Low trophic level diet of juvenile southern elephant seals *Mirounga leonina* from Marion Island: a stable isotope investigation using vibrissal regrowths

Nico Lübcker[1*], Ryan R. Reisinger[1+], W. Chris Oosthuizen[1], P.J. Nico de Bruyn[1], André van Tonder[1], Pierre A. Pistorius[2, 3], Marthán N. Bester[1]

[1]Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Hatfield 0028, Pretoria, South Africa.

[2]Marine Apex Predator Research Unit, Institute for Coastal and Marine Research, Nelson Mandela Metropolitan University, South Campus, Port Elizabeth 6031, South Africa.

[3]DST/NRF Centre of Excellence at the Percy FitzPatrick Institute, Department of Zoology, Nelson Mandela Metropolitan University, South Campus, Port Elizabeth 6031, South Africa.

[+]Centre d'Etudes Biologiques de Chizé, UMR 7372 du CNRS-Université de La Rochelle, 79360 Villiers-en-Bois, France.

*Corresponding author: nlubcker@zoology.up.ac.za

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ABSTRACT

Insight into the trophic ecology of marine predators is vital for understanding their ecosystem role and predicting their responses to environmental change. Juvenile southern elephant seals (SES) *Mirounga leonina* are considered generalist predators within the Southern Ocean. Although mesopelagic fish and squid dominate their stomach lavage samples, the stable isotope (SI) profile captured along the length of sampled vibrissae of young SES at Macquarie Island recently emphasized the contribution of crustaceans to their diet (likely *Euphausia superba*). Herein, we used the SI values of sampled vibrissal regrowths with known growth histories to assess the diet of juvenile SES at Marion Island on a temporally integrated basis. We specifically aimed to quantify the possible contribution of crustaceans to the diet of juvenile SES. Sequentially (chronologically) sampled vibrissal regrowths of 14 juvenile SES produced fine-scale dietary information spanning up to nine months. The depleted δ^{15}N (8.5 ± 0.6‰) and δ^{13}C (-20.3 ± 0.1‰) measured during the period of independent foraging suggested the use of a lower trophic level diet within the Polar Frontal Zone. A mixing model predicted that up to 76% of their diets comprised of crustaceans, consisting of two crustaceans groups, each contributing 26% (Credible Interval (CI): 13 – 39%) and 50% (CI: 35 – 64%) to their diets, presumably representing subantarctic krill species. This first utilisation of the isotopic signature captured along the length of vibrissal regrowths confirms the inclusion and importance of crustaceans in the diet of juvenile SES.

KEYWORDS: Crustaceans, Diet, Marine mammals, Pinnipeds, Stable isotopes, Vibrissae, Whiskers

INTRODUCTION

Populations of apex marine predators respond behaviourally and demographically to bottom-up processes, such as changes in prey abundance and distribution (Weimerskirch et al. 2003, Constable et al. 2014). Understanding their foraging ecology is pivotal for predicting their responses to environmental change (Hindell et al. 2003). Longitudinal dietary studies of marine mammals, however, remain notoriously difficult (Young et al. 2015). Southern elephant seals (SES) *Mirounga leonina*, for example, are often regarded as top marine mammal predators in the Southern Ocean (e.g. Hückstädt et al. 2012a). Yet, their extensive foraging migrations hinder fine-scale, longitudinal dietary assessments through conventional dietary reconstruction approaches, leading to uncertainty about their ecological role as predators.

Stomach lavage samples and stable isotope (hereafter, SI) values of blood and keratinous tissue, such as vibrissae (whiskers), suggest that juvenile and adult SES consume myctophids (lantern fishes) and cephalopods across their entire circumpolar distribution (Daneri and Carlini 2002, Field et al. 2007a, Cherel et al. 2008, Ducatez et al. 2008, Newland et al. 2011). Given the low abundance of crustaceans previously recovered in the stomach contents of SES, their significance has been questioned (Green and Burton 1993, Slip 1995, Burton and Van den Hoff 2002, Van den Hoff et al. 2003, Field et al. 2007b). The ingestion of krill was previously considered accidental or due to secondary consumption (Slip 1995). Nevertheless, stable isotope analysis of juvenile SES (< 1-year-old (yo)) vibrissae sampled at Macquarie Island (Fig. 1) recently led Walters et al. (2014) to recognise juvenile SES as "a new krill predator in the Southern Ocean". In that study, vibrissae were sampled once after returning from their first ca. 80 to 140-day foraging trip at sea, and 9.1 % of the total length of the vibrissae sampled from seven (out of 12) SES represented active foraging (Walters et al.

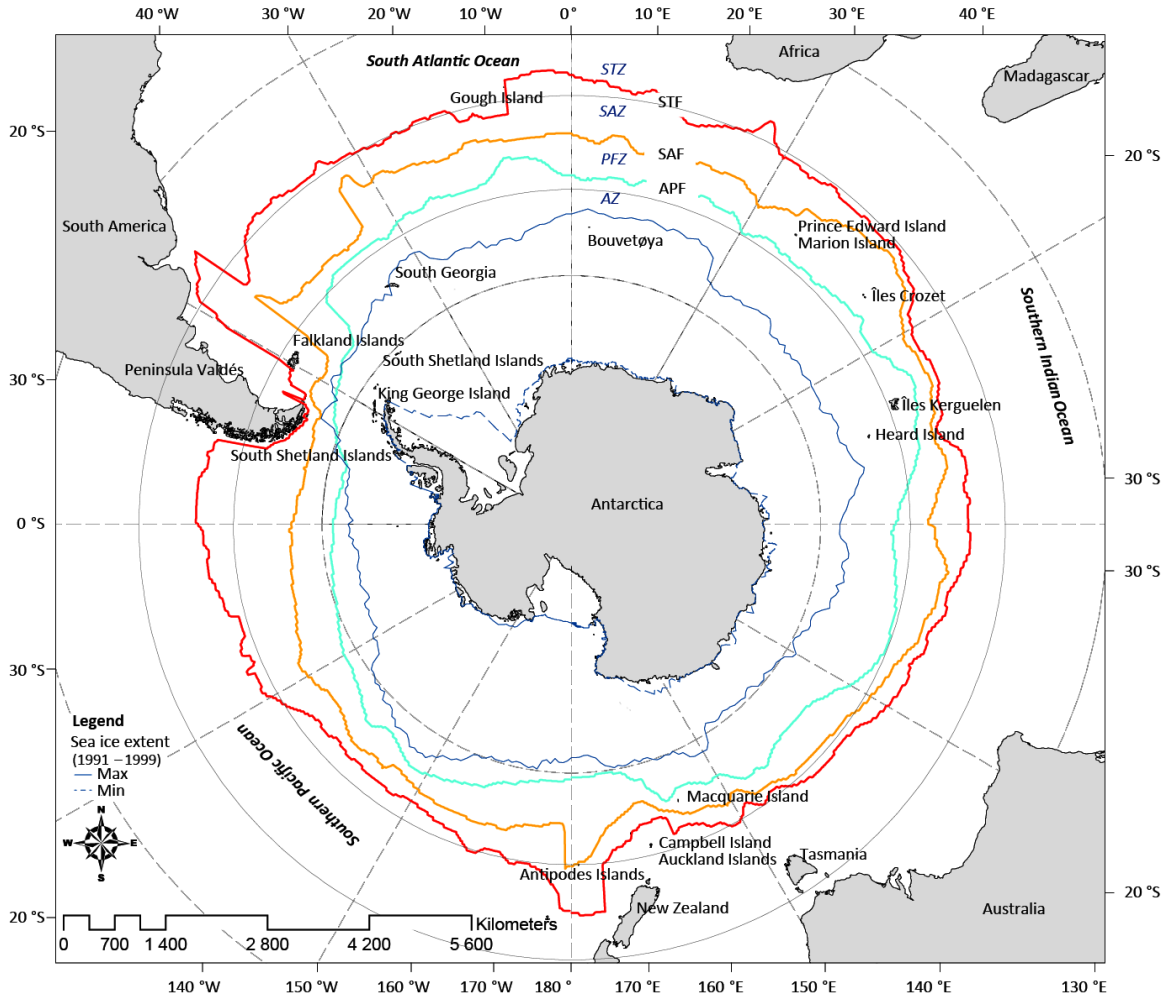

FIG. 1: Circumpolar map of the Southern Ocean displaying the localities (islands) where southern elephant seals *Mirounga leonina* populations are located, and from where prey isotopic values where obtained in relation to the circumpolar oceanic currents. The Subtropical Front (STF) represents the northern boundary of the Southern Ocean, and separates the Subtropical Zone (STZ) (north of the STF) from the Subantarctic Zone (SAZ). The SAZ is bounded to the south by the Subantarctic front (SAF). The water mass south of the SAF represents the Polar Frontal Zone (PFZ). The Antarctic Polar Front (APF) separates the Antarctic Zone (AZ) from the water masses in the north (Durgadoo et al. 2010).

2014). The low mean δ^{15}N (9.6‰) suggested a mixed diet of fish, squid, and crustaceans, presumably Antarctic krill *Euphausia superba* (Walters et al. 2014). However, the overall dietary contribution of crustaceans was not quantified. Moreover, temporal interpretations were biased as the recently described asymmetric growth rate of SES vibrissae (Lübcker et al. 2016) suggests that merely a small fraction of the Macquarie Island SES' first foraging trip was likely represented in the vibrissae analysed by Walters et al. (2014).

The Marion Island population of SES represents one of the most northerly breeding SES populations in the Southern Ocean, and unlike Macquarie Island, is situated outside the distributional range of *E. superba* (Pakhomov et al. 1994) (Fig. 1). Yet, even though this SES population has been subject to a multi-decade research programme (Bester et al. 2011), their diet remains unknown. The absence of dietary data impedes our ability to link observed environmental changes to fluctuations in this population's demographic parameters, including changes in weaning mass (Oosthuizen et al. 2015) and their survival rates (Pistorius and Bester 2002, Pistorius et al. 2004, McMahon et al. 2005).

The SI values captured along the length of vibrissae is biologically inert after biomolecule deposition (Cherel et al. 2009), providing a fine-scale chronology of dietary data that spans the growth period of the vibrissae (e.g. Beltran et al. 2015, Lübcker et al. 2016). Herein, we use a novel approach to extend the temporal resolution of dietary information obtained from vibrissae by sampling and resampling vibrissae from SES before and after their first foraging trip at sea. By cutting selected vibrissae of recently weaned SES and then sampling the regrowth on the seals' return to Marion Island, we extended the vibrissal growth period in comparison to Walters et al. (2014) and enabled a temporally integrated dietary assessment spanning their first year spent at sea (detailed in Lübcker et al. 2016).

The aim of this study was to assess the diet of juvenile SES from Marion Island using the SI values captured along the length of vibrissal regrowths with a known growth history. The study quantifies the dietary composition of juvenile SES during their first foraging trip, with special reference to the possible contribution of crustaceans to their diet. The use of vibrissal regrowths provides the highest resolution, temporally integrated dietary information of juvenile SES to date. Moreover, this study represents the first dietary assessment of SES at Marion Island.

MATERIALS AND METHODS

Study site

Marion Island is the largest of two islands in the Prince Edward Islands (PEIs) archipelago, situated within the Polar Frontal Zone (PFZ) (Fig. 1). Juvenile SES at Marion Island predominantly forage in pelagic waters more than 3000 meters deep to the south-west of the island, mainly within the PFZ (Tosh et al. 2012, 2015), while being physiologically restricted to the upper 100 to 200 m of the water column (Hindell et al. 1999). Most foraging occurs between 43º S and 56º S, around the Subantarctic Front (SAF) and Antarctic Polar Front (APF), respectively (Tosh et al. 2012). *Euphausia superba* are absent in the waters surrounding the PEIs; the food web is dominated by other euphausiids such as *E. vallentini*, salps, amphipods, and copepods (Pakhomov et al. 1994).

Sample collection

Vibrissae sampling from juvenile southern elephant seals

Vibrissae were sampled from 2011 to 2013, as part of a long-term mark-recapture programme on SES (Bester et al. 2011). Weaned SES pups (25 – 60 days old) were sexed, individually marked with hind-flipper tags (De Bruyn et al. 2008), and only the longest mystacial vibrissa

TABLE 1: The δ^{15}N and δ^{13}C (mean ± SD) of the independent foraging trip was obtained from the sequentially sampled vibrissal regrowths of n = 14 individual juvenile southern elephant seals *Mirounga leonina*, sampled during 2012 and 2013 at Marion Island. The percentage of the vibrissae representing independent foraging (Independent foraging (%)) and the isotopic niche breadth (Standard Ellipse Area (SEA_c)) utilized by each individual are indicated. The displayed δ^{15}N and δ^{13}C values represent the original values, before applying a trophic enrichment factor for the dietary reconstructions. M = male, F = female.

Individual	**Resampling date**	**Sex**	**Length (mm)**	**Days after initial sampling**	**Independent foraging (%)**	**δ^{15}N**	**δ^{13}C**	**SEA_c**
PG288	2012/10/23	F	81	374	42.9	8.6 ± 0.4	-21.0 ± 0.2	0.13
PG090	2012/12/13	F	74	397	32.4	8.9 ± 0.3	-20.0 ± 0.1	0.33
PG024	2012/12/18	F	73	419	21.9	8.1 ± 0.1	-19.7 ± 0.2	0.07
PG051	2012/09/08	M	81	316	17.1	8.1 ± 0.3	-20.4 ± 0.1	0.05
PG008	2012/11/14	M	65	388	18.1	8.8 ± 0.2	-20.1 ± 0.1	0.03
PG030	2012/11/17	M	47	266	19.6	8.5 ± 0.1	-20.0 ± 0.1	0.01
PG007	2012/11/27	M	94	401	33.3	9.1 ± 0.1	-20.2 ± 0.1	0.05
PG084	2012/12/13	M	46	405	4.3	9.0 ± 0.2	-21.2 ± 0.1	-
YO404	2013/11/29	F	60	380	20.0	8.6 ± 0.2	-20.3 ± 0.2	0.17
YO064	2013/11/30	F	71	397	22.9	8.4 ± 0.2	-20.0 ± 0.1	0.06
YO081	2013/07/14	M	79	250	30.8	8.9 ± 0.2	-20.1 ± 0.1	0.02
YO059	2013/11/30	M	58	392	11.5	6.9 ± 0.5	-20.5 ± 0.1	0.21
YO067	2013/12/03	M	63	397	15.2	8.6 ± 0.3	-20.7 ± 0.1	0.12
PG171	2012/08/28	M	75	387	66.7	9.6 ± 1.4	-20.3 ± 0.1	-
Mean ± SD		***n* = 14**	**69.0 ± 13. 4**	**362.4 ± 56.2**	**25.48 ± 15.4**	**8.6 ± 0.3**	**-20.3 ± 0.1**	**0.1 ± 0.1**

- not computed due to less than three segments representing independent foraging

on the right muzzle was cut as close to the skin as possible. Vibrissal regrowths are easily identifiable due to their blunt ends; these were subsequently cut when the seals returned after spending several months to a year foraging at sea (detailed in Lübcker et al. 2016). For the purpose of this study, 'juvenile' refers to individuals up to 15 months old. We analysed vibrissal regrowths of 14 juvenile SES (nine males, five females), collected 250 to 419 days (362 ± 56 days; mean ± standard deviation) after the initial sampling (Table 1). The length of the vibrissal regrowths sampled ranged from 46 to 94 mm (mean = 69.0 ± 13.4 mm). Resampling regrowths from juveniles required chemical immobilisation, administered through an intramuscular injection of ketamine hydrochloride (Bester 1988).

Isotopic values of potential prey

The remote foraging distribution of Marion Island SES prevented direct sampling of potential prey species. Isotopic values of potential prey were obtained from published cephalopod and fish isotopic values for Marion Island (Bushula et al. 2005), Îles Kerguelen (Cherel et al. 2010), and Îles Crozet (Guerreiro et al. 2015). We analysed all available, comprehensive prey SI datasets available for the south Indian sector of the Southern Ocean that fell within a similar latitudinal range as the foraging range of the Marion Island juvenile SES (43º S to 56º S) (Tosh et al. 2012, 2015).

Myctophids form the bulk of the fish biomass in the Southern Ocean south of the Subtropical front (Cherel et al. 2010), and supposedly comprise the largest portion of juvenile SES diets (Newland et al. 2011). In the isotopic baseline, we use SI values for the 14 myctophid species representative of the community of myctophids in the Southern Ocean (Cherel et al. 2010). These include the four most abundant species (*Electrona antarctica*, *E. carlsbergi*, G*ymnoscopelus nicholsi*, and *Krefftichthys anderssoni*)(Duhamel et al. 2000, Cherel et al. 2010). We additionally included the SI values of two Notothenioids, *Gobionotothen*

marionensis and *Lepidonotothen larseni*, sampled at Marion Island (Bushula et al. 2005, Supplementary table 1S).

Guerreiro et al. (2015) provide the most comprehensive description of the isotopic niches of cephalopod species occurring in the south Indian sector of the Southern Ocean. We used these published data in our analyses, specifically the lower beak isotopic values of 11 species obtained from diet samples of wandering albatross *Diomedea exulans* at Îles Crozet. We excluded two Subtropical front species (*Histioteuthis atlantica* and *Taonius sp.*), thereby including only cephalopods inhabiting the subantarctic region (δ^{13}C ranging from -22.9 to -19.5‰; Guerreiro et al. 2015). Unpublished isotopic values of *Kondakovia longimana* lower beaks from grey-headed albatross *Thalassarche chrysostoma* regurgitates at Marion Island nest sites were also included. For the dietary reconstruction, we used Guerreiro et al.'s (2015) adjusted beak isotopic values to represent the soft-tissue of squid. The *K. longimana* beaks sampled at Marion Island were adjusted to represent soft-tissue by adding 4.86‰ to the δ^{15}N values for beaks and subtracting 0.75‰ from the beak δ^{13}C values (Hobson and Cherel 2006), consistent with Guerreiro et al. (2015).

Krill samples were obtained from fresh macaroni *Eudyptes chrysolophus* and rockhopper *E. chrysocome filholi* penguin diet samples collected during April 2012 and 2013 at Marion Island, and identified using a published key (Baker et al. 1990). Three krill species were identified and included: *Euphausia vallentini*, *E. frigida*, and *Thysanoessa* sp. (*T. vicina* and *T. macrura* are difficult to distinguish morphologically, and classification to genus level sufficed). Partial digestion in the proventriculus of the penguins results in lower δ^{15}N values (Cherel et al. 2008) and we therefore only included undigested krill specimens; the SI values of these specimens are presumably unaffected by digestion (Cherel et al. 2010). Krill samples were stored at -20° C until analysed. Due to the predominantly pelagic SES foraging strategy

at Marion Island, we decided not include benthic crustaceans, such as the benthic shrimp *Nauticaris marionis*, in our analyses.

Stable isotope analysis

The isotopic values captured in the tip of the regrowths represents the pre-foraging period (gestation, lactation, and post-weaning fast), followed by the transition period when the isotopic turn-over from the maternally derived SI signature to the SI signature obtained from independent foraging occurs (Lübcker et al. 2016). The transition period is demonstrated by a ca. 3.7‰ δ^{15}N depletion (e.g. Walters et al. 2014). The base of the regrowths represents the diet consumed during the independent foraging period (Walters et al. 2014, Lübcker et al. 2016), and we only used segments immediately after the δ^{15}N depletion occurred for the dietary reconstruction (independent foraging) (Fig. 2). Sample preparation and analyses of vibrissae, hair, and feathers followed the procedures outlined in Lübcker et al. (2016). Samples were cleaned by sonication in a 1:2 chloroform:ethanol solution, repeated three times before rinsing with distilled water and oven-drying for 24 h at 70 ˚C. Vibrissae were sequentially sub-sampled into 2 mm (± 0.3 mm) sections from the proximal portion (base) to the distal portion (tip), obtaining an average of 33.9 ± 6.8 segments per vibrissa. Each 2 mm section was again sub-sampled, and 0.5 – 0.6 mg weighed into tin capsules (pre-cleaned in Toluene) for SI analysis. The remaining portion was also analysed as a duplicate if a 0.5 – 0.6 mg sample was still available. The distal end of hair samples was used for SI analysis. Whole, individual krill samples were homogenised, oven-dried, lipid extracted and decarbonised in a 1 mol HCl L^{-1} solution to remove inorganic carbonates (following Cherel et al. 2008). Cephalopod beaks were stored in 70% ethanol and cleaned with distilled water prior to isotopic analysis, similar to Guerreiro et al. (2015).

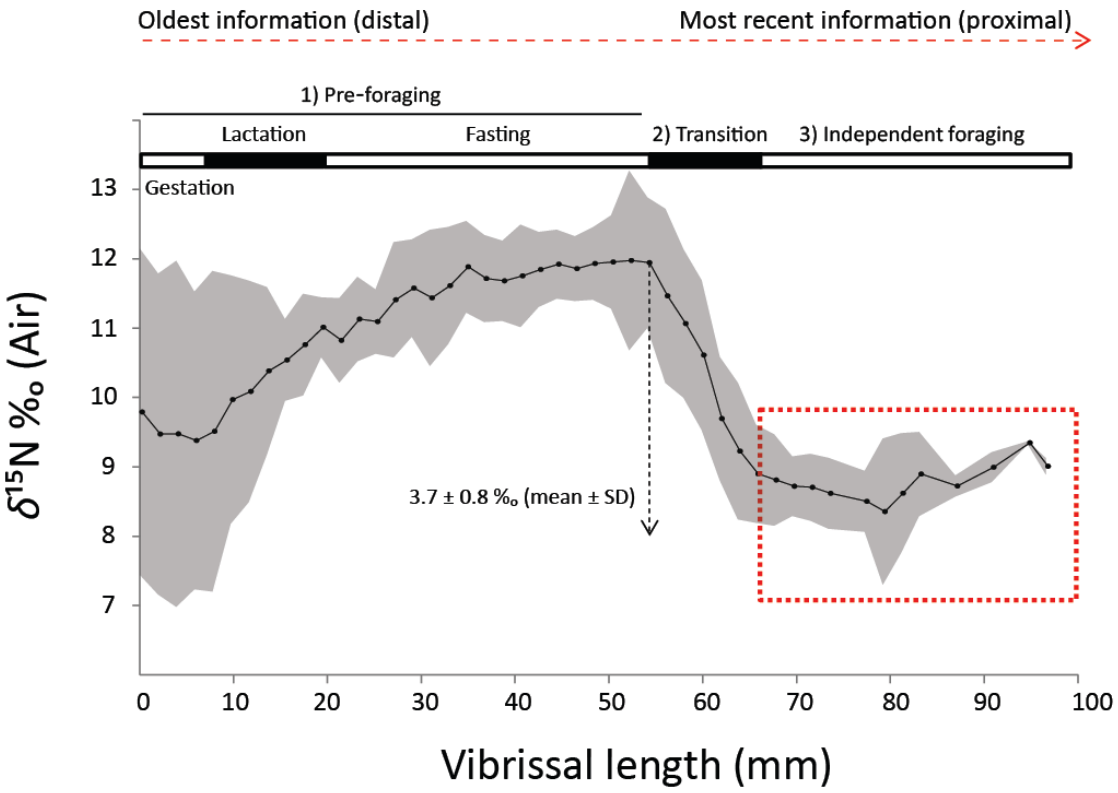

FIG. 2: Representation of data indicating the three isotopically distinct portions of the vibrissal regrowths collected from juvenile southern elephant seals *Mirounga leonina*. Life history events can be distinguished based on δ^{15}N (mean ± SD) measured along the length of the vibrissae. The onset of the independent foraging (transitioning period) is characterised by a 3.7 ‰ δ^{15}N depletion and we used only the period representing independent foraging for dietary reconstruction (red box).

Weighed sample aliquots were combusted in an elemental analyser (Flash EA, 1112 Series, Thermo™, Thermo Fisher Scientific), and the $\delta^{13}C$ and $\delta^{15}N$ isotopes were determined using a continuous-flow isotope ratio mass spectrometer (Delta V Plus, Thermo Finnigan) at the Stable Isotope Laboratory of the Mammal Research Institute, University of Pretoria, South Africa. Results are presented using standard delta notation in parts per thousand (‰) relative to an international standard: Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 (Air) for $\delta^{15}N$. Duplicate aliquots of n = 112 samples were interspersed with an in-house standard (Merck gel) and blank after every 10 samples to ensure reproducibility. Reproducibility of $\delta^{13}C$ and $\delta^{15}N$ values based on the standards was < 0.20‰, while the reproducibility of duplicate sample aliquots was ± 0.27‰ for $\delta^{15}N$ and ± 0.11‰ for $\delta^{13}C$.

Predicting the foraging range of juvenile southern elephant seals

The predictable latitudinal $\delta^{13}C$ gradient in particulate organic matter (POM) in the Southern Ocean allows differentiation between low latitudinal Subtropical Zone waters and Antarctic Zone (AZ) waters at high latitudes (Trull and Armand 2001). Oceanic fronts are characterised by steep temperature, salinity, and water density gradients, and the Polar Frontal Zone represents a major barrier against the mixing of subantarctic waters to the north and polar surface waters to the south (Durgadoo et al. 2010). The latitudinal gradient in $\delta^{13}C$ POM in the Southern Ocean is also reflected in the tissue of predators (Jaeger et al. 2010). Species- and tissue-specific $\delta^{13}C$ maps of gradients in stable isotopes ('isoscapes', Trull and Armand 2001, Jaeger et al. 2010) enable inference of the latitudes at which consumers foraged.

The latitudinal $\delta^{13}C$ gradient around Marion Island was previously characterised using the vibrissae of Antarctic fur seals sampled at the island. Walters (2014) found that the $\delta^{13}C$ of the AZ (57.7 ± 1.3° S; $\delta^{13}C$ = -21.5 ± 0.7‰) was more depleted relative to SAF (47.6 ± 1.6° S; $\delta^{13}C$ = -19.9 ± 1.2‰,), and that the APF was characterised by a $\delta^{13}C$ of -20.5 ± 1.2‰. A

δ^{13}C difference of ca. 1.6‰ corresponds to a 10° difference in foraging latitude (Walters 2014) and our inference was, therefore, restricted to the allocation of foraging locations to different oceanic fronts or water masses only, as based on the δ^{13}C values of the juvenile SES vibrissae, following Walters (2014). A Pearson's product-moment correlation test was used to determine the relationship between the vibrissal δ^{15}N and δ^{13}C values, which can be indicative of a shift in the δ^{15}N baseline values between different latitudes.

Statistical analyses

Dietary reconstruction

Bayesian stable isotope mixing models – fitted using the Stable Isotope Analysis in R (*Siar*, version 4.1.2) package (Parnell et al. 2010) (R Core Team 2015, version 3.2.3) – were used to reconstruct the juvenile SES diet. A diet-vibrissa specific trophic discrimination factor (hereafter, TDF) obtained from captive pinnipeds (Δ^{15}N = 2.8‰; Δ^{13}C = 3.2‰) (Hobson et al. 1996) was used to reconstruct SES diets. Southern elephant seal-specific TDFs are not available, and the TDFs from harp seals *Pagophilus groenlandicus*, harbour seals *Phoca vitulina*, and ringed seals *Phoca hispida* (Hobson et al. 1996; but also *c.f.* Beltran et al. 2016) are widely used instead (e.g. Eder et al. 2010, Newland et al. 2011, Hückstädt et al. 2012b, Walters et al. 2014). Possible prey items were identified based on their position in the isotope mixing polygon relative to the consumers ('isospace' in SIAR) (see Supplementary material for details). Based on the SIAR mixing polygon and the applied TDFs, prey species with δ^{15}N above or below 3.7 and 12.8‰ and a δ^{13}C above or below -16.2 and -24.5‰ (adjusted values), respectively, were excluded from further analyses (Supplementary Fig. 1S). Mixing models cannot differentiate between prey species with similar SI signatures (Phillips et al. 2014) and we clustered prey with similar SI values into groups using the *hclust* package in R (Müllner 2013, version 3.3.1) (Supplementary Fig. 2S). The identified prey groups were

considered unique if they differed significantly in $\delta^{15}N$, $\delta^{13}C$, or both, evaluated using an ANOVA (Supplementary table 2S). Mixing models will always attempt to fit a model to the data, even if the data are nonsensical, and the SIAR isospace plots were thus scrutinised to ensure that all relevant prey items (among those available) were included or excluded (Phillips et al. 2014).

The proportional contribution of each prey group to the diet of the juvenile SES was determined by running three chains of 100000 iterations, discarding the first 25000 iterations and then retaining every 25th iteration. A residual error term for $\delta^{15}N$ (SD $\delta^{15}N$) and $\delta^{13}C$ (SD $\delta^{13}C$) accounts for uncertainties or missing prey sources (Parnell et al. 2010). The mode of the mixing models represents the most likely solution, and we reported the mode, as well as the Bayesian credibility intervals (CI, analogue of frequentist confidence intervals) of the contribution of the different prey groups. Mixing models have the additional advantage of being able to incorporate uncertainty in the TDF and prey isotopic values (Parnell et al. 2010), thereby preventing biased model outputs (see Phillips et al. 2014). To account for the lack of SES-specific TDFs, a standard deviation of 0.3‰ for both $\Delta^{15}N$ and $\Delta^{13}C$ were assigned, thereby ensuring conservative data interpretations (Hobson et al. 1996). We included the individual identities of the juvenile SES as a random effect to account for multiple (but variable) number of vibrissal segments sampled per individual, thereby ensuring that each individual has equivalent weight in the analyses.

We assessed the individual juvenile SES isotopic niche breadths using the Stable Isotope Bayesian Ellipses (SIBER) model (Jackson et al. 2011) in the *Siar* package. The Standard Ellipse Area (SEA_c) provides a measure of the isotopic niche utilised by each individual (Layman et al. 2007, Jackson et al. 2011), and was used to assess the isotopic niche overlap between juvenile SES. The SEA_c contains around 40% of the isotopic data, thereby

representing the core isotopic niche of each species while correcting for variable sample sizes (Layman et al. 2007, 2012, Jackson et al. 2011). The isotopic niche overlap was calculated from the SEA_c output after 16 000 iterations (approach detailed in Jackson et al. 2011).

We estimated the daily resolution and temporal span of the dietary data using vibrissal regrowth rates of juvenile SES sampled at Marion Island (Lübcker et al. 2016). The time represented by each 2 mm vibrissal section was determined as:

$$T = \left[\frac{-1}{K}\ln\left(1 - \frac{S_T}{A}\right)\right] + T_0 \quad (2)$$

(from Beltran et al. (2015)), where S_T is the length of the regrowth at time T; A is the asymptotic length (maximum length that the vibrissa can reach); and K is the curvature parameter used to describe their vibrissal regrowth trajectories (Eq. 2). The vibrissal growth parameters (A and K) of the juvenile SES used in this study were estimated in a Bayesian framework, detailed in Lübcker et al. (2016). The time at which growth begins (T_0) was zero, because we cut the vibrissae down to the skin. The maximum growth rate occurs directly after cutting, and because we left a 12 mm portion of the vibrissa embedded when sampled, the maximum growth rate occurred 12 mm from the tip of the new regrowth (see Hall-Aspland et al. 2005). Vibrissae sampled from different positions in the vibrissal bed-map also have different A and K values (Beltran et al. 2015). We accounted for this, and for the 12 mm embedded remnant, by using the length of each regrowth plus 12 mm to obtain an estimate of the asymptotic length of each vibrissal regrowth analysed (Lübcker et al. 2016). The vibrissal regrowths were still actively growing when resampled and represented the entire period spent at sea (Lübcker et al. 2016). The 12 mm section again left embedded after cutting the regrowths represent the dietary information captured from September to December (Lübcker

et al. 2016). Our results thus refer to foraging from December/January to August during the first year of an elephant seal's life.

We tested for differences in stable isotope values using appropriate parametric or non-parametric tests, based on the distribution of the values. Values are presented as means ± one standard deviation (SD). Statistical significance was assumed at $p < 0.05$. All analyses were done in R (R Core Team 2015, version 3.2.3).

RESULTS

Isotopic signature of vibrissal regrowths

There were no significant inter-annual differences in the isotopic values measured along the length of vibrissae collected in 2012 and 2013 (Wilcoxon rank sum test: δ^{15}N; $W = 25472$, $p = 0.6$, and δ^{13}C; $W = 286466$, $p = 0.09$). The δ^{13}C for 2012 and 2013 differed by 0.05‰, while the δ^{15}N differed by 0.1‰. The δ^{13}C and δ^{15}N values differed significantly between males and females (Wilcoxon rank sum test: δ^{13}C; $W = 38820$, $p < 0.01$, and δ^{15}N; $W = 23423.5$, $p = 0.03$). However, the difference in the δ^{13}C and δ^{15}N between males and females was only 0.3‰ and 0.2‰, respectively, and we pooled the data across years and sexes for subsequent analyses.

The δ^{15}N and δ^{13}C values during the pre-foraging, transition, and independent foraging period were isotopically distinct from each other (δ^{15}N: Kruskal-Wallis $\chi^2 = 259.9$, $df = 2$, $p < 0.001$; δ^{13}C: Kruskal-Wallis $\chi^2 = 19.7$, $df = 2$, $p < 0.001$). During pre-foraging, δ^{15}N ranged from 10.9 to 12.2‰ (11.6 ± 0.4‰,), and δ^{13}C from -19.2 to -21.2‰ (-20.1 ± 0.5‰). The transition period was represented by a 2.7 to 5.7‰ δ^{15}N depletion (difference in δ^{15}N = -3.7 ± 0.8‰) (Fig. 3). The δ^{15}N during independent foraging was significantly lower than during the pre-

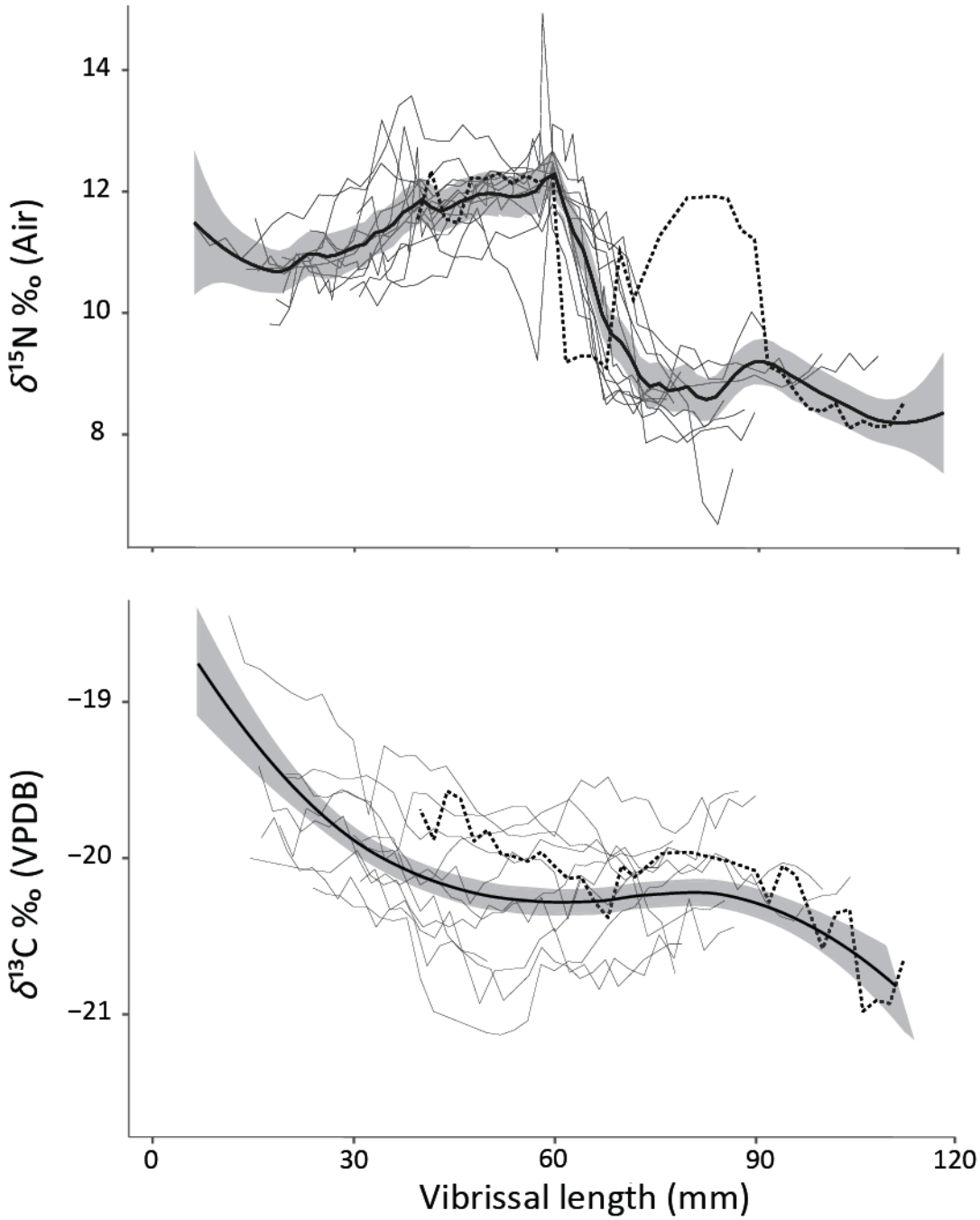

FIG. 3: Individual variation in δ^{15}N (top panel) and δ^{13}C (bottom panel) measured along the length of vibrissal regrowths collected from 14 juvenile southern elephant seals *Mirounga leonina* aged 0.75 – 1.21 years (1.05 ± 0.15 yo). The solid black lines with grey error bands represent the applied lowess smoothing algorithm. The dashed line represents the isotopic profile of PG171.

foraging period ($p < 0.001$), while the $\delta^{13}C$ was within 0.2‰ of the independent foraging value. The $\delta^{15}N$ during the independent foraging phase ranged from 6.9 to 9.6‰ (8.6 ± 0.3‰), while the $\delta^{13}C$ ranged from -19.7 to -21.2‰ (-20.3 ± 0.1‰). From 4.3 to 66.7% (or 1.9 – 50 mm) of the total vibrissal length represented independent foraging (Table 1).

One individual (PG171) was considered an outlier (Fig. 3) and was excluded from further analyses. Similar to that observed for most of the other individuals, the $\delta^{15}N$ of PG171 became depleted by 3.0‰ during the isotopic transition from resources obtained during lactation to active foraging (Fig. 3). In contrast to all other individuals, its $\delta^{15}N$ value then increased again by 2.6‰ during the independent foraging period, before again decreasing by 3.2‰.

During the period of independent foraging (Fig. 4), we detected significant inter-individual differences in both $\delta^{15}N$ (Kruskal-Wallis $\chi^2 = 76.6$, $df = 12$, $p < 0.001$) and $\delta^{13}C$ (Kruskal-Wallis $\chi^2 = 90.2$, $df = 12$, $p < 0.001$) values. The measured $\delta^{15}N$ and $\delta^{13}C$ ranged between 6.9‰ and 9.6‰. The maximum $\delta^{13}C$ (-19.4‰) differed by 1.9‰ from the minimum $\delta^{13}C$ ($\delta^{13}C$ = -21.3‰), defining the latitudinal extremes of their foraging range. Foraging occurred predominantly within the PFZ, south of the SAF (Fig. 4), corresponding to a $\delta^{13}C$ range of -19.7 to -21.2‰ (mode = -20.1‰). Foraging also occurred in the SAZ, and two individuals (PG090 and PG288) likely foraged in the AZ. The $\delta^{15}N$ increased with 0.19‰ for every 1‰ $\delta^{13}C$ increment (Supplementary Fig. 4S), but this correlation was not significant (Pearson's R^2 = 0.16 [95% Confidence Interval: -0.03- 0.34], $p = 0.09$).

A Wilcoxon rank sum test indicated that the $\delta^{15}N$ and $\delta^{13}C$ in the first half of vibrissal regrowths did not differ significantly from the second half ($\delta^{15}N$; $W = 1190$, $p = 0.14$; $\delta^{13}C$; $W = 1236.5$, $p = 0.23$), although disregarding growth rate differences between the two halves.

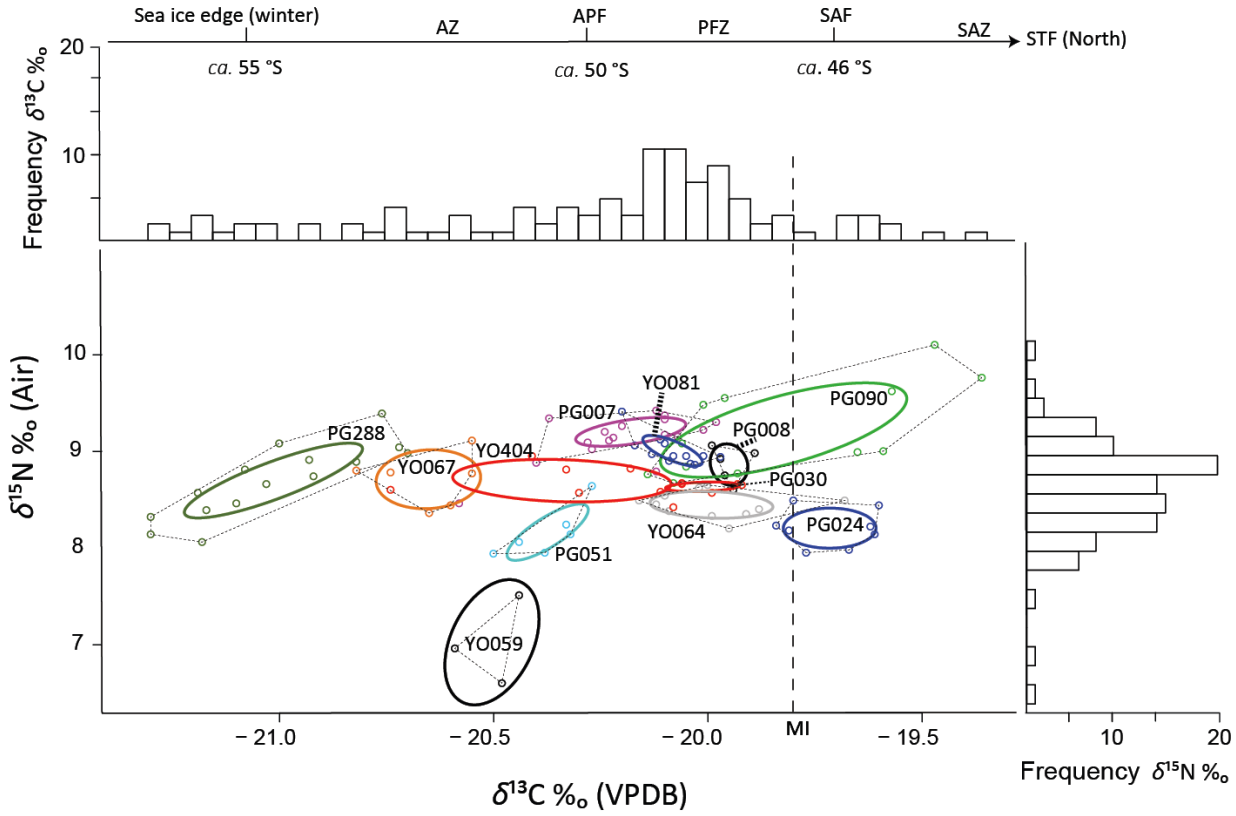

FIG. 4: Standard Ellipse Area (SEA_c) representing the core isotopic niche (solid lines) utilized by each juvenile southern elephant seal (SES) *Mirounga leonina*, during the period of independent foraging. Juvenile SES utilized a restricted niche, predominantly consuming prey from the same trophic level, as indicated by the unimodal $\delta^{15}N$ frequency distribution. The distribution of $\delta^{13}C$ suggests foraging over a wide geographical range, with most individuals foraging south of Marion Island, within the Polar Frontal Zone (PFZ). PG084 is not present in the isoplot due to a small number of segments representing independent foraging. The predicted position of Marion Island (MI; vertical dashed line) and the oceanic fronts relative to the $\delta^{13}C$ values are indicated. Dashed polygons refer to the total convex hull area utilized by each individual. STF = Subtropical Front; SAZ = Subantarctic Zone; SAF = Subantarctic Front; AZ = Antarctic Zones; APF = Antarctic Polar Front.

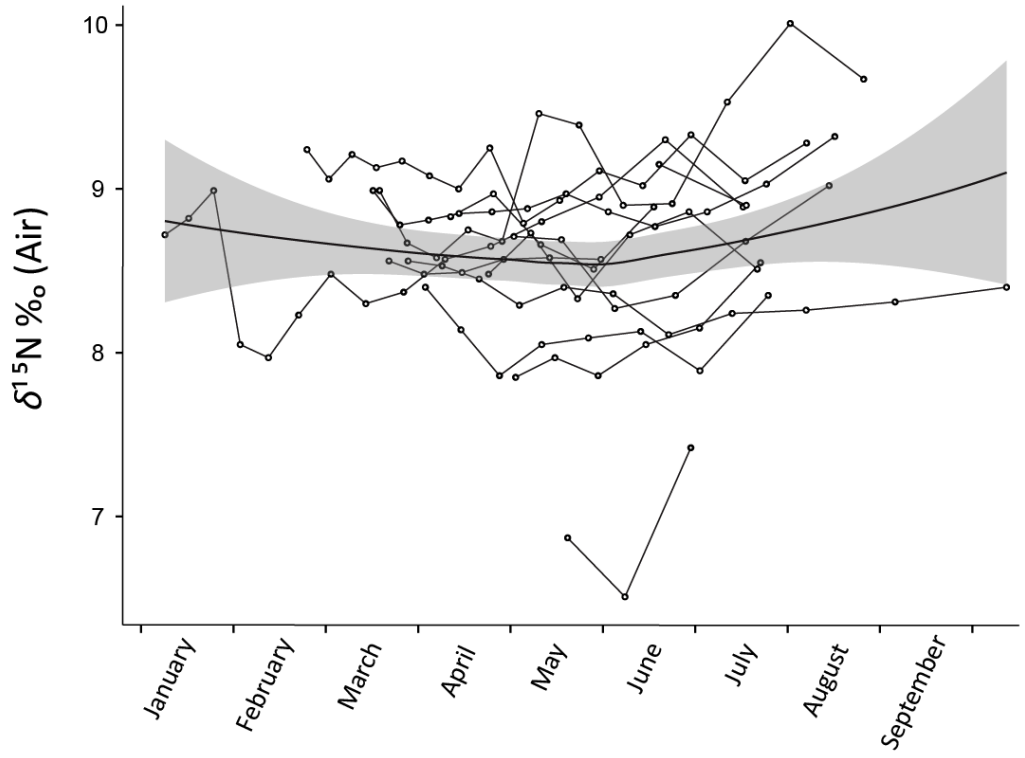

FIG. 5: Individual variation in δ^{15}N captured along vibrissal regrowths of juvenile southern elephant seals *Mirounga leonina* plotted against time. The growth rate of the vibrissal regrowths was used to plot the δ^{15}N at a weekly resolution. The plot shows that prey from a similar trophic level were consumed from January to September.

Individual SEA_c ranged from 0.01 to 0.33 (Table 1), each substantially smaller than the overall juvenile SES SEA_c of 0.8. In the portion of vibrissal regrowth that represented independent foraging, each 2 mm segment represented an average period of 14.3 ± 0.7 days. The period ranged from 6.7 days for longer, faster-growing regrowths, to 33.8 days for regrowths near their asymptotic lengths (e.g. YO064) (Fig. 5). The C:N ratio (% weight) was 3.15 ± 0.1 for all 474 vibrissal segments analysed.

Dietary reconstruction

Three prey groups were included in the final juvenile SES diet model (detailed in Supplementary material). Prey group 1 comprised *E. vallentini* and *E. frigida*, and these krill species were predicted to contribute 26% (13 – 39%) to the diet of juvenile SES. Prey group 2 also consisted of krill (*Thysanoessa sp.*) and contributed 50 % (35 – 64%) to the diet. Prey group 3, which contributed 23% (13 –35%) to the diet, included four myctophid fish species (*Protomyctophum gemmatum, P. bolini, Gymnoscopelus braueri, K. anderssoni*), a Notothenioid fish (*L. larseni*), and a cephalopod (*Martialia hyadesi*) (Supplementary table 1S) (Fig. 6). Prey groups 1 and 2, which both consisted of crustaceans sampled at Marion Island, were separated by a 2.1‰ $\delta^{15}N$ and 1.8‰ $\delta^{13}C$ difference in their isotopic values (Supplementary table 1S). The sum of the modes of the individual contributions of group 1 and 2 indicated a 76% contribution of crustaceans to the diet, compared to a 23% contribution of crustacean-predating cephalopods and myctophid fishes. The contribution of myctophid-predating cephalopods and myctophids occupying a higher trophic level (Group 4 and 5) was negligible (< 1.0%), and was excluded from the final model (detailed in Supplementary material).

The final model's modal residual error term for $\delta^{15}N$ (SD $\delta^{15}N$) and $\delta^{13}C$ (SD $\delta^{13}C$) were 0.13‰ and 0.15‰, respectively. This is lower than the reported standard reproducibility (<

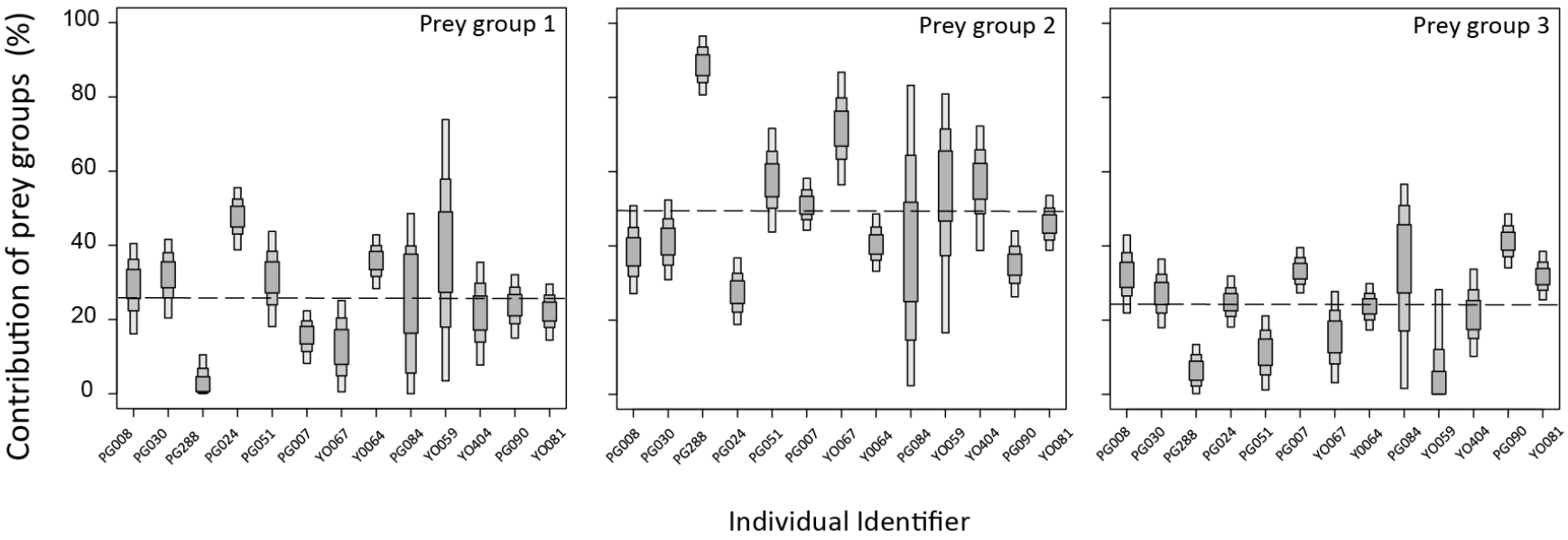

FIG. 6: Proportional contribution of each prey group to the diet of individual juvenile southern elephant seals *Mirounga leonina*. The 25, 75, and 95 % Bayesian credibility intervals (analogue of frequentist confidence intervals) of the contribution of the different prey groups are represented by the boxplots. The dashed line represents the predicted modal contribution of each prey group. Herein, we included the individual identities of the juvenile SES as a random effect to account for multiple (but variable) vibrissal segments sampled per individual. Prey group 1 comprised of two krill species, *E. vallentini* and *E. frigida*, Prey group 2 also consisted of krill (*Thysanoessa sp.*), while Prey group 3 included four myctophid fish species (*P. gemmatum, P. bolini, G. braueri, K. anderssoni*), a Notothenioid fish (*L. larseni*), and a cephalopod (*M. hyadesi*) (Supplementary table 1S) (Fig. 6).

0.20‰), indicating good model performance. Prey group 2 correlated negatively with prey group 1 (correlation coefficient = 0.64) and 3 (0.45), while prey group 1 correlated negatively with prey group 3 (0.40).

DISCUSSION

This study represents the first dietary investigation of SES at Marion Island. The low δ^{15}N (8.6 ± 0.3‰) values obtained indicated that both male and female juvenile SES fed on relatively low trophic level prey during their first year at sea (Fig. 6). Their δ^{15}N isotopic value was 1.0‰ lower than that reported for the krill-feeding juvenile SES from Macquarie Island (Walters et al. 2014) and 1.6‰ (ca. half a trophic level) lower than reported for myctophid-feeding adult female SES from, for example, the Antarctica Peninsula (δ^{15}N: 10.4 ± 0.8‰) (Hückstädt et al. 2012b) where the δ^{15}N baseline values are more depleted (Jaeger et al. 2010). Our data, therefore, supports the notion that juvenile SES include large proportions of crustaceans in their diets.

The isotopic dietary reconstruction presented in this study relies on the accurate representation of the prey isotopic values (baseline) consumed by the juvenile SES. We included all the comprehensive prey SI data available for the south Indian sector of the Southern Ocean, as well as prey sampled at Marion Island. However, the δ^{13}C values indicated that different individuals foraged at different latitudes, and associated changes in the δ^{15}N baseline values can influence isotopic dietary reconstructions (Phillips et al. 2014). The δ^{13}C values suggested that most of the individuals foraged within a narrow latitudinal range, with foraging occurring predominantly within the Polar Frontal Zone (PFZ) (Fig. 4), near the Antarctic Polar Front (APF) (Fig. 1). The predicted foraging range is consistent with satellite-linked tracking data for juvenile SES at Marion Island (Tosh et al. 2012, 2015). Two

individuals likely foraged in the Subantarctic Zone and Antarctic Zone. The SEA_c values of the juvenile SES also indicated that they utilised a restricted, specialised niche, consuming prey with similar $\delta^{15}N$ values, irrespective of their $\delta^{13}C$. We thus considered the variability in the baseline $\delta^{15}N$ values between the SAF and the northern edge of the APF negligible (see Jaeger et al. 2010).

The correlation between the $\delta^{15}N$ and $\delta^{13}C$ measured in the vibrissae of the juvenile SES (Eq. in Supplementary Fig. 4S) suggested that the $\delta^{15}N$ differs by 0.3‰ between the extremes of their foraging latitude, corresponding to a $\delta^{13}C$ range of -19.7‰ to -21.2‰. We included a standard deviation of 0.3‰ for both $\Delta^{15}N$ and $\Delta^{13}C$ to account for the lack of SES-specific TDFs, and we are confident that the latitudinal variation in the baseline $\delta^{15}N$ values would not have a large influence on our dietary model predictions. Yet, our correlation is based on the assumption that all the juveniles consumed similar prey while foraging at different latitudes. The $\delta^{13}C$ values of the Marion Island juvenile SES were more enriched than those of SES from Macquarie Island ($\delta^{13}C$: -21.2 ± 0.4‰, ranging from -20.6 to -21.8), that are known to consume *E. superba* further south (Walters et al. 2014, also see Newland et al. 2011). This suggests that our assumption that individuals foraging further south might also include crustaceans in their diets is reasonable. Our included prey isotopic baseline likely represented $\delta^{15}N$ baseline values of the SAF, PFZ, the APF, as well as the northern portion of the AZ. Nevertheless, both prey and predator are not strictly constrained by oceanic fronts and SES consume prey from multiple water masses. The latitudinal variation in the baseline $\delta^{15}N$ values, within the narrow foraging range of the juvenile SES, is unlikely to have adversely affected our dietary model predictions. The baseline $\delta^{15}N$ values, however, are known to decrease south of the APF (Jeager et al. 2010), and we advise caution when

interpreting the dietary results of individuals known to forage close to the sea ice edge within the Antarctic Zone.

The similarity in the δ^{15}N values between predators sampled at Marion Island, Îles Kerguelen (Cherel et al. 2010), and Îles Crozet (Guerreiro et al. 2015) in the south Indian sector of the Southern Ocean (Cherel and Hobson 2007, Cherel et al. 2007, Jaeger et al. 2010, 2013), supports the inclusion of the selected prey isotope values used herein to study the trophic ecology of juvenile SES at Marion Island.

Our results indicated that the diet of juvenile SES at Marion Island consist predominantly of crustaceans and crustacean-consuming cephalopods and myctophids (23%) (Fig. 6). Crustaceans from prey group 1 and 2 contributed 26% (13 – 39%) and 50% (35 – 64%), respectively, to the diet of juvenile SES. This suggests that the cumulative contribution of crustaceans (likely pelagic, subantarctic krill species) is 76%. Stable isotope analyses, however, are rarely capable of providing species-level dietary information (Post 2002). Nevertheless, the low modal residual error term of the model for both isotopes (SD < 0.16‰), suggests that low inter-individual variability occurred in their foraging strategy, and that the included prey species were sufficient to explain their diet. Our results are contrary to the expectation that recently weaned SES pups should have a broad dietary niche given their naivety (e.g. Bornemann et al. 2000).

Crustaceans have been found in the stomach contents of various age-class SES at various localities, including Windmill (in AZ), Heard and Macquarie islands (Green and Williams 1986, Green and Burton 1993, Slip 1995, reviewed by Burton and Van den Hoff 2002, Van den Hoff et al. 2003, Field et al. 2007b), but their significance was unclear (Slip 1995). Walters et al. (2014) were the first to show that crustaceans are important prey for juvenile

SES. However, the common occurrence of undigested *E. vallentini* in previous studies (Slip 1995, Burton and Van den Hoff 2002, Van den Hoff et al. 2003) should have suggested direct ingestion. The depleted δ^{15}N observed after weaning in the dentine growth layers of SES at Kerguelen Island is potentially also indicative of a crustacean-based diet at Kerguelen Island (Martin et al. 2011). Yet, the isotopic data and dietary models that we report show that juvenile SES from Marion Island clearly have a narrow diet, consisting of crustaceans during their first foraging trip. Moreover, this population represents one of the northernmost, pelagic feeding SES populations without proximate access to *E. superba*. Despite the foraging habitat differences between Macquarie (Walters et al. 2014) and Marion Island, the Marion juvenile SES seem to predominantly consumed crustaceans.

Copepods and amphipods (mainly *Themisto gaudichaudii*) were also identified previously in the stomach contents of SES (Green and Burton 1993, Slip 1995, Field et al. 2007b) and our results may have been affected by small contributions of such crustacean species. However, the lack of SI data for many crustacean species, and the expected similarity in the isotopic values among species prohibits distinguishing the species-specific contribution of various crustaceans using SI analysis.

Cephalopods and myctophid fishes constitute a major dietary component of a wide range of marine mammals (Slip 1995, Collins and Rodhouse 2006, Pakhomov et al. 2006, Cherel et al. 2010). Yet, the enriched δ^{15}N of the cephalopods and larger myctophid species, with available data (Cherel et al. 2011, Guerreiro et al. 2015), suggested a limited contribution to the diet of juvenile SES at Marion Island. The contribution of both lower trophic level myctophid fishes and cephalopods (Prey group 3) ranged from 13 to 35 % (Fig. 6). In contrast, stomach contents from yearling SES at Macquarie Island suggested that cephalopods dominated the diet of 1 to 3-year-old SES (Burton and Van den Hoff 2002, Van

den Hoff 2004, Field et al. 2007a). The difference can likely be attributed to the retention of cephalopod beaks, resulting in over-representation in stomach content analyses (Field et al. 2007a). *Kondakovia longimana* are known to contribute to the diet of older SES (Rodhouse et al. 1992, Slip 1995, Cherel et al. 2008), and although our measured δ^{15}N for *K. longimana* was 4.4‰ lower than that measured at Îles Crozet and Îles Kerguelen (Guerreiro et al. 2015), their contribution was still insignificant and formed part of the excluded Prey group 4.

One individual (PG288) that foraged further south than the other individuals (potentially to the sea ice edge), had a predicted diet consisting almost entirely (90%; 81 – 95% CI) of crustaceans, but, the difference in the δ^{15}N baseline values might have contributed to this prediction (Fig. 6). The δ^{15}N of the outlier (PG171) excluded from the dietary reconstruction, suggested a diet shift from crustaceans to higher trophic level prey, and then back to crustaceans, which is plausible (Fig. 3). However, the atypical, enriched δ^{15}N values after the initial δ^{15}N depletion were similar to the post-moult fasting δ^{15}N values, and it is also possible that this individual went into a catabolic state (starvation/fasting), inducing re-metabolised of stored reserves, which might still represent the post-weaning δ^{15}N values. Nevertheless, a similar pattern has not been observed in an additional n = 22 juvenile SES vibrissa regrowths, also sampled at Marion Island (MRI unpublished data).

We resampled vibrissal regrowths after 362 ± 56 days, and 25.5 ± 15.4 % of the vibrissae represented the independent foraging period from January to September (Fig. 5). This novel approach using vibrissal regrowths increased the portion of the vibrissa representing independent foraging by 2.8 times, compared to Walters et al. (2014) (Fig. 5). Nevertheless, we found no dietary differences based on the first and second half of the vibrissal regrowths.

Mixing models are sensitive to missing prey species and require accurate TDFs (Bond et al. 2011, Phillips et al. 2014). We acknowledge that the three krill species included in the model are not representative of all the zooplankton taxa found in the epipelagic zone of the Southern Ocean (Pakhomov et al. 1994). Sampling krill in the remote vicinity of Marion Island is logistically challenging. However, the δ^{15}N of the *E. vallentini* (δ^{15}N: 3.4 ± 0.5‰) included in this study was similar to that reported for adult *E. vallentini* (δ^{15}N: 3.7‰) sampled at Marion Island during 1998 (Gurney et al. 2001), suggesting that the SI values of the included krill species were reliable. We considered the included krill species sufficient for estimating the contribution of krill to the diets of juvenile SES. Clustering isotopically indistinguishable prey species into different groups reduces the risk of model over-parameterisation (reviewed in Phillips et al. 2014), while also reducing the potential of missing prey species to negatively affect the model performance (e.g. Hindell et al. 2012, Walters 2014). Adding additional crustacean species sampled at Marion Island may alter the mean δ^{15}N and δ^{13}C of the prey groups, but is unlikely to affect the broad qualitative outcome of the model.

Although SES-specific TDFs are still unavailable, the Δ^{13}C (3.5‰) and Δ^{15}N (2.8‰) of an adult female northern elephant seals *M. angustirostris,* was recently determined in the controlled feeding trail by Beltran et al. (2016). Our applied Δ^{15}N (2.8 ± 0.3‰) was identical to this earlier study, but our applied Δ^{13}C (3.2 ± 0.3‰) was 0.3‰ lower; although within range. The TDFs have been found to be similar within a tissue type, regardless of species (Hobson et al. 1996, Beltran et al. 2016), and are more likely to vary with age, or physiologically related changes, such as, e.g., reproduction, moult, or when nutritionally stressed (Beltran et al. 2016). The Δ^{15}N of five phocids (n = 6 individuals) published in Beltran et al. (2016, Table 4) was 2.96 ± 0.3‰, similar to our applied 2.8 ± 0.3‰, but the Δ^{15}N was 3.95 ± 0.1‰ for spotted seals *Phoca largha* (Beltran et al. 2016). The reason for

the higher $\Delta^{15}N$ in both spotted seals is unclear. Nevertheless, the use of the TDFs herein, provided by Hobson et al. (1996), follows the approach of Walters et al. (2014), who were the first to suggest that juvenile SES might consume krill, enabling a direct comparison with their findings. Still, as with previous studies (e.g. Eder et al. 2010, Newland et al. 2011, Hückstädt et al. 2012b, Walters et al. 2014), the validity of this study hinges on the utilised TDFs.

CONCLUSION

The depleted $\delta^{15}N$ values measured along the length of vibrissal regrowths collected from Marion Island juvenile SES clearly indicated that they are consuming relatively low trophic prey, despite foraging at different latitudes at Marion Island. This first dietary assessment of SES at Marion Island improves our understanding of the role and trophic position of juvenile SES in the Southern Ocean and clearly indicates the overwhelming importance of crustaceans as prey to juvenile SES. Dietary differences among different SES age-classes may govern their age-specific response to fluctuations in prey abundance. Furthermore, this study provides an example of how sequentially sampled vibrissal regrowths with a known growth history can be utilised to obtain high resolution, temporally integrated dietary information. By sampling actively growing vibrissae (regrowths), we maximised the temporal span and daily resolution of the dietary data obtainable for juvenile SES during their first foraging trip. However, increased isotopic characterisations of prey species around the foraging grounds of SES and SES-specific TDFs are required to enhance the model predictions.

The lack of available latitudinal prey isotopic baseline values for the Indian sector of the Southern Ocean hinders fine-scale dietary reconstructions incorporating differences in the $\delta^{15}N$ baseline values of animals that forage at different locations. Direct sampling of prey at the foraging localities of the juvenile SES, which is logistically and financially challenging, is required to account for the variation in the baseline $\delta^{15}N$ values. The advent of amino acid

δ^{15}N compound-specific stable isotopes (AA-CSIA) might be able to better characterise the trophic level utilised by individuals foraging at different locations. The advantage of AA-CSIA is that the δ^{15}N of source (or essential) amino acids reflects the δ^{15}N supporting the primary production at the base of the food web, eliminating the need to characterize the baseline δ^{15}N values to infer the trophic position of a predator (Vander Zanden et al. 2013).

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