



From warm to cold waters: new insights into the habitat and trophic ecology of Southern Ocean squids throughout their life cycle

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ABSTRACT: Cephalopods play a major role in marine ecosystems, yet very little is known about the dynamics of their habitat use and trophic ecology across the stages of their life cycle, particularly in the Southern Ocean. Here, we used stable isotope analyses of $\delta^{13}\text{C}$ (a proxy for foraging habitat) and $\delta^{15}\text{N}$ (a proxy for trophic position) to investigate the habitat use and trophic ecology of 10 squid species, collected from the diet of Antipodean (*Diomedea antipodensis antipodensis*) and Gibson's (*D. a. gibsoni*) albatrosses breeding at Antipodes and Adams Island (South Pacific), respectively. We analyzed isotopes in 2 sections of squid lower beaks, representing 2 stages of the life cycle: the tip of the rostrum (juvenile stage) and the wing (adult stage). Higher $\delta^{13}\text{C}$ values in early life stages ($-20.8 \pm 1.7\text{‰}$) than in adult life stages ($-21.6 \pm 1.9\text{‰}$) suggest that Southern Ocean squids tend to move southwards as they grow, with oceanic fronts being an important habitat for these species. Our results might indicate that adults may move southwards with climate change, possibly impacting top predators living on northern islands. Overall, $\delta^{15}\text{N}$ values revealed an increase in trophic position from early ($6.7 \pm 2.7\text{‰}$) to adult life stages ($9.0 \pm 2.5\text{‰}$) in all species. Nevertheless, significant differences between $\delta^{15}\text{N}$ values of the 10 species, in both beak sections, suggest different feeding strategies between species and life stages.

KEY WORDS: Ontogenetic changes · Antarctica · Cephalopods · Stable isotopes · Climate change · South Pacific

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1. INTRODUCTION

Cephalopods are ecologically important marine organisms in the marine environment, including in the Southern Ocean, defined here as water masses south of the subtropical front (STF; see Fig. 1) (Xavier et al. 1999, Collins & Rodhouse 2006, Xavier & Cherel

2009). Cephalopods act as a link between lower and higher trophic levels (Xavier & Peck 2015), preying on a wide range of species and generally occupying higher trophic position as they grow (Cherel & Hobson 2005, Cherel et al. 2009, Rodhouse 2013). Cephalopods are themselves important prey for many top predators, such as seabirds, fish and marine mam-

mals (Clarke 1996, Smale 1996, Cherel & Klages 1998). Moreover, Southern Ocean squids have the potential to be commercially exploited (Rodhouse 1990, Rintoul et al. 2018).

Despite the recognised importance of cephalopods in the Southern Ocean, relatively little is known about their habitat preferences and trophic ecology over different stages of their life cycle (Xavier et al. 1999, Collins & Rodhouse 2006, Rodhouse et al. 2014, Queirós et al. 2018, Xavier et al. 2018). Within the Southern Ocean, the latitudinal distribution of squids is apparently limited by the major frontal systems: the Antarctic polar front (APF), the subantarctic front and the STF (Collins & Rodhouse 2006, Rodhouse 2013, Rodhouse et al. 2014, Cherel 2020).

Common sampling methods, such as scientific nets, are easily avoided by large squids, and therefore do not provide reliable information, for example, on the distribution of these species (Cherel & Weimerskirch 1999, Collins & Rodhouse 2006, Rodhouse et al. 2014). Moreover, very few scientific cruises target these organisms in the Pacific sector of the Southern Ocean, resulting in an even larger gap in our knowledge of the cephalopod fauna inhabiting this sector, when compared to the Atlantic and Indian sectors (Alexeyev 1994, Rodhouse et al. 1996, Griffiths 2010, Xavier et al. 2014).

To fill this gap, top predators have been used as biological samplers (Xavier & Cherel 2009). Cephalopod beaks (chitinous jaws) extracted from the stomachs of their predators can reveal useful insights into squid ecology (Clarke 1962). Beaks are resistant to digestion and thus accumulate in predator stomachs for many months. Importantly, beak morphology allows identification of squids to species level and beak morphometry can be used to estimate size and biomass (Clarke 1986, Lu & Ickeringill 2002, Xavier & Cherel 2009). In addition, stable isotope analyses of beaks can provide insights into foraging habitat and trophic position of the species and individual (Cherel & Hobson 2005). Furthermore, because the beak is metabolically inert and grows throughout the individual's life, comparing isotopes in different parts of the beak enables tracking of changes in foraging habitat and trophic position across different life stages (Cherel & Hobson 2005, Guerra et al. 2010, Queirós et al. 2018). For example, the tip of the rostrum forms early in life, whereas the end of the hood (upper beak) or wing (lower beak) form as the squid ages (Cherel & Hobson 2005, Cherel et al. 2009, Guerra et al. 2010, Queirós et al. 2018). However, caution must be taken when interpreting the isotopic signature of the tip of the rostrum because this portion is con-

stantly abraded, with new beak material added on the top layers, and therefore may contain a mixture of old and new material, rather than exclusively reflecting juvenile foraging habitat and trophic position (Cherel & Hobson 2005, Queirós et al. 2018).

Stable isotope analysis applied to cephalopod beaks provides an indirect means of studying species habitat preferences and trophic ecology (Cherel & Hobson 2005, Queirós et al. 2018). Values of stable isotopes of carbon (i.e. $\delta^{13}\text{C}$), are relatively stable along the food chain and usually reflect the environmental characteristics of the consumers' foraging habitat. In the Southern Ocean, $\delta^{13}\text{C}$ values are typically negatively correlated with latitude and reflect the water mass where the individual lived (Cherel & Hobson 2005, Jaeger et al. 2010, Brault et al. 2018). Values of stable isotopes of nitrogen (i.e. $\delta^{15}\text{N}$) change along the food chain, with consumers being enriched stepwise relative to prey, typically increasing ca. 3‰ at each trophic level (Peterson & Fry 1987, Hobson et al. 1994, McCutchan et al. 2003). Thus, values of $\delta^{15}\text{N}$ are normally used to determine the relative trophic level of consumers (Cherel & Hobson 2005, Cherel et al. 2008).

Despite the potential of isotopic approaches, no studies have explored the distribution patterns of mid-trophic level pelagic organisms such as squids in the Southern Ocean, and how they change throughout the life cycle. Indeed, to our knowledge, how squids move between water masses at different life stages has thus far been overlooked (Xavier et al. 2015). Increasing our understanding of how squid species move between the different water masses of the Southern Ocean is of utmost importance in understanding squid response to present and future human-induced climate change. Furthermore, as organisms tend to disperse southwards with climate change and potentially cross the APF to Antarctic waters, with these waters falling under the management of the Commission for the Conservation of Antarctic Marine Living Resources (CCAMLR) (CAMLR 1980, Kock et al. 2007), identifying such ecological patterns is relevant for the assessment, management and conservation of Antarctic (and adjacent) waters.

The main objective of this study was to determine the habitat preferences and trophic ecology of 10 Southern Ocean squid species over 2 life stages (juveniles and adults). Although squid distribution is apparently limited by oceanic fronts (Collins & Rodhouse 2006, Cherel 2020), several studies have shown that some species are able to cross these barriers (e.g. *Moroteuthopsis longimana* (Queirós et al. 2018), *Slosarczykovia circumantarctica* and *Architeuthis dux* (Cherel & Hobson 2005). Some squid species are also

known to inhabit multiple water masses within the Southern Ocean (e.g. *Galiteuthis glacialis*, *Histioteuthis eltaninae* (Rodhouse et al. 2014, Chérel 2020)). Moreover, many cosmopolitan deep sea squid species are phylogenetically closely related to Southern Ocean species (Lindgren 2010, Bolstad et al. 2018). Therefore, we expect that (1) different squid species will employ different strategies, with some species using the same habitat as juveniles and adults and thus being confined to one geographic region (i.e. Antarctic, subantarctic or subtropical waters), while other species use different water masses throughout their lives; (2) all Southern Ocean squids will occupy higher trophic positions as they grow, as shown for other squid species in previous studies (Kear 1992, Phillips et al. 2003, Guerra et al. 2010, Golikov et al. 2018).

To test these hypotheses we applied stable isotope analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to 2 sections (tip of the rostrum and wing) of the lower beaks of 10 squid species occurring in the diet of Antipodean albatrosses *Diomedea antipodensis antipodensis* and Gibson's albatrosses *D. a. gibsoni* breeding at Antipodes Island and Adams Island in the New Zealand subantarctic, respectively (Xavier et al. 2014). These albatrosses are known to forage over vast, but different, areas of the Pacific sector of the Southern Ocean: Antipodean albatrosses forage to the east of New Zealand, whereas Gibson's albatrosses forage to the west (Walker & Elliot 2006). The 10 squid species selected for analysis are known to inhabit southern latitudes spanning from Antarctic to subtropical waters and are thus good models to test our hypotheses (Xavier et al. 2014, Chérel 2020). Finally, we examined the implications of our findings for squid fisheries in the Southern Ocean in the context of climate change.

2. MATERIALS AND METHODS

2.1. Sample collection

Squid lower beaks, identified as belonging to adults by fully darkened wings (Clarke 1986), were recovered from boluses regurgitated by Antipodean *Diomedea antipodensis antipodensis* and Gibson's

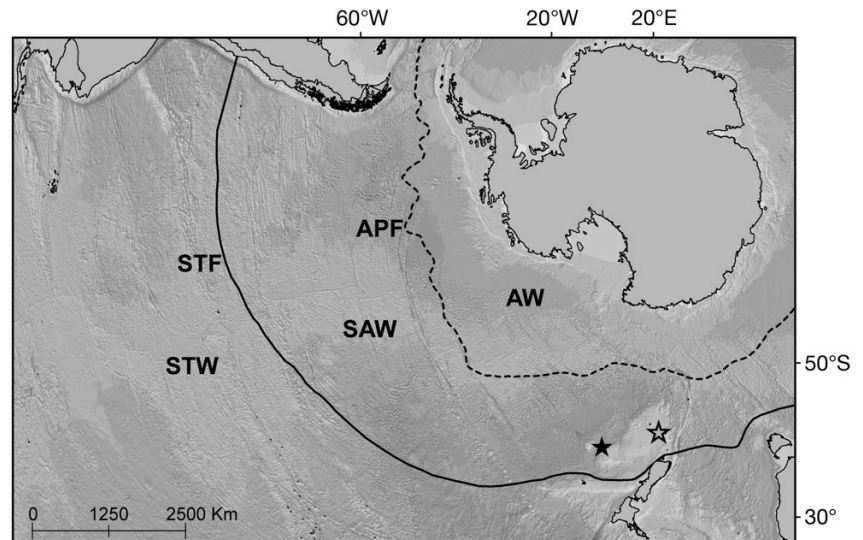


Fig. 1. The Pacific sector of the Southern Ocean, showing water masses and fronts. Filled star: Antipodes Islands (breeding site of Antipodean albatrosses *Diomedea antipodensis antipodensis*); empty star: Adams Islands (breeding site of Gibson's albatrosses *D. a. gibsoni*); solid line: subtropical front (STF); dashed line: Antarctic polar front (APF); STW: subtropical waters (north of the STF); SAW: subantarctic waters (between the APF and STF); AW: Antarctic waters (south of the APF)

D. a. gibsoni albatross chicks, following Xavier et al. (2014). Boluses were frozen after collection and beaks were preserved in 70 % ethanol upon identification (Xavier et al. 2014). All samples were collected at the end of the 2001 breeding season on Antipodes Island (49° S, 178° E) and Adam Island (Auckland Islands group, 51° S, 166° E), New Zealand (Fig. 1).

2.2. Beak selection and sectioning

Between 4 and 11 beaks of similar size and in good condition were randomly selected for each of the 10 squid species: *Batoteuthis skolops*, *Galiteuthis glacialis*, *Taonius notalia* (previously known as *Taonius sp. B* (Voss)), *Cycloteuthis sirventi*, *Histioteuthis atlantica* (small and large; as this species exhibits a bimodal beak size distribution), *Histioteuthis eltaninae*, *Alluroteuthis antarcticus*, *Octopoteuthis sp.*, *Moroteuthopsis longimana* and *Filippovia knipovitchi*. The lower rostral length (LRL, mm) of each beak was measured using a digital caliper to the nearest 0.01 mm (Xavier & Chérel 2009) (Table 1). Mantle length (ML, mm) and reconstructed mass (M, g) were estimated for each specimen using specific allometric equations (Xavier & Chérel 2009) (Table 1). Before sectioning, the transparent part of fresh beaks (i.e. beaks that spent little time in the stomachs) was removed because it pres-

Table 1. General characteristics of sampled squid species. LRL: measured lower rostral length, ML: estimated mantle length, M: estimated mass. Values are mean \pm SD

Family	Species	n	LRL (mm)	ML (mm)	M (g)
Batoteuthidae	<i>Batoteuthis skolops</i>	9	4.2 \pm 0.2	114 \pm 5	38 \pm 5
Cranchiidae	<i>Galiteuthis glacialis</i>	7	5.1 \pm 0.3	433 \pm 22	93 \pm 11
	<i>Taonius notalia</i>	7	10.8 \pm 0.5	648 \pm 32	400 \pm 42
Cycloteuthidae	<i>Cycloteuthis sirventi</i>	10	12.4 \pm 0.7	386 \pm 22	906 \pm 102
Histioteuthidae	<i>Histioteuthis atlantica</i> (small)	8	3.9 \pm 0.1	91 \pm 2	128 \pm 7
	<i>Histioteuthis atlantica</i> (large)	4	6.5 \pm 0.3	157 \pm 9	441 \pm 59
	<i>Histioteuthis eltaninae</i>	11	3.7 \pm 0.3	88 \pm 7	85 \pm 19
Neoteuthidae	<i>Alluroteuthis antarcticus</i>	4	5.4 \pm 0.5	183 \pm 19	487 \pm 138
Octopoteuthidae	<i>Octopoteuthis</i> sp.	10	13.2 \pm 0.6	243 \pm 11	878 \pm 100
Onychoteuthidae	<i>Moroteuthopsis longimana</i>	7	13.0 \pm 1.2	463 \pm 45	2380 \pm 724
	<i>Filippovia knipovitchi</i>	11	6.2 \pm 0.3	281 \pm 20	428 \pm 87

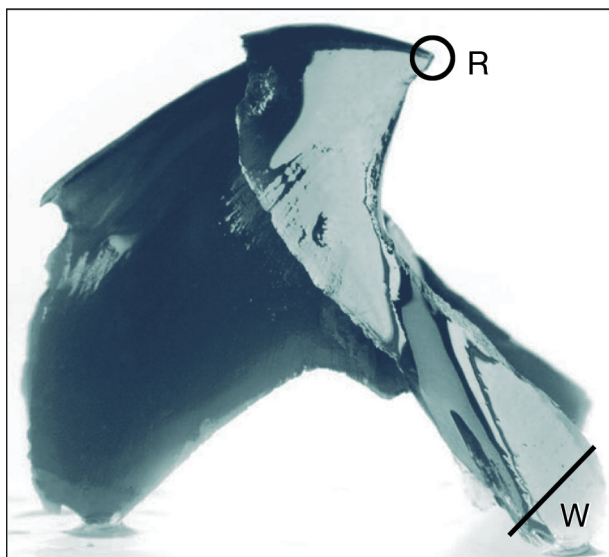


Fig. 2. Beak of *Filippovia knipovitchi* showing analysed sections. R: tip of the rostrum; W: wing. Adapted from Xavier & Cherel (2009)

ents less protein and more chitin than the rest of the beak, which can influence stable isotope results (Miserez et al. 2008, Cherel et al. 2009, Matias et al. 2019). The tip of the rostrum (R) and the wing (W) were cut using dissection scissors (Fig. 2). As most Southern Ocean squids have a short lifespan (Boyle & Rodhouse 2005, Collins & Rodhouse 2006), analyzing 2 periods of the life cycle, i.e. the juvenile (reflected by the tip of the rostrum) and adult (reflected by the wing) periods (Queirós et al. 2018), is sufficient to reflect habitat and trophic ecology throughout the entire life cycle.

2.3. Stable isotope analyses

After beaks were sectioned, the pieces were prepared for stable isotope analysis. Samples were cleaned with 80% ethanol and dried in an oven at 60°C. Samples were then reduced to a fine powder using a mixer mill (Retsch® MM400) for 10 min at a frequency of 30 Hz. Approximately 0.35 mg of milled powder from each sample was used for analyses. When there was insufficient material from a single individual (<0.10 mg), samples from 2 or 3 individuals with similar LRL were pooled (see Table 2).

Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured using a continuous flow isotope ratio mass spectrometer (CFIRMS) at Laboratório MAREFOZ, Figueira da Foz, Portugal. Results are presented using δ notation, in ‰, and were calculated using the equation

$$\delta X = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000 \quad (1)$$

where X represents C or N, and R the ratios of $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$. Isotope values for carbon and nitrogen are presented relative to Vienna PeeDee Belemnite and atmospheric N_2 , respectively. Throughout the analyses, internal reproducibility was measured as <0.1‰ for $\delta^{13}\text{C}$ and <0.3‰ for $\delta^{15}\text{N}$, using acetanilide as a standard.

2.4. Habitat preference

Squid species distributions were determined based on estimated isoscapes (i.e. isotopic geographic gradients) of the APF and STF (Cherel & Hobson 2007, Jaeger et al. 2010). Since no isoscapes exist based on

direct analyses of squid beaks, a conservative approach was adopted following Guerreiro et al. (2015). Values of $\delta^{13}\text{C}$ used to establish the position of the APF and STF were determined by analyses of penguin blood and albatross plasma (Cherel & Hobson 2007, Jaeger et al. 2010). Samples presenting values of $\delta^{13}\text{C}$ lower than -22.9‰ (the lowest value obtained for the APF in both studies) were considered to belong to individuals living in Antarctic waters, whereas values higher than -19.5‰ (the highest value obtained for the STF in both studies) were considered to belong to specimens living in subtropical waters (Cherel & Hobson 2007, Jaeger et al. 2010). Individuals with $\delta^{13}\text{C}$ values between -22.9 and -19.5‰ were considered to inhabit subantarctic waters.

2.5. Trophic ecology

Changes in squid trophic position between life stages were analysed using 3.3‰ as a reference value for one trophic level in cephalopod beaks (Hobson & Cherel 2006). $\delta^{15}\text{N}$ changes between the juvenile and adult stages of each species were then calculated using the equation

$$\Delta \text{TL} = \frac{(W - R)\text{‰}}{3.30\text{‰}} \quad (2)$$

where W and R are the mean $\delta^{15}\text{N}$ values measured in the wing (proxy for adult stage) and tip of the rostrum (proxy for juvenile stage), respectively.

2.6. Statistical and data analyses

Wilcoxon matched-pairs signed rank tests were used to test for significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between life stages (i.e. the tip of the rostrum and the wing) for each species. To test whether species differed in habitat use and trophic position as juveniles and adults, Kruskal-Wallis tests were used to evaluate differences in isotopic values of each beak section across all species. A Spearman correlation between wing $\delta^{15}\text{N}$ values and ML across all species was used to test whether body size related to trophic position. Chitin is impoverished in ^{15}N , but not in ^{13}N , in relation to proteins. As the ratio between both components varies across the beak (Miserez et al. 2008), which can lower the $\delta^{15}\text{N}$ values in the section with higher chitin amount (Cherel et al. 2009), we tested differences between the C:N mass ratio (higher C:N mass ratio means higher chitin amount) of the beak sections using a Wilcoxon matched-pairs

signed rank test. Differences between species in C:N mass ratio were tested using a Kruskal-Wallis test. These analyses (above, this subsection) were done using GraphPad Prism[®] v6.01. A cluster analysis (Euclidean distance; Ward's linkage) was performed using the `dist` and `hclust` functions in R (R Core Team 2019) to segregate the different species by water masses and feeding groups according to their average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. All statistical tests used $\alpha = 0.05$. Maps, images and graphs were prepared using ArcGis[®] ArcMap[™] v10.2 and Adobe Illustrator CC 2015[®].

3. RESULTS

Overall, $\delta^{13}\text{C}$ values varied between -24.1 and -17.8‰ in the tip of the rostrum and between -26.4 and -18.0‰ in the wings (Table 2, Fig. 3). Most of the studied species had wings depleted in ^{13}C relative to the tip of the rostrum, with the exception of *Histioteuthis atlantica* (large) and *Galiteuthis glacialis* which had similar $\delta^{13}\text{C}$ values in both beak sections, and *Moroteuthopsis longimana* whose wing had higher $\delta^{13}\text{C}$ values (Fig. 3, see Fig. A1 in the Appendix). Statistically significant differences in $\delta^{13}\text{C}$ between beak sections were found in 4 species (*M. longimana*, *Batoteuthis skolops*, *H. eltaninae* and *Cycloteuthis sirventi*; Fig. 3, see Table A1 in the Appendix). The largest difference between beak sections was found in *Filippovia knipovitchi*, which had wings depleted by 2.0‰ relative to the tip of the rostrum (Table A1, Fig. A1). Cluster analysis separated the species (and life stages) into 6 groups: Antarctic waters (AW), Antarctic polar front (APF), subantarctic waters (SAW), northern area of subantarctic waters (subantarctic waters closer to the STF; N-SAW), subtropical front (STF) and subtropical waters (STW) (Fig. 4). The dendrogram showed that 7 species moved south as adults (*B. skolops*, *H. eltaninae*, *Alluroteuthis antarcticus*, *C. sirventi*, *Taonius notalia*, *H. atlantica* [small] and *Octopoteuthis* sp.), 3 species stayed in the same area in both life stages (*F. knipovitchi*, *G. glacialis* and *H. atlantica* [large]) and one species moved northwards (*M. longimana*) (Fig. 4).

Values of $\delta^{15}\text{N}$ ranged from 2.2 to 13.3‰ in the tip of the rostrum and from 5.8 to 18.7‰ in the wing (Table 2, Fig. 5). Statistically significant differences in $\delta^{15}\text{N}$ values were found across all species in both beak regions (Table 2). The comparison between $\delta^{15}\text{N}$ values from the tip of the rostrum and wing of each species revealed significant differences in 6 species (*G. glacialis*, *M. longimana*, *H. eltaninae*, *B.*

Table 2. Isotopic values of both beak regions. N ind: number of individuals present in total samples analysed; N cap: number of samples analysed in IRMS. *H. atlantica* (s) and (l) represent small and large specimens, respectively. Values are mean \pm SD and (minimum; maximum)

Species	N ind	N cap	Tip of Rostrum			Wing		
			$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N mass ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N mass ratio
<i>Batoteuthis skolops</i>	9	9	-22.6 ± 0.4 (-23.4; -21.8)	6.0 ± 0.7 (4.9; 6.9)	3.5 ± 0.0	-23.8 ± 0.5 (-24.7; -23.0)	8.0 ± 0.5 (7.3; 8.8)	3.6 ± 0.1
<i>Galiteuthis glacialis</i>	7	7	-20.0 ± 1.1 (-21.3; -18.3)	3.6 ± 1.8 (2.2; 7.3)	3.9 ± 0.5	-20.1 ± 1.0 (-21.1; -18.2)	7.6 ± 1.1 (5.9; 9.2)	3.7 ± 0.4
<i>Taonius notalia</i>	10	10	-19.5 ± 1.2 (-21.8; -18.1)	7.5 ± 1.6 (5.7; 10.4)	3.4 ± 0.1	-19.9 ± 1.0 (-21.9; -18.5)	10.3 ± 0.9 (9.3; 11.9)	3.5 ± 0.1
<i>Cycloteuthis sirventi</i>	8	8	-20.3 ± 1.3 (-22.1; -17.8)	10.0 ± 1.3 (8.7; 12.2)	4.7 ± 0.8	-22.1 ± 0.7 (-23.3; -21.2)	11.1 ± 1.1 (9.6; 12.9)	6.6 ± 2.1
<i>Histioteuthis atlantica</i> (s)	11	6	-19.4 ± 0.8 (-20.5; -18.4)	9.3 ± 2.4 (7.1; 12.6)	3.6 ± 0.3	-20.1 ± 1.0 (-22.1; -19.6)	10.6 ± 1.2 (9.1; 11.8)	4.1 ± 1.3
<i>Histioteuthis atlantica</i> (l)	4	4	-18.5 ± 0.4 (-19.0; -18.1)	11.4 ± 2.4 (8.1; 13.3)	3.6 ± 0.1	-18.5 ± 0.6 (-19.4; -18.0)	15.8 ± 2.7 (12.5; 18.7)	3.6 ± 0.4
<i>Histioteuthis eltaninae</i>	10	10	-21.0 ± 0.5 (-21.8; -20.2)	4.9 ± 0.7 (3.8; 5.9)	3.7 ± 0.1	-21.8 ± 1.2 (-25.0; -20.4)	6.7 ± 0.6 (5.9; 7.8)	3.7 ± 0.2
<i>Alluroteuthis antarcticus</i>	7	4	-20.9 ± 0.6 (-21.5; -20.1)	4.4 ± 0.9 (3.7; 5.5)	3.7 ± 0.1	-21.5 ± 0.2 (-21.8; -21.3)	6.8 ± 0.6 (5.8; 7.3)	3.8 ± 0.2
<i>Octopoteuthis</i> sp.	3	3	-19.2 ± 0.4 (-19.7; -19.0)	8.4 ± 1.0 (7.4; 9.2)	3.6 ± 0.1	-20.7 ± 1.1 (-21.9; -19.8)	10.2 ± 0.6 (9.6; 10.7)	4.7 ± 0.7
<i>Moroteuthopsis longimana</i>	7	7	-23.3 ± 0.9 (-24.1; -21.7)	4.7 ± 0.6 (4.2; 5.7)	3.4 ± 0.1	-22.5 ± 0.8 (-23.9; -21.4)	7.1 ± 0.6 (6.4; 8.1)	3.5 ± 0.1
<i>Filippovia knipovitchi</i>	11	5	-23.1 ± 0.4 (-23.5; -22.6)	6.5 ± 1.5 (4.1; 7.8)	3.5 ± 0.2	-25.0 ± 0.8 (-26.4; -24.4)	8.2 ± 1.4 (6.9; 9.7)	4.6 ± 1.0
Statistics (Kruskal-Wallis test)			$H = 54.37$ $p < 0.001$	$H = 56.06$ $p < 0.001$	$H = 43.21$ $p < 0.001$	$H = 57.90$ $p < 0.001$	$H = 58.72$ $p < 0.001$	$H = 37.09$ $p < 0.001$

skolops, *Taonius notalia* and *C. sirventi*: Fig. 5, see Table A1 in the Appendix). Although some species (*A. antarcticus*, *F. knipovitchi*, *Octopoteuthis* sp. and *H. atlantica* [large]) did not show significant differences between beak sections, all species had higher $\delta^{15}\text{N}$ values in the wing than in the tip of the rostrum (Fig. 5, see Fig. A1 in the Appendix), with *H. atlantica* (large) and *G. glacialis* showing the largest increase (4.3 and 4.0‰, respectively; Table A1, Fig. A1). Cluster analysis of $\delta^{15}\text{N}$ values showed that the species are distributed into 9 different groups (considering a height of at least ~ 1), with all studied species changing group between beak sections (Fig. 6). No significant correlation was found between $\delta^{15}\text{N}$ values and estimated mantle length across species (Spearman correlation: $p = 0.09$).

There were significant differences in C:N mass ratios between all species in both beak sections (Table 2). In the tip of the rostrum the C:N mass ratios varied between 3.4 and 3.9, with the exception of *C. sirventi* (4.7). In the wing, C:N mass ratios ranged from 3.5 to

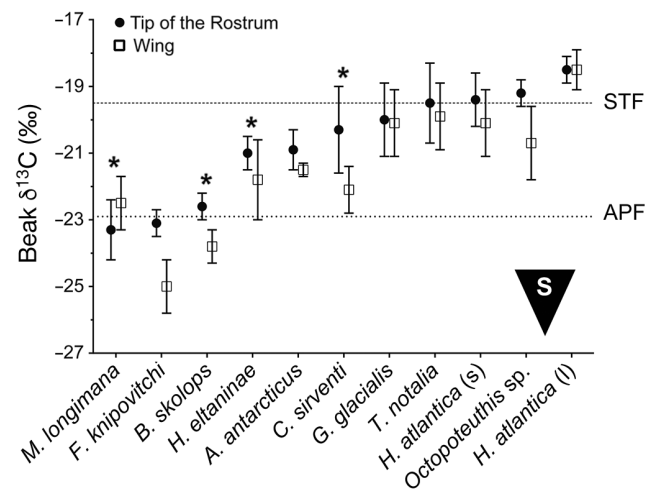


Fig. 3. Beak $\delta^{13}\text{C}$ values of squid species. *Significant differences between the tip of the rostrum and the wing. Values are mean \pm SD. APF: estimated isotopic position of the Antarctic polar front (-22.9‰); STF: estimated isotopic position of the subtropical front (-19.5‰). Species are organized from lowest to highest $\delta^{13}\text{C}$ value for the tip of the rostrum. *H. atlantica* (s) and (l) represent small and large specimens, respectively. Black triangle indicates south

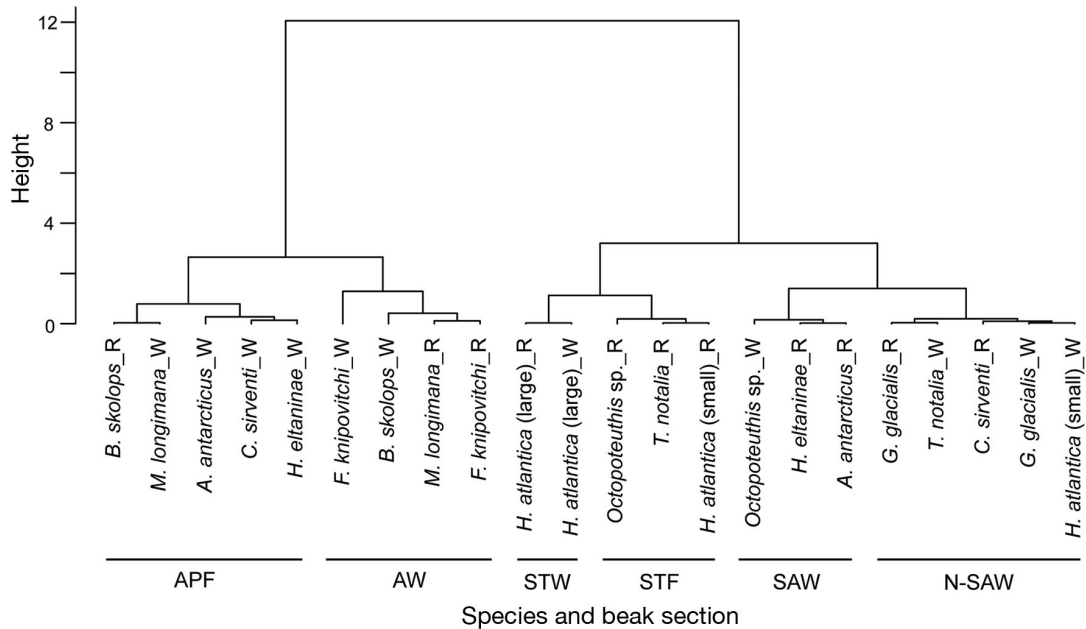


Fig. 4. Dendrogram produced by cluster analysis (Euclidean distance; Ward's linkage) of beak $\delta^{13}\text{C}$ values showing the distribution of species and life stages across the different water masses and oceanic fronts. R: tip of the rostrum; W: wing; APF: Antarctic polar front; AW: Antarctic waters; STW: subtropical waters; STF: subtropical front; SAW: subantarctic waters; N-SAW: northern area of the subantarctic area

3.8, except for *C. sirventi* (6.6), *Octopoteuthis* sp. (4.7), *F. knipovitchi* (4.6) and *H. atlantica* (small) (4.1). Differences between beak regions in C:N mass ratios were significant in *T. notalia*, *C. sirventi* and *M. longimana*, with the wing having higher values than the tip of the rostrum (Table A1). However, the C:N mass ratio differed by only 0.1 between beak regions in *T. notalia* and *M. longimana* (Table A1).

4. DISCUSSION

4.1. Temporal shifts in squid habitat preference

Values of $\delta^{13}\text{C}$ (a proxy for habitat) present a latitudinal gradient in the Southern Ocean, with high latitude waters exhibiting lower $\delta^{13}\text{C}$ values (Cherel & Hobson 2007, Jaeger et al. 2010). Although some species did not show significant differences in $\delta^{13}\text{C}$ values between beak regions, generally lower $\delta^{13}\text{C}$ values in adult squids (wing) than in juveniles (tip of the rostrum) suggest most species tend to migrate southwards as they grow. Further evidence for this movement was found using cluster analysis, including in some species that did not show significant differences between $\delta^{13}\text{C}$ values of beak regions (*Aluroteuthis antarcticus*, *Taonius notalia*, *Histioteuthis atlantica* (small) and *Octopoteuthis* sp.). The exceptions were *Fillipovia knipovitchi*, *Galiteuthis glacialis* and *H. atlantica* (large), which appear to inhabit the same region in both life stages, and *Moroteuthopsis longimana*, which had wings enriched in ^{13}C , suggesting a northward movement with age. Individuals of some species appeared to cross oceanic fronts during this movement: for example, *M. longimana* and *Batoteuthis skolops* crossed the APF heading north and south, respectively, and *H. atlantica* (small) and *Octopoteuthis* sp. crossed the STF heading south (see Fig. A1 in the Appendix). However, cluster analysis

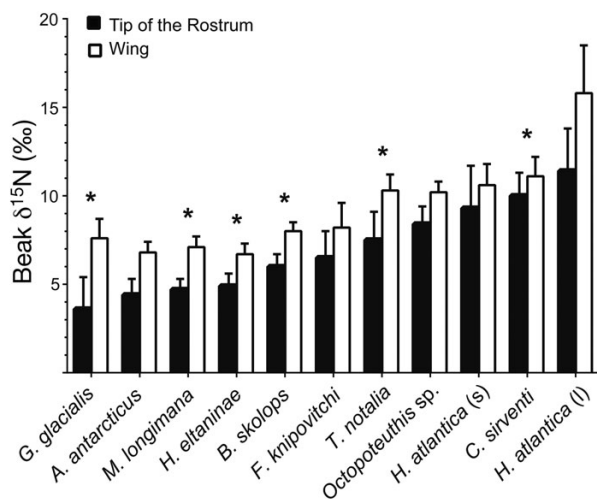


Fig. 5. Beak $\delta^{15}\text{N}$ values of squid species. *Significant differences between the tip of the rostrum and the wing. Values are mean \pm SD. Species are organized from lowest to highest $\delta^{15}\text{N}$ values for the tip of the rostrum. *H. atlantica* (s) and (l) represent small and large specimens, respectively

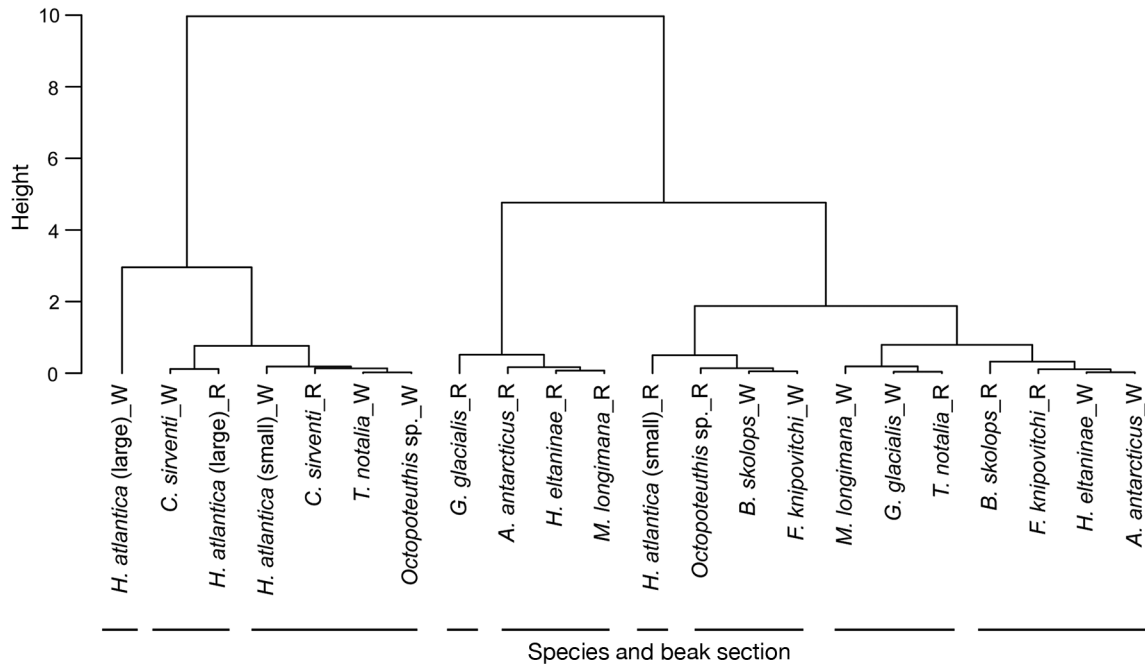


Fig. 6. Dendrogram produced by cluster analysis (Euclidean distance; Ward's linkage) of beak $\delta^{15}\text{N}$ values. Horizontal lines at the bottom show the separation of species by trophic groups. R: tip of the rostrum; W: wing

suggests that these individuals did not actually cross the oceanic front but rather moved to its immediate vicinity. This movement to and from the fronts support the importance of these oceanic features for squids (Collins & Rodhouse 2006, Xavier et al. 2016, Chérel 2020), and for the biodiversity of the Southern Ocean (Bost et al. 2009, De Broyer et al. 2014).

Dispersal movements are high risk and represent a large energetic cost to the individual (Zollner & Lima 2005), so squids presumably benefit from the movement to colder waters (even between fronts, the water temperature decreases towards the south (Griffiths 2010)) and to frontal regimes. In the Southern Ocean, such movements are usually driven by spatial or temporal variation (e.g. in food availability), ocean transportation (e.g. eddies associated with the APF) or behavioural processes (e.g. reproduction) (Huntley & Niiler 1995). We hypothesised 3 potential explanations for squid movements: (1) colder southern waters have higher concentrations of dissolved oxygen (Marshall & Speer 2012, Tripathy et al. 2015), providing better conditions for growth; (2) colder southern waters decrease squid metabolism, allowing adult squid to expend less energy, similar to the strategy used by jumbo squid *Dosidicus gigas* which perform daily vertical migrations to colder mesopelagic layers to save energy (Seibel 2013); or (3) Antarctic waters have high concentrations of nitrogen, sulphur and phosphorous (Tripathy et al. 2015), ele-

ments related to greater primary productivity at higher latitudes (Hiscock et al. 2003, Hunt & McKinnell 2006, Tripathy et al. 2015). Adult squid require more energy than juveniles, so adults may move south in summer to find more food and potentially larger prey in anticipation of winter breeding attempts (Xavier & Croxall 2007). As a counterpoint, this dispersal to frontal areas may increase the squids' risk of being preyed upon because the number of predators also increases during the austral summer, and they tend to congregate near oceanic fronts (Bost et al. 2009). As habitat suitability models predict (Xavier et al. 2016), we suggest that a combination of abiotic (water temperature, salinity, dissolved salts and high oxygen concentration) and biotic factors (higher productivity and more and larger prey) are the most likely explanation for the southward movement of oceanic squids in the Southern Ocean.

While Chérel & Hobson (2005) and Queirós et al. (2018) showed *M. longimana* can move southwards as they grow, we found no evidence to corroborate this, suggesting that, at least, this species may move freely between subantarctic and Antarctic regions. *F. knipovitchi*, *G. glacialis* and *H. atlantica* (large) had similar $\delta^{13}\text{C}$ values in both beak regions, thus suggesting they do not disperse latitudinally during their lifetime, and juveniles and adults use the same habitat. To determine whether *F. knipovitchi*, *G. glacialis*

and *H. atlantica* (large) show any type of movement throughout their life cycle, we suggest future studies analyze stable isotopes along the upper beak hood or crest (both beak sections allow for a more detailed analysis of ontogenetic changes than lower beaks), as recommended by Guerra et al. (2010) and Queirós et al. (2018).

Our analysis of $\delta^{13}\text{C}$ values places *H. atlantica* (large) exclusively in subtropical waters. However, previous studies have found this species in subantarctic waters, suggesting its distribution may range latitudinally from subantarctic to subtropical waters (Cherel 2020; Table 3). Nevertheless, the high $\delta^{13}\text{C}$ values of *H. atlantica* (large) that places this species in subtropical waters, may reflect the true habitat of our studied individuals, as supported by isotopic results in previous studies (Guerreiro et al. 2015) and the foraging area of both albatrosses species (Walker & Elliot 2006). However, the high $\delta^{13}\text{C}$ values might also be overestimated due to the high trophic position of this species, because while $\delta^{13}\text{C}$ values remain largely stable throughout the food chain, they do increase with trophic level (DeNiro & Epstein 1978). Overall, our results also suggest that Southern Ocean squids inhabit each of the different water masses south of the STF (i.e. Antarctic and subantarctic waters), but also occur in subtropical waters (Table 3).

A limitation of our work is that the tip of the rostrum contains both old and recent beak material (Cherel & Hobson 2005, Queirós et al. 2018); hence, its isotopic values do not solely reflect early life (Cherel & Hobson 2005). Lower $\delta^{13}\text{C}$ values in the wing compared to the tip of the rostrum might indicate that values for the tip of the rostrum were underestimated, suggesting that these individuals possibly inhabited northern waters as juveniles. In contrast, *M. longimana* showed higher $\delta^{13}\text{C}$ values in the wing than in the tip of the rostrum; thus values from the tip of the rostrum might be overestimated, suggesting that younger individuals might inhabit a more southern habitat than our results indicate. Thus, the latitudinal movements presented in this study are likely underestimated, and these squid species may perform larger movements than those presented here. Similar values in both beak regions for *G. glacialis*, *Taonius notalia* and *H. atlantica* (large) suggest that values for tip of the rostrum are almost the same as those of old beak material, and consequently refer to the exact habitat as juvenile.

Table 3. A review of biogeography (following Cherel 2020) and $\delta^{15}\text{N}$ values of squid lower beaks. AW: Antarctic waters (south of the APF). SAW: subantarctic waters (between the APF and the STF). STW: subtropical waters (north of the STF). \uparrow : this study recorded higher $\delta^{15}\text{N}$ values than previous studies; \downarrow : this study recorded lower $\delta^{15}\text{N}$ values than previous studies; =: our results do not change the previous known distribution and $\delta^{15}\text{N}$ range for this species; 0: no previous $\delta^{15}\text{N}$ values are available for this species. Known $\delta^{15}\text{N}$ is the minimum and maximum measured values for the species in previous studies. Parentheses in known distribution column indicate that the species is occasionally found in those waters

Species (Cherel 2020)	Known distribution	— This study — Juvenile	Adult	Known $\delta^{15}\text{N}$	Distribution range	Differences in $\delta^{15}\text{N}$	Source of $\delta^{15}\text{N}$ values
<i>Batoteuthis skolops</i>	AW + SAW ^a	APF	AW	7.8; 12.1	=	\downarrow	(Alvito et al. 2015, Guerreiro et al. 2015)
<i>Galiteuthis glacialis</i>	AW + SAW ^a	SAW	SAW	7.1; 11.0	=	\downarrow	(Alvito et al. 2015, Guerreiro et al. 2015)
<i>Taonius notalia</i>	(AW) + SAW	STF	SAW	8.9; 12.7	=	\downarrow	(Alvito et al. 2015, Guerreiro et al. 2015)
<i>Cycloteuthis sirventi</i>	(SAW) + STW	SAW	APF	11.0; 12.7	=	$\downarrow \uparrow$	(Guerreiro et al. 2015)
<i>Histioteuthis atlantica</i>	SAW + STW	STF	SAW	8.9; 12.3	=	$\downarrow \uparrow$	(Guerreiro et al. 2015)
<i>Histioteuthis atlantica</i> (small)	SAW + STW	STW	STW	10.2; 13.2	=	$\downarrow \uparrow$	(Guerreiro et al. 2015)
<i>Histioteuthis atlantica</i> (large)	SAW + STW	SAW	APF	7.9; 10.9	=	\downarrow	(Guerreiro et al. 2015)
<i>Histioteuthis ellaninae</i>	(AW) + SAW + (STW)	SAW	APF	7.4; 10.2	=	\downarrow	(Guerreiro et al. 2015)
<i>Alluroteuthis antarcticus</i>	AW + SAW ^a	SAW	APF		=	0	
<i>Octopoteuthis</i> sp.	SAW + STW	STF	SAW	5.0; 8.7	=	\downarrow	(Alvito et al. 2015, Guerreiro et al. 2015, Seco et al. 2016, Queirós et al. 2018)
<i>Moroteuthopsis longimana</i>	AW + SAW ^a	AW	APF	6.6; 9.7	=	\downarrow	(Alvito et al. 2015, Guerreiro et al. 2015, Seco et al. 2016)
<i>Filippovia knipovitchi</i>	AW + SAW ^a	AW	AW		=		

^aFound between polar front and subantarctic front

Climate change models predict that Southern Ocean fronts will move south (Constable et al. 2014), which, together with the southward movement found here, suggests that squids will inhabit higher latitudes in the future. This may have an impact on the ecology of southern ocean predators, but also on potential future squid fisheries (Rodhouse 1989) since squids will likely spend more time in Antarctic waters, where fisheries are strictly managed, than in less-regulated international waters further north (CAMLRL 1980, Kock et al. 2007).

4.2. Trophic ecology of squids

By determining $\delta^{15}\text{N}$ values in both beak regions, we can assess differences in squid trophic status over time, as an increase of $\sim 3.3\%$ between beak regions indicates a shift of approximately 1 trophic level (Hobson & Cherel 2006). We found that adult squids were enriched in ^{15}N , indicating that Southern Ocean squids occupy higher trophic positions as they grow, probably reflecting a general shift in diet from zooplankton to fish, or other zooplanktivorous species. Similar patterns have been found in other squid species using several methodologies, such as *Architeuthis dux* (stable isotopic analysis of upper beaks; Guerra et al. 2010), *Psychroteuthis glacialis* (visual and serological analyses of gut contents of juveniles and adults; Kear 1992), *M. (=Onykia) ingens* (stomach content and fatty acid analyses; Phillips et al. 2003), as well as in other squid species throughout the world's oceans (Hoving et al. 2014). This trophic increase has also been reported in octopod species such as *Muusoctopus (=Benthoctopus) thielei* (Cherel & Hobson 2005), and in *M. longimana* from the Indian and Atlantic sectors of the Southern Ocean (Cherel & Hobson 2005, Queirós et al. 2018), confirming that most cephalopods exhibit an increase in trophic status with age (Boyle & Rodhouse 2005, Golikov et al. 2018).

Many of the species in this study showed significant increases in $\delta^{15}\text{N}$ values from early to adult life stages. Although there was no significant difference between the $\delta^{15}\text{N}$ values of the beak sections in *H. atlantica* (large), these specimens showed the highest trophic increase between the juvenile and adult stages (1.3 trophic levels). *G. glacialis* increase by 1.2 trophic levels throughout their life cycle, thus showing the second largest increase of the study species. In most species, the differences in $\delta^{15}\text{N}$ values between early and adult life stages were smaller and did not reflect a complete trophic level shift, (e.g. an increase of only ~ 0.5 to ~ 0.8 trophic levels in *F.*

knipovitchi [1.7‰], *H. eltaninae* and *Octopoteuthis* sp. [1.8‰], *B. skolops* [2.0‰], *A. antarcticus* [2.4‰] and *Taonius notalia* [2.8‰]). These relatively small increases could be due to (1) individuals switching between prey items with similar feeding strategies (e.g. from zooplanktivorous fish to zooplanktivorous squid), (2) changes in the proportion of prey species in the diet (Bearhop et al. 2004), (3) changes in the plankton community, possibly related to change of habitat (discussed below) or (4) the deposition of newly formed beak material at the tip of the rostrum, increasing $\delta^{15}\text{N}$ values in this beak section. The absence of significant differences between $\delta^{15}\text{N}$ values of beak sections in several species can be explained by a larger prey diversity in the diet of juveniles. For example, *H. atlantica* (small and large) and *Octopoteuthis* sp. inhabit northern waters (see above), where there is a higher zooplankton biodiversity, including several carnivorous species (Pakhomov et al. 1999), leading to higher $\delta^{15}\text{N}$ values in the squid. However, the C:N mass ratios in the wing of *F. knipovitchi*, *Octopoteuthis* sp. and *H. atlantica* (small) show that the differences between wing and tip of the rostrum $\delta^{15}\text{N}$ values are lowered by the higher amount of chitin in the wing (Cherel et al. 2009), suggesting that these species might increase trophic position with age to a greater extent than our results suggest. High C:N mass ratios in both beak regions (higher in the wing) of *Cycloteuthis sirventi* indicate that both $\delta^{15}\text{N}$ values are lowered, suggesting a higher trophic position in both life stages and a larger trophic increase than reported.

There was greater variability in the $\delta^{15}\text{N}$ values of juveniles than adults, with the difference between the lowest and highest value in the tip of the rostrum varying from 1.5‰ in *M. longimana* to 5.5‰ in *H. atlantica* (small), whereas in the wing this difference ranged from 1.1‰ in *Octopoteuthis* sp. to 3.3‰ in *G. glacialis* and *C. sirventi*. These values suggest that juvenile squid might have a more generalist diet than adults, feeding over 2 to 3 trophic levels, while adults feed over 1 to 2 trophic levels (assuming an increase of $\sim 3.3\%$ per trophic level). However, these differences between life stages may not reflect differences in feeding strategy but rather arise as a result of differences in habitat, i.e. subtropical waters are known to have a higher $\delta^{15}\text{N}$ baseline (Jaeger et al. 2010) which is reflected in higher $\delta^{15}\text{N}$ values of predators. Squid species also have different vertical distributions which may influence their isotopic composition (Drazen & Sutton 2017). Furthermore, heterogeneity in the food chain length between waters, such as a higher num-

ber of trophic levels in subtropical waters than in waters south of the STF, due to the presence of several carnivorous zooplankton species, could lead to variability in squid $\delta^{15}\text{N}$ values.

Values of $\delta^{15}\text{N}$ suggest that feeding strategies vary in squid species in the Pacific sector of the Southern Ocean, either zooplanktivorous (e.g. *G. glacialis* as juvenile), piscivorous (e.g. *Octopoteuthis* sp.) or omnivorous (e.g. *H. atlantica* [large]). This heterogeneity in the community is supported by the 9 feeding groups identified in cluster analysis, and also occurs in other Southern Ocean regions, such as the subantarctic islands of Kerguelen in the Indian sector (Cherel & Hobson 2005). The absence of a significant correlation between estimated mantle length and $\delta^{15}\text{N}$ values suggest that squid trophic ecology is independent of size in these species. Although the lower $\delta^{15}\text{N}$ values at the tip of the rostrum were expected, the fact that tip of the rostrum is a pool of new and old beak material (see above) might indicate that $\delta^{15}\text{N}$ values were overestimated, meaning that juveniles may occupy a lower trophic position than suggested by our results.

With the exception of *C. sirventi* and *H. atlantica* (small and large), the $\delta^{15}\text{N}$ values obtained in this study are consistently lower than values from the Atlantic Sector (Alvito et al. 2015, Seco et al. 2016, Queirós et al. 2018) and from the Indian Sector (Cherel & Hobson 2005, Guerreiro et al. 2015) (Table 3). This difference may be due to variation in methods, as we analysed 2 sections of the beak, whereas the entire beak or a range of entire beak sizes were used in previous studies (Alvito et al. 2015, Guerreiro et al. 2015, Seco et al. 2016). This is supported by the similar values to ours obtained by Queirós et al. (2018) when sectioning beaks of *M. longimana*. This variation can also be related to the different isotopic baselines in the different Southern Ocean sectors (Brault et al. 2018). As has been shown in other species and regions, e.g. *Sthenoteuthis oualaniensis* in the Arabian Sea and in the Pacific and Indian oceans (Takai et al. 2000), differences in $\delta^{15}\text{N}$ values between squids from different Southern Ocean sectors could be due to geographical variation, for example, in prey availability. To clarify whether differences in $\delta^{15}\text{N}$ values of squid across the different Southern Ocean sectors are related to methodology, to $\delta^{15}\text{N}$ baselines, or whether they reflect different trophic ecology, we suggest that a future study perform stable isotope analysis of entire lower beaks in squids from the Pacific sector of the Southern Ocean, facilitating comparison with previous studies.

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Appendix. Detailed statistics (Table A1) and data (Fig. A1)

Table A1. Statistical results (Wilcoxon matched-pairs signed rank test) of comparisons between isotopic values and mass ratios from the tip of the rostrum and the wings. **Bold:** Significant differences ($p < 0.05$). Differences (mean \pm SD) are in relation to wing, e.g. if the difference value is negative, the wing is depleted in relation to the tip of the rostrum

Species	n	$\delta^{13}\text{C}$ values (‰)			$\delta^{15}\text{N}$ values (‰)			C:N mass ratios		
		Statistical value <i>W</i>	p	Difference (‰)	Statistical value <i>W</i>	p	Difference (‰)	Statistical value <i>W</i>	p	Difference
<i>Batoteuthis skolops</i>	9	-45.0	0.004	-1.2 \pm 0.4	45.0	0.004	+2.0 \pm 0.8	18.0	0.316	0.0 \pm 0.1
<i>Galiteuthis glacialis</i>	7	2.00	0.938	-0.1 \pm 1.1	28.0	0.016	+4.0 \pm 1.4	-12.0	0.375	-0.1 \pm 0.2
<i>Taonius notalia</i>	10	-37.0	0.065	-0.4 \pm 0.7	55.0	0.002	+2.8 \pm 1.4	39.0	0.020	+0.1 \pm 0.1
<i>Cycloteuthis sirventi</i>	8	-36.0	0.008	-1.8 \pm 1.7	30.0	0.039	+1.2 \pm 1.2	36.0	0.008	+1.9 \pm 1.9
<i>Histioteuthis atlantica</i> (small)	6	-15.0	0.156	-0.7 \pm 0.9	10.0	0.344	+1.3 \pm 2.4	3.00	0.844	+0.5 \pm 1.3
<i>Histioteuthis atlantica</i> (large)	4	-2.00	0.875	-0.1 \pm 0.3	10.0	0.125	+4.3 \pm 1.0	4.00	0.984	0.0 \pm 0.5
<i>Histioteuthis eltaninae</i>	10	-55.0	0.002	-0.9 \pm 1.1	55.0	0.002	+1.8 \pm 0.9	1.00	0.984	0.0 \pm 0.2
<i>Alluroteuthis antarcticus</i>	4	-10.0	0.125	-0.6 \pm 0.7	10.0	0.125	+2.4 \pm 0.8	4.00	0.625	+0.1 \pm 0.3
<i>Octopoteuthis</i> sp.	3	-6.00	0.250	-1.5 \pm 1.4	6.00	0.250	+1.8 \pm 1.1	6.00	0.250	+1.1 \pm 0.8
<i>Moroteuthopsis longimana</i>	7	28.0	0.016	+0.8 \pm 0.6	28.0	0.016	+2.4 \pm 0.9	28.0	0.016	+0.1 \pm 0.1
<i>Filippovia knipovitchi</i>	5	-15.0	0.063	-2.0 \pm 0.6	13.0	0.125	+1.7 \pm 1.5	15.0	0.063	+1.2 \pm 1.1

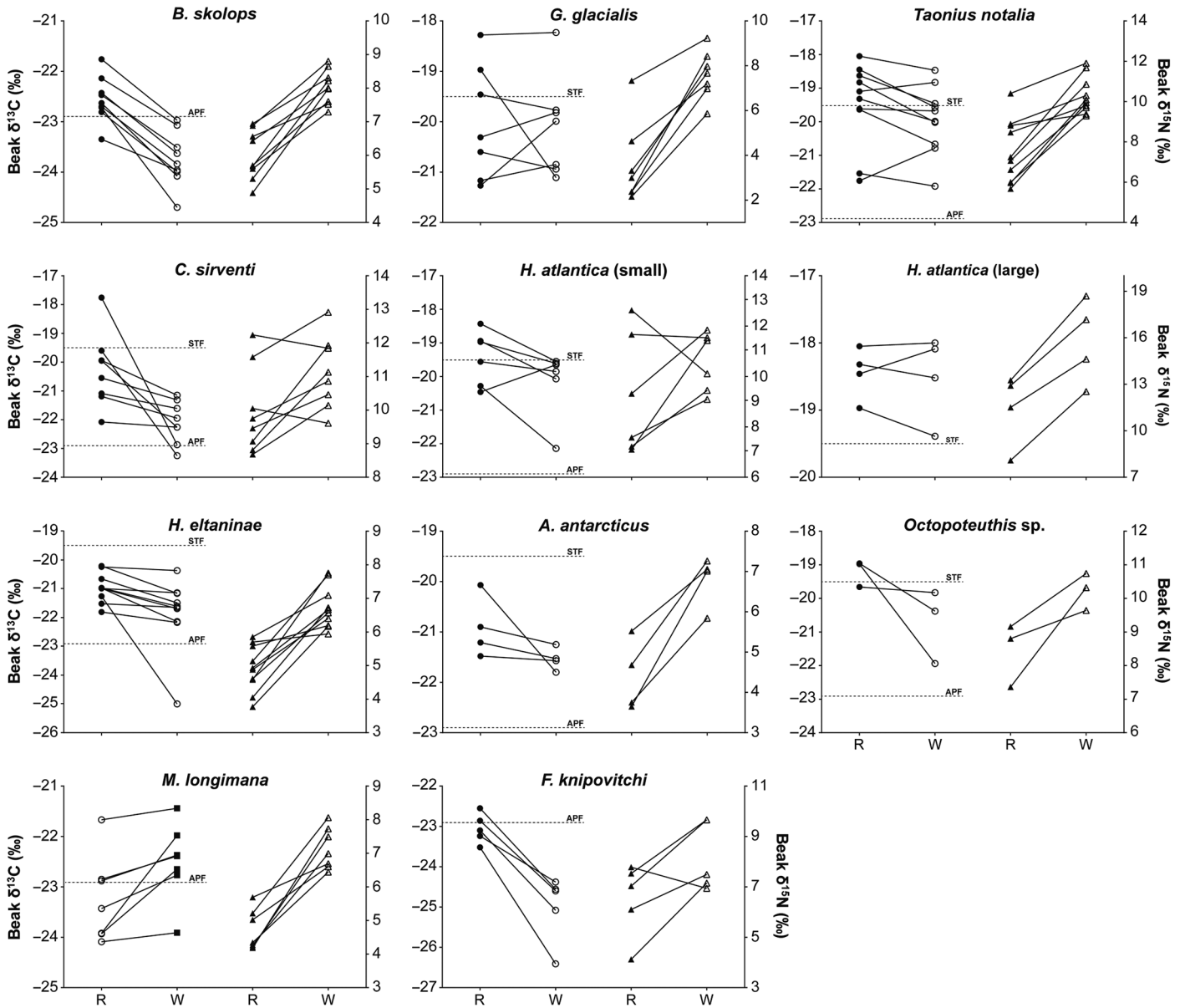


Fig. A1. Beak $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of tip of the rostrum and wing of all studied species and individuals. Filled symbols (circles and triangles) represent the tip of the rostrum values; Empty symbols (circles and triangles) represent the wing values. R: tip of the rostrum; W: wing; APF: Antarctic polar front; STF: subtropical front

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