Variation in the diet of killer whales
Orcinus orca at Marion Island, Southern Ocean

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ABSTRACT: Diet seems to be a key factor driving diversity and isolation among killer whale populations. Killer whales at Marion Island, Southern Ocean, have been observed preying on seals and penguins but are also know to depredate Patagonian toothfish from longline fishing vessels. However, their diet is poorly known especially when they occur offshore. We analysed carbon and nitrogen stable isotope ratios in 32 skin samples collected from 24 killer whales belonging to 8 social units. Adult males showed higher $\delta^{15}N$ values than adult females or subadults, indicating that they occupy a higher relative trophic level. There were no significant differences in $\delta^{13}C$ among social units, but $\delta^{15}N$ differed significantly and 2 individuals from social units which have been observed depredating Patagonian toothfish had higher $\delta^{15}N$ values. The inshore presence of killer whales at Marion Island was a significant predictor of $\delta^{13}C$ values, but not of $\delta^{15}N$ values. This suggests some foraging north of Marion Island, potentially on lower trophic level prey. We also analysed tissue samples from seal, penguin and Patagonian toothfish prey and used available values for Antarctic fur seals. Results show that killer whales around Marion Island are apex predators, but that they do not feed exclusively on other high trophic level predators such as elephant seals, fur seals, and Patagonian toothfish. Killer whales had $\delta^{15}N$ values similar to those of Patagonian toothfish and adult male elephant seals, implying that the diet of killer whales at Marion Island includes some lower trophic level prey such as cephalopods or fishes.

KEY WORDS: Predator · Stable isotopes · Carbon · $\delta^{13}C$ · Nitrogen · $\delta^{15}N$ · Foraging · Trophic level

INTRODUCTION

Large, mobile apex predators have the potential to affect the structure and function of ecosystems through multiple pathways (Heithaus et al. 2008). However, a better understanding of the ecological roles and importance of predators requires detailed knowledge of their trophic interactions, which can be challenging for marine and wide-ranging predators such as cetaceans (Kiszka et al. 2015).

Killer whales *Orcinus orca* are apex predators with a broad diet including over 140 prey species, ranging in size from herring *Clupea* spp. to baleen whales (Ford 2009). Killer whale populations tend to specialize on taxonomic prey resources and the most well-known example of this is in the coastal northeast Pacific where 3 sympatric populations (or ecotypes) of killer whales, termed transients, residents and offshores, specialize on marine mammals or fish. In the Antarctic, it has been suggested that 'Type A' killer

whales feed mainly on Antarctic minke whales *Balaenoptera bonaerensis*, large 'Type B' killer whales eat mainly seals, small 'Type Bs' feed on penguins, and 'Type C' killer whales specialize on fishes (Pitman & Ensor 2003, Pitman & Durban 2010, 2012). These dietary differences are accompanied by differences in the ecotypes' social structure, morphology, acoustic behaviour, and movement patterns, with many ecotypes being genetically distinct (reviewed by de Bruyn et al. 2013). Various authors have proposed evolutionary mechanisms by which dietary specialization may promote genetic differentiation in killer whales (e.g. Moura et al. 2015 and references therein; although see Foote et al. 2013) and there seem to be some parallels among top predators such as wolves *Canis lupus* (Pilot et al. 2012), arctic foxes *Alopex lagopus* (Dalén et al. 2005) and bottlenose dolphins *Tursiops* spp. (Louis et al. 2014). In killer whales, these resource use patterns are culturally transmitted within matrilineal social units (see Riesch et al. 2012 for an overview).

However, there are suggestions of killer whale populations pursuing multiple categories of prey (reviewed by de Bruyn et al. 2013) and Baird (2002) suggested that dietary specialization in killer whales should also be related to environmental productivity, in keeping with optimal foraging theory. That is to say, in low productivity areas killer whales should be less likely to specialize, and diet breadth should decrease as the availability of the most profitable prey increases. However, other mechanisms may be responsible for specialization (Araújo et al. 2011) and there is a paucity of data from low latitudes and oceanic areas. It has been speculated that killer whales have played a role in population declines of some marine mammals and penguins (e.g. Springer et al. 2003, Ainley et al. 2010), and interest in their roles in ecosystems (e.g. Williams et al. 2004) has added impetus to dietary studies in this species.

In species where direct observation is often impractical, such as marine mammals, stable isotope analysis has become widely used to study diet (Newsome et al. 2010). Stable isotopes can be used as tracers in food webs because the biomolecular composition of the predator reflects the isotopic composition of the prey consumed in a predictable manner (DeNiro & Epstein 1976). The most commonly measured stable isotopes in foraging ecology are those of carbon ($^{13}C:^{12}C$) and nitrogen ($^{15}N:^{14}N$), reflecting foraging habitat and relative trophic position, respectively (Layman et al. 2012). An advantage of stable isotope analysis over other methods such as stomach content and faecal analysis is that it provides dietary information integrated over time (Dalerum & Angerbjörn 2005). However, stable isotope analysis typically cannot resolve prey identity as definitively as other methods and the actual diet may be difficult to ascertain (Tollit et al. 2010). Nevertheless, killer whale foraging ecology has been investigated using stable isotope analysis in the North Atlantic (McHugh et al. 2007, Foote et al. 2012, 2013), Arctic (Matthews & Ferguson 2014), North Pacific (Herman et al. 2005, Krahn et al. 2007a,b, Newsome et al. 2009, Endo et al. 2014) and Antarctic (Krahn et al. 2008). No subantarctic killer whale stable isotope studies have been published.

The Prince Edward Islands—comprising Marion Island and the smaller Prince Edward Island—are an isolated pair of subantarctic islands in the Southern Ocean which provide a breeding and moulting site for millions of penguins and hundreds of thousands of seals (Ryan & Bester 2008). The abundance of penguins and seals attracts ~45 killer whales to the inshore waters of the islands annually (Reisinger et al. 2011a, Reisinger & de Bruyn 2014). These individuals have been classified into 9 social units, consisting of kin as well as non-kin (Reisinger 2015), although all individuals sampled share a single mitochondrial haplotype (Janse van Rensburg 2015). Social units regularly interact and dispersal of individuals between them has been recorded (Reisinger 2015). The Marion Island haplotype has been recorded among 13 haplotypes from 37 South African killer whales but no photographic identification matches have been made and the populations are significantly genetically different (Janse van Rensburg 2015). Marion Island killer whales have been directly observed preying on southern elephant seals, subantarctic fur seals *Arctocephalus tropicalis*, king penguins *Aptenodytes patagonicus*, macaroni penguins *Eudyptes chrysolophus* and rockhopper penguins *E. chrysocome filholi* (Reisinger et al. 2011b). Predation on Antarctic fur seals *Arctocephalus gazella* has not been documented, but is likely to occur.

Killer whales also depredate Patagonian toothfish *Dissostichus eleginoides* from 2 licenced longline fishing vessels which operate in the vicinity of the islands (Williams et al. 2009). It is unknown what proportion of Marion Island killer whales depredate toothfish, but 9 individuals which depredate toothfish from fishing vessels around the Crozet Islands (~1000 km due east of the Prince Edward Islands) have also been sighted inshore at Marion Island (Reisinger & de Bruyn 2014, Tixier et al. 2014).

Killer whales are most abundant at Marion Island from September to December, with a secondary

peak in April and May, periods which coincide with the breeding and moulting period of seals and penguins (Reisinger et al. 2011b). The abundance of seals and penguins inshore at Marion Island may change 10-fold or more within a year (e.g. Oosthuizen et al. 2012) and this is expected to cause a seasonal distribution and/or diet shift in killer whales in response.

All killer whale studies at Marion Island to date have relied on direct, shore-based observations of predation, which have allowed limited inferences about the diet of killer whales in this population, and about any intra-population or temporal dietary variations. We therefore used stable isotope analysis of carbon and nitrogen to provide information about the unseen foraging ecology of killer whales at Marion Island. More specifically, we investigated (1) whether there is any evidence for diet variation among individuals, social units and age- or sexclasses; and (2) if there is any variation in killer whale stable isotope values linked to their inshore presence, which would indicate diet and/or foraging distribution changes related to changing resource availability. In addition, we provide some inferences regarding the diet of Marion Island killer whales and the first concurrent isotopic description of seals, penguins, and Patagonian toothfish at Marion Island. Given the common ancestry of Marion Island killer whales and the relatively dynamic nature of their social organization (Reisinger 2015), we hypothesized that there should not be significant dietary differentiation among social units in this population. Secondly, given the seasonal fluctuations in prey distribution and abundance at Marion Island (Reisinger et al. 2011b) we hypothesized that killer whales should change their diet seasonally and/or forage in a different environment.

MATERIALS AND METHODS

Sample collection and preparation

Skin biopsy samples were remotely collected from live killer whales at Rockhopper Bay and Transvaal Cove, Marion Island (46.873° S, 37.859° E) from August 2011 to May 2013 (Table 1). Tissue samples were obtained using sterilized stainless steel biopsy tips (25 mm long, 7 mm inside diameter) attached to bolts which were fired from a 68 kg draw weight recurve crossbow (Barnett Panzer V). The region on the body directly below the dorsal fin was targeted. Individuals were identified from photographs, video

or by eye (later confirmed from photographs) following Reisinger et al. (2011a) and Reisinger & de Bruyn (2014) and assigned to one of 3 classes: adult male, adult female and subadult. The social unit designation follows Reisinger (2015); individuals from 8 social units were sampled, designated A to H. Sampling procedures, as well as the effects of sampling on killer whales, are discussed in detail in Reisinger et al. (2014). Samples were stored at −80°C (without preservatives) within 30 min of collection. We lyophilized the samples for 24 h before they were transported. Before homogenizing samples, we separated the skin from the blubber portion.

The presence of lipids in tissues introduces variation in $\delta^{13}C$ values, because the lipid content of organisms is heterogeneous and lipids have lower $\delta^{13}C$ values (DeNiro & Epstein 1977, Post et al. 2007). Accounting for lipids is important in lipid-rich tissues such as cetacean skin and blubber (Ryan et al. 2012). We took 2 aliquots from each skin sample: one aliquot was analysed without extracting lipids and the second aliquot was lipid extracted. To extract lipids we added 8 ml dicloromethane:methanol (3:1 volume:volume) to each sample and sonicated them for 15 min. We then centrifuged samples at 3000 rpm (1613 × g) for 10 min and discarded the supernatant. We repeated these steps twice (i.e. 3 times in total). Next, we added distilled water, agitated the samples and sonicated them for 15 min. We centrifuged the sample at 1613 × g for 10 min and discarded the supernatant. After repeating these latter steps (i.e. twice in total) and then oven drying the samples at 50°C for 48 h, we weighed 0.4−0.5 mg sample aliquots into tin capsules for carbon and nitrogen stable isotope ratio analysis. Further correction was unnecessary as almost all atomic carbon:nitrogen (C:N) ratios met the ~4.1 threshold (C:N mass ratio of 3.5) recommended by Post et al. (2007). Because lipid extraction altered $\delta^{15}N$ values as well as correcting $\delta^{13}C$ values (see 'Supplementary results – lipid extraction of killer whale samples' in the Supplement at www.int-res.com/articles/suppl/m549p263_supp.pdf), we used lipid extracted $\delta^{13}C$ values with non-lipid extracted $\delta^{15}N$ values for further analysis, as recommended by Ryan et al. (2012). Hereafter, unless stated otherwise, killer whale $\delta^{13}C$ values are from lipid extracted skin, and $\delta^{15}N$ values are from non-lipid extracted skin.

We used $\delta^{13}C$ and $\delta^{15}N$ values for all prey species recorded in the diet of Marion Island killer whales and all species were sampled at or (in the case of Patagonian toothfish) near Marion Island (Table 2, Tables S1–S3 in the Supplement). While we attemp-

Table 1. δ^{13}C and δ^{15}N values of skin collected via remote biopsy sampling from killer whales at Marion Island, Southern Ocean, before and after lipid extraction. Social units (A to H) follow Reisinger (2015). AM: adult male; AF: adult female; SA: subadult; U: unknown ID. Dates are shown as yyyy/mm/dd. C:N is the atomic carbon:nitrogen ratio. The average values of samples RR-19 and RR-20 were used for analyses as these samples were collected from the same individual on the same day. For two samples (RR-20 and RR-21) there was insufficient tissue for non-lipid extracted analyses

Social unit	Individual	Class	Sample	Date (yyyy/mm/dd)	Non-lipid extracted			Lipid extracted		
					δ^{13}C	δ^{15}N	C:N	δ^{13}C	δ^{15}N	C:N
A	M001	AM	RR-02	2011/08/23	−20.6	12.9	4.1	−18.9	13.0	3.5
A	M001	AM	RR-10	2011/10/22	−20.1	12.9	4.3	−18.5	13.2	3.4
A	M001	AM	DC-04	2012/10/17	−23.6	12.3	7.6	−18.0	14.1	3.4
A	M002	AF	RR-03	2011/09/06	−19.6	11.4	4.1	−18.4	11.7	3.5
A	M027	SA	RR-21	2011/12/14				−18.6	12.4	3.6
B	M004	AF	RR-08	2011/10/12	−19.9	13.4	4.2	−18.4	12.9	3.4
B	M007	AM	RR-01	2011/08/18	−21.2	13.0	4.2	−18.8	13.1	3.5
B	M028	AF	RR-25	2012/05/06	−19.9	11.8	4.4	−18.1	12.4	3.5
C	M005	AM	RR-05	2011/09/20	−20.3	12.2	4.1	−19.2	12.5	3.5
C	M013	AF	RR-17	2011/12/01	−20.0	12.1	4.1	−19.8	13.1	3.7
C	M013	AF	DC-06	2013/05/03	−21.6	11.6	5.6	−18.3	12.2	3.5
C	M015	AF	RR-06	2011/09/20	−20.7	12.1	4.5	−18.9	13.3	3.5
C	M015	AF	DC-07	2013/05/03	−20.4	11.6	4.7	−18.3	12.2	3.5
D	M008	AM	RR-14	2011/11/28	−19.2	13.2	3.9	−19.2	13.4	3.6
D	M008	AM	DC-05	2013/02/07	−20.4	13.0	4.4	−18.6	13.4	3.5
D	M009	SA	RR-22	2011/12/14	−19.3	13.5	4.0	−18.3	13.6	3.5
D	M033	AF	RR-18	2011/12/14	−20.0	12.9	4.2	−18.3	14.6	3.4
E	M031	AF	RR-09	2011/10/19	−21.5	12.3	5.0	−18.7	12.4	3.5
E	M031	AF	RR-11	2011/11/10	−20.5	12.2	4.3	−19.0	12.3	3.5
E	M040	SA	RR-04	2011/09/16	−21.3	11.9	4.7	−18.6	13.0	3.4
E	M050	SA	RR-23	2011/12/18	−20.7	11.8	4.5	−19.3	11.8	3.5
E	M051	SA	RR-13	2011/11/27	−21.3	12.5	5.0	−18.9	12.3	3.5
F	M012	AF	RR-19	2011/12/14	−20.1	11.7	4.3	−18.6	11.9	3.6
F	M012	AF	RR-20	2011/12/14				−18.3	12.4	3.5
F	M025	SA	RR-16	2011/11/29	−19.6	11.8	4.1	−18.4	12.4	3.5
F	M025	SA	DC-02	2012/08/28	−19.6	12.2	4.3	−18.5	12.7	3.5
F	M026	AF	DC-03	2012/10/09	−19.4	11.6	4.2	−18.1	12.1	3.5
G	M016	AM	RR-24	2012/01/03	−19.8	13.5	4.2	−19.3	13.8	3.5
H	M029	AF	RR-12	2011/11/26	−20.7	11.9	4.9	−18.5	12.2	3.5
H	M041	SA	DC-01	2012/08/05	−20.2	12.1	4.6	−18.0	12.7	3.5
–	U	SA	RR-07	2011/09/22	−19.6	12.1	4.1	−18.7	12.1	3.5
–	U	SA	RR-15	2011/11/28	−21.0	12.3	4.6	−19.2	12.6	3.5
n	24				30	30	30	32	32	32
Mean					−20.4	12.3	4.5	−18.6	12.8	3.5
SD					0.9	0.6	0.7	0.4	0.7	0.1

ted to match killer whale and prey samples closely in time, this was not always possible. Prey samples were collected in 4 years, and in 7 calendar months (November to May) (Tables S1–S3). Marked inter- or intra-annual variation in prey foraging behaviour or oceanographic conditions could thus influence our representation of the killer whale prey field. Sample collection and preparation is detailed in 'Supplementary methods—prey sample collection and preparation' in the Supplement. Additionally, we used δ^{13}C and δ^{15}N values for whole blood samples collected from 35 adult female Antarctic fur seals at Marion Island, reported in Walters (2014). The mean δ^{13}C value of these was −21.7 ± 0.5‰ and the mean δ^{15}N value was 11.2 ± 0.3‰.

Stable isotope analysis

We analysed killer whale, penguin and seal tissue at the Stable Isotope Biogeochemistry Laboratory, Durham University. We measured δ^{13}C and δ^{15}N using a Thermo-Finnigan Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher Scientific), coupled with a Costech ECS 4010 Elemental Analyser. Tin capsules containing samples were sequentially combusted in the elemental analyser; the resulting gases were passed through a gas chromatography column for separation and then measured in succession by the isotope ratio mass spectrometer. We monitored data accuracy by analysing in-house standards which are stringently calibrated

Table 2. Mean (±SD) δ^{13}C and δ^{15}N values (‰) of putative killer whale prey at Marion Island. Individual values (and further details, including C:N ratios) are given in Tables S1–S3 in the Supplement. RBC: red blood cells

Prey	n	Tissue	Non–lipid extracted		Lipid extracted		Adjusted	
			δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N	δ^{13}C	δ^{15}N
Aptenodytes patagonicus	8	RBC	−22.7 ± 0.1	10.4 ± 0.3	–	–	−22.3 ± 0.1	10.5 ± 0.3
Eudyptes spp.[a]	12	RBC	−22.9 ± 0.3	8.7 ± 0.6	–	–	−22.5 ± 0.3	8.8 ± 0.6
Arctocephalus tropicalis	13	Whole blood	−20.1 ± 0.5	11.4 ± 0.3	–	–	−20.5 ± 0.5	12.1 ± 0.3
Mirounga leonina[b]	20	Hair	−19.4 ± 0.7	12.0 ± 0.5	–	–	−20.9 ± 0.7	11.4 ±0.5
Mirounga leonina adult males	5	Hair	−18.3 ± 1.0	13.3 ± 0.6	–	–	−19.8 ± 1.0	12.7 ± 0.6
Dissostichus eleginoides	10	Muscle	−22.1 ± 1.1	12.7 ± 0.6	−20.2 ± 0.6	13.3 ± 1.1	–	–

[a]Values for rockhopper and macaroni penguins were combined for display purposes (for detailed results, see Table S1)
[b]Values for adult female, subadult, yearling and underyearling elephant seals were combined for display purposes (for detailed results, see Table S2)

against international standards (e.g. USGS 24 and 40, IAEA N1, N2 and 600 for δ^{15}N; USGS 40, IAEA 600, SPAR for δ^{13}C). Analytical uncertainty for δ values was typically ±0.1‰ for replicate analyses of the international standards and typically <0.2‰ on replicate sample analysis for both δ^{13}C and δ^{15}N.

We analysed Patagonian toothfish samples at the Mammal Research Institute, University of Pretoria. Tin cups containing the samples were combusted at 1020°C in an elemental analyser (Flash 1112 Series, Thermo Fisher Scientific). We determined δ^{13}C and δ^{15}N values using a Delta V Plus isotope ratio mass spectrometer (Thermo Fisher Scientific). We placed an in-house standard (Merck gel) and blank after every 10 samples to ensure reproducibility. Reproducibility in δ^{13}C and δ^{15}N was ±0.2‰.

We report stable isotope ratio values (^{13}C:^{12}C and ^{15}N:^{14}N) relative to the standards Vienna Peedee Belemnite (for carbon) and air (for nitrogen), expressed in parts per thousand (‰).

Statistical analysis

We performed statistical tests in the R environment (R Core Team 2013). We tested normality using a Shapiro-Wilk test, before applying an appropriate parametric (paired *t*-test, ANOVA) or non-parametric test (Welch's *t*-test, Mann-Whitney *U* test, Kruskal-Wallis test). To investigate the effect of class and social unit on killer whale δ-values, while taking into account multiple sampling of individuals, we fitted linear mixed effects models using the *lme4* (Bates et

al. 2014) and *MuMIn* (Barton 2013) packages in R. We included class and social unit as fixed terms, and individual as a random term. Fixed effects were assessed by comparing the null models to those with the fixed effect in question, using likelihood ratio tests. Where necessary, these were followed by a post-hoc Tukey's honest significant difference (HSD) test, using the *multcomp* package in R (Hothorn et al. 2008).

To assess the influence which the inshore presence of killer whales had on δ-values, we calculated a population level sighting rate in the 75 d prior to each biopsy sampling event. We assumed that the full profile skin samples we collected would represent diet integrated over the preceding 70 to 75 d (Hicks et al. 1985, St. Aubin et al. 1990). The sighting rate was calculated as the number of sightings of killer whales during shore-based dedicated killer whale search sessions at Marion Island (2008–2013; 1285 sightings in 5498 h) (see Reisinger et al. 2011a,c, 2014 for further details). The sighting rate was included as a fixed effect in linear mixed effects models, as for class and social unit (above).

Further, all models were compared using small sample size Akaike information criterion (AIC$_c$) and goodness of fit was assessed using the conditional coefficient of determination ($R^2_{GLMM(c)}$) (Nakagawa & Schielzeth 2013).

To visualize comparisons among classes and social units, for groups with more than 2 samples we calculated the small sample size corrected standard ellipse area (SEA$_c$) (Jackson et al. 2011) in the *SIAR* package (Parnell et al. 2010, Parnell & Jackson 2013) in R. The

SEA_c comprises approximately 40% of the data points, and can be interpreted as a representation of standard deviation for bivariate data (Jackson et al. 2011).

Inferring prey

As prey tissues are assimilated into a consumer's body following ingestion, δ-values change due to isotopic fractionation. $\delta^{13}C$ values change little (~0.75‰), while $\delta^{15}N$ values show greater change (~2.75‰), but these values vary with factors including age, body size and diet quality (Caut et al. 2009). These trophic discrimination factors (Δ-values) are tissue- and species-specific, and are typically measured in controlled feeding studies (e.g. Hobson et al. 1996, Browning et al. 2014). Three studies (Caut et al. 2011, Browning et al. 2014, Giménez et al. 2016) have estimated trophic discrimination factors in cetaceans experimentally. We used values reported in Browning et al. (2014) ($\Delta^{13}C_{diet-skin}$ = 1.1 ± 0.6‰ and $\Delta^{15}N_{diet-skin}$ = 2.1 ± 0.5‰), estimated for bottlenose dolphins, to visualize the potential prey 'mixing space' for killer whales in this study.

Different tissues have different diet-tissue discrimination factors and are not directly comparable without adjustment. To facilitate comparisons among prey and to visualize the putative prey of killer whales, we therefore adjusted $\delta^{13}C$ and $\delta^{15}N$ values of prey blood (fur seals and penguins) and hair (elephant seals) to represent muscle (Table 3), which we assumed would make up the largest proportion of protein assimilated by killer whales. We calculated adjustment values as the difference between the δ-values for the 2 tissues concerned, measured in controlled feeding studies (Hobson et al. 1996, Evans Ogden et al. 2004) (Table 3). Unless otherwise specified, values for prey hereafter are adjusted values which repre-

sent muscle (Table 3). Our simplistic, but necessary, adjustment should be interpreted cautiously as our adjustment is approximate and tissues differ in a number of respects which we could not take into account, such as isotopic turnover rate.

We attempted to use Bayesian stable isotope mixing models (Parnell et al. 2013) to estimate the proportion of each prey type in the diet of Marion Island killer whales. However, the caveats and assumptions involved in their use, particularly regarding the accuracy of available diet-tissue discrimination factors and the assumption that all potential prey are sampled (Bond & Diamond 2011, Parnell et al. 2013, Phillips et al. 2014), made them an unsatisfactory tool for this study, thus precluding inference about proportional diet composition.

RESULTS

The mean $\delta^{13}C$ value in lipid extracted killer whale skin samples was −18.6 ± 0.4‰. Mean $\delta^{15}N$ value in non-lipid extracted skin samples was 12.3 ± 0.6‰.

Skin $\delta^{13}C$ values were not significantly different among classes (χ^2 = 2.02, df = 2, p = 0.364) or among social units (χ^2 = 16.68, df = 9, p = 0.054). There was a significant among-class difference in $\delta^{15}N$ (χ^2 = 7.74, df = 2, p = 0.021); specifically, adult males had significantly higher values than adult females (p = 0.006) (Fig. 1). The difference among social units was also significant (χ^2 = 25.62, df = 9, p = 0.002); differences between several social units and Unit D showed the strongest differentiation (Table S5 in the Supplement).

We compared δ-values for individuals which have been observed interacting with longline fishing vessels around the Crozet Islands (5 samples from 4 individuals from Units D and G; Table 1) with those only observed at Marion Island. Skin $\delta^{13}C$ values did not

Table 3. Values (±SD) used in this study to adjust δ-values of tissue collected from putative killer whale prey species to represent muscle tissue

| Taxon in this study	Tissue collected	Adjustment value		Reference species	Reference
		$\delta^{13}C$	$\delta^{15}N$		
Penguins	Red blood cells	0.4 ± 0.1	0.1 ± 0.2	Dunlin *Calidris alpina pacifica*	Evans Ogden et al. (2004)
Fur seals	Whole blood	−0.4 ± 0.4	0.7 ± 0.6	Harp seals *Pagophilus groenlandicus* Harbour seals *Phoca vitulina* Ringed seals *Phoca hispida*	Hobson et al. (1996)
Elephant seal	Hair	−1.5 ± 0.5	−0.6 ± 0.4	*Pagophilus groenlandicus* *Phoca vitulina* *Phoca hispida*	Hobson et al. (1996)
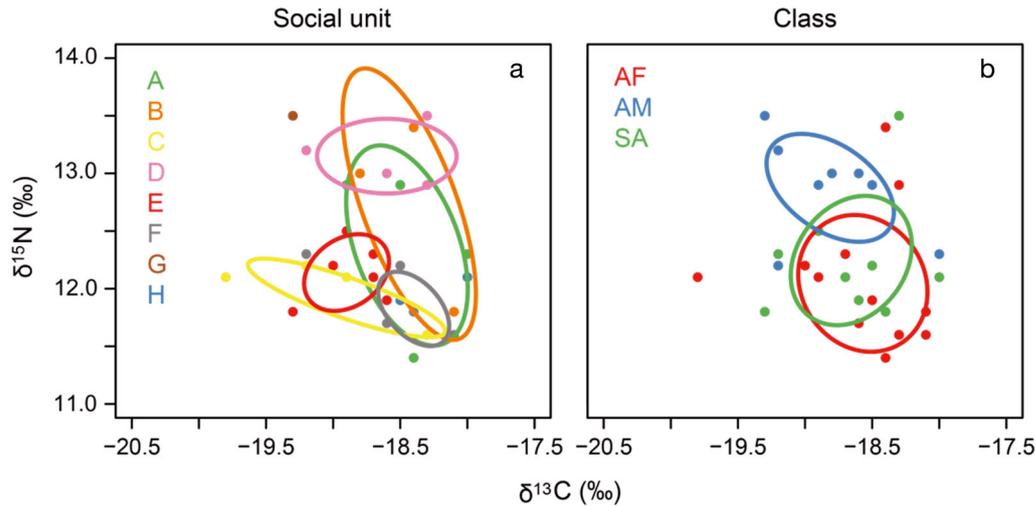
Fig. 1. Skin δ-values (points) of Marion Island killer whales coloured to differentiate (a) social unit and (b) class, with small-sample size corrected standard ellipse area (SEA$_c$) (Jackson et al. 2011) superimposed for groups with more than 2 samples. Social units (A to H) follow Reisinger (2015). AM: adult male; AF: adult female; SA: subadult

Table 4. Comparison of linear mixed effects models of the relationship between killer whale skin (a) δ^{13}C values and (b) δ^{15}N values. Three predictors were considered: age–sex class (*Class*), social unit (*Unit*) and sighting rate 75 d prior to sampling (*Rate*). Individual identity was included as a random effect in all models. Models are ranked by their small-sample size Akaike Information Criterion scores (AIC$_c$). R^2$_{GLMM(c)}$ is the conditional coefficient of determination (Nakagawa & Schielzeth 2013)

Model predictors	R^2$_{GLMM(c)}$	AIC$_c$	ΔAIC$_c$	Model weight
(a) δ^{13}C				
Rate	0.189	38.4	–	0.751
Null model	0.000	41.8	3.42	0.136
Class + Rate	0.241	42.5	4.14	0.095
Class	0.054	45.7	7.39	0.019
Rate + Unit	0.561	59.5	21.11	0.000
Unit	0.406	62	23.65	0.000
Class + Rate + Unit (full model)	0.681	65.6	27.23	0.000
Class + Unit	0.503	70.4	32.03	0.000
(b) δ^{15}N				
Class + Rate	0.467	52.0	–	0.709
Class	0.740	54.9	2.92	0.165
Rate	0.734	56.6	4.62	0.070
Null model	0.770	57.1	5.08	0.056
Unit	0.751	66.9	14.89	0.000
Rate + Unit	0.657	71.6	19.64	0.000
Class + Unit	0.714	73.9	21.95	0.000
Class + Rate + Unit (full model)	0.747	78.6	26.59	0.000

Sighting rate of killer whales in the 75 d prior to sampling was a significant predictor of δ^{13}C values (χ^2 = 22.71, df = 15, p = 0.030). Sighting rate was not a significant predictor of δ^{15}N values (χ^2 = 3.14, df = 4, p = 0.076), but models containing sighting rate were ranked above the null model according to AIC$_c$ (Table 4). δ^{13}C values decreased slightly with increased sighting rates, and δ^{15}N values showed the opposite pattern (Fig. 2). In both cases, however, the effect size was relatively small: based on the equivalent linear models, predicted change in δ^{13}C over the observed sighting rates was −0.5‰, and that for δ^{15}N was +0.7‰.

Overall, the model containing only sighting rate was the optimal one for δ^{13}C, but the variance explained was low (R^2$_{GLMM(c)}$ = 0.189) (Table 4). For δ^{15}N, the optimal model contained class and sighting rate, with higher variance explained (R^2$_{GLMM(c)}$ = 0.467) (Table 4).

Prey

There were significant differences among prey δ^{13}C values (ANOVA, F = 23.2, df = 9, p < 0.001) and among prey δ^{15}N values (ANOVA, F = 67.0, df = 9, p < 0.001), the latter showing a greater range of variation (Fig. 3a). A post-hoc multiple comparisons using adjusted values (Tukey's HSD) is shown in Table S4 and more detailed results are presented, and discussed, in the Supplement. Mean killer whale δ^{15}N (12.3 ± 0.6‰) was similar to,

differ significantly (Welch 2 sample *t*-test, t = −0.478, df = 5.23, p = 0.696) but δ^{15}N values did (t = −6.77, df = 9.92, p < 0.001). Killer whales sighted around Crozet had mean δ^{15}N values of 13.2 ± 0.3‰, while mean δ^{15}N for Marion individuals was 12.1 ± 0.5‰.
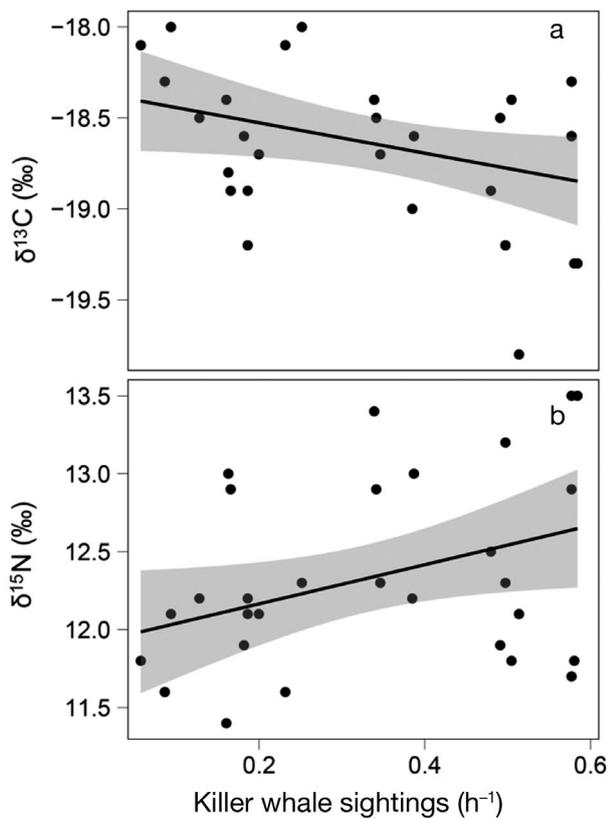
Fig. 2. (a) δ^{13}C and (b) δ^{15}N values in killer whale skin plotted against inshore sighting rate of killer whales in the 75 d prior to sampling. Fitted linear models (lines) are shown with 95% confidence intervals (grey shading)

but slightly lower than, those of adult male southern elephant seals (12.7 ± 0.6‰) and Patagonian toothfish (13.3 ± 1.1‰) (Fig. 3a). Mean killer whale δ^{13}C (−18.6 ± 0.4‰) was higher than that of all other prey, the nearest being southern elephant seal adult males (−18.3 ± 1.0‰) (Fig. 3a). After subtracting discrimination factors from killer whale δ-values, δ^{13}C values were similar to those of southern elephant seal adult males, and δ^{15}N values were slightly below those of king penguins (Fig. 3b). However, no prey values were close to the adjusted killer whale values in bivariate space (Fig. 3b).

DISCUSSION

Killer whale diet

We show that killer whales around Marion Island do not feed exclusively on other high trophic level predators such as elephant seals, fur seals, and Patagonian toothfish. Killer whales from Marion Island have δ^{15}N values similar to those of Patagonian toothfish and adult male elephant seals.

The diet of adult male elephant seals at Marion Island is unknown, but at other locations their diet consists largely of cephalopods and fishes (see 'Supplementary discussion — prey trophic structure' in the Supplement). The diet of toothfish sampled near Marion Island was dominated by cephalopods and
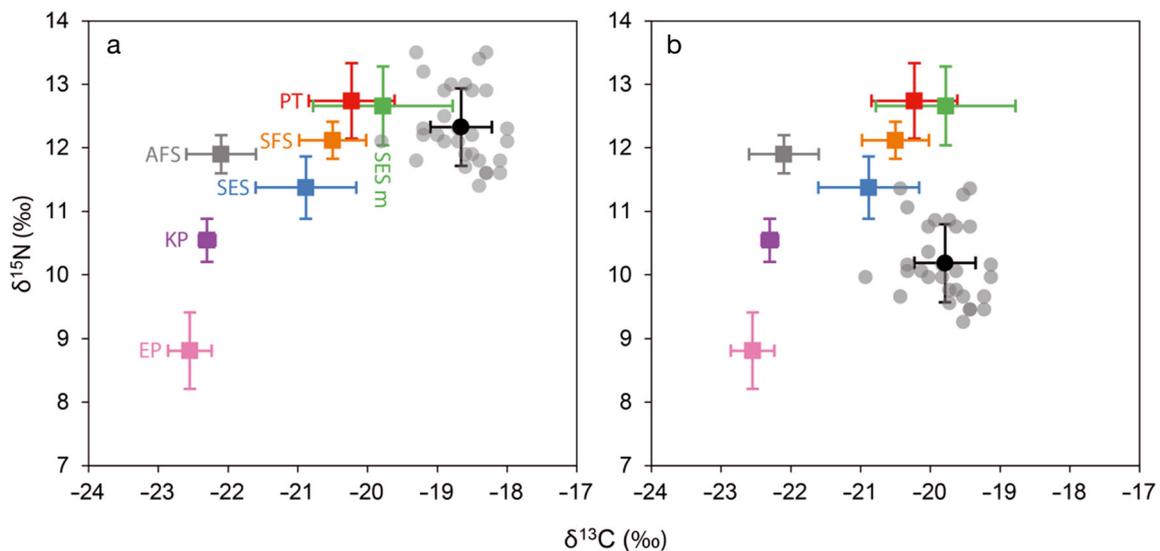

Fig. 3. Biplot of δ^{13}C against δ^{15}N showing mean values ± SD for killer whales (filled black circle) and their prey (squares). Individual values for killer whale skin are displayed as grey circles. In (b), discrimination factors have been subtracted from killer whale skin values. PT: Patagonian toothfish; AFS: Antarctic fur seal; SFS: subantarctic fur seal; SES: southern elephant seal (adult females, subadults and juveniles); SESm: southern elephant seal adult males; KP: king penguin; EP: *Eudyptes* spp. penguins

myctophids (Pakhomov et al. 2006). Cephalopods are an important element in the Southern Ocean marine ecosystem and are major components of the diets of toothed whales, seals, penguins, and large fishes (Rodhouse 2013). Killer whales feed on cephalopods elsewhere (see references in Reisinger et al. 2015) and Hanson & Walker (2014) suggest that cephalopods represent a previously underestimated component of transient (mammal-hunting) killer whales' diets. Reisinger et al. (2015) postulated that the depths (up to 499 and 767 m) and diel variation in the dive behaviour of 2 killer whales at Marion Island was related to predation on prey such as cephalopods. However, published isotope ratio values for the cephalopod community at the Crozet Islands (Guerreiro et al. 2015) did not lie near adjusted killer whale vales in bivariate space (cf. Fig. 3b). Various fish species could also be considered prey, based on their isotope ratio values (Pakhomov et al. 2006), but are too small to be energetically worthwhile prey. The lack of suitable prey values within the potential solution polygon of the adjusted killer whale values in bivariate space (Fig. 3b) suggests that potential prey species were missing from our analyses. Nevertheless, $\delta^{15}N$ values confirm the consumption of lower trophic level prey (such as cephalopods and fishes), by Marion Island killer whales.

Killer whale class, social unit and temporal variability in diet

The relationship between killer whale sighting rate and δ-values indicates that there is some variation in the foraging area, and possibly diet, of killer whales through the annual cycle. This is likely driven by the seasonal changes in prey abundance at Marion Island. The decrease of $\delta^{13}C$ values with increased inshore sighting rate is consistent with a shift from a diet including some prey consumption north of Marion Island to prey consumed primarily around Marion Island. There is a latitudinal gradient in baseline $\delta^{13}C$ values in the Southern Ocean (Trull & Armand 2001) and consequently consumer $\delta^{13}C$ values will reflect the latitudinal provenance of their diet (Jaeger et al. 2010). Based on Jaeger et al.'s (2010) regression of wandering albatross *Diomedea exulans* plasma $\delta^{13}C$ values against foraging latitude, and ignoring any effect of changing trophic level, our 0.5‰ predicted change in $\delta^{13}C$ would correspond to a 1.6° latitudinal shift in carbon provenance, a result corroborated by satellite tracking data of killer whales from Marion Island, showing restricted movement over seamounts

to the north of the islands by some individuals (Reisinger et al. 2015). However, because $\delta^{13}C$ values also increase with trophic level (~0.75‰), the size of this effect would be masked by the potential concomitant increase in prey trophic level (signalled by increasing $\delta^{15}N$ values; Fig. 2 and below) and the inferred latitudinal shift could be larger. Two of 9 killer whales satellite tracked from Marion Island moved north of the subtropical front. While their movements were rapid and directed, with no restricted movement patterns suggesting foraging behaviour (Reisinger et al. 2015), a different foraging mode is possible.

Although the increase in killer whale skin $\delta^{15}N$ with increased sighting rate was not significant, sighting rate was included in our best model predicting $\delta^{15}N$ values. We interpret the increase in $\delta^{15}N$ values as greater consumption of higher trophic level prey, notably southern elephant seals, when killer whales are present inshore. The decreased abundance of seals and penguins inshore primarily during the austral winter will result in increased search times for these prey, and should consequently lower their profitability for killer whales. Killer whales can respond by altering their diet (including more prey types, or switching to other prey types), by hunting in different habitats, or a combination of these. The relationship between δ-values and sighting rate, in conjunction with movement and dive data (Reisinger et al. 2015), suggests a combination of these strategies. However, the effect size is small and more killer whale samples throughout the year would be required to rigorously test to what degree foraging behaviour changes.

Although there were marginally significant differences in $\delta^{15}N$ values among social units, there was no obvious pattern of differentiation, evidence for broadly similar diets among social units at Marion Island. There was no evidence for divergent ecological specialization. The apparently similar resource-use strategies in this population may be related to the population's overall genetic relatedness and frequent social contact (Reisinger 2015).

Individuals which interacted with the Patagonian toothfish longline fisheries around the Crozet Islands showed $\delta^{15}N$ values about 1‰ higher than individuals which were only sighted at Marion Island, suggesting that this high trophic level prey may have had a detectable influence on killer whale $\delta^{15}N$ values and that Marion Island killer whales may depredate Patagonian toothfish to a lesser degree. This might be expected given that only 2 vessels operate legally in the vicinity of Marion Island (with zero esti-

mated illegal, unregulated and unreported fishing), compared to 7 around the Crozet Islands (Tixier et al. 2015).

However, 3 of the 5 samples from Units D and G were from adult males, and adult males had significantly higher δ^{15}N values than other classes. Thus the high sample proportion from adult males in the comparison of social units D and G to other units is a confounding factor, and could provide an alternative explanation to Patagonian toothfish depredation. The 3 samples from these toothfish depredating males may also have caused the significantly higher δ^{15}N values for all males compared to adult females and subadults; mean δ^{15}N for the 3 samples from 2 depredating males was 13.3 ‰ and that for the 3 non-depredating males was 12.7 ‰. Disentangling the effect is difficult with such a small number of samples, but the ranking of linear models using AIC_c (Table 4) suggests that class is a more parsimonious explanatory variable for δ^{15}N than social unit.

Since killer whales hunt cooperatively and often share prey with members of their social unit (e.g. Hoelzel 1991, Ford & Ellis 2006), the higher δ^{15}N values for adult males are unexpected. Newsome et al. (2009) found the same phenomenon among North Pacific transient killer whales and suggested that adult male killer whales may sometimes forage on their own at a higher trophic level, or that trophic discrimination factors become larger when growth rate slows (as less dietary protein is used directly for tissue synthesis to sustain growth). Endo et al. (2014) report a positive correlation between δ^{15}N values and body length. Given the dynamic social organization of Marion Island killer whales (Reisinger 2015; discussed above), it is possible that males do forage on their own, but we consider it unlikely that lone individuals successfully hunt large prey such as elephant seals. It may instead be that adult males take less lower trophic level prey if they forage on their own, or take more high trophic level prey (apart from adult male elephant seals), such as subantarctic fur seals or Patagonian toothfish.

Conclusions and further research

We show that killer whales are indeed apex predators in the Marion Island marine ecosystem, with mean δ^{15}N values similar to adult male southern elephant seals and Patagonian toothfish. However δ^{15}N values in killer whales were not high enough to suggest that they prey exclusively on high trophic level prey (seals and toothfish). Cephalopods, and perhaps some fishes, represent possible prey in addition to seals, penguins and toothfish. Killer whales in the Southern Ocean may thus have broader ecosystem impacts than previously suggested. The inclusion of lower trophic level prey is likely driven by seasonal changes in the abundance of seals and penguins. There was no strong differentiation in trophic ecology among social units, but individuals from 2 social units, known to depredate Patagonian toothfish around the Crozet Islands, had slightly higher δ^{15}N values. Adult male killer whales appeared to occupy a slightly higher relative trophic level than females and subadults.

The lack of accurate trophic discrimination factors, and possibly missing δ-values for unknown prey of killer whales precluded diet reconstructions using mixing models. Sampling all possible prey is rarely possible, especially in a versatile predator such as the killer whale, but we suggest further work to characterize potential cephalopod and fish prey around Marion Island. Accurate, species-specific trophic discrimination factors will continue to be rare for cetaceans. Compound specific stable isotope analysis or fatty acid analysis can be used as additional, and perhaps more informative, dietary tracers. We recommend further biopsy sampling so that more robust inferences can be made, but the 24 individuals we sampled already represent approximately half of the Marion Island population of killer whales.

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