

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^{1,4}, W. Chris Oosthuizen¹, P. J. Nico de Bruyn¹,
André van Tonder¹, Pierre A. Pistorius^{2,3}, Marthán N. Bester¹

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

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

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

⁴Present address: Centre d'Etudes Biologiques de Chizé, UMR 7372 du CNRS-Université de La Rochelle, 79360 Villiers-en-Bois, France

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 profile captured along the length of sampled vibrissae of young SES at Macquarie Island, southwest Pacific Ocean (54.5° S, 158.9° E) recently emphasized the contribution of crustaceans to their diet (likely *Euphausia superba*). Herein, we used the stable isotope values of sampled vibrissal regrowths with known growth histories to assess the diet of juvenile SES at Marion Island, southern Indian Ocean (46.8° S, 37.8° E) 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 9 mo. The depleted stable isotope signatures of nitrogen ($\delta^{15}\text{N}$) ($8.5 \pm 0.6\text{‰}$) and carbon ($\delta^{13}\text{C}$) ($-20.3 \pm 0.1\text{‰}$) 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 juvenile SES diet comprised crustaceans, consisting of 2 crustacean 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.

KEY WORDS: Crustaceans · Diet · Marine mammals · Pinnipeds · Stable isotopes · Vibrissae · Whiskers

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Populations of apex marine predators respond behaviourally and demographically to bottom-up processes, such as changes in prey abundance and dis-

tribution (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 notori-

ously 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 & 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 & Burton 1993, Slip 1995, Burton & 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, SI analysis of juvenile SES (<1 yr old) vibrissae sampled at Macquarie Island (see 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 the juvenile SES returned from their first ca. 80 to 140 d foraging trip at sea, and 9.1% of the total length of the vibrissae sampled from 7 (out of 12) SES represented active foraging (Walters et al. 2014). The low mean nitrogen stable isotope ($\delta^{15}\text{N}$) values (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 & Bester 2002, Pistorius et al. 2004, McMahon & Burton 2005).

The SI values captured along the length of vibrissae are 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 used 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 compared with Walters et al. (2014) and enabled a temporally integrated dietary assessment spanning the 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 2 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 m deep to the southwest 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 43° S and 56° 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).

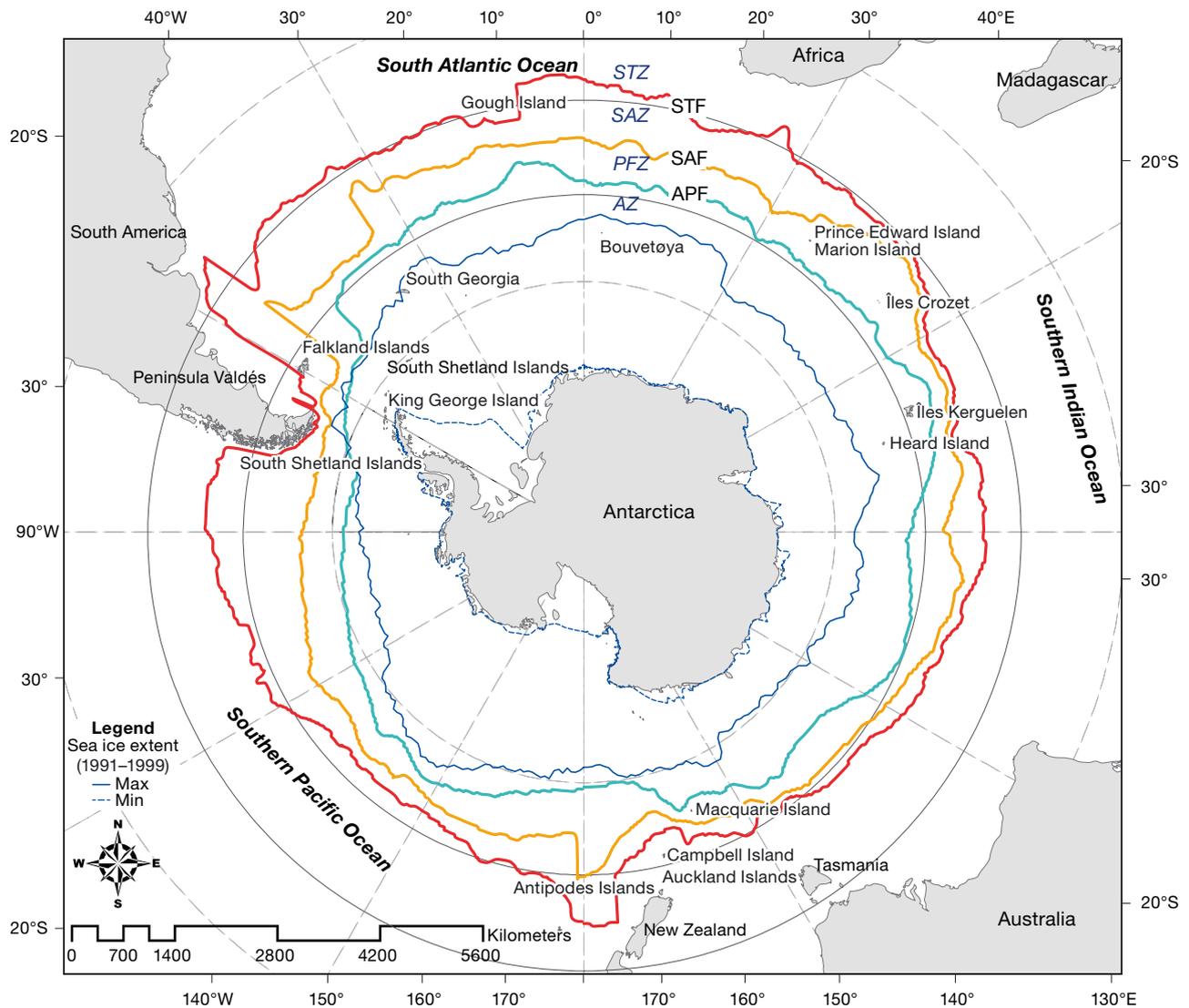


Fig. 1. Circumpolar map of the Southern Ocean displaying the localities (islands) where southern elephant seal *Mirounga leonina* populations are located and from where prey isotopic values were obtained in relation to the circumpolar oceanic currents. The Subtropical Front (STF) represents the northern 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)

Sample collection

Vibrissae sampling from juvenile SES

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 d old) were sexed, individually marked with hind-flipper tags (de Bruyn et al. 2008), and only the longest mystacial vibrissa 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 subse-

quently cut when the seals returned after spending between several months and a year foraging at sea (detailed in Lübcker et al. 2016). For the purpose of this study, ‘juvenile’ refers to individuals up to 15 mo old. We analysed vibrissal regrowths of 14 juvenile SES (9 males, 5 females), collected 250 to 419 d (362 ± 56 d; mean \pm SD) after the initial sampling (Table 1). The length of the vibrissal regrowths sampled ranged from 46 to 94 mm (mean \pm SD = 69.0 ± 13.4 mm). Sampling regrowths from juveniles required chemical immobilisation, administered through an intramuscular injection of ketamine hydrochloride (Bester 1988).

Table 1. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (mean \pm 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (\pm SD) represent the original values, before applying a trophic enrichment factor for the dietary reconstructions. Resampling dates given as yyyy/mm/dd. M = male; F = female; nd = not computed due to less than 3 segments representing independent foraging

Individual	Resampling date	Sex	Length (mm)	Days after initial sampling	Independent foraging (%)	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	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	nd
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	nd
Mean \pm SD			69.0 ± 13.4	362.4 ± 56.2	25.48 ± 15.4	8.6 ± 0.3	-20.3 ± 0.1	0.1 ± 0.1

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 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 (43°S to 56°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 used SI values for the 14 myctophid species representative of the community of myctophids in the Southern Ocean (Cherel et al. 2010). These include the 4 most abundant species: *Electrona antarctica*, *E. carlsbergi*, *Gymnoscopelus nicholsi*, and *Krefflichthys anderssoni* (Duhamel et al. 2000, Cherel et al. 2010). We also included the SI values of 2 notothenioids, *Gobionotothen marionensis* and *Lepidonotothen larseni*, sampled at Marion Island (Bushula et al. 2005; Table S1 in the Supplement at www.int-res.com/articles/suppl/m577p237_supp.pdf).

Guerreiro et al. (2015) provided 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 2 Subtropical Front species (*Histioteuthis atlantica* and *Taonius* sp.), thereby including only cephalopods inhabiting the subantarctic region (carbon stable isotope ($\delta^{13}\text{C}$) values 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 $\delta^{15}\text{N}$ values for beaks and subtracting 0.75‰ from the beak $\delta^{13}\text{C}$ values (Hobson & Cherel 2006), consistent with Guerreiro et al. (2015).

Krill samples were obtained from fresh macaroni penguin *Eudyptes chrysolophus* and rockhopper penguin *E. chrysocome filholi* 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: *Euphausia valentini*, *E. frigida*, and *Thysanoessa* spp. (*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 $\delta^{15}\text{N}$ 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 to include benthic crustaceans, such as the benthic shrimp *Nauticaris marionis*, in our analyses.

SI analysis

The isotopic values captured in the tip of the regrowths represent the pre-foraging period (gestation, lactation, and post-weaning fast), followed by the transition period when the isotopic turnover from the maternally derived SI signature to the SI signature obtained from independent foraging occurs (Lübcker et al. 2016). The transition period is indicated by a ca. 3.7% $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ depletion occurred for the dietary reconstruction (independent foraging) (Fig. 2). Sample preparation and analyses of vibrissae followed the procedures outlined in Lübcker et al. (2016). Samples were cleaned by sonication in a 1:2 chloroform:ethanol solution, repeated 3 times before rinsing with distilled water and oven-drying for 24 h at 70°C . Vibrissae were sequentially sub-sampled into 2 mm ($\text{SD} = \pm 0.3$ 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. 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

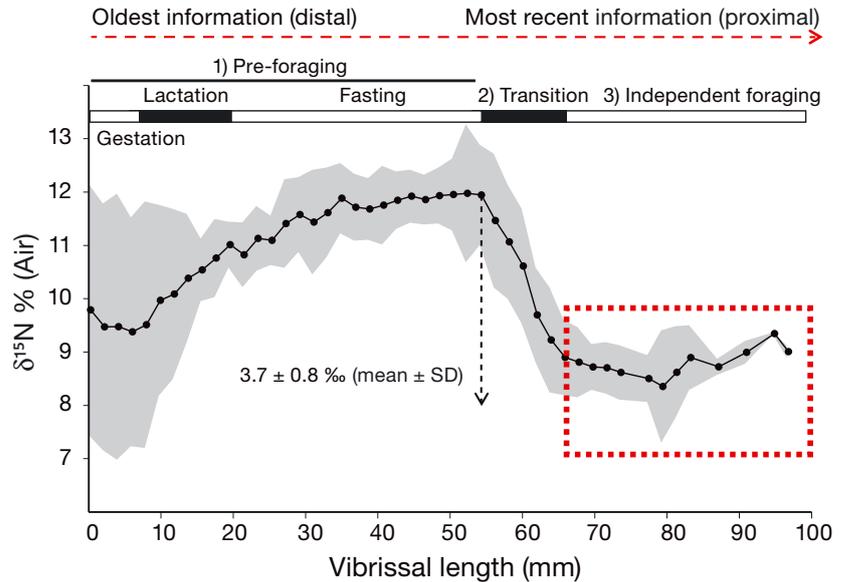


Fig. 2. Representation of data indicating the 3 isotopically distinct portions of the vibrissal regrowths collected from juvenile southern elephant seals *Mirovunga leonina*. Life history events can be distinguished based on $\delta^{15}\text{N}$ (mean \pm SD) measured along the length of the vibrissae. The onset (indicated by the arrow) of the independent foraging (transitioning period) is characterised by a 3.7% $\delta^{15}\text{N}$ depletion and we used only the period representing independent foraging for dietary reconstruction (red box). The solid black line with grey error bands represents the ^{15}N mean \pm SD of all the sampled juveniles

distilled water prior to isotopic analysis, similar to Guerreiro et al. (2015).

Weighed sample aliquots were combusted in an elemental analyser (Flash EA, 1112 Series, Thermo™, Thermo Fisher Scientific), and the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 PeeDee Belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 (air) for $\delta^{15}\text{N}$. 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values based on the standards was $<0.20\%$, while the reproducibility of duplicate sample aliquots was $\pm 0.27\%$ for $\delta^{15}\text{N}$ and $\pm 0.11\%$ for $\delta^{13}\text{C}$.

Predicting the foraging range of juvenile SES

The predictable latitudinal $\delta^{13}\text{C}$ gradient in particulate organic matter (POM) in the Southern Ocean allows differentiation between low latitudinal Subtropi-

cal Zone waters and Antarctic Zone (AZ) waters at high latitudes (Trull & Armand 2001). Oceanic fronts are characterised by steep temperature, salinity, and water density gradients, and the PFZ 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 $\delta^{13}\text{C}$ POM in the Southern Ocean is also reflected in the tissue of predators (Jaeger et al. 2010). Species- and tissue-specific $\delta^{13}\text{C}$ maps of gradients in SIs ('isoscapescapes'; Trull & Armand 2001, Jaeger et al. 2010) enable inference of the latitudes at which consumers foraged.

The latitudinal $\delta^{13}\text{C}$ gradient around Marion Island was previously characterised using the vibrissae of Antarctic fur seals sampled at the island. Walters (2014) found that the $\delta^{13}\text{C}$ of the AZ ($57.7 \pm 1.3^\circ\text{S}$; $\delta^{13}\text{C} = -21.5 \pm 0.7\text{‰}$) was more depleted relative to the SAF ($47.6 \pm 1.6^\circ\text{S}$; $\delta^{13}\text{C} = -19.9 \pm 1.2\text{‰}$), and that the APF was characterised by a $\delta^{13}\text{C}$ of $-20.5 \pm 1.2\text{‰}$. A $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, which can be indicative of a shift in the $\delta^{15}\text{N}$ baseline values between different latitudes.

Statistical analyses

Dietary reconstruction

Bayesian stable isotope mixing models—fitted in R using the Stable Isotope Analysis (SIAR; version 4.1.2) package (Parnell et al. 2010)—were used to reconstruct the juvenile SES diet. A diet to vibrissa-specific trophic discrimination factor (hereafter, TDF) obtained from captive pinnipeds ($\delta^{15}\text{N} = 2.8\text{‰}$; $\delta^{13}\text{C} = 3.2\text{‰}$) (Hobson et al. 1996) was used to reconstruct SES diets. SES-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 the Supplement for details). Based on the SIAR mixing polygon and the applied TDFs, prey species with

$\delta^{15}\text{N}$ above or below 3.7 and 12.8‰ and a $\delta^{13}\text{C}$ above or below -16.2 and -24.5‰ (adjusted values), respectively, were excluded from further analyses (Fig. S1 in the Supplement). 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 version 3.3.1 (Müllner 2013) (Fig. S2 in the Supplement). The identified prey groups were considered unique if they differed significantly in $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, or both, evaluated using an ANOVA (Table S2 and Fig. S3 in the Supplement). 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 3 chains of 100 000 iterations, discarding the first 25 000 iterations and then retaining every 25th iteration. A residual error term for $\delta^{15}\text{N}$ (SD $\delta^{15}\text{N}$) and $\delta^{13}\text{C}$ (SD $\delta^{13}\text{C}$) 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ 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 individual 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:

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

(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 the vibrissal regrowth trajectories. 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 represents 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 SI values using appropriate parametric or non-parametric tests, based on the distribution of the values. Values are presented as means \pm 1 SD. Statistical significance was assumed at $p < 0.05$. All analyses were done in R version 3.2.3 (R Core Team 2015).

RESULTS

Isotopic signature of vibrissal regrowths

There were no significant interannual differences in the isotopic values measured along the length of

vibrissae collected in 2012 and 2013 (Wilcoxon rank sum test: $\delta^{15}\text{N}$, $W = 25\,472$, $p = 0.6$; $\delta^{13}\text{C}$, $W = 286\,466$, $p = 0.09$). The $\delta^{13}\text{C}$ for 2012 and 2013 differed by 0.05‰, while the $\delta^{15}\text{N}$ differed by 0.1‰. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values differed significantly between males and females (Wilcoxon rank sum test: $\delta^{13}\text{C}$, $W = 38\,820$, $p < 0.01$; $\delta^{15}\text{N}$, $W = 23\,423.5$, $p = 0.03$). However, the difference in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values during the pre-foraging, transition, and independent foraging periods were isotopically distinct from each other ($\delta^{15}\text{N}$: Kruskal-Wallis $\chi^2 = 259.9$, $df = 2$, $p < 0.001$; $\delta^{13}\text{C}$: Kruskal-Wallis $\chi^2 = 19.7$, $df = 2$, $p < 0.001$). During the pre-foraging period, $\delta^{15}\text{N}$ values ranged from 10.9 to 12.2‰ (11.6 ± 0.4 ‰), and $\delta^{13}\text{C}$ values from -19.2 to -21.2 ‰ (-20.1 ± 0.5 ‰). The transition period was represented by a 2.7 to 5.7‰ $\delta^{15}\text{N}$ depletion (difference in $\delta^{15}\text{N} = -3.7 \pm 0.8$ ‰) (Fig. 3). The mean $\delta^{15}\text{N}$

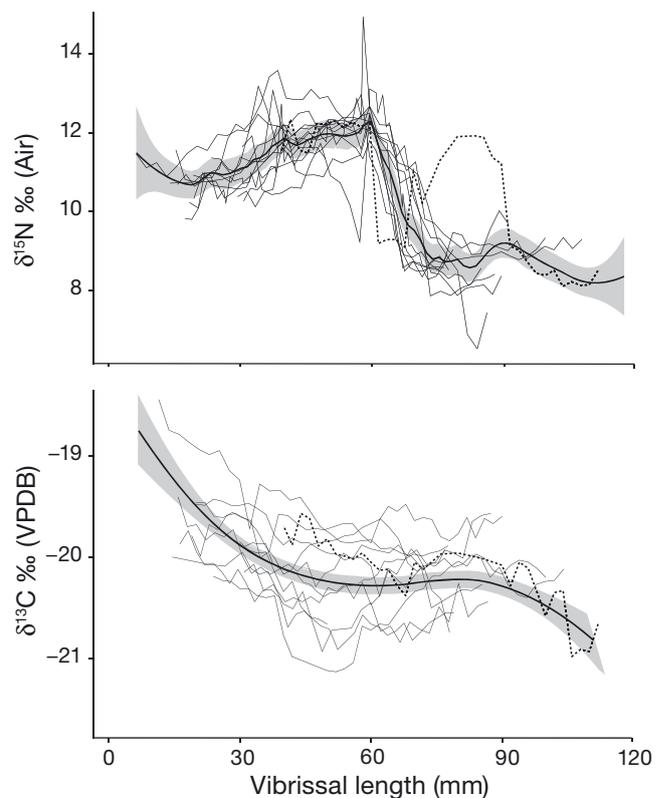


Fig. 3. Individual variation in $\delta^{15}\text{N}$ (top) and $\delta^{13}\text{C}$ (bottom) values measured along the length of vibrissal regrowths collected from 14 juvenile southern elephant seals *Mirounga leonina* aged 0.75–1.21 yr (mean \pm SD: 1.05 ± 0.15 yr). The solid black lines with grey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

values during the independent foraging period were significantly lower than during the pre-foraging period ($p < 0.001$), while the $\delta^{13}\text{C}$ values were within 0.2‰ of the independent foraging $\delta^{13}\text{C}$ values. The $\delta^{15}\text{N}$ values during the independent foraging phase ranged from 6.9 to 9.6‰ ($8.6 \pm 0.3\text{‰}$), while the $\delta^{13}\text{C}$ values ranged from -19.7 to -21.2‰ ($-20.3 \pm 0.1\text{‰}$). 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ value then increased again by 2.6‰ during the independent foraging period, before again decreasing by 3.2‰.

During the independent foraging period (Fig. 4), we detected significant inter-individual differences in both $\delta^{15}\text{N}$ (Kruskal-Wallis $\chi^2 = 76.6$, $df = 12$, $p <$

0.001) and $\delta^{13}\text{C}$ (Kruskal-Wallis $\chi^2 = 90.2$, $df = 12$, $p < 0.001$) values. The measured $\delta^{15}\text{N}$ values ranged between 6.9‰ and 9.6‰. The maximum $\delta^{13}\text{C}$ value (-19.4‰) differed by 1.9‰ from the minimum $\delta^{13}\text{C}$ value (-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 $\delta^{13}\text{C}$ range of -19.7 to -21.2‰ (mode = -20.1‰). Foraging also occurred in the Subantarctic Zone (SAZ), and 2 individuals (PG090 and PG288) likely foraged in the AZ. The $\delta^{15}\text{N}$ increased by 0.19‰ for every 1‰ $\delta^{13}\text{C}$ increment (Fig. S4 in the Supplement), 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the first half of vibrissal regrowths did not differ significantly from the second half ($\delta^{15}\text{N}$: $W = 1190$, $p = 0.14$; $\delta^{13}\text{C}$: $W = 1236.5$, $p = 0.23$), although growth rate differences between the 2 halves were disregarded. Individual SEA_c values ranged

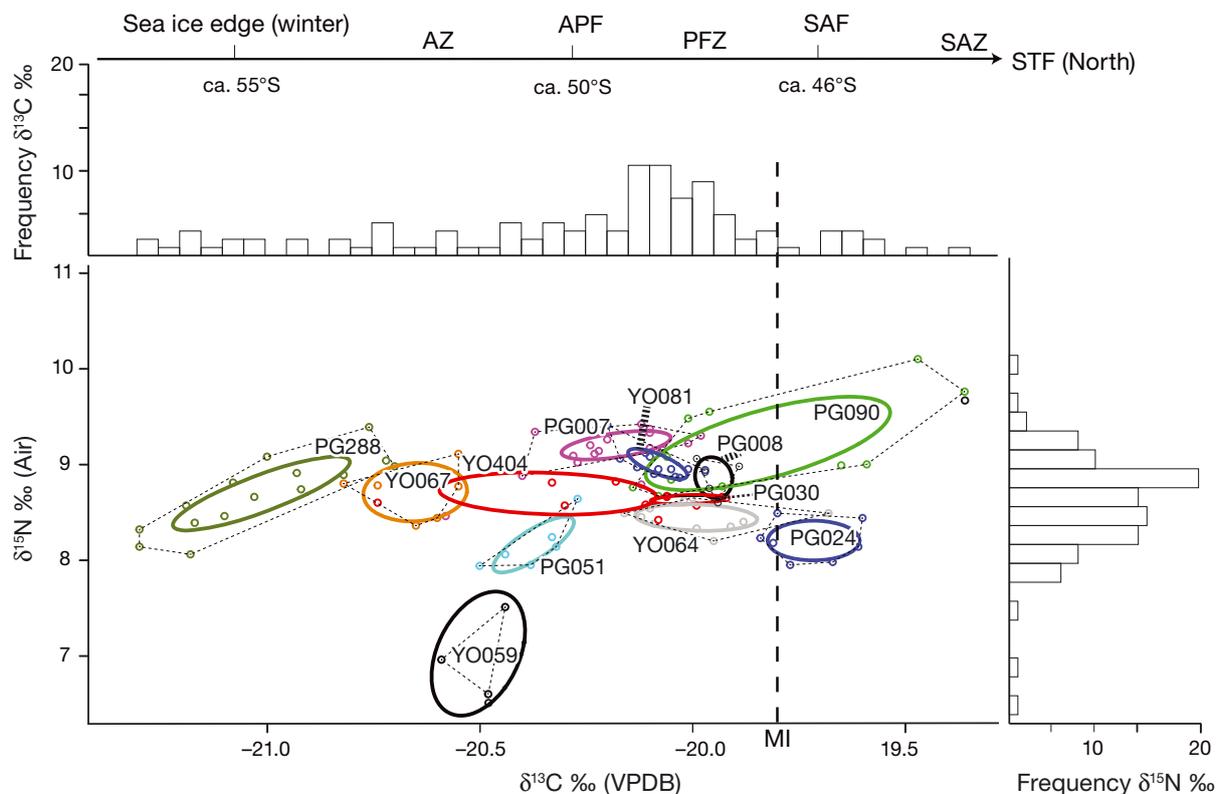


Fig. 4. Standard ellipse area (SEA_c) representing the core isotopic niche (solid lines) utilized by each juvenile southern elephant seal (SES) *Mirounga leonina* during the independent foraging period. Juvenile SES utilized a restricted niche, predominantly consuming prey from the same trophic level, as indicated by the unimodal $\delta^{15}\text{N}$ frequency distribution. The distribution of $\delta^{13}\text{C}$ 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 isopleth 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 $\delta^{13}\text{C}$ 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 Zone; APF = Antarctic Polar Front; VPDB = Vienna PeeDee Belemnite

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 d. The period ranged from 6.7 d for longer, faster-growing regrowths, to 33.8 d for regrowths near their asymptotic lengths

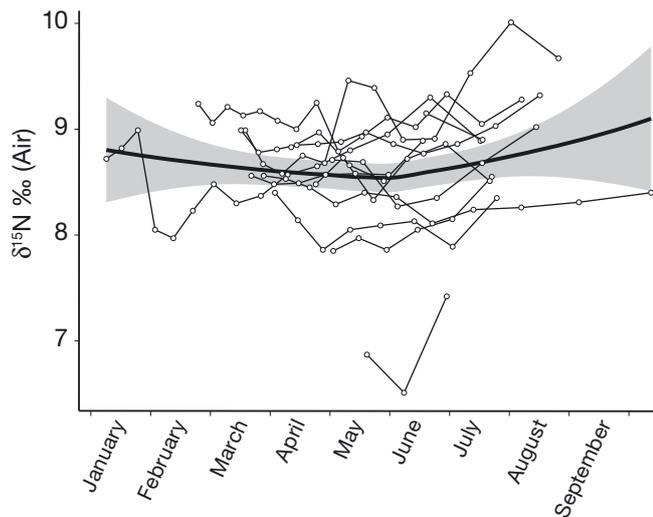


Fig. 5. Individual variation in $\delta^{15}\text{N}$ captured along vibrissal regrowths of juvenile southern elephant seals *Mirounga leonina* plotted against time. The growth rate of the vibrissal regrowths was used to plot the $\delta^{15}\text{N}$ at a weekly resolution. The plot shows that prey from a similar trophic level were consumed from January to September. The solid black line with grey error bands represents the applied lowest smoothing algorithm

(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 the Supplement). Prey group 1 comprised *Euphausia 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* spp.) and contributed 50% (35–64%) to the diet. Prey group 3, which contributed 23% (13–35%) to the diet, included 4 myctophid fish species (*Protomyctophum gemmatum*, *P. bolini*, *Gymnoscopelus braueri*, *Krefflichthys anderssoni*), a notothenioid fish (*Lepidonotothen larseni*), and a cephalopod (*Martialia hyadesi*) (Table S1 in the Supplement) (Fig. 6). Prey groups 1 and 2, which both consisted of crustaceans sampled at Marion Island, were separated by a 2.1‰ $\delta^{15}\text{N}$ and 1.8‰ $\delta^{13}\text{C}$ difference in their isotopic values (Table S1 in the Supplement). The sum of the modes of the individual contributions of groups 1 and 2 indicated a 76% contribution of crustaceans to the diet, compared with a 23% contribution of crustacean-predating cephalopods and myctophid fishes. The contribution of myctophid-predating cephalopods and myctophids occupying a higher trophic level (groups 4 and 5) was negligible (<1.0%), and was excluded from the final model (detailed in the Supplement).

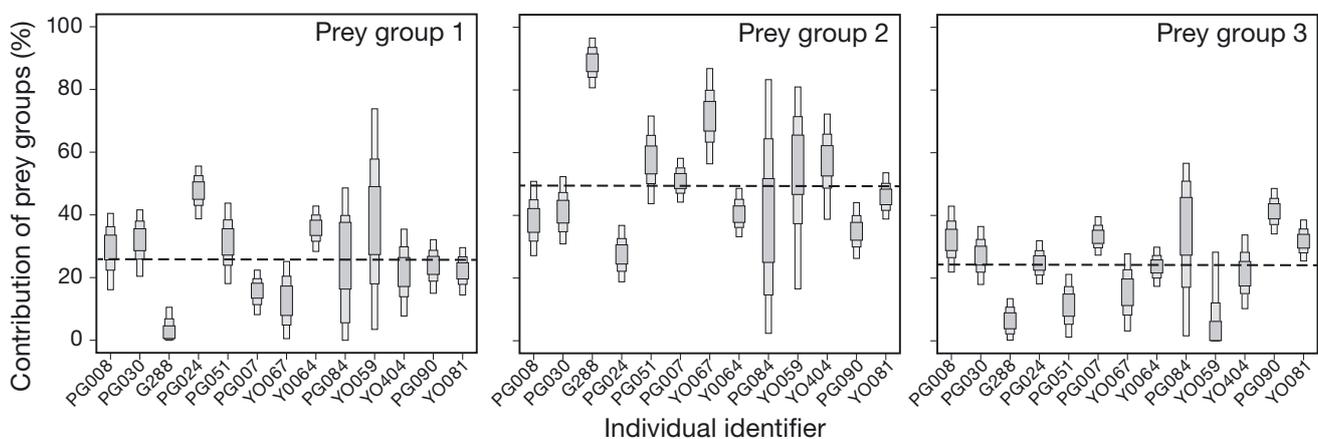


Fig. 6. Proportional contribution of each prey group to the diet of individual juvenile southern elephant seals *Mirounga leonina*. The 25, 75, and 95% Bayesian credibility intervals (analogue of frequentist confidence intervals) of the contribution of the different prey groups are represented by the boxplots. The dashed line represents the predicted modal contribution of each prey group. 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 2 krill species, *Euphausia vallentini* and *E. frigida*, prey group 2 also consisted of krill (*Thysanoessa* spp.), while prey group 3 included 4 myctophid fish species (*Protomyctophum gemmatum*, *P. bolini*, *Gymnoscopelus braueri*, *Krefflichthys anderssoni*), a notothenioid fish (*Lepidonotothen larseni*), and a cephalopod (*Martialia hyadesi*) (Table S1 in the Supplement) (Fig. 6)

The final model's modal residual error term for $\delta^{15}\text{N}$ (SD $\delta^{15}\text{N}$) and $\delta^{13}\text{C}$ (SD $\delta^{13}\text{C}$) was 0.13‰ and 0.15‰, respectively. This is lower than the reported standard reproducibility (<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 $\delta^{15}\text{N}$ ($8.6 \pm 0.3\text{‰}$) 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 $\delta^{15}\text{N}$ 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 Antarctic Peninsula ($\delta^{15}\text{N}$: $10.4 \pm 0.8\text{‰}$) (Hückstädt et al. 2012b) where the $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values indicated that different individuals foraged at different latitudes, and associated changes in the $\delta^{15}\text{N}$ baseline values can influence isotopic dietary reconstructions (Phillips et al. 2014). The $\delta^{13}\text{C}$ values suggested that most of the individuals foraged within a narrow latitudinal range, with foraging occurring predominantly within the PFZ (Fig. 4), near the 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 SAZ and AZ. The SEA_c values of the juvenile SES also indicated that they utilised a restricted, specialised niche, consuming prey with similar $\delta^{15}\text{N}$ values, irrespective of their $\delta^{13}\text{C}$. We thus considered the variability in the baseline $\delta^{15}\text{N}$ values between the SAF and the northern edge of the APF negligible (see Jaeger et al. 2010).

The correlation between the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ measured in the vibrissae of the juvenile SES (equation in

Fig. S4 in the Supplement) suggested that the $\delta^{15}\text{N}$ value differs by 0.3‰ between the extremes of their foraging latitude, corresponding to a $\delta^{13}\text{C}$ range of -19.7‰ to -21.2‰ . We included a standard deviation of 0.3‰ for both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ to account for the lack of SES-specific TDFs, and we are confident that the latitudinal variation in the baseline $\delta^{15}\text{N}$ 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 $\delta^{13}\text{C}$ values of the Marion Island juvenile SES were more enriched than those of SES from Macquarie Island ($\delta^{13}\text{C}$: $-21.2 \pm 0.4\text{‰}$, ranging from -20.6 to -21.8) that are known to consume *Euphausia 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 $\delta^{15}\text{N}$ baseline values of the SAF, PFZ, 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 $\delta^{15}\text{N}$ values, within the narrow foraging range of the juvenile SES, is unlikely to have adversely affected our dietary model predictions. The baseline $\delta^{15}\text{N}$ values, however, are known to decrease south of the APF (Jaeger 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 AZ.

The similarity in the $\delta^{15}\text{N}$ 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 & 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 consists predominantly of crustaceans and crustacean-consuming cephalopods and myctophids (23%) (Fig. 6). Crustaceans from prey groups 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%. SI 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 occur-

red in SES 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 naïvety (e.g. Bornemann et al. 2000).

Crustaceans have been found in the stomach contents of various age-class SES at numerous localities, including Windmill (in the AZ), Heard, and Macquarie islands (Green & Williams 1986, Green & Burton 1993, Slip 1995, reviewed by Burton & 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 *Euphausia vallentini* in previous studies (Slip 1995, Burton & van den Hoff 2002, van den Hoff et al. 2003) should have suggested direct ingestion. The depleted $\delta^{15}\text{N}$ values observed after weaning in the dentine growth layers of SES at Kerguelen Island is potentially also indicative of a crustacean-based diet there (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 islands, the Marion juvenile SES seem to predominantly consume crustaceans.

Copepods and amphipods (mainly *Themisto gaudichaudii*) were also identified previously in the stomach contents of SES (Green & 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 & Rodhouse 2006, Pakhomov et al. 2006, Cherel et al. 2010). Yet, the enriched $\delta^{15}\text{N}$ values 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 juvenile SES at Macquarie Island suggested

that cephalopods dominated the diet of 1- to 3-yr-old SES (Burton & 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ baseline values might have contributed to this prediction (Fig. 6). The $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values after the initial $\delta^{15}\text{N}$ depletion were similar to the post-moult fasting $\delta^{15}\text{N}$ values, and it is also possible that this individual went into a catabolic state (starvation or fasting), inducing re-metabolisation of stored reserves, which might still represent the post-weaning $\delta^{15}\text{N}$ values. Nevertheless, a similar pattern has not been observed in an additional $n = 22$ juvenile SES vibrissa regrowths, also sampled at Marion Island (Mammal Research Institute unpubl data).

We resampled vibrissal regrowths after 362 ± 56 d, and $25.5 \pm 15.4\%$ 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 with Walters et al. (2014) (Fig. 5). Nevertheless, we found no dietary differences between the first and second portion of the vibrissal regrowths representing the independent foraging period.

Mixing models are sensitive to missing prey species and require accurate TDFs (Bond & Diamond 2011, Phillips et al. 2014). We acknowledge that the 3 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 $\delta^{15}\text{N}$ of the *E. vallentini* ($\delta^{15}\text{N}$: $3.4 \pm 0.5\text{‰}$) included in this study was similar to that reported for adult *E. vallen-*

tini ($\delta^{15}\text{N}$: 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 $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of the prey groups, but is unlikely to affect the broad qualitative outcome of the model.

Although SES-specific TDFs are still unavailable, the $\delta^{13}\text{C}$ (3.5‰) and $\delta^{15}\text{N}$ (2.8‰) of an adult female northern elephant seal *Mirounga angustirostris* was recently determined in a controlled feeding trial by Beltran et al. (2016). Our applied $\delta^{15}\text{N}$ ($2.8 \pm 0.3\text{‰}$) was identical to this earlier study, but our applied $\delta^{13}\text{C}$ ($3.2 \pm 0.3\text{‰}$) 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, e.g. reproduction, moult, or when nutritionally stressed (Beltran et al. 2016). The $\delta^{15}\text{N}$ of 5 phocids ($n = 6$ individuals) published in Table 4 of Beltran et al. (2016) was $2.96 \pm 0.3\text{‰}$, similar to our applied $2.8 \pm 0.3\text{‰}$, but the $\delta^{15}\text{N}$ was $3.95 \pm 0.1\text{‰}$ for spotted seals *Phoca largha* (Beltran et al. 2016). The reason for the higher $\delta^{15}\text{N}$ in 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.

CONCLUSIONS

The depleted $\delta^{15}\text{N}$ values measured along the length of vibrissal regrowths collected from Marion Island juvenile SES clearly indicated that they are consuming relatively low trophic level 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 at 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 $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ values. The advent of amino acid $\delta^{15}\text{N}$ 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 $\delta^{15}\text{N}$ of source (or essential) amino acids reflects the $\delta^{15}\text{N}$ supporting the primary production at the base of the food web, eliminating the need to characterise the baseline $\delta^{15}\text{N}$ values to infer the trophic position of a predator (Vander Zanden et al. 2013).

Acknowledgements. The project has ethics clearance from the Animal Use and Care Committee (AUCC) of the Faculty of Veterinary Science, University of Pretoria, under AUCC 040827-022, AUCC 040827-023, AUCC 040827-024 and EC030602-016, and was carried out under permit from the Director-General: Department of Environmental Affairs, South Africa. Funding for the project was obtained from the South African Department of Science and Technology (DST) and National Research Foundation (NRF), grant number 93071. The conclusions drawn and discussed are attributed to the authors and not necessarily to the NRF. The Department of Environmental Affairs provided logistical support for ongoing fieldwork at Marion Island, under the South African National Antarctic Programme (SANAP). The 'Grant in Aid of Research' provided additional funding, obtained from the Society for Marine Mammalogy. We are also grateful to Prof. Yves Cherel for his comments on an earlier version of the manuscript, as well as to all the Marion Island personnel that faithfully performed their duties beyond expectation. Special thanks to William Haddad, Mia Wege and Nicolas Prinsloo, as well as to Dr. Grant Hall, Stable Isotope Laboratory, University of Pretoria.

LITERATURE CITED

- Baker A de C, Boden BP, Brinton E (1990) A practical guide to the euphausiids of the world. Natural History Museum Publications, London
- ✦ Beltran RS, Sadou MC, Condit R, Peterson SH, Reichmuth C, Costa DP (2015) Fine-scale whisker growth measurements can reveal temporal foraging patterns from stable isotope signatures. *Mar Ecol Prog Ser* 523:243–253
- ✦ Beltran RS, Peterson SH, McHuron EA, Reichmuth C, Hückstädt LA, Costa DP (2016) Seals and sea lions are what they eat, plus what? Determination of trophic discrimination factors for seven pinniped species. *Rapid Commun Mass Spectrom* 30:1115–1122
- Bester MN (1988) Chemical restraint of Antarctic fur seals and southern elephant seals. *S Afr J Wildl Res* 18:57–60
- ✦ Bester MN, de Bruyn PJN, Oosthuizen WC, Tosh CA, McIntyre T, Reisinger RR, Wege M (2011) The Marine Mammal Programme at the Prince Edward Islands: 38 years of research. *Afr J Mar Sci* 33:511–521
- ✦ Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. *Ecol Appl* 21:1017–1023
- ✦ Bornemann H, Kreyscher M, Ramdohr S, Martin T, Carlini A, Sellmann L, Plötz J (2000) Southern elephant seal movements and Antarctic sea ice. *Antarct Sci* 12:3–5
- ✦ Burton H, van den Hoff J (2002) Humans and the southern elephant seal *Mirounga leonina*. *Aust Mammal* 24: 127–139
- ✦ Bushula T, Pakhomov EA, Kaehler S, Davis S, Kalin RM (2005) Diet and daily ration of two small nototheniid fish on the shelf of the sub-Antarctic Prince Edward Islands. *Polar Biol* 28:585–593
- ✦ Cherel Y, Hobson KA (2007) Geographical variation in carbon stable isotope signatures of marine predators: a tool to investigate their foraging areas in the Southern Ocean. *Mar Ecol Prog Ser* 329:281–287
- ✦ Cherel Y, Hobson KA, Guinet C, Vanpe C (2007) Stable isotopes document seasonal changes in trophic niches and winter foraging individual specialization in diving predators from the Southern Ocean. *J Anim Ecol* 76:826–836
- ✦ Cherel Y, Ducatez S, Fontaine C, Richard P, Guinet C (2008) Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands. *Mar Ecol Prog Ser* 370: 239–247
- ✦ Cherel Y, Kernaléguen L, Richard P, Guinet C (2009) Whisker isotopic signature depicts migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals. *Biol Lett* 5:830–832
- ✦ Cherel Y, Fontaine C, Richard P, Labat J (2010) Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. *Limnol Oceanogr* 55: 324–332
- Cherel Y, Gasco N, Duhamel G (2011) Top predators and stable isotopes document the cephalopod fauna and its trophic relationships in Kerguelen waters. In: *The Kerguelen Plateau: marine ecosystem and fisheries*. Société Française d'Ichtyologie, p 99–108
- Collins MA, Rodhouse PGK (2006) Southern Ocean cephalopods. In: Alan CMY, Southward J, Lee AF (eds) *Advances in marine biology*. Elsevier, Amsterdam, p 191–265
- ✦ Constable AJ, Melbourne-Thomas J, Corney SP, Arrigo KR and others (2014) Climate change and Southern Ocean ecosystems I: how changes in physical habitats directly affect marine biota. *Glob Change Biol* 20:3004–3025
- Daneri G, Carlini A (2002) Fish prey of southern elephant seals, *Mirounga leonina*, at King George Island. *Polar Biol* 25:739–743
- ✦ de Bruyn PJN, Tosh CA, Oosthuizen WC, Phalanndwa MV, Bester MN (2008) Temporary marking of unweaned southern elephant seal (*Mirounga leonina* L.) pups. *S Afr J Wildl Res* 38:133–137
- ✦ Ducatez S, Dalloyau S, Richard P, Guinet C, Cherel Y (2008) Stable isotopes document winter trophic ecology and maternal investment of adult female southern elephant seals (*Mirounga leonina*) breeding at the Kerguelen Islands. *Mar Biol* 155:413–420
- ✦ Duhamel G, Koubbi P, Ravier C (2000) Day and night mesopelagic fish assemblages off the Kerguelen Islands (Southern Ocean). *Polar Biol* 23:106–112
- ✦ Durgadoo JV, Ansoorge IJ, Lutjeharms JRE (2010) Oceanographic observations of eddies impacting the Prince Edward Islands, South Africa. *Antarct Sci* 22:211–219
- ✦ Eder EB, Lewis MN, Campagna C, Koch PL (2010) Evidence of demersal foraging from stable isotope analysis of juvenile elephant seals from Patagonia. *Mar Mamm Sci* 26: 430–442
- ✦ Field IC, Bradshaw CJA, Burton HR, Hindell MA (2007a) Differential resource allocation strategies in juvenile elephant seals in the highly seasonal Southern Ocean. *Mar Ecol Prog Ser* 331:281–290
- ✦ Field IC, Bradshaw CJA, van den Hoff J, Burton HR, Hindell MA (2007b) Age-related shifts in the diet composition of southern elephant seals expand overall foraging niche. *Mar Biol* 150:1441–1452
- ✦ Green K, Burton HR (1993) Comparison of the stomach contents of southern elephant seals, *Mirounga leonina*, at Macquarie and Heard islands. *Mar Mamm Sci* 9:10–22
- ✦ Green K, Williams R (1986) Observations on food remains in faeces of elephant, leopard and crabeater seals. *Polar Biol* 6:43–45
- ✦ Guerreiro M, Phillips RA, Cherel Y, Ceia FR, Alvito P, Rosa R, Xavier JC (2015) Habitat and trophic ecology of Southern Ocean cephalopods from stable isotope analyses. *Mar Ecol Prog Ser* 530:119–134
- ✦ Gurney LJ, Froneman PW, Pakhomov EA, McQuaid CD (2001) Trophic positions of three euphausiid species from the Prince Edward Islands (Southern Ocean): implications for the pelagic food web structure. *Mar Ecol Prog Ser* 217:167–174
- ✦ Hall-Aspland SA, Rogers TL, Canfield RB (2005) Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals. *Mar Ecol Prog Ser* 305: 249–259
- ✦ Hindell MA, McConnell BJ, Fedak MA, Slip DJ, Burton HR, Reijnders PJ, McMahon CR (1999) Environmental and physiological determinants of successful foraging by naive southern elephant seal pups during their first trip to sea. *Can J Zool* 77:1807–1821
- Hindell MA, Bradshaw CJA, Harcourt RG, Guinet C (2003) Ecosystem monitoring: Are seals a potential tool for monitoring change in marine systems? In: Gales NJ, Hindell MA, Kirkwood R (eds) *Marine mammals: fisheries, tourism and management issues*. CSIRO Publishing, Collingwood, p 318–331
- ✦ Hindell MA, Lyderson C, Hop H, Kovacs KM (2012) Pre-partum diet of adult female bearded seals in years of contrasting ice conditions. *PLOS ONE* 7:e38307
- ✦ Hobson KA, Cherel Y (2006) Isotopic reconstruction of mar-

- ine food webs using cephalopod beaks: new insight from captive raised *Sepia officinalis*. *Can J Zool* 84:766–770
- Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Can J Fish Aquat Sci* 53:528–533
- Hückstädt LA, Burns JM, Koch PL, McDonald BI, Crocker DE, Costa DP (2012a) Diet of a specialist in a changing environment: the crabeater seal along the western Antarctic Peninsula. *Mar Ecol Prog Ser* 455:287–301
- Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE, Costa DP (2012b) Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. *Oecologia* 169: 395–406
- Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *J Anim Ecol* 80:595–602
- Jaeger A, Lecomte VJ, Weimerskirch H, Richard P, Cherel Y (2010) Seabird satellite tracking validates the use of latitudinal isoscapes to depict predators' foraging areas in the Southern Ocean. *Rapid Commun Mass Spectrom* 24: 3456–3460
- Jaeger A, Jaquemet S, Phillips RA, Wanless RM, Richard P, Cherel Y (2013) Stable isotopes document inter- and intraspecific variation in feeding ecology of nine large southern Procellariiformes. *Mar Ecol Prog Ser* 490:255–266
- Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide quantitative measures of trophic diversity within food webs? *Ecology* 88:42–48
- Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Bearhop S (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol Rev Camb Philos Soc* 87:545–562
- Lübcker N, Condit R, Beltran RS, de Bruyn PJN, Bester MN (2016) Vibrissal growth parameters of southern elephant seals *Mirounga leonina*: obtaining fine-scale, time-based stable isotope data. *Mar Ecol Prog Ser* 559:243–255
- Martin C, Bentaleb I, Steelandt S, Guinet C (2011) Stable carbon and nitrogen isotope variations in canine dentine growth layers of Kerguelen southern elephant seals. *Mar Ecol Prog Ser* 439:295–305
- McMahon CR, Burton HR (2005) Climate change and seal survival: evidence for environmentally mediated changes in elephant seal, *Mirounga leonina*, pup survival. *Proc Biol Sci* 272:923–928
- Müllner D (2013) fastcluster: fast hierarchical, agglomerative clustering routines for R and Python. *J Stat Softw* 53:1–18
- Newland C, Field IC, Cherel Y, Guinet C, Bradshaw CJA, McMahon CR, Hindell MA (2011) Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. *Mar Ecol Prog Ser* 424:247–258
- Oosthuizen WC, Bester MN, Altwegg R, McIntyre T, De Bruyn PJN (2015) Decomposing the variance in southern elephant seal weaning mass: partitioning environmental signals and maternal effects. *Ecosphere* 6:139
- Pakhomov EA, Perissinotto R, McQuaid CD (1994) Comparative structure of the macrozooplankton/micronekton communities of the Subtropical and Antarctic Polar Fronts. *Mar Ecol Prog Ser* 111:155–169
- Pakhomov EA, Bushula T, Kaehler S, Watkins BP, Leslie RW (2006) Structure and distribution of the slope fish community in the vicinity of the sub-Antarctic Prince Edward Archipelago. *J Fish Biol* 68:1834–1866
- Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLOS ONE* 5:e9672
- Phillips DL, Inger R, Bearhop S, Jackson AL and others (2014) Best practices for use of stable isotope mixing models in food-web studies. *Can J Zool* 92:823–835
- Pistorius PA, Bester MN (2002) Juvenile survival and population regulation in southern elephant seals at Marion Island. *Afr Zool* 37:35–41
- Pistorius PA, Bester MN, Lewis MN, Taylor FE, Campagna C, Kirkman SP (2004) Adult female survival, population trend, and the implications of early primiparity in a capital breeder, the southern elephant seal (*Mirounga leonina*). *J Zool (Lond)* 263:107–119
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718
- R Core Team (2015) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Rodhouse PG, Arnobom T, Fedak MA, Yeatman J, Murray AWA (1992) Cephalopod prey of the southern elephant seal, *Mirounga leonina* L. *Can J Zool* 70:1007–1015
- Slip DJ (1995) The diet of southern elephant seals (*Mirounga leonina*) from Heard Island. *Can J Zool* 73:1519–1528
- Tosh CA, Steyn J, Bornemann H, Van den Hoff J, Stewart BS, Plötz J, Bester MN (2012) Marine habitats of juvenile southern elephant seals from Marion Island. *Aquat Biol* 17:71–79
- Tosh CA, de Bruyn PJN, Steyn J, Bornemann H and others (2015) The importance of seasonal sea surface height anomalies for foraging juvenile southern elephant seals. *Mar Biol* 162:2131–2140
- Trull TW, Armand L (2001) Insights into Southern Ocean carbon export from the $\delta^{13}\text{C}$ of particles and dissolved inorganic carbon during the SOIREE iron release experiment. *Deep Sea Res II* 48:2655–2680
- van den Hoff J (2004) Comparative study of the cephalopod prey of Patagonian toothfish (*Dissostichus eleginoides*) and southern elephant seals (*Mirounga leonina*) near Macquarie Island. *Polar Biol* 27:604–612
- van den Hoff J, Burton H, Davies R (2003) Diet of male southern elephant seals (*Mirounga leonina* L.) hauled out at Vincennes Bay, East Antarctica. *Polar Biol* 26:27–31
- Vander Zanden HB, Arthur KE, Bolten AB, Popp BN and others (2013) Trophic ecology of a green turtle breeding population. *Mar Ecol Prog Ser* 476:237–249
- Walters A (2014) Quantifying the trophic linkages of Antarctic marine predators. PhD thesis, University of Tasmania
- Walters A, Lea M–A, van den Hoff J, Field IC, Virtue P, Sokolov S, Pinkerton MH, Hindell MA (2014) Spatially explicit estimates of prey consumption reveal a new krill predator in the Southern Ocean. *PLOS ONE* 9:e86452
- Weimerskirch H, Inchausti P, Guinet C, Barbraud C (2003) Trends in bird and seal populations as indicators of a system shift in the Southern Ocean. *Antarct Sci* 15:249–256
- Young JW, Hunt BPV, Cook TR, Llopiz JK and others (2015) The trophodynamics of marine top predators: current knowledge, recent advances and challenges. *Deep Sea Res II* 113:170–187