The following supplement accompanies the article

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

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Marine Ecology Progress Series 577: 237–250 (2017)

PREY SELECTION

A Bayesian statistical mixing modelling approach was used for the dietary reconstruction, using the Stable Isotope Analysis in R (SIAR v. 4.2) package (Parnell et al. 2010). The SIAR method utilises a tissue- and species-specific trophic discrimination factor (TDF) to adjust prey isotopic values to the isotopic values obtained from the vibrissae of juvenile SES, before computing the proportional contribution of each prey group. A diet-vibrissa specific TDF obtained from captive pinnipeds ($\Delta^{15}N = 2.8$ ‰; $\Delta^{13}C = 3.2$ ‰) (Hobson et al. 1996) was used to reconstruct their diets.

Prey selection for the mixing models followed the recommendations of Phillips et al. (2014). Firstly, we selected possible prey items based on their position in the isotope mixing polygon ('isospace' in SIAR) relative to the consumers, after applying the TDF to the isotopic values of the prey (for a review of SIAR method, see Phillips et al. 2014). Prey should be nested inside the potential solution polygon (possible range) (Phillips et al. 2005, 2014), and prey with $\delta^{15}N$ and $\delta^{13}C$ ranging below or above the minimum and maximum of the mixing polygon were not considered in the model (Fig. S1). Based on the SIAR mixing polygon and the applied TDF, prey species with $\delta^{15}N$ above 12.8 or below 3.7 ‰ and a $\delta^{13}C$ above -16.2 or below -24.5 ‰ (adjusted values) were excluded from further analyses. The excluded prey was predominantly myctophid-predating cephalopod species and one myctophid fish, *Gymnoscopelus nicholsi*.

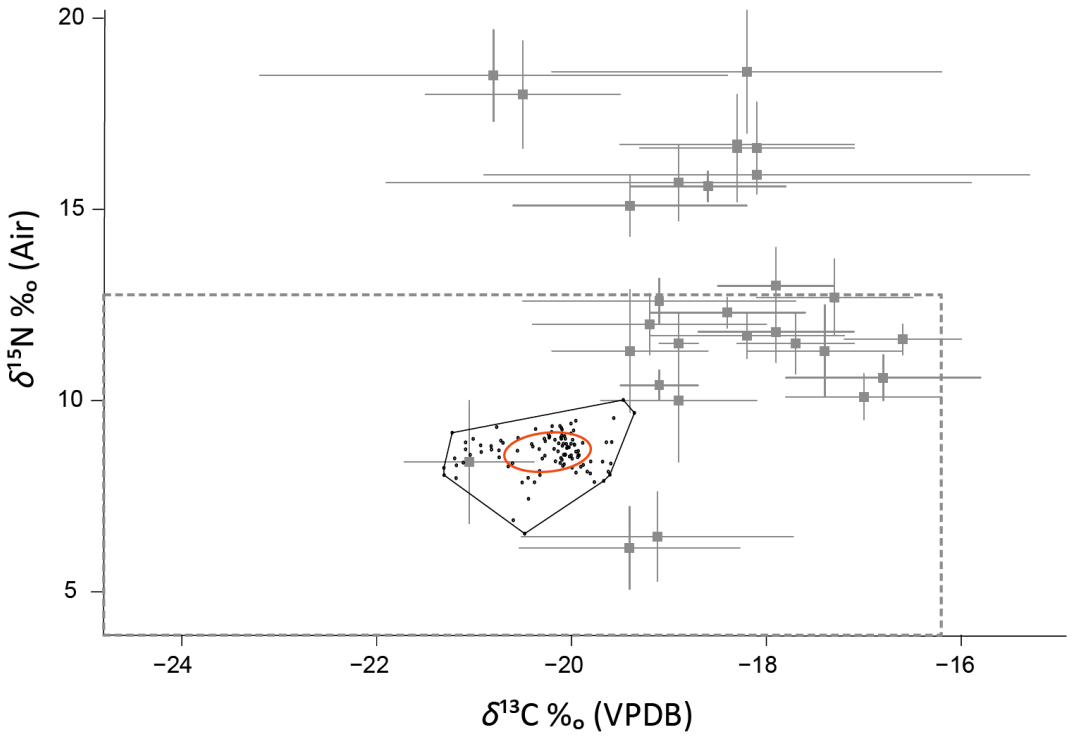

FIG. S1: Potential prey items consumed by juvenile southern elephant seals *Mirounga leonina* were selected based on their position in the isotope mixing polygon ('isospace') relative to the consumers, after applying the appropriate trophic discrimination factors to prey isotope values, as recommended by Phillips et al. (2014). Prey with $\delta^{15}N$ and $\delta^{13}C$ ranging below or above the minimum and maximum of the mixing polygon (dashed rectangle) were not considered in the modelling. The red ellipse represents the core isotopic niche (Standard Ellipse Area; Jackson et al. 2011) utilised, while the surrounding black polygon (solid line) represents the total niche area utilised.

Secondly, a hierarchical, agglomerative clustering approach was used to group the potential prey species with similar mean $\delta^{15}N$ and $\delta^{13}C$ values (e.g. Hindell et al. 2012, Walters 2014, Rossman et al. 2015), following the recommendation of Phillips et al. (2014). Mixing models cannot differentiate between prey species with similar isotopic signatures, and the inclusions of similar prey species result in model over-parameterisation and the production of uninformative models (Layman et al. 2012, Phillips et al. 2014). We used the *hclust* package in R (Müllner 2013, version 3.3.1) to cluster prey *a priori*, based on the dissimilarity matrix produced from Euclidean distances (999 permutations), while applying the average linkage as the agglomeration method (Clarke 1993) (Fig. S2). The identified prey groups were considered as separate groups if either $\delta^{15}N$ and/or $\delta^{13}C$ differed significantly. There was a significant difference in either $\delta^{15}N$ and/or $\delta^{13}C$ between each clustered prey group ($\delta^{15}N$: ANOVA, $F = 23.0$, df = 4, $p < 0.001$; and $\delta^{13}C$: ANOVA, $F = 56.6$, df = 4, $p < 0.001$), confirmed using a *post-hoc* Tukey's HSD test) (Table S2). All potential prey species were reduced to five prey groups (Fig. S2; Table S1).

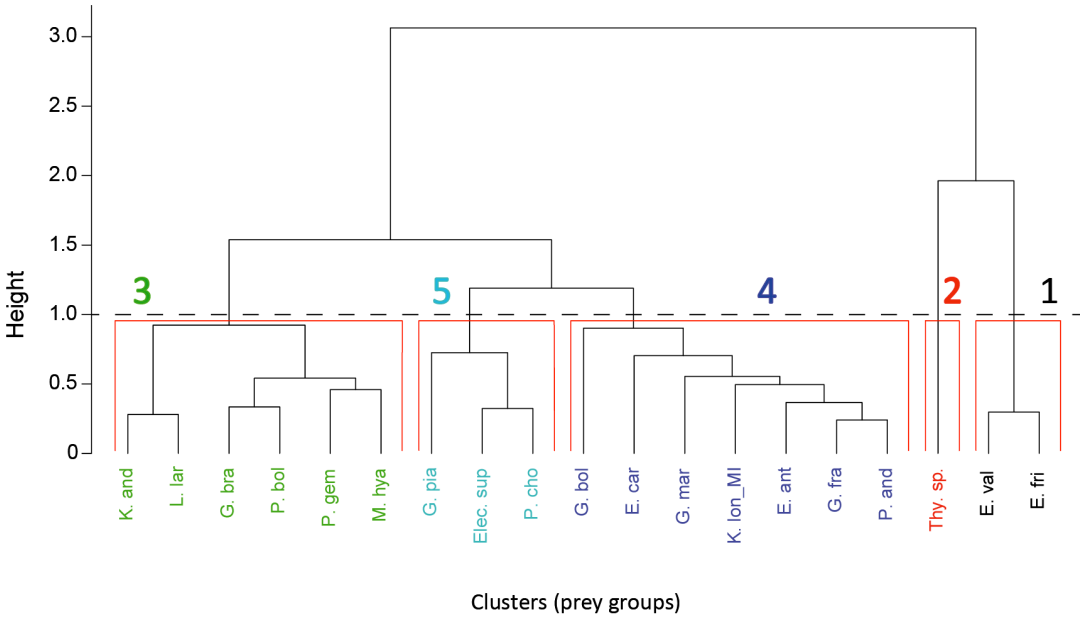

FIG. S2: A hierarchical, agglomerative clustering approach was utilised to group the potential prey with similar mean $\delta^{15}N$ and $\delta^{13}C$ values. A description of the abbreviations of the different prey species can be found in Table S1.

TABLE S1: Summary of the included prey species after clustering potential prey with similar $\delta^{15}N$ and $\delta^{13}C$ (mean ± SD) values together. Prey species were collected from Marion Island (MI), and published isotopic values for different prey species were obtained from Îles Kerguelen (KI) and Îles Crozet (CI). Abbr. = abbreviations for each species. The displayed $\delta^{15}N$ and $\delta^{13}C$ values represent the original values obtained from the published literature. The utilised diet-vibrissa specific trophic discrimination factor obtained from captive pinnipeds ($\Delta^{15}N$ = 2.8‰; $\Delta^{13}C$ = 3.2‰) can be subtracted from the $\delta^{15}N$ and $\delta^{13}C$ values presented to obtain the corrected isotopic values used for the dietary reconstruction.

Group	Species	Abbr.	Tissue	Year collected	Site	*n*	$\delta^{15}N$	$\delta^{13}C$	Source
Prey group 1									
Crustacean	*Euphausia vallentini*	*E. val*	Whole	2012	MI	19	3.4 ± 0.5	-22.6 ± 0.6	This study
Crustacean	*Euphausia frigida*	*E. fri*	Whole	2012	MI	18	3.7 ± 0.6	-22.3 ± 0.7	This study
Mean ± SD							**3.5 ± 0.2**	**-22.5 ± 0.2**	
Prey group 2									
Crustacean	*Thysanoessa sp.*	*Thy. sp.*	Whole	2012	MI	18	**5.6 ± 0.8**	**-24.3 ± 0.3**	This study
Prey group 3									
Fish	*Protomyctophum gemmatum*	*P. gem*	Muscle	2005	KI	4	8.7 ± 0.4	-22.1 ± 0.1	Cherel et al. 2010
Fish	*Protomyctophum bolini*	*P. bol*	Muscle	2005	KI	12	9.2 ± 0.4	-22.4 ± 0.6	Cherel et al. 2010
Fish	*Gymnoscopelus braueri*	*G. bra*	Muscle	2005	KI	12	9.8 ± 0.3	-22.3 ± 0.7	Cherel et al. 2010
Fish	*Lepidonotothen larseni*	*L. lar*	Muscle	1999 ─ 2003	MI	5	7.2 ± 0.8	-22.1 ± 0.4	Bushula et al. 2005
Fish	*Krefftichthys anderssoni*	*K. and*	Muscle	2005	KI	12	7.6 ± 0.2	-22.3 ± 0.2	Cherel et al. 2010
Cephalopods	*Martialia hyadesi*	*M. hya*	Beak	1997 ─ 2001	CI	10	8.5 ± 0.8	-22.6 ± 0.4	Guerreiro et al. 2015
Mean ± SD							**8.5 ± 1.0**	**-22.3 ± 0.2**	
Prey group 4									
Cephalopods	*Kondakovia longimana*	*K. lon_MI*	Beak	2012 ─ 2013	MI	10	8.1 ± 0.7	-12.3 ± 0.9	This study
Fish	*Gobionotothen marionensis*	*G. mar*	Muscle	1999 ─ 2003	MI	5	8.5 ± 0.6	-20.6 ± 0.4	Bushula et al. 2005
Fish	*Protomyctophum andriashevi*	*P. and*	Muscle	2005	KI	7	8.7 ± 0.4	-20.9 ± 0.1	Cherel et al. 2010
Fish	*Gymnoscopelus fraseri*	*G. fra*	Muscle	2005	KI	12	9.0 ± 0.4	-21.1 ± 0.4	Cherel et al. 2010
Fish	*Electrona antarctica*	*E. ant*	Muscle	2005	KI	12	8.9 ± 0.3	-21.4 ± 0.5	Cherel et al. 2010
Fish	*Electrona carlsbergi*	*E. car*	Muscle	2005	KI	12	9.5 ± 0.2	-21.6 ± 0.4	Cherel et al. 2010
Fish	*Gymnoscopelus bolini*	*G. bol*	Muscle	2005	KI	12	9.9 ± 0.5	-20.5 ± 0.4	Cherel et al. 2010
Mean ± SD							**8.9 ± 0.6**	**-21.0 ± 0.4**	
Prey group 5									
Fish	*Electrona subaspera*	*Elec. sup*	Muscle	2005	KI	14	7.3 ± 0.3	-20.2 ± 0.4	Cherel et al. 2010
Fish	*Protomyctophum choriodon*	*P. cho*	Muscle	2005	KI	12	7.8 ± 0.3	-20.0 ± 0.5	Cherel et al. 2010
Fish	*Gymnoscopelus piablis*	*G. pia*	Muscle	2005	KI	12	8.8 ± 0.2	-19.8 ± 0.3	Cherel et al. 2010
Mean ± SD							**8.0 ± 0.8**	**--20.0 ± 0.2**	

TABLE S2: A *post-hoc* Tukey's HSD test confirmed that the $\delta^{15}N$ and/or $\delta^{13}C$ values of all the clustered prey groups differed significantly. Reported values represent the difference in $\delta^{15}N$ (below diagonal) and/ $\delta^{13}C$ values (above diagonal) between the different groups.

	Group 1	Group 2	Group 3	Group 4	Group 5
Group 1	-	-1.79	0.16	1.40	2.46
Group 2	2.09	-	1.95	3.19	4.25
Group 3	5.00	2.91	-	1.24	2.30
Group 4	5.44	3.35	0.44	-	1.06
Group 5	4.47	2.38	-0.53	-0.97	-

$p > 0.001$
$p > 0.01$
$p > 0.05$

Following prey clustering, we utilised the SIAR Bayesian modelling approach to determine the proportional contribution of each prey group to the diet of the juvenile SES as described in the main manuscript. Based on the position of prey group 4 and 5 in the isotope mixing polygon (Fig. S3), it was evident that their contribution would be negligible. After running the SIAR model with all five groups, the model confirmed that prey group 4 and 5 contributed < 1% (mode), and they were consequently omitted from the final model. Only prey groups 1-3 were included in the modeling. Mixing models will always attempt to fit a model even if the data are nonsensical, and the SIAR isospace plots were carefully scrutinised to ensure that all relevant prey items were included or excluded (Phillips et al. 2014). Reducing the prey groups from five to three improved the model fit and reduced the SD of the $\delta^{15}N$ and $\delta^{13}C$ from 0.22‰ (0 – 2.20‰) to 0.13‰ (0 – 1.54‰), and from 0.26‰ (0 – 4.07‰) and 0.15‰ (0 – 1.95‰), respectively.

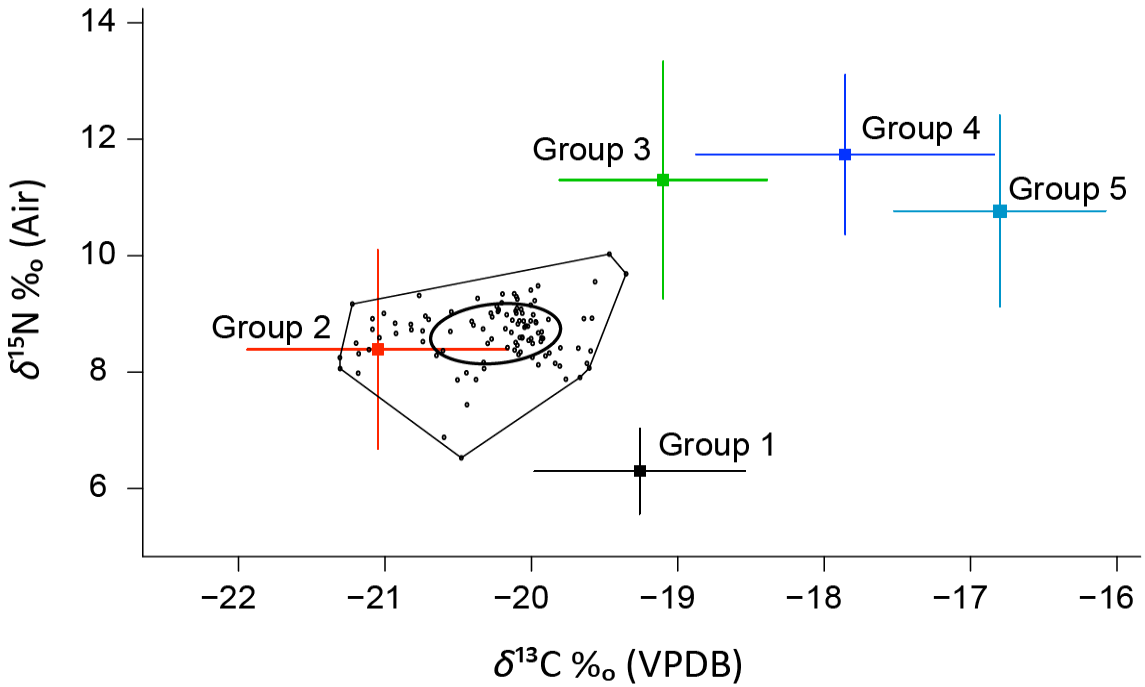

FIG. S3: Biplot of juvenile southern elephant seals (SES) *Mirounga leonina* isotopic values (black dots) relative to the potential prey groups; grouped using a cluster analysis. The δ^{15}N and δ^{13}C of the prey groups were corrected relative to SES by utilising the appropriate tissue-specific trophic discrimination factor (TDF) (Δ^{15}N = 2.8 ‰ and Δ^{13}C = 3.2 ‰; Hobson et al. 1996). The error bars represent two times the standard deviation of the prey groups, as well as the uncertainty regarding the applied TDF (± 0.3 ‰). The position of the excluded prey groups 4 and 5 relative to the juvenile SES sampled at Marion Island, suggested little contribution to their diet and was excluded from further analyses, after confirming their negligible contribution. Prey groups 1 and 2 consisted exclusively of krill species.

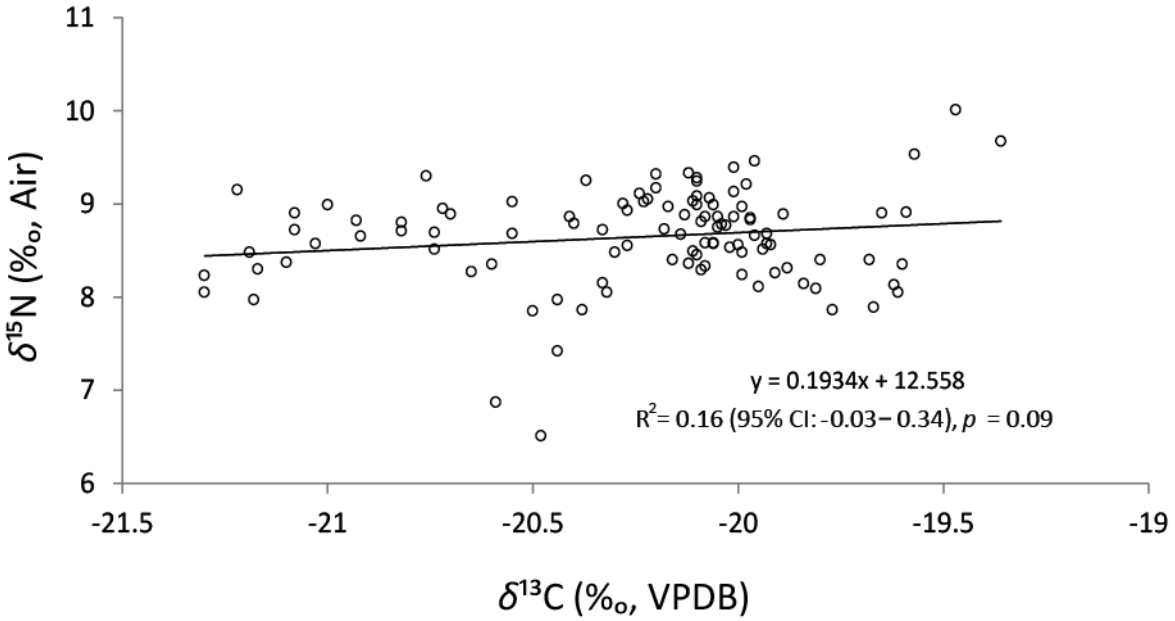

FIG. S4: Correlation between the $\delta^{15}N$ and $\delta^{13}C$ values measured in 2 mm vibrissal segments sampled from juvenile southern elephant seals *Mirounga leonina*, at Marion Island. A Pearson's product-moment correlation test indicated that the relationship between the $\delta^{15}N$ and $\delta^{13}C$ is not significant.

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