



# Age and growth estimation of Southern Ocean squid *Moroteuthopsis longimana*: can we use beaks collected from predators' stomachs?

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## Abstract

Squid play a major role in the Southern Ocean food web. However, their age and growth remain poorly studied. Here, using upper and lower beaks of *Moroteuthopsis longimana* collected from the diet of *Dissostichus mawsoni* from Pacific and Atlantic sectors of the Southern Ocean, we studied: (1) Feasibility of using beaks collected from predators' stomachs to study the age of Southern Ocean oceanic squid; and (2) Age estimation and growth patterns of *M. longimana*. The rostrum sagittal section (RSS) of both beaks had micro-increments, with the lower beak being the best to observe and count a readable sequence of increments to estimate the age. Assuming a daily deposition of increments, our results suggest that *M. longimana* can live up to 820 days and may hatch throughout the year. Studied individuals presented a consistent growth rate from hatching to death but with, at least, one period of faster growth. A novel pattern of regular cycles, composed of 7–10 lighter increments followed by a darker one, was found in the medium-anterior region of the RSS. Differences were found in the growth rate and size reached at the same age between individuals from the Pacific and Atlantic sectors, which might be related with different environmental conditions between both capture sites. This study shows that lower beaks from predators' stomachs can be used to study the age of Southern Ocean squids and that *M. longimana* hatches in all seasons, being available year round to predators that feed of this species.

**Keywords** Antarctica · Cephalopods · Growing · Onychoteuthidae · Reproduction · Sclerochronology

## Introduction

Squid play a major role in the Southern Ocean ecosystem (Xavier et al. 2018; Cherel 2020). Most of the 60 squid species inhabiting the Southern Ocean (waters south of the Subtropical front) present a circumpolar distribution (Xavier et al. 2014; Cherel 2020). They act as a major link between the lower trophic levels, e.g. zooplankton and small zooplanktivorous species, and the top predators, such as sea-birds, marine mammals and toothfish (Collins and Rodhouse 2006; Xavier and Croxall 2007; Queirós et al. 2022). Annually, it is estimated that Southern Ocean predators consume between 12.5 and 24.0 million tonnes of squid (Santos et al. 2001). However, and despite this importance in the ecosystem, very few studies evaluated abundance, age and growth of Southern Ocean squid (Jackson and Lu 1994; Schwarz and Perez 2010). In contrast to Southern Ocean octopods, whose lifespan and growth was studied for several species (Barratt and Allcock 2010; Schwarz et al. 2019), the life history of only six Southern Ocean squid species (*Doryteuthis gahi*, *Galiteuthis glacialis*, *Illex argentinus*, *Martialia*

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*hyadesi*, *Moroteuthopsis ingens* and *Moroteuthopsis longimana*) was studied (Clarke 1965; Patterson 1988; Jackson 1993; Jackson and Lu 1994; Rodhouse et al. 1994; Arkhipkin et al. 2018). The study of age and growth of a species in the wild requires a technique that enables an accurate estimation of the absolute age (Perales-Raya and Hernández-González 1998). In cephalopods, this has been widely done by counting the increments of statoliths, but also using stylets, gladius, beaks, lipofuscin and eye lenses (Arkhipkin et al. 2018). In order to analyse most of these structures, it is necessary to capture the individual squid. Oceanic squid, however, are highly mobile and can easily avoid the scientific nets (Wormuth and Roper 1983). Furthermore, the low number of research surveys targeting these organisms in remote areas of the world (Clarke 1996), suggests that it might be appropriate to find ways that do not require the capture of the live individual.

Cephalopod beaks, chitinous mandibles, grow throughout the life of individuals without replacement (Guerra et al. 2010; Perales-Raya et al. 2014b; Xavier et al. 2022). They resist to digestion and accumulate in predators' stomachs, and being easily sampled from seabird boluses, seal scats and predator stomach contents (Xavier et al. 2011; Abreu et al. 2019; Cherel 2021; Queirós et al. 2022). Beak morphology varies among species which allows its identification (Clarke 1986; Xavier and Cherel 2021; Xavier et al. 2022). Furthermore, beaks sampled from predators' stomachs can be used to study the ecology of squid (Xavier et al. 2016) and, as beaks sampled from the individuals, might also be used to study the age and growth patterns of cephalopod specimens (Clarke 1965; Perales-Raya and Hernández-González 1998; Hernández-López et al. 2001).

The age determination of cephalopods using upper and lower beaks has been extensively studied in the common octopus *Octopus vulgaris* (Perales-Raya and Hernández-González 1998; Perales-Raya et al. 2010, 2014a, b, 2018; Canali et al. 2011; Cuccu et al. 2013; Franco-Santos et al. 2015; Garrido et al. 2016; Armelloni et al. 2020). More recently, this technique has also been applied in other cephalopod species, e.g. the octopods *Megaleledone setebos* (Schwarz et al. 2019), *Octopus maya* (Bárcenas et al. 2014), the sepiid *Sepia officinalis* (Lishchenko et al. 2018) and the oegopsid squid *Dosidicus gigas* (Liu et al. 2015, 2017; Hu et al. 2016), *Ommastrephes bartramii* (Liu et al. 2015; Fang et al. 2016), *O. caroli* (Agus et al. 2021), *Sthenoteuthis oualaniensis* (Liu et al. 2015; Lu et al. 2022) and *Architeuthis dux* (Perales-Raya et al. 2020). Age determination in beaks have been mainly performed in two beak regions: the rostrum sagittal section (RSS) and the inner lateral wall surface (LWS) (Perales-Raya et al. 2010). However, due to its role in predation, the RSS tend to be eroded and care should be taken to avoid underestimating the age (Perales-Raya et al. 2010, 2020). Beaks also present environmental

stress marks in the RSS, such as temperature changes and capture (Perales-Raya et al. 2014a; b). Using chemical and environmental marking, as well as known-age specimens, these studies confirmed that beak increments are daily deposited (Bárcenas et al. 2014; Perales-Raya et al. 2014b), and later cross-verification with statoliths was performed in the RSS for several oegopsid squids (Liu et al. 2015, 2017). Nevertheless, we must be aware that a recent study suggests that cold-water deep-sea species may have a different periodicity in the deposition of these increments (Schwarz et al. 2019). Unfortunately, those authors did not explore RSS and used only beak LWS, which showed a nonconstant sequence of increments for ageing *M. longimana* and other cold-water living species (e. g., *Architeuthis dux*, see Perales-Raya et al. 2020). Malcolm Clarke was the first to note the presence of micro-increments in beaks of Southern Ocean squid (Clarke 1965). His study was based on beaks of *M. longimana* (initially identified as *M. ingens* (Clarke 1965), but later confirmed as *M. longimana* (Cherel 2020)) sampled from sperm whales' stomachs, but it only analysed the LWS (Clarke 1965). In that study, a pattern of temporal deposition could not be established since some areas of the beak had less rings than expected. The author hypothesised that it could be an effect of the predator's gastric acids acting on the beak (Clarke 1965). However, to our knowledge, no studies performed a careful analysis of beaks in/from predators' stomachs, and whether gastric acids could affect the micro-increments in the RSS of the beaks.

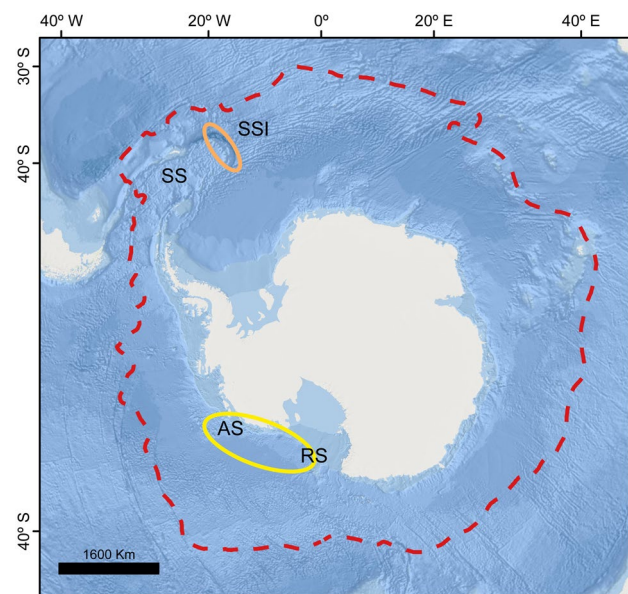
The giant warty squid *M. longimana* is a major prey for Southern Ocean's top predators (Xavier et al. 2018; Cherel 2020), and one of the most studied Antarctic squid (Clarke 1980; Lu and Williams 1994; Lynnes and Rodhouse 2002; Cherel and Hobson 2005; Laptikhovskiy et al. 2009, 2013; Seco et al. 2016; Laptikhovskiy and Xavier 2017; Queirós et al. 2021a, b, 2018, 2020; Abreu et al. 2020; van Tonder et al. 2020). This species inhabits both Antarctic and Subantarctic waters in all the three sectors of the Southern Ocean (Cherel 2020). It can reach large sizes (more than 2 m in total length (Lynnes and Rodhouse 2002)) and it has an ontogenetic diet change with juveniles feeding in zooplankton and adults in fish and squids (Nemoto et al. 1985; Collins and Rodhouse 2006; Queirós et al. 2018). Age and growth of this species have been previously studied using micro-increments in the lateral wall of lower beaks (Clarke 1965), gladius cross-sections (Bizikov 1991) and beaks' size frequency (Jarre et al. 1991). These studies suggested a lifespan of up to 10 years, a low growth rate, and slower and faster growing periods in winter and summer, respectively. Clarke (1965) also found patterns of cycles in the micro-increments of the lateral wall and, similar to other squid species, a faster growing during the first year in comparison to the following years where the growth rate was lower. This previous knowledge on the growing patterns of

*M. longimana* suggests this species as a good model to study the use of beaks from predators' stomachs to determine age and growth of oceanic squid from the Southern Ocean. Furthermore, due to its ecological role in the Southern Ocean ecosystem, it is important to re-evaluate its growth patterns and maximum age as previous studies suggest different lifespans for the species (i.e. Bizikov (1991) hypothesised that *M. longimana* can live up to 5 years while Jarre et al. (1991) and Clarke (1965) suggests a lifespan of 8–10 years) and growth can be different in different Southern Ocean regions (Constable et al. 2014).

Here, we used lower and upper beaks of *M. longimana* collected from the stomachs of *Dissostichus mawsoni* from the Ross/Amundsen Sea (Pacific Sector) and South Sandwich Islands (Atlantic Sector), in order to (i) evaluate whether it is feasible to use beaks' micro-increments from predators' stomachs to study age and growth pattern of cephalopods; and to (ii) estimate the age and growth pattern of the giant warty squid *M. longimana* from different areas of the Southern Ocean. As *D. mawsoni* is a sedentary species that does not perform long-distance movements (tag-recapture show that only ~7% of individuals perform a long-distance movement and took years) and cephalopod beaks are retained by just a couple of days in its stomachs, it is possible to confirm that the squid individuals were predated in the same sea basin as the *D. mawsoni* was captured (Hanchet et al. 2008; Grilly et al. 2022).

## Materials and methods

Ten lower beaks (ML) and five upper beaks (MU) of mature [fully darkened wings (Clarke 1986)] *Moroteuthopsis longimana* in a range of sizes were sampled from the stomachs of *Dissostichus mawsoni* captured in Antarctic waters, i.e. South of Antarctic Polar Front, in the Ross/Amundsen Sea (Pacific Sector) between December and February (Austral summer 2016/17) and South Sandwich Islands (Atlantic Sector) between March and April (Austral autumn 2009; only ML8, ML9 and ML10) (Fig. 1). Due to space limitation onboard, we opened the *D. mawsoni*'s stomachs and kept all beaks together instead of separating them which preclude the determination of the exact capture date. To estimate the date of birth of the different individuals we assigned a capture date in the middle of the sampling period, i.e. 15th January 2017 for beaks sampled in the Ross/Amundsen Seas and 30th March 2009 for those from South Sandwich Islands (Table 1). Though we need to be aware that the squid might have been eaten earlier or later than this date which influences the estimated hatching date. The beaks were frozen onboard at  $-30\text{ }^{\circ}\text{C}$  and posteriorly preserved in ethanol 70%. Before their analysis for age estimation, beaks were rehydrated in distilled water at  $4\text{--}5\text{ }^{\circ}\text{C}$  for 5 days. The crest



**Fig. 1** Sampling locations of *Moroteuthopsis longimana*. Yellow circle: Ross/Amundsen Seas in the Pacific Sector (from beak ML1 to ML7); Orange circle: South Sandwich Islands in the Atlantic Sector (from beak ML8 to ML10); Red dashed line: position of Antarctic Polar Front. AS Amundsen Sea, RS Ross Sea, SS Scotia Sea, SSI South Sandwich Islands

length, hood length, rostral length and height in both upper and lower beaks were measured using a digital calliper ( $\pm 0.01\text{ mm}$ , Table 1, Supplementary Fig. 1, Supplementary Table 1). Beaks' weight (W) was determined using a high precision balance ( $\pm 0.1\text{ mg}$ ) (Table 1). Estimated dorsal mantle length (DML) and mass (M) of the individual were calculated for lower beaks using the following allometric equations:  $\text{DML} = -22.348 + 37.318\text{LRL}$  and  $\text{M} = 0.713\text{LRL}^{3.152}$  (Adams and Klages 1987).

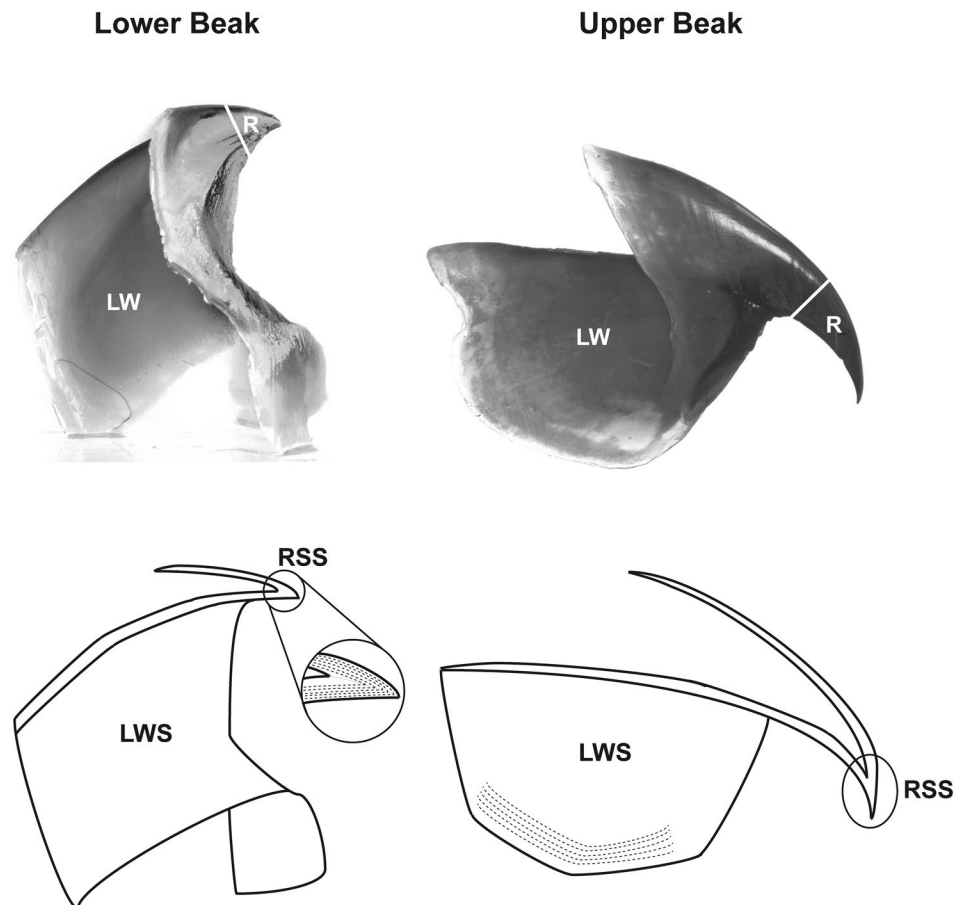
The age of the individuals was studied through the analysis of micro-increments in the rostrum sagittal section (RSS) and the lateral wall surface (LWS) of beaks, following the methodologies described in Perales-Raya et al. (2010) (Fig. 2). Briefly, the RSS were obtained by sectioning the beak into rostrum area and lateral wall using a stainless scissors. The rostrum area was laterally mounted in a microscope slide using an acrylic resin ClaroCit Struers®. After drying, the piece was ground using a 1200 grit carborundum sandpaper to obtain the sagittal section, and posteriorly polished using a  $1\text{ }\mu\text{m}$  diamond paste to remove the scratches of the surface. The RSS surface was examined under a Nikon AZ100 microscope with violet and ultraviolet epi-illumination, and magnifications between  $100\times$  and  $200\times$ . All RSS were photographed using an attached Nikon digital camera 5MP and the software NIS-Elements F3.2, Microscope Imaging Software, Nikon®. Two readings were performed by experienced readers and the precision between both readings estimated through the coefficient of

**Table 1** Sampling and measurements of *Moroteuthopsis longimana* lower beaks collected from the stomachs of Antarctic toothfish

Beak	Sampling season	Place of catch	Individual		Lower beak				
			DML (mm)	M (g)	LRL (mm)	LCL (mm)	LHL (mm)	H (mm)	W (g)
ML1	Summer	Ross/Amundsen Sea	442	2019	12.5	20.6	7.1	19.2	0.3
ML2	Summer	Ross/Amundsen Sea	445	2049	12.5	20.4	7.8	21.0	0.2
ML3	Summer	Ross/Amundsen Sea	582	4629	16.2	27.2	9.3	29.0	0.8
ML4	Summer	Ross/Amundsen Sea	369	1180	10.5	17.5	7.3	15.7	0.3
ML5	Summer	Ross/Amundsen Sea	644	6284	17.9	37.4	13.6	31.6	1.2
ML6	Summer	Ross/Amundsen Sea	470	2416	13.2	21.3	7.6	19.8	0.3
ML7	Summer	Ross/Amundsen Sea	486	2679	13.6	27.7	10.4	25.4	0.6
ML8	Winter	South Sandwich Islands	325	808	9.3	16.5	6.0	15.4	0.1
ML9	Winter	South Sandwich Islands	820	13,165	22.6	24.0	9.9	22.4	0.4
ML10	Winter	South Sandwich Islands	680	7412	18.8	33.4	12.8	33.6	1.3

DML estimated mantle length, M estimated mass, LRL lower rostral length, LCL lower crest length, LHL lower hood length, H height, W weight

**Fig. 2** *Moroteuthopsis longimana* beaks and analysed sections. Dashed lines are expected format of the increments in the reading zones of LWS and RSS (Perales-Raya et al. 2014b). R rostrum, LW lateral wall, RSS rostrum sagittal section, LWS inner lateral wall surface



variation, calculated as the ratio of the standard deviation over the mean (Chang 1982; Campana 2001) as follows:

$$CV(\%) = \frac{\sqrt{(R1 - R)^2 + (R2 - R)^2}}{R} \times 100,$$

where R1 and R2 are the number of increments counted in both 1st and 2nd reading and R the average number of increments of both readings. The distance between increments was measured using the software Age and Shape 1.0 (Infaimon S.L.®) preceded by a calibration for the used magnification. In areas of the RSS where the sequence of increments was not clear, an extrapolation was performed

using the previous sequence of the same size. Erosion in the tip of the beak was mitigated by using the next increments counted in the dorsal area of the RSS, where thin increments were visible. This number of lateral increments was used to estimate the eroded length in the reading axis by extrapolating the widths of the same number of previous increments, as described in Perales-Raya et al. (2020). The estimated eroded length percentage was calculated using the following equation:

$$\% \text{ of Eroded Length} = \frac{\text{Eroded length}}{\text{Total reading length}} \times 100$$

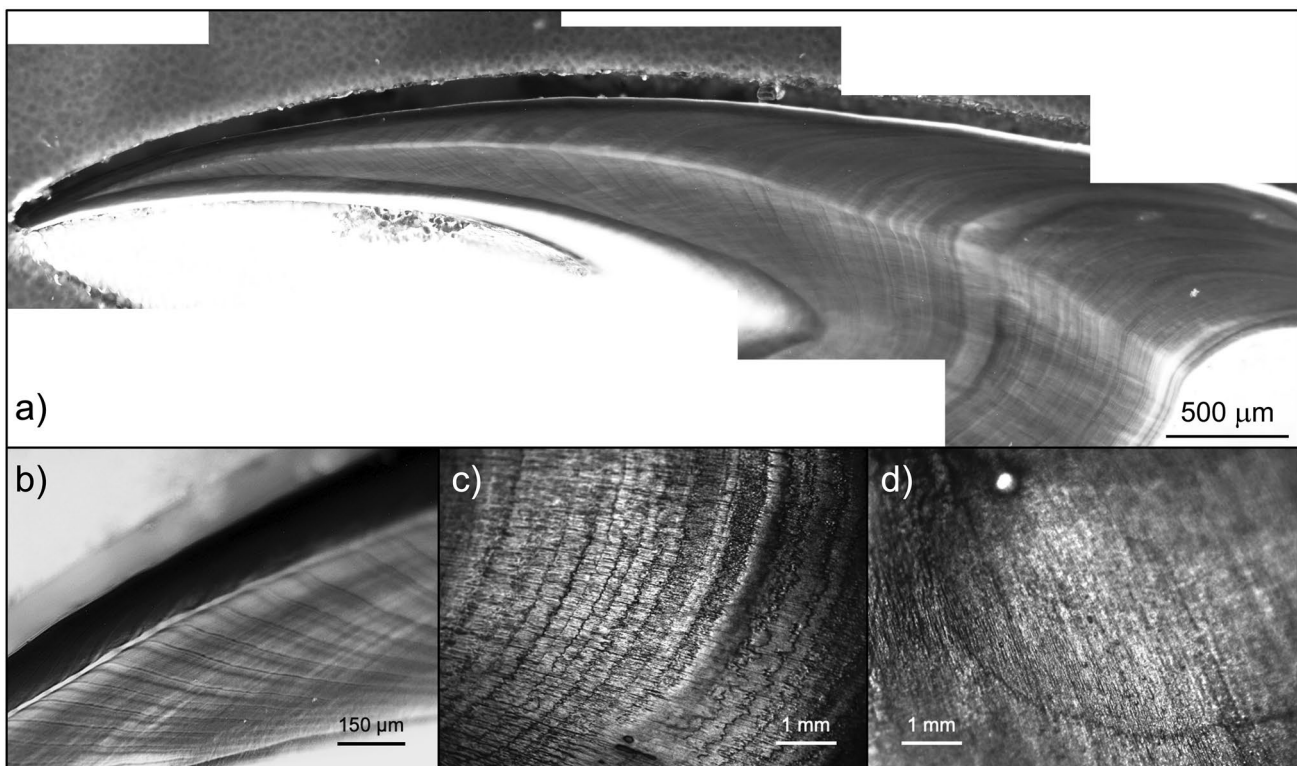
RSS length encompasses the cumulative width of all increments including those in the eroded zone. In zones with a clear sequence of cycles (7–10 lighter increments followed by a darker increment), the number of increments of adjacent areas where only the dark increment of the cycle was visible were estimated using the mean number of increments in the closed cycles (Fig. 3b).

The growth rate (GR) was calculated using the DML and the number of increments counted in R1 as follows:

$$\text{GR}(\text{mm.d}^{-1}) = \frac{\text{Dorsal Mantle Length}}{\text{Number of increments}}$$

Regarding the LWS, beaks were divided into two symmetric halves through a longitudinal cut in the crest. The LWS was placed in a petri dish covered with distilled water and micro-increments observed under a stereomicroscope in the inner side of the lateral walls.

To test if the number of increments in the beak is related with the size of the individual or beak, Spearman's rank correlations were performed between the two readings (R1 and R2) and an average of both readings (R) with the lower beak measurements (lower rostral length (proxy for DML and M), lower crest length, lower hood length, height, and weight) and the size of the RSS. To compare the mean increment width between individuals a Kruskal–Wallis test was performed, preceded by a Shapiro–Wilk normality test. Statistical analyses were performed in Graphpad Prism v.6.0. using  $\alpha = 0.05$  and graphs prepared using Adobe® Photoshop CC 2015.



**Fig. 3** Growth increments in the beaks of *Moroteuthopsis longimana*. **a** composition image of the increments on the rostrum sagittal section (RSS) of lower beaks; **b** lower beak's RSS with cycles of 7–10 light

increments followed by a dark increment; **c** lateral wall showing a zone with regular increments; **d** lateral wall with only one increment

## Results

Increments were only possible to count and measure in the RSS (Fig. 3a). RSS from lower beaks showed to be the most reliable section to analyse as the upper beaks presented highly compacted increments with many of them being indistinguishable. A pattern of cycles was clearly visible in some areas of the RSS from lower beaks. These cycles are composed of 7–10 lighter increments followed by a darker one, as shown in Fig. 3b. These “cycled zones” were mostly located in the medium and anterior regions where older increments are laid down and they showed a more constant sequence. They were used, in some cases, to extrapolate missing adjacent parts of the rostrum tip where only the dark increment of the cycle was visible. Regarding the LWS from both upper and lower beaks, this beak region did not present a regular pattern, with some areas not presenting increments (Fig. 3c, d).

All the beaks used in this study belonged to adult individuals whose DML varied between 325 (ML8) and 820 mm (ML9) (Table 1). The coefficient of variation between both readings in lower beaks' RSS was  $2.7 \pm 2.1\%$  (Table 2), showing that both readings were similar. Thus, we considered the 1st reading to the age estimation. In specimens ML1, ML3, ML4, ML6, ML8, ML9 and ML10, there were eroded increments, but no erosion was observed in the rostrum tips of ML2, ML5 and ML7 (Table 2), with a mean value for the whole sample of 1.87% of eroded length.

The number of increments in the ten lower beaks of *Moroteuthopsis longimana* varied from 464 in ML1 to 820 in ML9 (Table 2). Significant differences were found between the mean increment width of the 10 studied individuals ( $H = 808.8$ ,  $p < 0.0001$ ), with ML6 and ML10 presenting the lowest and highest value, respectively (Table 2). The growth rate of all studied individuals was of  $0.86 \pm 0.30$  mm  $d^{-1}$ , with the smallest growth rate being found in ML8 ( $0.53$  mm  $d^{-1}$ ) and the largest in ML9 ( $1.41$  mm  $d^{-1}$ ) (Table 2). No significant correlations were found between the number of increments and either the individual's size or the beak measurements (Table 3). Considering all beaks together, the mean increment width was of  $15.9 \pm 8.1$   $\mu$ m (Table 2). The mean increment width slightly increased towards the end of the RSS, with a trendline slope of  $0.0023 \pm 0.0004$  (Fig. 4). Considering a daily deposition of increments, it was estimated that four individuals hatched during the winter, whilst two individuals hatched in spring, summer and autumn (Fig. 5, Table 3).

Cumulative curves of increment widths showed that *M. longimana* tend to have a constant growth throughout the entire life, however with at least one period of faster

**Table 2** Number of increments and estimated hatching date of the ten studied *Moroteuthopsis longimana*

Beak	RSS length ( $\mu$ m)	Number of increments		Mean increment width ( $\mu$ m)	Number of eroded increments	% of eroded distance	Growth rate (mm $d^{-1}$ )	Estimated hatching date	Hatching season
		Beak reading 1	Beak reading 2						
ML1	8093	464	468	$17.4 \pm 6.2$	35	4.95	0.95	09/10/2015	Spring
ML2	9629	590	578	$16.3 \pm 9.5$	0	0	0.75	05/06/2015	Autumn
ML3	9576	515	481	$18.6 \pm 8.7$	45	7.74	1.13	19/08/2015	Winter
ML4	9830	624	600	$15.8 \pm 6.8$	9	1.95	0.59	02/05/2015	Autumn
ML5	10,150	556	533	$18.6 \pm 8.3$	0	0	1.15	09/07/2015	Winter
ML6	9112	741	708	$12.3 \pm 5.6$	4	0.57	0.63	05/01/2015	Summer
ML7	10,888	820	782	$13.3 \pm 5.7$	0	0	0.59	18/10/2014	Spring
ML8	7461	604	605	$12.4 \pm 5.2$	15	1.26	0.53	05/08/2007	Winter
ML9	9674	579	639	$16.7 \pm 10.1$	4	0.88	1.41	30/08/2007	Winter
ML10	16,056	819	808	$19.6 \pm 9.4$	20	1.31	0.83	02/01/2007	Summer

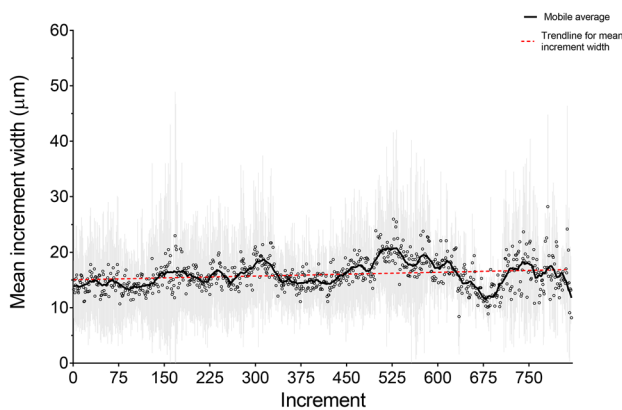
Individuals from ML1 to ML7 were sampled in the Austral Summer 2016/17 in the Pacific Sector and from ML8 to ML10 during the Austral autumn in the Atlantic Sector. Values are mean  $\pm$  standard deviation

RSS length of the rostrum sagittal section, CV coefficient of variation

**Table 3** Spearman’s rank correlation results between the number of increments and the beak measurements

Measurement	R1	R2	R
Lower rostral length (mm)	$p > 0.999$	$p = 0.407$	$p = 0.560$
Lower crest length (mm)	$p = 0.865$	$p = 0.560$	$p = 0.632$
Lower hood length (mm)	$p = 0.632$	$p = 0.387$	$p = 0.427$
Beak height (mm)	$p = 0.892$	$p = 0.584$	$p = 0.657$
Beak weight (g)	$p = 0.946$	$p = 0.785$	$p = 0.759$
RSS length ( $\mu\text{m}$ )	$p = 0.166$	$p = 0.166$	$p = 0.096$

RSS Rostrum sagittal section, R1 first reading, R2 second reading, R mean of both R1 and R2



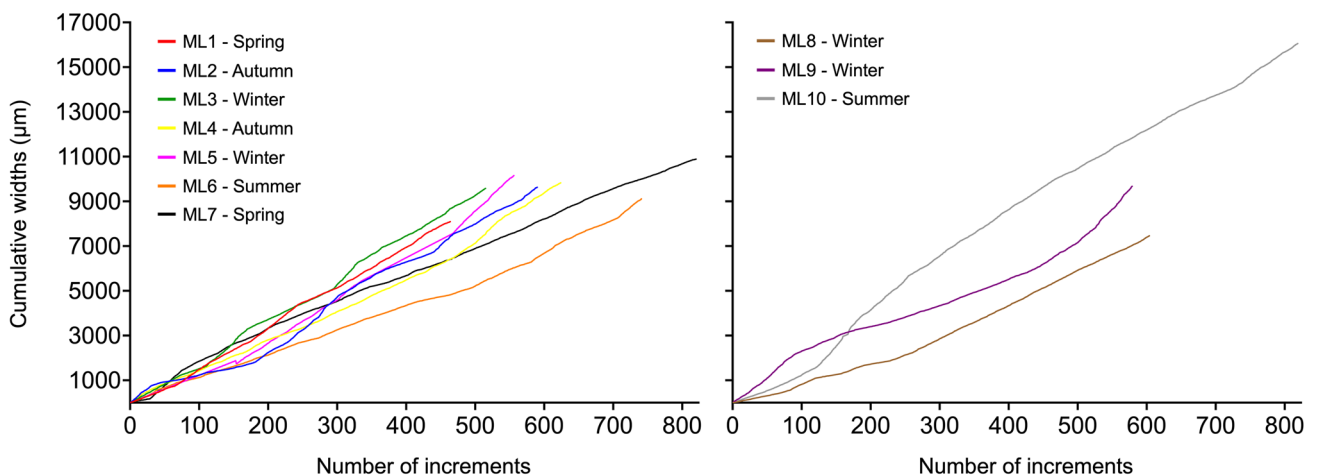
**Fig. 4** Mean increment width of the ten *Moroteuthopsis longimana*’s lower beaks. Straight black line shows the mobile average considering the 20 neighbour increments. Dashed red line shows the trendline. Error bars in grey are shown for all increments

growth (Figs. 5 and 6). In the Pacific Sector, individuals born in summer showed the lowest growth, whereas those born during the winter had larger beaks at the same age (Fig. 5, Table 2). In the Atlantic Sector it was found the opposite, as the two individuals born in winter grew less than the specimen born in summer, which was the largest RSS of the whole sample (Fig. 5, Table 2). The faster growth periods varied between the studied individuals both in number, from 1 to 3, and in seasons (Fig. 6). All individuals from the Pacific sector had, not exclusively, a faster growth period during the summer (Fig. 6). Individuals that hatched in the summer, autumn and winter (ML2–ML6) had at least two periods of fast growth mainly in summer and winter, with exception to ML5 that presented fast growing period in the autumn (Fig. 6). Those hatched during spring (ML1 and ML7) presented only one fast growing period and both in late spring/summer of the first year (Fig. 6). In contrast, individuals from the Atlantic sector had their fast-growing period mostly on spring (ML8 and ML9) and in autumn (ML10) (Fig. 6). ML8 and ML10 had just one period of fast growing, while ML9 had two periods of fast growing in the spring and summer (Fig. 6).

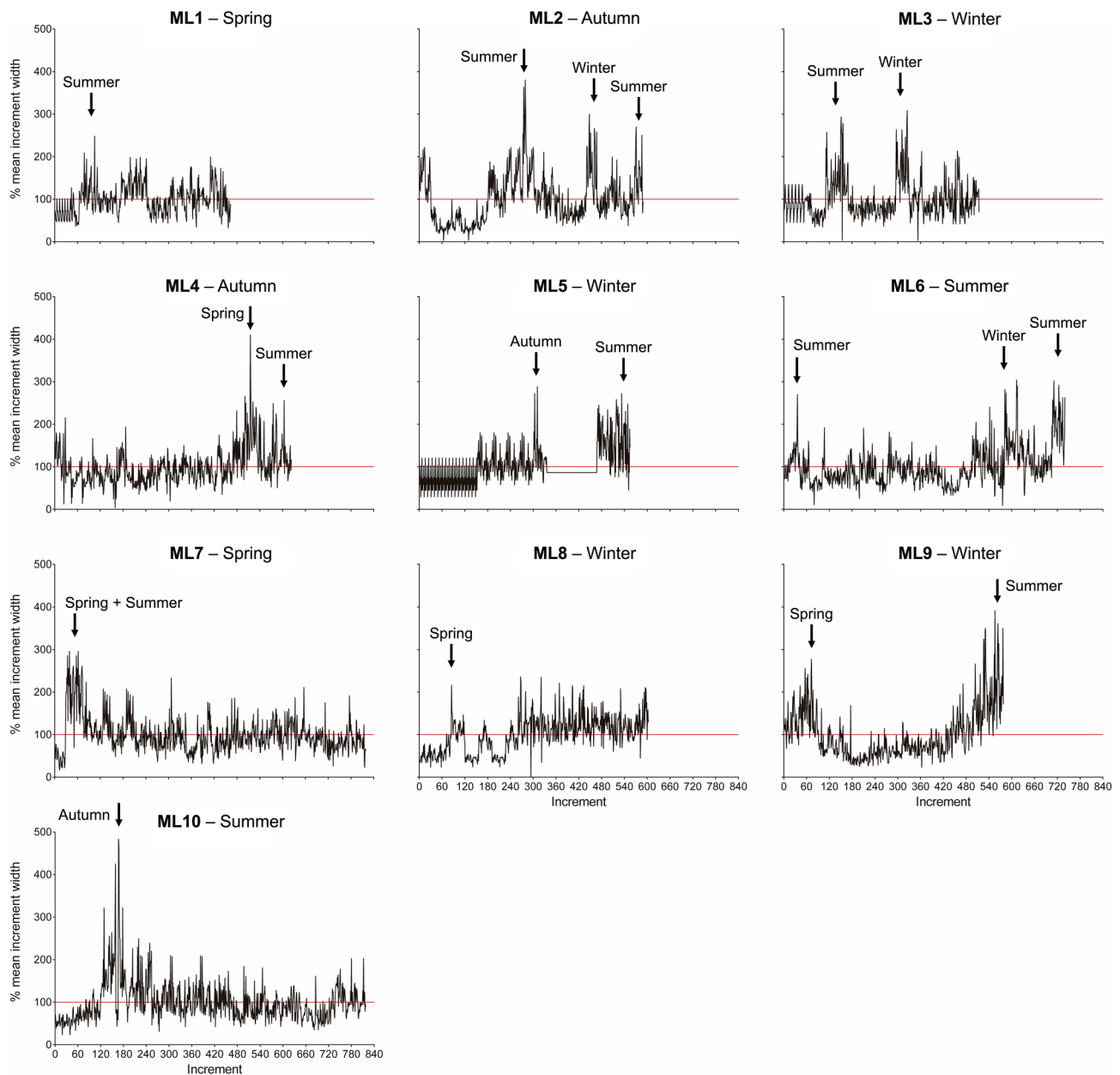
## Discussion

### Use of cephalopod beaks from predators’ stomachs for age and growth studies

Cephalopod beaks have been used to study age and growth of octopods, squid and sepiids, mostly using beaks sampled directly from the individual (e. g., Perales-Raya et al. 2010, 2014b, 2020; Fang et al. 2016; Lishchenko et al.



**Fig. 5** Cumulative curves of increment widths in the ten *Moroteuthopsis longimana*. Left panel: beaks sampled in the Ross/Amundsen Seas (Pacific Sector); Right panel: beaks sampled at South Sandwich Islands (Atlantic Sector)



**Fig. 6** Deviation from the average of mean increment width of each analysed individual. Red line marks 100% (i.e., the % correspondent to the mean width of the individual)

2018; Lu et al. 2022). In our study, we used beaks of the squid *Moroteuthopsis longimana* collected from the stomachs of *Dissostichus mawsoni* from two Southern Ocean regions to test whether cephalopod beaks, obtained from the stomach of predators, can also be used for studies on age and growth of cephalopods. Our results indicate that the LWS and RSS of both lower and upper beaks present increments. However, the RSS of lower beaks is the only beak section where it is possible to observe and count a readable sequence of increments to estimate the age of

oceanic squid using beaks collected from predators' stomachs. Despite the RSS of upper beaks showing more precise results in *Octopus vulgaris* (Perales-Raya et al. 2010), the tight agglomeration of increments in *M. longimana*, due to the shape of this beak in relation to the lower beak, preclude a better reading of RSS in the upper beak. Similar difficulties were found in a previous study that compares the increments in both lower and upper beak of the oceanic squid *Architeuthis dux* (Perales-Raya et al. 2020).



In contrast to the RSS, the absence of increments or a regular pattern in the LWS in both lower and upper beaks, as hypothesised by Clarke (1965), might be related with the period that beaks spent in the stomach of predators. Beaks accumulate in the stomachs of predators from days to months (Piatkowski and Pütz 1994; Xavier et al. 2003; Cherel 2020; Xavier and Cherel 2021). Unfortunately, the specific amount of time the beaks spent in *D. mawsoni*'s stomach is unknown, but the presence of several eroded beaks in the stomachs of this predator suggest that beaks accumulate for, at least, several days before being regurgitated (Queirós, personal observation). The time beaks spent on the stomach can influence the estimation of the hatching date. Nevertheless, as *D. mawsoni* accumulates beaks for just a couple of days, our estimate hatching dates are not highly influenced by this. Even though beaks resist digestion (Clarke 1962), the action of gastric acids in the stomachs of predators can wear the beaks (Adams and Klages 1987; Hills and Fiscus 1988; Piatkowski and Pütz 1994), removing increments from the surface of lateral walls. Since the RSS is not on the surface of the beak in direct contact with the gastric acids, the increments observed in RSS are not affected by this process. Furthermore, a gradient of increasing stiffness, pigmentation, and protein, together with a decreasing content of water and chitin, has been reported from the posterior to the anterior region of the beak (Miserere et al. 2008; Tan et al. 2015). The low content of water in the rostrum compared with lateral walls could provide the rostrum microstructure with more resistance to gastric acids, and therefore, the increment sequence in RSS is less or non-affected by the time elapsed in the predator stomach, comparing with the alteration suffered in the microstructure of LWS.

### Age and growth of *Moroteuthopsis longimana*

Micro-increments in cephalopod beaks are able to record stress events related with physiological and/or environmental changes (Perales-Raya et al. 2014a; Franco-Santos et al. 2015). Such influence is known for other age recording structures, e.g. statoliths in cephalopods (Arkhinpin et al. 2015) and otoliths in fish (Campana and Neilson 1985). The presence of constant “cycled areas” in the RSS, which were not previously observed in other species studied by the authors (e.g. Architeuthidae, Octopodidae, Loliginidae, Sepiidae), can be related with physiological or environmental factors. The presence of several lighter increments followed by one darker may be related with periods of stable food supply experienced by the individuals that enabled the constant deposition of beak material. It may also be related to specific environmental conditions found in the Southern Ocean. During summer and winter seasons there is an absence of a day/night period which may influence the

deposition. Furthermore, *M. longimana* is a pelagic species for which daily vertical migrations have not been confirmed (Collins and Rodhouse 2006), and other daily environmental cycles that may influence other species worldwide, e.g. tides, are also absent. Nevertheless, the presence of lighter increments during these periods did not affect the age estimation of the individual because it was still possible to see and count these increments. Previous studies on Antarctic octopods also did not find such patterns (Schwarz et al. 2019), however it did not analysed RSS and studied coastal species where, for example, tides create a daily environmental cycle. Nevertheless, the analyses of LWS using 32–50× magnification may have precluded the identification of these soft increments in Schwarz et al. (2019). Cycles of micro-increments were also recognized in the LWS of *M. longimana* by Clarke (1965). Although the author did not analyse RSS and no constant pattern was found in the LWS cycles, the mean number of micro-increments by cycle was between 8 and 12, which is not far from those obtained in the present study (7–10) and, therefore, it seems a specific feature of the growth in *M. longimana*.

The rostrum (and hence the RSS) is exposed to erosion during feeding activity of the animal, however, with different species showing higher erosion in lower beaks while others in the upper beak (Liu et al. 2015; Fang et al. 2016; Perales-Raya et al. 2020). In our study the RSS of the upper beak did not present the most favourable beak section to count increments, and we were not able to determine which beak showed the highest percentage of an eroded tip. However, using the technique of Perales-Raya et al. (2020) it might be possible to determine the original length of an eroded RSS and to estimate the number of increments in such area, overcoming such issue.

Previous studies in temperate waters using beaks of *O. vulgaris* and *O. maya* validated the daily deposition of increments in octopod beaks (Bárceñas et al. 2014; Perales-Raya et al. 2014b). The beaks of *M. longimana* were sampled south of the Antarctic Polar Front where the sea surface temperature ranges from  $-1.5$  °C near the continent to  $\sim 4$  °C at the frontal zone (Griffiths 2010). A recent study on four Antarctic cold-water ( $-2$  to  $3$  °C) octopods (i.e. *Megaleledone setebos*, *Muusoctopus rigbyae*, *Pareledone aequipapillae*, *Pareledone charcoti*) showed that in such cold environments the periodicity of increment deposition might be longer than one day, at least in the LWS where those authors estimated the age (Schwarz et al. 2019). Considering a daily deposition, our results from the RSS suggest that adult *M. longimana* can live up to 820 days, i.e. more than 2 years. This result was obtained for an individual sampled in the Pacific sector with 486 mm of DML. A similar life span (819 days) was estimated for an individual captured in the Atlantic sector, however, with a DML of 680 mm. Our results are in accordance with Bizikov (1991) who, using the

gladius, suggested that *M. longimana* measuring between 420 to 470 mm of DML had an age of 21–22 months and a life span of 5 years, yet it considered a linear and similar growth pattern of individuals throughout their life and to maximum size of 1100 mm of DML (Bizikov 1991; Lynnes and Rodhouse 2002). However, our results do not support this constant and equal growth.

The absence of a correlation between the number of increments and the LRL (proxy for DML) of individuals and beak measurements, as well the presence of, for example, two individuals with 820 and 680 mm of estimated DML presenting 579 and 819 increments in the RSS suggest that different individuals present different growth rates and individuals of similar size might not have the same age. This is supported by significant differences between increment widths of the studied individuals, varying from 12.3 to 19.6  $\mu\text{m}$  and with growth rates ranging between 0.53 and 1.41  $\text{mm d}^{-1}$  of DML. These differences are reinforced when comparing individuals from different locations, with individuals captured in the Ross/Amundsen Sea in the Pacific sector and around South Sandwich Islands in the Atlantic sector showing differences in terms of growth rate in different seasons, but also in the size reached at similar ages (number of days). Similar variability was also found in the *Architeuthis dux* [mean increment width from 8.3 to 20.7  $\mu\text{m}$  (Perales-Raya et al. 2020)] and the *Dosidicus gigas* [daily growth rate from 0.65 to 2.12  $\text{mm d}^{-1}$  (Hu et al. 2016)] as well as in several cold-water octopods (Schwarz et al. 2019). This is supported by the similar mean increment width of the ten studied individuals throughout the beak (trend line with a slope of approximately zero; see Fig. 4). Nevertheless, mobile average shows that the increment width variation between individuals slightly increases towards the end of their life, suggesting that growth rates might be slightly larger towards the end of the individual's life. Nevertheless, this pattern can arise from the lower number of individuals older than 600 days that increases the variability of increment width, as well the different hatching seasons. Indeed, previous studies in cold-water squid showed that growth rate differ between squids of the same species but from different locations (Rodhouse et al. 1994). This pattern differs from *A. dux*, and from other oceanic squid, inhabiting warmer waters whose increment width decreases throughout life (Perales-Raya et al. 2020). Though it is similar with other Southern Ocean species that maintain similar growth rate throughout their entire life, e.g. *Illex argentinus* and *Martialia hyadesi* (Rodhouse and Hatfield 1990; Rodhouse et al. 1994), these differences between cold- and warmer-water species might be related with environmental conditions, where oxygen-enriched cold waters may enable the maintenance of growing rates throughout the squid's life (Chapelle and Peck 1999).

Estimated hatching dates from our study suggest that *M. longimana* hatches throughout the year with individuals born in all the four seasons, rather than only between autumn and spring (Bizikov 1991) or just in the autumn/winter (Clarke 1980; Cherel and Weimerskirch 1999; Filippova 2002; Xavier and Croxall 2007). Nevertheless, the absence of a specific death date for each of the studied individuals might influence the estimated hatching date and, ultimately, the hatching season. For example, the estimated hatching date in early January for individuals hatched in the summer may suggest that, if predated earlier, these would hatch in the autumn rather than in the summer. This would support the hypothesis of a hatching season between autumn and spring proposed by Bizikov (1991).

Despite all individuals present a constant growth rate throughout their life cycle (discussed above), between one and three periods of faster growing was also observed for every studied individual. In samples from the Pacific sector with just one fast-growing period, this occurred in the summer. In the individuals with three fast growing periods, two of these were in the summer. Moreover, the winter also seems to be an important season for *M. longimana* in the Pacific sector since three of the six studied squid also had a faster growing period in that season. Similar results were found by Bizikov (1991) in individuals studied from the northern Weddell Sea (Atlantic Sector), which presented a faster growth rate in the summer and early winter. In contrast, individuals from the South Sandwich Islands in the Atlantic Sector had these faster growing periods earlier in the year. Two of the three studied individuals had these faster growing periods in spring, whilst the third grew faster in autumn. Notwithstanding, we must have in mind that this can also be influenced by the back calculation of the hatching date.

The differences between individuals from the Pacific and Atlantic sectors in terms of growth rate and seasons of fast growth might relate with the different conditions found between both seas, but also within each sea across seasons, and their productivity that reflects in the *M. longimana*'s prey biomass. Both seas are located south of the Antarctic Polar Front, however, with the South Sandwich Islands being located within the Antarctic Circumpolar Current whilst the Ross Sea is placed south of the Southern Boundary of this current (Whitehouse et al. 2008; Murphy et al. 2013; Smith et al. 2014; Trathan et al. 2014). Apart from their location in relation to the Antarctic Polar Front, there are several differences between both areas. The Ross and Amundsen Seas are two of the most active cyclogenetic areas in the Southern Ocean with an annual cycle of sea ice abundance (Smith et al. 2014). A phytoplankton bloom occurs in spring. However, due to iron limitation, it only lasts a several weeks, and it is not followed by a great increase in zooplankton abundance (Smith et al. 2014). Indeed, zooplankton abundances

are of approximately 15% of those found in the Scotia Sea where the South Sandwich Islands are located (Smith et al. 2014). Waters at the South Sandwich Islands are slightly warmer with winter sea-ice not covering the entire archipelago [with the exception of some years, e.g. 2014, when it extends up to the northern islands (Hart and Convey 2018)] and are highly productive in the spring and summer, being one of the most productive areas in the Southern Ocean (Murphy et al. 2013; Trathan et al. 2014). The phytoplankton bloom induces an increase of Antarctic krill *Euphausia superba* and other zooplankton species' abundances (Atkinson et al. 2004; Murphy et al. 2013; Trathan et al. 2014). As *M. longimana* mostly feeds on zooplankton and zooplanktivorous species (Nemoto et al. 1985, 1988; Collins and Rodhouse 2006), this higher food availability might explain the faster growth of squid born in summer. Furthermore, it also explains the differences between individuals from the Ross/Amundsen Seas and those from South Sandwich Islands. The limited period of enhanced productivity during spring in the Ross/Amundsen Seas forces individuals from this area to use all the available resources to grow in this season, whilst those from South Sandwich Islands, as the zooplankton bloom extends to the summer, can invest in growth in this season. Nevertheless, the phytoplankton bloom in the Scotia Sea is known to influence regions downstream the Antarctic Circumpolar Current during 4–5 months towards late autumn and winter (Murphy et al. 2013; Trathan et al. 2014). As result, there is a higher food availability in these seasons in the Ross/Amundsen Seas region, enhancing the growth of *M. longimana* breeding in winter. Furthermore, different species of phytoplankton exist in both regions, with the phytoplankton community from the Ross and Amundsen Sea being dominated by smaller species (Korb et al. 2008; Smith et al. 2014) than those in the Scotia Sea (South Sandwich Islands) where it is mainly composed by large diatom species (Korb et al. 2008). Notwithstanding, it is important to have in mind that there is no evidence that Southern Ocean squid spend their entire life in the same sector thus it is possible that these individuals spent part of their life cycle in other Southern Ocean sector.

Jarre et al. (1991) also found differences in the growth rate of *M. longimana* individuals from different locations when re-examining the size of beaks collected from the stomachs of sperm whales at Durban and Donkergat in the Indian and Atlantic coasts of South Africa, respectively, by Clarke (1980). The similarities in the growth rates between individuals from the Pacific sector in this study and those from the Weddell Sea in the Atlantic Sector studied by Bizikov (1991) can be explained by similar environmental conditions. Indeed, the Weddell Sea is also located south of the Antarctic Circumpolar Current and has lower primary productivity in the spring/summer in comparison to the neighbouring waters of the South Sandwich Islands and

South Georgia archipelagos (Holm-Hansen et al. 2004; Murphy et al. 2007). This suggests that *M. longimana* presents different growth patterns in the regions they inhabit in the Southern Ocean and further studies on the life-history of this squid species should consider this variability.

## Conclusion

Using beaks of the Southern Ocean squid *Moroteuthopsis longimana* collected from the diet of *Dissostichus mawsoni* in both the Ross/Amundsen Seas (Pacific sector) and the South Sandwich Islands (Atlantic sector) we showed that the rostrum sagittal section (RSS) of lower beaks collected from predators' stomachs allows the estimation of the age and growth of squids. In contrast, the RSS of upper beaks and the lateral wall of both lower and upper beaks, despite presenting increments, did not show a regular pattern along the full growth axis that is necessary to age estimation of *M. longimana*.

Regarding the life history of *M. longimana*, we showed that this squid species can live for more than 2 years. Yet, future studies should try to validate the periodicity of increment formation in cold-water and deep-sea squid to confirm the daily deposition as in temperate and tropical species. Furthermore, our results indicate that different individuals present different growth rates and individuals of similar size can have different ages. *M. longimana* presents a nearly constant growth pattern throughout its life, with at least one period of faster growth during summer in the Pacific sector and during spring in the Atlantic sector. Differences in growth rate and seasonal fast-growing periods found between these areas in the Southern Ocean suggest that environmental conditions affect the growth of this species and future studies on the life history of *M. longimana* from different locations should consider this.

Further work should also focus on studying the age and growth of other Southern Ocean squid species that typically avoid net sampling, but whose beaks can be sampled from predators' stomachs. Increasing the knowledge on the life history of these cephalopods will allow us to better understand the functioning of this ecosystem. Furthermore, experimental studies on Southern Ocean squids are required to confirm if their beaks present daily depositions as in cephalopods of temperate seas or, as has been suggested in studies of cold-water octopods, the deposition of these increments can take a longer period.

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**Data availability** The datasets generated during and/or analysed during the current study are available at <https://doi.org/10.5281/zenodo.7351679>

## Declarations

**Conflict of interest** The authors have no relevant financial or non-financial interests to disclose.

**Ethics approval** No approval of research ethics committees was required to accomplish the goals of this study because we used squid beaks sampled from the stomachs of Antarctic toothfish captured in licensed fishing vessels by the Commission for the Conservation of Antarctic Marine Living Resources (FV Antarctic Discovery) and the Government of South Georgia and the South Sandwich Islands (FV San Aspiring).

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