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Another New Zealand centenarian: age validation of black cardinalfish (*Epigonus telescopus*) using lead–radium and bomb radiocarbon dating

Dianne M. Tracey[A,C], *Allen H. Andrews*[B], *Peter L. Horn*[A] *and Helen L. Neil*[A]

[A]National Institute of Water and Atmospheric Research, Private Bag 14-901,
Kilbirnie, Wellington, New Zealand.
[B]NOAA Fisheries – Pacific Islands Fisheries Science Center, 1845 Wasp Boulevard,
Honolulu, HI 96818, USA.
[C]Corresponding author. Email: di.tracey@niwa.co.nz

Abstract. Black cardinalfish (*Epigonus telescopus*, Apogonidae) is an important component of deepsea commercial fishing activity in the New Zealand region. It is estimated to live longer than 100 years on the basis of counts of unvalidated annual growth zones in otoliths. Age-validation procedures for long-lived fishes are often one of the following two techniques: (1) lead–radium disequilibria, which uses the natural decay of radium-226 into lead-210 as a natural clock; or (2) bomb radiocarbon (Δ^{14}C) dating, which relies on the marine signal created by nuclear testing. The high estimated lifespan, as well as the large size of the otolith core region, make *E. telescopus* an excellent candidate for a combined application of these two independent age-validation techniques. The lead–radium dating using otolith cores indicated that growth-zone counts less than ~60 years were consistent with radiometric ages, whereas higher counts appeared to be under-estimates. There was 95% confidence that maximum age was at least 95 years. The validation indicated that fish aged over 60 years tended to be under-aged by up to 30%. The bomb radiocarbon levels in otolith cores supported age estimates up to ~40 years made from zone counts, and by inference from the zone counts validated with lead–radium dating, longevity exceeds 100 years.

Additional keywords: Apogonidae, lead–radium disequilibria, carbon-14, micromilling, otolith.

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Introduction

Several species of *Epigonus* (Apogonidae) are widely distributed in New Zealand waters, but only black cardinalfish (*E. telescopus*) reaches a marketable size and is an important component of deepsea commercial fishing activity. *Epigonus telescopus* occurs at depths of 300–1100 m and mostly in very mobile schools up to 150 m off the bottom (above hills, seamounts and rough ground). This species has been caught commercially in New Zealand waters since 1981, initially as a by-catch of target trawling for other high-value species, and, since 1986, as a target species and mostly from the eastern coast of the North Island (Fig. 1). Annual commercial landings peaked at ~4000 tonnes (t) in the mid-1990s, but have been less than 2000 t since 2008, probably as a consequence of stock depletion owing to fishing effort (Dunn 2009; Ministry for Primary Industries 2014).

The stock assessment of *E. telescopus* in the New Zealand region depends on estimates of biomass and species productivity, with the latter being regularly determined by age and growth analyses. Length–frequency analyses showed that the average size of *E. telescopus* landed by the commercial fishery is between 50- and 60-cm fork length (FL). Length–frequency distributions from research surveys are unimodal with a peak at 55–65 cm FL and a maximum length of ~75 cm FL. Larger juveniles of ~12 cm and aged to 5 years have been caught in bottom trawls at depths of 400–700 m, with ontogenetic movement to deeper water, and adult fish have been caught primarily at 800–1000 m (Dunn 2009). Early juveniles are known to be pelagic (Maugé and Mayer 1990). Very small ~1-year-old juvenile samples have not yet been recorded in New Zealand waters, and they probably occur in depths shallower than ~400 m.

In most stock assessments, productivity is determined using ageing analyses to estimate growth, age at maturity, longevity, rate of natural mortality and recruitment variability. Many age estimates are based solely on sagittal otolith growth-zone counts. In some cases, the lack of a convincing age validation associated with these counts has led to disbelief in the age interpretations (e.g. see Andrews *et al.* 2009, for the debate on orange roughy longevity). As with several of the deepwater fish ageing studies, the age-estimation studies for *E. telescopus* have been based on otolith growth-zone counts assuming annual zone formation. A study of age and growth was carried out by Tracey *et al.* (2000) where interpretation of the zonation pattern in whole juvenile otoliths aided interpretation of the zones in

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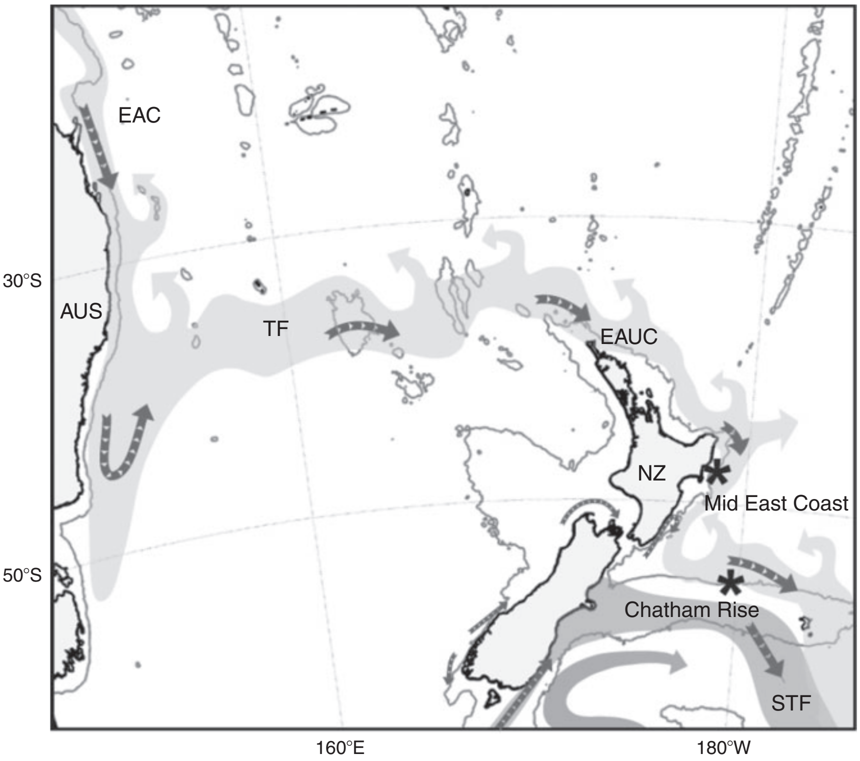

Fig. 1. Map showing sampled area in the New Zealand region (asterisk denotes sampling areas of juvenile and adult black cardinalfish samples) along with key physical oceanography (adapted from Chiswell *et al.* 2015) between Australia (AUS) and New Zealand (NZ) (East Australian Current (EAC), Tasman Front (TF) and East Auckland Current (EAUC).

cross-sectioned adult otoliths. Zone patterns were reasonably clear, although, towards the edge of the otolith, counting was difficult owing to the narrow zone spacing. Otolith readings from 722 fish indicated that the species was slow growing and long lived. A maximum age of 104 years was reported, with most of the commercial-catch age data ranging from 35 and 55 years. The estimated von Bertalanffy growth parameters reported in Tracey *et al.* (2000) were $L_\infty = 70.8$ cm (the average length of the oldest fish), $k = 0.035$ (the average growth rate) and $t_0 = -6.32$ years (the theoretical age at which length $= 0$), and, similar to *H. atlanticus*, a low value of instantaneous natural mortality (*M*) was estimated (0.034). There was some doubt about the age data presented for this species as the between-reader variability had a measured coefficient of variation (cv) of 16.7%, indicating precision lower than that found for other deepwater species (Doonan et al. 1997 Doonan and Tracey 1997). Also, it had been difficult to interpret zones on the margins of the adult otoliths. Hence, although the otoliths were interpreted similarly to those of other long-lived deepwater species, the initial estimates of *M* were based on otolith growth-zone counts that remained unvalidated. Because of the importance of precise and accurate age data needed for stock assessment in the New Zealand region, it was recommended that the high age estimates for this species required validation.

A longevity of over 100 years and large otoliths make *E. telescopus* an excellent candidate for the application of the following two age-validation methods: (1) bomb radiocarbon dating (Kalish 1993; Andrews *et al.* 2012) and (2) lead–radium dating (Smith *et al.* 1991; Andrews *et al.* 2009). Each technique can independently corroborate age estimates and the combined application can provide an opportunity to cross-validate the radioisotope proxy methods. Lead–radium dating uses the natural decay of radium-226 (^{226}Ra) into lead-210 (^{210}Pb) as a natural clock and can provide an estimate of age that is independent of growth-zone counting. For fishes, lead–radium dating depends on the incorporation of radium-226 into the otolith from the environment where it subsequently decays to lead-210 at a known rate (Smith *et al.* 1991; Panfili *et al.* 2002). This validation method is unique because it is strictly regulated by the passage of time and can serve as a form of validation for age-estimation procedures, such as traditional growth-zone counting (Kastelle *et al.* 1994; Andrews *et al.* 1999*a*, 2009; Andrews 2016).

Bomb radiocarbon dating is based on levels of radiocarbon measured in the core of otoliths in a direct comparison with regional radiocarbon reference records (Kalish 1993; Campana 2001; Andrews *et al.* 2011). Changes over recent decades in marine radiocarbon, reported as Δ^{14}C in reference to a pre-nuclear standard (Stuiver and Polach 1977), as a result of atmospheric testing of thermonuclear devices have provided a time-specific marker in the 1950s and 1960s that can be used to age marine fishes (Kastelle *et al.* 2008; Horn *et al.* 2010, 2012; Grammer *et al.* 2015; Andrews 2016).

Two reference curves can be used to validate fish-age data in the New Zealand region. They are (1) from shallow-water hermatypic zooxanthellae (with symbiotic algae) coral cores at the southern end of the Great Barrier Reef, Australia (Andrews 2016), from where surface waters from the Coral Sea reach the eastern coast of New Zealand via the East Australian Current, the Tasman Front and the East Auckland Current (Ganachaud *et al.* 2014; Chiswell *et al.* 2015; Fig. 1) and (2) the otolith-core Δ^{14}C curve for the demersal fish species snapper (*Pagrus auratus*) caught off the eastern coast of North Island, New Zealand (Kalish 1993), the same area that produced the cardinalfish otoliths analysed here. Annual growth was previously validated using other methods in the Δ^{14}C references chosen for the present study. For the coral records, the use of annual temperature cycles by δ^{18}O or Sr/Ca ratio proxy validated the timeline (Grottoli and Eakin 2007), and for the snapper record, the ages were validated using oxytetracycline marking and tagging (Francis *et al.* 1992). The applicability of the hermatypic corals of the Great Barrier Reef (shallower than 50 m) and the snapper record off New Zealand (occurring at depths down to 200 m) is supported by a good temporal representation of marine chemistry for the mixed surface layer of the region where *E. telescopus* resides as a juvenile.

Organisms living in the surface region will be in isotopic equilibrium with dissolved inorganic carbon (DIC) because DIC enters the food chain through phytoplankton photosynthesis. For this reason, regardless of the pathway taken by carbon from DIC to the formation of otolith carbonate (air-sea diffusion or trophic sequestration), the references provide proxies of DIC Δ^{14}C in the ambient waters surrounding New Zealand. Grammer *et al.* (2015) investigated bomb radiocarbon transport in the south-western Pacific Ocean and discussed how stratification and current patterns may affect the sequestration of ^{14}C. From the otolith radiocarbon data measured in ocean perch (*Helicolenus barathri*), Grammer *et al.* (2015) concluded that their near-surface data matched the Δ^{14}C in reference sets described for earlier studies in the New Zealand and Australian region where strong ocean currents efficiently transfer water in the South Pacific region (Chiswell *et al.* 2015). As such, the reference curves used in the current study are likely to be valid for *E. telescopus*, a species with juveniles known to occur in depths of 400–700 m, and probably in even shallower waters when very young. The focus of the present study is to apply both the lead–radium and bomb radiocarbon age-validation techniques to determine the validity of *E. telescopus* age estimates. The specific goals of the study were to apply both the lead–radium and bomb radiocarbon age-validation techniques to determine the validity of *E. telescopus* age estimates determined in previous studies.

Materials and methods

The first otolith from each fish had been sectioned for growth-zone counting and the second of the otolith pair, the 'sister' otoliths of the fish aged previously by Tracey *et al.* (2000), were selected for use in one of two age-validation techniques based on criteria that are unique to each method. Adult *E. telescopus* otoliths were obtained in 1998 and 1999 from commercial fishing off the eastern coast of North Island, New Zealand (Fig. 1). Estimated age from zone counts was used to back-calculate estimated birth years, an important first step in each of the validation methods for different reasons, including the following: (1) otoliths chosen for bomb radiocarbon dating provided a range of birth years covering the periods before, during and after the bomb-produced radiocarbon increase in surface waters; and (2) otoliths chosen for lead–radium dating covered as much of the potential lifespan as measurable and were pooled on the basis of similar estimated birth years. In addition, juvenile *E. telescopus* otoliths were sourced from research trawl surveys of the Chatham Rise region (Tracey *et al.* 2000) to assist with various steps required in each of the age-validation methods. For lead–radium dating, the amount of material formed in the first few years of growth (the otolith core) provided information on the amount of material to be extracted by hand grinding the exterior parts away. For the bomb radiocarbon dating method, juveniles also provided samples for which the earliest growth (birth year) was known with reasonable certainty and were used in guiding proper extraction of the birth-year material in the micromilling process.

Lead–radium dating

The approach for lead–radium dating was to compose a series of age-group samples (youngest to oldest fish) consisting of otolith cores from adult *E. telescopus*, as well as whole juvenile otoliths, to measure the disequilibria of lead-210 and radium-226 activities as an independent measure of age. To determine a suitable sample size for the adult age groups, the whole juvenile otolith group was analysed first to provide baseline lead–radium levels. All adult otoliths used for lead–radium dating were ground down by hand to produce an otolith core with the dimensions and weight of a juvenile *E. telescopus* otolith. Cores were isolated with a Buehler Ecomet III lapping wheel with 120–320-grit wet–dry silicon-carbide paper and each was individually measured and weighed for consistency. Target core dimensions and weight were based on measurements of whole 5-year-old otoliths at length × width × depth of 8.0 × 5.5–6.0 × 1.1–1.2 mm (tapering to 0.9 mm at the edges) and a weight of 0.105 g. During the grinding process, regular comparisons were made microscopically and macroscopically between the extracted core and the juvenile reference otoliths to ensure that the target dimensions were closely met and that the extraction was centred on the core of the adult otolith. The weights of the cleaned cores and pooled age groups were measured to the nearest 0.1 mg.

Dried and weighed samples (otolith core age-groups and the whole juvenile group) were dissolved and prepared for α-spectrometry in a series of steps described in detail elsewhere (Andrews *et al.* 1999*a*, 2009). In brief, the α-decay of polonium-210 was used as a daughter-proxy to determine lead-210 activity. A yield tracer (polonium-208) provided a basis for measuring the sample polonium-210 activity, and, consequently, the activity of lead-210. The remaining solution after polonium assays was dried and saved for radium-226 analyses, the details of which are described elsewhere (Andrews *et al.* 1999*b*, 2009).

Radiometric age was calculated using the measured lead-210 and radium-226 activities in a standardised equation derived from Smith *et al.* (1991). This model compensates for the ingrowth gradient of lead-210 from radium-226 in the otolith core. A radiometric age range was calculated for each sample by using error propagation through to the final age determinations (2 s.e.). Calculated and measured error included standard sources (i.e. pipetting, spike and calibration uncertainties), α-counting statistics and the inductively coupled plasma–mass spectrometry (ICP–MS) analysis routine.

Traditional (otolith growth-zone counts) and radiometric (lead–radium dating) age estimations were compared indirectly relative to the expected lead-210 : radium-226 ingrowth curve (measured ratio relative to the total age of the group) and by direct comparison of age estimates corrected for time since capture. The 95% confidence interval for radiometric age was used to interpret the validity of mean growth-zone counting ages.

Bomb radiocarbon dating

A group of 18 sister otoliths from aged fish was selected for bomb radiocarbon analysis. The estimated age of the samples ranged from 14 to 89 years, with projected birth years of 1910–1984 (Table 1). This ensured that the sample series covered part of the pre-bomb period, through the $\Delta^{14}C$ rise and peak periods (typically ~1955–1970), and potentially into the post-peak decline period. Because $\Delta^{14}C$ uptake by the mixed layer of the oceans can vary regionally, the reference for validating estimates of age from measured $\Delta^{14}C$ values in otoliths was carefully considered.

The two references selected here were inferred geographically and based on recently published current circulation patterns (Chiswell *et al.* 2015), and have been described above (Kalish 1993; Andrews 2016). Validation of *E. telescopus* age was limited to the most diagnostic part of the regional reference record, the $\Delta^{14}C$ rise period, with support for the growth-zone counting provided by potential concordance of the data series with the full $\Delta^{14}C$ reference (pre-bomb to post-peak).

Each otolith was sampled by extracting core material, defined as material of earliest growth, comprising the primordium up to the first transluscent growth zone. This part of the otolith was formed when *E. telescopus* juveniles were high in the water column. Extractions were performed with a New Wave Research (ESI–NWR Division; Fremont, CA, USA) micromilling machine using a 200-µm Brasseler (Savannah, GA, USA) bur. Approximately 2 mg of powderised core material was collected in a clean vial and analysed for radiocarbon by using standard methods adopted at Rafter Radiocarbon Laboratory, New Zealand (www.RafterRadiocarbon.co.nz, accessed 10 March 2016). The quantity determined from accelerator mass spectrometry was the ratio of the ^{14}C counting rate in the gridded ionisation chamber used to detect the ^{14}C ions to the corresponding ^{13}C current transmitted through the accelerator. The $^{14}C : ^{13}C$ ratios were corrected for chemical isotopic fractionation effects by determining the $\delta^{13}C$ for each sample by stable isotope mass spectrometry, then normalising the measured $^{14}C : ^{13}C$ ratio to a standard $\delta^{13}C$ of −25‰. The $^{14}C : ^{13}C$ ratios for each sample were calibrated against a similar measurement by using a sample of National Bureau of Standards (NBS) standard oxalic acid (HOx-I). Measurements were provided as $\Delta^{14}C$, defined as the deviation of the sample ^{14}C activity (i.e. the normalised ^{14}C isotopic ratio) from 0.95 times the activity of the oxalic acid standard, expressed in parts per million (Karlen *et al.* 1964; Stuiver and Polach 1977).

Results

Lead–radium dating

Twelve samples of pooled sets of otolith cores of *E. telescopus* were successfully prepared for lead–radium dating (Table 2). The age groups within each set covered an estimated age range from young fish (3–5 years) at 14–20 cm FL to old adults up to 76 cm FL (maximum estimated age of 102 years). The initial

Table 1. Biological and capture data, and otolith-core radiocarbon measurements for the *Epigonus telescopus* specimens chosen for ^{14}C analysis
Errors are ± 2 s.e. Fish marked with asterisks are specimens where the estimated birth year (from age) does not match the $\Delta^{14}C$ reference curve. F, female; M, male

Fish number	Catch date	Fork length (cm); sex	Estimated age (years)	Estimated birth year	$\Delta^{14}C$ (‰)
991807-91	8 Dec. 1999	71; F	89	1910	−32.5 ± 3.7
991803-05	1 June 1999	61; M	69	1930	−48.7 ± 3.9
991803-06	1 June 1999	63; F	56	1943	−40.7 ± 3.7
991803-60	1 June 1999	63; F	50	1949	−43.6 ± 3.7
991803-80	1 June 1999	70; F	50*	1949	−25.2 ± 3.7
991811-48	20 Sep. 1999	61; M	45	1954	−37.8 ± 3.8
991811-09	20 Sep. 1999	63; F	40	1959	−37.9 ± 4.3
991803-31	1 June 1999	57; F	38	1961	−23.6 ± 3.6
991802-08	28 May 1999	49; M	35	1964	45.5 ± 4.5
991803-89	1 June 1999	54; F	32*	1967	−14.4 ± 4.0
991803-93	1 June 1999	57; F	32	1967	36.9 ± 3.8
991803-14	1 June 1999	58; F	30*	1969	−14.4 ± 3.8
991803-25	1 June 1999	50; M	30	1969	80.6 ± 4.4
991811-47	20 Sep. 1999	52; F	28	1971	101.5 ± 4.3
991803-99	1 June 1999	50; F	25*	1974	44.0 ± 4.5
991802-02	28 May 1999	42; F	18	1981	109.4 ± 4.9
991802-16	28 May 1999	40; F	17	1982	93.2 ± 4.4
981801-53	10 Nov. 1998	34; F	14	1984	136.6 ± 4.3

analysis of the juvenile age group (EPT 0) produced a measured ^{226}Ra value of 0.222 ± 4.1% disintegrations per minute per gram (dpm g^{-1}); a relatively high activity when compared with other otolith-based studies (see 'Radium-226 in otoliths: synopsis and review' in Andrews 2009), indicating that sample weights of 0.5–1.0 g were needed to provide useful results. Consequently, adult *E. telescopus* otolith samples were selected and placed into the following age groups (replicates denoted as A and B): 20–23 years (EPT 1A/B), 35–40 years (EPT 2A/B), 50–55 years (EPT 3 A/B), 60–65/66 years (EPT 4A/B), 70–78 years (EPT 5A/B) and 80–102 years (EPT 6). Replicate groups for EPT 1A/B and EPT 5A/B consisted of otoliths that were randomly selected. Age groups for EPT 2 through EPT 4 consisted of low (A) and high (B) otolith weight groups because the range of whole otolith weight varied considerably within each age group. This approach was used to potentially help elucidate age-estimation problems that may be indicated by a wide otolith weight range at a given estimated age. Mean whole otolith weights for the high-weight groups were 25–42% greater than the corresponding weights for the low-weight groups. The oldest age-group sample was not replicated because of low amount of core sample available. Adult age-group samples consisted of 7–12 otolith cores with pooled sample weights of 0.681–1.174 g. Average core weight was consistent and close to the target weight of a 5-year-old otolith (~0.105 g).

The activity of lead-210 and radium-226 was measured for all samples and radiometric age was calculated (Table 3). The activity of lead-210 did not increase as expected with an increasing age, with the greatest lead-210 activity from the middle-aged adult samples and lower for the oldest samples. However, the activity of radium-226 compensated for this disparity, with a steady increase of the lead-210 : radium-226 activity ratio with an increasing age (uptake of radium-226 was greater for birth years in 1950s and 1960s for unknown reasons; see Chapter 5 of Andrews 2009). As expected, the lead-210 : radium-226 activity ratio increased from 0.159 for the juveniles to near secular equilibrium at 1.0 for the oldest groups. A comparison of the estimated age *v.* the measured lead-210 : radium-226 activity ratio, plotted with

Table 2. Summary of characteristics for the *Epigonus telescopus* specimens and samples processed for lead–radium dating in the present study

Estimated age composition, mean fish length (fork length) and the resultant number of otoliths with total sample weight are given, with mean values in parentheses. Replication method applies to A/B sample sets where some were randomly pooled and the others were separated by low and high otolith-weight groups

Sample number	Estimated age (years)	Fork length (cm)	Otolith weight (g)	Otolith number	Sample weight (g)	Replication method
EPT 0	3–5 (4.3)	14.0–20.5 (17.4)	0.050–0.087 (0.071)	7[A]	0.484	n.a.
EPT 1A	20–23 (21.6)	39.0–49.0 (44.0)	0.243–0.312 (0.277)	12	1.173	Random
EPT 1B	20–23 (21.5)	41.0–49.0 (44.5)	0.260–0.337 (0.291)	12	1.174	Random
EPT 2A	35–40 (37.3)	50.0–60.0 (54.8)	0.328–0.462 (0.409)	10	1.053	Low weight
EPT 2B	35–40 (36.8)	54.0–64.0 (57.6)	0.467–0.571 (0.521)	10	1.062	High weight
EPT 3A	50–55 (52.0)	57.0–63.0 (60.4)	0.483–0.560 (0.524)	7	0.681	Low weight
EPT 3B	50–55 (52.8)	60.0–69.0 (64.4)	0.592–0.704 (0.653)	9	0.940	High weight
EPT 4A	60–65 (62.0)	48.0–65.0 (58.3)	0.352–0.587 (0.502)	8	0.785	Low weight
EPT 4B	60–66 (61.4)	58.0–70.0 (66.1)	0.637–0.792 (0.711)	8	0.761	High weight
EPT 5A	70–78 (74.1)	60.0–71.0 (66.4)	0.585–0.812 (0.728)	9	0.907	Random
EPT 5B	70–78 (73.2)	64.0–72.0 (67.7)	0.621–0.869 (0.733)	9	0.897	Random
EPT 6	80–102 (89.6)	62.0–76.0 (67.6)	0.669–0.826 (0.746)	9	0.917	n.a.

[A] Whole juvenile otoliths.

Table 3. Comparison of estimated age and radiometric age for *Epigonus telescopus*

Radiometric age was calculated from the measured lead-210 : radium-226 activity ratios (measured in disintegrations per minute per gram, dpm g^{-1}) and corrected for time since capture. Time between capture and lead–radium dating was 3 years for juveniles (EPT 0) and 7 years for all adults (EPT 1–6). Radiometric age range was based on the analytical uncertainty and error propagation (2 s.e.)

Sample	^{210}Pb (dpm g^{-1}) ± % error	^{226}Ra (dpm g^{-1}) ± % error	^{210}Pb : ^{226}Ra (2 s.e.)	Radiometric age (years)	Mean zone count age (years)
EPT 0	0.0353 ± 7.0	0.2220 ± 4.1	0.159 (0.028)	5 (4–6)	4.0
EPT 1A	0.0297 ± 9.3	0.0659 ± 2.1	0.450 (0.043)	15 (12–17)	21.6
EPT 1B	0.0468 ± 9.4	0.0889 ± 2.1	0.527 (0.051)	19 (16–23)	21.5
EPT 2A	0.0760 ± 8.4	0.1084 ± 2.1	0.701 (0.061)	34 (28–42)	37.3
EPT 2B	0.1446 ± 7.8	0.1790 ± 2.1	0.808 (0.065)	48 (39–62)	36.8
EPT 3A	0.1297 ± 8.6	0.1727 ± 2.1	0.751 (0.066)	40 (33–50)	52.0
EPT 3B	0.1170 ± 7.8	0.1430 ± 2.1	0.818 (0.066)	50 (40–65)	52.8
EPT 4A	0.1162 ± 8.3	0.1380 ± 2.1	0.842 (0.072)	55 (43–74)	62.0
EPT 4B	0.1058 ± 7.9	0.1103 ± 2.1	0.959 (0.078)	98 (64–undef.)	61.4
EPT 5A	0.0718 ± 7.7	0.0738 ± 2.1	0.974 (0.078)	112 (68–undef.)	74.1
EPT 5B	0.0890 ± 8.8	0.0848 ± 2.1	1.050 (0.095)	Undef. (95–undef.)	73.2
EPT 6	0.0797 ± 8.1	0.0825 ± 2.1	0.965 (0.080)	103 (65–undef.)	90.0

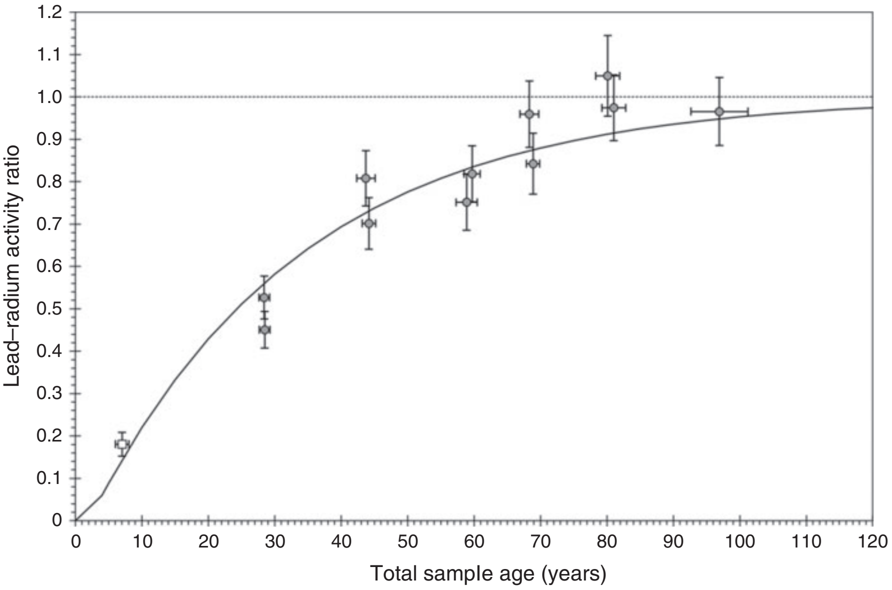

Fig. 2. Plot of the measured lead-210 : radium-226 activity ratios with respect to total sample age (age estimate plus the time since capture) for *Epigonus telescopus*, plotted with the expected lead-210 : radium-226 ingrowth curve. The open square is the juvenile sample plotted at total sample age. Horizontal error bars represent 2 s.e. for the age group. The vertical error bars represent the analytical uncertainty associated with measuring lead-210 : radium-226 (2 s.e.).

the expected lead–radium ingrowth curve (building in of lead-210 from the decay of radium-226; Smith *et al.* 1991) provided a visual means to assess the level of agreement between the zone counts and lead–radium data (Fig. 2). The distribution of the measured lead-210 : radium-226 ratios compared with the expected ingrowth curve indicated that age estimates were reasonably precise for some age groups and older than expected for others. It must be noted that the ingrowth plot is possible only for the actual sample age (age from growth-zone counts plus the time since capture). In most cases, the measured ratio falls close to the expected ingrowth curve and usually within the margin of error (2 s.e.). This margin of error translates into a radiometric age-estimation error that becomes larger as the ratio approaches the top of the curve (Table 3). The juvenile sample (total sample age of ~8 years, includes time since collection) was in close agreement with the expected ingrowth ratio, indicating that the effect of exogenous lead-210 was negligible and that offsets in age were likely to be due to real differences in the age composition of otolith-weight age groups and most of the oldest age groups (Table 3).

The findings provided the most support for the growth-zone counting criteria up to ~50 or 60 years, with significant age discrepancies for the oldest fish. There was an indication that age was greater, on average, for the high otolith-weight age groups and that the oldest age groups were generally under-estimated. The low-weight age groups had lead-210 : radium-226 activity ratios that were consistently lower than the corresponding high-weight age groups, and each differed by 2–3.4 standard errors (EPT 3A/B, 4A/B and 5A/B; Table 3). The oldest radiometric ages were from lead-210 : radium-226 activity ratios that were all approaching secular equilibrium (ratio of 1.0).

Bomb radiocarbon dating

Measured Δ^{14}C values were obtained for each otolith sample and could be compared with the birth year inferred from growth-zone counting (Table 1). The Δ^{14}C values were corrected to the year of formation and plotted against calibrated growth year (Fig. 3). Although there was some scatter in the data, the *E. telescopus* core Δ^{14}C values for the birth years were closely distributed and similar in magnitude to the two reference curves. This was expected for a fish species that spends its initial period of life in the mixed layer. In general, the cardinalfish data were consistent with the ages inferred from growth-zone counts, thus encouraging the qualitative judgement that any bias in the age estimates for the fish analysed for ^{14}C must be relatively small. However, three otoliths with Δ^{14}C values that place them on the Δ^{14}C rise period may have been under-aged by 5–8 years, and one over-aged by ~12 years (Fig. 3).

Discussion

Epigonus telescopus was estimated to be a long-lived species; however, age remained unvalidated and, as a result, there were significant uncertainties in the estimates of *M* (Tracey *et al.* 2000). It was thought the preparation method used to age *E. telescopus* might have obscured annuli, particularly in the oldest fish, resulting in decreased between-reader precision. Tracey *et al.* (2000) suggested that the use of the thin otolith-section technique used for ageing other deepwater species might improve the accuracy of the zone counts. The findings of the present study have effectively validated the concerns expressed for age-estimate discrepancies and that age-estimation methods for the oldest fish must be developed to address the greater-longevity scenario.

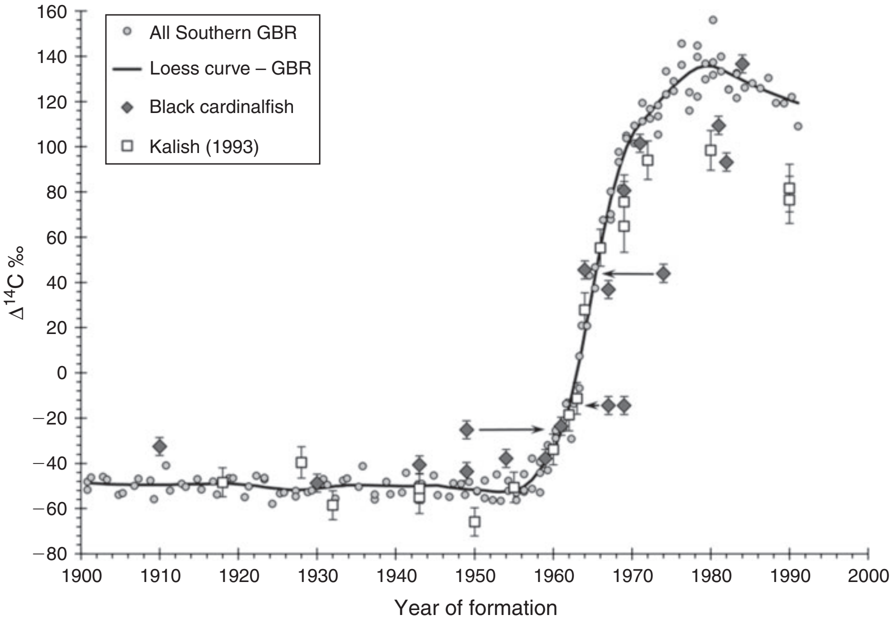

Fig. 3. Results of the Δ^{14}C measurements from black cardinalfish (*Epigonus telescopus*) otolith cores corrected for decay since growth, and plotted with bomb radiocarbon reference records from the southern Great Barrier Reef (GBR), composite of three coral cores with loess curve, Andrews *et al.* (2015), as well as from Kalish (1993) for snapper (*Pagrus auratus*) off eastern North Island, New Zealand. Error bars are ± 1 s.d. (Kalish 1993) or 2 s.e. (black cardinalfish). Horizontal arrows indicate samples that may have been substantially over- or under-aged.

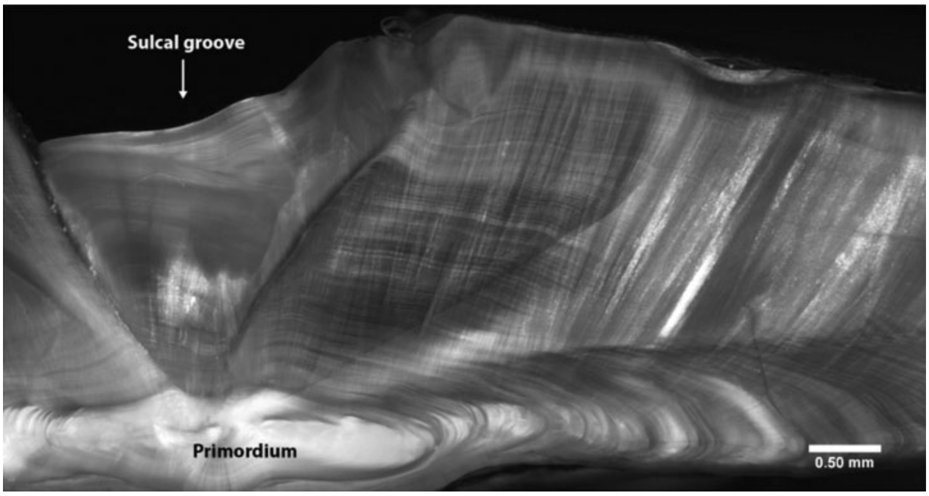

Fig. 4. Section through a black cardinalfish (*Epigonus telescopus*) otolith photographed with reflected light and estimated to an age of ~90 years. Note the wide inner dark zones close to the primordium or nucleus area followed by the formation of progressively finer growth zones. Counts past the first five zones are made adjacent to sulcal groove. Growth zones near the primordium region are reasonably easy to interpret; however, assumed annual growth zones radiating outward to the otolith margin are not as well defined and interpretation is often difficult and highly variable. Some annuli may be obscured at the edge and this is a particular problem for older fish.

Lead–radium dating of *E. telescopus* was successful by providing validation of growth-zone counting to a point and then revealing age discrepancies for larger and oldest fish. There was good agreement between estimated age and lead–radium estimates for the early age groups and the low otolith-weight groups up to ~50–60 years. Although the margin of error for radiometric age overlapped between the corresponding low and high otolith-weight groups, there was a systematic bias towards older ages for the higher otolith-weight groups. The oldest estimated-age groups were either in agreement or under-aged within a wide margin of uncertainty. Hence, the implication is that some of the heaviest otoliths were older than originally estimated from growth-zone counting and that otolith weight provided a good additional check for determining whether ages had been inaccurately assigned using zone counts. The group with the greatest minimum age from lead–radium dating was 95 years old, effectively validating that the age of some fish in the group exceed 95 years, indicating that centenarian longevity is attainable for *E. telescopus* (Fig. 2).

The work also provided an indication of where improvements can be made in the growth-zone counting criteria. Age estimation of *E. telescopus* was described as difficult at high ages (Tracey *et al.* 2000). The findings of the current study indicated that age was under-estimated by more than 20% for one of the oldest age groups. Further investigation is necessary in the analysis of otolith sections and it is suggested that thin-section ageing (rather than baking, embedding and cross-sectioning, see cross-section image, Fig. 4) be applied to increase the chances of detecting growth zones that were missed. In addition, otoliths used in the high otolith-weight age groups should be reinvestigated. The probable bias in ageing older fish is likely to have little effect on the estimated growth parameters because these fish are on the flat section of the growth curve and close to asymptotic length; however, the bias would influence the estimate of the natural mortality rate as the maximum age would change.

The age validation performed for black cardinalfish by using bomb radiocarbon dating was supported not only by the two reference curves (Kalish 1993; Andrews 2016), but also by some recently completed work on ocean stratification and ^{14}C sources (Grammer *et al.* 2015). They provided evidence that ^{14}C in surface-water reference sets was consistent and propagated over large distances. In addition, Grammer *et al.* (2015) showed that earlier studies using otoliths in New Zealand and Australian waters provided information about the time-lag before surface ^{14}C reaches deep waters, and that the juvenile otolith core ^{14}C values from some deepwater fishes were in alignment with this time-lag. Hence, it is likely that the alignment of the *E. telescopus* pre-bomb levels with the surface-layer references provides a verification that the ^{14}C sequestered by the juvenile otoliths was from mixed surface waters, given that their presence in these waters around New Zealand has been inferred from Maugé and Mayer (1990).

The radiocarbon results provided validated ages up to ~40 years, but provided limited support for growth zone-derived ages up to ~90 years, this being the oldest age estimate for a fish used in the bomb radiocarbon dating. Pre-bomb Δ^{14}C levels are not time-specific because of the plateau in levels over a period of many decades. However, by inference from lead–radium dating, growth-zone counts to the maximum estimated age of 104 years are supported, but with uncertainty apparent mainly for the older age class. These ages may seem surprisingly high; however, adult *E. telescopus* lives in depths of 700–1000 m and it is increasingly common to find that deepwater species are long-lived (Allain and Lorance 2000; Doonan *et al.* 1997; Cailliet *et al.* 2001; (Andrews *et al.* 2009; Horn *et al.* 2010, 2012; Sutton *et al*. 2010). In addition, a study describing mercury levels in black cardinalfish provided evidence that suggested that the species is slow-growing and long-lived (Tracey 1993). The validation of a centenarian longevity places emphasis on the difficulty of counting growth zones in the oldest of long-lived fishes, such as those seen in *E. telescopus* otoliths. These otoliths are particularly complex and a high degree of subjectivity is required in defining the narrow bands as an annual zone. A re-examination of the four otoliths identified as having incorrect otolith-zone counts, on the basis of the disagreement with the Δ^{14}C rise period (Fig. 3), may provide a beginning to determining the source of age-reading discrepancies.

Lead–radium and bomb radiocarbon dating were successful and complementary in validating the estimates of *E. telescopus* age to more than 100 years. This is the first instance of applying both methods in tandem to validate age-reader interpretations of a New Zealand fish species. A key outcome of the study is that the validated age estimates provide confidence in the age data from zone counts for all but the oldest fish, and have since been incorporated to stock-assessment models for fisheries management purposes (Ministry for Primary Industries 2014). Currently, the estimate of *M* remains at 0.034; however, because of the ageing bias for older fish, a range of *M*s are used in the stock assessment for black cardinalfish (Ministry for Primary Industries 2014).

The combined approach provides an opportunity to combine the advantages, as well as to overcome some of the disadvantages of each technique when applied individually. For bomb radiocarbon dating, individual fish up to ~55 years are suitable candidates for this method, but fish with birth years before the Δ^{14}C rise (*c.* 1957) can provide only a minimum age (e.g. Andrews *et al.* 2013). Adding lead–radium dating to the study increases the range of possibilities for age determination beyond this limitation and can cover a full 100-year lifespan.

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