data presented here. In particular, we would like to acknowledge Joe Tarnecki and Miaya Taylor for aid in collecting samples. We would also like to thank Kathy Elder, Susan Handwork, and others at NOSAMS for their support and expertise in ^{14}C analysis. The scientific results and conclusions, as well

as any views or opinions expressed herein, are those of the author(s) and do not necessarily reflect those of NOAA or the U.S. Department of Commerce.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fishres.2024.107123.

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