

Stable isotope values of Marion Island myctophids using otoliths from fur seal faecal samples

by

André van Tonder

13004744

Submitted in partial fulfilment of the requirements for the degree

MSc (Zoology)

Department of Zoology and Entomology

Faculty of the Natural and Agricultural Sciences

University of Pretoria

Pretoria

South Africa

9 June 2023

Supervisors:

Prof P.J. Nico de Bruyn

Dr N. Lübcker

Declaration

I, André van Tonder, declare that the dissertation, which I hereby submit for the degree, MSc Zoology, at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

I, the author, have obtained the required research ethics approval for the research described in this work, under ethics code EC077-15. I declare that I have observed the ethical standards required in terms of the University of Pretoria's Code of ethics for scholarly activities.

The dissertation's data chapters are formatted as independent manuscripts for publication. Therefore, some repetition occurs, particularly with introductions, and should please be taken into consideration while reading the dissertation.

André van Tonder

9 June 2023

This dissertation should be cited as:

Van Tonder A (2023) Stable isotope values of Marion Island myctophids using otoliths from fur seal faecal samples. MSc Dissertation, University of Pretoria.

Title: Stable isotope values of Marion Island myctophids using otoliths from fur seal faecal samples

Student: André van Tonder

Supervisors: Prof P.J. Nico de Bruyn

Dr N. Lübcker

Degree: MSc (Zoology)

Department: Zoology and Entomology, University of Pretoria

Summary

Lanternfish (Myctophidae) are abundant mesopelagic fishes in the Southern Ocean ecosystem and represent a large dietary component for sentinel marine predators, such as seals and seabirds. Their contribution to diets of predators can be quantified indirectly using stable isotope mixing models. However, bivariate isotope mixing models require nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope values of potential prey items, which is often lacking. Published stable isotope values of potential prey from geographically dissimilar sites are often used to parameterise the isotope-based mixing models, which might not be adequate, especially when variation in the isotope values at the base of food web exists. Acknowledging the inherent, but often unavoidable spatial assumption using extrinsic data for local modelling, in this dissertation I aimed to generate isotope values for myctophids, specific to the sub-Antarctic Prince Edward Islands (PEIs; Chapter 2), which can be used to refine previous dietary models of sentinel marine predators at PEIs (Chapter 3).

Firstly (Chapter 2), muscle values were required for myctophids utilised by marine predators at PEIs. Fur seals (*Arctocephalus* spp.) with known foraging areas were

used as bio-samplers of the myctophid community, whereby myctophid otoliths were recovered from faecal samples collected around seal colonies breeding at the PEIs. The required stable isotope difference between muscle and otoliths (otolith-to-muscle offset) was established using pairs of fresh muscle and otolith samples of *Electrona antarctica* ($n = 6$) and *Gymnoscopelus opisthopterus* ($n = 2$) from Dronning Maud Land, Antarctica. The average otolith-to-muscle offset used was $-1.3 \pm 0.4\text{‰}$ for $\delta^{13}\text{C}$ and $1.6 \pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. Using these otolith-to-muscle offsets, the muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of myctophids could be estimated where only faecal otoliths are available, provided that an additional oxidative cleaning step was used to account for faecal contamination. An oxidative cleaning oversight was corrected for and the associated uncertainty incorporated.

The otolith-to-muscle offset was applied to oxidant-cleaned otoliths from fur seal faecal samples at the PEIs. The myctophid species under study were *E. subaspera*, *G. fraseri*, *G. nicholsi* and *G. piabilis*. Stable isotope values of fish from the PEIs were positively correlated to their Îles Kerguelen counterparts, illustrating similar relative stable isotope ecology of species across sites. The absolute difference between stable isotope values of PEI and Îles Kerguelen fish could be explained by a depleted baseline at the PEIs relative to Îles Kerguelen. Detecting these patterns indicate that the oxidative cleaning and offset method is a realistic way to estimate fish muscle from faecal otoliths, despite, in this case, uncertainty brought about by cleaning correction.

The novel otolith-derived muscle isotope values of the myctophids (Chapter 2) were then used to revise a previous dietary model of juvenile southern elephant seals (*Mirounga leonina*), in addition to construction of novel models for sub-adults, adult females and adult males, as well as killer whales (*Orcinus orca*; Chapter 3). Remodelling for juvenile southern elephant seals revised krill consumption from 76%

to 58%, and improved modelling performance. Squid, which only included *Martialia hyadesi* for juveniles, went from a maximal contribution of 35% to a maximum of 28%. Older elephant seal age classes indicated an expected decreasing trend for crustacean consumption with age, but remained substantial for each age class specifically, 35% [0% - 65%] to 24% [0% - 72%] and 19% [0% - 28%] from sub-adults to adult females and adult males. Results also indicated that all classes fed on >30% squid, while fish, surprisingly, played a predominant role in adult male diets (sum of fish containing clusters' modes 88% [23% - 100%]). The results vary from findings at the PEIs and other sites which predicted that squid predominate diets over fish. While variation may be due to differences in prey abundances or historical estimate biases due to retention of hard squid remains in seal stomachs, it is additionally evident (Chapter 2) that variation in stable isotope values at the base of food web exists between the neighbouring PEIs and Îles Kerguelen. Baseline variation needs to be considered, as it affects the source values considered and modelling outcomes. Wide credibility intervals also suggest varied niche use and fluid combinations of dietary resources within age-sex classes.

The killer whale isotope-based dietary model remained unresolved due to a lack of potential prey with high enough $\delta^{13}\text{C}$ values to generate a mixing space around the consumer values of these top predators. In addition to highlighting the requirement for local prey isotope values to better characterise southern elephant seal diets, this dissertation reiterates the need for sampling of more northerly prey sources with higher $\delta^{13}\text{C}$ values to parameterise killer whale models. To this end, this study's validation of using degraded otoliths and the myctophid-specific, otolith-to-muscle isotopic offset provides means to obtain myctophid isotope values while using predators as samplers of their environments (bio-samplers).

Acknowledgements

I wish to thank the crew and research teams aboard the Kronprins Haakon during the Dronning Maud Land ecosystem survey, led by the Norwegian Polar Institute's Dr Harald Steen, for their assistance. Especially Dr Elvar Hallfredsson for allowing me to aid the fish station in exchange for samples - it became a central part of the study. Bianca Coulson, thank you for the late nights' assistance dissecting fish and for sharing your elephant seal data. The National Research Foundation (RSA) provided funding, and the Department of Forestry, Fisheries and the Environment (DFFE), through the South African National Antarctic Program (SANAP), provided logistical support for the collection of otolith samples at Marion Island. Thanks to fellow Marion Island "sealers" for the dirty work of scat collection and preparation. Various contributors at the Mammal Research Institute, University of Pretoria, and elsewhere provided invaluable input to this project, both logistically and conceptually. In particular, Dr Grant Hall at the Stable Isotope Facility, University of Pretoria for his passionate dedication to the lab. To my supervisors, thank you for your patience and frank critiques. I am thankful to be sharpened by your standards. The team of technicians at the Marin Økologi research at Aarhus University, Susanne Vase Petersen and Inge Lacour, with Prof Peter Grønkjær, for their kind and eager assistance and training. In addition, the forthcoming financial assistance with analyses was invaluable.

To my mom and uncles, thank you for instilling a passion for natural science and a drive for exploration. I am a product of your dedication and commitment, always.

"Following our will and whim we may just go where no one's been." "Every footprint returns to the dirt."

Table of contents

Declaration-----	ii
Summary -----	iii
Acknowledgements-----	vi
Table of contents -----	vii
List of tables -----	ix
List of figures -----	xi
Manuscript and co-author contributions-----	xiv
Author contributions during candidature -----	xv
CHAPTER 1: Introductory chapter -----	1
TOP PREDATORS USED TO MONITOR ECOSYSTEMS-----	1
STABLE ISOTOPE ANALYSIS -----	2
MODELLING DIETS USING STABLE ISOTOPE ANALYSIS-----	3
THE PRINCE EDWARD ISLANDS -----	4
MYCTOPHIDS AT THE PRINCE EDWARD ISLANDS -----	5
OTOLITHS-----	6
DISSERTATION STRUCTURE-----	8
CHAPTER 2: Estimating carbon and nitrogen stable isotope values of lanternfish muscle from faecal otoliths -----	11
ABSTRACT -----	12
1. INTRODUCTION-----	13
2. MATERIALS AND METHODS -----	16
3. RESULTS -----	22
4. DISCUSSION -----	29
5. CONCLUSION-----	36
CHAPTER 3: Use of hard faecal prey remains to contribute to isotope-based dietary models of sentinel marine predators-----	38
ABSTRACT -----	39
1. INTRODUCTION-----	40
2. MATERIALS AND METHODS -----	43
3. RESULTS -----	49
4. DISCUSSION -----	55
5. CONCLUSION-----	63

CHAPTER 4: General conclusions -----	65
Supplementary material to Chapter 2-----	68
LIPID EXTRACTION COMPARISON -----	69
MATHEMATICAL AVERAGE VS PHYSICAL POOLING FOR MUSCLE-----	70
UNCERTAINTY PROPAGATION FOR MUSCLE ESTIMATES-----	72
Appendix to Chapter 2: Correcting stable isotope values for insufficient oxidative cleaning of otolith material-----	76
ABSTRACT -----	77
1. INTRODUCTION-----	78
2. MATERIALS AND METHODS -----	79
3. RESULTS -----	80
4. DISCUSSION -----	83
5. CONCLUSION-----	85
Supplementary material to Chapter 3-----	90
SOURCE VALUES USED FOR MODELLING-----	91
Literature cited-----	100

List of tables

Table 1: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of myctophids sampled near Dronning Maud Land, Antarctica from February to March 2019. Individual (conspecific) fish were randomly assigned to samples, with 16 – 17 fish pooled per sample. The offset (Δ) between muscle and otolith (muscle minus otolith) values for the samples are also shown. (EA = <i>Electrona antarctica</i> , GO = <i>Gymnoscopelus opisthopterus</i> .-----	23
Table 2: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of selected sampling units of myctophids at the Prince Edward Islands (PEIs). ES denotes <i>Electrona subaspera</i> , GF – <i>Gymnoscopelus fraseri</i> , GN – <i>G. nicholsi</i> , GP – <i>G. piabilis</i> . The sampling periods were around January 2005 and December 2010. The values are of otoliths collected from faecal samples of various fur seal colonies on Marion Island, part of the PEIs. The otoliths were cleaned with NaOCl before measurement. Applying the otolith-to-muscle offset established for myctophids provided estimates of muscle values. The uncertainty, final standard deviation (SD), is the sum of both measurand uncertainty (including cleaning correction and muscle estimation) as well as the standard deviation of sampling units. -----	24
Table 3: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of muscle of myctophids compared between different study sites. KAR – Îles Kerguelen (Cherel et al. 2010). BB-KPL – Kerguelen Plateau (Woods et al. 2020). MI– Marion Island – part of the Prince Edward Islands (PEIs), ~ values estimated from figure, and <i>Gymnoscopelus</i> sp. here only include <i>G. fraseri</i> and <i>G. piabilis</i> and exclude <i>G. braueri</i> (Pakhomov et al. 2006). PEIs – this study. DML – Dronning Maud Land (this study). The values for this study (PEIs) were estimated using offsets from organic material of otoliths from fur seal faecal samples. Final standard deviation (SD) is the sum of analytical and measurand uncertainty. -----	26
Table 4: Proportional contributions of clusters of prey to the diets of juvenile (J), sub-adult (SA) and adult female (AF) southern elephant seals (<i>Mirounga leonina</i>). Proportional contributions were determined using the R package <i>siar</i> using stable isotope values of elephant seal whiskers sampled at the sub-Antarctic Prince Edward Islands. Prey values were sourced from the literature and means $\pm$ standard deviations (SD) are reported here. The constituent species for each cluster and its reference can be found in Table 10 of the Supplementary Material to Chapter 3. SDs for the isotopic tracers relative to measurement reproducibility for standards are a measure of model performance, with lower variability indicative of better performance. <i>M. hya</i> = <i>Martialia hyadesi</i> ; <i>Thy. sp.</i> = <i>Thysanoessa</i> sp. and <i>M. lon</i> = <i>Moroteuthopsis longimana</i> . The model for adult male southern elephant seals (AM) had different cluster constituents as other classes (Table 11). -----	50
Table 5: A comparison of stable isotope values for myctophids from Dronning Maud Land, Antarctica sampled in March - April 2019. Multiple fish (~17) were combined into six units for <i>Electrona antarctica</i> (EA) and two for <i>Gymnoscopelus opisthopterus</i> (GO). The comparison is where the stable isotope values of muscle samples of individual fish were averaged relative to physically combining the muscle samples of fish and making a single, pooled measurement. -----	71

Table 6: Summary of propagated uncertainty for estimates of myctophid muscle stable isotope values from oxidant-cleaned faecal otoliths (ES = <i>Electrona subaspera</i> , GF = <i>Gymnoscopelus fraseri</i> , GN = <i>G. nicholsi</i> , GP = <i>G. piabilis</i>). Roman numerals denote the sources of uncertainty listed in the current supplement propagated up to that point. -----	75
Table 7: Correction values (‰) for stable carbon isotope ratios ($\delta^{13}\text{C}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The correction values and associated error were derived from nearest neighbour regression (<i>loess</i>) curves at a 95% confidence level, span = 0.75, using R (R Core Team 2020, RStudio Team 2020). The curves were established using otolith material of <i>Gymnoscopelus piabilis</i> from Antarctic fur seal (<i>Arctocephalus gazella</i>) faecal samples collected at the Prince Edward Islands. -----	86
Table 8: Correction values (‰) for stable nitrogen isotope ratios ($\delta^{15}\text{N}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The correction values and associated error were derived from nearest neighbour regression (<i>loess</i>) curves at a 95% confidence level, span = 0.75, using R (R Core Team 2020, RStudio Team 2020). The curves were established using otolith material of <i>Gymnoscopelus piabilis</i> from Antarctic fur seal (<i>Arctocephalus gazella</i>) faecal samples collected at the Prince Edward Islands. -----	88
Table 9: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of clustered potential prey items of juvenile, sub-adults and adult female southern elephant seals (<i>Mirounga leonina</i>) at the sub-Antarctic Prince Edward Islands. Prey items include novel data for myctophids from the Prince Edward Islands (PEI; Chapter 2) that replace conspecific data from other sites (Îles Crozet = CI and Îles Kerguelen = KI), as found in Lübcker et al. (2017). All values are either for whole organisms (krill) or estimates of muscle values for fish and squid. -----	92
Table 10: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of clustered potential prey items of adult male southern elephant seals (<i>Mirounga leonina</i>), for which considered prey was different than other classes. Prey items include novel data for myctophids from the Prince Edward Islands (PEI; Chapter 2) that replace conspecific data from other sites (Îles Crozet = CI and Îles Kerguelen = KI), as found in Lübcker et al. (2017). All values are either for whole organisms (krill) or estimates of muscle values for fish and squid. -----	94
Table 11: Stable isotope values of potential prey items of killer whales (<i>Orcinus orca</i>) as found in Reisinger et al. (2016). AM = adult male, UY = under yearling, Y = yearling, SA = sub-adult, AF = adult female. Prey items include novel data for myctophids from the Prince Edward Islands (PEI; Chapter 2) that replace conspecific data from other sites (Îles Crozet = CI and Îles Kerguelen = KI) -----	96

List of figures

- Figure 1: Estimated stable isotope values of muscle values of myctophids (*Electrona* and *Gymnoscopelus*) around the sub-Antarctic Prince Edward Islands around January 2005 (blue) and December 2010 (red). Estimates were obtained by applying an otolith-to-muscle offset to values of oxidant-cleaned otoliths obtained from fur seal faecal samples. The mean and final standard deviations are shown. The final standard deviation includes uncertainty from measurements, correction for insufficient oxidative cleaning, and application of the otolith-to-muscle offset, as well as variance within sampling units. The asterisk (*) indicates that *G. piabilis* 2005 had significantly different $\delta^{13}\text{C}$ values compared other species. There was also a significant $\delta^{13}\text{C}$ difference for *G. nicholsi* 2010 and *G. piabilis* 2010 (#). Black points and bars represent mean and standard deviations of conspecific values from Îles Kerguelen samples in 2005 (Cherel et al. 2010). 3.4 Sampling units at the PEIs, and conspecific comparison with Îles Kerguelen (uncertainty propagated)----- 28
- Figure 2: Representation of frontal and bathymetric features in the Indian Ocean sector of the Southern Ocean. STZ = Sub-Tropical Zone; STF = Sub-Tropical Front; SAZ = Sub-Antarctic Zone; APF = Antarctic Polar Front; AZ = Antarctic Zone. SWIR = Southwest Indian Ridge and archipelagos shown are Prince Edward Islands (PEI), Îles Crozet (CI) and Îles Kerguelen (KI). Frontal data is available online (Park & Durand 2019, Park et al. 2019). ----- 44
- Figure 3: Dendrograms indicating the composition of clusters for (a) juvenile, (b) sub-adults and adult female and (c) adult male southern elephant seals (*Mirounga leonina*). Clusters in (a) are the same as (b), aside from the absence of *Moroteuthopsis longimana*. Sources include values from the Prince Edward Islands (PEIs), Îles Crozet and Îles Kerguelen. Sources preceded with an asterisk (*) are adopted from Chapter 2 (PEIs) of this dissertation. Red coloured sources are krill species, green sources are fish and orange sources are squid. ----- 53
- Figure 4: Graphical representation of the bivariate stable isotope mixing space for (a) juvenile, (b) sub-adult, (c) adult female and (d) adult male southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands during foraging (fasting excluded). The black squares represent clusters of potential prey values and one standard deviation, after applying the appropriate trophic discrimination factor (TDF), but not incorporating TDF uncertainty (Hobson et al. 1996, Lübcker et al. 2017). The sources in these models include values for fish sampled around the PEIs specifically, and other sources from Îles Crozet and Îles Kerguelen. The dashed grey boxes are the bounds within which potential source values, adjusted for TDF, were considered. The numbers assigned to clusters and its constituents can be found in Table 5 and 10, with source clustering consistent for juveniles (aside from having no cluster 6), sub-adults and adult females. The adult male model considered a different set of sources (Table 11). ----- 54
- Figure 5: Percentage contributions, mode and 95% credibility intervals, of prey groups to different age-sex classes of southern elephant seal (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. The distributions are the frequentist-results of posteriors generated using the *siar* package in R. Modelling for juveniles did not consider a sixth cluster. Adult males are not depicted here, as the clusters for that

- class were made up differently than for juveniles, sub-adults and adult females (see Figure 7 and Tables 10 and 11 for cluster make-up). ----- 61
- Figure 6: Percentage contributions, mode and 95% credibility intervals, of prey groups to adult male southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. The distributions are the frequentist-results of posteriors generated using the *siar* package in R. Clusters are made up differently to clusters for other age-sex classes, see Figure 6 and Table 10, hence the 'AM' (adult male) denotation in this figure. (See Table 11 for cluster make-up of adult males). When determining relative contribution using simulation modelling, models only indicate the range of mathematical solutions that exist with the sources that are provided and needs to be validated against what is known (Smith et al. 2013, Phillips et al. 2014). As our knowledge of consumers' diets grow, particularly poignant for squid here (Bester & Connan 2022), we can spot and fill gaps in our modelling efforts. The simulation method also weights the outer sources that make up the mixing geometry and devalues 'inner' sources that may be important in reality (Smith et al. 2013). E.g., the contribution of inner clusters 2 and 3 to the diet of sub-adult southern elephant seals may be underestimated relative to outer vertices like clusters 4, 5 and 6 because it is less important in shaping the borders of the mixing space. Yet, it stands to reason that the sources plotting closest to the consumer values are ecologically more closely related, either by overlapping niches or, as inferred in this case, by a strong trophic link (Phillips & Gregg 2003, Moore & Semmens 2008, Parnell et al. 2010, Jackson et al. 2011). ----- 62
- Figure 7: Correction curve for stable carbon isotope ratios ($\delta^{13}\text{C}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The curve is a nearest neighbour regression curve (*loess*) at a 95% confidence level span = 0.75, and was produced using R (R Core Team 2020, RStudio Team 2020). It represents otolith material of *Gymnoscopelus piabilis* from Antarctic fur seal faecal (*Arctocephalus gazella*) samples collected at the Prince Edward Islands. ----- 81
- Figure 8: Correction curve for stable nitrogen isotope ratios ($\delta^{15}\text{N}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The curve is a nearest neighbour regression curve (*loess*) at a 95% confidence level, span = 0.75, and was produced using R (R Core Team 2020, RStudio Team 2020). It represents otolith material of *Gymnoscopelus piabilis* from Antarctic fur seal faecal (*Arctocephalus gazella*) samples collected at the Prince Edward Islands ----- 82
- Figure 9: Probability distributions of mixing polygons produced by source clusters of (a) juvenile, (b) sub-adult, (c) adult female and (d) adult male southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. Mixing spaces make use of source values adjusted for trophic discrimination factors (TDFs) between diet and the sampled whiskers tissue (black dots) of seals. Sources were clustered into groups (white crosses), which were similar for juvenile, sub-adults and adult female seals (though juveniles had only five clusters). The composition of clusters is reported in Table 5 and 10.----- 97
- Figure 10: Dendrograms indicating the composition of potential prey clusters for killer whales (*Orcinus orca*) at the sub-Antarctic Prince Edward Islands. The constituent species of each cluster and numbers assigned to the clusters can be found in Table 12. ----- 98

Figure 11: Graphical representation of the stable isotope mixing model for killer whales (*Orcinus orca*) at the sub-Antarctic Prince Edward Islands (PEIs). The black squares represent clusters of potential prey values and one standard deviation, after applying the appropriate trophic discrimination factor (TDF), but not incorporating TDF uncertainty (Hobson et al. 1996, L  b  cker et al. 2017). The sources in these models include values for prey sampled around the PEIs specifically. The dashed grey boxes are the bounds within which potential source values, adjusted for TDF, were considered. The numbers assigned to the clusters can be found in Table 12. The lower image shows probability distributions of mixing polygons produced by source clusters of killer whales at the PEIs. Mixing spaces make use of source values adjusted for trophic discrimination factors between diet and the sampled skin tissue (black dots) of killer whales, incorporating uncertainty (1 standard deviation) in both sources and TDFs. Sources were clustered into groups (white crosses), the composition of which is reported in Table 12. ----- 99

Manuscript and co-author contributions

Chapter 2:

Nico Lübcker and P.J. Nico de Bruyn participated in conceptual input and reviewed multiple drafts of the work, taking on supervisory roles. Peter Grønkjær provided conceptual input and corrections to data. Jessica Lueders-Dumont provided aid and conceptual input into solving the systematic cleaning error. Elvar Hallfredsson was head of fish analysis aboard Kronprins Haakon and assisted with data by identifying myctophids for this study.

Chapter 3:

Nico Lübcker and Ryan Reisinger provided consumer and source data for dietary modelling of juvenile southern elephant seals and killer whales, respectively. Bianca Coulson provided consumer data for modelling sub-adult, adult female and adult male southern elephant seals. Nico Lübcker and P.J. Nico de Bruyn took on a conceptual and supervisory role and reviewed drafts of the work.

Author contributions during candidature

Moreau S, Hattermann T, Laura de Steur L, Kauko HM, Ahonen H, Ardelan M, Assmy P, Chierici M, Descamps S, Dinter T, Falkenhaus T, Fransson A, Grønningsæter E, Hallfredsson EH, Huhn O, Lebrun A, Lowther A, Lübcker N, Monteiro P, Peeken I, Roychoudhury A, Róžańska M, Ryan-Keogh T, Sanchez N, Singh A, Simonsen JH, Steiger N, Thomalla SJ, van Tonder A, Wiktor JM, Steen H (2023) Wind-driven upwelling of iron sustains dense blooms and food webs in the eastern Weddell Gyre. *Nature Communications* 14:1303

Van Tonder A, Lübcker N, Guerreiro M, Xavier JC, Cherel Y, de Bruyn PJN (2021) Ecology of *Moroteuthopsis longimana* at the sub-Antarctic Prince Edward Islands, revealed through stable isotope analysis of squid beaks. *Marine Ecology Progress Series* 658:105-115.

CHAPTER 1: Introductory chapter

TOP PREDATORS USED TO MONITOR ECOSYSTEMS

Top predator populations have been shown to respond to climate warming (Weimerskirch et al. 2003, Reisinger et al. 2022) and changes in food abundance (Cury et al. 2011) by shifting foraging areas, demonstrating their utility as ecosystem indicators. The use of top predators as indicators, however, requires an understanding of the underlying mechanisms (functional relationships, e.g., trophic interactions) that determine their responses to environmental changes (Grémillet & Charmantier 2010).

The aforementioned authors also discuss the use of indicator species or sentinels by entities like the Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR). They further iterate how a sentinel approach requires intimate knowledge of the functional relationships between sentinels' responses, e.g., dietary shifts, and environmental parameters like food abundance. When looking at responses to food abundance, knowing which prey is exploited is important (Österblom et al. 2008).

Methods for dietary estimation of marine predators traditionally involve gastric lavage and/or scat analysis (Croxall et al. 1988, Lea et al. 2002, Makhado et al. 2007, 2013, Waluda et al. 2012, Reisinger et al. 2018a). For some species these methods are not always feasible, particularly for larger marine mammals like killer whales (*Orcinus orca*) and southern elephant seals, where stomach sampling or scat analysis is unethical or logistically challenging (Antonelis Jr et al. 1987, Daneri & Carlini 2002, Best et al. 2010). Besides for rare direct feeding observations (e.g., killer whales, Condry et al. 1978, Reisinger et al. 2011), indirect methods for dietary analysis therefore provides a valuable alternative, with stable isotope analysis (SIA) being one of the favoured methods (Richoux et al. 2010, Karnovsky et al. 2012, Reisinger et al. 2016, Lübcker et al. 2017).

26 STABLE ISOTOPE ANALYSIS

27 The use of SIA for dietary reconstruction exploits the assimilation of ingested material
28 in consumers' tissue. The ratios of naturally occurring stable isotopes in a tissue are
29 determined by the ratios in the consumer's environment (isoscape) and by physical or
30 metabolic processes that discriminate for lighter or heavier stable isotopes. In this way,
31 examining the isotopic ratios in sampled consumer tissue provides insight to the
32 consumer's ecological niche (de Niro & Epstein 1978, 1981, Eggers & Jones 2000).
33 The stable isotope ratios (δ) of carbon ($^{13}\text{C}:^{12}\text{C}$) and nitrogen ($^{15}\text{N}:^{14}\text{N}$) are commonly
34 used for dietary studies (Boecklen et al. 2011).

35 The $\delta^{13}\text{C}$ have been shown to vary broadly, and inversely, with latitude at the base of
36 the food web in the Southern Ocean, due in part to differences in dissolved carbon
37 dioxide concentrations and growth rates of phytoplankton between areas with different
38 water temperatures (Goericke & Fry 1994, MacKenzie et al. 2011, Kurle & McWorther
39 2017). The $\delta^{13}\text{C}$ values of primary producers are reflected in the tissues of consumers
40 because the $\delta^{13}\text{C}$ show limited fractionation ($+0.4 \pm 1.3\text{‰}$ (mean $\pm$ SD)) as material is
41 passed along a food chain (Peterson & Fry 1987, Post 2002, Layman et al. 2012). The
42 $\delta^{13}\text{C}$ measured in the tissues (e.g., muscle, hair, feathers) sampled from top predators
43 can be used to determine the broad latitudinal origins of the consumed prey (Cherel &
44 Hobson 2007, Jaeger et al. 2010, Guerreiro et al. 2015, Carpenter-Kling et al. 2020,
45 St John Glew et al. 2021). Local island effects can also influence the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
46 values due to, for example, increased kelp derived organic material (Kaehler et al.
47 2006), pointing out the importance of scale when making conclusions using isotopic
48 information.

49 The baseline $\delta^{15}\text{N}$ is predominantly related to the $\delta^{15}\text{N}$ of upwelled nitrate to the
50 euphotic zone, as well as the nitrogen demand of primary producers (uptake) (Graham

et al. 2010), which may vary seasonally (Mengesha et al. 1998, Lourey et al. 2003). Values of $\delta^{15}\text{N}$ are used to determine trophic level, as these isotopes discriminate incrementally from prey to predator due to the preferential excretion of lighter ^{14}N in urea (Vander Zanden & Rasmussen 2001, Mill et al. 2007). The remaining heavier ^{15}N are concentrated along food chains (Minagawa & Wada 1984, Peterson & Fry 1987, Layman et al. 2012). These trophic discrimination factors (TDFs) range between 2‰ and 5‰, averaging $3.4 \pm 1.0\text{‰}$ (Minagawa & Wada 1984, Vander Zanden & Rasmussen 2001, Post 2002), but has been shown to vary substantially between species, tissues, differences in diet quality and physiological factors like nutritional stress and reproductive status (Caut et al. 2009, Pecquerie et al. 2010, Blanchet-Aurigny et al. 2012, Healy et al. 2018). Determining the trophic level (Cherel et al. 2008) occupied by a consumer requires known isotopic values at the base of the food web and the species- and tissue-specific incremental TDFs between trophic levels.

MODELLING DIETS USING STABLE ISOTOPE ANALYSIS

When stable isotope signatures of tissues are plotted against each other graphically, with different numerical axes represented by the stable isotope ratios of different elements, the space can be used as a representation of the ecological niche of the individuals or groups in question (Jackson et al. 2011). Bayesian mixing models make use of stable isotope values from a consumer group and values from its potential prey sources to estimate the relative contribution of food sources to the consumer's diet. The relative proximity of source values to consumer values in the niche space is indicative of importance (Phillips & Gregg 2003, Moore & Semmens 2008, Parnell et al. 2010, Jackson et al. 2011). In addition to requirements like correcting for isotope discrimination occurring during metabolic processing (Caut et al. 2009, Bond & Diamond 2011), sufficient potential source (i.e., prey) isotope values are central to

76 successful dietary models (Phillips & Gregg 2003, Moore & Semmens 2008, Parnell
77 et al. 2010, Jackson et al. 2011).

78 THE PRINCE EDWARD ISLANDS

79 The Prince Edward Archipelago consists of two islands, a smaller namesake and the
80 larger Marion Island. The islands are bathymetrically steep volcanic elevations located
81 in sub-Antarctic southern Indian Ocean at 46°44'S and 37°49'E (Prince Edward
82 Islands Management Plan Working Group 1996). Oceanographically, the archipelago
83 finds itself near the sub-Antarctic front and lacks an extensive shelf, features
84 determining biological and physical interactions that affect local biota (Prince Edward
85 Islands Management Plan Working Group 1996, Perissinotto et al. 2000).

86 Historically (19th to mid-20th century), natural resources like fur seal (*Arctocephalus*
87 spp.) pelts and southern elephant seal (*Mirounga leonina*) blubber were sourced from
88 the Prince Edward Islands (PEIs). Since 1995, the PEIs were managed as a Special
89 Nature Reserve (Prince Edward Islands Management Plan Working Group 1996), and
90 a Marine Protected Area was declared around the islands in 2013, aiming in part to
91 protect the food resources of predators on and around the island (Lombard et al.
92 2007). Management of protected areas is dynamic and dependent on changing natural
93 conditions; current information on trophic interrelations in the area's ecosystem are
94 required to make adaptive conservation decisions. Research on top predators is used
95 to monitor and inform these protection measures (Lea et al. 2002, Waluda et al. 2012,
96 Ansorge et al. 2014, Kirkman et al. 2016, Reisinger et al. 2018a b).

97 Only two published attempts at modelling the diet of PEI mammals with SIA have been
98 made, one for juvenile southern elephant seals (Lübcker et al. 2017), and one for killer
99 whales (Reisinger et al. 2016). Stable isotope analysis for fur seals is also progressing

(I. Mukutyu unpubl. data). The diet modelling attempt for killer whales was precluded by, among others, a lack of sufficient potential source values, particularly for potential prey like cephalopods and lanternfish (Myctophidae). The juvenile southern elephant seal model had viable solutions through adopting the majority of source values, mainly myctophids, from a study of Îles Kerguelen fishes (Cherel et al. 2010). The spatial assumption was made that Kerguelen prey would be isotopically similar to prey localised around the PEIs. While this may well be appropriate given the close proximity of the islands, similar latitudes (PEIs: 46°44'S, 37°49'E; Îles Kerguelen: 49°20'S, 69°30'E) and sitting within similar sub-Antarctic circumpolar currents (Figure 4), the model may benefit from the use of more localised source values to test this inherent spatial assumption.

MYCTOPHIDS AT THE PRINCE EDWARD ISLANDS

As gleaned from cases where dietary or faecal samples could be collected and the prey identified, fish make up a large part of the diets of several PEI predators (Adams & Klages 1987, Brown & Klages 1987, Reisinger et al. 2018a). The remains of myctophids, a high energy resource (van de Putte et al. 2006), are predominant in the diet (faeces) of fur seals (Adams & Klages 1987, Brown & Klages 1987, Reisinger et al. 2018a). This predominance lends further support that the isotopic characterisation of myctophids would be valuable to estimate the diets of more elusive mammals like southern elephant seals and killer whales, as these mammals may well share the myctophid resource. Myctophids are widely distributed in the Southern Ocean and are an important link in pelagic food webs (Saunders et al. 2019, Woods et al. 2022). In the Indian sector of the Southern Ocean, myctophids were recorded much more frequently in the sub-Antarctic as opposed to in the Antarctic waters (Woods et al. 2022).

Finding appropriate fish samples for SIA has proven challenging. There are hardly any scientific cruises that source fish around the PEI archipelago. Analysis of stomach contents of seabirds breeding on the island yield low frequencies of fish remains, which are often degraded, so their use for SIA is considered dubious. Fish data from previous studies focussed around the PEIs are also limited in species richness with small sample sizes (Kaehler et al. 2000, Bushula et al. 2005, Pakhomov et al. 2006). Even though muscle samples of fish are typically sought after for SIA, the fur seal faecal samples alluded to above may provide an alternative.

OTOLITHS

Using fur seals as bio-samplers requires knowledge of their foraging areas. Main foraging for fur seals from the PEIs occurs from ~43° to 55°S and ~40° to 50°E (Reisinger et al. 2018b). Core areas are to the south of the PEIs (Antarctic fur seals, *A. gazella*) and north near the sub-Antarctic Front, and the Del Caño Rise and Gallieni Rise (sub-Antarctic fur seals, *A. tropicalis*) (de Bruyn et al. 2009, Kirkman et al. 2016, Wege et al. 2016, Wege 2017).

Fur seal faecal samples are collected systematically around breeding colonies on Marion Island and yield a number of myctophid otoliths (Makhado et al. 2007, 2013, Reisinger et al. 2018a). Otoliths are the bony carbonate structures that form inside the crania of fish. These otoliths, in addition to being identifiable to species level (Hecht 1987, Williams & McEldowney 1990, Smale et al. 1995) and serving as important platforms to estimate metabolic rates and fishes' role in biogeochemical cycling (Alewijnse et al. 2021), also house organic material (OM) that, once extracted, can provide reliable ecological information when using SIA of carbon and nitrogen (Grønkjær et al. 2013, von Biela et al. 2015). The OM is an endolymph-deposited composition of protein, glycoprotein and collagens (Lowen 2016). The generally acidic

protein component is made up mostly of otolin-1, starmaker and OMP-1 (Murayama et al. 2002, 2005, Lowen 2016), but compositions may differ between species (Dauphin & Dufour 2003).

While the ecological utility of OM stable isotope ratios has been demonstrated, these signatures cannot be used directly in dietary models, as otolith OM is not of dietary importance; otoliths are defecated, and its organic component is minute. The stable isotope ratios of OM are not necessarily identical to that of the ingested and assimilated fish's muscle, which is required to construct isotope-based dietary models. The difference between isotopic values of otolith OM and muscle can be applied to otolith values to estimate muscle, and offsets have been published for a variety of fish species (Vandermyde & Whitley 2008, Elsdon et al. 2010, Rowell et al. 2010, Ankjær et al. 2012, Grønkjær et al. 2013, Sirot et al. 2017). However, the variability of this relationship between taxa suggests that more taxon-specific muscle-otolith isotope discrimination factors are required. Establishing the otolith-to-muscle offset for myctophids may provide a reliable means of estimating myctophid muscle values where only otoliths are available.

If faecal otoliths are to be used reliably, the effects of digestion and faecal contamination need to be controlled for. Oxidative cleaning using sodium hypochlorite (NaOCl) of otoliths has been used successfully to control for diagenetic effects on sedimentary samples (Lueders-Dumont et al. 2018). The NaOCl solvent relies on the oxidising capacity of Cl^- and OCl^- ions to remove organic material (Spanó et al. 2001, Estrela et al. 2002, Wang et al. 2018). The basis of its use in cleaning of fouled otoliths is by removing exposed organic material that may have been altered or contaminated, leaving only organic material protected within the inorganic aragonite (CaCO_3) structures of the otolith (Lueders-Dumont et al. 2018).

In addition to controlling for extraneous effects on the otolith values, the effects of high-carbon lipids on muscle values also need to be considered. Lipid content varies between individual fish and its removal from samples leads to less variable $\delta^{13}\text{C}$ responses within sampling units (Cherel et al. 2010). Cyclohexane is the solvent opted for in Cherel et al. (2010) to extract lipids from myctophid muscle samples. Considering that muscle estimates will be compared to values from Cherel et al. (2010), the use of cyclohexane for the current study was considered to be justified.

DISSERTATION STRUCTURE

If faecal otolith-derived muscle isotope values are to be used as prey values in the isotope-based dietary mixing models of myctophid-feeding predators, reliable otolith-to-muscle isotopic offsets need to be established. Concerns regarding the reliability of using stable isotope values measured in partially digested otoliths should also be addressed. The dissertation is structured as follows:

Chapter 2 - Estimate muscle isotope values for PEI myctophids by establishing the isotopic difference between myctophid otolith OM and muscle from fresh fish collected in Dronning Maud Land. The isotopic offsets are then applied to oxidant-cleaned myctophid otoliths taken from resident fur seal faecal samples collected at PEIs around January 2005 and December 2010.

Chapter 3 - Use the faecal otolith-derived estimated muscle isotope values (Chapter 2) to re-evaluate the diet of killer whales (Reisinger et al. 2016) and juvenile southern elephant seals (Lübcker et al. 2017). Novel source values (Chapter 2) of local prey that replace some sources from neighbouring islands, and recent consumer values from whiskers (B. Coulson unpubl. data), were used to model diets of older age-sex classes of southern elephant seals – sub-adults, adult females and adult males.

A synthesis of findings with drawbacks and suggestions for future work is then provided in Chapter 4. The major findings were that myctophid species' stable isotope values were correlated between the PEIs and Îles Kerguelen, albeit with lower absolute values due to baseline depletion at the PEIs. The consistent ecological patterns illustrate the utility of oxidative cleaning and otolith-to-muscle offset values to estimate muscle values of fish from faecal otoliths. These tools could be directed at further characterising prey stable isotope values to enable and improve dietary models of elusive sentinel predators.

To this end, newly established myctophid muscle estimates were introduced to previous modelling efforts of juvenile southern elephant seals (improving model performance), and used to construct novel models for older age-sex classes. Krill (*Thysanoessa* sp. and *Euphausia* spp.) in this study was found to be a predominant resource for juveniles and sub-adult seals, and potentially a substantial component for adult females and adult males. Squid (mainly *Martialia hyadesi* and *Moroteuthopsis longimana*) was low in contribution relative to findings in the literature, and an almost exclusive contribution of fish to adult male southern elephant seals was surprising. However, while frequentist outputs are reported (modes), wide credibility intervals do allow for fluid interpretation of resource use, as many combinations of sources can be utilised (within and between seal individuals) and still fall within modelling intervals. Further, the models would benefit from the further characterisation of PEI-specific prey, as only some of the extraneous sources could be replaced with values from Chapter 2. Squid would be especially useful in this regard, as recent direct sampling indicated a host of species not considered for modelling here. Killer whale modelling was not possible and requires sources with higher $\delta^{13}\text{C}$ values, expected to occur to the north of the PEIs.

224 Bio-sampling is a cost-efficient way to sample prey in isolated ecosystems, and this
225 study showed that prey remains of bio-samplers can be used to parameterise stable
226 isotope mixing models for more elusive sentinel predators. Careful indirect modelling
227 also fills gaps brought about by known biases in traditional, direct diet sampling
228 methods.

CHAPTER 2: Estimating carbon and nitrogen stable isotope values of lanternfish muscle from faecal otoliths

André van Tonder^{1*}, Nico Lübcker^{1,2}, Peter Grønkjær³, Jessica A. Lueders-Dumont⁴, Elvar H. Hallfredsson⁵, P.J. Nico de Bruyn¹.

^{1.} Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag X2320, Hatfield, South Africa

^{2.} Center for Stable Isotopes, University of New Mexico, Albuquerque NM 87131, USA

^{3.} Aquatic Biology, Department of Biology, Aarhus University, 8000 Aarhus C, Denmark

^{4.} Ward Research Laboratory, Department of Geosciences, Princeton University, Guyot Hall, Princeton NJ 08544, USA

^{5.} Deepwater and Cartilaginous Fish, Institute of Marine Research, Fram Centre, P.O. Box 6606, Tromsø, Norway

Running head: Faecal otolith stable isotope values as proxy for myctophid muscle

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

ABSTRACT

Prey-predator interactions are important parameters to consider when using marine predators as bio-indicators of ecosystem health. Characterizing the diet of elusive taxa, however, often require indirect methods. Carbon and nitrogen stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of consumers and potential prey items are used to parameterise stable isotope mixing models used for dietary reconstructions. Lanternfish (Myctophidae) are a substantial component in the diets of various sentinel species. Yet, the paucity in available stable isotope values for myctophid species at localities like the sub-Antarctic Prince Edward Islands (PEIs) precluded/hampered previous dietary modelling efforts. Herein, we assessed the possibility of deriving muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from myctophid otoliths, which were collected from fur seal faeces, after controlling for digestion with oxidative cleaning and applying a myctophid-specific otolith-to-muscle isotope offset to PEI faecal otoliths. The fresh myctophid otolith-to-muscle isotope offset determined and used was $-1.3 \pm 0.4\text{‰}$ for $\delta^{13}\text{C}$ and $1.6 \pm 0.3\text{‰}$ for $\delta^{15}\text{N}$. PEI prey muscle estimates correlated positively ($r = 0.65$ for $\delta^{13}\text{C}$ and $r = 0.90$ for $\delta^{15}\text{N}$) with that of Îles Kerguelen conspecifics, but were lower in absolute values for $\delta^{13}\text{C}$ (mean difference $\pm$ final SD for all four species combined: $-2.8 \pm 1.5\text{‰}$) and $\delta^{15}\text{N}$ (mean difference $\pm$ final SD $-1.4 \pm 2.2\text{‰}$), due to differences in baselines of the respective food webs. Detecting correlated species patterns and the expected PEI baseline depletion suggest that the cleaning and offset method for muscle estimation is viable, despite propagation of measurement uncertainty for correction of otolith oxidative cleaning. The newly established myctophid otolith-muscle $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ can be used in other studies with only otolith tissue available, and, moreover, can be applied to archival faecal otoliths to perform historic dietary reconstructions.

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

KEY WORDS: Stable isotope analysis, otoliths, myctophids, Prince Edward Islands, Antarctic Circumpolar Current, Kerguelen Plateau, bio-sampling

1. INTRODUCTION

Understanding ecosystems' underlying trophic relationships and energy transfer between species are of regional and global significance for, among others, fisheries management and marine conservation (McCormack et al. 2020, Murphy et al. 2021). In marine ecosystems, the movement of predators, and any related changes in diet, forms part of the dynamic management of protected areas under changing natural conditions (Ansorge et al. 2014, Kirkman et al. 2016). The Prince Edward Islands (PEIs) in the Southern Ocean (SO) have been managed as a Special Nature Reserve since 1995. A Marine Protected Area was declared around the islands in 2013, aiming in part to protect the food resources of apex predators on and around the island (Lombard et al. 2007). Marine studies often rely on satellite tracking data and stomach or faecal content analyses of apex predators, as trophic interactions are rarely directly observed (Lea et al. 2002, Waluda et al. 2012, Reisinger et al. 2018a b). In some cases, collecting faecal or stomach contents remains unfeasible (Antonelis Jr et al. 1987, Daneri & Carlini 2002, Best et al. 2010). Indirect methods, such as stable isotope analysis (SIA), are valuable for assessing the trophic ecology of animals (Richoux et al. 2010, Karnovsky et al. 2012, Reisinger et al. 2016, Lübcker et al. 2017, de Lima et al. 2022). The use of SIA for dietary reconstruction exploits the assimilation of ingested material in consumers' tissue; a consumer's stable isotope values are determined by the ingested stable isotope ratios and any mass-based diffractions that occur during metabolism, quantified as trophic discrimination factors (TDFs) (de Niro & Epstein 1978, 1981, Eggers & Jones 2000, Post 2002, McCutchan et al. 2003). Mixing models make use of consumer (predator) and sufficient potential source (prey) stable isotope

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

values (Phillips & Gregg 2003, Moore & Semmens 2008, Parnell et al. 2010, Jackson et al. 2011) as well as appropriate TDFs (Caut et al. 2009, Bond & Diamond 2011) to quantify trophic relationships. Knowing how stable isotopes are distributed in the environment makes isotope values of sources and consumers predictable.

In the SO environmental $\delta^{13}\text{C}$ isotopes vary broadly, and inversely with latitude, due in part to differences in dissolved carbon dioxide concentrations and growth rates of phytoplankton between areas with different water temperatures (Goericke & Fry 1994, MacKenzie et al. 2011, Kurle & McWorther 2017). The baseline $\delta^{15}\text{N}$ is predominantly related to the $\delta^{15}\text{N}$ of upwelled nitrate to the euphotic zone, as well as the nitrogen demand of primary producers (uptake) (Mengesha et al. 1998, Lourey et al. 2003, Graham et al. 2010). Stable isotope values change as material is passed along a food chain, TDFs are $+0.4 \pm 1.3\text{‰}$ (mean $\pm$ standard deviation) for $\delta^{13}\text{C}$ (Peterson & Fry 1987, Post 2002, Layman et al. 2012) and $3.4 \pm 1.0\text{‰}$ for $\delta^{15}\text{N}$ (Minagawa & Wada 1984, Vander Zanden & Rasmussen 2001, Post 2002), but this diffraction is dependent on various factors including species, stress and diet quality (Caut et al. 2009, Pecquerie et al. 2010, Blanchet-Aurigny et al. 2012, Healy et al. 2018). With this information, a SO consumer's stable isotope values can be used to determine its broad latitudinal position and trophic level (Cherel & Hobson 2007, Cherel et al. 2008, Jaeger et al. 2010, Carpenter-Kling et al. 2020, St John Glew et al. 2021).

Fish of the family Myctophidae (lanternfish) occur globally and play an important role in carbon export down the water column (Belcher et al. 2019, Alewijnse et al. 2021) and as links between trophic levels in the SO (Saunders et al. 2019). Myctophids make up a substantial part of the diet of the PEIs' predators (Adams & Klages 1987, Brown & Klages 1987, Reisinger et al. 2018a). The isotopic values of fish species from the PEIs specifically are limited in species richness and with small sample sizes (Kaehler

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

et al. 2000, Bushula et al. 2005, Pakhomov et al. 2006). In cases where SIA has been used to attempt to estimate PEI predators' diets, the stable isotope values of potential fish prey were either taken from neighbouring islands (Lübcker et al. 2017), or could not be included in the mixing model (Reisinger et al. 2016).

Using predators as bio-samplers can overcome the logistical challenges of sampling fish directly, as prey hard remains, such as otoliths, can be retrieved from faecal or dietary samples from, for example, seals with known geographical foraging areas, and used to identify fish prey to species level (Hecht 1987, Williams & McEldowney 1990, Smale et al. 1995). Though otoliths are independently useful for ecological studies with SIA (Grønkjær et al. 2013, von Biela et al. 2015), including estimating metabolic rates with bearing on oceanic carbon fluxes (Alewijnse et al. 2021), they also serve as a proxy for muscle stable isotope values (Vandermyde & Whitledge 2008, Elsdon et al. 2010, Rowell et al. 2010, Grønkjær et al. 2013, Sirot et al. 2017), which are useful for dietary modelling (Cherel et al. 2010, Lübcker et al. 2017). The relationship (offset) between proxy otolith and muscle stable isotope values varies between the above cited studies, making a generalised application of previously published offsets to myctophid otoliths dubious.

This study aimed to obtain a reliable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ otolith-to-muscle offset ($\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$) for myctophids, using paired otolith and muscle values of Antarctic myctophids. The second aim was to apply the offsets to oxidant-cleaned otoliths from faecal samples recovered from fur seal (*Arctocephalus* spp.) colonies at the PEIs, that typically forage from $\sim 43^\circ$ to 55°S and $\sim 40^\circ$ to 50°E , (Reisinger et al. 2018b), to estimate muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of PEI myctophids. A comparison of estimated muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between PEI species, and for PEI species with directly measured

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

conspecific values from Îles Kerguelen (Cherel et al. 2010), gauged the feasibility of obtaining reliable stable isotope values from oxidatively cleaned faecal otoliths.

2. MATERIALS AND METHODS

2.1 Study site and Sample Collection

To establish the otolith-muscle offsets, whole myctophids were caught during pelagic trawls of an ecosystem survey in the Norwegian Antarctic territory of Dronning Maud Land (DML) during March – April 2019 (68° to 69°S, 1° to 7 °E, with depth up to 562m). These myctophids were identified (Gon & Heemstra 1990) and immediately frozen until thawed for dissection. The species used here were *Electrona antarctica* (total length: 65 – 93 mm) and *Gymnoscopelus opisthopterus* (total length: 80 – 101 mm). Multiple otolith pairs (from 16 to 17 fish, Table 1) were pooled randomly and noted carefully before preparation to make up samples with sufficient otolith organic material for SIA. The otoliths were paired with muscle samples dissected from the same fish noted when pooling otoliths (Table 1).

Samples for the SIA of PEI myctophid otolith organic matter were obtained from fur seal faecal samples collected on Marion Island (part of the PEIs, 46°47'S and 37°47'E) from December 2004 to February 2005 (Jan 2005), and from November 2010 to January 2011 (Dec 2010). The faecal sampling occurs systematically at fur seal colonies (Reisinger et al. 2018a). The colonies that were searched, included one Antarctic fur seal (*Arctocephalus gazella*) colony named 'Watertunnel', and two sub-Antarctic fur seal (*A. tropicalis*) colonies named 'Cape Davis' and 'Mixed Pickle'. (But only one *A. tropicalis* colony, Cape Davis, was searched around January 2005.) The faecal samples were stored frozen awaiting preparation, which involves rinsing the sample through sieves under running tap water (de Bruyn et al. 2003). The hard remains were then dried before sorting, which included the retrieval of all otoliths.

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

The otoliths obtained at the PEIs were identified using various guides (Hecht 1987, Gon & Heemstra 1990, Smale et al. 1995) and otoliths were selected for analysis if identified without ambiguity. Species targeted were both important components of fur seal diets (Reisinger et al. 2018a), and with reasonably large otoliths to allow SIA of organic matrices: *E. subaspera*, *G. fraseri*, *G. nicholsi* and *G. piabilis*. Obtaining enough organic material for SIA still required the pooling of otoliths together, which was done with conspecific otoliths, strictly within the same time frame and sampling colony, and within the same faecal sample where possible, to minimise variance.

2.2 Otolith preparation

Following Grønkjær et al. (2013), 0.2 mol.L⁻¹ sodium hydroxide (NaOH) was used for the surface cleaning of the otoliths, done by soaking the samples for an hour, and ultrasonic agitation for one minute. Samples were then rinsed three times with distilled water and dried at 30°C for up to 48h. This concluded preparation of otoliths from DML, which were not digested.

The use of partially digested or faecal otoliths may, however, introduce unwanted variation or bias due to the effect of digestion on otolith organic material, and contamination by faecal material. To remove any remaining faecal matter or organic material affected by digestion, the otoliths from the PEIs were subjected to oxidative cleaning. Otoliths were ground to powder using a pestle and mortar which was cleaned with 70% ethanol between grinds. The otolith powder was then soaked in 1.5 ml sodium hypochlorite (NaOCl), diluted to 10-15% available chlorine. (This was a systematic error; 15 ml was required (Lueders-Dumont et al. 2018). The systematic error was corrected for, see Chapter 2's Supplementary Material and Appendix.) Samples were soaked for 24h and rinsed with distilled water using a centrifuge at 2900 rpm for three minutes. The rinse was conducted three times, drawing off the

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

supernatant and replacing it with new distilled water between rinses. The cleaned samples were then dried at 30°C for up to 48h.

2.3 Extraction of organic matrices from otoliths

The extraction method followed was that of Grønkjær et al. (2013) but, to maximise workable organic material, the soluble and insoluble components of the organic matrices were not split. The otolith aragonite (CaCO_3) was dissolved gradually with hydrochloric acid (HCl), after which the remaining organic material underwent a series of centrifugal ultra-filtrations. The dissolved samples were transferred to Amicon® ultra-4 10K filter devices for centrifugation at 2400 rpm for 17 minutes. Twice, the filters were then topped up with MilliQ water and centrifuged again, decanting the spun-off water each time. The remaining ~70 µl samples containing concentrated organic material were then pipetted into pre-weighed tin boats and frozen for 24h. The frozen samples were freeze-dried for 48h and the remaining organic material weighed on a microbalance before SIA.

2.4 Muscle preparation

Muscle collected during the dissection of DML myctophids were dried at 30°C for 48h. The individual samples were then split twice. The initial split was to allow for one unit of samples to be pooled together the same way the randomised otolith samples were, and the other unit remaining separate. The samples in these two units were then split again to allow for lipids of one group to be extracted with cyclohexane (Cherel et al. 2010), and the other group left with lipids. (I.e., pooled-lipid-extracted; pooled-non-lipid-extracted; individual-lipid-extracted; individual-non-lipid-extracted. See Supplementary Material to Chapter 2.)

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

The samples for lipid extraction were placed in polyethylene tubes with 1.5 ml cyclohexane. The cyclohexane was replaced every second day and treatment continued until the yellow discolouration of the cyclohexane by lipids were no longer visible, indicating complete lipid extraction (typically after three rounds, approximately six days). The samples were then dried at 30°C for 48h. Approximately 0.5 mg of the dried samples, both lipid extracted and non-lipid-extracted, were weighed off into tin cups pending SIA.

2.5 Stable isotope analysis

The otolith and muscle samples were run by elemental analysers at the Bioscience Department at Aarhus University in Denmark (Thermo Electron Corporation Flash EA 1112), and the Stable Isotope Facility at the University of Pretoria (Flash EA 1112, Thermo Finnigan) in South Africa, respectively. The otolith material used to correct for the systematic error was also analysed at the University of Pretoria.

Standard material for the three sets of samples were weighed off and interspersed with the samples. The standards at Aarhus University were Gel A and in-house cod otolith powder. At the University of Pretoria, the standard materials Merck Gel and DL-Valine were used. For the sake of continuity, Gel A from Aarhus University was added to the set of standards at the University of Pretoria for the analysis of otoliths for correction.

Stable isotope values are reported in parts per thousand, per mille (‰), relative to universal standards Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric nitrogen for $\delta^{15}\text{N}$ (see Equation below, where 'X' represents a periodic element, e.g., C, and 'R' denotes the ratio of the element's heavy to light isotopes in the sample or standard material).

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

$$\delta X = \left[\frac{R_{sample}}{R_{standard}} \right] * 1000$$

The mean and standard deviation among standards were as follows: Gel A $\delta^{13}\text{C} = -21.8 \pm 0.6\text{‰}$ and $\delta^{15}\text{N} = 5.4 \pm 0.4\text{‰}$; cod otolith $\delta^{13}\text{C} = -18.0 \pm 0.7\text{‰}$ and $\delta^{15}\text{N} = 11.8 \pm 0.9\text{‰}$; Merck Gel $\delta^{13}\text{C} = -20.3 \pm 0.1\text{‰}$ and $\delta^{15}\text{N} = 7.9 \pm 0.1\text{‰}$; DL-Valine $\delta^{13}\text{C} = -10.6 \pm 0.1\text{‰}$ and $\delta^{15}\text{N} = -6.2 \pm 0.1\text{‰}$. The values for Gel A run at the University of Pretoria for continuity were $\delta^{13}\text{C} = -21.8 \pm 0.2\text{‰}$ and $\delta^{15}\text{N} = 5.5 \pm 0.1\text{‰}$. Duplicates were not run for otolith samples, but the duplicate reproducibility for the pooled muscle samples $\delta^{13}\text{C}$ was $\pm 0.1\text{‰}$ and $\delta^{15}\text{N} \pm 0.2$ ($n = 48$).

2.6 Statistical analyses

2.6.1 Otolith-to-muscle offsets

The DML otolith stable isotope values were obtained by randomly pooling together otolith pairs from individual fish, with the fish that made up the otolith samples noted. Randomisation was to avoid size bias and obtain offsets not specific to size-classes. Obtaining the difference in the stable isotope values from these otolith samples to their respective muscle values was done by physically combining and measuring muscle samples of the fish species in the same way that the otolith samples were compiled, and then subtracting the otolith values from their paired pooled muscle value counterparts. (The pooled muscle measurements fell within one standard deviation of the mathematical averages of individual muscle samples, see Supplementary Material to Chapter 2.) Lipid extraction, however, also affects $\delta^{15}\text{N}$ values (Boecklen et al. 2011), and comparisons of data made use of lipid extracted $\delta^{13}\text{C}$ values and non-lipid-extracted $\delta^{15}\text{N}$ values (Reisinger et al. 2016).

2.6.2 Estimating muscle values

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

The mean of these $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ otolith-to-muscle offsets (Table 1) were then used to estimate the muscle values for the PEI myctophids, for which only faecal otolith samples were available. This was done by adding the mean otolith-to-muscle offset for *E. antarctica* ($n = 6$) to the cleaned and corrected PEI otolith values for all of the PEI species, as a unique depth effect was expected to drive *G. opisthopterus* offset values ($n = 2$, see Discussion). Hereafter, the estimated muscle values could be compared between sampling units of interest. Of particular interest was the stable isotope values of different myctophid species, across the two sampling periods (Table 2) - Jan 2005 and Dec 2010 (species-year), with sampling of Lübcker et al. (2017) and Reisinger et al. (2016) in mind (Chapter 3). This means species' otoliths were pooled from both *A. gazella* and *A. tropicalis* colonies.

2.6.3 Sampling units at the PEIs, and conspecific comparison with Îles Kerguelen (uncertainty not propagated)

The normality and homoscedasticity were tested for the species-year sampling units using Shapiro-Wilk and Levene's tests, respectively. Non-normal data distributions were found for some of the sampling units – *G. fraseri* Jan 2005 $\delta^{15}\text{N}$ ($W = 0.73$, $p < 0.01$) and *G. piabilis* Dec 2010 $\delta^{13}\text{C}$ ($W = 0.80$, $p < 0.01$). Variance was distributed equally across sampling units, $\delta^{13}\text{C}$: $df = 7$, $F = 0.92$, $p = 0.49$; $\delta^{15}\text{N}$: $df = 7$, $F = 0.93$, $p = 0.49$. Non-parametric Kruskal-Wallis χ^2 and post-hoc Nemenyi analyses were used for species comparison across both sampling periods.

Welch tests were used to compare summary statistics between the PEI and Îles Kerguelen conspecifics. As a simple, illustrative assessment of the similarity in relative stable isotope values between species across study sites, correlation between mean

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

stable isotope values of conspecifics in 2005 was tested with Pearson's correlation tests ($n = 4$).

2.6.4 Sampling units at the PEIs, with propagated uncertainty, and conspecific comparison with Îles Kerguelen

Note that the assumption testing and statistical comparisons considered only the analytical standard deviation (SD) associated with sampling units. The uncertainty around individual muscle estimates that has been introduced from various sources, chiefly the systematic cleaning error correction and otolith-to-muscle offset application, is not incorporated yet (Farrance & Frenkel 2012). These uncertainties were propagated to properly account for the cleaning correction, and other sources of uncertainty to the muscle estimates (see Supplementary Material to Chapter 2). The reported final SD is the product of this propagation and its combination with the SD of sampling units (Farrance & Frenkel 2012), and is shown after the final step of estimating sampling units' mean muscle values (Table 2, Figure 2).

For the incorporation of widened uncertainty, and to allow the use of summary statistics, one-way ANOVA and post hoc Tukey analyses were used to compare sampling units within the PEI dataset (Table 2) and Welch tests for conspecifics between the PEIs and Îles Kerguelen (Table 4). Pearson's correlations (see Section 2.6.3) between conspecifics ($n = 4$) did not consider measurand or analytical uncertainty, and is merely used for illustrative purposes.

3. RESULTS

3.1 Otolith-to-muscle offsets

The otolith and muscle $\delta^{13}\text{C}$ values of the DML samples ranged from -25.4‰ to -23.6‰ and from -26.1‰ to -25.1‰, respectively, while the $\delta^{15}\text{N}$ of the otoliths ranged

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

from 7.3‰ to 8.1‰ and the $\delta^{15}\text{N}$ of muscle ranged from 9.1‰ to 10.7‰ (Table 1). The average $\pm$ analytical SD of the otolith-to-muscle offset (muscle minus otolith) for *Electrona antarctica* ($n = 6$) was -1.3 ± 0.4 ‰ for $\delta^{13}\text{C}$ and 1.6 ± 0.3 ‰ for $\delta^{15}\text{N}$. For *Gymnoscopelus opisthopterus* the difference ($n = 2$) was -1.4 ± 0.6 ‰ for $\delta^{13}\text{C}$ and 2.6 ± 0.2 ‰ for $\delta^{15}\text{N}$.

Table 1: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of myctophids sampled near Dronning Maud Land, Antarctica from February to March 2019. Individual (conspecific) fish were randomly assigned to samples, with 16 – 17 fish pooled per sample. The offset (Δ) between muscle and otolith (muscle minus otolith) values for the samples are also shown. (EA = *Electrona antarctica*, GO = *Gymnoscopelus opisthopterus*).

Sample (number of fish)	Fish total length (mean $\pm$ SD, mm)	Fish wet mass (mean $\pm$ SD, g)	Otolith value (‰)		Muscle value (‰)		Otolith-to- muscle offset (‰)	
			$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\Delta^{13}\text{C}$	$\Delta^{15}\text{N}$
EA1 (17)	77 $\pm$ 7	4 $\pm$ 1	-24.5	7.7	-25.9	9.3	-1.4	1.6
EA2 (16)	79 $\pm$ 14	5 $\pm$ 3	-25.0	7.9	-26.0	9.1	-1.0	1.2
EA3 (17)	77 $\pm$ 11	4 $\pm$ 2	-25.4	7.8	-26.1	9.5	-0.7	1.7
EA4 (17)	79 $\pm$ 8	4 $\pm$ 2	-24.6	7.9	-26.0	9.2	-1.4	1.3
EA5 (16)	77 $\pm$ 8	4 $\pm$ 1	-24.0	7.4	-25.7	9.2	-1.7	1.8
EA6 (16)	77 $\pm$ 11	5 $\pm$ 2	-24.3	7.3	-25.8	9.3	-1.5	2.0
Mean $\pm$ SD	78 $\pm$ 10	4 $\pm$ 2	-24.6	7.7 $\pm$	-25.9	9.3 $\pm$	-1.3 $\pm$	1.6 $\pm$
			$\pm$ 0.5	0.2	$\pm$ 0.1	0.1	0.4	0.3
GO1 (17)	88 $\pm$ 8	4 $\pm$ 1	-24.2	8.0	-25.1	10.7	-0.9	2.7
GO2 (17)	91 $\pm$ 10	4 $\pm$ 2	-23.6	8.1	-25.5	10.5	-1.9	2.4
Mean $\pm$ SD	90 $\pm$ 9	4 $\pm$ 2	-23.9	8.1 $\pm$	-25.3	10.6	-1.4 $\pm$	2.6 $\pm$
			$\pm$ 0.4	0.1	$\pm$ 0.3	$\pm$ 0.1	0.6	0.2

3.2 Otolith and estimated muscle stable isotope values

The muscle stable isotope values for the PEIs by species-year sampling units, estimated from values of oxidant-cleaned and corrected faecal otoliths, ranged (mean $\pm$ analytical SD only) from -24.5 ± 0.7 ‰ to -21.8 ± 0.9 ‰ for $\delta^{13}\text{C}$ and from 6.5 ± 0.9 ‰ to 8.3 ± 1.4 ‰ for $\delta^{15}\text{N}$ (Table 2 for summary with final SD). Overall, the myctophids sampled around January 2005 had estimated muscle stable isotope values (mean $\pm$ analytical SD only) of -23.0 ± 1.2 ‰ for $\delta^{13}\text{C}$ and 7.1 ± 1.4 ‰ for $\delta^{15}\text{N}$. Around December 2010 these values were -23.4 ± 0.9 ‰ for $\delta^{13}\text{C}$ and 7.3 ± 1.5 ‰ for $\delta^{15}\text{N}$.

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

Table 2: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of selected sampling units of myctophids at the Prince Edward Islands (PEIs). ES denotes *Electrona subaspera*, GF – *Gymnoscopelus fraseri*, GN – *G. nicholsi*, GP – *G. piabilis*. The sampling periods were around January 2005 and December 2010. The values are of otoliths collected from faecal samples of various fur seal colonies on Marion Island, part of the PEIs. The otoliths were cleaned with NaOCl before measurement. Applying the otolith-to-muscle offset established for myctophids provided estimates of muscle values. The uncertainty, final standard deviation (SD), is the sum of both measurand uncertainty (including cleaning correction and muscle estimation) as well as the standard deviation of sampling units.

Sampling unit	$n \delta^{13}\text{C}; n \delta^{15}\text{N}$	Cleaned and corrected otolith value (‰, mean $\pm$ SD)		Estimated muscle value (‰, mean $\pm$ final SD)	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
ES2005	10; 5	-22.5 ± 1.2	4.9 ± 1.9	-23.7 ± 1.4	6.5 ± 1.9
ES2010	10; 8	-22.2 ± 1.0	6.1 ± 1.9	-23.5 ± 1.3	7.7 ± 1.9
GF2005	14; 10	-22.2 ± 1.1	5.7 ± 2.0	-23.4 ± 1.4	7.3 ± 2.0
GF2010	18; 11	-22.1 ± 1.1	5.5 ± 2.2	-23.4 ± 1.3	7.1 ± 2.2
GN2005	18; 9	-22.1 ± 1.4	6.7 ± 1.9	-23.4 ± 1.6	8.3 ± 1.9
GN2010	15; 14	-23.2 ± 1.1	6.7 ± 2.2	-24.5 ± 1.4	8.3 ± 2.2
GP2005	19; 19	-20.5 ± 1.3	5.1 ± 2.5	-21.8 ± 1.5	6.7 ± 2.5
GP2010	30; 29	-21.7 ± 1.1	5.1 ± 2.2	-22.9 ± 1.4	6.7 ± 2.2

3.3 Sampling units at the PEIs, and conspecific comparison with Îles Kerguelen (uncertainty not propagated)

The species-year sampling units differed significantly for $\delta^{13}\text{C}$ ($\chi^2 = 65.79$, $df = 7$, $p < 0.01$), and for $\delta^{15}\text{N}$ ($\chi^2 = 22.84$, $df = 7$, $p < 0.01$). The differences in $\delta^{13}\text{C}$ were driven by higher values for *G. piabilis* around January 2005 at $-21.8 \pm 0.9\text{‰}$ (mean $\pm$ analytical SD only) relative to all other species ($p \leq 0.01$). Lower $\delta^{13}\text{C}$ values for *G. nicholsi* around December 2010 relative to *G. piabilis* 2010 also contributed significantly ($p < 0.01$). Post-hoc Nemenyi analysis could not determine significant differences between particular pairs of sampling units $\delta^{15}\text{N}$. Post-hoc tests suffer significance penalties when comparing many groups (Kim 2017).

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

324 All species at the PEIs were significantly lower in $\delta^{13}\text{C}$ than Îles Kerguelen conspecifics
325 ($t \leq -13.93$, $df \geq 31.63$, $p < 0.01$), with a mean difference and analytical SD, for all four
326 species combined, of $-2.8 \pm 0.8\text{‰}$. Aside from *E. subaspera* ($t = -0.33$, $df = 13.83$, $p =$
327 0.74), the other (*Gymnoscopelus*) species were also significantly lower in $\delta^{15}\text{N}$ at the
328 PEIs than at Îles Kerguelen ($t \leq -6.55$, $df \geq 27.36$, $p < 0.01$), mean and analytical SD
329 for all species combined = $-1.4 \pm 1.3\text{‰}$, and for combined *Gymnoscopelus* spp. = -1.9
330 $\pm 1.4\text{‰}$.

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

Table 3: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of muscle of myctophids compared between different study sites. KAR – Îles Kerguelen (Cherel et al. 2010). BB-KPL – Kerguelen Plateau (Woods et al. 2020). MI– Marion Island – part of the Prince Edward Islands (PEIs), ~ values estimated from figure, and *Gymnoscopelus* sp. here only include *G. fraseri* and *G. piabilis* and exclude *G. braueri* (Pakhomov et al. 2006). PEIs – this study. DML – Dronning Maud Land (this study). The values for this study (PEIs) were estimated using offsets from organic material of otoliths from fur seal faecal samples. Final standard deviation (SD) is the sum of analytical and measurand uncertainty.

Species	This study (‰, mean $\pm$ analytical SD; final SD)	Location	$\delta^{13}\text{C}$ (‰, mean $\pm$ SD)	$\delta^{15}\text{N}$ (‰, mean $\pm$ SD)
<i>E. antarctica</i>	DML $\delta^{13}\text{C} = -25.9 \pm 0.1$ $\delta^{15}\text{N} = 9.3 \pm 0.1$	KAR	-21.4 ± 0.5	8.9 ± 0.3
		BB-KPL	-25.2 ± 0.7	8.2 ± 0.6
<i>E. subaspera</i>	PEIs $\delta^{13}\text{C} = -23.6 \pm 0.6$; 1.3 $\delta^{15}\text{N} = 7.2 \pm 1.0$; 2.1	KAR	-20.2 ± 0.4 ($t = -10.98$, $df = 23.85$, $p < 0.01$)	7.3 ± 0.3 ($t = -0.17$, $df = 12.46$, $p = 0.87$)
<i>Electrona</i> sp.	PEIs $\delta^{13}\text{C} = -23.6 \pm 1.3$ $\delta^{15}\text{N} = 7.2 \pm 2.1$	MI	~ -24.7	~ 6.6
<i>G. fraseri</i>	PEIs $\delta^{13}\text{C} = -23.4 \pm 0.6$; 1.3 $\delta^{15}\text{N} = 7.2 \pm 1.1$; 2.1	KAR	-21.1 ± 0.4 ($t = -8.94$, $df = 41.22$, $p < 0.01$)	9.0 ± 0.4 ($t = -3.81$, $df = 22.46$, $p < 0.01$)
<i>G. opisthopterus</i>	DML $\delta^{13}\text{C} = -25.3 \pm 0.3$ $\delta^{15}\text{N} = 10.6 \pm 0.1$	BB-KPL	-25.4 ± 0.6	9.2 ± 0.6
<i>G. nicholsi</i>	PEIs $\delta^{13}\text{C} = -23.9 \pm 1.0$; 1.6 $\delta^{15}\text{N} = 8.3 \pm 1.2$; 2.2	KAR	-21.1 ± 0.3 ($t = -9.60$, $df = 37.47$, $p < 0.01$)	10.2 ± 0.5 ($t = -3.95$, $df = 26.06$, $p < 0.01$)
		BB-KPL	-22.7 ± 0.7	9.0 ± 0.8
<i>G. piabilis</i>	PEIs $\delta^{13}\text{C} = -22.5 \pm 0.9$; 1.5 $\delta^{15}\text{N} = 6.7 \pm 2.4$	KAR	-19.8 ± 0.3 ($t = -11.68$, $df = 58.19$, $p < 0.01$)	8.8 ± 0.2 ($t = -5.98$, $df = 49.48$, $p < 0.01$)
<i>Gymnoscopelus</i> sp.	PEIs $\delta^{13}\text{C} = -23.2 \pm 1.6$ $\delta^{15}\text{N} = 7.2 \pm 1.5$; 2.4	MI	~ -20.8 to -24.5	~ 9.0 to 9.5

Positive Pearson's correlations were found between conspecifics of the different sites for both $\delta^{13}\text{C}$ ($r = 0.65$, $t = 1.20$, $df = 2$, $p = 0.35$) and $\delta^{15}\text{N}$ ($r = 0.90$, $t = 3.00$, $df = 2$, p

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

343 = 0.10), though statistical significance could not be established with small sample sizes
344 ($n = 4$).

345 Importantly, the above statistical differences emerge before propagating the
346 uncertainty around, chiefly, otolith cleaning correction. The incorporation of
347 measurement uncertainty was used to make more robust conclusions in light of
348 cleaning correction (see Supplementary Material to Chapter 2).

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

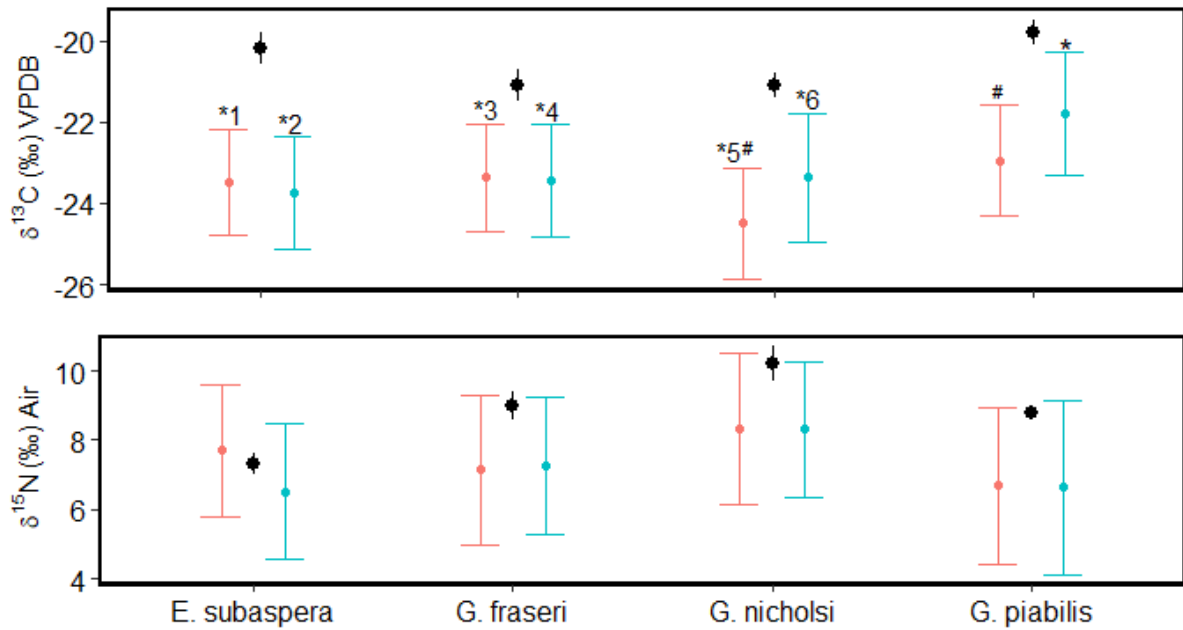


Figure 1: Estimated stable isotope values of muscle values of myctophids (*Electrona* and *Gymnoscopelus*) around the sub-Antarctic Prince Edward Islands around January 2005 (blue) and December 2010 (red). Estimates were obtained by applying an otolith-to-muscle offset to values of oxidant-cleaned otoliths obtained from fur seal faecal samples. The mean and final standard deviations are shown. The final standard deviation includes uncertainty from measurements, correction for insufficient oxidative cleaning, and application of the otolith-to-muscle offset, as well as variance within sampling units. The asterisk (*) indicates that *G. piabilis* 2005 had significantly different $\delta^{13}\text{C}$ values compared other species. There was also a significant $\delta^{13}\text{C}$ difference for *G. nicholsi* 2010 and *G. piabilis* 2010 (#). Black points and bars represent mean and standard deviations of conspecific values from Îles Kerguelen samples in 2005 (Cherel et al. 2010). 3.4 Sampling units at the PEIs, and conspecific comparison with Îles Kerguelen (uncertainty propagated)

ANOVA analysis using summary statistics, to be able to incorporate the widened uncertainty, indicated a persisting significant difference between PEI sampling units for $\delta^{13}\text{C}$ ($F = 4.98$, $df = 7$, $p < 0.01$), driven by higher values for *G. piabilis* 2005 relative to *E. subaspera* 2005 ($p = 0.02$) and 2010 ($p = 0.05$), *G. fraseri* 2005 ($p = 0.04$) and 2010 ($p = 0.02$), *G. nicholsi* 2005 ($p = 0.02$) and 2010 ($p < 0.01$), and between *G. nicholsi* 2010 and *G. piabilis* 2010 ($p = 0.01$). For $\delta^{15}\text{N}$ the propagated measurand

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

uncertainty made sampling units statistically indistinguishable even before post-hoc analyses ($F = 1.33$, $df = 7$, $p = 0.25$, Figure 2).

Comparison between conspecifics from the PEIs (with final SD) and Îles Kerguelen using Welch tests indicated persistent significantly lower $\delta^{13}\text{C}$ values, mean difference for all species combined $\pm$ final SD: $-2.8 \pm 1.5\text{‰}$ (Welch tests: $t \leq -8.94$, $df \geq 23.85$, $p < 0.01$, Table 4). Values for *Gymnoscopelus* species' $\delta^{15}\text{N}$ remained distinguishable between the PEIs and Îles Kerguelen (Welch tests: $t \leq 3.81$, $df \geq 22.46$, $p < 0.01$, Table 4). Mean $\delta^{15}\text{N}$ difference and final SD for all species = $-1.4 \pm 2.2\text{‰}$, and for *Gymnoscopelus* spp. = $-1.9 \pm 2.3\text{‰}$.

4. DISCUSSION

4.1 Otolith-to-muscle offsets

The establishment of otolith-to-muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ offsets allowed for the estimation of muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for myctophids from faecal otoliths. In this case otolith-to-muscle offsets were applied to faecal otoliths from the PEIs for comparison of interspecies differences between 2005 and 2010 within the PEI data, as well as comparison of myctophids from the PEIs with other study sites like Îles Kerguelen (Cherel et al. 2010). These comparisons illustrate the utility of oxidant-cleaned faecal otoliths as proxy for the muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of prey to be used in isotope-based dietary reconstructions, after applying the otolith-to-muscle offsets.

There are differences in otolith-to-muscle isotopic offsets between species, with muscle values relative to otolith values ranging from -10.7‰ (*Fundulus heteroclitus*) to approximately $+1.3\text{‰}$ (*Gadus morhua*) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranging from -3.1‰ (*Salvelinus namaycush*) to $+7.3\text{‰}$ (*Gadus morhua*; see Vandermyde & Whitledge 2008, Elsdon et al. 2010, Rowell et al. 2010, Grønkjær et al. 2013, Sirot et al. 2017,

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

Lueders-Dumont et al. 2020). In this study the differences were $-1.3 \pm 0.4\text{‰}$ for $\delta^{13}\text{C}$ and $1.6 \pm 0.3\text{‰}$ for $\delta^{15}\text{N}$ for *Electrona antarctica* ($n = 6$), and $-1.4 \pm 0.6\text{‰}$ for $\delta^{13}\text{C}$ and $2.6 \pm 0.2\text{‰}$ for $\delta^{15}\text{N}$ of *Gymnoscopelus opisthopterus* ($n = 2$; Table 1).

Findings published after this study's data were generated, indicate differences in otolith and muscle offsets are driven by variation in how otolith size and shape affect metabolic protein degradation at the otolith surface, with small-otolith species showing increased otolith $\delta^{15}\text{N}$ values (Lueders-Dumont et al. 2020). Muscle $\delta^{15}\text{N}$ minus otolith $\delta^{15}\text{N}$ (how the otolith-to-muscle offset is defined in this study, unlike otolith minus muscle in the above cited study) would thus be smaller (less positive or more negative) than for large otolith species. The otoliths for *E. antarctica* and *G. opisthopterus* are smaller and rounder than the PEI species, except *G. fraseri* (Gon & Heemstra 1990). If a size-effect is present in the current data, it is expected that the otolith-to-muscle offset has been underestimated for PEI species, at least for $\delta^{15}\text{N}$ (Lueders-Dumont et al. 2020). Accordingly, the muscle values estimated when applying (adding) this offset to PEI otolith data would also be underestimated.

Given that otolith protein carbon would be degraded along with nitrogen, an otolith size-related effect on offset and estimation is expected for $\delta^{13}\text{C}$ as well. The less positive (more negative) offset values in this study, coupled with opposing effects of oxidative cleaning on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on cleaning load (see Appendix to Chapter 2), provides exploratory evidence that protein degradation would affect $\delta^{13}\text{C}$ in the opposite direction too. The implication is that smaller otoliths have lower $\delta^{13}\text{C}$ values and the offset (muscle minus otolith) is then more positive (less negative). If so, the offset for $\delta^{13}\text{C}$ was overestimated, inflating the respective muscle $\delta^{13}\text{C}$ estimates for large-otolith PEI species.

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

However, the cited correlation between otolith size and otolith-to-muscle offset was established with otoliths from multiple species and ranging to otoliths heavier than 900 mg (Lueders-Dumont et al. 2020). In the PEI data individual otolith mass ranged only from 3 mg to a maximum of 22 mg [mean $\pm$ SD for otolith mass: *E. subaspera* 6 ± 1 mg, *G. fraseri* 6 ± 1 mg; *G. nicholsi* 7 ± 2 mg; *G. piabilis* 15 ± 3 mg (A. van Tonder unpubl. data)]. There was no effect of otolith size on offsets when limiting analysis to within species, where size variation was restricted (Lueders-Dumont et al. 2020), and low size variation in this study is expected to limit size-based $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ variation.

Relative turnover rates between otolith and muscle tissue may contribute to variation in the offset values, as inert otolith tissue captures isotopic profiles across the entire life history, but muscle profiles reflect only the latest depositions (Sirot et al. 2017). Lueders-Dumont et al. (2020) suggest that, in many species, differences in offsets are driven by differences in diet-otolith and diet-muscle TDFs, not changes in feeding ecology, but note that changes in baseline (primary producers in food web) stable isotope values cannot be ignored.

The available samples could not control for the effect of e.g., historical migrations through isotopically distinct water bodies - related to latitude in the SO (Jaeger et al. 2010, Carpenter-Kling et al. 2020, St John Glew et al. 2021). Myctophids, notably including *E. antarctica* and excluding *G. opisthopterus*, generally tend to follow Bergmann's rule, where fish occupy increasingly cold, higher latitude water (with decreased $\delta^{13}\text{C}$) as they grow (Saunders & Tarling 2018). The distinction made was between species that track increasingly cold water with growth, and species that reside in water with uniform temperature. The discrepancy is not reflected in $\delta^{13}\text{C}$ offset values between *E. antarctica* and *G. opisthopterus* in the current study, indicating that the scale of differences in migration between myctophid species is insufficient to cause

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

differences in otolith-to-muscle offset values between species. While the finding does not prove that even sharper changes in baselines would not affect offsets, it lends confidence to the generalisability of the $\delta^{13}\text{C}$ offset established in this study.

Species variation in the $\delta^{15}\text{N}$ offset was consistent within families (Lueders-Dumont et al. 2020), but the higher offset in $\delta^{15}\text{N}$ for *G. opisthopterus* than its con-familial, *E. antarctica*, in this study (Table 1) does not adhere. The greater offset value is an indication that *G. opisthopterus* make rapid increases in dietary $\delta^{15}\text{N}$ with growth relative to *E. antarctica*. The discrepant offsets have emerged already when *G. opisthopterus* reaches 90 mm and *E. subaspera* 78 mm (Table 1), corresponding to three to four and two to three years old, respectively (Saunders et al. 2014, 2015). A surge in dietary $\delta^{15}\text{N}$ would have to occur before *G. opisthopterus* ages three years, after sufficient earlier time given for otoliths to accrete low $\delta^{15}\text{N}$ signals. No relatable surge for *E. subaspera* before age two should occur. Increases in dietary $\delta^{15}\text{N}$ is linked to feeding on larger and/or higher $\delta^{15}\text{N}$ prey, dependent on body size for myctophids (Gurney et al. 2001, Richoux & Froneman 2009, Shreeve et al. 2009, Saunders et al. 2014, 2015). Alternatively, $\delta^{15}\text{N}$ increases are linked to feeding at greater depths, as suspended organic matter (baseline) increases for $\delta^{15}\text{N}$ with depth due to heterotrophic reworking (Hannides et al. 2013, Choy et al. 2015, Romero-Romero et al. 2019). The early growth rate for *E. subaspera* is linear (Greely et al. 1999) and, while *G. opisthopterus* attained slightly greater size (+8 mm) by age two (Saunders et al. 2014, 2015), no information was found that early *G. opisthopterus* grow exponentially (surge) and not linearly (stable). However, *G. opisthopterus* has predominantly been found at depths greater than 400 m and described as a deep-water resident (Hulley & Duhamel 2011, Koubbi et al. 2011, Saunders et al. 2015), a strong suggestion that the depth-effect may be driving a higher $\delta^{15}\text{N}$ offset value. If so,

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

it would mean that early development occurs at the surface or with surface related resources and that older, deep-water stages do not feed on surface-related resources (Choy et al. 2015, Gloeckler et al. 2018). There is evidence that larvae and small juveniles generally occur in shallower water, and that adults occur in deeper water while not undergoing diel migration to feed at the surface (Hulley & Duhamel 2011, Koubbi et al. 2011, Saunders et al. 2015).

Given the putative effect of depth on offset values, care should be taken to avoid applying depth-dependent offsets to vertical migrators and vice versa. All of the PEI study species undergo vertical diel migrations (Saunders et al. 2014, 2015), which mitigates the effect of depth-related population structure (juveniles and adults at different depths) on stable isotope values (Choy et al. 2015, Gloeckler et al. 2018). It is therefore considered most appropriate to only apply the offset values determined for *E. antarctica* to the PEI species in this study. Note, though, that a fast grower in the study species is *G. nicholsi* (Saunders et al. 2015), which means this species could have an offset similar to *G. opisthopterus*, though due to growth and not depth-dependent population structure.

4.2 Estimated muscle SI values at PEI and comparisons with Îles Kerguelen

After establishing the otolith-muscle offsets, the offset was applied to stable isotope values of oxidant-cleaned otoliths at the PEIs, resulting in muscle estimates for PEI myctophids which, despite the far-ranging feeding of fur seal bio-samplers, is still expected to have been sourced >1000 km west of Îles Kerguelen (Wege et al. 2019). Within the PEI dataset, there were statistically significant differences in $\delta^{13}\text{C}$ between species-year sampling units, but $\delta^{15}\text{N}$ could not be differentiated between species in the sampling periods (Table 2, Figure 2). The simple interpretation for $\delta^{13}\text{C}$ would be that *G. piabilis* were generally bio-sampled at higher latitudes, and *G. nicholsi* at a

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

lower latitude in 2010. Myctophids at the PEIs were distinctly lower in $\delta^{13}\text{C}$ and (except for *E. subaspera*) in $\delta^{15}\text{N}$ relative to conspecifics at Îles Kerguelen (Table 4), supposing that PEI myctophids were bio-sampled in higher latitude habitats than their Îles Kerguelen counterparts. Given the wide foraging area of fur seal bio-samplers from the PEIs (Wege et al. 2019), a strictly higher latitude explanation for differences between sites is not expected. However, these structural differences may be strongly affected by differences in sampling design, discussed below.

While changes in stable isotope values in the SO are typically interpreted as north-south ($\delta^{13}\text{C}$) or trophic level ($\delta^{15}\text{N}$) distinctions, the marked differences in stable isotope values of suspended particulate organic matter (POM) in the Antarctic Circumpolar Current (ACC) upstream ($\delta^{13}\text{C} = -23.5 \pm 0.6\text{‰}$; $\delta^{15}\text{N} = -2.8 \pm 0.8\text{‰}$) and downstream ($\delta^{13}\text{C} = -19.2 \pm 2.8\text{‰}$; $\delta^{15}\text{N} = 3.4 \pm 0.7\text{‰}$) of the PEIs may have had considerable effects on the stable isotope values of myctophids (Kaehler et al. 2000, 2006, Bushula et al. 2005, Pakhomov et al. 2006). Due to the introduction of decaying kelp material downstream, there is significant local west-east variation (Kaehler et al. 2000, 2006, Bushula et al. 2005, Pakhomov et al. 2006), which should be appreciated alongside the larger-scale, and more generally considered, north-south variation (Jaeger et al. 2010, Carpenter-Kling et al. 2020, St John Glew et al. 2021). Satellite-linked telemetry data showed some PEI fur seal (*Arctocephalus* spp.) foraging (bio-sampling) directly eastward (Wege et al. 2019), but for a fur seal colony not sampled in this study. Also, it is uncertain how far the kelp-effect persists downstream; the extent of the Kaehler et al. (2006) study was 25 km, whereas fur seals frequently forage at much greater distances (Wege et al. 2019). Therefore, the effect of kelp is not expected to strongly drive local differences between species and sampling periods at the PEIs. Species were positively correlated in their stable isotope values between

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

the PEIs and Îles Kerguelen ($\delta^{13}\text{C}$ $r = 0.65$ and $\delta^{15}\text{N}$ $r = 0.90$). These correlations indicate ecological consistencies of species across sites and that ecological patterns could be established using otolith-to-muscle offset values applied to otolith values. While the relative stable isotope pattern between species at the PEIs is similar to the pattern at Îles Kerguelen, the absolute values are markedly lower at the PEIs.

The larger scale differences in myctophid stable isotope values between the PEIs and Îles Kerguelen (mean $\pm$ final SD, $\delta^{13}\text{C}$: $-2.8 \pm 1.5\text{‰}$, $\delta^{15}\text{N}$: $-1.4 \pm 2.2\text{‰}$) is hypothesised to be affected by factors like upstream kelp effect oceanographic features (bathymetry). The myctophids from Îles Kerguelen were sampled in the lee of the ACC, with the potential for kelp derived POM (Kaehler et al. 2000, 2006, Bushula et al. 2005, Pakhomov et al. 2006), and on the shelf (Cherel et al. 2010), where POM $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expected to be enriched (Trull et al. 2008). The PEIs do not have such an extensive shelf or as high chlorophyll-a concentrations (Perissinotto et al. 2000, de Bruyn et al. 2009). Taken together these two baseline factors are expected to contribute to differences in stable isotope values of consumers between the PEIs and Îles Kerguelen. Differences between PEI-Îles Kerguelen conspecifics was recently also observed for giant warty squid (*Moroteuthopsis longimana*); PEI squid had lower values for $\delta^{13}\text{C}$: $-2.5 \pm 2.1\text{‰}$ and $\delta^{15}\text{N}$: $-2.5 \pm 1.1\text{‰}$ (van Tonder et al. 2021). The pattern of lower PEI values relative to eastern counterparts emerged despite PEI bio-samplers, grey-headed albatrosses, *Thalassarche chrysostoma* (van Tonder et al. 2021) and fur seals (this study), foraging well within the sub-Antarctic zone during the sampling periods (Nel et al. 2000, 2001, Wege et al. 2019). Sub-Antarctic bio-sampling rules out a strictly more southerly (Antarctic zone) foraging of PEI predators as an explanation for the lower isotopic values (van Tonder et al. 2021), in favour of kelp-and/or oceanography affected baseline differences between island groups.

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

The hypothesised effect of structural differences in baseline values between study sites emerged in myctophids for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, even after propagating uncertainty in correction of systematic otolith cleaning. Positive conspecific correlations suggest that species patterns are consistent across sites. (Sufficient) oxidative cleaning of partially digested, faecal or fossil otoliths, in conjunction with the provided otolith-to-muscle offsets, are considered reliable tools for ecological studies using SIA. Given the ecological importance and wide extent of myctophids (Irigoien et al. 2014, Saunders et al. 2019), these tools are also widely applicable. The observed differences in consumer stable isotope values between study sites has important implications for the use of prey values of sites other than the PEIs, e.g., Îles Kerguelen, for estimating contributions to the diet of higher consumers using stable isotope mixing models (Lübcker et al. 2017). These newly estimated myctophid values can be used to revisit such models and may provide sufficient source values for dietary models that have been precluded for lack of sufficient potential prey sources (Reisinger et al. 2016).

5. CONCLUSION

This study established the otolith-to-muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ offsets for myctophids, which we subsequently used to estimate the prey muscle isotope values from faecal otoliths recovered from fur seal (*Arctocephalus* spp.) scat at PEIs. While direct sampling of myctophids is logistically challenging, the importance of broadening the species for which offsets like these are established, and with greater sample sizes, would increase the confidence with which offsets can be applied to sedimentary or faecal otoliths to estimate muscle values. Additionally, the drivers of differences in offsets between species (ecology or physiology) could also be further explored (as in Lueders-Dumont et al. 2020) to aid in making informed estimations using offsets, in the same way that understanding differences in ecology of this study's DML species

Chapter 2: Faecal otolith stable isotope values as proxy for myctophid muscle

prompted discretion in applying the established offsets to PEI otoliths. Prey isotope values around the PEIs are sparse, but are required to construct accurate isotope-based dietary models of sentinel marine predators. We demonstrated that oxidant-cleaned myctophid otoliths and a myctophid specific otolith-to-muscle offset allow for realistic estimates of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of myctophid muscle. Moreover, faecal otoliths can be readily and cost-effectively bio-sampled at high resolution for decades, which is not feasible using ship-based sampling strategies in the SO. Additionally, archived faecal or fossil myctophid otoliths can now be used to estimate historical muscle values to assess past myctophid ecology and its importance in food webs. Inferring trophic relationships between predators and myctophids (with stable isotope mixing models) would aid in understanding predator monitoring data used to meet conservation objectives.

CHAPTER 3: Use of hard faecal prey remains to contribute to isotope-based dietary models of sentinel marine predators

André van Tonder^{1*}, Bianca Coulson¹, Nico Lübcker^{1,2}, Ryan R. Reisinger^{1,3}, P.J. Nico de Bruyn¹

^{1.} Mammal Research Institute, Department of Zoology and Entomology, University of Pretoria, Private Bag X2320, Hatfield, South Africa

^{2.} Center for Stable Isotopes, University of New Mexico, Albuquerque NM 87131, USA

^{3.} National Oceanography Centre, University of Southampton Waterfront Campus SO14 3ZH, England

Running head: Revised and novel stable isotope dietary models at Marion Island

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

ABSTRACT

The accuracy of indirect approaches, such as stable isotope analysis, to study the diet of consumers hinge on the availability of stable isotope (SI) values of potential prey. Paucity of such data in remote ecosystems may lead to erroneous isotope-based dietary reconstructions of study species. Herein, we used novel Prince Edward Island (PEI) specific source stable isotope values for sub-Antarctic myctophids (lanternfish) to revise previous dietary modelling attempts of juvenile southern elephant seals, *Mirounga leonina*, and construct novel models for other classes. Prey isotope values were previously sourced from the neighbouring Îles Kerguelen and Îles Crozet. The contribution (mode [95% CI]) of krill to the diet of juvenile southern elephant seals was revised from 78% to 58% [18% - 100%], with improved modelling performance. Fish and squid contributed 26% [0% – 83%] and 9% [0% - 28%], respectively. Modelled contribution to sub-adults: krill 35% [0% - 65%], fish 20% [0% - 87%], squid 25% [0% - 87%]. For adult females: krill 24% [2% - 72%], fish 20% [0% - 96%] and squid 29% [2% - 62%]. Fish was predominant for adult males: 88% [22% - 100%], squid 25% [1% - 65%] and krill 19% [0% - 28%]. Findings glean new information regarding squid and fish importance to older age-sex classes, which may be confounded by biases using direct sampling. Added PEI-specific source (bio)sampling is still required. The available source values were too ^{13}C depleted to contribute to a dietary model of killer whales, *Orcinus orca*, suggesting a need for more northerly sampled source values. Extended (bio)sampling will also improve modelling for southern elephant seal consumers.

KEY WORDS: Mixing models, source values, myctophids, southern elephant seals, killer whales

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

1. INTRODUCTION

Indirect methods, such as carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis (SIA), are increasingly used to describe the diet of consumers with cryptic life histories. The underlying principle is that isotopic values of consumers' tissue reflect the stable isotope values of their diet in a predictable manner, therefore enabling retrospective dietary reconstructions (de Niro & Epstein 1978, 1981, Eggers & Jones 2000). By comparing predator (consumer) stable isotope values with those of potential prey (source) values, the relative importance of different prey items can be estimated (Phillips & Gregg 2003, Moore & Semmens 2008, Parnell et al. 2010, Jackson et al. 2011). Given the various sources of variation for stable isotope values, the utility of these values for dietary models is constrained by the availability of accurate source values and cautious knowledge of various discrimination factors (Caut et al. 2009, Bond & Diamond 2011).

Trophic discrimination occurs where stable isotope ratios ($^{13}\text{C}:^{12}\text{C}$ and $^{15}\text{N}:^{14}\text{N}$) diffract between food and consumer, as metabolic reactions energetically select for lighter or heavier isotopes, accumulating along food chains. The trophic discrimination of $\delta^{13}\text{C}$ is $0.4 \pm 1.3\text{‰}$ (mean $\pm$ standard deviation (SD)) (Peterson & Fry 1987, Post 2002, Layman et al. 2012). The trophic discrimination of $\delta^{15}\text{N}$ is more pronounced, $3.4 \pm 1.0\text{‰}$ (Post 2002) due to the preferential excretion of the lighter ^{14}N isotope which leaves the tissue of the consumer enriched in ^{15}N with every trophic level increment (Minagawa & Wada 1984, Peterson & Fry 1987, Layman et al. 2012). Trophic discrimination factors (TDFs) represent quantified, predictable diffractions from diets to consumers, but vary between different consumers, between tissues of a particular consumer, and with physiological condition (Caut et al. 2009, Pecquerie et al. 2010, Blanchet-Aurigny et al. 2012, Healy et al. 2018).

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

Variation at the base of food webs (primary producers) may distinguish communities even before investigating trophic discrimination. In the Southern Ocean (SO) ecosystem, $\delta^{13}\text{C}$ gradually increases from south to north, ~ 0.21 to 0.31‰ per degree latitude (Jaeger et al. 2010, Carpenter-Kling et al. 2020, St John Glew et al. 2021). This is owed to differences in bicarbonate dissolution and variable phytoplankton growth rates as a function of water temperature (MacKenzie et al. 2011). This latitudinal variation allows researchers to establish the broad latitudinal position of the base of the food web for a given consumer and, therefore, the foraging latitude of consumers themselves (Guerreiro et al. 2015). A latitudinal effect for $\delta^{15}\text{N}$ also exists in the SO, ~ 0.12 to 0.20‰ per degree latitude (Jaeger et al. 2010, Carpenter-Kling et al. 2020, St John Glew et al. 2021). Other factors expected to influence the baseline stable isotope values are bathymetry and the proximity to land (Kaehler et al. 2000, 2006, Trull et al. 2008, van Tonder et al. 2021).

Isotope-based dietary reconstructions require prey sampling at multiple foraging locations at different times and should include all potential prey types to be informative, which can be challenging and costly. At the sub-Antarctic Prince Edward Islands (PEIs), dietary estimation of juvenile southern elephant seals (*Mirounga leonina*) found that krill was an important resource during the seals' first year at sea (Lübcker et al. 2017). At the time, stable isotope values for fish and most cephalopods around the PEIs were unavailable, and prey isotope values from the nearest Îles Kerguelen ($\sim 2300\text{km}$ east-south-east) and Îles Crozet ($\sim 1000\text{km}$ east-north-east) (Cherel et al. 2010, Guerreiro et al. 2015) were used to parameterise the models. The expectation was that the prey stable isotope values would be comparable to the PEIs, due to the sites being at a similar latitude. However, prey isotope values are lower at the PEIs than Îles Kerguelen for myctophid fish, $\Delta^{13}\text{C}$: $-2.7 \pm 1.9\text{‰}$ and $\Delta^{15}\text{N}$: $-1.5 \pm 2.3\text{‰}$, and

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

for *Moroteuthopsis longimana* squid $\Delta^{13}\text{C}$: $-2.5 \pm 2.1\text{‰}$ and $\Delta^{15}\text{N}$: $-2.5 \pm 1.1\text{‰}$ (Chapter 2, Cherel et al. 2010, Guerreiro et al. 2015, van Tonder et al. 2021). It was suggested (Chapter 2) that these differences arose from sampling in the lee of the Îles Kerguelen group, and on a shelf, factors expected to enrich $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Kaehler et al. 2000, 2006, Trull et al. 2008).

Another modelling attempt, for killer whales (*Orcinus orca*), was precluded due to the lack of source values that would sufficiently contribute to the consumer values (Reisinger et al. 2016). Addressing the lack of mid-trophic level source values (fish and squid) was recommended. Stable isotope values for lanternfish (family Myctophidae), a widely consumed resource, were recently estimated for the PEIs (Chapter 2).

The aim of this study is to apply the novel source information (Chapter 2) to the aforementioned modelling efforts (Reisinger et al. 2016, Lübcker et al. 2017), in an attempt to improve the accuracy of previous isotope-based dietary attempts for juvenile southern elephant seals and killer whales at the PEIs. Additional, novel models for other local top predators, like different age-sex classes of southern elephant seals, were also generated using the novel information. In addition to advancing the characterisation of PEI predators' diets, and in conjunction with preceding work in Chapter 2 of this dissertation, this part of the study aims to illustrate the utility of fish source values estimated from faecal otoliths for modelling purposes more generally. The use of faecal remains for source value estimation aids modelling efforts as remains are often easier and cheaper to obtain than fresh samples. While muscle estimates from squid beaks have been used before (e.g., Lübcker et al. 2017),

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

to my knowledge, this source estimation from dietary remains is novel for faecal otoliths.

2. MATERIALS AND METHODS

2.1 Study site as used by bio-samplers, potential prey and model-consumers

The PEIs (46° 47' S and 37° 47' E) are a pair of volcanic islands rising with a steep bathymetry and, other than a shallow saddle between the two islands, lacks an extensive shelf (Ansorge et al. 2012), so predators feed almost exclusively in the pelagic zone. It is situated within the Antarctic Circumpolar Current, to the north of the Antarctic Polar Front (Figure 3). Local consumers have varied ranges (Reisinger et al. 2018b), which leads to a potentially broad geographical bio-sampling area. The extent of overlap between bio-samplers, consumers under study and their potential prey needs to be kept in mind when modelling diets. In Reisinger et al. (2018b); fur seals (*Arctocephalus* spp.), bio-samplers of PEI myctophids (A. van Tonder, unpubl. data Chapter 2) and potential prey to killer whales (*Orcinus orca*, Table 12 in Supplementary Material to Chapter 3), and crested penguins (*Eudyptes* spp.), potential prey to killer whales, were localised at ~43° to 55° S and ~40° to 50° E in summers. Grey-headed albatrosses (*Thalassarche chrysostoma*), bio-samplers for PEI *Moroteuthopsis longimana* (van Tonder et al. 2021), ranged from ~40° to 53° S and ~15° to 70° E. Available summer tracking data for killer whales, a consumer for dietary modelling herein, indicated an extended northward range ~34° to 50° S and ~43° to 47° E. Southern elephant seals (*Mirounga leonina*), another consumer under consideration had much wider ranges, both in summer and winter, extending from ~40° to 69° S and ~10° W to 50° E.

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

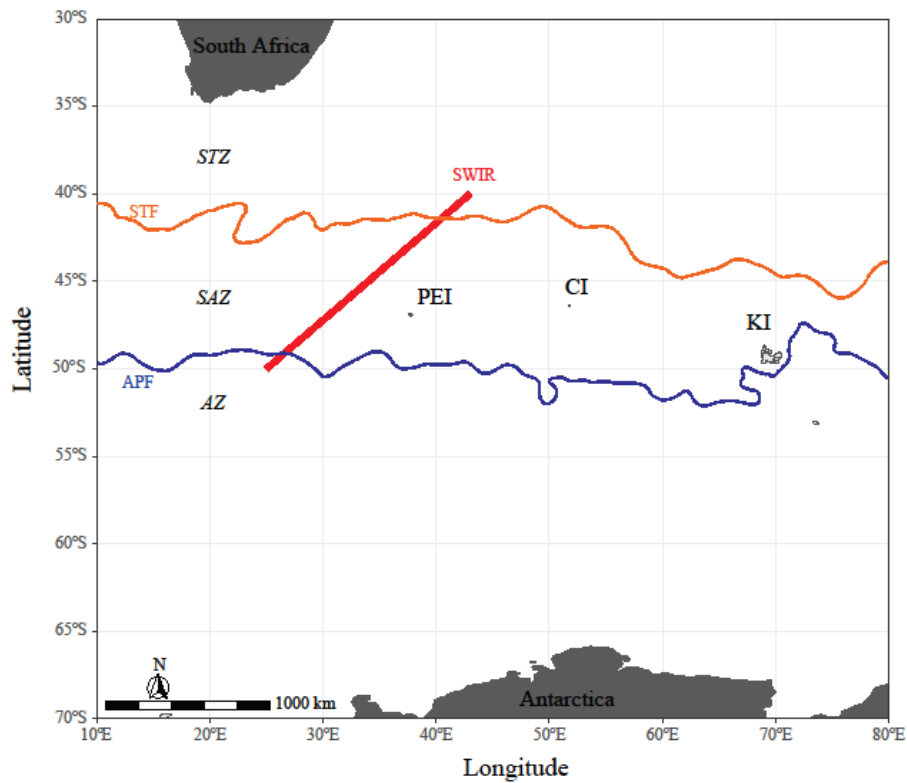


Figure 2: Representation of frontal and bathymetric features in the Indian Ocean sector of the Southern Ocean. STZ = Sub-Tropical Zone; STF = Sub-Tropical Front; SAZ = Sub-Antarctic Zone; APF = Antarctic Polar Front; AZ = Antarctic Zone. SWIR = Southwest Indian Ridge and archipelagos shown are Prince Edward Islands (PEI), Îles Crozet (CI) and Îles Kerguelen (KI). Frontal data is available online (Park & Durand 2019, Park et al. 2019).

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

2.2 Isotopic values of consumers and potential prey

The isotope values of juvenile (age <15 months) southern elephant seals at the PEIs used in Lübcker et al. (2017)) were obtained from serially sampled whiskers (2.0 ± 0.3 mm resolution). We used the same isotope data captured in the portion of the whiskers that represented at-sea foraging only, excluding nursing or fasting (Lübcker et al. 2017, 2020). The number of segments for juveniles ranged from two to 15 per individual, totalling 107 segments representing nine males and five females.

Consumer values for other southern elephant seal age-sex classes were obtained from whiskers sampled similarly to that of juveniles. Chronological whisker bulk tissue isotopes values were obtained for each 2.0 ± 0.3 mm whisker section, based on the methods and instrumentation discussed in Lübcker et al. (2017), using known individuals marked with colour-coded plastic Dal 008 Jumbotags®, Dalton Supplies Ltd. (de Bruyn et al. 2008, Oosthuizen et al. 2010, Pistorius et al. 2011). Whisker sampling required immobilisation with ketamine hydrochloride (Bester 1988). Isotope data were selected that fasting (increased $\delta^{15}\text{N}$) was excluded (Lübcker et al. 2020).

Samples from nine sub-adult seals were used in the analysis, seven female (data here range from two to four years old, that have not bred) and two males (these data only include two-year-old non-breeders). Sub-adult data ranged in number of segments from three to 31 per individual, totalling 155.

Adult females analysed ($n = 16$) were four to 13 years old in the data set used and have bred, and analysed adult male seals ($n = 4$) were six to 12 years old that have bred. The number of segments for the adult groups, respectively, ranged from 11 to 87 per individual totalling 411, and from 12 to 28 totalling 75.

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

Analyses were performed using a Thermo Scientific Flash 1112 Series elemental analyzer (Thermo™, Thermo Fisher Scientific, Bremen, Germany) coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (EA-IRMS; Thermo Finnigan, Bremen, Germany) at the Stable Isotope Facility, University of Pretoria, South Africa.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed in parts per mil (‰) relative to international standards Vienna Pee Dee Belemnite for $\delta^{13}\text{C}$ and atmosphere (air) for $\delta^{15}\text{N}$. See below where R is the ratio of the abundance of the heavy to light isotope in the sample and standards.

$$\delta X = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} \right] * 1000$$

Standard reproducibility (Merck Gel) was $<0.2\text{‰}$ and sample reproducibility 0.1‰ for $\delta^{13}\text{C}$ and 0.3‰ for $\delta^{15}\text{N}$. The standard reproducibility (Merck Gel and DL Valine) for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in analyses of older age-classes was 0.1‰ and 0.3‰ , respectively.

The source (prey) values utilised were the same as Lübcker et al. (2017), with the addition of more extensive *Moroteuthopsis longimana* $\delta^{13}\text{C}$ ($-24.8 \pm 1.0\text{‰}$) and $\delta^{15}\text{N}$ ($10.2 \pm 0.7\text{‰}$) values ($n = 40$) obtained from van Tonder et al. (2021). The newly estimated myctophid muscle values for the PEIs around January 2005 were added, *Electrona subaspera* ($n = 5$), *Gymnoscopelus fraseri* ($n = 10$), *G. nicholsi* ($n = 9$), and *G. piabilis* ($n = 19$), with $\delta^{13}\text{C}$ ranging from $-23.7 \pm 1.4\text{‰}$ to $-21.8 \pm 1.5\text{‰}$ and $\delta^{15}\text{N}$ from $6.5 \pm 2.5\text{‰}$ to $8.3 \pm 1.9\text{‰}$ (Chapter 2).

The consumer values for 23 killer whales (five adult males, 10 adult females and eight sub-adults) are the stable isotope values of skin sampled at the PEIs, having removed blubber and accounted for the effect of lipids on skin $\delta^{13}\text{C}$ (Reisinger et al. 2016). The

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

number of samples ranged from one to three (different events) per individual and totalled 30, taken mostly during the summers of 2011 to 2013.

The values were obtained using a Thermo-Finnigan Delta V Advantage (Thermo Fischer Scientific) mass spectrometer, and Costech ECS 4010 elemental analyser, at the Stable Isotope Biogeochemistry Laboratory, Durham University. Analytical reproducibility for standards was 0.1‰ and <0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The source values for this model are the same as in Reisinger et al. (2016), and includes myctophid muscle values for the PEIs around December 2010; *E. subaspera* ($n = 8$), *G. fraseri* ($n = 11$), *G. nicholsi* ($n = 14$), and *G. piabilis* ($n = 29$), with $\delta^{13}\text{C}$ ranging from $-24.5 \pm 1.4\text{‰}$ to $-22.9 \pm 1.4\text{‰}$ and $\delta^{15}\text{N}$ from $6.7 \pm 2.2\text{‰}$ to $8.3 \pm 2.2\text{‰}$ (Chapter 2).

2.3 Dietary reconstruction

The dietary reconstruction was conducted using the Stable Isotope Analysis in R (SIAR) package (Parnell 2008, Parnell et al. 2010, R Core Team 2020, RStudio Team 2020). The TDFs used were the same as in the original publications; for juvenile southern elephant seals the diet to whisker discrimination of harp seals (*Pagophilus groenlandicus*) was used, $\Delta^{13}\text{C}$: $3.2 \pm 0.9\text{‰}$ and $\Delta^{15}\text{N}$: $2.8 \pm 0.5\text{‰}$ (Hobson et al. 1996, Lübcker et al. 2017), and for killer whales, the diet to skin discrimination values of bottlenose dolphins (*Tursiops truncatus*), $\Delta^{13}\text{C}$: $1.1 \pm 0.6\text{‰}$ and $\Delta^{15}\text{N}$: $2.1 \pm 0.5\text{‰}$ (Browning et al. 2014, Reisinger et al. 2016). An additional 0.3‰ was added to the uncertainty (SD) of both TDFs to account for the use of different seal and cetacean species' TDFs (Lübcker et al. 2017).

Prey were selected firstly by determining the limits of prey isotope values that could contribute to the consumers' isotope values based on the TDF of consumers (see Phillips et al. 2014). The resulting bounds were used to exclude prey species for which

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

the mean stable isotope values did not fall within the bounds (uncertainty wasn't considered). In the case of juvenile southern elephant seals, species were included if their TDF-adjusted means were in the $\delta^{13}\text{C}$ range of -24.5‰ to -16.2‰, and in the $\delta^{15}\text{N}$ range of 3.7‰ to 12.8‰, sub-adults $\delta^{13}\text{C}$ -25.6‰ to -14.9‰ and $\delta^{15}\text{N}$ 6.3‰ to 15.1‰, adult females $\delta^{13}\text{C}$ -25.4‰ to -13.9‰ and $\delta^{15}\text{N}$ 5.7‰ to 15.5‰, adult males $\delta^{13}\text{C}$ -22.8‰ to -13.6‰ and $\delta^{15}\text{N}$ 7.8‰ to 16.7‰. For killer whales, the included values could range from -20.9‰ to -16.9‰ for $\delta^{13}\text{C}$, and from 9.3‰ to 15.6‰ for $\delta^{15}\text{N}$.

Potential source values were then clustered to reduce the modelling parameters, following cited recommendations (Phillips et al. 2014). The source clusters were determined using *hclust* in R by agglomerative, average linkage clustering based on Euclidean distances between individual sources. The optimal number of clusters was determined using the *k*-means elbow method, and taken as the number of clusters where total within cluster sum of squares start to stabilise (999 permutations). The distinctness of clusters at the optima were confirmed with descriptive ANOVA and post hoc Tukey tests, able to use summary statistics (combined means, SDs and sample sizes) for sources in each cluster. Clusters were considered distinct if either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ differed significantly at a 95% confidence level.

The proportional contributions estimated for clusters were the *siar* products of 500000 iterations, with a burn-in of 50000, resulting in sets of posterior values for each prey cluster. No thinning was used, so the distributions contain 450000 posteriors. The modal value of these posteriors was considered the proportional contribution of the prey cluster to the consumer's diet, couched within 95% credibility intervals. Modal residual error, measured as SD of predictions across seal or killer whale individuals, is reported as an indicator of model performance. Wider residual error implies poorer

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

model fit, and performance can be compared to the measurement error of standards.

All analyses were performed using R in the RStudio interface (R Core Team 2020,

RStudio Team 2020) at a significance level α of 0.05.

3. RESULTS

The dietary model for juvenile southern elephant seals (*Mirounga leonina*) consisted

of five clusters of prey (Table 5, Table 10 in Supplementary Material to Chapter 3),

which were statistically distinct (ANOVA); for $\delta^{13}\text{C}$: $F = 72.14$, $df = 4$, $p < 0.01$ and for

$\delta^{15}\text{N}$: $F = 106.36$, $df = 4$, $p < 0.01$. Post hoc Tukey analyses could not distinguish

clusters 3 and 4 based on $\delta^{13}\text{C}$ ($p = 0.59$), but this was resolved for $\delta^{15}\text{N}$ ($p < 0.01$).

The cluster contributions ranged from 5% for cluster 1 to 32% for cluster 5, with

credibility intervals ranging from 25% to 53%, see Table 5. The two krill clusters'

modes (clusters 4 and 5) made up a majority 58% of diets, but could collectively

contribute minimum 18% and maximum 100% based on summed lower and upper

credibility values. Fish made up 26% based on the summed modes of clusters 1, 2

and 3 (to a maximum summed upper credibility value of 83%), and squid 9%

(maximum 28% if cluster 3 is occupied entirely by the squid *Martialia hyadesi* and no

fish). Model performance was adequate, with SD of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (0.1‰ for

either tracer) being equal or lower than standard and sample reproducibility.

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

Table 4: Proportional contributions of clusters of prey to the diets of juvenile (J), sub-adult (SA) and adult female (AF) southern elephant seals (*Mirounga leonina*). Proportional contributions were determined using the R package *siar* using stable isotope values of elephant seal whiskers sampled at the sub-Antarctic Prince Edward Islands. Prey values were sourced from the literature and means $\pm$ standard deviations (SD) are reported here. The constituent species for each cluster and its reference can be found in Table 10 of the Supplementary Material to Chapter 3. SDs for the isotopic tracers relative to measurement reproducibility for standards are a measure of model performance, with lower variability indicative of better performance. *M. hya* = *Martialia hyadesi*; *Thy. sp.* = *Thysanoessa* sp. and *M. lon* = *Moroteuthopsis longimana*. The model for adult male southern elephant seals (AM) had different cluster constituents as other classes (Table 11).

Cluster	Type	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	J	SA	AF
1	Fish	60	-20.9 ± 0.7	8.9 ± 0.8	5% [0%-25%]	5% [0%-35%]	1% [0%-53%]
2	Fish	24	-23.5 ± 1.5	7.5 ± 2.1	12% [0%-30%]	7% [0%-25%]	7% [0%-19%]
3	Fish + <i>M.hya</i>	74	-22.2 ± 0.9	8.1 ± 1.8	9% [0%-28%]	8% [0%-27%]	12% [0%-24%]
4	Krill	37	-22.5 ± 0.6	3.5 ± 0.6	26% [8%-48%]	14% [0%-29%]	15% [2%-29%]
5	Krill (<i>Thy. sp.</i>)	18	-24.3 ± 0.3	5.6 ± 0.8	32% [10%-63%]	21% [0%-36%]	9% [0%-43%]
6	Squid (<i>M. lon</i>)	40	-24.8 ± 1.0	10.2 ± 0.7	NA	17% [3%-39%]	17% [2%-38%]
SD $\delta^{13}\text{C}$ (‰)					0.1	<0.1	<0.1
SD $\delta^{15}\text{N}$ (‰)					0.1	0.1	<0.1
Cluster	Type	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	AM		
1	Fish	60	-20.9 ± 0.7	8.9 ± 0.8	48% [22%-66%]		
2	Fish + <i>Thy. sp.</i>	42	-23.8 ± 1.2	6.7 ± 1.9	19% [0%-28%]		
3	Fish + <i>M.hya</i>	74	-22.2 ± 0.9	8.1 ± 1.8	21% [1%-37%]		
4	Squid	60	-21.5 ± 1.0	13.4 ± 0.7	3% [0%-14%]		
5	Squid (<i>M. lon</i>)	40	-24.8 ± 1.0	10.2 ± 0.7	1% [0%-14%]		
SD $\delta^{13}\text{C}$ (‰)					<0.1		
SD $\delta^{15}\text{N}$ (‰)					<0.1		

Sub-adult elephant seal modelling made use of six clusters, after adding a cluster for the squid *Moroteuthopsis longimana* (Table 5, Table 10 in Supplementary Material to Chapter 3), and ANOVA could distinguish the clusters; $\delta^{13}\text{C}$: $F = 111.84$, $df = 5$, $p < 0.01$ and for $\delta^{15}\text{N}$: $F = 130.98$, $df = 5$, $p < 0.01$. Post hoc Tukey analyses could not distinguish clusters 2 and 5 ($p = 0.06$), 3 and 4 ($p = 0.71$) or 5 and 6 ($p = 0.27$) based on $\delta^{13}\text{C}$, but resolved these pairs for $\delta^{15}\text{N}$ ($p < 0.01$ each). Cluster contributions ranged

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

from 5% for cluster 1 to 21% for cluster 5, with credibility intervals spanning 25% (cluster 1) to 36% (clusters 5 and 6), see Table 5. Krill could collectively contribute the modal majority 35% (sum clusters 4 and 5) with summed credibility intervals 0% to 65%. Squid modes (clusters 3 and 6, if cluster 3 exclusively contained *M. hyadesi*) sum to 25% with a collective credibility interval from 3% to 66%. Fish (clusters 1 to 3) could contribute 20% with summed intervals of 0% to 87%, with the theoretical assumption that cluster 3 contains no *M. hyadesi* squid. Model performance was adequate with SD for both tracers $\leq 0.1\%$.

The same six clusters for sub-adults were used to model adult females and the clusters had the same statistical comparability. Proportional contributions ranged from 1% (cluster 1) to 17% (cluster 6), couched in credibility intervals spanning 19% (cluster 2) to 53% (cluster 1), see Table 5. Modal contribution of squid (a *M. hyadesi* exclusive cluster 3 and *M. longimana* cluster 6) summed to majority 29% with a collective credibility interval from 2% to 62%. Krill summed to 24% (clusters 4 and 5), collective credibility interval 2% to 72%. Fish (cluster 1 to a zero-*M. hyadesi* cluster 3) could contribute 20% of adult female diets, though the collective credibility interval is 0% to 96%. Model performance indicated modal residual error of $<0.1\%$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

Five clusters were considered optimal for the adult male elephant seal modelling but, because new sources were considered, the make-up of the clusters are different from the other age-sex classes (Table 5 main, Table 11 in Supplementary Material to Chapter 3). ANOVA revealed the clusters as distinguishable; $\delta^{13}\text{C}$: $F = 136.22$, $df = 4$, $p < 0.01$ and $\delta^{15}\text{N}$: $F = 353.08$, $df = 4$, $p < 0.01$. Post hoc Tukey analyses indicated that all cluster pairs could be discerned for both tracers (all $p < 0.01$). The clusters'

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

297 proportional contributions ranged from 1% (cluster 5) to 48% (cluster 1), with credibility
298 intervals ranging in width from 14% (clusters 4 and 5) to 44% (cluster 1), see Table 5.
299 Adult males were, by mode, overwhelmingly piscivorous; clusters 1, a *Thysanoessa*
300 spp. free cluster 2, and a *M. hyadesi* free cluster 3 sum to 88% with collective credibility
301 at 23% to 100%. Squid, clusters 4, 5 and a fish-free cluster 3, sum to 25% with
302 collective 0.05 credibility in 1% to 65% contributions. Krill could contribute 19% [0% -
303 28%] if cluster 2 contains only *Thysanoessa* spp. and no fish. Modal residual error was
304 $\leq 0.1\text{‰}$ for both tracers.

305

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

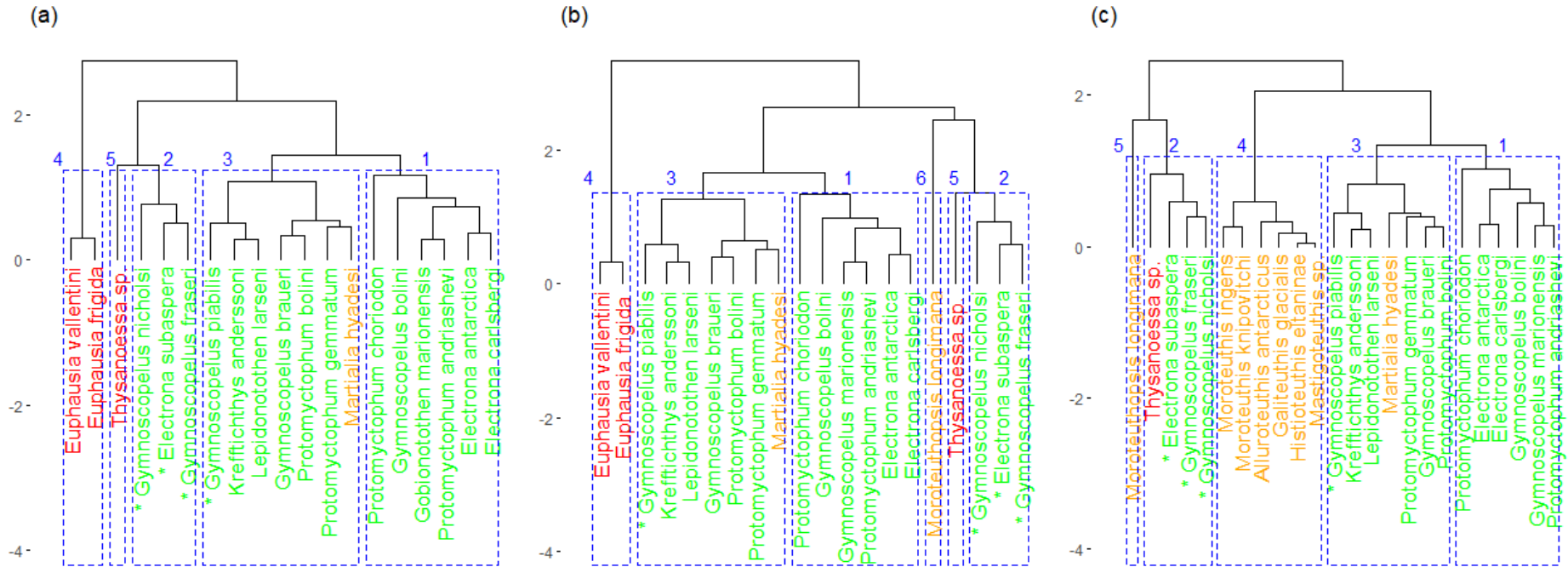


Figure 3: Dendrograms indicating the composition of clusters for (a) juvenile, (b) sub-adults and adult female and (c) adult male southern elephant seals (*Mirounga leonina*). Clusters in (a) are the same as (b), aside from the absence of *Moroteuthopsis longimana*. Sources include values from the Prince Edward Islands (PEIs), Îles Crozet and Îles Kerguelen. Sources preceded with an asterisk (*) are adopted from Chapter 2 (PEIs) of this dissertation. Red coloured sources are krill species, green sources are fish and orange sources are squid.

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

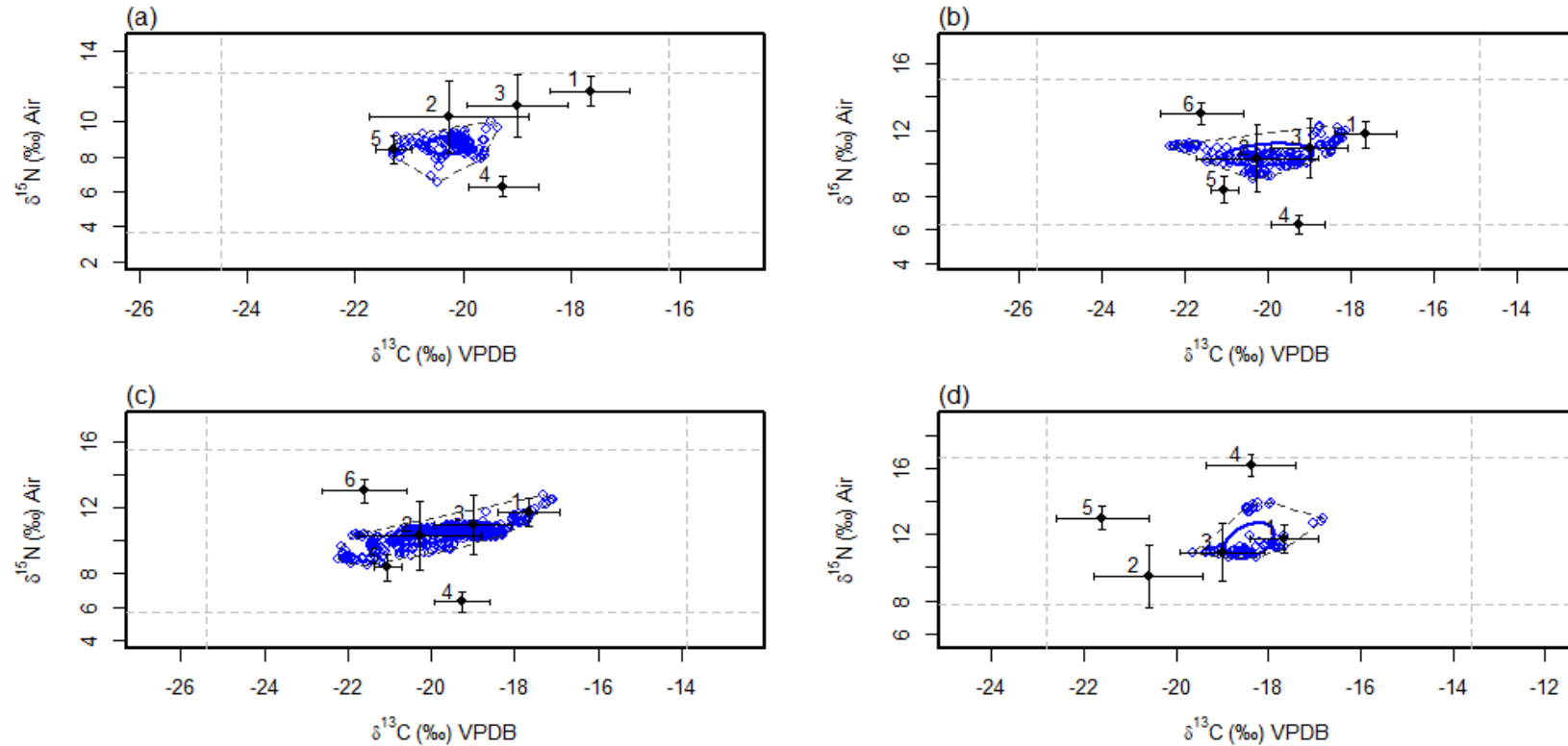


Figure 4: Graphical representation of the bivariate stable isotope mixing space for (a) juvenile, (b) sub-adult, (c) adult female and (d) adult male southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands during foraging (fasting excluded). The black squares represent clusters of potential prey values and one standard deviation, after applying the appropriate trophic discrimination factor (TDF), but not incorporating TDF uncertainty (Hobson et al. 1996, Lúbcker et al. 2017). The sources in these models include values for fish sampled around the PEIs specifically, and other sources from îles Crozet and îles Kerguelen. The dashed grey boxes are the bounds within which potential source values, adjusted for TDF, were considered. The numbers assigned to clusters and its constituents can be found in Table 5 and 10, with source clustering consistent for juveniles (aside from having no cluster 6), sub-adults and adult females. The adult male model considered a different set of sources (Table 11).

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

For killer whales (*Orcinus orca*) the source values were also different than other models (Table 12 Supplementary Material to Chapter 3). The majority of sources, including the additional myctophid values, fell outside the bounds for killer whales and, additionally, its positioning in the bivariate isotopic space precluded the generation of a source mixing space (Figure 9 and 10 Supplementary Material to Chapter 3) that encapsulates the consumer values (Smith et al. 2013). The killer whale model could not be resolved with the available source values.

4. DISCUSSION

Understanding the diet of predators has implications for their conservation and the ecosystems they use (Lea et al. 2002, Österblom et al. 2008, Grémillet & Charmantier 2010, Waluda et al. 2012, Ansorge et al. 2014, Kirkman et al. 2016). Improving the accuracy of dietary models using SIA with more appropriate source values therefore makes for more informed management decisions. Newly established source values (Chapter 2) were applied to revise previously modelled juvenile southern elephant seal (*Mirounga leonina*) diets (Lübcker et al. 2017), and parameterise novel models for other age- and sex categories of southern elephant seals.

The novel information revised the previous predicted contribution of krill (*Euphausia* spp. and *Thysanoessa* sp.) to the diet of juveniles from 76% (Lübcker et al. 2017) to 58% (sum of modes for clusters 4 and 5, Table 5). Fish contribution was similar in the revised model (26% sum of modes for clusters 1, 2 and 3, which also includes the squid *Martialia hyadesi*, Table 5). The only squid within modelling bounds was *M. hyadesi*, and could theoretically, based on modal modelling output, contribute 9% of the diet. The 95% credibility intervals are wide and mean that, theoretically, diets could be very fluid. Based on upper credibility limits for different cluster types (Table 5), juveniles could feed 100% on krill, or up to 83% on fish with the remainder made up

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

of krill, or squid up to 28% (if cluster 3 contains only *M. hyadesi*). By looking at modes, the most frequent modelling outcomes based on 450000 permutations, krill remained predominant to the diet of juvenile southern elephant seals, concordant with recent suggestions (Walters 2014, Walters et al. 2014, Lübcker et al. 2017). Other findings suggest a predominance of myctophids (Newland et al. 2011), but may be due to varied foraging between sites or not controlling for nursing and fasting in the stable isotope data of the cited study. Further, Newland et al. (2011) looked at seals aged 1 to 4 that have not bred, a group that includes juveniles and sub-adults according to definitions herein. Direct stomach sampling for seals aged >3 years at the PEIs indicated the presence of various taxa of squid, and only degraded fish and crustaceans (Bester & Connan 2022), although stomach sampling incorporates bias for digestion-resistant remains. Nonetheless, the presence of various squid taxa indicates that squid species additional to *M. hyadesi* and *Moroteuthopsis longimana* are important to parameterise diets using SIA for perhaps juvenile (>1 year olds), but certainly sub-adult seals. That modelling performance improved for $\delta^{13}\text{C}$ from 0.2‰ (Lübcker et al. 2017) to 0.1‰ (Table 5) after replacing only four fish species with specifically PEI counterpart values, it confirms the importance of using the local source data for modelling, and the need for further characterisation of PEI specific sources.

The proportional contribution of krill (sum of krill modes in Table 5) to the diet of southern elephant seals decreased from 58% in juveniles to 35% for sub-adults and 24% for adult females. The only krill species considered for adult males was *Thysanoessa* sp., clustered together with fish (see Cluster 2, Table 11 in Supplementary Material Chapter 3), and this cluster contributed 19% (Table 5) to adult male diets. The decreasing trend with seal age aligns with findings of dietary krill occurrence (Field et al. 2007). Note that results in Field et al. 2007 were limited to

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

seals up to 3 years old. The credibility intervals in our modelling do suggest variable dependence on krill within seal age-sex classes. As such, adult females could feed more heavily on krill than sub-adults, based on collective upper credibility limits for krill clusters (adult females 72% and sub-adults 65%). It is likely from our frequentist approach with the available sources that all seal classes could exploit krill to some extent (Table 5). Krill feeding is an interesting finding for adult classes, as the accurate contribution of krill is often difficult to establish with direct stomach sampling (Slip 1995, Bester & Connan 2022), and not accordant with stable isotope findings for Îles Kerguelen seals (Cherel et al. 2008).

The contribution of fish decreased from 26% for juveniles to 20% for both sub-adults and adult females (Table 5). Fish (adult male clusters 1, 2 and 3) dominated the modelled diet of adult male southern elephant seals at 88%. Stable fish contributions between sub-adults (2-4 years old, that haven't bred) and adult females (4-13 years old, that have bred) is supported by findings in Slip (1995), albeit by frequency of occurrence in diet and not contribution by mass. However, the sharp increase in fish-feeding inferred for adult males in this study is not supported by findings by Slip (1995). The findings may be due to differences in foraging between sites, as differences in fish consumption (by frequency of occurrence) between southern elephant seals (of various classes) from King George, Heard and Macquarie Islands have been found (Green & Burton 1993, Slip 1995, Daneri & Carlini 2002). However, the bias for digestion-resistant remains in stomach sampling also confounds comparisons with the current stable isotope study. At the very least, though, fish feeding is a likely characteristic for all age classes in this study, and concurs with the presence of fish in directly sampled seal diets elsewhere in the SO (Green & Burton 1993, Slip 1995, Daneri & Carlini 2002). If the sharp increase in fish-feeding for adult males is real, it

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

represents novel insight gleaned from stable isotope analysis that could not be established with traditional methods. Previous studies using direct sampling suggest a predominance of squid (Green & Burton 1993, Slip 1995), but given the accumulation of beaks in seal stomachs, fish feeding could well have been underestimated. Fish was considered the predominant resource for adult females at Îles Kerguelen (Cherel et al. 2008). Note also that, based on our 95% limits, sums for fish clusters could collectively contribute 0%-83%, 0%-87%, 0%-96% and 23%-100% to the juveniles, sub-adults, adult females and adult males, respectively, so the credibility intervals in this study provide for substantial variability in fish-feeding.

The modal contributions for collective squid clusters to the diet of seal classes range from 9% for juvenile seals, to 29% for adult females (Table 5). The model for juvenile southern elephant seals did not consider squid-specific clusters to be resolved, and the only included squid is *M. hyadesi*, which forms part of cluster 3. Squid of various taxa feature prominently in the diet of juveniles elsewhere, as for sub-adults and adult females (Green & Burton 1993, Slip 1995, Burton & van den Hoff 2002, van den Hoff 2004, Field et al. 2007). Multiple squid taxa have been observed in the diet of sub-adults at the PEIs itself, interestingly excluding *M. hyadesi* (Bester & Connan 2022), but isotopic data for other taxa within the delineated modelling range (bounds) were not available. The only other squid taxon considered for sub-adults and adult females was *M. longimana*, and contributed 17% to the diet of these two classes. Cherel et al. (2008) also suggested that squid is not a staple in the southern elephant seal diets, at least for adult females. Further, the collective contribution of *M. longimana* and other purely squid taxa (Table 11) to adult males in this study is comparably low (4%, 25% if *M. hyadesi* considered) compared to suggestions by other studies, 77% and 38% by frequency of occurrence (Green & Burton 1993, Slip 1995). While these differences

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

may also be due to variation between seal populations at different sites (Green & Burton 1993), or biased results due to retention of squid beaks, it is possible that squid-containing clusters are underrepresented for models in the present study. Seeing that data obtained for Îles Kerguelen (and possibly Îles Crozet) are structurally different from the PEIs due to bathymetry-related enrichment (Chapter 2, van Tonder et al. 2021), it implies that species not replaced for PEI specific data (Chapter 2, van Tonder et al. 2021) were potentially excluded by modelling bounds because of enrichment.

Available sources were unable to resolve a model for killer whales (*Orcinus orca*), due to insufficient source values with higher $\delta^{13}\text{C}$ values. Given the increases in $\delta^{13}\text{C}$ with decreased latitude (Jaeger et al. 2010, Carpenter-Kling et al. 2020, St John Glew et al. 2021), increased depth (Hannides et al. 2013, Choy et al. 2015, Romero-Romero et al. 2019), and bathymetry-associated $\delta^{13}\text{C}$ enrichment (Perissinotto et al. 2000, Trull et al. 2008, de Bruyn et al. 2009), the more positive source values required for the killer whale model would likely come from more northerly sources, deeper sources and/or sources at areas with distinctly high productivity due to bathymetry (e.g., shelves). The notion of northerly foraging during the time of skin formation is supported by Reisinger et al. (2016), who reported increased $\delta^{13}\text{C}$ values with decreased sighting rates around the PEI. The premise is, however, that killer whales do in fact travel north when sighted less frequently. Tracking data suggests that this occurs (Reisinger et al. 2015, 2018b). The concomitant reduction in $\delta^{15}\text{N}$ values (Reisinger et al. 2016) would mean feeding on lower-level prey. Consequentially, lower $\delta^{15}\text{N}$ source values would further aid in the establishment of an appropriate mixing space for killer whales (Figure 10), and means mid-trophic level prey, which probably occurs further north, is required for future modelling efforts.

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

446 The credibility intervals in the current models span 14% to 53% for individual clusters.
447 While the frequentist (mode and 95% credibility interval) is a powerful illustration of
448 most likely solutions, the width of the credibility intervals does show that the true
449 structure of seal diets could vary widely given the parameters used. While the variation
450 may be reduced by more complete source sampling, the wide intervals also illustrate
451 a very fluid trophic system at the PEIs, where resources can be utilised in widely varied
452 combinations. Niche separation was found (B. Coulson, unpubl. data) for sub-adults
453 based on sex (a delineation not considered herein), and such a delineation may tighten
454 credibility limits around modal contributions, while dietary modelling for individuals
455 over time may even show individual specialisations (Hückstädt et al. 2012,
456 Kernaléguen et al. 2016).

457

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

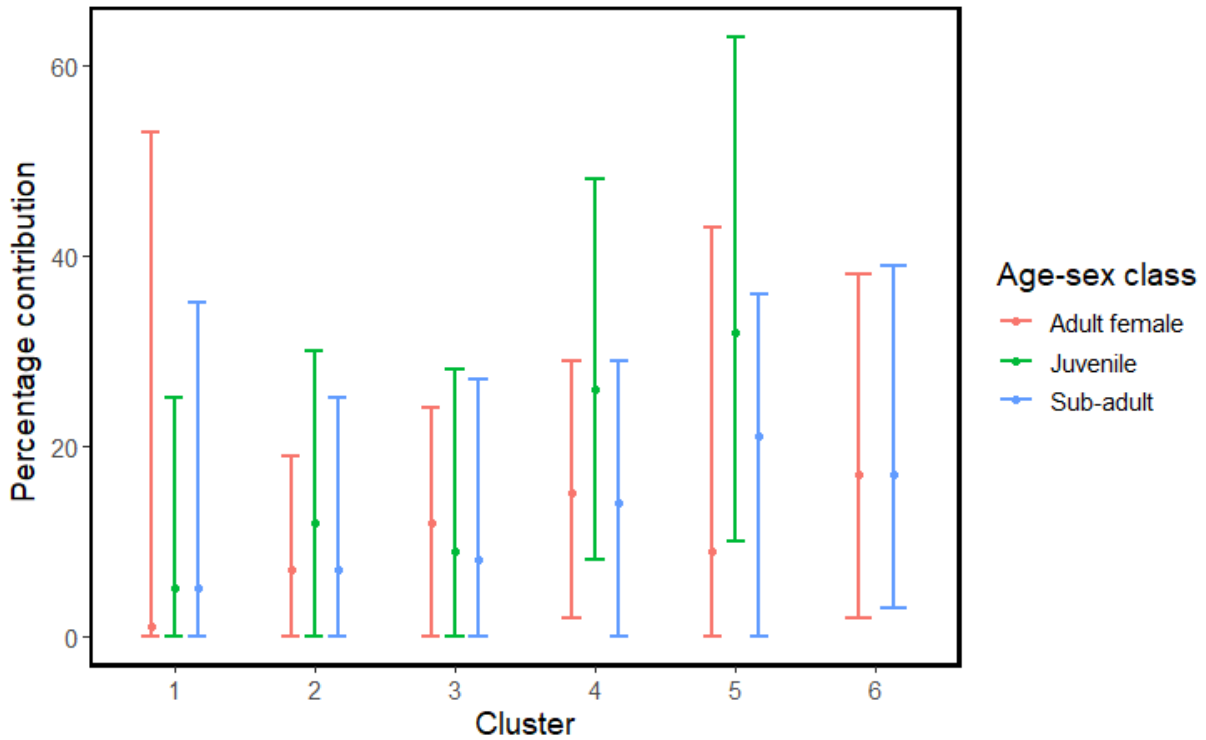


Figure 5: Percentage contributions, mode and 95% credibility intervals, of prey groups to different age-sex classes of southern elephant seal (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. The distributions are the frequentist-results of posteriors generated using the *siar* package in R. Modelling for juveniles did not consider a sixth cluster. Adult males are not depicted here, as the clusters for that class were made up differently than for juveniles, sub-adults and adult females (see Figure 7 and Tables 10 and 11 for cluster make-up).

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

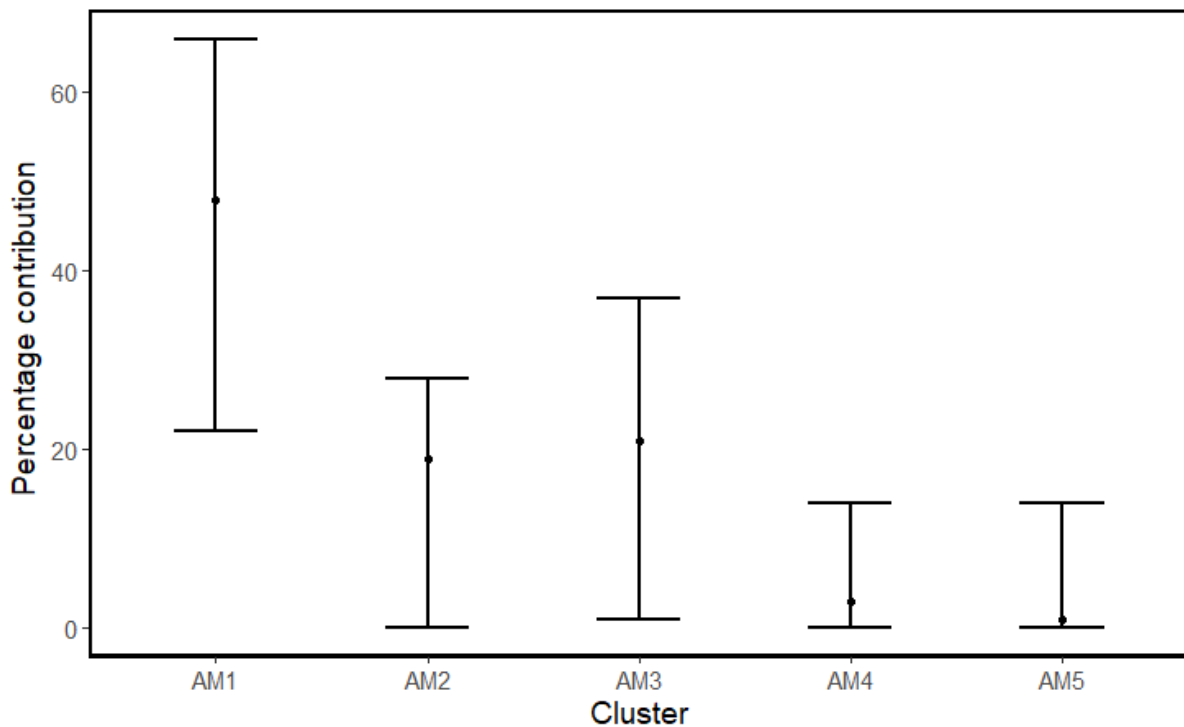


Figure 6: Percentage contributions, mode and 95% credibility intervals, of prey groups to adult male southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. The distributions are the frequentist-results of posteriors generated using the *siar* package in R. Clusters are made up differently to clusters for other age-sex classes, see Figure 6 and Table 10, hence the ‘AM’ (adult male) denotation in this figure. (See Table 11 for cluster make-up of adult males). When determining relative contribution using simulation modelling, models only indicate the range of mathematical solutions that exist with the sources that are provided and needs to be validated against what is known (Smith et al. 2013, Phillips et al. 2014). As our knowledge of consumers’ diets grow, particularly poignant for squid here (Bester & Connan 2022), we can spot and fill gaps in our modelling efforts. The simulation method also weights the outer sources that make up the mixing geometry and devalues ‘inner’ sources that may be important in reality (Smith et al. 2013). E.g., the contribution of inner clusters 2 and 3 to the diet of sub-adult southern elephant seals may be underestimated relative to outer vertices like clusters 4, 5 and 6 because it is less important in shaping the borders of the mixing space. Yet, it stands to reason that the sources plotting closest to the consumer values are ecologically more closely related, either by overlapping niches or, as inferred in this case, by a strong trophic link (Phillips & Gregg 2003, Moore & Semmens 2008, Parnell et al. 2010, Jackson et al. 2011).

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

Further, modelling outcomes here are interpreted based on taxa, but it should be kept in mind that outputs are made using stable isotope values of taxa. Modelling output would thus be similar for different taxa with the same stable isotope values. This means, for example, that the relative importance of fish for adult male southern elephant seals may actually reflect the relative importance of different, yet unsampled sources with similar stable isotope values to the sampled fish.

5. CONCLUSION

This stable isotope modelling study assessed the feasibility of using faecal fish remains (otoliths) to estimate source values for predators' dietary models. Stable isotope models have important bearing on the understanding of predator-prey interactions in the ecosystems they represent. Source values for myctophids, derived from faecal otoliths, improved modelling performance for juvenile southern elephant seals, while reiterating the predominance of krill to their diets. Interestingly, modelling using our parameters and available source values indicated substantial krill feeding for all age-sex classes (lowest 19% [0% - 28%] for adult males). Fish feeding was characterised and found to be predominant in adult males' diets, and squid contributions were less than 30% for any class. While these may represent insights gleaned from stable isotope studies that do not reflect biases for squid beaks the way direct stomach sampling does, this study was still constrained by unavailable source values for the PEIs specifically, especially for squid.

Given that data from other islands do not accurately reflect PEI values (Chapter 2, van Tonder et al. 2021), increased (bio)sampling effort for specifically PEI source values is vital. Specific values for the PEIs may lead to the inclusion and reappraisal of more squid species to the modelling efforts of juveniles, sub-adults and adult females, more accurately reflect potential squid and fish sources for seals (particularly adult males),

Chapter 3: Revised and novel stable isotope dietary models at Marion Island

513 and provide the sources necessary for resolved killer whale models. More extensive
514 bio-sampling for krill, fish and squid is possible using dietary sampling of birds and
515 faecal sampling of seals. Some samplers are known to travel north (Reisinger et al.
516 2018b), so targeting their diets may aid in characterising required source values for
517 killer whale modelling and possibly further efforts for southern elephant seals. While
518 whole krill would still be necessary, degraded fish (myctophid) and squid samples are
519 useful for dietary modelling, given known relationships between hard remains and
520 muscle (Hobson & Cherel 2006, Chapter 2).

521

1 CHAPTER 4: General conclusions

2 This study aimed to provide tools for establishing the stable isotope values of
3 lanternfish (Myctophidae) muscle from faecal otoliths, and using the new stable
4 isotope characterisation to model diets of marine mammal predators at the sub-
5 Antarctic Prince Edward Islands (PEIs). In Chapter 2, stable isotope values of
6 myctophids were generated from otoliths found in fur seal faecal samples. Myctophids
7 are an important prey item for marine predators and its stable isotope values are
8 valuable to parameterise stable isotope dietary models. In Chapter 3, the new
9 myctophid values were used to parameterise models for juvenile, sub-adults, adult
10 female and adult male southern elephant seals at the PEIs, as well as in an attempt
11 to model killer whale diets.

12 Otolith-to-muscle offsets in stable isotope values for myctophids were established
13 using directly sampled myctophids. The offsets were then applied to stable isotope
14 values of oxidant-cleaned otolith values (after correcting for insufficient cleaning) to
15 estimate muscle values, as muscle is of dietary modelling importance. Offsets are
16 considered useful for myctophids with similar life histories, and in this case offsets for
17 the diel migrant *Electrona antarctica* were used to estimate muscle values for four PEI
18 study species (for modelling) that are also diel migrants. Offsets for *Gymnoscopelus*
19 *opisthopterus* was considered inappropriate for the PEI species as there is scant
20 evidence for diel migration in this species, which would mitigate for ontogenetic, depth
21 related increases in stable isotope values that affect the otolith-to-muscle offsets.
22 Given the widespread occurrence of myctophids, the offsets are also a widely
23 applicable tool to use where myctophid muscle values are sought, but only otoliths are
24 available. With the added oxidative cleaning step, this factor could even be applied to
25 archived sedimentary or faecal samples. The cleaning correction did contribute

considerable uncertainty in the measurands, but comparisons between island groups and patterns among species showed that species were correlated in their relative stable isotope values across study sites. This is an indication that the correction method was adequate, and that the muscle estimates for the PEI species using the offsets are realistic. Research aimed at increasing the sample sizes for offsets with direct sampling and twin analyses of fish otoliths and muscle, and squid beak and muscle, would increase the confidence with which offsets are applied to hard remains and sharpen the discretion with which this is done.

When introducing the novel myctophid estimates to a dietary model of juvenile southern elephant seals (*Mirounga leonina*), the inferred contribution of krill to their diet was revised downward by 18%, but still represented the predominant dietary component. Modelling performance improved after using the four myctophids from Chapter 2 to replace conspecifics from Îles Kerguelen. Krill consumption was shown to decrease in novel models for sub-adults, adult females and adult males, a pattern consistent with previous findings and expectations for ontogenetic size increases and dietary requirements for seals. Fish and squid contributions were not consistent with other findings in literature and may be due to differences in sampling methods and biases during direct sampling, or in prey abundances and seal foraging strategies between sites. However, with increasing evidence that stable isotope values at the PEIs differ from neighbouring islands, more (bio)sampling effort is required to continue improving our modelling efforts. Also, directly testing for the hypothesised baseline differences between the PEIs and archipelagos further east, with greater sample sizes than the four study species herein, is expected to resolve problems that could only be illustrated from consumers and literature review in this study.

50 This study in Chapter 3, therefore, highlights the need for more extensive source
51 sampling to parameterise dietary models of local predators with PEI-specific values.
52 The variety of predators (bio-samplers) at the PEIs represent a valuable means of
53 extending source sampling around the PEIs. With the validation of methods to
54 estimate source values from hard prey remains, like with faecal otoliths in Chapter 2
55 of this study, the resolution of source data could be optimised even at large spatial
56 and temporal scales.

57 The unresolved killer whale model also presents an opportunity to test the underlying
58 assumption of ~70 days turnover (and the associated TDF) for killer whale skin, which
59 is currently based on findings for captive dolphins (Browning et al. 2014, Reisinger et
60 al. 2016). Given that the timeframe does not resolve models using prey directly around
61 the PEIs, there is scope for testing hypotheses around maintenance migration in PEI
62 killer whales, where skin is retained for longer and replaced in warmer water, with
63 assimilation of more northerly prey, to avoid thermal costs further south. 'Skin age'
64 could be estimated with photograph comparison and/or more analytical techniques
65 like measuring telomere lengths

Supplementary material to Chapter 2

This supplement accompanies the manuscript entitled:

**Estimating carbon and nitrogen stable isotope value of lanternfish muscle
from faecal otoliths**

**André van Tonder^{1*}, Nico Lübcker^{1,2}, Peter Grønkjær³, Jessica A. Lueders-
Dumont⁴, Elvar H. Hallfredsson⁵, P.J. Nico de Bruyn¹.**

^{6.} Mammal Research Institute, Department of Zoology and Entomology,
University of Pretoria, Private Bag X2320, Hatfield, South Africa

^{7.} Center for Stable Isotopes, University of New Mexico, Albuquerque NM 87131,
USA

^{8.} Aquatic Biology, Department of Biology, Aarhus University, 8000 Aarhus C,
Denmark

^{9.} Ward Research Laboratory, Department of Geosciences, Princeton University,
Guyot Hall, Princeton NJ 08544, USA

^{10.} Deepwater and Cartilaginous Fish, Institute of Marine Research, Fram Centre,
P.O. Box 6606, Tromsø, Norway

LIPID EXTRACTION COMPARISON

Two muscle samples were taken from each fish, one of which had its lipids extracted, and one analysed without extraction. Lipid extraction was done by soaking samples in cyclohexane replaced every two days until the solvent remained clear, indicating no lipids remaining, typically after approximately three rounds (i.e., six days). The C:N ratio decreased from 5.5 ± 0.2 to 3.4 ± 0.1 with lipid extraction. The extraction is done to control for variation brought about by differences in fat content between individual samples, and the use of cyclohexane for the extraction makes the data comparable to that of published data (Cherel et al. 2010). The effect of lipid extraction on both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are shown below for samples that made up the otolith-to-muscle offset (lipid-extracted $n = 24$, and non-lipid-extracted $n = 24$).

Shapiro-Wilk tests revealed non-normal distributions for lipid-extracted $\delta^{13}\text{C}$ ($W = 0.87$, $p < 0.01$), lipid-extracted $\delta^{15}\text{N}$ ($W = 0.77$, $p < 0.01$) and non-lipid-extracted $\delta^{15}\text{N}$ ($W = 0.78$, $p < 0.01$). The variance was spread equally across the two treatments for both $\delta^{13}\text{C}$ (Levene's test: $F = 0$, $df = 46$, $p = 1.00$) and $\delta^{15}\text{N}$ (Levene's test: $F = 0.72$, $df = 46$, $p = 0.40$).

Non-parametric comparison, using Mann-Whitney U analyses, indicated a significant effect of lipid extraction on $\delta^{13}\text{C}$ ($W = 576$, $p < 0.01$) and a small, significant effect on $\delta^{15}\text{N}$ ($W = 391$, $p = 0.03$). The mean and standard deviation (SD) of $\delta^{13}\text{C}$ increased from $-28.9 \pm 0.3\text{‰}$ to $-25.7 \pm 0.3\text{‰}$ after lipid extraction, and $\delta^{15}\text{N}$ from $9.6 \pm 0.6\text{‰}$ to $9.7 \pm 0.4\text{‰}$.

The $\delta^{13}\text{C}$ estimations of myctophid muscle for the Prince Edward Islands (PEIs) were done by making use of the otolith-to-muscle offset where lipids were extracted. But, in

order to avoid incorporating the effect of lipid extraction on $\delta^{15}\text{N}$ values, the non-lipid-extracted otolith-to-muscle offset was used for $\delta^{15}\text{N}$.

MATHEMATICAL AVERAGE VS PHYSICAL POOLING FOR MUSCLE

A comparison was made to establish whether making use of mathematical averages of measurements for individual muscle samples is different from pooling muscle samples together and measuring the pooled sample. Fish were randomly assigned to groups that would be used to make up randomised samples of otoliths (small otolith sizes required the lumping of otolith pairs of different individuals together). Twin muscle samples were taken from these fish along with the otolith pair, and one of each muscle sample twin was subjected to lipid extraction.

Prior to stable isotope measurement, all samples were dried. Muscle samples were compiled according to the fish groups that made up the otolith samples by taking a portion of each of the twin samples (lipid extracted and non-lipid-extracted) and pooling these together according to the respective grouping. The remaining portions would then be measured individually for later mathematical averaging.

There were eight groups of fish that made up otolith samples, and the direct muscle stable isotope measurements are reported alongside the otolith stable isotope values (Table 1 main manuscript). The averages made up of individual sample measurements were not different from these direct, pooled measurements, as the direct measurements fell within one SD of the mathematical averages (Table 6).

Table 5: A comparison of stable isotope values for myctophids from Dronning Maud Land, Antarctica sampled in March - April 2019. Multiple fish (~17) were combined into six units for *Electrona antarctica* (EA) and two for *Gymnoscopelus opisthopterus* (GO). The comparison is where the stable isotope values of muscle samples of individual fish were averaged relative to physically combining the muscle samples of fish and making a single, pooled measurement.

Unit	<i>n</i>	Mathematical average		Physically pooled measurement	
		$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
EA1	17	-26.0 ± 0.5	9.0 ± 0.6	-25.9	9.3
EA2	16	-26.2 ± 0.7	9.0 ± 0.6	-26.0	9.1
EA3	17	-26.1 ± 0.5	9.2 ± 0.3	-26.1	9.5
EA4	17	-26.0 ± 0.6	9.1 ± 0.6	-26.0	9.2
EA5	16	-25.8 ± 0.4	9.1 ± 0.4	-25.7	9.2
EA6	16	-26.0 ± 0.5	9.1 ± 0.5	-25.8	9.3
GO1	17	-25.2 ± 0.7	10.8 ± 0.3	-25.1	10.7
GO2	17	-25.2 ± 0.7	10.7 ± 0.5	-25.5	10.5

CLEANING CORRECTION SUMMARY

There was an oversight during the oxidative cleaning of faecal otolith samples from the PEIs where only 1.5 ml of NaOCl was used, as opposed to 15 ml (Lueders-Dumont et al. 2018). The oxidative potential of the NaOCl would have depleted under higher relative cleaning loads (amount of material to clean). For this reason, a means of correction was established (see Appendix to Chapter 2).

Briefly summarised, faecal otoliths were surface cleaned and powdered to homogeneity. A subset of $n = 6$ was taken, as a control and cleaned in excess NaOCl to obtain the true stable isotope value of the faecal otolith powder after oxidative cleaning. Experimental samples ($n = 27$) were cleaned at a range of cleaning load (mg

powder to ml NaOCl) by making up samples of varied mass, all cleaned in 1.5 ml of NaOCl to simulate conditions in the main data set.

The value of each experimental unit was subtracted from the average of the control unit, and this difference plotted as the response to cleaning load. Nearest neighbour, local regression (*loess* in R (R Core Team 2020, RStudio Team 2020)) was used to describe the relationships and to predict correction values for the main data set. Local regression described the complexity of the stable isotope response within the range of cleaning load.

The correction values were applied to the stable isotope values of the main PEI data set, as predicted by the cleaning load of each sample. The uncertainty surrounding this prediction as standard deviation was determined by multiplying the predicted standard error around each updated PEI value with the square-root of the correction data sample size ($n = 27$). This increased uncertainty was then propagated into the reported final SD, along with other sources of uncertainty (Table 6).

UNCERTAINTY PROPAGATION FOR MUSCLE ESTIMATES

Various sources of uncertainty arise throughout the process of creating data. This study aimed to incorporate all of these sources into a final value of uncertainty (final SD). Sources include estimations of measurement accuracy, the aforementioned standard error around correction values, the application of otolith-to-muscle offsets for muscle value estimation and the SD within sampling units of the PEI data. The final SD reported combines dispersion around sampling unit means (Type A uncertainties) as well as determined measurement precision, otolith cleaning correction values and otolith-to-muscle offsets (Type B uncertainties) (Farrance & Frenkel 2012).

The variances (square of SDs below) of all sources of uncertainty were summed, and the root of this sum-total determined as the final SD. The following sources were included:

- i. Measurement uncertainty (accuracy of mass spectrometer) for the PEI samples analysed at Aarhus University. Reproducibility of the standards (SD) was 0.6‰ for $\delta^{13}\text{C}$ and 0.4‰ for $\delta^{15}\text{N}$.
- ii. The measurement uncertainty (machine accuracy) for correction samples analysed at the University of Pretoria, where standard reproducibility (SD) was 0.2‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$.
- iii. The SD of the control unit (Unit A) during the establishment of correction curves, as the values of individual experimental samples were subtracted from an averaged value for the control unit: 0.2‰ for $\delta^{13}\text{C}$ and 0.6‰ for $\delta^{15}\text{N}$.
- iv. The uncertainty around the correction curves, with predicted standard error multiplied by the root of the sample size of experimental units ($n = 27$). The standard error varied along the correction curve, with each predicted value associated with its own estimate of uncertainty.
- v. The measurement error (machine accuracy) of the mass spectrometer at the University of Pretoria, used for muscle measurement as well; standard reproducibility was 0.2‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$.
- vi. When establishing the otolith-to-muscle offset the muscle value counterparts to otolith values were the average of duplicate measurements of muscle samples, duplicate reproducibility was 0.1‰ for $\delta^{13}\text{C}$ and 0.2‰ for $\delta^{15}\text{N}$.
- vii. The measurement uncertainty (machine accuracy) for the otolith samples used to establish the otolith-to-muscle offset. Standard reproducibility at Aarhus University: $\delta^{13}\text{C}$ 0.6‰ and $\delta^{15}\text{N}$ 0.4‰.

viii. The uncertainty for the otolith-to-muscle offset (SD) for *Electrona antarctica* (*Gymnoscopelus opisthopterus* not used): $\delta^{13}\text{C}$ 0.4‰, $\delta^{15}\text{N}$ 0.3‰.

ix. Finally, the standard deviation (analytical uncertainty) within the sampling units of interest. Each of the sampling unit SDs below were propagated alongside the above listed sources of measurand uncertainty:

- a. *E. subaspera* 2005: $\delta^{13}\text{C}$ 0.7‰; $\delta^{15}\text{N}$ 0.9‰
- b. *E. subaspera* 2010: $\delta^{13}\text{C}$ 0.5‰; $\delta^{15}\text{N}$ 0.9‰
- c. *G. fraseri* 2005: $\delta^{13}\text{C}$ 0.7‰; $\delta^{15}\text{N}$ 0.9‰
- d. *G. fraseri* 2010: $\delta^{13}\text{C}$ 0.6‰; $\delta^{15}\text{N}$ 1.3‰
- e. *G. nicholsi* 2005: $\delta^{13}\text{C}$ 1.0‰; $\delta^{15}\text{N}$ 1.0‰
- f. *G. nicholsi* 2010: $\delta^{13}\text{C}$ 0.7‰; $\delta^{15}\text{N}$ 1.4‰
- g. *G. piabilis* 2005: $\delta^{13}\text{C}$ 0.9‰; $\delta^{15}\text{N}$ 1.5‰
- h. *G. piabilis* 2010: $\delta^{13}\text{C}$ 0.7‰; $\delta^{15}\text{N}$ 1.4‰
- i. All myctophids 2005: $\delta^{13}\text{C}$ 1.2‰; $\delta^{15}\text{N}$ 1.4‰
- j. All myctophids 2010: $\delta^{13}\text{C}$ 0.9‰; $\delta^{15}\text{N}$ 1.5‰
- k. All *E. subaspera*: $\delta^{13}\text{C}$ 0.6‰; $\delta^{15}\text{N}$ 1.0‰
- l. All *G. fraseri*: $\delta^{13}\text{C}$ 0.6‰; $\delta^{15}\text{N}$ 1.1‰
- m. All *G. nicholsi*: $\delta^{13}\text{C}$ 1.0‰; $\delta^{15}\text{N}$ 1.2‰
- n. All *G. piabilis*: $\delta^{13}\text{C}$ 0.9‰; $\delta^{15}\text{N}$ 1.5‰

The final SD reported in the main manuscript therefore incorporates sources of uncertainty over the entire analysis of the samples, chiefly the uncertainty associated with correction of incompletely cleaned otolith samples, application of the otolith-to-muscle offset and regular standard deviation within sampling units.

Table 6: Summary of propagated uncertainty for estimates of myctophid muscle stable isotope values from oxidant-cleaned faecal otoliths (ES = *Electrona subaspera*, GF = *Gymnoscopelus fraseri*, GN = *G. nicholsi*, GP = *G. piabilis*). Roman numerals denote the sources of uncertainty listed in the current supplement propagated up to that point.

Sampling unit	n ($\delta^{13}\text{C}$; $\delta^{15}\text{N}$)	Analytical SD only (ix)		Otolith SD* (i – iv)		Final SD* (i - ix)	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
ES05	10; 5	0.7	0.9	1.2	1.9	1.4	1.9
ES10	10; 8	0.5	0.9	1.0	1.9	1.3	1.9
GF05	14; 10	0.7	0.9	1.1	2.0	1.4	2.0
GF10	18; 11	0.6	1.3	1.1	2.2	1.3	2.2
GN05	18; 9	1.0	1.0	1.4	1.9	1.6	1.9
GN10	15; 14	0.7	1.4	1.1	2.2	1.4	2.2
GP05	19; 19	0.9	1.5	1.3	2.5	1.5	2.5
GP10	30; 29	0.7	1.4	1.1	2.2	1.4	2.2

* Based on average measurand uncertainty of measurands (n) in each sampling unit

**Appendix to Chapter 2: Correcting stable isotope values for insufficient
oxidative cleaning of otolith material**

**André van Tonder^{1*}, Nico Lübcker^{1,2}, Peter Grønkjær³, Jessica A. Lueders-
Dumont⁴, Elvar H. Hallfredsson⁵, P.J. Nico de Bruyn¹.**

^{11.} Mammal Research Institute, Department of Zoology and Entomology,
University of Pretoria, Private Bag X2320, Hatfield, South Africa

^{12.} Center for Stable Isotopes, University of New Mexico, Albuquerque NM 87131,
USA

^{13.} Aquatic Biology, Department of Biology, Aarhus University, 8000 Aarhus C,
Denmark

^{14.} Ward Research Laboratory, Department of Geosciences, Princeton University,
Guyot Hall, Princeton NJ 08544, USA

^{15.} Deepwater and Cartilaginous Fish, Institute of Marine Research, Fram Centre,
P.O. Box 6606, Tromsø, Norway

Running head: Correcting stable isotope values for insufficiently cleaned otoliths

18 ABSTRACT

19 Oxidative cleaning with bleach (NaOCl) is an important step to control for contaminant
20 or altered organic material in a variety of calcareous samples like corals and fish
21 otoliths. It is an important step for samples used for stable isotope analysis. Oversight
22 in the cleaning step may lead to the incomplete oxidation of contaminant material and
23 affect stable isotope measurements. This short study established corrections for
24 isotopic values of myctophid otolith powder samples that had been cleaned
25 insufficiently. Corrections are based on the amount of powder relative to solvent, as
26 greater cleaning loads are associated with lower oxidative potential. Generally, as the
27 cleaning load increases, $\delta^{13}\text{C}$ correction values increased, and $\delta^{15}\text{N}$ correction values
28 decreased. Such application should also be cautious that the amount of contaminant
29 organic material is similar to the content in this study, which could only be established
30 broadly. The observed stable isotope correction values suggest that oxidative cleaning
31 preferentially affects organic material with the heavier ^{13}C isotope and the lighter ^{15}N
32 isotope, though this may be very specific to this study and those similar.

33 KEY WORDS:

34 Oxidative cleaning, stable isotope analysis, bleach, otoliths, correction

1. INTRODUCTION

Oxidative cleaning viably removes contaminant diagenetic or altered organic material from calcium carbonate bound organic samples, including coral skeletons, ostracods, foraminifera and fish otoliths (Ingalls et al. 2003, Ren et al. 2009, Bright & Kaufman 2011, Wang et al. 2014, Lueders-Dumont et al. 2018, 2020). The aforementioned studies all use sodium hypochlorite solutions, bleach ($\text{NaOCl} + \text{H}_2\text{O}$), as part of their respective oxidative cleaning processes. The method of action of NaOCl is by oxidising and hydrolysing organic material, with OCl^- , Cl^- or OH^- species deprotonating organic molecules, making it useful even in fields like dentistry or treating of fouled filters (Spanó et al. 2001, Estrela et al. 2002, Wang et al. 2018). Bleach treatment is especially effective in high pH (alkaline) conditions with excess oxidising species (Wang et al. 2018).

The stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) of prey remains provides a useful way to characterise the ecology of prey stocks, using predators as bio-samplers (Chapter 2, van Tonder et al. 2021). Due to the method of action of bleach oxidative cleaning, it can be used to control for digestive altering and fouling by faecal matter of calcareous prey remains (Reisinger et al. 2018a), in a similar way that it controls for diagenetic effects (Ingalls et al. 2003, Ren et al. 2009, Bright & Kaufman 2011, Lueders-Dumont et al. 2018).

While insufficient cleaning of samples should much rather be avoided, it is not known how such an oversight can be corrected. It is unknown how decreased oxidative potential of NaOCl affects the stable isotope values of samples, be it from too much contaminant material relative to solvent or a too low solvent concentration. The more pointed question is therefore whether there is any preference for OCl^- , Cl^- or OH^-

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

species to deprotonate lighter or heavier C and N stable isotopes in contaminant organic material.

The aim of this study is primarily to provide a means of correcting organic material stable isotope values for insufficiently cleaned calcareous samples. In doing so though, it provides insight into how NaOCl interacts with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in organic contaminants of these samples.

2. MATERIALS AND METHODS

Faecal otoliths of *Gymnoscopelus piabilis* were compiled from Antarctic fur seal (*Arctocephalus gazella*) scats collected during January 2005 and December 2010. The otoliths were surface cleaned in 0.2 M NaOH according to Grønkjær et al. (2013) and homogenised into powder as a whole. The powder was then subsampled into a control unit ($n = 6$) and experimental units ($n = 27$) of differing masses. The control unit was cleaned in excess NaOCl to ensure complete oxidative cleaning. The experimental samples were cleaned in 1.5 ml NaOCl (10-15 % available chlorine) to simulate a consistent systematic error of using too little oxidant. This results in a range of cleaning load (mg powder to ml NaOCl); 19 – 57 mg.ml⁻¹.

The powder samples were then demineralised in HCl and the organic material was extracted using centrifugal ultrafiltration of the dissolved product (Grønkjær et al. 2013). The stable isotope values of the extracted organic material were measured at the Stable Isotope Facility at the University of Pretoria in South Africa. The in-house standards used throughout this analysis were Merck Gel; $\delta^{13}\text{C} = -20.3 \pm 0.1\text{‰}$ and $\delta^{15}\text{N} = 7.9 \pm 0.1\text{‰}$ and DL-Valine $\delta^{13}\text{C} = -10.6 \pm 0.1\text{‰}$ and $\delta^{15}\text{N} = -6.2 \pm 0.1\text{‰}$, as well as Gel A; $\delta^{13}\text{C} = -21.8 \pm 0.2\text{‰}$ and $\delta^{15}\text{N} = 5.5 \pm 0.1\text{‰}$.

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

Correction curves were established by obtaining the difference between the mean isotope values of Unit A (control, $n = 6$) and the individual sample values. These differences were plotted as the response to the cleaning load of the experimental units. These response curves would make it possible to predict the correction values required for insufficiently cleaned otolith samples based on their cleaning load.

The response curves for each isotopic tracer were established and produced using RStudio (R Core Team 2020, RStudio Team 2020) as a *loess* function, a local or nearest neighbour regression that appreciates non-linearity in responses. All curves were set at a 95% confidence level and a span of 0.75.

3. RESULTS

The six samples for the control unit (Unit A) averaged at $-21.2 \pm 0.2\text{‰}$ for $\delta^{13}\text{C}$ and $4.7 \pm 0.6\text{‰}$ for $\delta^{15}\text{N}$, considered the true isotopic values of the homogenised otolith powder. Subtracting the individual isotopic values of the experimental units from this value produced correction values.

There was a complex increase in $\delta^{13}\text{C}$ correction values as cleaning load increased (mean $\pm$ SD; SD = SE*sqrt($n = 27$)) from $0.1 \pm 0.6\text{‰}$ to $1.0 \pm 1.5\text{‰}$. Conversely, there was a complex decrease in $\delta^{15}\text{N}$ from $2.2 \pm 1.6\text{‰}$ to $0.3 \pm 3.9\text{‰}$. In all cases this is greater than instrumental measurement uncertainty (maximum 0.2‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$).

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

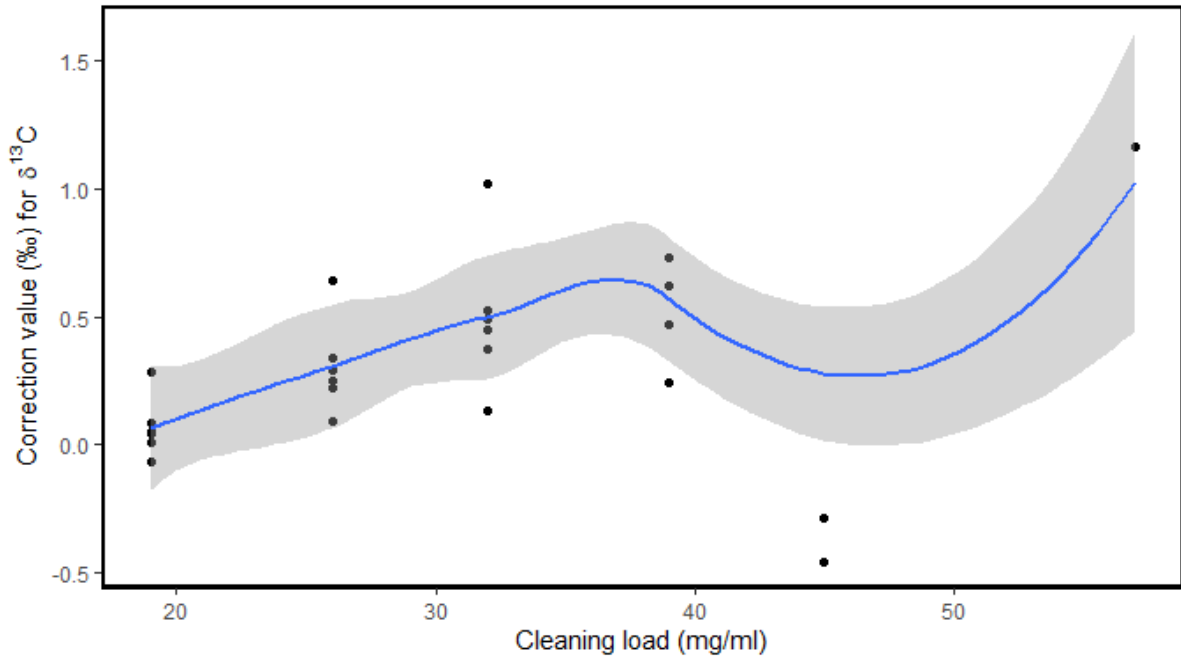


Figure 7: Correction curve for stable carbon isotope ratios ($\delta^{13}\text{C}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The curve is a nearest neighbour regression curve (*loess*) at a 95% confidence level span = 0.75, and was produced using R (R Core Team 2020, RStudio Team 2020). It represents otolith material of *Gymnoscopelus piabilis* from Antarctic fur seal faecal (*Arctocephalus gazella*) samples collected at the Prince Edward Islands.

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

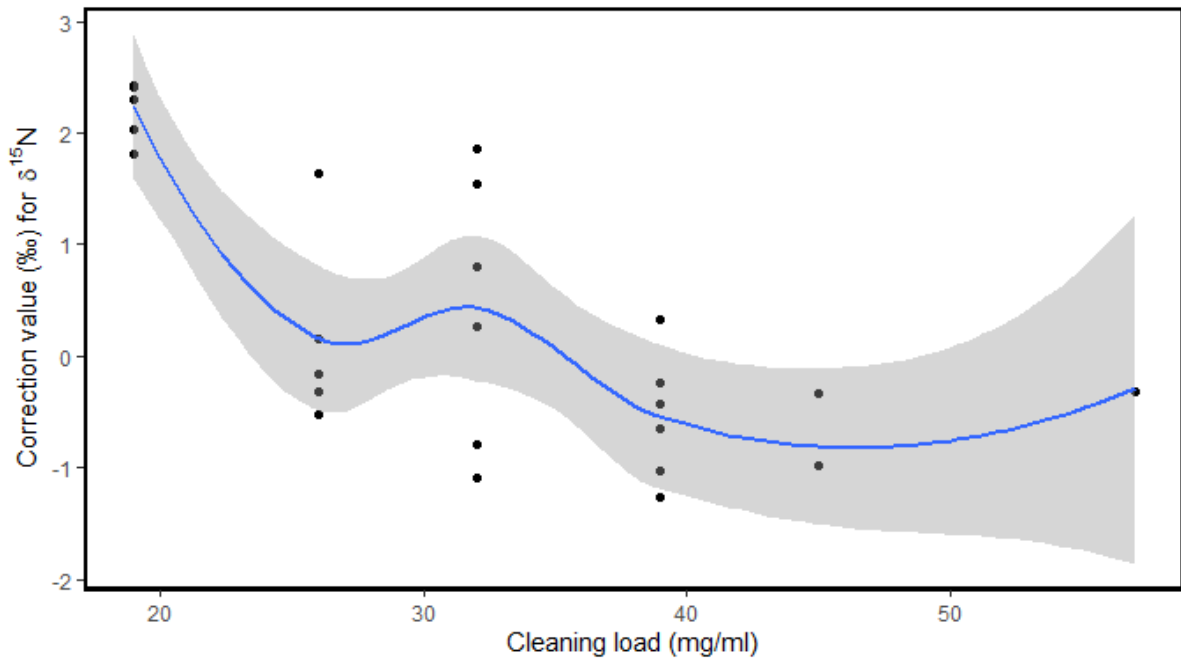


Figure 8: Correction curve for stable nitrogen isotope ratios ($\delta^{15}\text{N}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The curve is a nearest neighbour regression curve (*loess*) at a 95% confidence level, span = 0.75, and was produced using R (R Core Team 2020, RStudio Team 2020). It represents otolith material of *Gymnoscopelus piabilis* from Antarctic fur seal faecal (*Arctocephalus gazella*) samples collected at the Prince Edward Islands

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

Expressed as ordinary linear regression (OLR) functions, which do not weight for reduced variance, these relationships are:

Correction $\delta^{13}\text{C}$ (‰) = $0.01 \times \text{Cleaning load (mg.ml}^{-1}) - 0.10$; (Adjusted $R^2 = 0.10$)

Correction $\delta^{15}\text{N}$ (‰) = $-0.09 \times \text{Cleaning load (mg.ml}^{-1}) + 3.21$; (Adjusted $R^2 = 0.45$)

And when expressed as weighted linear functions (WLR):

Correction $\delta^{13}\text{C}$ (‰) = $-0.00 \times \text{Cleaning load (mg.ml}^{-1}) + 0.19$; (Adjusted $R^2 = -0.04$)

Correction $\delta^{15}\text{N}$ (‰) = $-0.13 \times \text{Cleaning load (mg.ml}^{-1}) + 4.57$; (Adjusted $R^2 = 0.82$)

As the corrections are not linear, the correction values of the nearest neighbour regression *loess* curves (Figure 7 and 8) are presented in Table 8 and 9. Additionally, the standard errors surrounding the corrections are also provided (Table 8 and 9), as this is a source of uncertainty that can be propagated with the established correction values.

4. DISCUSSION

An increase in cleaning load (more organic material relative to solvent) in this study was associated with decreasing oxidative potential. Because the response value is the difference between the true value and experimental values, a decreasing correction trend means the actual measured stable isotope values increased with cleaning load and vice versa.

The implication for the main study is that measured otolith $\delta^{13}\text{C}$ values will be increased as powder mass (per 1.5 ml NaOCl) increased, and $\delta^{15}\text{N}$ will decrease most for the heaviest samples. Correction also contributes significant uncertainty to measurand values when applied to data of incompletely cleaned samples, which needs to be propagated robustly and reported along with analytical uncertainty

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

(standard deviation - SD) within sampling units (Farrance & Frenkel 2012). To establish correction uncertainty as SD, the predicted standard error (Table 8 and 9) is multiplied by the root of the correction sample size ($n = 27$).

While cleaning load in this study may reasonably be associated with reduced oxidative potential of NaOCl due more material with contaminant organic content, the amount of organic material in fouled calcareous samples may vary with taxa and sampling conditions. Any application of these corrections would be under the assumption that the amount of contaminant organic material in powdered samples is similar to that of the faecal *Gymnoscopelus piabilis* otolith powder in this study. In that regard, the total organic matter (contaminant plus native) of myctophid otolith powder was measured in two ways (A. van Tonder, unpubl. data): using absorbance of light at 280 nm, and physically measuring the mass of organic matter after dissolution and freeze-drying. Respectively, these estimates are 0.005 ± 0.002 and 0.004 ± 0.002 mg per mg powder. The strictly contaminant organic material content for myctophid otolith powder would be lower than these estimates.

Some insight into how oxidation discriminates for heavier or lighter C and N stable isotopes can be gleaned from the relationship between the cleaning concentration and stable isotope value. Given the increasing trend for $\delta^{13}\text{C}$ correction values and a decreasing trend for $\delta^{15}\text{N}$ correction values, it means that as oxidative potential decreased the stable isotope values of $\delta^{13}\text{C}$ decreased and that of $\delta^{15}\text{N}$ increased. Oxidation favoured molecules that contained the heavier ^{13}C stable isotopes and the lighter ^{14}N stable isotopes. The effect of pre-treatment on stable isotope values is not very predictable (Mannella et al. 2019), so the meaningfulness of this observation is limited to studies with similar material. Still, the effect oxidative potential has on stable

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

165 isotope values provides insight into how values of organic material changes as it is
166 naturally degraded (Lueders-Dumont et al. 2018, 2020).

167 5. CONCLUSION

168 This research note provides data useful for the correction of calcareous samples that
169 have been insufficiently cleaned using NaOCl as an oxidant. While incomplete
170 cleaning should much rather be avoided, the correction data is applicable to samples
171 with similar composition to otoliths from fur seal faecal samples. Application of these
172 corrections need to be cognisant of the associated uncertainty and how to incorporate
173 it to uncertainty of predicted measurands. For the purpose of this dissertation, the
174 corrections are reliable for use in predicting stable isotope values of PEI myctophids,
175 which also relied on data of otoliths from fur seal faecal samples. Furthermore, insight
176 into how oxidative potential affects C and N stable isotope values are useful in
177 understanding natural degradation of organic proteins. The observed complexity in
178 correction curves would be resolved by increased sample sizes for heavier cleaning
179 loads and validations of the corrections using measurements without correction would
180 evaluate the accuracy of estimates made using the established corrections.

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

Table 7: Correction values (‰) for stable carbon isotope ratios ($\delta^{13}\text{C}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The correction values and associated error were derived from nearest neighbour regression (*loess*) curves at a 95% confidence level, span = 0.75, using R (R Core Team 2020, RStudio Team 2020). The curves were established using otolith material of *Gymnoscopelus piabilis* from Antarctic fur seal (*Arctocephalus gazella*) faecal samples collected at the Prince Edward Islands.

Cleaning load (mg.ml ⁻¹)	Correction value	Minimum correction value	Maximum correction value	Standard error (SE)
19.0000	0.0667	-0.1753	0.3086	0.1165
19.4810	0.0837	-0.1362	0.3037	0.1059
19.9620	0.1007	-0.1045	0.3059	0.0988
20.4430	0.1176	-0.0799	0.3151	0.0951
20.9241	0.1344	-0.0614	0.3302	0.0943
21.4051	0.1511	-0.0476	0.3498	0.0957
21.8861	0.1678	-0.0369	0.3724	0.0985
22.3671	0.1843	-0.0278	0.3965	0.1021
22.8481	0.2008	-0.0193	0.4209	0.1060
23.3291	0.2172	-0.0105	0.4448	0.1096
23.8101	0.2335	-0.0007	0.4677	0.1127
24.2911	0.2497	0.0105	0.4889	0.1152
24.7722	0.2658	0.0234	0.5083	0.1167
25.2532	0.2819	0.0381	0.5257	0.1174
25.7342	0.2979	0.0547	0.5410	0.1171
26.2152	0.3138	0.0737	0.5539	0.1156
26.6962	0.3302	0.0978	0.5626	0.1119
27.1772	0.3470	0.1257	0.5682	0.1065
27.6582	0.3639	0.1550	0.5728	0.1006
28.1392	0.3809	0.1830	0.5788	0.0953
28.6203	0.3977	0.2071	0.5884	0.0918
29.1013	0.4143	0.2254	0.6032	0.0909
29.5823	0.4304	0.2373	0.6235	0.0930
30.0633	0.4458	0.2436	0.6480	0.0974
30.5443	0.4605	0.2464	0.6746	0.1031
31.0253	0.4742	0.2480	0.7004	0.1089
31.5063	0.4868	0.2506	0.7230	0.1137
31.9873	0.4981	0.2562	0.7399	0.1164
32.4684	0.5105	0.2685	0.7525	0.1165
32.9494	0.5264	0.2890	0.7638	0.1143
33.4304	0.5445	0.3150	0.7741	0.1105
33.9114	0.5639	0.3437	0.7842	0.1060
34.3924	0.5835	0.3722	0.7948	0.1017
34.8734	0.6021	0.3975	0.8067	0.0985
35.3544	0.6188	0.4172	0.8204	0.0971
35.8354	0.6324	0.4295	0.8354	0.0977
36.3165	0.6420	0.4336	0.8503	0.1003

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

36.7975	0.6463	0.4298	0.8628	0.1042
37.2785	0.6444	0.4186	0.8702	0.1087
37.7595	0.6351	0.4008	0.8695	0.1128
38.2405	0.6175	0.3771	0.8579	0.1158
38.7215	0.5904	0.3477	0.8331	0.1169
39.2025	0.5540	0.3131	0.7949	0.1160
39.6835	0.5177	0.2785	0.7568	0.1151
40.1646	0.4836	0.2452	0.7221	0.1148
40.6456	0.4519	0.2133	0.6905	0.1149
41.1266	0.4226	0.1831	0.6621	0.1153
41.6076	0.3957	0.1547	0.6367	0.1160
42.0886	0.3713	0.1283	0.6142	0.1170
42.5696	0.3493	0.1041	0.5946	0.1181
43.0506	0.3299	0.0820	0.5777	0.1193
43.5316	0.3130	0.0624	0.5637	0.1207
44.0127	0.2988	0.0451	0.5524	0.1221
44.4937	0.2871	0.0303	0.5439	0.1236
44.9747	0.2782	0.0181	0.5382	0.1252
45.4557	0.2719	0.0085	0.5354	0.1269
45.9367	0.2684	0.0014	0.5355	0.1286
46.4177	0.2677	-0.0031	0.5385	0.1304
46.8987	0.2698	-0.0051	0.5447	0.1323
47.3797	0.2747	-0.0045	0.5540	0.1344
47.8608	0.2825	-0.0015	0.5666	0.1368
48.3418	0.2933	0.0040	0.5826	0.1393
48.8228	0.3070	0.0118	0.6022	0.1421
49.3038	0.3237	0.0219	0.6255	0.1453
49.7848	0.3435	0.0342	0.6527	0.1489
50.2658	0.3663	0.0487	0.6839	0.1529
50.7468	0.3922	0.0652	0.7192	0.1575
51.2278	0.4212	0.0836	0.7588	0.1626
51.7089	0.4535	0.1040	0.8029	0.1683
52.1899	0.4889	0.1262	0.8516	0.1746
52.6709	0.5276	0.1502	0.9050	0.1817
53.1519	0.5696	0.1760	0.9632	0.1895
53.6329	0.6149	0.2035	1.0263	0.1981
54.1139	0.6635	0.2327	1.0943	0.2074
54.5949	0.7156	0.2636	1.1675	0.2176
55.0759	0.7711	0.2963	1.2458	0.2286
55.5570	0.8300	0.3307	1.3293	0.2404
56.0380	0.8924	0.3668	1.4180	0.2531
56.5190	0.9584	0.4048	1.5121	0.2666
57.0000	1.0280	0.4445	1.6115	0.2809

188

189

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

Table 8: Correction values (‰) for stable nitrogen isotope ratios ($\delta^{15}\text{N}$) of otolith material based on cleaning load – mass of otolith powder (mg) to volume of NaOCl (ml). The correction values and associated error were derived from nearest neighbour regression (*loess*) curves at a 95% confidence level, span = 0.75, using R (R Core Team 2020, RStudio Team 2020). The curves were established using otolith material of *Gymnoscopelus piabilis* from Antarctic fur seal (*Arctocephalus gazella*) faecal samples collected at the Prince Edward Islands.

Cleaning load (mg.ml ⁻¹)	Correction value	Minimum correction value	Maximum correction value	Standard error (SE)
19.0000	2.2350	1.5851	2.8849	0.3129
19.4810	2.0100	1.4191	2.6008	0.2845
19.9620	1.7971	1.2458	2.3485	0.2654
20.4430	1.5965	1.0659	2.1270	0.2554
20.9241	1.4079	0.8820	1.9339	0.2532
21.4051	1.2316	0.6979	1.7653	0.2570
21.8861	1.0674	0.5177	1.6171	0.2646
22.3671	0.9154	0.3455	1.4853	0.2744
22.8481	0.7755	0.1842	1.3668	0.2847
23.3291	0.6478	0.0362	1.2594	0.2945
23.8101	0.5323	-0.0968	1.1613	0.3029
24.2911	0.4289	-0.2137	1.0715	0.3094
24.7722	0.3377	-0.3137	0.9891	0.3136
25.2532	0.2586	-0.3964	0.9136	0.3154
25.7342	0.1917	-0.4615	0.8450	0.3145
26.2152	0.1388	-0.5062	0.7839	0.3106
26.6962	0.1118	-0.5125	0.7361	0.3006
27.1772	0.1092	-0.4852	0.7036	0.2862
27.6582	0.1264	-0.4348	0.6877	0.2702
28.1392	0.1590	-0.3726	0.6907	0.2560
28.6203	0.2025	-0.3096	0.7146	0.2466
29.1013	0.2523	-0.2551	0.7597	0.2443
29.5823	0.3040	-0.2148	0.8227	0.2498
30.0633	0.3530	-0.1903	0.8962	0.2616
30.5443	0.3948	-0.1804	0.9700	0.2769
31.0253	0.4249	-0.1828	1.0327	0.2926
31.5063	0.4389	-0.1956	1.0734	0.3055
31.9873	0.4322	-0.2176	1.0819	0.3128
32.4684	0.4050	-0.2451	1.0551	0.3130
32.9494	0.3626	-0.2752	1.0004	0.3071
33.4304	0.3071	-0.3096	0.9239	0.2969
33.9114	0.2409	-0.3507	0.8326	0.2849
34.3924	0.1661	-0.4015	0.7338	0.2733
34.8734	0.0851	-0.4646	0.6347	0.2647
35.3544	-0.0001	-0.5417	0.5415	0.2608
35.8354	-0.0870	-0.6323	0.4582	0.2625
36.3165	-0.1736	-0.7332	0.3861	0.2695

Appendix to Chapter 2: Correcting stable isotope values for insufficiently cleaned otoliths

36.7975	-0.2575	-0.8391	0.3241	0.2800
37.2785	-0.3365	-0.9430	0.2701	0.2920
37.7595	-0.4083	-1.0378	0.2211	0.3031
38.2405	-0.4708	-1.1167	0.1750	0.3110
38.7215	-0.5217	-1.1738	0.1303	0.3139
39.2025	-0.5599	-1.2070	0.0872	0.3116
39.6835	-0.5937	-1.2361	0.0488	0.3093
40.1646	-0.6250	-1.2656	0.0155	0.3084
40.6456	-0.6541	-1.2951	-0.0130	0.3086
41.1266	-0.6807	-1.3241	-0.0373	0.3098
41.6076	-0.7050	-1.3524	-0.0577	0.3117
42.0886	-0.7270	-1.3796	-0.0744	0.3142
42.5696	-0.7466	-1.4055	-0.0878	0.3172
43.0506	-0.7640	-1.4298	-0.0982	0.3206
43.5316	-0.7790	-1.4524	-0.1056	0.3242
44.0127	-0.7917	-1.4731	-0.1103	0.3281
44.4937	-0.8021	-1.4919	-0.1123	0.3321
44.9747	-0.8102	-1.5088	-0.1116	0.3364
45.4557	-0.8161	-1.5239	-0.1083	0.3408
45.9367	-0.8196	-1.5370	-0.1022	0.3454
46.4177	-0.8209	-1.5485	-0.0934	0.3503
46.8987	-0.8200	-1.5584	-0.0816	0.3555
47.3797	-0.8168	-1.5669	-0.0666	0.3612
47.8608	-0.8114	-1.5744	-0.0484	0.3674
48.3418	-0.8037	-1.5809	-0.0265	0.3742
48.8228	-0.7938	-1.5868	-0.0008	0.3818
49.3038	-0.7817	-1.5925	0.0290	0.3903
49.7848	-0.7674	-1.5981	0.0633	0.4000
50.2658	-0.7509	-1.6041	0.1023	0.4108
50.7468	-0.7322	-1.6108	0.1463	0.4230
51.2278	-0.7114	-1.6184	0.1956	0.4367
51.7089	-0.6883	-1.6272	0.2505	0.4520
52.1899	-0.6632	-1.6375	0.3112	0.4691
52.6709	-0.6358	-1.6496	0.3780	0.4881
53.1519	-0.6063	-1.6637	0.4511	0.5091
53.6329	-0.5747	-1.6798	0.5305	0.5321
54.1139	-0.5409	-1.6983	0.6164	0.5572
54.5949	-0.5050	-1.7191	0.7090	0.5845
55.0759	-0.4670	-1.7424	0.8083	0.6141
55.5570	-0.4269	-1.7682	0.9144	0.6458
56.0380	-0.3847	-1.7967	1.0272	0.6798
56.5190	-0.3404	-1.8278	1.1469	0.7161
57.0000	-0.2941	-1.8616	1.2734	0.7547

Supplementary material to Chapter 3

This supplement accompanies the manuscript entitled:

**Use of hard faecal prey remains to contribute to isotope-based dietary models
of sentinel marine predators**

**André van Tonder^{1*}, Bianca Coulson¹, Nico Lübcker^{1,2}, Ryan R. Reisinger^{1,3}, P.J.
Nico de Bruyn¹**

^{1.} Mammal Research Institute, Department of Zoology and Entomology,
University of Pretoria, Private Bag X2320, Hatfield, South Africa

^{2.} Center for Stable Isotopes, University of New Mexico, Albuquerque NM 87131,
USA

^{3.} National Oceanography Centre, University of Southampton Waterfront Campus
SO14 3ZH, England

SOURCE VALUES USED FOR MODELLING

The majority of sources used for the modelling of southern elephant seals at the Prince Edward Islands (PEIs) were obtained from published literature (Table 10, 11 and 12).

Sources and clustering of sources for juvenile, sub-adult and adult female southern elephant seals (*Mirounga leonina*) were similar and is combined in Table 10, while adult male southern elephant seals and killer whales (*Orcinus orca*) had distinct sources and clustering (Table 11 and 12).

The geometry of considered sources for killer whales did not allow for appropriate modelling, as some of the consumer values fell outside the simulated mixing space (Figure 10). For illustrative purposes the clustering and mixing geometry is shown in Figures 9 and 10.

Table 9: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of clustered potential prey items of juvenile, sub-adults and adult female southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. Prey items include novel data for myctophids from the Prince Edward Islands (PEI; Chapter 2) that replace conspecific data from other sites (Îles Crozet = CI and Îles Kerguelen = KI), as found in Lübcker et al. (2017). All values are either for whole organisms (krill) or estimates of muscle values for fish and squid.

	Type	n	Site	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Reference
Prey cluster 1						
<i>Electrona antarctica</i>	Fish	12	KI	-21.4 ± 0.5	8.9 ± 0.3	Cherel et al. 2010
<i>Electrona carlsbergi</i>	Fish	12	KI	-21.6 ± 0.4	9.5 ± 0.2	Cherel et al. 2010
<i>Gymnoscopelus bolini</i>	Fish	12	KI	-20.5 ± 0.4	9.9 ± 0.5	Cherel et al. 2010
<i>Gobionotothen marionensis</i>	Fish	5	PEI	-20.6 ± 0.4	8.5 ± 0.6	Bushula et al. 2005
<i>Protomyctophum andriashevi</i>	Fish	7	KI	-20.9 ± 0.3	8.7 ± 0.4	Cherel et al. 2010
<i>Protomyctophum choriodon</i>	Fish	12	KI	-20.0 ± 0.5	7.8 ± 0.3	Cherel et al. 2010
		60		-20.9 ± 0.7	8.9 ± 0.8	
Prey cluster 2						
<i>Electrona subaspera</i>	Fish	5	PEI	-23.7 ± 1.4	6.5 ± 1.9	Chapter 2
<i>Gymnoscopelus fraseri</i>	Fish	10	PEI	-23.4 ± 1.4	7.3 ± 2.0	Chapter 2
<i>Gymnoscopelus nicholsi</i>	Fish	9	PEI	-23.4 ± 1.6	8.3 ± 1.9	Chapter 2
		24		-23.5 ± 1.5	7.5 ± 2.1	
Prey cluster 3						
<i>Gymnoscopelus braueri</i>	Fish	12	KI	-22.3 ± 0.7	9.8 ± 0.3	Cherel et al. 2010
<i>Gymnoscopelus piabilis</i>	Fish	19	PEI	-21.8 ± 1.5	6.7 ± 2.5	Chapter 2
<i>Kreftichthys anderssoni</i>	Fish	12	KI	-22.3 ± 0.2	7.6 ± 0.2	Cherel et al. 2010
<i>Lepidonotothen larseni</i>	Fish	5	PEI	-22.1 ± 0.4	7.2 ± 0.8	Bushula et al. 2005
<i>Protomyctophum bolini</i>	Fish	12	KI	-22.4 ± 0.6	9.2 ± 0.4	Cherel et al. 2010
<i>Protomyctophum gemmatum</i>	Fish	4	KI	-22.1 ± 0.1	8.7 ± 0.4	Cherel et al. 2010
<i>Martialia hyadesi</i>	Squid	10	CI	-22.6 ± 0.4	8.5 ± 0.8	Guerreiro et al. 2015
		74		-22.2 ± 0.9	8.1 ± 1.8	
Prey cluster 4						
<i>Euphausia vallentini</i>	Krill	19	PEI	-22.6 ± 0.6	3.4 ± 0.5	Lübcker et al. 2017
<i>Euphausia frigida</i>	Krill	18	PEI	-22.3 ± 0.7	3.7 ± 0.6	Lübcker et al. 2017
		37		-22.5 ± 0.6	3.5 ± 0.6	
Prey cluster 5						
<i>Thysanoessa</i> sp.	Krill	18	PEI	-24.3 ± 0.3	5.6 ± 0.8	Lübcker et al. 2017

				18	-24.3 ± 0.3	5.6 ± 0.8	
	Prey cluster 6						
	<i>Moroteuthopsis longimana</i>	Squid	40	PEI	-24.8 ± 1.0	10.2 ± 0.7	van Tonder et al. 2021
			40		-24.8 ± 1.0	10.2 ± 0.7	

35

36

Table 10: Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope values of clustered potential prey items of adult male southern elephant seals (*Mirounga leonina*), for which considered prey was different than other classes. Prey items include novel data for myctophids from the Prince Edward Islands (PEI; Chapter 2) that replace conspecific data from other sites (Îles Crozet = CI and Îles Kerguelen = KI), as found in Lübcker et al. (2017). All values are either for whole organisms (krill) or estimates of muscle values for fish and squid.

	Type	n	Site	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Reference
Prey cluster 1						
<i>Electrona antarctica</i>	Fish	12	KI	-21.4 ± 0.5	8.9 ± 0.3	Cherel et al. 2010
<i>Electrona carlsbergi</i>	Fish	12	KI	-21.6 ± 0.4	9.5 ± 0.2	Cherel et al. 2010
<i>Gymnoscopelus bolini</i>	Fish	12	KI	-20.5 ± 0.4	9.9 ± 0.5	Cherel et al. 2010
<i>Gobionotothen marionensis</i>	Fish	5	PEI	-20.6 ± 0.4	8.5 ± 0.6	Bushula et al. 2005
<i>Protomyctophum andriashevi</i>	Fish	7	KI	-20.9 ± 0.3	8.7 ± 0.4	Cherel et al. 2010
<i>Protomyctophum choriodon</i>	Fish	12	KI	-20.0 ± 0.5	7.8 ± 0.3	Cherel et al. 2010
		60		-20.9 ± 0.7	8.9 ± 0.8	
Prey cluster 2						
<i>Electrona subaspera</i>	Fish	5	PEI	-23.7 ± 1.4	6.5 ± 1.9	Chapter 2
<i>Gymnoscopelus fraseri</i>	Fish	10	PEI	-23.4 ± 1.4	7.3 ± 2.0	Chapter 2
<i>Gymnoscopelus nicholsi</i>	Fish	9	PEI	-23.4 ± 1.6	8.3 ± 1.9	Chapter 2
<i>Thysanoessa</i> sp.	Krill	18	PEI	-24.3 ± 0.3	5.6 ± 0.8	Lübcker et al. 2017
		42		-23.8 ± 1.2	6.7 ± 1.9	
Prey cluster 3						
<i>Gymnoscopelus braueri</i>	Fish	12	KI	-22.3 ± 0.7	9.8 ± 0.3	Cherel et al. 2010
<i>Gymnoscopelus piabilis</i>	Fish	19	PEI	-21.8 ± 1.5	6.7 ± 2.5	Chapter 2
<i>Krefftichthys anderssoni</i>	Fish	12	KI	-22.3 ± 0.2	7.6 ± 0.2	Cherel et al. 2010
<i>Lepidonotothen larseni</i>	Fish	5	PEI	-22.1 ± 0.4	7.2 ± 0.8	Bushula et al. 2005
<i>Protomyctophum bolini</i>	Fish	12	KI	-22.4 ± 0.6	9.2 ± 0.4	Cherel et al. 2010
<i>Protomyctophum gemmatum</i>	Fish	4	KI	-22.1 ± 0.1	8.7 ± 0.4	Cherel et al. 2010
<i>Martialia hyadesi</i>	Squid	10	CI	-22.6 ± 0.4	8.5 ± 0.8	Guerreiro et al. 2015
		74		-22.2 ± 0.9	8.1 ± 1.8	
Prey cluster 4						
<i>Galiteuthis glacialis</i>	Squid	10	CI	-21.3 ± 0.5	13.8 ± 0.6	Guerreiro et al. 2015
<i>Histioteuthis eltaninae</i>	Squid	10	CI	-21.5 ± 0.6	13.9 ± 0.3	Guerreiro et al. 2015
<i>Mastigoteuthis</i> sp. A (Clarke)	Squid	10	CI	-21.5 ± 0.5	13.8 ± 0.7	Guerreiro et al. 2015

<i>Alluroteuthis antarcticus</i>	Squid	10	CI	-21.3 ± 1.4	13.1 ± 0.2	Guerreiro et al. 2015
<i>Moroteuthis ingens</i>	Squid	10	CI	-21.8 ± 0.4	12.8 ± 0.2	Guerreiro et al. 2015
<i>Moroteuthis knipovitchi</i>	Squid	10	CI	-22.1 ± 1.5	12.9 ± 0.5	Guerreiro et al. 2015
		60		-21.6 ± 1.0	13.4 ± 0.7	
Prey cluster 5						
<i>Moroteuthopsis longimana</i>	Squid	40	PEI	-24.8 ± 1.0	10.2 ± 0.7	van Tonder et al. 2021
		40		-24.8 ± 1.0	10.2 ± 0.7	

Table 11: Stable isotope values of potential prey items of killer whales (*Orcinus orca*) as found in Reisinger et al. (2016). AM = adult male, UY = under yearling, Y = yearling, SA = sub-adult, AF = adult female. Prey items include novel data for myctophids from the Prince Edward Islands (PEI; Chapter 2) that replace conspecific data from other sites (Îles Crozet = CI and Îles Kerguelen = KI)

	Type	n	Site	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Reference
Prey cluster 1						
<i>Arctocephalus tropicalis</i>	Seal	13	PEI	-20.5 ± 0.5	12.1 ± 0.3	Reisinger et al. 2016
<i>Mirounga leonina</i> UY	Seal	5	PEI	-21.5 ± 0.4	11.9 ± 0.2	Reisinger et al. 2016
<i>Mirounga leonina</i> Y	Seal	5	PEI	-21.1 ± 0.7	11.3 ± 0.5	Reisinger et al. 2016
<i>Mirounga leonina</i> SA	Seal	5	PEI	-20.6 ± 0.4	11.2 ± 0.3	Reisinger et al. 2016
<i>Mirounga leonina</i> AF	Seal	5	PEI	-20.4 ± 0.8	11.1 ± 0.6	Reisinger et al. 2016
<i>Mirounga leonina</i> AM	Seal	5	PEI	-19.8 ± 1.0	12.7 ± 0.6	Reisinger et al. 2016
<i>Dissostychus elegenoides</i>	Fish	10	PEI	-20.2 ± 0.6	12.7 ± 0.6	Reisinger et al. 2016
		48		-20.5 ± 0.8	12.0 ± 0.7	
Prey cluster 2						
<i>Aptenodytes patagonicus</i>	Penguin	8	PEI	-22.3 ± 0.1	10.5 ± 0.3	Reisinger et al. 2016
<i>Eudyptes chrysolophus</i>	Penguin	5	PEI	-22.5 ± 0.1	9.2 ± 0.3	Reisinger et al. 2016
<i>E. c. filholi</i>	Penguin	7	PEI	-22.6 ± 0.4	8.5 ± 0.6	Reisinger et al. 2016
		20		-22.5 ± 0.3	9.5 ± 1.0	
Prey cluster 3						
<i>Electrona subaspera</i>	Fish	8	PEI	-23.5 ± 1.3	7.7 ± 1.9	Chapter 2
<i>Gymnoscopelus fraseri</i>	Fish	11	PEI	-23.4 ± 1.3	7.1 ± 2.2	Chapter 2
<i>Gymnoscopelus piabilis</i>	Fish	29	PEI	-22.9 ± 1.4	6.7 ± 2.2	Chapter 2
		48		-23.1 ± 1.4	7.0 ± 2.2	
Prey cluster 4						
<i>Gymnoscopelus nicholsi</i>	Fish	14	PEI	-24.5 ± 1.4	8.3 ± 2.2	Chapter 2
		14		-24.5 ± 1.4	8.3 ± 2.2	

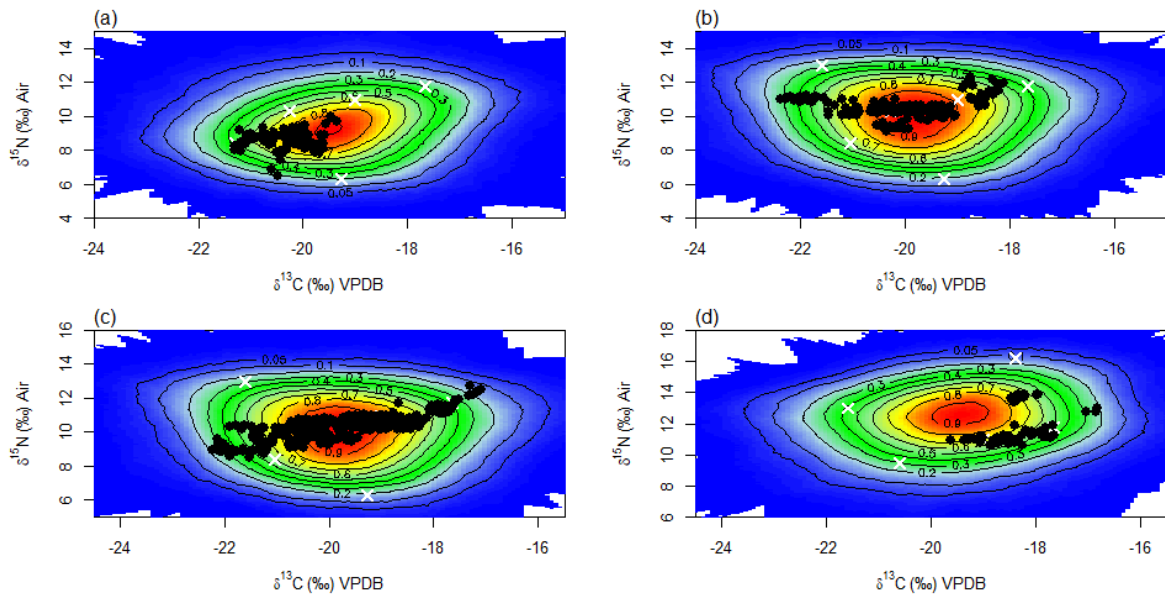


Figure 9: Probability distributions of mixing polygons produced by source clusters of (a) juvenile, (b) sub-adult, (c) adult female and (d) adult male southern elephant seals (*Mirounga leonina*) at the sub-Antarctic Prince Edward Islands. Mixing spaces make use of source values adjusted for trophic discrimination factors (TDFs) between diet and the sampled whiskers tissue (black dots) of seals. Sources were clustered into groups (white crosses), which were similar for juvenile, sub-adults and adult female seals (though juveniles had only five clusters). The composition of clusters is reported in Table 5 and 10.

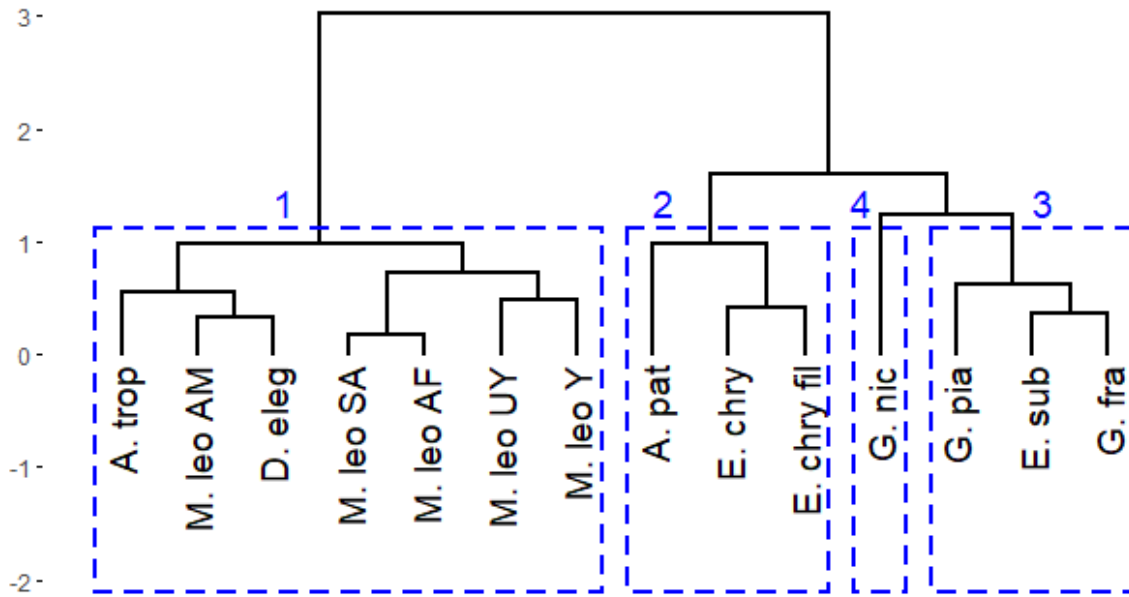
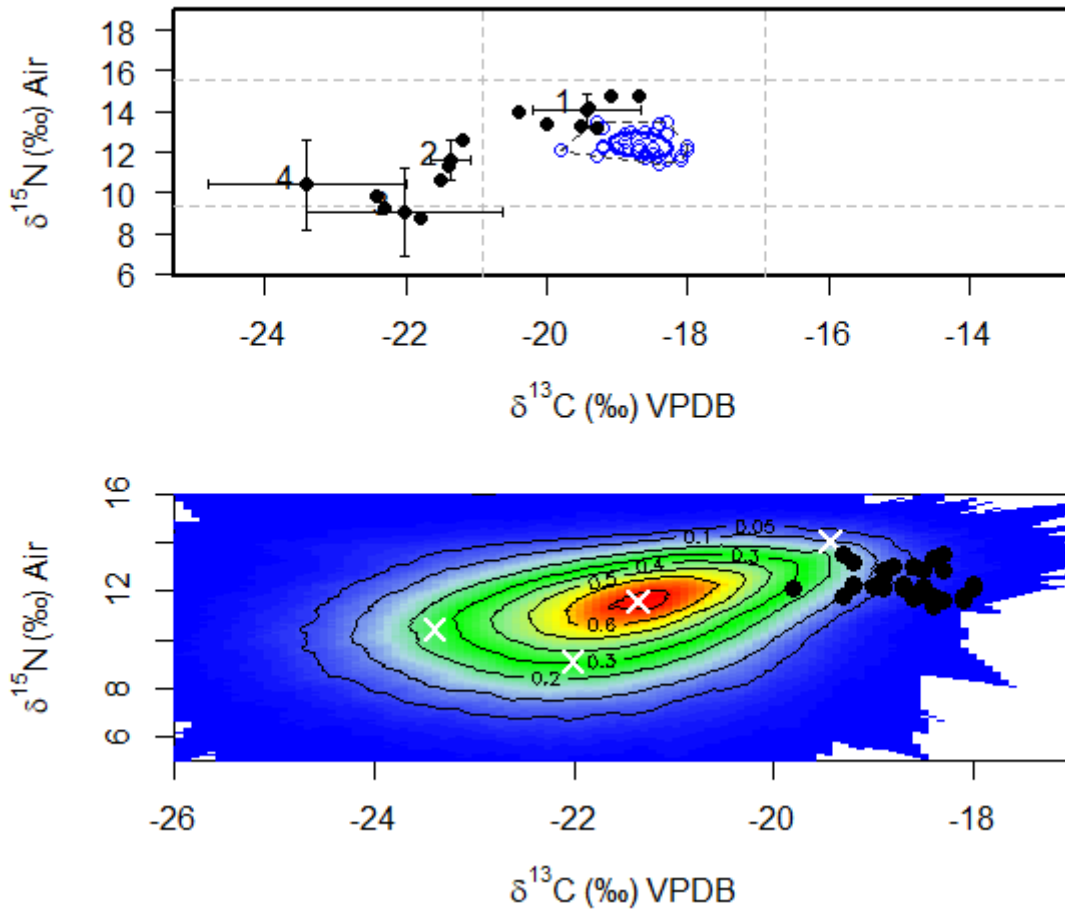


Figure 10: Dendrograms indicating the composition of potential prey clusters for killer whales (*Orcinus orca*) at the sub-Antarctic Prince Edward Islands. The constituent species of each cluster and numbers assigned to the clusters can be found in Table 12.

68



69

Figure 11: Graphical representation of the stable isotope mixing model for killer whales (*Orcinus orca*) at the sub-Antarctic Prince Edward Islands (PEIs). The black squares represent clusters of potential prey values and one standard deviation, after applying the appropriate trophic discrimination factor (TDF), but not incorporating TDF uncertainty (Hobson et al. 1996, L  bcker et al. 2017). The sources in these models include values for prey sampled around the PEIs specifically. The dashed grey boxes are the bounds within which potential source values, adjusted for TDF, were considered. The numbers assigned to the clusters can be found in Table 12.

The lower image shows probability distributions of mixing polygons produced by source clusters of killer whales at the PEIs. Mixing spaces make use of source values adjusted for trophic discrimination factors between diet and the sampled skin tissue (black dots) of killer whales, incorporating uncertainty (1 standard deviation) in both sources and TDFs. Sources were clustered into groups (white crosses), the composition of which is reported in Table 12.

83

Literature cited

- Adams NJ, Klages NT (1987) Seasonal variation in the diet of the king penguin (*Aptenodytes patagonicus*) at sub-Antarctic Marion Island. *J Zool* 212:303–324.
- Alewijnse SR, Stowasser G, Saunders RA, Belcher A, Crimmen OA, Cooper N, Trueman C (2021) Otolith-derived field metabolic rates of myctophids (Family Myctophidae) from the Scotia Sea (Southern Ocean). *Mar Ecol Prog Ser* 675:113-131.
- Ankjærø T, Christensen JT, Grønkjær P (2012) Tissue-specific turnover rates and trophic enrichment of stable N and C isotopes in juvenile Atlantic cod *Gadus morhua* fed three different diets. *Mar Ecol Prog Ser* 461:197–209.
- Ansorge IJ, Durgadoo JV, Treasure AM (2014) Sentinels to climate change. The need for monitoring at South Africa's Subantarctic laboratory. *S Afr J Sci* 110:1– 4.
- Ansorge IJ, Froneman PW, Durgadoo JV (2012) The marine ecosystem of the sub- Antarctic, Prince Edward Islands. In: *Marine Ecosystems*. Cruzado A (ed) InTech Press, Rijeka
- Antonelis Jr GA, Lowry MS, de Master DP, Fiscus CH (1987) Assessing northern elephant seal feeding habits by stomach lavage. *Mar Mamm Sci* 3:308–322.
- Belcher A, Saunders RA, Tarling GA (2019) Respiration rates and active carbon flux of mesopelagic fishes (Family Myctophidae) in the Scotia Sea, Southern Ocean. *Mar Ecol Prog Ser* 610:149-162.
- Best PB, Meýer MA, Lockyer C (2010) Killer whales in South African waters — a review of their biology. *Afr J Mar Sci* 32:171–186.
- Bester MN (1988) Chemical restraint of Antarctic fur seals and southern elephant seals. *S Afr J Wildl Res* 18:57–60.

- Bester MN, Connan M (2022) Cephalopod diet of juvenile male southern elephant seals *Mirounga leonina* at Marion Island, South Indian Ocean. Polar Biol <https://doi.org/10.1007/s00300-022-03070-z> S.
- Blanchet-Aurigny A, Guillou M, Pernet F, Gaffet J, Dubois SF (2012) Tissue-diet discrimination factors of isotopic ratios ($\Delta\delta^{13}\text{C}$ and $\Delta\delta^{15}\text{N}$) in two brittle star species: Effect of reproductive state, diet and tissue composition. J Exp Mar Bio Ecol 426–427:68–77.
- Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42.
- Bond AL, Diamond AW (2011) Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol Appl 21:1017–1023.
- Bright J, Kaufman DS (2011) Amino acid racemization in lacustrine ostracodes, part I: effect of oxidizing pre-treatments on amino acid composition. Quat Geochronol 6:154–173.
- Brown CR, Klages NT (1987) Seasonal and annual variation in diets of Macaroni (*Eudyptes chrysolophus chrysolophus*) and Southern rockhopper (*E. chrysocome chrysocome*) penguins at sub-Antarctic Marion Island. J Zool 212:7–28.
- Browning NE, Dold C, I-Fan J, Worthy GAJ (2014) Isotope turnover rates and diet- tissue discrimination in skin of ex situ bottlenose dolphins (*Tursiops truncatus*). J Exp Biol 217:214–221.
- 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 nototheniid fish on the shelf of the sub-Antarctic Prince Edward Islands. *Polar Biol* 28:585–593.
- Carpenter-Kling T, Pistorius P, Reisinger R, Cherel Y, Connan M (2020) A critical assessment of marine predator isoscapes within the southern Indian Ocean. *Mov Ecol* 8:<https://doi.org/10.1186/s40462-40020-00208-40468>.
- Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *J Appl Ecol* 46:443–453.
- 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, Fontaine C, Richard P, Labat JP (2010) Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean. *Limnol Oceanogr* 55:324–332.
- 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.
- Choy CA, Popp BN, Hannides CCS, Drazen JC (2015) Trophic structure and food resources of epipelagic and mesopelagic fishes in the North Pacific Subtropical Gyre ecosystem inferred from nitrogen isotopic compositions. *Limnol Oceanogr* 60:1156–1171.
- Condy PR, van Aarde RJ, Bester MN (1978) The seasonal occurrence and behaviour of Killer whales *Orcinus orca*, at Marion Island. *J Zool* 184:449–464.

- Croxall JP, Davis RW, O'Connell MJ (1988) Diving patterns in relation to diet of gentoo and macaroni penguins at South Georgia. *Condor* 90:157–167.
- Cury PM, Boyd IL, Bonhommeau S, Anker-Nilssen T, Crawford RJM, Furness RW, Mills JA, Murphy EJ, Österblom H, Paleczny M, Piatt JF, Roux JP, Shannon L, Sydeman WJ (2011) Seabird response to forage fish depletion - one-third for the birds. *Science* 334:1703–1706.
- Daneri GA, Carlini AR (2002) Fish prey of southern elephant seals, *Mirounga leonina*, at King George Island. *Polar Biol* 25:739–743.
- Dauphin Y, Dufour E (2003) Composition and properties of the soluble organic matrix of the otolith of a marine fish: *Gadus morhua* Linne, 1758 (Teleostei, Gadidae). *Comp Biochem Physiol Part A* 134:551–561.
- de Bruyn PJN, Bester MN, Mecenero S, Kirkman SP, Roux JP, Klages NTW (2003) Temporal variation of cephalopods in the diet of Cape fur seals in Namibia. *S Afr J Wildl Res* 33:85–96.
- de Bruyn PJN, Tosh CA, Oosthuizen C, Bester MN, Arnould JPY (2009) Bathymetry and frontal system interactions influence seasonal foraging movements of lactating subantarctic fur seals from Marion Island. *Mar Ecol Prog Ser* 394:263–276.
- 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.
- de Lima RC, Cebuhar JD, Negrete J, Ferreira A, Secchi ER, Botta S (2022) Ecosystem shifts inferred from long-term stable isotope analysis of male Antarctic fur seal *Arctocephalus gazella* teeth. *Mar Ecol Prog Ser* 695:203-216.

- de Niro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim Cosmochim Acta* 45:341–351.
- de Niro MJ, Epstein S (1978) Influence of diet on the distribution of carbon isotopes in animals. *Geochim Cosmochim Acta* 42:495–506.
- Eggers T, Jones TH (2000) You are what you eat...or are you? *Trends Ecol Evol* 5:265–266.
- Elsdon TS, Ayvazian S, McMahon KW, Thorrold SR (2010) Experimental evaluation of stable isotope fractionation in fish muscle and otoliths. *Mar Ecol Prog Ser* 408:195–205.
- Estrela C, Estrela CRA, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD (2002) Mechanism of action of sodium hypochlorite. *Braz Dent J* 13:113–117.
- Farrance I, Frenkel R (2012) Uncertainty of measurement: A review of the rules for calculating uncertainty components through functional relationships. *Clin Biochem Rev* 33:49–75.
- Field IC, Bradshaw CJA, van den Hoff J, Burton HR, Hindell MA (2007) Age-related shifts in the diet composition of southern elephant seals expand overall foraging niche. *Mar Biol* 150:1441–1452.
- Gloeckler K, Choy CA, Hannides CCS, Close HG, Coetze E, Popp BN, Drazen JC (2018) Stable isotope analysis of micronekton around Hawaii reveals suspended particles are an important nutritional source in the lower mesopelagic and upper bathypelagic zones. *Limnol Oceanogr* 63:1168–1180.
- Goericke R, Fry B (1994) Variations of marine plankton $\delta^{13}\text{C}$ with latitude, temperature, and dissolved CO_2 in the world ocean. *Global Biogeochem Cycles* 8:85–90.
- Gon O, Heemstra P (1990) *Fishes of the Southern Ocean*. J.L.B. Smith Institute of Ichthyology, Grahamstown.

- Graham BS, Koch PL, Newsome SD, McMahon KW, Aurioles D (2010) Using isoscapes to trace the movements and foraging behavior of top predators in oceanic ecosystems. In: *Isoscapes*. West JB, Bowen GJ, Dawson TE, Tu KP (eds) Springer, Dordrecht
- Greely TM, Gartner JV, Torres JJ (1999) Age and growth of *Electrona antarctica* (Pisces: Myctophidae), the dominant mesopelagic fish of the Southern Ocean. *Mar Biol* 133:145–158.
- 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.
- Grémillet D, Charmantier A (2010) Shifts in phenotypic plasticity constrain the value of seabirds as ecological indicators of marine ecosystems. *Ecol Appl* 20:1498–1503.
- Grønkvær P, Pedersen JB, Ankjærø TT, Kjeldsen H, Heinemeier J, Steingrund P, Nielsen JM, Christensen JT (2013) Stable N and C isotopes in the organic matrix of fish otoliths: validation of a new approach for studying spatial and temporal changes in the trophic structure of aquatic ecosystems. *Can J Fish Aquat Sci* 70:143–146.
- 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.
- Hannides CCS, Popp BN, Choy CA, Drazen JC (2013) Mid-water zooplankton and suspended particle dynamics in the North Pacific Subtropical Gyre: a stable isotope perspective. *Limnol Oceanogr* 58:1931–1946.

- Healy K, Guillerme T, Kelly SBA, Inger R, Bearhop S, Jackson AL (2018) SIDER: an R package for predicting trophic discrimination factors of consumers based on their ecology and phylogenetic relatedness. *Ecography (Cop)* 41:1396–1400.
- Hecht T (1987) A guide to the otoliths of Southern Ocean fishes. *S Afr J Antarct Res* 17:2–85.
- Hobson KA, Cherel Y (2006) Isotopic reconstruction of marine food webs using cephalopod beaks: new insight from captively 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*:528– 533.
- Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE, Costa DP (2012) Stable isotope analyses reveal individual variability in the trophic ecology of a top marine predator, the southern elephant seal. *Oecologia* 169:395–406.
- Hulley PA, Duhamel G (2011) Aspects of lanternfish distribution in the Kerguelen Plateau region. *Kerguelen Plateau Mar Ecosyst Fish*:183–195.
- Ingalls AE, Lee C, Druffel RM (2003) Preservation of organic matter in mound- forming coral skeletons. *Geochim Cosmochim Acta* 67:2827–2841.
- Irigoiien X, Klevjer TA, Røstad A, Martinez U, Boyra G, Acuña JL, Bode A, Echevarria F, Gonzalez-Gordillo JI, Hernandez-Leon S, Agusti S, Aksnes DL, Duarte CM, Kaartvedt S (2014) Large mesopelagic fishes biomass and trophic efficiency in the open ocean. *Nat Commun* 5:DOI: 10.1038/ncomms4271.

- 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* 24:3373–3497.
- Kaehler S, Pakhomov EA, Kalin RM, Davis S (2006) Trophic importance of kelp- derived suspended particulate matter in a through-flow sub-Antarctic system. *Mar Ecol Prog Ser* 316:17–22.
- Kaehler S, Pakhomov EA, McQuaid CD (2000) Trophic structure of the marine food web at the Prince Edward Islands (Southern Ocean) determined by $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar Ecol Prog Ser* 208:13–20.
- Karnovsky NJ, Hobson KA, Iverson SJ (2012) From lavage to lipids: estimating diets of seabirds. *Mar Ecol Prog Ser* 451:263–284.
- Kernaléguen L, Dorville N, Ierodiaconou D, Hoskins AJ, Baylis AMM, Hindell MA, Semmens J, Abernathy K, Marshall GJ, Cherel Y, Arnould JPY (2016) From video recordings to whisker stable isotopes: a critical evaluation of timescale in assessing individual foraging specialisation in Australian fur seals. *Oecologia* 180:657–670.
- Kim TK (2017) Understanding one-way ANOVA using conceptual figures. *Korean J Anaesthesiol* 70:22–26.
- Kirkman SP, Yemane DG, Lamont T, Meýer MA, Pistorius PA (2016) Foraging behaviour of Subantarctic fur seals supports efficiency of a marine reserve's design. *PLoS One* 11:e0152370.

- Koubbi P, Hulley P., Pruvost P, Henri P, Labat JP, Wadley V, Hirano D, Moteki M (2011) Size distribution of meso- and bathypelagic fish in the Dumont d'Urville Sea (East Antarctica) during the CEAMARC surveys. *Polar Sci* 5:195–210.
- Kurle CM, McWorther JK (2017) Spatial and temporal variability within marine isoscapes: implications for interpreting stable isotope data from marine systems. *Mar Ecol Prog Ser* 568:31–45.
- Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol Rev* 87:545–562.
- Lea MA, Cherel Y, Guinet C, Nichols PD (2002) Antarctic fur seals foraging in the Polar Frontal Zone: inter-annual shifts in diet as shown from fecal and fatty acid analyses. *Mar Ecol Prog Ser* 245:281–297.
- Lombard AT, Reyers B, Schonegevel L, Cooper J, Smith-Adao L, Nel D, Froneman W, Ansorge I, Bester M, Tosh C, Strauss T, Akkers T, Gon O, Leslie R, Chown S (2007) Conserving pattern and process in the Southern Ocean: designing a Marine Protected Area for the Prince Edward Islands. *Antarct Sci* 19:39–54.
- Lourey MJ, Trull TW, Sigman DM (2003) Sensitivity of $\delta^{15}\text{N}$ of nitrate, surface suspended and deep sinking particulate nitrogen to seasonal nitrate depletion in the Southern Ocean. *Global Biogeochem Cycles* 17:doi:10.1029/2002GB001973.
- Lowen T (2016) The use of otolith micro-chemical techniques to examine trace element residence time, migration, and population discrimination of teleost fishes in the Canadian polar north. PhD dissertation, University of Manitoba, Winnipeg, Canada

- Lübcker N, Reisinger RR, Oosthuizen WC, de Bruyn PJN, van Tonder A, Pistorius PA, Bester MN (2017) Low trophic level diet of juvenile southern elephant seals *Mirounga leonina* from Marion Island: a stable isotope investigation using vibrissal regrowths. *Mar Ecol Prog Ser* 577:237–225.
- Lübcker N, Whiteman JP, Millar RP, de Bruyn PJN, Newsome SD (2020) Fasting affects amino acid nitrogen isotope values: a new tool for identifying nitrogen balance of free-ranging mammals. *Oecologia* 193:53–65.
- Lueders-Dumont JA, Sigman DM, Johnson BJ, Jensen OP, Oleynik S, Ward BB (2020) Comparison of the isotopic composition of fish otolith-bound organic N with host tissue. *Can J Fish Aquat Sci* 77:264–275.
- Lueders-Dumont JA, Wang XT, Jensen OP, Sigman DM, Ward BB (2018) Nitrogen isotopic analysis of carbonate-bound organic matter in modern and fossil fish otoliths. *Geochim Cosmochim Acta* 224:200–222.
- MacKenzie KM, Palmer MR, Moore A, Ibbotson AT, Beaumont WRC, Poulter DJS, Trueman CN (2011) Locations of marine animals revealed by carbon isotopes. *Sci Rep* 1:DOI: 10.1038/srep00021.
- Makhado AB, Bester MN, Kirkman SP, Pistorius PA, Ferguson JWH, Klages NTW (2007) Prey of the Antarctic fur seal *Arctocephalus gazella* at Marion Island. *Polar Biol* 31:575–581.
- Makhado AB, Bester MN, Somhlaba S, Crawford RJM (2013) The diet of the subantarctic fur seal *Arctocephalus tropicalis* at Marion Island. *Polar Biol* 36:1609–1617.
- Mannella G, Zanchetta G, Regattieri E, Perchiazzi N, Drysdale NR, Giaccio B, Leng MJ, Wagner B (2019) Effects of organic removal techniques prior to carbonate stable

isotope analysis of lacustrine marls: A case study from palaeo-lake Fucino (central Italy). *Rapid Commun Mass Spectrom* 34:e8623.

McCormack SA, Melbourne-Thomas J, Trebilco R, Blanchard JL, Raymond B, Constable A (2020) Decades of dietary data demonstrate regional food web structures in the Southern Ocean. *Ecol Evol* 11:227–241.

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378–390.

Mengesha S, Dehairs F, Fiala M, Elskens M, Goeyens L (1998) Seasonal variation of phytoplankton community structure and nitrogen uptake regime in the Indian Sector of the Southern Ocean. *Polar Biol* 20:259–272.

Mill AC, Pinnegar JK, Polunin NVC (2007) Explaining isotope trophic-step fractionation: why herbivorous fish are different. *Funct Ecol* 21:1137–1145.

Minagawa M, Wada E (1984) Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. *Geochim Cosmochim Acta* 48:1135–1140.

Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into stable isotope mixing model. *Ecol Lett* 11:470–480.

Murayama E, Herbomel P, Kawakami A, Takeda H, Nagasawa H (2005) Otolith matrix proteins OMP-1 and Otolin-1 are necessary for normal otolith growth and their correct anchoring onto the sensory maculae. *Mech Dev* 122:791–803.

Murayama E, Takagi Y, Ohira T, Davis JG, Greene MI, Nagasawa H (2002) Fish otolith contains a unique structural protein, otolin-1. *Eur J Biochem* 269:688–696.

- Murphy EJ, Johnston NM, Hofmann EE, Phillips RA, Jackson JA, Constable AJ, Henley SF, Melbourne-Thomas J, Trebilco R, Cavanagh RD, Tarling GA, Saunders RA, Barnes DKA, Costa DP, Corney SP, Fraser CI, Höfer J, Hughes KA, Sands CJ, Thorpe SE, Trathan PN, Xavier XC (2021) Global connectivity of Southern Ocean ecosystems. *Front Ecol Evol* 9:doi: 10.3389/fevo.2021.624451.
- Nel DC, Lutjeharms JRE, Pakhomov EA, Ansorge IJ, Ryan PG, Klages NTW (2001) Exploitation of mesoscale oceanographic features by grey-headed albatross *Thalassarche chrysostoma* in the southern Indian Ocean. *Mar Ecol Prog Ser* 217:15–26.
- Nel DC, Nel JL, Ryan PG, Klages NTW, Wilson RP, Robertson G (2000) Foraging ecology of grey-headed mollymawks at Marion Island, southern Indian Ocean, in relation to longline fishing activity. *Biol Conserv* 96:219–231.
- Newland C, Field IC, Cherel Y, Guinet C, Bradshaw CJA, McMahon C, Hindell MA (2011) Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. *Mar Ecol Prog Ser* 424:247–258.
- Oosthuizen WC, de Bruyn PJN, Bester MN, Girondot M (2010) Cohort and tag-site- specific tag-loss rates in mark recapture studies: A southern elephant seal cautionary case. *Mar Mamm Sci* 26:350–369.
- Österblom H, Olsson O, Blenckner T, Furness RW (2008) Junk-food in marine ecosystems. *Oikos* 117:967–977.
- 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.

- Park JH, Durand I (2019) Altimetry-derived Antarctic Circumpolar Current fronts. DOI: 10.17882/59800. <https://www.seanoe.org/data/00486/59800/> (accessed 21 July 2022)
- Park JH, Park T, Kim T, Lee SH, Hong CS, Lee JH, Rio MH, Pujol MI, Ballarotta M, Durand I, Provost C (2019) Observations of the Antarctic Circumpolar Current over the Udintsev Fracture Zone, the narrowest choke point in the Southern Ocean. *J Geophys Res Ocean* 124:4511–4528.
- Parnell A (2008) SIAR: stable isotope analysis in R.
- Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. *PLoS One* 5:e9672.
- Pecquerie L, Nisbet RM, Fablet R, Lorrain A, Kooijman SALM (2010) The impact of metabolism on stable isotope dynamics: a theoretical framework. *Philos Trans R Soc B* 365:3455–3468.
- Perissinotto R, Lutjeharms JRE, van Ballegooyen RC (2000) Biological–physical interactions and pelagic productivity at the Prince Edward Islands, Southern Ocean. *J Mar Syst* 24:327–341.
- Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18:293–320.
- Phillips DL, Gregg JW (2003) Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136:261–269.
- Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food- web studies. *Can J Fish Aquat Sci* 92:823–835.

- Pistorius PA, de Bruyn PJN, Bester MN (2011) Population dynamics of southern elephant seals: a synthesis of three decades of demographic research at Marion Island. *Afr J Mar Sci* 33:523–534.
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718.
- Prince Edward Islands Management Plan Working Group (1996) Prince Edward Islands Management Plan. Cameron CM (ed) Department of Environmental Affairs and Tourism, Pretoria.
- R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing.
- Reisinger RR, de Bruyn PJN, Tosh CA, Oosthuizen WC, Mufanadzo NT, Bester MN (2011) Prey and seasonal abundance of killer whales at sub-Antarctic Marion Island. *Afr J Mar Sci* 33:99–105.
- Reisinger RR, Corney S, Raymond B, Lombard AT, Bester MN, Crawford RJM, Davies D, de Bruyn PJN, Dilley BJ, Kirkman SP, Makhado AB, Ryan PG, Schoombie S, Stevens KL, Tosh CA, Wege M, Whitehead TO, Sumner MD, Wotherspoon S, Friedlander AS, Cotté C, Hindell MA, Ropert-Coudert Y, Pistorius PA (2022) Habitat model forecasts suggest potential redistribution of marine predators in the southern Indian Ocean. *Divers Distrib* 28:142–159.
- Reisinger RR, Gröcke DR, Lübcker N, McClymont EL, Hoelzel AR, de Bruyn PJN (2016) Variation in the diet of killer whales *Orcinus orca* at Marion Island, Southern Ocean. *Mar Ecol Prog Ser* 549:263–274.

- Reisinger RR, Keith M, Andrews RD, de Bruyn PJN (2015) Movement and diving of killer whales (*Orcinus orca*) at a Southern Ocean archipelago. *J Exp Mar Bio Ecol* 473:90–102.
- Reisinger RR, Landman M, Bester MN, de Bruyn N (2018a) Overlap and temporal variation in the diets of sympatric Antarctic and Subantarctic fur seals (*Arctocephalus* spp.) at Marion Island, Prince Edward Islands. *Polar Res* 37:1451142.
- Reisinger RR, Raymond B, Hindell MA, Bester MN, Crawford RJM, Davies D, de Bruyn PJN, Dilley BJ, Kirkman SP, Makhado AB, Ryan PG, Schoombie S, Stevens K, Sumner MD, Tosh C, Wege M, Whitehead TO, Wotherspoon S, Pistorius PA (2018b) Habitat modelling of tracking data from multiple marine predators identifies important areas in the Southern Indian Ocean. *Divers Distrib* 24:535–550.
- Ren H, Sigman DM, Meckler AN, Plessen B, Robinson RS, Rosenthal Y, Haug GH (2009) Foraminiferal isotope evidence of reduced nitrogen fixation in the Ice Age Atlantic Ocean. *Science* 323:244–248.
- Richoux NB, Froneman PW (2009) Plankton trophodynamics at the subtropical convergence, Southern Ocean. *J Plankton Res* 31:1059–1073.
- Richoux NB, Jaquemet S, Bonnevie BT, Cherel Y, McQuaid CD (2010) Trophic ecology of Grey-headed albatrosses from Marion Island, Southern Ocean: insights from stomach contents and diet tracers. *Mar Biol* 157:1755–1766.
- Romero-Romero S, Choy CA, Hannides CCS, Popp BN, Drazen JC (2019) Differences in the trophic ecology of micronekton driven by diel vertical migration. *Limnol Oceanogr* 64:1473–1483.

- Rowell K, Dettman DL, Dietz R (2010) Nitrogen isotopes in otoliths reconstruct ancient trophic position. *Environ Biol Fishes* 89:415–425.
- RStudio Team (2020) RStudio: Integrated Development for R.
- Saunders RA, Collins MA, Foster E, Shreeve R, Stowasser G, Ward P, Tarling GA (2014) The trophodynamics of Southern Ocean *Electrona* (Myctophidae) in the Scotia Sea. *Polar Biol* 37:789–807.
- Saunders RA, Collins MA, Ward P, Stowasser G, Shreeve R, Tarling GA (2015) Distribution, population structure and trophodynamics of Southern Ocean *Gymnoscopelus* (Myctophidae) in the Scotia Sea. *Polar Biol* 38:287–308.
- Saunders RA, Hill SL, Tarling GA, Murphy EJ (2019) Myctophid fish (Family Myctophidae) are central consumers in the food web of the Scotia Sea (Southern Ocean). *Front Mar Sci* 6:<https://doi.org/10.3389/fmars.2019.0053>.
- Saunders RA, Tarling GA (2018) Southern Ocean mesopelagic fish comply with Bergmann's Rule. *Am Nat* 191:343–351.
- Shreeve RS, Collins MA, Tarling GA, Main CE, Ward P, Johnston NM (2009) Feeding ecology of myctophid fishes in the northern Scotia Sea. *Mar Ecol Prog Ser* 386:221–236.
- Sirot C, Grønkjær P, Pedersen JB, Panfili J, Zetina-Rejon M, Tripp-Valdez A, Ramos-Miranda J, Flores-Hernandez D, Sosa-Lopez A, Darnaude AM (2017) Using otolith organic matter to detect diet shifts in *Bardiella chrysoura*, during a period of environmental changes. *Mar Ecol Prog Ser* 575:137–152.
- Slip D (1995) The diet of southern elephant seals (*Mirounga leonina*) from Heard Island. *Can J Zool* 73:1519–1528.

- Smale M, Hecht T, Watson GW (1995) Otolith atlas of southern African marine fishes. J.L.B. Smith Institute of Ichthyology, Grahamstown.
- Smith JA, Mazumder D, Suthers IM, Taylor MD (2013) To fit or not to fit: evaluating stable isotope mixing models using simulated mixing polygons. *Methods Ecol Evol* 4:612–618.
- Spanó JCE, Barbin EL, Santos TC, Guimarães LF, Pécora JD (2001) Solvent action of sodium hypochlorite on bovine pulp and physico-chemical properties of resulting liquid. *Braz Dent J* 12:154–157.
- St John Glew K, Espinasse B, Hunt BP, Pakhomov EA, Bury SJ, Pinkerton M, Nodder SD, Gutiérrez-Rodríguez A, Safi K, Brown JC, Graham L (2021) Isoscape models of the Southern Ocean: Predicting spatial and temporal variability in carbon and nitrogen isotope compositions of particulate organic matter. *Global Biogeochem Cycles* 35:e2020GB006901.
- Trull TW, Davies D, Casciotti K (2008) Insights into nutrient assimilation and export in naturally iron-fertilized waters of the Southern Ocean from nitrogen, carbon and oxygen isotopes. *Deep Sea Res II* 55:820–840.
- van den Hoff J (2004) A 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 de Putte A, Flores H, Volckaert F, van Franeker JA (2006) Energy content of Antarctic mesopelagic fishes: implications for the marine food web. *Polar Biol* 29:1045–1051.
- Vandermyde JM, Whitledge GW (2008) Otolith $\delta^{15}\text{N}$ distinguishes fish from forested and agricultural streams in southern Illinois. *J Freshw Ecol* 23:333–336.

- Vander Zanden MJ, Rasmussen JB (2001) Variation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ trophic fractionation: Implications for aquatic food web studies. *Limnol Oceanogr* 46:2061–2066.
- van Tonder A, Lübcker N, Guerreiro M, Xavier JC, Cherel Y, de Bruyn PJN (2021) Ecology of *Moroteuthopsis longimana* at the sub-Antarctic Prince Edward Islands, revealed through stable isotope analysis of squid beaks. *Mar Ecol Prog Ser* 658:105–115.
- von Biela VR, Newsome SD, Zimmerman CE (2015) Examining the utility of bulk otolith $\delta^{13}\text{C}$ to describe diet in wild-caught black rockfish *Sebastes melanops*. *Aquat Biol* 23:201–208.
- Walters A (2014) Quantifying the trophic linkages of Antarctic marine predators. PhD dissertation, University of Tasmania, Hobart, Australia
- Walters A, Lea M, Van den Hoff J, Field IC, Virtue P, Sokolov S, Pinkerton HP, Hindell MA (2014) Spatially explicit estimates of prey consumption reveal a new krill predator in the Southern Ocean. *PLoS One* 9:e86452.
- Waluda CM, Hill SL, Peat HJ, Trathan PN (2012) Diet variability and reproductive performance of macaroni penguins *Eudyptes chrysolophus* at Bird Island, South Georgia. *Mar Ecol Prog Ser* 466:261–274.
- Wang X, Ma J, Wang Z, Chen H, Liu M, Wu Z (2018) Reinvestigation of membrane cleaning mechanisms using NaOCl: Role of reagent diffusion. *J Memb Sci* 550:278–285.
- Wang XT, Prokopenko MG, Sigman DM, Adkins JF, Robinson LF, Ren H, Oleynik S, Williams B, Haug GH (2014) Isotopic composition of carbonate-bound organic nitrogen in deep-sea scleractinian corals: A new window into past biogeochemical change. *Earth Planet Sci Lett* 400:243–250.

- Wege M (2017) Population trend and foraging ecology of Antarctic and Subantarctic fur seals at Marion Island. PhD dissertation, University of Pretoria, Pretoria, South Africa
- Wege M, de Bruyn PJN, Hindell MA, Lea M, Bester MN (2019) Preferred, small- scale foraging areas of two Southern Ocean fur seal species are not determined by habitat characteristics. *BMC Ecol* 19:<https://doi.org/10.1186/s12898-019-0252-x>.
- Wege M, Tosh CA, de Bruyn PJN, Bester MN (2016) Cross-seasonal foraging site fidelity of subantarctic fur seals: Implications for marine conservation areas. *Mar Ecol Prog Ser* 554:225–239.
- 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.
- Williams R, McEldowney A (1990) A guide to the fish otoliths from waters off the Australian Antarctic Territory, Heard and Macquarie Islands. ANARE Res Notes 75.
- Woods B, Trebilco R, Walters A, Hindell M, Duhamel G, Flores H, Moteki M, Pruvost P, Reiss C, Saunders RA, Sutton C, Gan YM, van de Putte A (2022) Myctobase, a circumpolar database of mesopelagic fishes for new insights into deep pelagic prey fields. *Sci Data* 9:<https://doi.org/10.1038/s41597-022-01496-y>.
- Woods B, Walters A, Hindell M, Trebilco R (2020) Isotopic insights into mesopelagic niche space and energy pathways on the southern Kerguelen Plateau. *Deep Sea Res II* 174:104657.
-