

## Age estimation and lead–radium dating of Antarctic toothfish (*Dissostichus mawsoni*) in the Ross Sea

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**Abstract** Antarctic toothfish (*Dissostichus mawsoni*) are the target of an important commercial fishery in the Southern Ocean, yet age data used for management have not been comprehensively tested for accuracy. In this study, Antarctic toothfish were aged using counts of otolith growth zones based on criteria established for Patagonian toothfish, *D. eleginoides*, a closely related species. To validate these ages, the radioactive disequilibrium of lead-210 and radium-226 in otolith cores was measured and used as an independent chronometer to accurately determine age across the range of fish caught in large numbers by the fishery. Growth-zone counts indicated Antarctic

toothfish live to at least 39 years of age, and were in close agreement with the chronometer, validating the age estimation criteria and the accuracy of age estimates. Von Bertalanffy growth function parameters indicated Antarctic toothfish were relatively slow-growing ( $k = 0.111$ ), especially in relation to their maximum size ( $L_{\infty} = 158.9$  cm).

**Keywords** Antarctic toothfish · *Dissostichus mawsoni* · Nototheniidae · Ross Sea · Age validation · Lead–radium dating · Radiometric dating

### Introduction

The Antarctic toothfish (*Dissostichus mawsoni*) is a large piscivorous, benthic-pelagic notothenioid with a circum-polar distribution along the shelves, slopes, and ridges off the Antarctic continent. They have been collected down to a depth of 3,000 m (Hanchet et al. 2003) with juveniles occupying the shallow end of the depth distribution (typically less than 1,000 m), but as adults they achieve neutral buoyancy and descend to deeper waters along the shelf slope (Eastman and DeVries 2000; Near et al. 2003). Age at first maturity is estimated at between 13 and 16 years of age and roughly 100 cm TL (Hanchet et al. 2003; Parker and Grimes, in press). Adults can grow to more than 2 m in length and can weigh in excess of 100 kg. Together with its sister species, the Patagonian toothfish (*Dissostichus eleginoides*), they are the largest fishes in the Southern Ocean (Eastman and DeVries 2000).

Toothfish are the target of the largest and most valuable commercial fin-fisheries in the Southern Ocean (CCAMLR 2009). Both species are commonly known by the market name “Chilean sea bass.” Patagonian toothfish, which usually occur north of 60°S latitude, have been fished since the

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mid-1980s with large-scale commercial fishing efforts beginning in the early 1990s (Lack and Sant 2001). Despite international efforts to regulate this fishery, extensive illegal, unreported, and unregulated (IUU) fishing compromised management and led to substantial population declines and stock closures by the mid-1990s (Agnew et al. 2002). High prices in world markets combined with population depletions, pushed fishing vessels further south, initiating exploitation of Antarctic toothfish which are most abundant south of 60°S latitude. New Zealand officially started an exploratory fishery for Antarctic toothfish in the Ross Sea in 1997. Since then it has grown steadily into an international fishery with annual landings that increased from 42 t in 1997 to more than 4,000 t in 2007 (CCAMLR 2009).

Exploitation of Antarctic toothfish falls within the jurisdiction of the Commission established by the Convention for the Conservation of Antarctic Marine Living Resources (CCAMLR 2009). The objective set by the convention is to conserve Antarctic marine living resources, including their rational use, by preserving harvested populations at levels that ensure stable recruitment while maintaining ecological relationships and preventing irreversible changes in the marine ecosystem. To achieve this end, it is imperative to understand population age structure and generate age-based estimates of growth, recruitment, mortality, and age at maturity (Jones 1992), and the CCAMLR Commission has called for accurate age data to support sustainable management of the Antarctic toothfish fishery. Age estimates for fishes are traditionally obtained by counting growth zones in calcified structures, most commonly otoliths. In traditional aging studies, the annual periodicity of growth zones has often been assumed without validation (Beamish and McFarlane 1983; Campana 2001). Underestimation of age has resulted in the overexploitation of other important fisheries, including the Pacific ocean perch (*Sebastes alutus*; Beamish 1979; Archibald et al. 1983) and orange roughy (*Hoplostethus atlanticus*; Beamish and McFarlane 1983; Mace et al. 1990), with little evidence of recovery even after decades of conservative management practices (Clark et al. 2007).

Age and growth estimates for Antarctic toothfish are limited to a few published studies (Burchett et al. 1984; Horn 2002; Horn et al. 2003). Although otoliths were determined as the best structure for age estimation, they have proved difficult to interpret because of irregular growth zones in the first few years of growth and narrow zones that become considerably compressed in older fish. Hence, testing the accuracy of age estimates and age estimation criteria for these fish is especially important. Yet Antarctic toothfish have a depth distribution and geographical location that make most age validation techniques difficult to apply. A tag-recapture study using oxytetracycline (OTC) marking in otoliths provided support for the

accuracy of age estimates that were made using thin otolith sections (Horn et al. 2003), but sample sizes were small and did not cover the full lifespan. Although the technique addressed annual periodicity of growth-zone formation for early growth, it did not provide an independent estimate of age that could be used to test the accuracy of growth-zone counts. As was the case with this approach, many other methods have limited applicability to deep-water or long-lived species (Campana 2001). An alternative that is suitable for potentially long-lived and deep-dwelling species is a radiometric technique called lead–radium dating (Fenton et al. 1991; Andrews et al. 2009).

Lead–radium dating has been widely successful with other deep-dwelling and long-lived fishes as a means to validate aging criteria and longevity (e.g., Campana et al. 1990; Kestelle et al. 1994; Stevens et al. 2004; Andrews 2009). This technique relies on the incorporation of radium-226 ( $^{226}\text{Ra}$ ) from the environment to the otolith as a natural occurring analog to calcium. Radium-226 subsequently decays to lead-210 ( $^{210}\text{Pb}$ ), building into secular equilibrium over time (Ivanovich and Harmon 1992). By measuring the disequilibria of these radioisotopes from within the otolith core (representing the first few years of life), an independent estimate of age can be determined based on the known ingrowth rate of  $^{210}\text{Pb}$  from  $^{226}\text{Ra}$ , which can then be compared to age estimates from growth-zone counting (Smith et al. 1991; Andrews et al. 1999a).

In this study, Antarctic toothfish age was estimated from counting growth zones in otoliths and the accuracy of these age estimates were tested using lead–radium dating. The objectives of the study were to (1) estimate fish age using otolith growth-zone counting and criteria agreed upon by CCAMLR for the Patagonian toothfish; (2) test the accuracy of age estimates and age estimation procedures with lead–radium dating; (3) provide age and growth function parameters from age-validated growth-zone counting; and (4) provide other groups challenged with age determination of Antarctic toothfish otoliths with an otolith reference set and understandable growth-zone counting criteria.

## Methods

### Estimation of age and precision

Otoliths were collected by fishery observers aboard the American commercial fishing vessels *American Warrior* and *America I* in the Ross Sea, Antarctica during the 2004 fishing season (January to March). Fish were randomly sampled from the long-line catch, in accordance with the CCAMLR fisheries observer protocol (CCAMLR 2006). All intact and completely paired otoliths were used ( $n = 1,508$ ), with one otolith (left or right) randomly

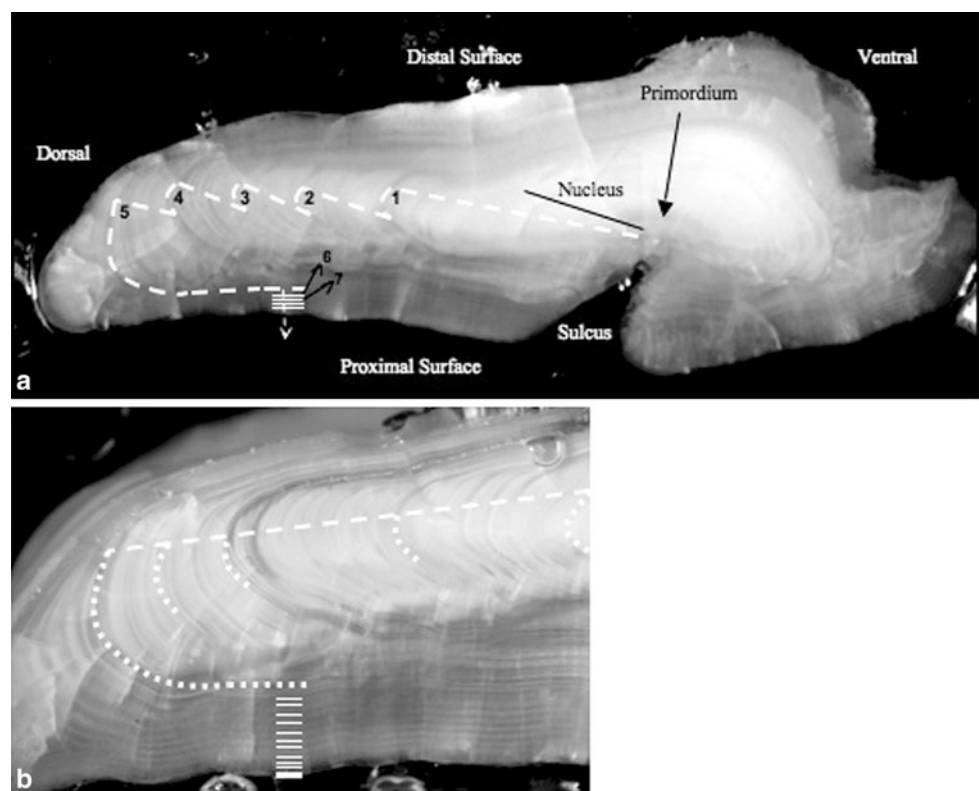
selected from each fish and prepared for reading. To estimate age, otoliths were processed to reveal growth zones (opaque and translucent rings) that could be counted (Fig. 1a). The randomly selected otoliths were baked at 365°F for about 5 min to obtain a caramel color, which enhanced otolith growth zones and facilitated growth-zone counting. Once otoliths cooled, a Hillquist® Thin Section Machine was used to grind away the anterior end of the otolith to within 2 mm of the nucleus. The exposed edge was then polished with a Crystalmaster 8 Machine® with 30M polishing film. The otolith portion was mounted on labeled slides using Loctite® adhesive and placed under a UV light for approximately an hour until the adhesive hardened. The posterior end of the otolith was ground down to a thickness of 0.5 mm using the Hillquist® Thin Section Machine. The exposed edge was polished on the Diamond® polisher until the transverse plane became visible, revealing the primordium surrounded by the nucleus, with the translucent and opaque zones clearly exposed. Dry otoliths were covered in a layer of Flo-Texx®, which helped preserve the reading surface of the otolith for long-term use.

The order of sectioned otoliths was randomized and sections were aged independently by two readers, the lead author (Reader 1) and a reader at CQFE (Reader 2). Otoliths were read according to criteria established at the CCAMLR Workshop on Estimating Age in Patagonian toothfish (SC-CAMLR 2001). Because Patagonian toothfish and

Antarctic toothfish are closely related and have otoliths that are similar in structure, the same criteria were adopted. An annulus consisted of one opaque and one translucent zone and was read at magnifications ranging from 10 to 40×. The count path began at the nucleus and followed the annuli along the dorsal-distal axis of the transverse section, until the axis growth zones became compressed, at which point the reader followed the annulus pattern around to the proximal side of the otolith section (Fig. 1a, b). A subset of 200 otoliths was randomly selected as a reference set and was used in the initial training of the independent readers. Once consistency was established between readers, the reference set was randomly mixed back into the full set of otoliths to monitor any bias in age reading over time. Each reader read the full sample set once ( $n = 1,508$ ).

Precision was assessed by calculating average percent error (APE; Beamish and Fournier 1981) and coefficient of variation (CV; Chang 1982). Age bias plots were also used to determine whether systematic differences were present between readers (Campana et al. 1995). A von Bertalanffy growth function (VBGF) was fitted to age data to estimate growth parameters. This function was chosen because of the suitable fit and to allow for comparisons with the VBGF parameters of other toothfish studies (Burchett et al. 1984; Ashford 2001; Horn 2002). The ages from Reader 1, which achieved higher precision, were used to estimate VBGF parameters and for the validation study.

**Fig. 1** **a** Transverse section of Antarctic toothfish otolith viewed under reflected light. Diagram illustrates growth-zone count path utilized in this study. **b** Magnified image of an older Antarctic toothfish otolith featuring compression of growth zones with age



### Age validation: sample preparation

Otoliths were selected for radiometric analysis based on estimated age from growth-zone counts and pooled into age classes 3–5, 6–7, 10–11, 14–15, and 18–19 years and 22–24 years, covering the age range exploited in large numbers by the fishery. Within each of these age levels, otoliths were randomly allocated into three experimental units, except 3–5 (two experimental units) and 22–24 (one experimental unit).

The otolith core, representing the first few years of growth, was the portion of the otolith targeted for lead–radium analyses. Core size was based on the average size of whole juvenile Antarctic toothfish otoliths aged at 5 years ( $n = 46$ ). Core sizes were approximately 5.5 mm wide by 7 mm long and 1 mm thick with an average weight of 0.14 g. Each experimental unit contained 35 otoliths to obtain a sample weight of approximately 1.5 g, except for the 3–5 year unit ( $n = 22$ ). The number of otoliths was based on the results from a parallel study on lead–radium dating of Patagonian toothfish (Andrews 2009).

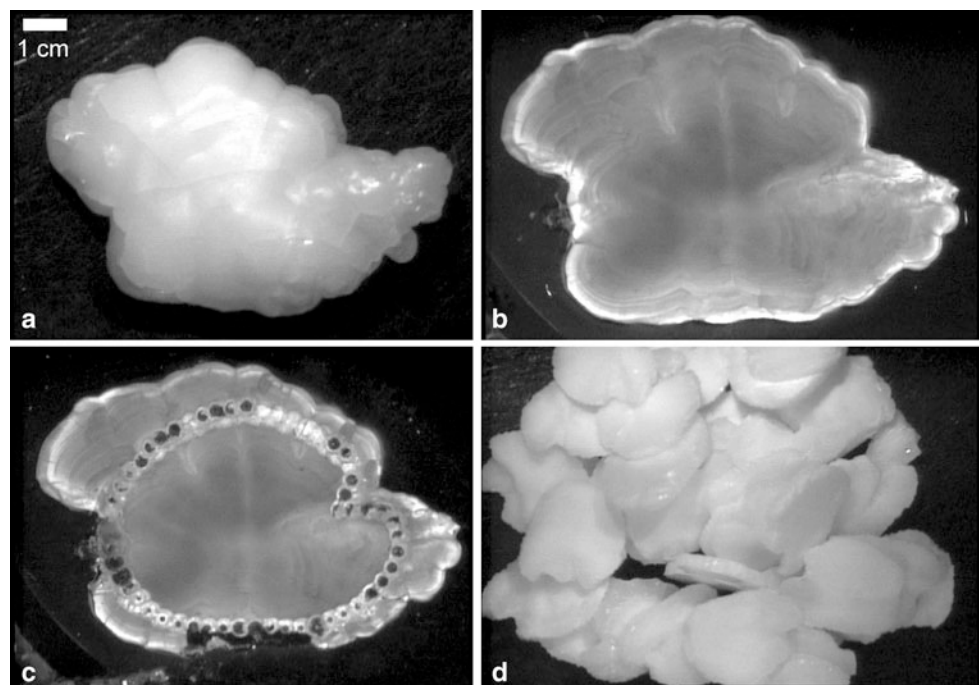
To extract the otolith core, whole otoliths were ground down on the proximal using a Buehler Ecomet III<sup>®</sup> lapping wheel with 600 grit silicon-carbide paper. The sulcus provided a visual cue to determine when to stop grinding. Prepared otoliths were mounted with cytoseal (ground side down) onto a glass slide. Mounted otoliths were further ground down on the distal surface to the measured juvenile otolith thickness (1 mm), revealing sagittal-plane growth zones that were instrumental in selecting the core

extraction location. A New Wave<sup>®</sup> micromilling machine was used to extract the 5-year core, according to the dimensions of 5-year juvenile otoliths and modified as necessary to agree with sagittal growth zones visible in the section (Fig. 2). Otolith cores were extracted by drilling a series of holes in the otolith section that traced out the outer edge of the 5-year core. This was accomplished using a 500  $\mu\text{m}$  carbide Brasseler<sup>®</sup> drill bit tip (no. 005) at 35% speed, 400  $\mu\text{m}$  spacing, and a plunge rate of 150  $\mu\text{m}/\text{s}$  (Fig. 2c). Otolith cores were removed from the slides by soaking them in mass-spectrometry grade toluene and allocated into experimental units within each age level (Fig. 2d). Cores in each experimental unit were thoroughly cleaned, dried and weighed to the nearest 0.0001 g. To assess whether measurable exogenous  $^{210}\text{Pb}$  was present in the otolith cores and to verify that  $^{226}\text{Ra}$  levels that were sufficient for measuring cored adult otoliths, the whole juvenile otoliths (3–5 years) were analyzed as a trial before the main analysis.

### Age validation: radiochemical protocol

Radiometric procedures and analysis for  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  followed methods previously described in Andrews et al. (1999a, b). In this study, we followed these same procedures with two exceptions: (1) radium recovery was improved by shifting the collection interval on the final chromatograph column to begin after the first 200  $\mu\text{L}$  (instead of after 250  $\mu\text{L}$ ); and (2) purified radium samples were analyzed using an improved inductively coupled plasma mass spectrometry (ICPMS) technique, which is

**Fig. 2** Otolith from Antarctic toothfish showing **a** the distal surface, **b** the sagittal plane, and **c** the sagittal plane after coring with the micromill. **d** Group of cored otoliths ready for dissolution and radiometric analyses



less prone to ionization suppression from variable levels of barium. Trace metal precautions were used throughout sample processing (Watters et al. 2006), and all acids used were double distilled (GFS Chemicals®) and dilutions were made using Millipore®-filtered Milli-Q (MQ) water.

A radiometric age, including analytical uncertainty for each experimental unit, was calculated using the measured  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  activities based on the following equation derived from Smith et al. (1991):

$$t_{\text{age}} = \frac{\ln\left(\frac{1 - \left(\frac{A^{210}\text{Pb}}{A^{226}\text{Ra}}\right)}{(1-R_0)\left(\frac{1 - e^{-\lambda T}}{\lambda T}\right)}\right)}{-\lambda} + T$$

where  $t_{\text{age}}$  was the radiometric age at the time of analysis,  $A^{210}\text{Pb}$  was the measured activity of  $^{210}\text{Pb}$  at the time of analysis (reported as disintegrations per minute per gram; dpm),  $A^{226}\text{Ra}$  was the activity of  $^{226}\text{Ra}$  measured using ICPMS (dpm/g),  $R_0$  was the initial activity ratio of  $^{210}\text{Pb}$ : $^{226}\text{Ra}$ ,  $\lambda$  was the decay constant for  $^{210}\text{Pb}$  ( $\ln(2)/22.26$  years), and  $T$  = estimated age of otolith core based on the first 5 years. A radiometric age range, based on the analytical uncertainty, was calculated for each experimental unit by using error propagation in 2 standard errors (2SE) through to the final age determinations. Calculated error included the standard sources of error (i.e., pipetting, spike, and calibration uncertainties), alpha-counting statistics for  $^{210}\text{Pb}$ , and ICPMS analysis.

The radiometric age, including analytical uncertainty (2SE), was compared to growth-zone-derived age estimates. Lead-210:radium-226 activities for each experimental unit

were plotted against the total sample age (estimated age plus the time since capture) with respect to the expected  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  ingrowth curve. To describe the trend between age estimation from growth-zone counts and lead–radium dating, we applied a simple linear Model I regression and tested for departure from the 1:1 line of agreement by  $t$ -tests of the slope and intercept.

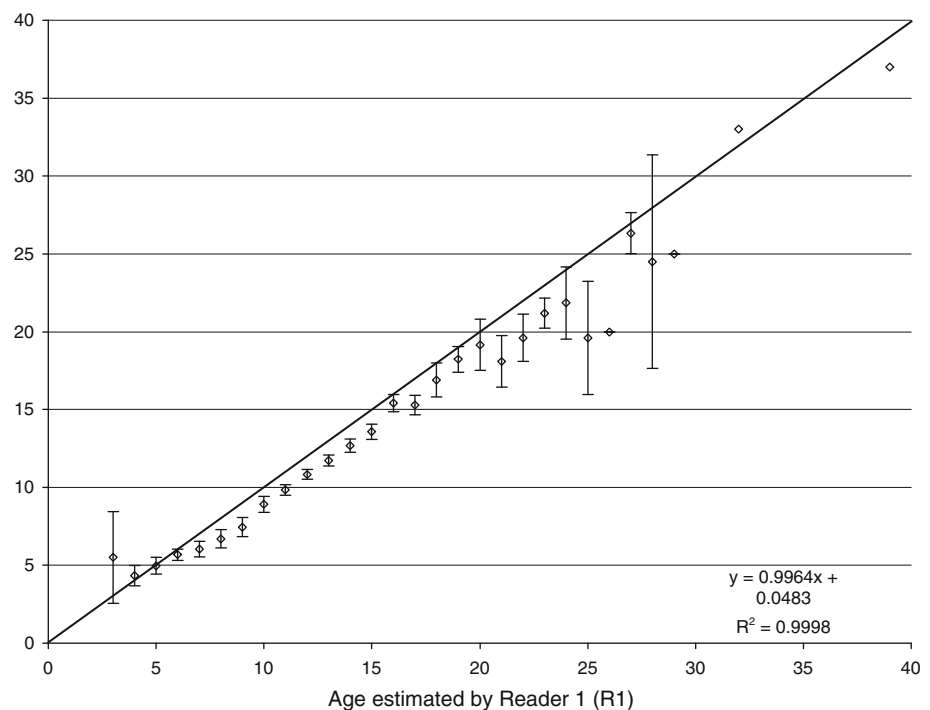
## Results

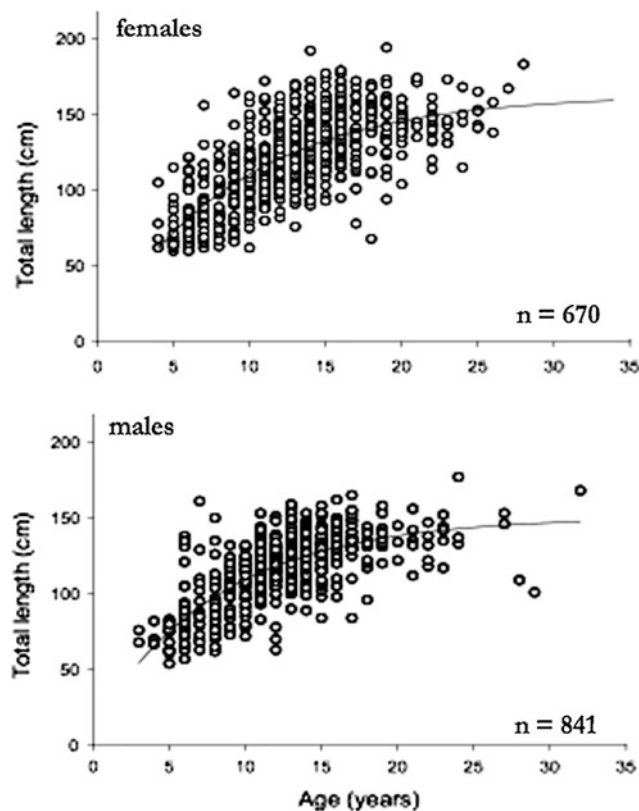
### Estimation of age and precision

Estimated ages were determined for 1,508 Antarctic toothfish by Reader 1 and Reader 2, and precision estimates indicated there was agreement within and between readers. Overall, age estimates between readers were within  $\pm 1$  year 43% of the time, and within  $\pm 5$  years 95% of the time. Between the repeated readings of Reader 1, age estimates were within  $\pm 1$  year 73% of the time and  $\pm 3$  years 96% of the time. Disagreement between readers was measured as APE at 4.6% and CV at 6.5%. Within reader error values for Reader 1 were 4.5% APE and 6.4% CV; and for Reader 2, 5.8% APE and 8.2% CV.

An age bias plot between readers indicated a slight positive bias for Reader 1 over Reader 2 (Fig. 3). A two-tailed  $t$ -test showed that the age estimated by Reader 1 were significantly different from those estimated by Reader 2 (two-tailed  $t$ -test,  $df = 27$ ,  $t = 3.127$ ,  $P = 0.0001$ ) and that Reader 2 systematically under-aged by about 2 years

**Fig. 3** Age bias plot comparing average estimated age of Reader 1 versus Reader 2 showing overall agreement with a slight positive bias of Reader 1





**Fig. 4** Age–length data and the calculated von Bertalanffy growth curves for male and female Antarctic toothfish in the Ross Sea. For curve equations, see Table 1

in relation to Reader 1. Variability between readers increased with fish age and may have been caused by fewer old age replicates or because growth zones were more difficult to interpret due to compression at older ages (Fig. 1).

Estimated ages using growth-zone counts of Antarctic toothfish were between 3 and 39 years for fish ranging in size from 54 to 194 cm total length (TL). Fitting the VBGF to age–length data resulted in slightly different growth curves and parameters for males and females (Fig. 4). The

growth coefficient,  $k$ , was  $0.124 (\pm 0.03)$  for males and  $0.111 (\pm 0.03)$  for females. The asymptotic length was  $150 (\pm 8.8)$  cm TL for males and  $162 (\pm 10.4)$  cm TL for females (Table 1).

#### Age validation using radiometrics

Radiometric analysis resulted in successful determination of  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  activities (Table 2). Sample weights for each experimental unit ranged from 1.519 to 1.913 grams, with average core weights ranging from 0.0434 to 0.0546 g. For fish aged 3–5 years, each experimental unit (3–5A and B) had 22 otoliths with total sample weight near 1.6 g and average otolith weights near 0.07 g, indicating the extraction of 5-year cores was conservative.

All samples resulted in measured lead–radium activities (Table 3). Activities for  $^{210}\text{Pb}$  were slightly variable, but increased in general with estimated age, ranging from  $0.00364 \pm 21\%$  dpm/g to  $0.0135 \pm 12\%$  dpm/g. Radium values were fairly consistent for most age groups ranging between  $0.0192 \pm 14\%$  dpm/g to  $0.0271 \pm 3.7\%$  dpm/g. A few samples (14–15G, 18–19A and B, 22–24) had poor radium recovery and an average radium value derived from the ten samples that did have good recovery was used based on the relative consistency of  $^{226}\text{Ra}$  levels ( $0.0231 \pm 7.1\%$ ; Table 3). This procedure can reliably be used if uptake is relatively consistent (e.g., Stevens et al. 2004), and the average core value was based on other cores from the same sampled population. The ratio of the  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  activities increased with growth-zone-derived age as expected and ranged from  $0.151 \pm 0.03$  to  $0.584 \pm 0.08$  (Table 3). The radiometric age for the oldest fish (22–24 years; mean =  $22.6 \pm 0.8$  year) was 27.3 year [range of 21.7–34.1 year (2 SE)]. Although the asymptotic nature of the ingrowth curve can cause the radiometric uncertainty of the oldest samples to increase relative to younger samples, our data fell along the steepest part of the curve. Within level, experimental units had similar  $^{210}\text{Pb}$  values and lead–radium ratios, as would be expected for

**Table 1** Von Bertalanffy growth function parameters (with 95% confidence intervals) for this study compared with Horn (2002) for combined sexes, females, and males

	$n$	$L_{\infty}$	$k$	$t_0$
This study				
All	1,508	158.9 (151.5–166.3)	0.111 (0.091–0.130)	–0.605 (–1.511 to 0.301)
Female	841	162.3 (151.9–172.7)	0.111 (0.085–0.134)	–0.278 (–1.491 to 0.935)
Male	670	149.7 (141.0–158.5)	0.124 (0.094–0.154)	–0.605 (–1.830 to 0.620)
Horn (2002)				
Female	864	184.5 (178.3–190.8)	0.103 (0.092–0.114)	1.02 (0.52 to 1.51)
Male	661	165.0 (159.3–170.6)	0.119 (0.104–0.134)	1.19 (0.64 to 1.74)

All lengths are in centimeters total length (TL)

**Table 2** Age groups utilized in this study, indicating the number of replicates in each age group and the number of otoliths in each replicate

Age group (year)	Average age (year)	No. of Otoliths	Sample wt (g)	Average core wt (g)
3–5A*	4.7	22	1.644	0.0747
3–5B*	4.7	22	1.608	0.0731
6–7B	6.4	35	1.576	0.0450
6–7C	6.5	35	1.632	0.0466
6–7D	6.4	35	1.623	0.0464
10–11A	10.4	35	1.662	0.0475
10–11B	10.6	35	1.810	0.0517
10–11C	10.6	35	1.708	0.0488
14–15B	14.5	35	1.803	0.0515
14–15D	14.4	35	1.913	0.0546
14–15G	14.5	35	1.728	0.0494
18–19A	18.5	35	1.534	0.0438
18–19B	18.5	35	1.576	0.0450
22–24	22.6	35	1.519	0.0434

\* Indicates juvenile samples that were not cored

**Table 3** Measured  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  activities and ratios for each sample

Age group (year)	$^{210}\text{Pb}$ activity (dpm/g) $\pm$ error	$^{226}\text{Ra}$ activity (dpm/g) $\pm$ error	$^{210}\text{Pb}$ : $^{226}\text{Ra}$ activity ratio	Average growth zone age (year)	Radiometric age (year)	Radiometric age range (year)
3–5A*	0.0042 $\pm$ 18	0.0265 $\pm$ 5.8	0.1605	4.7	5	3.9–6.2
3–5B*	0.004 $\pm$ 20	0.0246 $\pm$ 5.2	0.1642	4.7	5.2	3.9–6.5
6–7B	0.0048 $\pm$ 18	0.0194 $\pm$ 7.2	0.2483	6.4	8.5	6.5–10.6
6–7C	0.0036 $\pm$ 21	0.0241 $\pm$ 5.1	0.1513	6.5	4.6	3.4–5.8
6–7D	0.0046 $\pm$ 17	0.0271 $\pm$ 3.7	0.1685	6.4	5.2	4.1–6.3
10–11A	0.0074 $\pm$ 15	0.0216 $\pm$ 5.0	0.3433	10.4	12.8	10.3–15.5
10–11B	0.0062 $\pm$ 15	0.0230 $\pm$ 5.8	0.2717	10.6	9.4	7.6–11.4
10–11C	0.0072 $\pm$ 14	0.0222 $\pm$ 9.2	0.3246	10.6	11.9	9.4–14.5
14–15B	0.0089 $\pm$ 13	0.0192 $\pm$ 14.5	0.4608	14.5	19.1	14.1–24.9
14–15D	0.0096 $\pm$ 12	0.0229 $\pm$ 8.5	0.4204	14.4	16.8	13.5–20.4
14–15G	0.0081 $\pm$ 13	0.0231 $\pm$ 7.1	0.3533	14.5	13.2	10.7–16.0
18–19A	0.0113 $\pm$ 12	0.0231 $\pm$ 7.1	0.4898	18.5	20.7	16.8–25.2
18–19B	0.0109 $\pm$ 12	0.0231 $\pm$ 7.1	0.4748	18.5	19.8	15.9–24.2
22–24	0.0135 $\pm$ 12	0.0231 $\pm$ 7.1	0.5844	22.6	27.3	21.7–34.1

Error is expressed as two standard errors (SE). Activities are expressed as disintegrations per minute per gram (dpm/g). The measured activity ratio of  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  was used to determine the overall age of the sample, which was corrected for elapsed time between collection and sample processing (3 years)

\* Indicates juvenile samples that were not cored

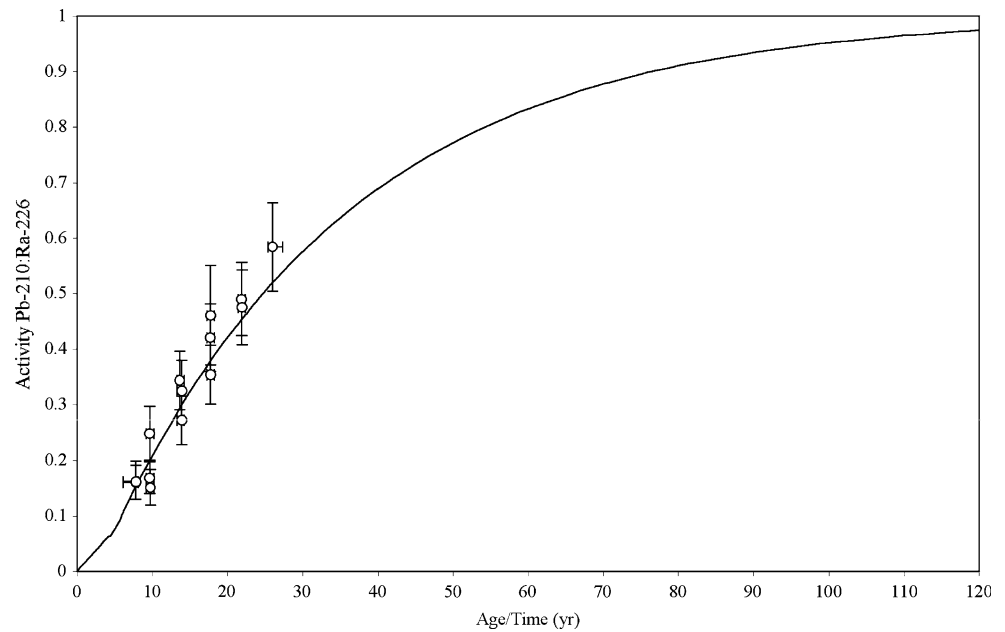
samples with relatively consistent uptake of  $^{226}\text{Ra}$  and of similar age from randomized selection (Table 3).

The growth-zone-derived age estimates were similar to the radiometric ages determined from the measured  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  activity ratios (Table 3). Agreement of the measured  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  ratios with the expected ingrowth curve indicated that growth-zone-derived age estimates were accurate (Fig. 5) and provides support for the age estimation procedures. The measured ratio fell within 2 SE of the expected ingrowth curve for the fish aged

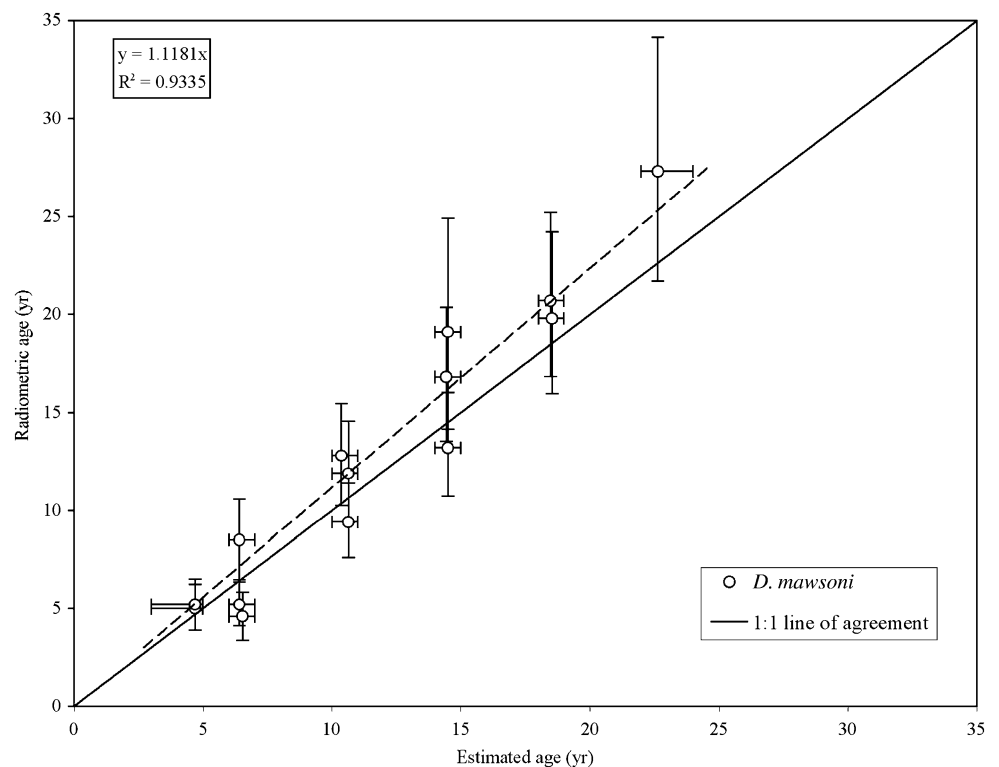
22–24 years, and for all but three of the experimental units in the design (Fig. 5).

Correlation between the ages from growth-zone count and radiometric ages was good ( $R^2 = 0.934$ , Fig. 6), and the slope of the regression was close to 1.0 (slope = 1.12), indicating that there was general agreement between estimated and radiometric ages. The regression of the radiometric ages against the estimated ages showed no significant differences from the 1:1 line of agreement with respect to intercept (two-tailed  $t$ -test,  $df = 4$ ,  $t = -1.123$ ,

**Fig. 5** Measured  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  ratios with respect to total sample age (growth-zone counts and the time since capture), with the expected  $^{210}\text{Pb}$ : $^{226}\text{Ra}$  ratio (ingrowth curve) for the Antarctic toothfish. *Horizontal error bars* represent the age range for each group. *Vertical error bars* represent analytical uncertainty associated with measuring  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  measured in 2 SE



**Fig. 6** Antarctic toothfish age agreement plot of growth-zone-derived age estimates versus radiometric age estimates (corrected for date of capture). *Horizontal error bars* represent the age range associated with each age group, while vertical error bars represent 2 SE for the analytical uncertainty associated with measuring  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$ . The *solid line* represents a hypothetical 1:1 line of agreement, and the *dashed line* is the line generated by the data



$P = 0.283$ ) or slope (two-tailed  $t$ -test,  $df = 4$ ,  $t = 2.3583$ ,  $P = 0.0781$ ).

## Discussion

Age estimation using otolith growth zones showed no departure from radiometry, indicating age estimates and

estimation criteria were accurate. The application of lead-radium dating to Antarctic toothfish otolith cores was successful with good agreement between the mean age from growth-zone counts and the lead-radium technique. This agreement validated the estimated age of fish up to 24 years and provided support for ages up to 39 years based on use of the same age estimation criteria. This application of lead-radium dating contained an unprecedented number of

samples and included multiple experimental units at each age level, comparable only to the recent parallel study on Patagonian toothfish (Andrews 2009).

The otolith samples in this study had particularly low  $^{226}\text{Ra}$  levels, which were compensated for by increasing the sample size. By using more than 30 otoliths per sample, roughly 1.5 g of cored otolith material was obtained, resulting in successful  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  measurements. Because otoliths were cored using a micromill machine, the cores were a repeatable size, shape, and mass, increasing the overall precision and confidence in the age determinations. This advance in technology provides a strong basis for future applications of lead–radium dating with other Southern Ocean fishes, which also may have low  $^{226}\text{Ra}$  levels in their otoliths (Andrews 2009).

Previous age and growth studies of Antarctic toothfish indicate the otoliths are difficult to interpret (Burchett et al. 1984; Horn 2002). In this study, the interpretation of the first three to eight growth zones was complicated because of the presence of “checks,” or false annual growth rings, which have also been described in Patagonian toothfish otoliths (Ashford et al. 2005). Readability of the older growth zones was difficult because of growth-zone compression with increasing age. This compression was especially apparent in growth zones greater than age twenty. Despite these difficulties, between and within reader precision values were better than other deep-dwelling species with difficult to age otoliths (e.g., Burton 1999).

Age estimates from transverse otolith sections indicated Antarctic toothfish are relatively slow-growing and long-lived. The results of this study indicate they may live to 39 years of age, which is similar to a reported maximum age of 35 years (Horn 2002). The growth coefficient generated in this study ( $k = 0.11 \pm 0.02$ ) indicated Antarctic toothfish are a moderately slow-growing species in relation to their maximum size ( $L_{\infty} = 158.9 \pm 7.4$ ) and is similar to the findings of Horn (2002). While Antarctic toothfish are not as slow-growing as other deep-dwelling species, such as yelloweye rockfish ( $k = 0.046$ ; Andrews et al. 2002), they are among the slowest growing Antarctic notothenioid fishes (La Mesa and Vacchi 2001) and have a growth rate similar to Patagonian toothfish ( $k = 0.085 \pm 0.23$ ; Horn 2002; Ashford et al. 2005).

Antarctic toothfish VBGF parameters  $k$  and  $t_0$  generated in this study were similar to those derived by Horn (2002), but  $L_{\infty}$  values had non-overlapping 95% CI. The mean  $L_{\infty}$  value in this study was 15 and 22 cm smaller for males and females, respectively. Because both studies sampled populations in the Ross Sea using commercial long-line operations, the observed reduction in  $L_{\infty}$  may reflect changes in the size distribution of Antarctic toothfish. Alternatively these differences may be a result of temporal and spatial differences in commercial fishing operations,

which vary considerably from year to year in the Ross Sea. Spatial variation of fishing effort is largely driven by the distribution of sea-ice, and the samples in this study were collected in 2004, which was a fishing season reported as limited by extensive sea-ice conditions (Hanchet et al. 2007).

The sustainability of deep-sea fisheries has been questioned because of life history parameters that characterize many deep-sea fishes, including slow growth, moderate to high longevity and late maturity (Koslow et al. 2000; Cailliet et al. 2001). These factors make them highly vulnerable to overfishing with little resilience once over-exploited (Koslow et al. 2000; Clark 2001; Haedrich et al. 2001; Morato et al. 2006). In this context, the data from this study suggest that Antarctic toothfish, as a long-lived, moderately slow-growing species, may be vulnerable to overfishing. To help prevent this outcome, this study provides accurate age and growth data for use in stock assessment models, as well as a validated otolith reference set for the CCAMLR Otolith Network (CON). Age-based life history characteristics and vital rates can now be estimated based on accurate age information, validated across the age range in the fishery, and incorporated in management decisions in support of sustainable harvest practices.

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