Marine Biology (2020) 167:56
https://doi.org/10.1007/s00227-020-3666-2

ORIGINAL PAPER

Ontogenetic changes in habitat and trophic ecology of the giant Antarctic octopus *Megaleledone setebos* inferred from stable isotope analyses in beaks

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Received: 11 December 2019 / Accepted: 6 February 2020

Abstract

The giant Antarctic Octopus *Megaleledone setebos* is the largest Southern Ocean octopod whose ecology is poorly known. Here, we study ontogenetic shifts of habitat and trophic ecology of *M. setebos* throughout its life cycle by stable isotopic analysis of δ^{13}C and δ^{15}N on its beaks collected from the diet of Antarctic toothfish in Amundsen and Ross Seas (Antarctica). Values of δ^{13}C (from − 24.3 to − 19.4‰) differed between beaks of individuals from different capture locations, thus reflecting the ability of *M. setebos* living in different habitats. Despite sequential sampling along beaks showed a small (< 2.3‰), but significant variation in lower beak's δ^{13}C values, a relation with δ^{15}N values suggests that such differences are related to changes in the diet with *M. setebos* inhabiting the same area its entire life. Values of δ^{15}N differed between beaks of individuals from different capture locations, suggesting that different habitats of *M. setebos* are associated with different diets. Serial sampling along the beaks (from + 4.2 to + 10.7‰) suggests an ontogenetic change of, at least, one trophic level from juvenile to adult. We also report a capture of two large intact specimens from Dumont D'Urville Sea (Antarctica): a male with 1150 mm of total length and 18,300 g of mass and a female with 1030 mm of total length and 10,061 g of mass. The beaks of these both specimens, confirmed to be of *M. setebos* through genetic analysis, were also used to confirm the identification of *M. setebos* collected from Antarctic toothfish stomachs.

Introduction

The Southern Ocean is home to some of the largest animals found in the world's oceans (Chapelle and Peck 1999) including the giant Antarctic octopus *Megaleledone setebos* (Robson 1932), which is the largest Southern Ocean octopod. *M. setebos* is currently placed in the genus *Megaleledone* (family: Megaleledonidae), but was originally described in the genus *Graneledone* (Robson 1932). Prior to the work of Allcock et al. (2003), it was also referred to under the synonym *M. senoi* (Taki 1961). *M. setebos* is endemic to the Southern Ocean with the biggest specimen had ≈ 280 mm of mantle length and weighted ≈ 24,000 g of total weight (Piatkowski et al. 2003). It is a benthic species with a circumpolar distribution restricted to the continental shelf and slope of Antarctic waters between 32 and 850 m deep (Lu and Stranks 1994; Allcock et al. 2003).

Compared to oceanic squid, coastal benthic octopods are slower moving and easier to catch with traditional scientific methods [e.g., using demersal trawls (Piatkowski et al. 2003)]. However, despite its large size, *M. setebos* is relatively rarely captured and poorly studied. *M. setebos* has

Responsible Editor: R. Villanueva.

Reviewed by undisclosed experts.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00227-020-3666-2) contains supplementary material, which is available to authorized users.

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Published online: 08 April 2020

been reported from the diet of predators, including Antarctic toothfish (*Dissostichus mawsoni)* and Weddell seals (*Leptonychotes weddellii)* (Lipinski and Woyciechowski 1981; Stevens et al. 2014). As predator, preliminary diet data suggest that *M. setebos* feeds mainly on smaller organisms, such as ophiuroids, amphipods, and fish (Piatkowski et al. 2003). All these diet studies used a conventional methodology (stomach contents), which provide a snapshot of the diets (Barrett et al. 2007), but do not give longer term trophic information.

Beaks, undigestible chitinous structures that continuously grow through individuals' life cycle (Clarke 1986), collected from predator diets can provide important information for several characteristics of its entire life cycle (Xavier et al. 2015).

Stable isotope analysis of δ^{13}C (ratio ^{12}C/^{13}C) and δ^{15}N (ratio ^{14}N/^{15}N) in beaks are now largely used to study the habitat and trophic ecology of cephalopods (Cherel and Hobson 2005; Cherel et al. 2011; Golikov et al. 2018). As δ^{13}C values relate to the carbon source in the food chain, and are relatively stable along the trophic levels, they are used as a proxy for the habitat location of organisms (Cherel and Hobson 2007), e.g., offshore/inshore and benthic/pelagic (Newsome et al. 2007). Conversely, values of δ^{15}N increase along the food web and are a good proxy for the trophic level of organisms (McCutchan et al. 2003). When applied to different regions of a cephalopod beak, stable isotope analysis has demonstrated that is a valuable technique to study the habitat and trophic ecology throughout the life cycle of individual cephalopods (Guerra et al. 2010; Queirós et al. 2018, 2019).

The aim of this study is to investigate the habitat and trophic ecology of *M. setebos* throughout its life cycle. This is achieved by applying stable isotopic analyses along the lower and upper beaks of *M. setebos* collected from Antarctic toothfish stomachs. Due to the scarcity of reports of the capture of specimens of *M.* setebos, we also report the capture of two large intact specimens.

Materials and methods

Sample collection

The upper and lower beaks of *M. setebos*, identified by comparison with the beaks of the two captured specimens (see below), were collected from the stomachs of Antarctic toothfish *D. mawsoni* captured in the Amundsen Sea [from ~ 68º to ~ 71ºS—beaks ML1 (beak code: *Megaleledone* Lower 1) to ML7 and MU1 (beak code: *Megaleledone* Upper 1) and MU2, Table 1] and the Ross Sea (at ~ 75ºS—beaks ML8, ML9, MU3, and MU4, Table 1) (CCAMLR subareas 88.2

Table 1 Beak measurements for *Megaleledone setebos* from Antarctic toothfish stomachs

Beak	Lower/upper	LHL/UHL (mm)	LCL/UCL (mm)	Analysed subsections	δ^{13}C values (‰)	δ^{15}N values (‰)	C:N mass ratios
ML1	Lower	13.2	27.6	11	− 22.5 ± 0.5	+ 6.9 ± 1.0	3.5 ± 0.3
ML2	Lower	18.4	34.2	14	− 22.2 ± 0.5	+ 6.2 ± 1.2	3.4 ± 0.2
ML3	Lower	14.5	27.7	9	− 22.1 ± 0.4	+ 7.4 ± 1.0	3.5 ± 0.4
ML4	Lower	12.6	25.3	11	− 22.5 ± 0.7	+ 6.9 ± 1.0	3.5 ± 0.4
ML5	Lower	15.4	26.8	10	− 23.4 ± 0.3	+ 5.8 ± 1.2	3.4 ± 0.3
ML6	Lower	14.1	27.8	12	− 23.0 ± 0.5	+ 6.4 ± 1.4	3.4 ± 0.3
ML7	Lower	14.1	25.3	12	− 22.8 ± 0.3	+ 6.1 ± 0.8	3.5 ± 0.2
ML8	Lower	19.6	39.4	16	− 21.4 ± 0.3	+ 6.5 ± 1.0	3.5 ± 0.2
ML9	Lower	13.1	27.7	16	− 20.3 ± 0.6	+ 7.5 ± 1.2	3.5 ± 0.2
Kruskal–Wallis test					$H_9 = 88.12$ $p < 0.001$	$H_9 = 22.58$ $p = 0.003$	$H_9 = 8.169$ $p = 0.417$
MU1	Upper	20.7	39.9	20	− 22.2 ± 0.3	+ 8.7 ± 1.1	3.7 ± 0.2
MU2	Upper	20.1	34.0	16	− 22.9 ± 0.5	+ 7.3 ± 0.8	3.7 ± 0.2
MU3	Upper	27.9	56.0	25	− 20.9 ± 0.4	+ 8.7 ± 1.3	3.5 ± 0.2
MU4	Upper	18.9	35.1	16	− 20.6 ± 0.3	+ 8.6 ± 1.1	3.5 ± 0.2
Kruskal–Wallis test					$H_4 = 62.18$ $p < 0.001$	$H_4 = 18.50$ $p < 0.001$	$H_4 = 11.94$ $p = 0.007$

LHL lower hood length (lower beaks), *UHL* upper hood length (upper beaks), *LCL* lower crest length (lower beaks), *UCL* upper crest length (upper beaks). Values are mean ± SD.

ML8, ML9, MU3, and MU4 were collected onboard the *FV* Janas at higher latitudes than the remaining specimens that were captured onboard the *FV* Antarctic Discovery.

and 88.1, respectively) onboard the *Fishing Vessel* Antarctic Discovery (fishing season 2016/17) and *Fishing Vessel* Janas (fishing season 2005/06), respectively. Beaks were initially frozen at – 40 °C onboard and later preserved in 70% ethanol in the laboratory.

Stable isotopic analyses

Nine lower and four upper beaks of *M. setebos* from different toothfish stomachs were identified by comparison with the beaks removed from whole individuals (see below). LHL (lower hood length) and LCL (lower crest length) in lower beaks and UHL (upper crest length) and UCL (upper crest length) in upper beaks were measured using digital callipers (± 0.1 mm, Fig. 1). Allometric equations are not available, so mantle length and mass could not be estimated. Beaks were sectioned, using a small saw blade, from the tip of the rostrum (R) to the end of the crest (C) in a section along the crest in both lower and upper beaks (Fig. 1). Each subsection of the crest was 1 mm thick in the anterior part of the section and 2 mm thick posteriorly. We used both thicknesses (1 and 2 mm), because previous studies that sectioned cephalopod beaks found a *plateau* in δ^{13}C and δ^{15}N values in the posterior region of the beaks' hood [upper beaks (Guerra et al. 2010; Queirós et al. 2018)].

Each subsection was cleaned in 80% ethanol, stored in a separate microtube, and dried in an oven at 60 °C overnight. Subsections were ground to a fine powder using a mixer mill Retsch® MM400 for 10 min with a frequency of 30 s^{-1}. Approximately 0.35 mg of each subsection were weighed in a tin capsule using a Mettler Toledo® UMX2 ultra-microbalance. Values of δ^{13}C and δ^{15}N were determined using a Continuous Flow Isotope Ratio Mass Spectrometer (Delta V™ Advantage Thermo Scientific®) with an organic elemental analyser (Flash™ EA 1112 Thermo Scientific®) at MAREFOZ (Figueira da Foz, Portugal), following Queirós et al. (2018).

Results are presented in delta (δ) notation in per mil (‰) using the equation:

$$\delta X = \left(\left[R_{sample} - R_{standard}\right] - 1\right) \times 1000,$$

where X represents ^{13}C or ^{15} N, and R the ratio between light and heavy isotope, i.e., $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively. Vienna Pee-Dee Belemnite (V-PDB) and Atmospheric N^2 (AIR) were used as standard for δ^{13}C and δ^{15}N, respectively. Throughout the analysis, machine internal errors were calculated (< 0.1‰ for δ^{13}C and < 0.3‰ for δ^{15}N) using an isotopic reference material (Thermo® acetanilide STD: 71.09% of C and 10.36% of N).

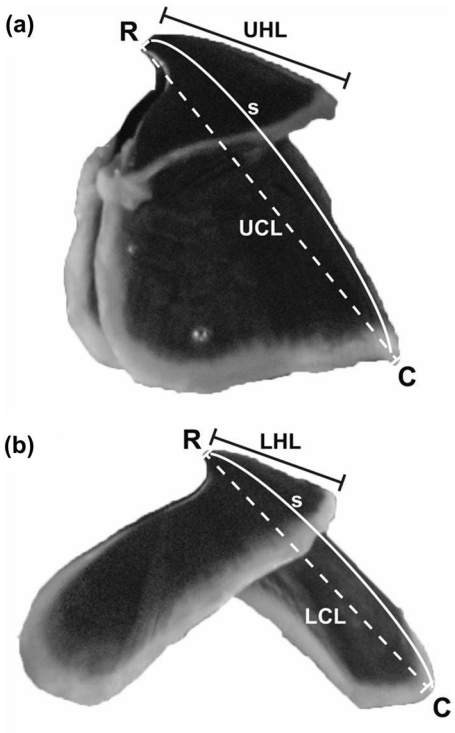

Fig. 1 Beaks of *Megaleledone setebos*. **a** Upper beak, **b** Lower beak. R is the tip of the rostrum, C the end of crest and S the section analysed. *UHL* upper hood length, *UCL* upper crest length, *LHL* lower hood length, *LCL* lower crest length

Genetic and beak identification of two *Megaleledone setebos* specimens

Two specimens of *M. setebos*, one male and one female, were captured at the Dumont D'Urville Sea onboard of the *FV* Antarctic Discovery during the Austral Summer (24th and 25th of January of 2017) using bottom set longlines with an autoline system (Fenaughty 2008) baited with ommastrephid squid to catch Antarctic toothfish. The two octopuses were frozen onboard and kept at – 40 °C for later analysis. In the laboratory, each specimen was measured (mantle and total length) using a ruler (± 1 mm), sexed, and weighed using a digital balance (± 1 g). As the larger specimen exceeded the balance limit (15 kg), the onboard capture weight was used with a larger error (± 0.1 kg). Beaks from both specimens were removed and photographed (Fig. 1), and the LHL and UHL measured using digital callipers (± 0.01 mm) (Fig. 1). Beaks were measured twice, once including the transparent (fresh) material and a second time for only the sclerotinized (older) material. The specimens were refrozen, and their beaks were preserved in 70% ethanol. The specimens and beaks were donated to the Museum of New Zealand Te Papa Tongarewa (NMNZ). Specimens were catalogued and numbered NMNZ M.328333 and NMNZ M.328334.

To confirm species identification, both individuals (NMNZ M.328333 and NMNZ M.328334) were sampled for genetic analysis. Tissue snips from specimens were fixed in 100% EtOH and stored at – 20 °C until analysis. Samples were extracted using EconoSpin (Epoch Life Science) spin columns with QIAGEN reagents following the protocols for the DNeasy Blood and Tissue Kit (QIAGEN).

The DNA barcode region—648 bp from the 5′ end of cytochrome *c* oxidase subunit 1 (COI)—was amplified using 12.5 µl reaction volumes with the following reaction volumes: 6.25 µl 10% trehalose, 2 µl ddH_2O, 1.25 µl 10X buffer, 0.625 µl $MgCl_2$ (50 mM), 0.1 µl primer LCO1490 (Folmer et al. 1994; 10 µm), 0.1 µl primer HCO2198 (Folmer et al. 1994; 10 µm), 0.0625 µl 10 mM dNTPs, 0.06 µl Platinum Taq polymerase (5 U/µl), and 2 µl of DNA (~ 50 ng/µl). The reaction profile was as follows: hot start of 94 °C for 1 min; 5 cycles of 94 °C for 40 s, 45 °C for 40 s, 72 °C for 1 min; 35 cycles of 94 °C for 40 s, 51 °C for 40 s, 72 °C for 1 min; extension at 72 °C for 5 min, hold 4 °C indefinitely.

PCR products were visualised using a 1% agarose gel stained with GelRed (Biotium). Products that showed a single, clear band were bi-directionally sequenced by Macrogen (Korea) using the same primers used for the PCR. Sequences were edited in CodonCode Aligner (v 8.0.1) (CodonCode Corporation, Dedham, MA) and uploaded to the Barcode of Life Data System [BOLD (Ratnasingham and Hebert 2007)] in a public project titled 'DNA barcoding the largest specimens of *Megaleledone setebos*' (project code: DBMS) and submitted to GenBank (Accession numbers MT048392 and MT048393 for NMNZ M.328333 and NMNZ M.328334, respectively).

Sequences were screened for contamination using the Basic Local Alignment Search Tool (BLAST) through GenBank. Specimen IDs were confirmed using the BOLD 'All Barcode Records on BOLD' identification engine in May 2018 (Ratnasingham and Hebert 2013). Specimen IDs were also confirmed using the Barcode Index Number (BIN) analysis in BOLD, which uses a clustering algorithm to automatically generate operational taxonomic units based on COI, which have a high concordance with species (Ratnasingham and Hebert 2013).

Vertical distribution of *Megaleledone setebos*

To study if bigger specimens of *M. setebos* are found in deeper waters, a correlation between the size (total and mantle length) of *M. setebos* specimens [analysed in Allcock et al. (2003) and both intact specimens in this study] and the depth of their capture (Table 2), plus the two captured here, was performed to test if bigger specimens are captured in deeper waters (Table 2).

When a specimen was captured using a longline, the average depth of both longline ends was used.

Table 2 Total length, mantle length, and depth of capture of individuals used in the correlation octopus' size and depth

Total length (mm)	Mantle length (mm)	Depth (m)
	105	200
335	105	202
350	110	120
410	110	154
420	115	190
460	135	655
470	145	120
527	190	800
542	169	387
	170	280
590		750
601	181	431
620	150	312
650	200	322
696	234	465
715	195	33
748	207	435
750	230	476.5
830	200	219
900	280	867
1030	**300**	**775**
1150	**290**	**865.5**

These values are from Allcock et al. (2003) except the both individuals in bold that are from this study.

Statistical analysis

Regarding stable isotopic analysis, all the subsections with a C:N mass ratio above 4.1 were discarded from the study as it was likely that these subsections had higher levels of chitin than the others, thus precluding comparison of $\delta^{15}N$ values between sections (Cherel et al. 2009).

Differences in the values of $\delta^{13}C$ and $\delta^{15}N$ and of C:N mass ratios between the nine lower beaks and the four upper beaks were tested using Kruskal–Wallis test followed by Dunn's multiple comparison test. As all the beaks had a similar pattern [i.e., continuous increase of $\delta^{13}C$ and $\delta^{15}N$ values (see Online Resource Fig. A1)], differences in $\delta^{13}C$ and $\delta^{15}N$ values between subsections were also tested using a Skillings–Mack test proceeded by the Nemenyi post hoc test [only the subsections common to all the beaks, i.e., until the last subsection of the beak with the lower number (ML3 and MU2), were tested]. A Spearman rank correlation was performed between $\delta^{13}C$ values and $\delta^{15}N$ values of all individuals and all subsections analysed. All tests were performed using $\alpha = 5\%$ and in R [version 3.5.2; (R core team 2019)] using the packages "Car" (Fox and Weisberg 2019), "PMCMR" (Pohlert 2014), and "Skillings.Mack"

(Srisuradetchai 2015). Values are provided as mean ± standard deviation (SD) (Fig. 2).

Results

Stable isotope analysis

All beaks used in this study had fully darkened wings. Values of LHL ranged from 12.6 to 19.6 mm, LCL from 25.3 to 39.4 mm, UHL from 18.9 to 27.9 mm, and UCL from 34.0 to 56.0 mm (Table 1). Values of δ^{13}C in lower beaks varied from − 24.3 to − 19.4‰ with beak ML5 presenting the lowest values and ML9 the highest, and from − 23.9 to − 19.9‰ in the upper beaks, with MU2 and MU4 presenting the smallest and highest values, respectively (Table 1). Analysing δ^{13}C values in the different subsections, the tip of the rostrum (R) and its adjacent were the subsections presenting the lowest δ^{13}C values in both upper and lower beaks, with the highest values being found in subsections from the posterior area of the crest (Fig. 3). Significant differences were found between δ^{13}C values of lower beak subsections (Skilling–Mack test, $_{SM}$ = 38.90, df = 12, $p < 0.001$). Multiple comparisons revealed significant differences between the second subsection and the seventh and eighth subsections (from R to C; see Online resource Table A1). No significant differences were found between upper beak subsections (Skilling–Mack test, $_{SM}$ = 13.93, df = 17, $p = 0.716$).

Fig. 2 Large specimen of *Megaleledone setebos*. M. 328,333; Male; TL = 1150 mm; ML = 290 mm; M = 18,300 kg. (Photo kindly given by D. Allen from NIWA)

Values of δ^{15}N varied from + 3.3 to + 10.5‰ in the lower beaks, with ML5 and ML9 presenting the lowest and highest values, respectively, and in upper beaks between + 6.0 and + 10.7‰, with MU2 presenting the smallest value and MU1 and MU3 the highest (Table 1). The lowest δ^{15}N values were found in the adjacent subsection to R in the lower beaks and in R in upper beaks, with the highest values being found in the posterior subsections of the crest in both beaks (Fig. 3). Significant differences were found between the subsections δ^{15}N values in both lower and upper beaks (Skilling–Mack test, lower beaks: $_{SM}$ = 66.73, df = 12, $p < 0.001$; upper beaks: $_{SM}$ = 45.37, df = 17, $p < 0.001$). Multiple comparison tests showed that the second subsection differs significantly from the seventh and the thirteenth subsection (the last subsection statistically analysed) in lower beaks, and between the fifth and nineteenth (the last statistically analysed) subsections in upper beaks (from R to C; see Online Resource Table A1).

No significant differences were found between C:N ratios of lower beaks, whilst significant differences were found in upper beaks (Table 1). A significant correlation was found between δ^{13}C and δ^{15}N values from all beaks (Spearman's rank correlation, $r_s = 0.512$, $p < 0.001$, $n = 189$) (Fig. 4).

Capture site, morphometrics, and genetic identification of two *Megaleledone setebos*

The specimens reached a mantle length of 300 mm and 18,300 g of weight (Table 3, Fig. 2). A significant positive correlation were found between the fishing depth and both total and mantle lengths (Spearman's rank correlations, total length: $r_s = 0.531$, $p = 0.016$, $n = 20$; mantle length: $r_s = 0.684$, $p < 0.001$, $n = 21$) of specimens summarized in Allcock et al. (2003) plus the two larger specimens reported here.

For both specimens, sequences of 658 bp were recovered without indels or stop codons. In the GenBank BLAST search, they showed a 99.39–100% match to *M. setebos* and *M. senoi* sequences (GU073512.1, GU073581.1, AF377977.1, and EF102174.1). In BOLD, both sequences matched 98.78–100% to *M. setebos*. There is currently a single BIN for *M. setebos*, which has a maximum intraspecific distance of 1.28% (*p* distance), and is 3.18% (*p* distance) from its nearest neighbour, *Pareledone turqueti*. The two specimens sequenced in the present study have been placed in the same BIN as the other sequences of *M. setebos* (BOLD:AAD7551).

Discussion

The stable isotopic analysis showed that *M. setebos* is capable of inhabit different habitats, with differences between δ^{13}C values of the studied lower beaks reflecting the different

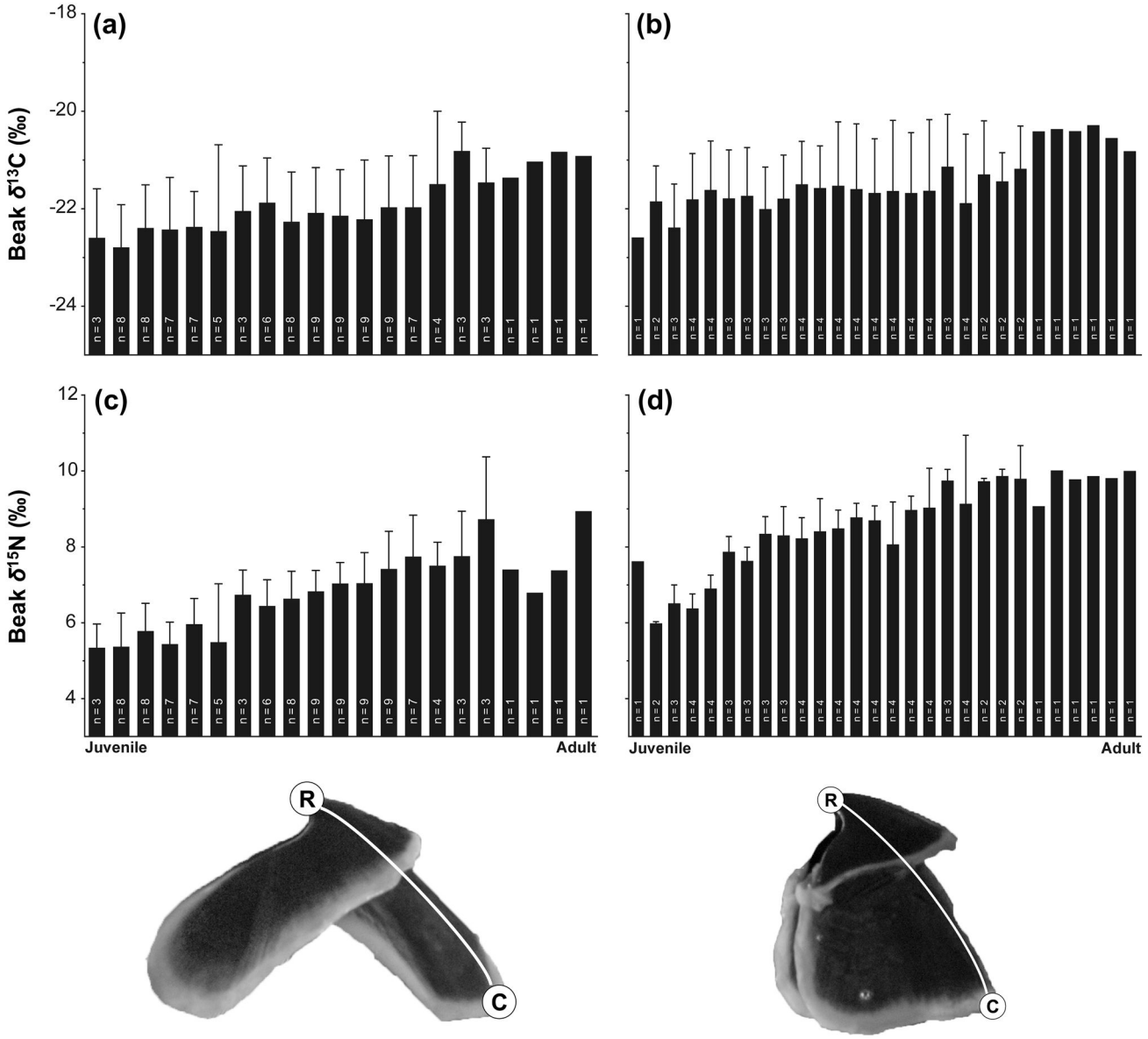

Fig. 3 Values of δ^{13}C and δ^{15}N for the different subsections along the beaks. **a** δ^{13}C values of lower beaks; **b** δ^{13}C values of upper beaks; **c** δ^{15}N values of lower beaks; **d** δ^{15}N values of upper beaks. R: tip of the rostrum reflects juvenile life stage; C: end of the crest reflects adult life stage. Values are mean ± SD

capture locations. Sequential sampling along the beak revealed that *M. setebos* does not perform large migratory movements inhabiting the same area its entire life. This analysis also revealed that *M. setebos* present different δ^{15}N values in the different study areas, suggesting that this species adapts its diet to the region. An increase of δ^{15}N values from the tip of the rostrum suggests a change of ≈1 trophic level throughout the individuals' life. Genetic analysis performed in this study confirmed the captured specimens as *M. setebos* which confirmed the beak morphology for this species allowing the posterior stable isotopic analysis using beaks collected from Antarctic toothfish stomachs.

Ontogenetic changes in habitat and trophic ecology

All *M. setebos* beaks recovered from stomachs of Antarctic toothfish used in this study had fully darkened wings and slightly smaller LHL than the captured female; therefore, they were from mature individuals (Clarke 1986), and are likely to have had a mantle length of, at least, 200 mm (i.e., minimum size to maturation (Allcock et al. 2003)). The beaks were comparable in size to those recovered from the two fresh specimens, indicating that they were from large individuals (Tables 1 and 3). This suggests that the analysis of subsections throughout these beaks reflects

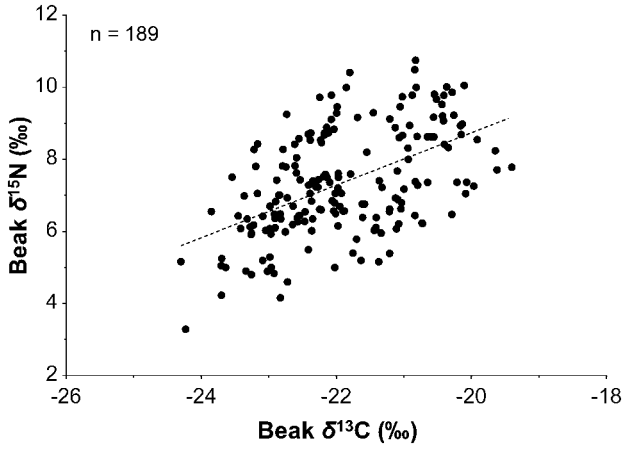

Fig. 4 Correlation between δ^{13}C and δ^{15}N values in all the studied subsections ($r_s = 0.512$)

the majority of their life cycle (Guerra et al. 2010; Queirós et al. 2018).

In the marine environment, consumers' δ^{13}C values vary with latitudes in oceanic waters and also along an inshore–offshore gradient (Cherel and Hobson 2007). Since *M. setebos* is a benthic species, it is likely that differences between beak δ^{13}C values reflect primarily the different benthic habitats at sampling sites, with higher δ^{13}C values in the Ross Sea reflecting the proximity of the Antarctic shelf. When analysed along the beak, δ^{13}C values can provide information on ontogenetic changes in habitat, with subsections near the tip of the rostrum being a proxy for earlier periods of the individuals' life (Guerra et al. 2010; Queirós et al. 2018). Yet, we must be cautious when analysing this section, because the rostrum tip is a beak region with continuous wearing of old material and deposition of new material, thus altering the early life isotopic values of the cephalopods (Cherel et al. 2009). Differences between the subsections in the lower beaks suggest either that individuals occupy different habitats in different periods of their life cycle and/or that the isotopic differences are linked to different diets. Multiple comparison test showed no differences between adjacent subsections revealing a continuous enrichment in ^{13}C along the beaks which indicates a progressive habitat shift. However, the positive relation between δ^{13}C and δ^{15}N values indicates that the continuous enrichment of δ^{13}C along the beak most probably reflects an ontogenetic change in the diet [despite being considered non-trophic accumulated, δ^{13}C values increase of ~ 1‰ per trophic level (Cherel and Hobson 2005)], suggesting that *M. setebos* does not change the habitat but use different feeding sources throughout its life cycle. This sedentary behaviour is similar to other giant octopod species in the world (e.g., the giant Pacific octopus, *Enteroctopus dofleini* (Scheel and Bisson 2012), that only performs small movements throughout its life.

Values of δ^{15}N are used to study the trophic ecology of organisms because consumers are step wised enriched in ^{15}N when compared to their prey (McCutchan et al. 2003; Cherel and Hobson 2005). In marine systems in general and more specifically in cephalopod beaks, this increase in δ^{15}N amounts to ~ 3.3‰ (McCutchan et al. 2003; Hobson and Cherel 2006). Analysis of different beak subsections enables the study of the octopus trophic ecology from early to later life stages (Guerra et al. 2010; Queirós et al. 2018). Differences between the subsections with the lowest and highest δ^{15}N values (3.9‰ in lower beaks and 4.2‰ in upper beaks) suggest an increase of ≈1 trophic level (≈ 1.2 and ≈ 1.3 trophic levels in lower and upper beaks, respectively) from early to later life stages. This increase is continuous throughout the lifetime of the individual, as suggested by the absence of differences between adjacent subsections. As far as we know, only two studies did a stable isotopic analysis in sectioned beaks of Antarctic octopods (Cherel and Hobson 2005; Matias et al. 2019), with our results suggesting that, as juvenile, *M. setebos* (6.0‰ and 3.3‰ are the lowest δ^{15}N values in upper and lower beaks, respectively) feeds in the same trophic position as *Adelieledone polymorpha*, *Pareleledone turqueti* (≈ 6.0‰ and ≈ 6.1‰ δ^{15}N in the rostrum of upper beaks, respectively), and *Benthoctopus thielei* (≈ 3.0‰ δ^{15}N in lower beak's rostrum) (Cherel and Hobson 2005; Matias et al. 2019). However, when adult, *M. setebos* feeds in a higher trophic position than these species (*M. setebos*: 10.5 ‰ and 10.7 ‰ in lower and upper beaks; *A. polymorpha*: ≈ 7.0‰ in upper beak's crest; *P.* turqueti:

Table 3 Measurements of the two captured specimens of *Megaleledone setebos*

NMNZ	Capture site			Sex	Total length (mm)	Mantle length (mm)	Total wet weight (g)	LHL (mm)		UHL (mm)	
	Latitude	Longitude	Depth (m)					w/clear	w/out clear	w/clear	w/out clear
M. 328333	64° S	132° E	821–910	Male	1150	290	18300	23.0	21.8	32.1	28.6
M. 328334	64° S	132° E	700–850	Female	1030	300	10061	17.2	16.5	29.1	27.4

Capture site is the mean position of the longline

LHL lower hood length, and *UHL* upper hood length of the beaks. *W/clear* measurements including the edge of darkened zone and the newly formed undarkened margin of the beak, *w/out clear* measure without the newly formed undarkened margin of the beak that is easily digested, thus allowing comparison with beaks collected from predators' stomachs.

≈ 7.8‰ in upper beak's crest; *B. thielei*: ≈ 8‰ in lower beak's wing) (Cherel and Hobson 2005; Matias et al. 2019). Nevertheless, as the differences for *B. thielei* and *P. turqueti* are lower than 3.3‰, both species might feed in the same trophic level but in different prey or in the same prey but in different ratios. Because most cephalopods macerate the food before swallowing or digest it previous to the ingestion (Collins and Rodhouse 2006), the analysis of stomach contents to identify prey items is not easy. Piatkowski et al. (2003) is the only study analysing stomach contents of *M. setebos,* finding that smaller individuals feed mainly in ophiuroids (62%), with fish and amphipods (8% each) being the next preferred prey. As *M. setebos* increase the trophic level throughout their life cycle, it is hypothesised that (1) adults may feed in ophiuroids' predators [e.g., cephalopods (Collins and Rodhouse 2006)]; or (2) feed in the same group of prey but changing their % [e.g., fish becoming the main prey and ophiuroids decrease their importance (discussed above)] or (3) maintain the same diet composition just changing the species within each group (e.g., different ophiuroid species present different diets, thus different δ^{15}N values (Gutt et al. 2014)]. To confirm these hypotheses, future studies should be conducted to analyse stomach contents of *M. setebos* with different sizes and/or apply new emergent techniques that allow prey species identification without hard structures, e.g., molecular analysis of stomach contents (Carreon-Martinez et al. 2011).

As expected, the continuous enrichment of *M. setebos* beaks differs from oceanic squid species [e.g., *Architeuthis dux* (Guerra et al. 2010) and *Moroteuthopsis longimana* (Queirós et al. 2018)] that show an abrupt increase in the beginning of life, before maintaining the same trophic position during their adult lives. These differences between squids and octopuses suggest that different cephalopods present, besides different reproductive strategies (Lipiński 1998; Boyle and Rodhouse 2005), different ontogenetic strategies.

Vertical distribution of *Megaleledone setebos*

The positive correlation between the depth of capture and specimen size suggests that larger specimens inhabit deeper waters [specimens with total length ≥ 900 mm were all captured below 800 m depth (Allcock et al. 2003; this study)] suggesting an ontogenetic descend through the slope as individuals grow. Nevertheless, individuals from smaller sizes can be found in deeper waters and bigger animals in shallower waters (see Table 2). Despite beaks recovered from Antarctic toothfish stomachs (this study; Stevens et al. 2014; Hanchet et al. 2015) can be used to provide insights into the habitat and trophic ecology of *M. setebos*, the absence of allometric equations precluded the estimation of the size of individuals from these beaks; however, all the beaks collected from the stomachs were smaller (LHL, Tables 1 and 3) than those from the two large captured specimens. Moreover, allometric equations may be established using the beaks and specimens preserved in several collections, to estimate the size of individuals inhabiting deeper waters.

Acknowledgements Authors would like to give a special thanks to the crew of the *FV* Antarctic Discovery, Antarctic toothfish fishing season 2016/2017, for keeping the two *M. setebos* specimens onboard for JQ, allowing the amazing discovery of these large animals. We would also like to thank the crew and observers on board of the *FV* Janas for collecting the Antarctic toothfish stomachs. Thanks to the National Institute of Water and Atmospheric Research (NIWA) scientists and staff especially Heather Braid for working in the genetic analysis, Sarah Allen for helping with the permits, Diana Macpherson, and all the team from NIWA's Invertebrate Collection for the help in storing both specimens and Bruce Marshall from Te Papa (National Museum of New Zealand Te Papa Tongarewa). Thanks to Dave Allen for the *M. setebos* photo. This project was financially supported by NHK Japan. This work is an international collaboration under the Scientific Committee on Antarctic Research (SCAR) associated programs, expert and action groups, namely SCAR AnT-ERA (Antarctic Thresholds—Ecosystems Resilience and Adaptation), SCAR EGBAMM (Expert Group in Birds and Marine Mammals), and ICED (Integrating Climate and Ecosystems Dynamics). JQ would like to thank Australian Longline for allowing his presence onboard of *FV* Antarctic Discovery. JQ was supported by FCT/MCTES through national funds (PIDDAC), Portuguese Polar Program PROPOLAR, and SCAR AnT-ERA. This study benefited from the strategic program of MARE (Marine and Environmental Sciences Centre), financed by FCT [Foundation for Science and Technology (UIDB/04292/2020)]. Authors would like to thank to both reviewers that helped to improve the quality of the manuscript.

Funding NHK Japan financed this study allowing the boarding of JQ in the *FV* Antarctic Discovery. JQ was supported by FCT/MCTES through national funds (PIDDAC), Portuguese Polar Program PROPOLAR. JQ was supported by SCAR AnT-ERA scholarship. The study benefited from the strategic program of MARE (Marine and Environmental Sciences Centre), financed by FCT [Foundation for Science and Technology (UIDB/04292/2020)].

Availability of data The data sets during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest The authors declare that they have no conflict of interest.

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