

# Determination and precision of otolith growth zone estimates of *Electrona antarctica* in the Southern Kerguelen Plateau region in the Indian sector of the Southern Ocean

Guoping Zhu<sup>a,b,c,d,\*</sup>, Mi Duan<sup>a,b</sup>, Lian Wei<sup>a,b</sup>, Rowan Trebilco<sup>e,f</sup>, Sophie Bestley<sup>g,a</sup>, Andrea Walters<sup>f</sup>

<sup>a</sup> College of Marine Sciences, Shanghai Ocean University, Shanghai, 201306, China

<sup>b</sup> Center for Polar Research, Shanghai Ocean University, Shanghai, 201306, China

<sup>c</sup> Polar Marine Ecosystem Group, Key Laboratory of Sustainable Exploitation of Oceanic Fisheries Resources, 999 Huchenghuan Road, Pudong New District, Shanghai, 201306, China

<sup>d</sup> National Engineering Research Center for Oceanic Fisheries, Shanghai, 201306, China

<sup>e</sup> Antarctic Climate & Ecosystems Cooperative Research Centre, University of Tasmania, Private Bag 80, Hobart, Tasmania, 7001, Australia

<sup>f</sup> CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart, TAS, 7001, Australia

<sup>g</sup> Institute for Marine and Antarctic Studies, University of Tasmania, Private Bag 129, Hobart, TAS, 7001, Australia

## ABSTRACT

Antarctic lanternfish (*Electrona antarctica*) is the dominant mesopelagic fish species in the Southern Ocean. It is the most common myctophid occurring south of the Antarctic Polar Front, and an important component of Southern Ocean food webs, both as a zooplankton predator and as a major prey item for higher predators. Despite its importance, there are major gaps in current understanding of the life history of *E. antarctica*, with previous ageing studies having produced inconclusive results, hampering our understanding of the population structure in this species and its role in marine ecosystems. This is particularly relevant around the Kerguelen Plateau, a highly productive region in the Indian sector of the Southern Ocean, where *E. antarctica* is a key mid-trophic level species. The present study took advantage of mesopelagic sampling efforts in the southern Kerguelen Plateau region on the 2015–2016 Kerguelen Axis study, and enumerated growth zones in the otoliths of a sample of 100 *E. antarctica* collected during the voyage. Growth zones were counted using both sectioned and whole otoliths and the precision of estimates was estimated by repeat counts. The estimated number of growth zones in the sectioned otoliths of *E. antarctica* (standard length 53–97 mm) ranging from 2 to 9. The average coefficient of variation (CV) and average percentage error (APE) for the estimated growth zones were 6.78% and 4.51%, respectively based on counts of sectioned otoliths. The number of growth zones in sectioned otoliths is positively related to weight of otolith and standard length of fish. The results presented here can inform future work on age structure and life history of *E. antarctica* in the Southern Ocean as understanding of the nature of growth rings improves.

## 1. Introduction

Mesopelagic fish, which occupy the upper 1000 m of the world's oceans, play a key role in linking primary consumers and macrozooplankton to higher trophic levels (Collins et al., 2012). Antarctic lanternfish (*Electrona antarctica* Greely et al., 1999) are endemic to the offshore oceanic waters of the Southern Ocean, have a circumpolar distribution south of the Antarctic Polar Front (Andriashev, 1965; Collins et al., 2012; Hulley, 1981; McGinnis, 1982), and are the most numerically abundant myctophids in this region. *Electrona antarctica* are important predators of mesozooplankton (Hureau, 1994; Clarke et al., 2018; Riaz et al., 2019; Saunders et al., 2019), and are frequently observed in the diet of top predators, including many seabirds and seals (Ainley et al., 1986; Greely et al., 1999; Knox, 2006; Loots et al., 2007;

Rowedder, 1979a, b; Ward et al., 2012). The large potential economic significance of this species was also recorded (Linkowski, 1987).

Although they are important as predators and prey (Van de Putte et al., 2010; Saunders et al., 2019), the biology and ecology of *E. antarctica* remains poorly understood. Previous work on this species has considered aspects of population structure (Collins et al., 2012; Zhu et al., 2018), genetic diversity (Van De Putte et al., 2012) and trophodynamics (Collins et al., 2012; Saunders et al., 2014; Waluda et al., 2010). Compared to the information available on tropical, temperate, and subarctic species, data on age and growth of mesopelagic fish in the Antarctic are remarkably lacking (Greely et al., 1999; Linkowski, 1987).

Existing studies on age and growth of *E. antarctica* are inconclusive and contradictory. Early work using size-frequency distributions (Rowedder, 1979a,b) estimated a maximum lifespan of up to 3 years. This was

\* Corresponding author. College of Marine Sciences, Shanghai Ocean University, Shanghai, 201306, China.

E-mail address: [gpzhu@shou.edu.cn](mailto:gpzhu@shou.edu.cn) (G. Zhu).

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contested by Linkowski (1987) who, based on sectioned otoliths, reported maximum lifespans of male and female *E. antarctica* as 8 and 11 years, respectively. Hulley et al. (1989), also using sectioned otoliths, found a maximum lifespan of 6 years. Liu and Chen (1995) supported the findings of Linkowski (1987) and Hulley et al. (1989) using size frequency distributions, finding at least six age groups for fish with standard lengths ranging from 39.4 mm to 118.0 mm. However, Greely et al. (1999), using the microstructure of sectioned otoliths, again estimated the lifespan of this species as only 3 years.

Otoliths are formed during the embryogenesis period and continue to grow throughout the life cycle of fish (Campana and Neilson, 1985; Tohse and Mugiya, 2002). Periodic environmental variation leads to the formation of bands of varying density that can be visually counted in sectioned otoliths, providing a widely-used method for assessing fish age (Campana, 2001). Age data forms the basis for estimation of growth and mortality rates (Campana, 2001). Otoliths have been used in age determination of several Antarctic fish species including the Patagonian toothfish (*Dissostichus eleginoides*) (Ashford et al., 2005; Cassia, 1998; Horn, 2002), Scotia Sea icefish (*Chaenocephalus aceratus*) (La Mesa et al., 2004), and ocellated icefish (*Chionodraco rastrospinosus*) (La Mesa and Ashford, 2008).

The precision of aging methods has not been estimated in previous ageing studies of *E. antarctica*. Moreover, existing information on the age and lifespan of *E. antarctica* is mainly limited to the Atlantic sector of the Southern Ocean and equivalent studies have not been performed in the Indian sector of that Ocean.

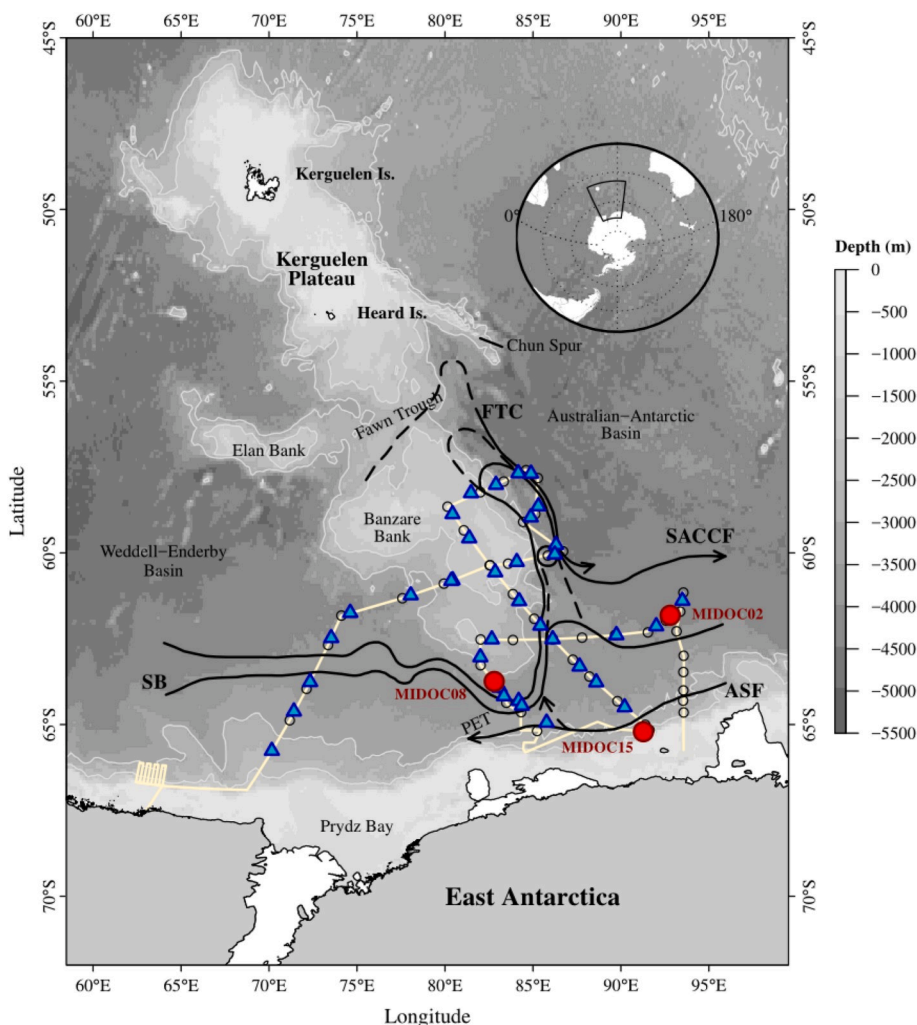
Here, we take advantage of mesopelagic sampling in a rarely-

surveyed area – the Southern Kerguelen Plateau – conducted during the 2015–2016 Kerguelen Axis study (Trebilco et al., 2019) to provide new information to support improved understanding of the age and growth of *E. antarctica*. We examined growth zones in otoliths of *E. antarctica*, using both whole and sectioned otoliths, and their precision.

## 2. Material and methods

### 2.1. Sample collection and processing

A marine survey was undertaken during the austral summer of 2015–2016 across the southern Kerguelen Plateau region, within the Indian Sector of the Southern Ocean (sampling locations are shown in Fig. 1). Sampling of the mesopelagic fishes in the upper 1000 m of the water column was undertaken using an International Young Gadoid Pelagic Trawl (IYGPT) net equipped with a midwater opening/closing (MIDOC) multiple cod-end device net configuration onboard the RV *Aurora Australis* between 25 January and 2 February 2016. A total of 147 individual *E. antarctica* were collected from 3 sampling stations (MIDOC stations 15, 8 and 2, Fig. 1), with 95, 42, and 10 individuals from each station, respectively. From these, we selected a random subset of 70, 25 and 5 individuals (total  $n = 100$ ) to estimate the growth zone (Table 1) because of budget constraint. The standard length (SL, mm) and wet body weight (BW, g) of specimens measured in the wet lab ranged from 53 to 97 mm (mean  $\pm$  SD:  $71.68 \pm 9.68$  mm) (Fig. 2) and 2–13 g ( $5.01 \pm 2.38$  g).



**Fig. 1.** Regional map used in Zhu et al. (2018, Fig. 1) showing cruise track (white lines) and CTD sampling stations (black circles) along transects occupied during the KAXIS survey off East Antarctica (inset). Stations sampled using the Mid-water Opening-Closing Net (MIDOC) shown as blue triangles; MIDOC sampling stations (MIDOC 2, 8, 15) for *Electrona antarctica* in this study highlighted as red circles. Major oceanographic features: Southern Antarctic Circumpolar Current Front (SACCF); Fawn Trough Current (FTC); Southern Boundary of the ACC (black line without arrow); and Antarctic Slope Front (southern line with westward arrow). Bathymetry from Weatherall et al. (2015) shows 500 m intervals, with 2000m and 3000 m isobaths highlighted as white contours. Antarctic continent and major ice shelves respectively in grey and white. PET = Princess Elizabeth Trough. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

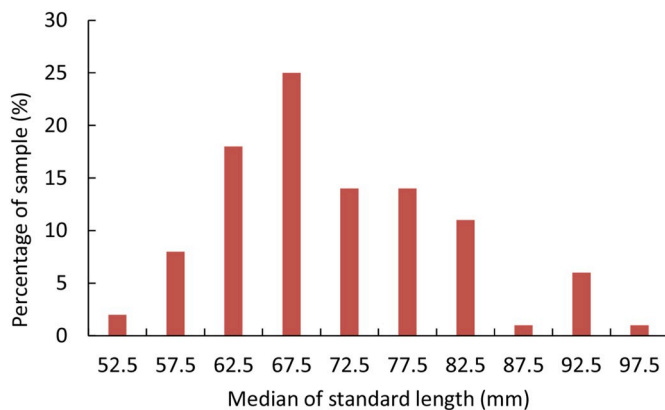
**Table 1**

Sample location, size, and summary statistics for *E. antarctica* included in this study. For a map of the locations, see Fig. 1.

Trawl No.	Location		n	Fish length	
	Longitude (E)	Latitude (S)		Mean $\pm$ SD (mm)	Range (mm)
MIDOC 2	92.78	61.81	5	68.00 $\pm$ 10.37	54–84
MIDOC 8	82.81	63.76	25	82.04 $\pm$ 8.49	68–97
MIDOC 15	85.43	65.17	70	68.40 $\pm$ 7.14	53–88

Sagittal otoliths were extracted and rinsed with ultrapure water to remove any attached tissue. After drying at room temperature, the otoliths were stored in plastic vials for processing at the Center for Aging Fish and Invertebrates (CAFI) at the Shanghai Ocean University. The right or left otolith from each fish sample was selected randomly, weighed on a 1/10000 precision balance and photographed using stereo microscope with Camera (OLYMPUS, SZ61-ILST), which was interfaced to a desktop computer. Otoliths were then embedded in Crystalbond embedding medium for 24 h before grinding. Initial examinations of otolith sections using the stereo microscope with reflected light indicated that growth zones were clearest on the ventral part of the transverse sections. The transverse section of the otolith was ground by hand using a grinder (REMET LS2) with 600 grit and 734 grit waterproof sandpapers. Then, 1200 grit and 2000 grit waterproof sandpapers were used to grind the sections until the nuclei were clearly exposed. Finally, the ground sagittal sections were polished with 0.3  $\mu$ m alumina powder to about 1 mm thick.

Two readers counted the growth zones in transverse sections using a stereo microscope with reflected light. Reader 1 counted the dark (translucent) zones on each section to produce a set of reference zones, and subsequently trained Reader 2 to similarly interpret the zones. Both readers then produced zone counts from the transverse sections on three occasions at two-week intervals. In addition, Reader 1, who was well-experienced in evaluating otoliths, also counted zones on the distal surface of whole sagittal otoliths (i.e. the sister otoliths of the 100 transverse section samples) three times to enable a comparison between section growth zone and whole otolith growth zone. Whole otoliths were immersed in ultrapure water in a clear dimple dish and placed on a black plate. The same stereo microscope was used to view otoliths using reflected light. The final determined growth zone is the result of the three counts done by the two readers with the lowest coefficient of variation (CV) after determining which structure was found to be most suitable for detecting the growth zone. The order of the otoliths was randomised during each counting run.



**Fig. 2.** Length frequency distribution of *E. antarctica* specimens (n = 100) used in the analyses.

## 2.2. Data analysis

The precision of growth zone estimates of the two readers or of growth zone estimates of one reader for reading the same otolith at different counting runs were calculated using the coefficient of variation (CV), which is expressed as the ratio of the standard deviation to the mean, as follows:

$$CV_j = 100\% \times \frac{\sqrt{\sum_{i=1}^R (X_{ij} - \bar{X}_j)^2 / (R-1)}}{\bar{X}_j}$$

where  $CV_j$  = the growth zone precision estimate for the  $j$ th fish;  $R$  = number of readers or counts;  $X_{ij}$  = the  $i$ th growth zone estimation for the  $j$ th fish;  $\bar{X}_j$  = the mean growth zone of the  $j$ th fish. Beamish and Fournier (1981) also used the average percent error (APE) to evaluate precision in growth zone determination, as follows:

$$APE_j = 100\% \times \frac{1}{R} \sum_{i=1}^R \frac{|X_{ij} - \bar{X}_j|}{\bar{X}_j}$$

The length-weight relationship (LWR) of *E. antarctica* is described by the following equation:

$$BW = aSL^b$$

To confirm whether the  $a$  and  $b$  parameters obtained from the above function were significantly different from those reported in FishBase and further to support the comparison of growth zone between studies, a test with a confidence level of  $\pm 95\%$  ( $\alpha = 0.05$ ) was applied (Sokal and Rohlf, 1987):

$$t_s = (x - xF) / xSE$$

where  $t_s$  is the  $t$ -test value,  $x$  is the parameter,  $xSE$  is the standard error, and  $xF$  is the value reported in FishBase.

The relationship between number of growth zone and standard length of fish was fitted by general linear regression. The one-way Analysis of variance (ANOVA) was used to examine the significance of the relationship ( $p = 0.05$ ).

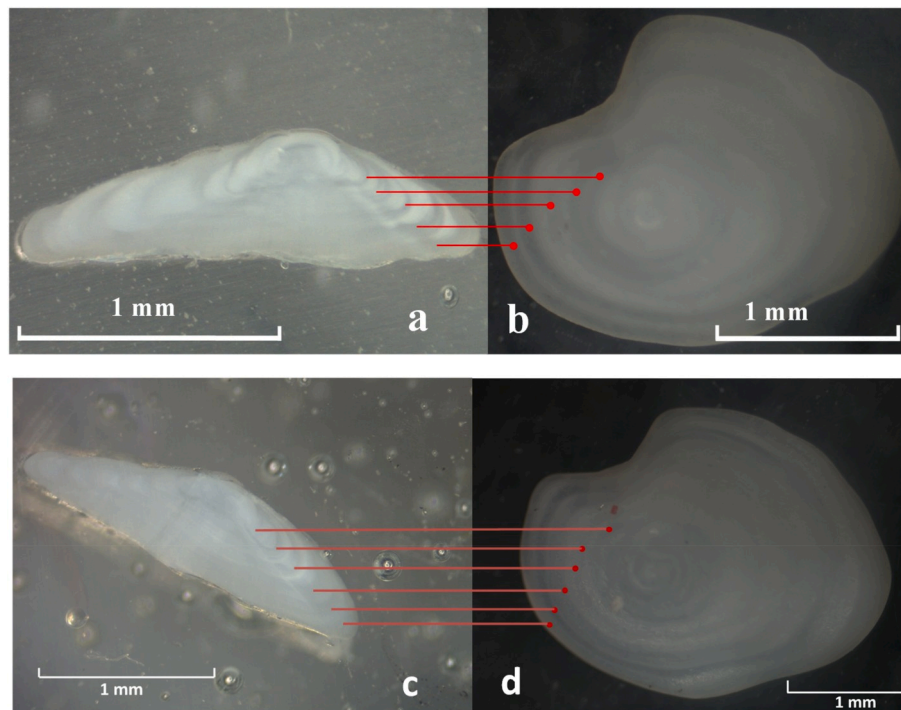
## 3. Results

### 3.1. Otolith shape and growth

Sagittal otoliths of *E. antarctica* were approximately elliptical, with the otolith core easily located close to the center (Fig. 3b). The transverse section had an approximately triangular shape, with the otolith core located at the top of the triangle (Fig. 3a). Translucent zones were easily observed in the transverse sections, but were more difficult to determine on the distal surfaces of whole otoliths. Beyond the core, very clear translucent and opaque zones could be observed from both the sagittal plate and transverse section (Fig. 3). The shape of the first and second growth zones surrounding the core of the sagittal plates were nearly circular, whereas the shape of the third growth zone and above were more analogous to the marginal zone of sagittal plate. The shapes of zones on the transverse sections were quite different to those of the whole otolith, but were analogous to the triangular shape of transverse sections.

### 3.2. Average percent error (APE) and coefficient of variation (CV) of growth zone estimations

The paired  $t$ -tests indicated that there were no significant differences between different counting runs for either sectioned otolith counts or whole otolith counts (Table 2). Compared to the whole otolith, the APEs and CVs of Reader 1 was more significantly decreased for counting the growth zone in the sectioned otoliths between different counts of same



**Fig. 3.** Two examples of sagittal plane (a) and transverse section (b) (SL = 74 mm, BW = 6 g) and sagittal plane (c) and transverse section (d) (SL = 93 mm, BW = 13 g) of *E. antarctica* otoliths, respectively.

**Table 2**

Growth zone count precision parameters calculated using the average percent error (APE) and the coefficient of variation (CV) to examine the biases between estimates from different counts of the same otolith by same reader. *p* values are reported for *t*-values from paired *t*-tests, *df* = degrees of freedom. Values given as mean  $\pm$  SD.

Items	Comparison	APE (%) Average $\pm$ SD	CV (%) Average $\pm$ SD	<i>t</i>	<i>df</i>	<i>p</i>
Reader 1 transverse section	Count 1 & Count 2	7.19 $\pm$ 8.21	10.16 $\pm$ 11.61	1.91	99	0.06
	Count 1 & Count 3	5.69 $\pm$ 7.39	8.05 $\pm$ 8.21	1.26	99	0.21
	Count 2 & Count 3	3.20 $\pm$ 6.10	4.52 $\pm$ 8.62	1.26	99	0.21
	Count 1 & Count 2	6.10 $\pm$ 7.93	8.63 $\pm$ 11.22	0.00	99	1.00
Reader 2 transverse section	Count 1 & Count 3	5.08 $\pm$ 8.13	7.18 $\pm$ 11.50	0.55	99	0.58
	Count 2 & Count 3	4.65 $\pm$ 7.07	6.58 $\pm$ 10.00	0.63	99	0.52
	Count 1 & Count 2	6.50 $\pm$ 6.65	9.20 $\pm$ 9.41	0.39	99	0.70
	Count 1 & Count 3	6.05 $\pm$ 6.60	8.56 $\pm$ 9.33	0.00	99	1.00
Reader 1 whole otolith	Count 2 & Count 3	3.83 $\pm$ 6.88	5.41 $\pm$ 9.73	0.49	99	0.62

otolith, and lower values were found for the APE and CV in between Count 2 and Count 3 of growth zone in the sectioned otoliths. The APEs and CVs among the three replicate counts conducted by the same reader (on transverse sections and whole otoliths) were relatively stable (Table 3). The APEs and CVs overall indicate that the experienced reader (Reader 1) was able to effectively train the less experienced reader (Reader 2), which facilitated repeatable counts in transverse sectioned otoliths. No bias was detected between growth zone estimates from different readers or different counts of the same otolith, whether growth zones were counted on the transverse section or whole otolith. With the

**Table 3**

Growth zone count precision parameters calculated using the average percent error (APE) and the coefficient of variation (CV), to examine the biases between estimates from different readers or different counts of the same otolith. Values given as mean  $\pm$  SD.

Comparison	Otolith method	Counting run	APE (%)	CV (%)
Within readers	Transverse section	Reader 1: counts 1 to 3	4.73 $\pm$ 1.28	6.68 $\pm$ 1.80
	Transverse section	Reader 2: counts 1 to 3	5.88 $\pm$ 1.78	8.31 $\pm$ 2.52
	Whole otolith	Reader 1: counts 1 to 3	5.61 $\pm$ 1.26	7.72 $\pm$ 1.66
	Whole otolith	Reader 2: counts 1 to 3	6.27 $\pm$ 7.75	8.87 $\pm$ 10.96
Between readers 1 & 2	Transverse section	2	5.38 $\pm$ 7.46	7.60 $\pm$ 10.55
	Transverse section	3	1.88 $\pm$ 5.40	2.65 $\pm$ 7.64
	The average across 6 counts on sectioned otolith conducted by two readers		4.51 $\pm$ 7.20	6.78 $\pm$ 10.50

repeated counting runs, APEs (and CVs) of sectioned otolith counts decreased between Readers 1 and Reader 2 (i.e. counts became more similar with repetition). Generally, the average APE and CV values were  $4.51 \pm 7.20$  and  $6.78 \pm 10.50$ , respectively across 6 counts conducted by the two readers (Table 3).

### 3.3. Growth zone and growth determination

Two to nine growth zones were detected for the fish collected in our study, with the dominant growth zone group being 3 to 5 (Fig. 4). Only a few fish had 7 growth zones and above ( $n = 6$ ). The relationship between SL and BW was fitted as an exponential curve, expressed as  $BW = 0.0088 \times SL^{3.1828}$  ( $R^2 = 0.9171$ ,  $p < 0.05$ ). There were no significant differences between values of parameters *a* and *b* of LWRs between FishBase and this study.



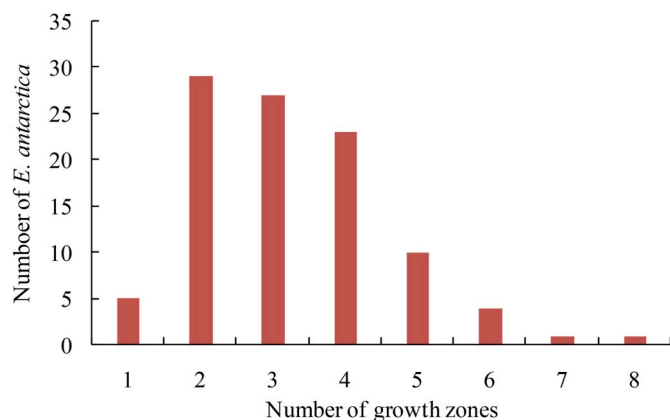


Fig. 4. The number of growth zones distribution of *E. antarctica* based on estimates from otolith sections. Samples were counted from 2 to 9 growth zones, with the dominant growth zone group being 3 to 5.

The estimated number of growth zone was significantly positive related to standard length of fish ( $y = 5.783x + 47.09$ ,  $R^2 = 0.652$ ,  $p < 0.001$ ) (Fig. 5). The  $t$ -test indicated that there was no significant difference between the weight of the left and right otoliths ( $p > 0.05$ ). A linear relationship between the estimated growth zone of *E. antarctica* (T) and the weight of the otolith (OW) was found, and could be expressed as  $OW = 0.0004 T + 0.0006$  ( $R^2 = 0.6276$ ,  $p < 0.001$ ).

## 4. Discussion

### 4.1. Growth zones on otolith sections

Otolith zones record the growth history of an individual fish from date of birth (Campana and Neilson, 1985). *E. antarctica* spawns during the austral fall or early winter (April to June) (Lubimova et al., 1987) and hatches during the austral winter (June to August) (Kellermann, 1989a, b). Fish otoliths form during embryogenesis (Campana and Neilson, 1985; Tohse and Mugiya, 2002), so the core zone of *E. antarctica* otoliths is presumably formed around the austral winter. A translucent core zone was evident in otoliths examined in our study, which would be consistent with slow growth as a result of low winter food availability and temperatures (Linkowski, 1987), and is also consistent with the dark

larval zone observed (under transmitted light) by Greely et al. (1999). In contrast, the wider opaque zones along the outer area of the core are likely representative of the fish developing to the juvenile stage, and growing more rapidly during the austral summer (January to April) (Efremenko, 1986; McGinnis, 1982). Hence, we agree with Linkowski (1987) in assuming that the pattern of paired narrow translucent zones and wide opaque zones (observed in all otoliths in this study) are representative of annual growth zone formation in *E. antarctica* (Fig. 3). The otoliths of *E. antarctica* examined in the present study were sampled during the austral summer (January to February). Thus, there was little scope for examining the seasonality of growth zone formation in most age classes, although an opaque zone was still observed on the outer margins of most otolith samples, when more protein was deposited in the otolith during faster growth rate of fish in summer.

While the translucent core zone and surrounding growth zones that we observed in otoliths seems consistent with a larval core zone and annual growth rings respectively, examination of microstructure using scanning electron microscopy and/or repeat sampling throughout the annual cycle would be required to validate this interpretation of ring structure.

### 4.2. Growth zone determination and precision

The growth zone range inferred here from counting otolith growth zones (i.e. two to nine zones) is congruent with a previous study that aged this species using otolith growth zones (Linkowski, 1987), and indicates a greater maximum age than studies using length-frequency analysis (three and six years; Rowedder, 1979a, and Lin and Chen, 1995; respectively). Slow or even stagnant growth of fish at high latitudes has been noted as being problematic for the use of length frequency analysis for fish aging (Burchett, 1983; Hemmingsen, 1991; Kochkin, 1980), and our results, together with those of previous studies, indicate that this method is unlikely to be suitable for *E. antarctica*.

The coefficient of variation (CV) and the average percent error (APE) values of three counts on sectioned otoliths for this study (Tables 2 and 3) are typical expected values for bony fishes in general ( $<10\%$ ; Campana et al., 2011), and comparable to the range of CVs of  $<5\%$  reported in a previous study on Myctophidae (Young et al., 1988), although a slightly higher CV value was found in this study. The CV values were similar for counts on sectioned otoliths between the two readers (Table 3), although the counts were conducted by each reader independently. The relatively clear ventral section in the otoliths of *E. antarctica* made the counts easier than for other Antarctic fish species, such as Antarctic dragonfish (*Parachaenichthys charcoti*) (La Mesa et al., 2012) and Patagonian toothfish (Ashford et al., 2005; Cassia, 1998; Horn, 2002). However, with repeating counting runs, the rate of decreasing for CV and APE values for Reader 1 (well-experienced reader) is greater than that for Reader 2 (less experienced reader), meaning the importance of experience in reading the counts on the sectioned otolith for this species. The CV and APE values of counts on sectioned and whole otoliths for the two readers demonstrated that the transverse sections are optimal for growth zone determination of *E. antarctica*, while the whole otolith can be considered as a supplementary approach for the growth zone determination of this species (Fig. 3). It is important to note that presenting estimates of within- and between-reader precision does not validate an ageing method; it simply shows that readers can consistently interpret a zonation pattern in the otoliths of a species. To validate that the interpretation of the zonation pattern is producing accurate ages would require additional effort (such as otolith markers, estimation of periodicity of growth rings) that as yet remain challenging for species unlikely to be recaptured or to be monitored monthly in the Southern Ocean.

In some Antarctic fish species, such as sub-Antarctic notothenioid (*Patagonotothen ramsayi*) (Brickle et al., 2006), Scotia Sea icefish (La Mesa and Ashford, 2008), and Antarctic dragonfish (La Mesa et al., 2012), it is possible to use daily growth zones to estimate age. However,

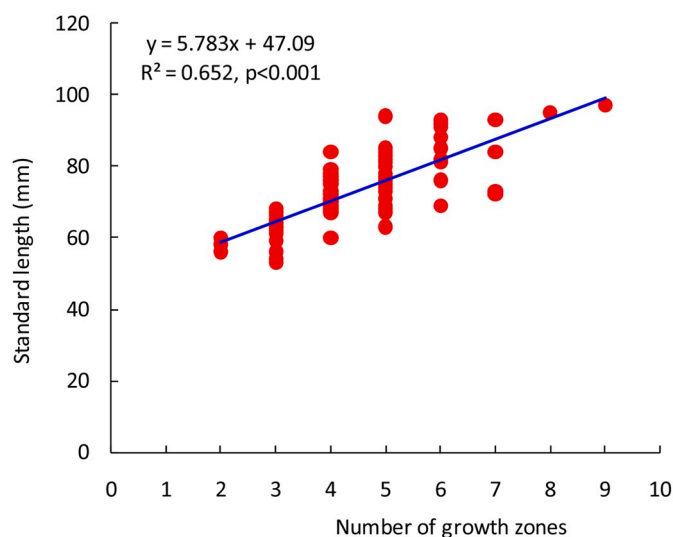


Fig. 5. The relationship between the number of growth zones and standard length of *E. antarctica* (the data from the final determined transverse section reading was used).

those species are demersal, in contrast to the mesopelagic habit of *E. antarctica*. Adult *E. antarctica* undertake diel vertical migration, whereas the larvae seem to stay at the surface layer of ocean (5–200 m below the sea surface) (Moteki et al., 2017). Hence, the habitats are significantly different between the early life stage and adult for *E. antarctica*. The formation of growth zones on the otolith section is affected by the photoperiod and marine environment, such as feeding frequency and temperature fluctuations (Campana and Neilson, 1985; Radtke and Dean, 1982). Sub-daily or non-daily increments were visible throughout the larval zone (Greely et al., 1999), implying for *E. antarctica* growth ring estimation using daily growth zones could introduce uncertainty. However, validation of first annuli formation is needed to understand whether the growth zone of otolith was formed annually.

#### 4.3. Otolith weight and growth zone

Fish otoliths are formed throughout the fish life cycle, and continually increase in weight (Fowler and Doherty, 1992). As such, otolith weight may also provide an index for fish age (Lou et al., 2007). However, the rate of change for otolith weight is dependent upon the growth rate (Pawson, 1990) and habitat (Metin and Ilkyaz, 2008), so aging fish based solely on otolith weight is likely to be problematic. Here we found that otolith weight had a positive and linear relationship to growth zone. While this does not suggest that otolith weight alone should be used for aging *E. antarctica* in future studies (for the reasons described above), it does provide some support for the zone-based age estimates.

#### 5. Conclusions

Data on age and growth of fish forms the basis of research on population dynamics, age-based stock assessment and movement history (Greely et al., 1999; Zhu et al., 2018). Earlier age and growth studies (Rowedder, 1979a, b; Linkowski, 1987; Hulley et al., 1989; Liu and Chen, 1995; Greely et al., 1999) have provided inconclusive and contradictory maximum lifespans for *E. antarctica* in the order of 3, 6 or 8–11 years. Further, these studies did not include estimates of the precision of the aging methods used. Our results provide important additional information to support estimation of growth rate of this species, and are consistent with earlier studies finding at least six age classes (Linkowski, 1987; Liu and Chen, 1995; Hulley et al., 1989) with our observation of up to 9 growth zones. An important priority for future work will be to definitely ascertain the age of the first ring and the period between rings, particularly in the early years, for this species mesopelagic species more generally. As knowledge of early ring formation in myctophids continues to progress, we hope that the results presented here can be incorporated into future work to understand broader patterns of age and growth for this species and myctophids more broadly in Southern Ocean ecosystems.

#### Declaration of competing interest

We have no conflicts of interest.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.dsr2.2020.104778>.

#### References

- Ainley, D.G., Fraser, W.R., Sullivan, C.W., Torres, J.J., Hopkins, T.L., Smith, W.O., 1986. Antarctic mesopelagic micronekton: evidence from seabirds that pack ice affects community structure. *Science* 232, 847–849.
- Andriashev, A.P., 1965. A General Review of the Antarctic Fish Fauna. *Biogeography and Ecology in Antarctica*. Springer, Netherlands, pp. 491–550.
- Ashford, J., Duhamel, G., Jones, C., Bonko, S., 2005. Age, growth and mortality of Patagonian toothfish (*Dissostichus eleginoides*) caught off Kerguelen. *CCAMLR Sci.* 12 (1), 29–41.
- Beamish, R.J., Fournier, D.A., 1981. A method for comparing the precision of a set of age determinations. *Can. J. Fish. Aquat. Sci.* 38, 982–983.
- Brickle, P., Arkhipkin, A., Shcherbich, Z., 2006. Age and growth of a sub-Antarctic notothenioid, *Patagonotothen ramsayi*, (Regan 1913), from the Falkland Islands. *Polar Biol.* 29 (8), 633–639.
- Burchett, M.S., 1983. Age and growth of the antarctic fish *Notothenia rossii* from south Georgia. *Bull. Br. Antarct. Surv.* 60, 45–61.
- Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish. Biol.* 59, 197–142.
- Campana, S.E., Neilson, J.D., 1985. Microstructure of fish otoliths. *Can. J. Fish. Aquat. Sci.* 42 (5), 1014–1032.
- Campana, S.E., Annand, M.C., McMillan, J.I., 2011. Graphical and statistical methods for determining the consistency of age determinations. *Trans. Am. Fish. Soc.* 124 (1), 131–138.
- Cassia, M.C., 1998. Comparison of age readings from scales and otoliths of the Patagonian toothfish (*Dissostichus eleginoides*) from South Georgia. *CCAMLR Sci.* 5, 191–203.
- Clarke, L.J., Trebilco, R., Walters, A., Polanowski, A.M., Deagle, B.E., 2018. DNA-based diet analysis of mesopelagic fish from the southern kerguelen Axis. *Deep Sea Res. Part II Top. Stud. Oceanogr.* S0967064517304046 <https://doi.org/10.1016/j.dsr2.2018.09.001>.
- Collins, M.A., Stowasser, G., Fielding, S., Shreeve, R., Xavier, J.C., Venables, H.J., Enderlein, P., Cherel, Y., Van de Putte, A., 2012. Latitudinal and bathymetric patterns in the distribution and abundance of mesopelagic fish in the Scotia Sea. *Deep-Sea Res. II* 59–60, 189–198.
- Efremenko, V.N., 1986. Distribution of eggs and larvae of Myctophidae in the southern Atlantic. *J. Ichthyol.* 26, 141–147.
- Fowler, A.J., Doherty, P.J., 1992. Validation of annual growth increments in the otoliths of two species of Damsel fish from the Southern Great Barrier Reef. *Mar. Freshw. Res.* 43 (5), 1057–1068.
- Greely, T.M., Jr, J.V.G., Torres, J.J., 1999. Age and growth of *Electrona antarctica* (pisces: Myctophidae), the dominant mesopelagic fish of the Southern Ocean. *Mar. Biol.* 133 (1), 145–158.
- Hemmingsen, E.A., 1991. *Biology of Antarctic Fish*. Springer-Verlag, Berlin Heidelberg, pp. 87–96.
- Horn, P.L., 2002. Age and growth of Patagonian toothfish (*Dissostichus eleginoides*) and Antarctic toothfish (*D. mawsoni*) in waters from the New Zealand sub-Antarctic to the Ross Sea, Antarctica. *Fish. Res.* 56 (3), 275–287.
- Hulley, P.A., 1981. Results of the research cruises of FRV 'walther herwig' to south America. LVIII. Family Myctophidae (*osteichthyes*, myctophiformes). *Arch. Fischwiss.* 31, 1–300.
- Hulley, P.A., Camus, P., Duhamel, G., 1989. Ichthyological results of cruise MD-42/SIBEX-II. Part 1. Fishes from RMT-8 stations, with additional records of lanternfishes (Myctophidae: osteichthyes) from the Indian and Atlantic sectors of the Southern Ocean. *Cybius* 13 (1), 83–99.
- Hureau, J.C., 1994. The significance of fish in the marine Antarctic ecosystems. *Polar Biol.* 14 (5), 307–313.
- Kellermann, A., 1989a. Fish eggs and larvae. *Ber. Polarforsch.* 62, 157–158.
- Kellermann, A., 1989b. The larval fish community in the zone of seasonal pack-ice cover and its seasonal and interannual variability. *Arch. Fisch. Wiss. Beih.* 39, 81–109.
- Knox, G.A., 2006. *Biology of the Southern Ocean*, second ed. CRC Press, New York.
- Kochkin, K.H., 1980. Graphical analysis of length frequency of *Champscephalus gunnari* lonnberg (*channichthyidae*) from south Georgia. *Cybius* 8, 33–42.
- La Mesa, M., Ashford, J.R., Larson, E., Vacchi, M., 2004. Age and growth of Scotia Sea icefish, *Chaenocephalus aceratus*, from the south shetland islands. *Antarct. Sci.* 16 (3), 253–262.
- La Mesa, M., Ashford, J., 2008. Age and growth of ocellated icefish, *Chionodraco rastrospinosus* DeWitt and hureau, 1979, from the south shetland islands. *Polar Biol.* 31 (11), 1333–1342.

- La Mesa, M., Catalano, B., Kock, K.H., Jones, C.D., 2012. Age and growth of the antarctic dragonfish *Parachaenichthys charcoti* (pisces, bathydraconidae) from the southern Scotia arc. *Polar Biol.* 35 (10), 1545–1553.
- Linkowski, T.B., 1987. Age and growth of four species of *Electrona* (teleostei, Myctophidae). In: Kullander, S.O., Fernholm, B. (Eds.), *Proceedings of the 5<sup>th</sup> Congress of European Ichthyologists*. Swedish Museum of Natural History Stockholm, Swedish, pp. 435–442.
- Liu, Q., Chen, D.G., 1995. Length frequency analysis of *Pleuragramma antarcticum*, *Electrona antarctica*, *Protomyctophum bolini*. *Chin. J. Oceanol. Limnol.* 13 (4), 380–384.
- Loots, C., Koubbi, P., Duhamel, G., 2007. Habitat modelling of *Electrona antarctica*, (Myctophidae, Pisces) in Kerguelen by generalized additive models and geographic information systems. *Polar Biol.* 30 (8), 951–959.
- Lou, D.C., Mapstone, B.D., Russ, G.R., Begg, G.A., Davies, C.R., 2007. Using otolith weight–age relationships to predict age based metrics of coral reef fish populations across different temporal scales. *Fish. Res.* 83 (2–3), 216–227.
- Lubimova, T.G., Shust, K.V., Popkov, V.V., 1987. Specific Features in the Ecology of Southern Ocean Mesopelagic Fish of the Family Myctophidae. *Biological Resources of the Arctic and Antarctic*. Nauka Press, pp. 320–337.
- McGinnis, R.F., 1982. Biogeography of lanternfishes (Myctophidae) south of 30°S. *Antarct. Res.* 35, 1–110.
- Metin, G., Ilkyaz, A.T., 2008. Use of otolith length and weight in age determination of poor cod (*Trisopterus minutus* linn. 1758). *Turk. J. Zool.* 32 (3), 293–297.
- Moteki, M., Fujii, K., Amakasu, K., Shimada, K., Tanimura, A., Odate, T., 2017. Distributions of larval and juvenile/adult stages of the Antarctic myctophid fish, *Electrona antarctica*, off Wilkes Land in East Antarctica. *Pol. Sci.* 12, 99–108.
- Pawson, M.G., 1990. Using otolith weight to age fish. *J. Fish. Biol.* 36, 521–531.
- Radtke, R.L., Dean, J.M., 1982. Increment formation in the otoliths of embryos, larvae, and juveniles of the mummichog, *Fundulus heteroclitus*. *Fish. Bull.* 80, 201–215.
- Riaz, J., Walters, A., Trebilco, R., Bestley, S., Lea, M.-A., 2019. Stomach content analysis of mesopelagic fish from the southern kerguelen Axis. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 104659 <https://doi.org/10.1016/j.dsr2.2019.104659>.
- Rowedder, U., 1979a. Some aspects of the biology of *Electrona antarctica* (günther, 1878) (family Myctophidae). *Meeresforsch.-Rep. Mar. Res.* 27 (4), 244–251.
- Rowedder, U., 1979b. Feeding ecology of *Electrona antarctica* (günther, 1878) (teleostei). *Meeresforsch.-Rep. Mar. Res.* 27 (4), 252–263.
- Saunders, R.A., Collins, M.A., Foster, E., Shreeve, R., Stowasser, G., Ward, P., Tarling, G. A., 2014. The trophodynamics of Southern Ocean *Electrona*, (Myctophidae) in the Scotia Sea. *Polar Biol.* 37 (6), 789–807.
- Saunders, R.A., Hill, S.L., Tarling, G.A., Murphy, E.J., 2019. Myctophid fish (family Myctophidae) are central consumers in the food web of the Scotia Sea (Southern Ocean). *Front Mar. Sci.* 6, 530. <https://doi.org/10.3389/fmars.2019.00530>.
- Sokal, R.R., Rohlf, F.J., 1987. *Introduction to Biostatistics*, second ed. Freeman, New York, p. 363.
- Tohse, H., Mugiya, Y., 2002. Diel variations in carbonate incorporation into otoliths in goldfish. *J. Fish. Biol.* 61 (1), 199–206.
- Trebilco, R., Walters, A., Melbourne-Thomas, J., Bestley, S., Cox, M., Gastauer, S., Constable, A., 2019. Mesopelagic community structure on the southern Kerguelen Axis. In: Welsford, D.C., Dell, J., Duhamel, G. (Eds.), *The Kerguelen Plateau: Marine Ecosystem and Fisheries*. *Proceedings of the Second Symposium*. Australian Antarctic Division, Kingston, Tasmania, pp. 49–54.
- Van de Putte, A.P., Jackson, G.D., Pakhomov, E., Flores, H., Volckaert, F.A.M., 2010. Distribution of squid and fish in the pelagic zone of the Cosmonaut Sea and Prydz Bay region during the BROKE-West campaign. *Deep-Sea Res.* 57, 956–967.
- Van De Putte, A.P., Van Houdt, J.K.J., Maes, G.E., Hellemans, B., Collins, M.A., Volckaert, F.A.M., 2012. High genetic diversity and connectivity in a common mesopelagic fish of the Southern Ocean: the myctophid *Electrona antarctica*. *Deep-Sea Res.* 59, 199–207.
- Waluda, C.M., Collins, M.A., Black, A.D., Staniland, I.J., Trathan, P.N., 2010. Linking predator and prey behaviour: contrasts between Antarctic Fur seals and macaroni penguins at South Georgia. *Mar. Biol.* 157 (1), 99–112.
- Ward, P., Atkinson, A., Venables, H.J., Tarling, G.A., Whitehouse, M.J., Fielding, S., Collins, M.A., Korb, R., Black, A., Stowasser, G., Schmidt, K., Thorpe, S.E., Enderlein, P., 2012. Food web structure and bioregions in the Scotia Sea: a seasonal synthesis. *Deep-Sea Res. II* 59–60, 253–266.
- Weatherall, P., Marks, K.M., Jakobsson, M., Schmitt, T., Tani, S., Arndt, J.E., Rovere, M., Chayes, D., Ferrini, V., Wigley, R., 2015. A new digital bathymetric model of the world's oceans. *Earth Space Sci.* 2, 331–345.
- Young, J.W., Bulman, C.M., Blaber, S.J.M., Wayte, S.E., 1988. Age and growth of the lanternfish *Lampanyctodes hectoris* (Myctophidae) from eastern Tasmania, Australia. *Mar. Biol.* 99, 569–576.
- Zhu, G.P., Duan, M., Ashford, J.R., Wei, L., Bestley, S., 2018. Otolith nucleus chemistry distinguishes *Electrona antarctica* in the westward-flowing antarctic Slope Front current and eastward-flowing antarctic circumpolar current off East Antarctica. *Mar. Environ. Res.* 142, 7–20.