

Stable isotopes reveal the trophic position and mesopelagic fish diet of female southern elephant seals breeding on the Kerguelen Islands

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ABSTRACT: Trophic interactions between organisms are the main drivers of ecosystem dynamics, but scant dietary information is available for wide-ranging predators during migration. We investigated feeding habits of a key consumer of the Southern Ocean, the southern elephant seal *Mirounga leonina*, by comparing its blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values with those of various marine organisms, including crustaceans, squid, fishes, seabirds and fur seals. At the end of winter, $\delta^{13}\text{C}$ values (-23.1 to -20.1‰) indicate that female elephant seals forage mainly in the vicinity of the Polar Front and in the Polar Frontal Zone. Trophic levels derived from $\delta^{15}\text{N}$ values (trophic level = 4.6) show that the southern elephant seal is a top consumer in the pelagic ecosystem that is dominated by colossal squid. The mean $\delta^{15}\text{N}$ value of seals ($10.1 \pm 0.3\text{‰}$) indicates that they are not crustacean eaters, but instead feed on crustacean-eating prey. Surprisingly, most of the previously identified prey species have isotope $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that do not fit with those of potential food items. The most singular pattern to emerge from $\delta^{15}\text{N}$ values of predators and prey is that female seals are likely to feed on myctophid fishes, not squid or Patagonian toothfish. We therefore suggest that they occupy a unique trophic niche amongst the guild of air-breathing, diving vertebrates by feeding on mesopelagic fish at great depths. In turn, this finding emphasizes the role of myctophids in oceanic waters and will help to quantify and model fluxes of matter and energy within the pelagic ecosystem of the Southern Ocean.

KEY WORDS: Cephalopod · Mesopelagic fish · Myctophid · Pelagic ecosystem · Southern Ocean · Squid

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INTRODUCTION

The southern elephant seal *Mirounga leonina* is the largest phocid species and its abundance and body mass make it a major consumer of marine resources in the Southern Ocean (Guinet et al. 1996, Hindell et al. 2003). The use of sophisticated electronic devices over the last decade has shown that, depending on age and sex, southern elephant seals forage in deep waters (300 to 600 m) over wide marine areas during their postmolt and postbreeding trips (Hindell et al. 1991, 2003, Biuw et al. 2007). The identities of their prey are still largely

unknown because *M. leonina* forage far away from land and have almost empty stomachs when they return. Indeed, stomach lavage has collected only hard parts of prey species, mainly cephalopod beaks, thus suggesting a strong bias and overestimation towards items that resist digestion and accumulate over time (Rodhouse et al. 1992, Green & Burton 1993, Slip 1995). Moreover, dietary samples are only representative of the very last portion of the trip, not of prey consumed over the entire foraging area. These biases lead to the common, but poorly supported, thought that southern elephant seals are mainly teuthivorous (Whitehead et

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al. 2003), and thus to the estimation that their total annual consumption was about 4.5 million tonnes of prey, primarily oceanic squid (Santos et al. 2001).

Since disentangling the trophodynamics within food webs is an important first step for a better overall understanding of ecosystem functioning, recently developed methods of diet analysis, e.g. fatty acids and stable isotopes, are now increasingly used to investigate the feeding habits of major consumers. These methods are based on the common principle that 'you are what you eat', i.e. the biochemical composition of molecules and tissues of consumers reflects that of their food in a predictable manner (Kelly 2000). For example, consumer tissues are enriched in ^{15}N relative to their food and, consequently, $\delta^{15}\text{N}$ ($^{15}\text{N}/^{14}\text{N}$) measurements serve as indicators of a consumer trophic position. In contrast, $\delta^{13}\text{C}$ ($^{13}\text{C}/^{12}\text{C}$) values vary little along the food chain and are mainly used to determine primary sources in a trophic network. In the marine environment, $\delta^{13}\text{C}$ values indicate consumer foraging areas (i.e. inshore versus offshore, pelagic versus benthic) or latitudinal variation in the contribution to food intake (Kelly 2000, Cherel & Hobson 2007).

In contrast to previous dietary analyses based on examination of stomach contents, the use of fatty acids as trophic markers was consistent with elephant seals having a fish-based diet (Brown et al. 1999) or a fish and squid diet, depending on the location of the foraging grounds (Bradshaw et al. 2003), hence the call for further analyses to determine the relative importance of squid versus fish in its food (Brown et al. 1999). To our knowledge, the stable isotope method was only used once for southern elephant seals, but, due to the lack of appropriate reference collections, prey species were not identified (Lewis et al. 2006). In the present study, we determine whether blood $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can indicate the feeding habits of adult female southern elephant seal (hereafter elephant seal) breeding on the Kerguelen Islands, southern Indian Ocean. We built up 3 unique datasets to interpret the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of seals relative to those of various marine organisms from the Southern Ocean.

The first dataset comprised isotopic data from Kerguelen penguins and fur seals, i.e. the guild of air-breathing diving vertebrates to which elephant seals belong. Penguins and fur seals breeding in the southern Indian Ocean occupy a wide range of trophic niches, including pelagic/oceanic and benthic/neritic species and both fish and crustacean eaters (Lescroel & Bost 2005, Lea et al. 2006, Cherel et al. 2007). The second set of data included $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from soft tissues of the main known pelagic resources from the Southern Ocean targeted by marine mammals and seabirds. Since female elephant seals dive pelagically from Kerguelen to the Polar Front and Antarctic waters

(Biuw et al. 2007), we focused on subantarctic and Antarctic swarming crustaceans (2 euphausiids and 1 hyperiid amphipod), 2 main subantarctic and Antarctic squid, and the 4 most abundant myctophid fishes that form the bulk of mesopelagic fish biomass in the Southern Ocean (Kozlov 1995). Remains from all these species were previously found in variable amounts in stomach contents of elephant seals (Slip 1995, Field et al. 2007). We also determined the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Patagonian toothfish living in slope waters, because the species was suggested to be a main prey of elephant seals (Brown et al. 1999). The third dataset was built using recently published $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of cephalopods living in Kerguelen waters, based on measurements of squid beaks (Cherel & Hobson 2005). The Kerguelen community is composed of almost all the main oceanic squid occurring in the Southern Ocean, including the giant and colossal squid. Finally, we estimated the trophic level (TL) of elephant seals using their $\delta^{15}\text{N}$ values and compared it with those of other consumers to verify the common assumption that marine mammals are top predators in marine ecosystems (Bradshaw et al. 2003, Field et al. 2007).

MATERIALS AND METHODS

Fieldwork was carried out at Kerguelen Islands (49° 21' S, 70° 18' E), located in the southern part of the Polar Frontal Zone in the immediate vicinity of the Polar Front (Park & Gambéroni 1997). A blood sample was taken from randomly chosen female southern elephant seals during the breeding period (early spring 2006), and from lactating female Antarctic fur seals *Arctocephalus gazella* and adults of each species of penguin in spring 2002 (first dataset). Macaroni and rockhopper penguins (*Eudyptes chrysolophus* and *E. chrysolophus filholi*, respectively) were captured just after their arrival in the colony to breed, while king and gentoo penguins (*Aptenodytes patagonicus* and *Pygoscelis papua*, respectively) were parent birds feeding chicks. The second dataset included pelagic crustaceans and fish that were collected at night in January 2005 in oceanic waters east of the Kerguelen Archipelago using an International Young Gadoid Pelagic Trawl. Patagonian toothfish *Dissostichus eleginoides* were caught on bottom longlines in Kerguelen slope waters in May 2006, and warty squid *Moroteuthis ingens* during trawl operations in September 2006.

To further constrain the foraging ecology of highly migratory elephant seals, values of species spanning a latitudinal gradient in carbon stable isotopes (Cherel & Hobson 2007) were used. First, a subtropical species, lactating female subantarctic fur seals *Arctocephalus tropicalis* from Amsterdam Island (37° 51' S, 77° 31' E),

and second, a high-Antarctic species, Adélie penguins *Pygoscelis adeliae* from Adélie Land (66° 40' S, 140° 01' E) at the beginning of their breeding cycle. Finally, barely digested Antarctic krill *Euphausia superba* and the squid *Psychroteuthis glacialis* were sorted from emperor penguin vomits found on the sea-ice in Adélie Land (Cherel 2008).

Once specimens of invertebrates and fish were identified, whole crustaceans and pieces of squid and fish muscle (see Table 1) were collected for isotopic analysis and kept at -20°C. Whole blood of pinnipeds and penguins was kept in 70% ethanol. After being dried in an oven at 60°C, crustaceans and muscle samples were ground to a fine powder and lipids were extracted using cyclohexane. The low blood lipid content does not typically necessitate lipid extraction (Cherel et al. 2005a,b). Carbonates were removed prior to lipids from crustaceans using 1 mol HCl l⁻¹. Relative abundance of ¹³C and ¹⁵N were determined using an Isoprime (Micromass) continuous-flow isotope-ratio mass spectrometer. Results are presented in the usual δ notation relative to Pee Dee Belemnite and atmospheric N₂ (air) for δ¹³C and δ¹⁵N, respectively.

To compare TL of pelagic organisms based on isotopic measurement, the following equation was used for invertebrates and fish:

$$TL_x = [(\delta^{15}N_x - 3.4)/3.2] + 2.0$$

where δ¹⁵N_x is the nitrogen isotope ratio of animal 'x', 3.4‰ is the average value for *Salpa thompsoni*, a dominant herbivorous salp (Perissinotto & Pakhomov 1998) in Kerguelen oceanic waters with an assumed TL of 2.0, and 3.2‰ is the average trophic enrichment factor between the muscle of fish and their food (Sweeting et al. 2007). The latter value is close to that of the cuttlefish *Sepia officinalis*, the only cephalopod species so far investigated (3.3‰, Hobson & Cherel 2006). Since enrichment factors vary with prey and predator tissue types, we used 1.7‰ as the enrichment factor between prey muscle and pinniped and penguin blood (Hobson et al. 1996, Lesage et al. 2002, Cherel et al. 2005b), and the following equation for estimated TL of mammals and birds:

$$TL_x = [(\delta^{15}N_x - 3.4 - 1.7)/3.2] + 3.0$$

The isotopic values of elephant seals were not corrected when compared to the δ¹⁵N and the δ¹³C values of marine organisms (second and third datasets) because (1) the enrichment factors are small relative to the spread of the data, and (2) statistical results were used not only to define the seal diet but also to look at the overall structure of the food web. Cephalopod beaks were depleted in ¹⁵N and enriched in ¹³C when compared to muscle tissue. The δ¹³C and δ¹⁵N values of beaks were consequently corrected in the third dataset by adding 4.9‰ to and subtracting 0.8‰ from their

δ¹⁵N and δ¹³C values, respectively, which correspond to the mean isotopic differences between lower beak and muscle of cephalopods (Cherel & Hobson 2005, Hobson & Cherel 2006). Data were statistically analysed using SYSTAT 9. Values are mean ± SD.

RESULTS

At Kerguelen, the 6 species of sympatric pinnipeds and penguins (first dataset) were segregated by their overall carbon and nitrogen stable isotope values (MANOVA, Wilk's lambda, $F_{10,176} = 67.04$, $p < 0.0001$) and, in univariate analysis, in their δ¹³C (ANOVA, $F_{5,89} = 45.05$, $p < 0.0001$) and δ¹⁵N values for blood ($F_{5,89} = 107.53$, $p < 0.0001$) (Table 1). Post hoc Tukey's HSD multiple comparison tests indicated that (1) gentoo penguins had a significantly higher δ¹³C value than the other 5 species (all $p < 0.0001$), and (2) female southern elephant seals had an isotopic value not statistically different from those of female Antarctic fur seals, king penguins and the 2 species of crested penguins (Fig. 1). Comparison tests showed that δ¹⁵N values of macaroni and rockhopper penguins were significantly lower than those of the other species (all $p < 0.0001$), and, interestingly, that the δ¹⁵N value of elephant seals was slightly, but significantly, different from that of Antarctic fur seals ($p = 0.023$), but not from that of king penguins. Elephant seals also segregated from species known to forage in either high-Antarctic (Adélie penguins) or subtropical (subantarctic fur seals) waters by both their δ¹³C and δ¹⁵N values (2-sample *t*-tests, $t = 15.44$ and 19.45 , and 12.71 and 25.38 , respectively, all $p < 0.0001$).

Elephant seals and subantarctic and Antarctic crustaceans, fish and squid (second dataset) were segregated by both δ¹³C (ANOVA, $F_{10,136} = 128.85$, $p < 0.0001$) and δ¹⁵N values ($F_{10,136} = 237.48$, $p < 0.0001$). Multiple comparison tests indicated that Patagonian toothfish, *Psychroteuthis glacialis* and Antarctic krill had higher and lower δ¹³C values than the other species, respectively (all $p < 0.004$). Otherwise, the δ¹³C value of elephant seals was not statistically different from those of the myctophids *Electrona antarctica*, *E. carlsbergi* and *Gymnoscopelus nicholsi*, but it was significantly different from that of *Krefftichthys anderssoni* ($p = 0.001$). Its higher δ¹⁵N value segregated toothfish from other marine organisms (all $p < 0.0001$), while the 3 crustacean species segregated from fishes, squid and seal by their lower δ¹⁵N values (all $p < 0.0001$). The δ¹⁵N value of elephant seals was higher than those of *E. antarctica* ($p < 0.0001$), *E. carlsbergi* ($p = 0.017$) and *K. anderssoni* ($p < 0.0001$), but not statistically different from the values of *G. nicholsi* and warty squid (Fig. 2).

Table 1. Stable carbon and nitrogen isotope values of female elephant seals and of various marine organisms from the Southern Ocean

Species	Location	Tissue	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Estimated trophic level
Female southern elephant seal <i>Mirounga leonina</i>	Kerguelen	Whole blood	32	-21.4 ± 0.8	10.1 ± 0.3	4.6
FIRST DATASET (predator community)						
Pinnipeds						
Female Antarctic fur seal <i>Arctocephalus gazella</i>	Kerguelen	Whole blood	10	-20.8 ± 0.4	10.7 ± 0.2	4.8
Female subantarctic fur seal <i>Arctocephalus tropicalis</i>	Amsterdam	Whole blood	10	-18.1 ± 0.2	13.5 ± 0.4	–
Penguins						
King penguin <i>Aptenodytes patagonicus</i>	Kerguelen	Whole blood	12	-21.6 ± 0.3	9.8 ± 0.2	4.5
Gentoo penguin <i>Pygoscelis papua</i>	Kerguelen	Whole blood	10	-18.1 ± 1.1	10.4 ± 1.3	–
Southern rockhopper penguin <i>Eudyptes chrysocome filholi</i>	Kerguelen	Whole blood	20	-21.6 ± 0.6	8.1 ± 0.3	4.0
Macaroni penguin <i>Eudyptes chrysolophus</i>	Kerguelen	Whole blood	11	-21.3 ± 0.3	7.0 ± 0.5	3.6
Adélie penguin <i>Pygoscelis adeliae</i>	Adélie Land	Whole blood	10	-25.4 ± 0.2	7.9 ± 0.1	3.9
SECOND DATASET (marine resources)						
Amphipod						
<i>Themisto gaudichaudii</i>	Kerguelen	Whole individuals	10	-22.8 ± 0.7	5.0 ± 1.2	2.5
Euphausiids						
<i>Euphausia vallentini</i> (subantarctic krill)	Kerguelen	Whole individuals	10	-20.0 ± 1.2	5.4 ± 0.8	2.6
<i>Euphausia superba</i> (Antarctic krill)	Adélie Land	Whole individuals	12	-25.8 ± 0.4	5.5 ± 0.4	–
Squid						
<i>Moroteuthis ingens</i> (warty squid)	Kerguelen	Mantle	10	-20.1 ± 0.4	10.0 ± 0.4	4.1
<i>Psychroteuthis glacialis</i>	Adélie Land	Arms	10	-25.5 ± 0.3	10.9 ± 1.1	–
Fish						
<i>Krefflichthys anderssoni</i>	Kerguelen	White muscle	12	-22.3 ± 0.2	7.6 ± 0.2	3.3
<i>Electrona antarctica</i>	Kerguelen	White muscle	12	-21.4 ± 0.5	8.9 ± 0.3	3.8
<i>Electrona carlsbergi</i>	Kerguelen	White muscle	12	-21.6 ± 0.4	9.5 ± 0.2	3.9
<i>Gymnoscopelus nicholsi</i>	Kerguelen	White muscle	12	-21.1 ± 0.3	10.2 ± 0.5	4.2
<i>Dissostichus eleginoides</i> (Patagonian toothfish)	Kerguelen	White muscle	15	-19.0 ± 0.7	12.9 ± 0.6	5.0
THIRD DATASET (squid, corrected values)						
<i>Architeuthis dux</i> (giant squid)	Kerguelen	Beaks	4	-18.1 ± 0.3	11.6 ± 1.3	4.6
<i>Mesonychotheuthis hamiltoni</i> (colossal squid)	Kerguelen	Beaks	10	-20.4 ± 1.4	16.3 ± 0.8	6.1
<i>Martalia hyadesi</i>	Kerguelen	Beaks	10	-20.9 ± 0.5	7.7 ± 0.6	3.4
<i>Todarodes</i> sp.	Kerguelen	Beaks	10	-20.4 ± 0.4	8.2 ± 0.8	3.6
<i>Moroteuthis knipovitchi</i>	Kerguelen	Beaks	5	-21.5 ± 0.5	11.1 ± 0.4	4.4
<i>Kondakovia longimana</i>	Kerguelen	Beaks	3	-21.1 ± 0.5	9.2 ± 1.1	3.8
<i>Slosarczykovia circumantarctica</i>	Kerguelen	Beaks	10	-25.8 ± 0.3	8.8 ± 0.5	3.7
<i>Gonatus antarcticus</i>	Kerguelen	Beaks	10	-21.0 ± 0.8	13.3 ± 0.5	5.2
<i>Taningia danae</i>	Kerguelen	Beaks	3	-20.8 ± 0.4	11.6 ± 0.1	4.6
<i>Histioteuthis eltaninae</i>	Kerguelen	Beaks	10	-20.7 ± 0.4	10.4 ± 0.7	4.2
<i>Nototeuthis dimegacotyle</i>	Kerguelen	Beaks	10	-20.3 ± 0.7	11.4 ± 0.4	4.6
<i>Mastigoteuthis psychrophila</i>	Kerguelen	Beaks	10	-21.0 ± 0.6	12.8 ± 0.6	5.0
<i>Chiroteuthis veranyi</i>	Kerguelen	Beaks	10	-20.0 ± 0.6	12.9 ± 1.3	5.0

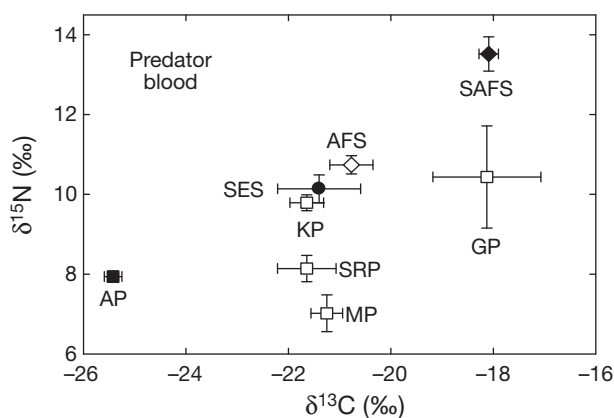


Fig. 1. Stable carbon and nitrogen isotope values in whole blood of elephant seals and other air-breathing diving predators. AFS: female Antarctic fur seal (Kerguelen); AP: Adélie penguin (Adélie Land, Antarctica); GP: gentoo penguin (Kerguelen); KP: king penguin (Kerguelen); MP: macaroni penguin (Kerguelen); SAFS: subantarctic fur seal (Amsterdam, subtropics); SES: female southern elephant seal (Kerguelen); SRP: southern rockhopper penguin (Kerguelen)

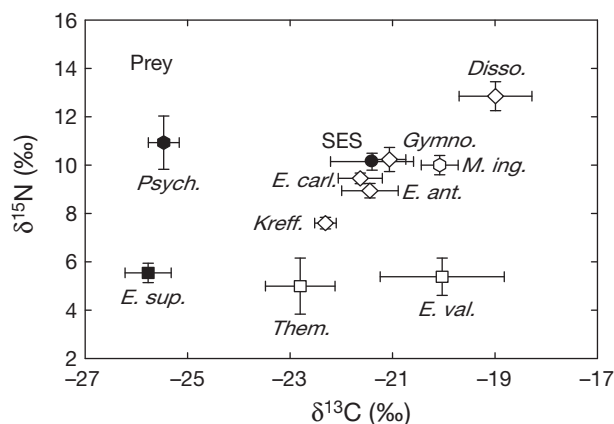


Fig. 2. Stable carbon and nitrogen isotope values in whole blood of elephant seals and in muscle (fish) and whole specimens (crustaceans) of potential prey from Kerguelen Islands (open symbols) and Antarctica (filled symbols). *Disso.*: *Dissostichus eleginoides* (Patagonian toothfish); *E. ant.*: *Electrona antarctica* (myctophid); *E. carl.*: *Electrona carlsbergi* (myctophid); *E. sup.*: *Euphausia superba* (Antarctic krill); *E. val.*: *Euphausia vallentini* (subantarctic krill); *Gymno.*: *Gymnosopelus nicholsi* (myctophid); *Kreff.*: *Krefflichthys anderssoni* (myctophid); *M. ing.*: *Moroteuthis ingens* (warty squid); *Psych.*: *Psychroteuthis glacialis* (squid); SES: female southern elephant seal; *Them.*: *Themisto gaudichaudii* (amphipod)

Elephant seals and Kerguelen squid (third dataset) were segregated by their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Kruskal-Wallis, $H = 71$ and 123 , respectively, both $p < 0.0001$). Except *Slosarczykovia circumantarctica* and the giant squid *Architeuthis dux* (all $p < 0.0001$), squid species showed a restricted range in $\delta^{13}\text{C}$ values, with elephant seals having a $\delta^{13}\text{C}$ value that was not statistically different from those of *Gonatus antarcticus*, *His-*

toteuthis eltaninae, *Kondakovia longimana*, *Martialia hyadesi*, *Mastigoteuthis psychrophila*, *Moroteuthis knipovitchi* and *Taningia danae*. The colossal squid *Mesonychoteuthis hamiltoni* had the highest $\delta^{15}\text{N}$ value (all $p < 0.0001$), which was much higher than that of elephant seals (Fig. 3). Only 3 squid species (*S. circumantarctica* and juvenile *Martialia hyadesi* and *Todarodes* sp.) had $\delta^{15}\text{N}$ values significantly lower than elephant seals (all $p < 0.0001$), with 3 other species (*Histioteuthis eltaninae*, *K. longimana* and *Moroteuthis knipovitchi*) having similar values to seals.

Estimated TLs within the community of Kerguelen consumers ranged from 2.5 (the amphipod *Themisto gaudichaudii*) to 6.1 (colossal squid), with southern elephant seals having an intermediate position (TL = 4.6). Four squid species and the Patagonian toothfish had a much higher TL (≥ 5.0) than elephant seals, which shared the same TL (4.5 to 4.6) as 3 squid species and the king penguin (Table 1).

DISCUSSION

This study is the first, to our knowledge, to investigate the TL and dietary habits of elephant seals by comparing their blood isotopic values with the values for a large range of marine organisms from the Southern Ocean. The stable isotope method is based on time-integrated assimilated food, thus contrasting with the snapshot of stomach content analysis. Blood of

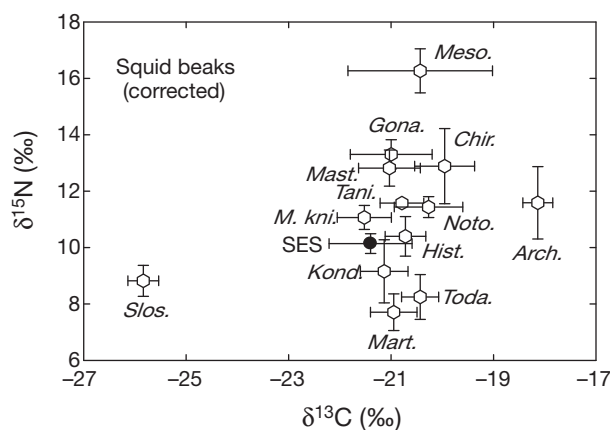


Fig. 3. Stable carbon and nitrogen isotope values in whole blood of elephant seals (SES, filled symbol) and in chitinized beaks of potential squid prey from Kerguelen Islands. Isotope values of beaks were corrected (see 'Materials and methods'). Arch.: *Architeuthis dux* (giant squid); Chir.: *Chiroteuthis veranyi*; Gona.: *Gonatus antarcticus*; Hist.: *Histioteuthis eltaninae*; Kond.: *Kondakovia longimana*; Mart.: *Martialia hyadesi*; Mast.: *Mastigoteuthis psychrophila*; Meso.: *Mesonychoteuthis hamiltoni* (colossal squid); M. kni.: *Moroteuthis knipovitchi*; Noto.: *Nototeuthis dimegacotyle*; SES: female southern elephant seal; Slos.: *Slosarczykovia circumantarctica*; Tani.: *Taningia danae*; Toda.: *Todarodes* sp.

large mammals integrates a period of 2 to 3 mo (Hilderbrand et al. 1996), corresponding here to the food consumed by female elephant seals at the end of the austral winter, during the last part of their 8 mo postmolt foraging trip. The stable isotope values of consumers may be affected by various extrinsic and intrinsic processes, including ecological gradients at the base of the food webs and physiological status of the animals. Seals fast during lactation but, in agreement with little changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of penguin blood with fasting (Cherel et al. 2005a), preliminary investigations on female elephant seals have shown no significant differences in blood carbon and nitrogen values between the beginning and the end of the lactation period (Ducatez et al. 2008). Variation in $\delta^{15}\text{N}$ values in consumers may result not only from the TL at which they feed, but also from changes at the base of the food web. $\delta^{15}\text{N}$ values of oceanic particulate organic matter (POM) vary spatiotemporally in the Southern Ocean (Altabet & François 1994, Lourey et al. 2003), and these changes can potentially affect our calculation of TL. For example, the large $\delta^{15}\text{N}$ gradients at the Subtropical Front (Altabet & François 1994) and between neritic and oceanic waters at Kerguelen (Cherel & Hobson 2007) precluded the accurate estimation of TL for the neritic gentoo penguin and subantarctic fur seals foraging in subtropical waters (Table 1). However, latitudinal variation in POM $\delta^{15}\text{N}$ is relatively low in oceanic waters of the Southern Ocean, and seasonal variation is integrated and buffered all along the food web, from short-lived phytoplankton to long-lived predators. Finally, little effect of temporal variation in POM isotope baseline levels was also indicated by the consistency of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of female seals over time, since seals sampled in other years (2004–2007) and seasons (pre- and postmolt trips) had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values not significantly different from those sampled in 2006 (authors' unpubl. data).

Inshore/offshore and latitudinal gradients in $\delta^{13}\text{C}$ values helped us to delineate foraging areas of female elephant seals from Kerguelen. Elephant seal carbon values were much more positive than those of Antarctic organisms and more negative than those of fur seals breeding in the subtropics, showing that they foraged neither in cold waters surrounding the Antarctic continent nor in warmer subtropical waters north of the Subtropical Front. Instead, their $\delta^{13}\text{C}$ values (from -23.1 to -20.1 ‰) indicated that, depending on individuals, female elephant seals fed from medium and/or low latitudes of the Antarctic Zone to the Subantarctic Zone, including the Polar Frontal Zone. The $\delta^{13}\text{C}$ values of elephant seals were also much more negative than those of the neritic forager, the gentoo penguin, showing that they did not forage over the Kerguelen shelf (<200 m). The seal $\delta^{13}\text{C}$ value was thus in general

agreement with tracking data showing that adult females travel quickly over the shelf to forage over wide oceanic areas of the Southern Ocean in winter (Hindell et al. 2003, Lewis et al. 2006).

Pinnipeds, including elephant seals, are generally considered to be top predators, sharing a place at the highest TLs of the food web with seabirds and some large pelagic fish. However, evidence from stable isotopes indicates that this common paradigm is questionable. When compared to other consumers from the Southern Ocean, including those considered as from lower TLs (e.g. some mesopelagic fish and squid), there was significant overlap. Moreover, the most singular pattern to emerge from estimated TLs was that the colossal squid (TL = 6.1), and, to a lesser extent, the Patagonian toothfish (5.0), were the highest top consumers in Kerguelen waters, but not giant squid (4.6), pinnipeds (4.6 to 4.8) or penguins (3.6 to 4.5). Derived TLs from $\delta^{15}\text{N}$ values showed that marine mammals (including elephant seals) and seabirds belong to the upper levels of the trophic web, but most of these species do not occupy the highest levels, as does the polar bear in the high-Arctic (Hobson et al. 2002).

Nitrogen values of penguins and fur seals at Kerguelen (first dataset) agreed with the known trophic structure of the community and feeding ecology of the species, i.e. the fish-eating king penguins and female Antarctic fur seals were one TL higher than the crustacean-eating macaroni and rockhopper penguins and the Antarctic Adélie penguin (Ridoux & Offredo 1989, Lea et al. 2006, Cherel et al. 2007). Within that community, the $\delta^{15}\text{N}$ value of elephant seals was higher (about 1 TL) than those of both species of crested penguins, and was much higher (about 2 TL) than those of euphausiids and amphipods. Taken together, these data indicated that elephant seals fed on crustacean-eating prey and were thus not primarily crustacean eaters, which is in agreement with both stomach content and blubber and milk lipid analyses (Slip 1995, Brown et al. 1999, Bradshaw et al. 2003).

The 3 isotopic datasets shed new light on the old debate about the relative proportions of squid versus fish in the diet of elephant seals. The mean nitrogen isotope value of female seals was not statistically different from that of king penguins and was close to that of Antarctic fur seals, 2 consumers that feed primarily on lanternfish (Myctophidae) (Cherel et al. 2002, Lea et al. 2006). Myctophids (*Electrona antarctica*, *E. carlsbergi* and *Krefflichthys anderssoni*) are crustacean eaters (Gaskett et al. 2001), and their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values fit well with those of theoretical prey of elephant seals (second dataset). Myctophids were the commonest fish identified from stomach contents of elephant seals, but, owing to their small size, they amounted to a low biomass and were sometimes con-

sidered as secondary prey of squid (Slip 1995, Daneri & Carlini 2002, Field et al. 2007). Milk fatty acid concentration analysis indicated that elephant seal females may indeed feed on myctophids and/or on the larger Patagonian toothfish (Brown et al. 1999). A few otoliths of toothfish were recovered from seal stomach contents (Slip 1995, Field et al. 2007), but its high $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values precluded the species being a significant prey of female elephant seals at Kerguelen.

In the same way, most of the main species of cephalopods eaten by elephant seals (third dataset) had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values precluding them as important dietary items of seals at the end of winter. $\delta^{13}\text{C}$ values for *Psychroteuthis glacialis* and *Slosarczykovia circumantarctica* were too low, and $\delta^{15}\text{N}$ values for *Gonatus antarcticus*, onychoteuthids (the major squid prey at Heard Island on the Kerguelen Plateau) and *Histioteuthis eltaninae* were too high when compared to seal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, respectively. Such a high $\delta^{15}\text{N}$ value of oceanic squid is the likely explanation of the elevated blood $\delta^{15}\text{N}$ value of the most specialized cephalopod-eating seabird from the Southern Ocean, the wandering albatross, which, accordingly, shows a higher TL than elephant seals (4.6 versus 5.7, authors' unpubl. data). Among squid, only ommastrephid juveniles (*Martialia hyadesi* and *Todarodes* sp.) had $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values that fit well with those of theoretical prey of elephant seals. Ommastrephids are common (but not the main) food items of seals from South Georgia and Macquarie, but not at Heard Island (Rodhouse et al. 1992, Green & Burton 1993, Slip 1995). Accordingly, only one ommastrephid beak (of a total of 57 beaks retrieved from 7 seals, authors' unpubl. data) was identified in a preliminary dietary investigation, suggesting that they are not important elephant seal prey at Kerguelen, at least at the very end of their foraging trip.

Isotopic data of elephant seals and of their potential prey items are consistent with a diet dominated by myctophids, but, theoretically, they cannot preclude the possibility that seals fed on a mixed diet including fish and squid, or even solely a variety of squid (e.g. a mixture of species having high and low $\delta^{15}\text{N}$ values leading to an integrated intermediate value for elephant seals). However, a reassessment of both stomach content and fatty acid data also suggests that cephalopods are not the staple food of female elephant seals. The 2 main arguments supporting the hypothesis that seals are mainly teuthivorous consumers are the long-recognized presence of cephalopod beaks in their stomach (Rodhouse et al. 1992, Green & Burton 1993), and the recent finding that some of their blubber fatty acid profiles classify with those of squid (Bradshaw et al. 2003). As previously discussed, chitinized beaks of cephalopods resist digestion better than fish items;

consequently, they accumulate over time, leading to an overestimation of cephalopods in the diet of consumers (Rodhouse et al. 1992). Another key, but previously unrecognized, issue is that analyzed stomach contents of elephant seals contain fewer beaks than those of the squid-eating toothed whales (usually 10s versus 100s to 1000s) (Whitehead et al. 2003), but about the same order of magnitude as food samples of many crustacean- and fish-eating predators, including king penguins (Cherel et al. 2002). Interestingly, king penguins were initially considered squid consumers on the basis of beaks collected from fasting individuals (Croxall & Prince 1980) before subsequent dietary and lipid analyses recognized them as the most specialized myctophid-eating seabird from the Southern Ocean (Raclot et al. 1998, Cherel et al. 2002).

The second argument is that some elephant seal lipid profiles correlate better with squid than with fish profiles (Bradshaw et al. 2003). However, the taxonomic resolution of statistical analysis of fatty acid patterns in the study was coarse (i.e. fish versus squid, Bradshaw et al. 2003), while fish lipid patterns show considerable taxonomic variation (at the species and family levels), thus allowing separation of fishes into functional groups for a better predator-prey analysis (Connan et al. 2007a,b). Moreover, due to the importance of prey lipids in the digestive gland of squid (that exceed mantle lipid content by an order of magnitude), it is important to consider the type of squid fatty acid data included in dietary studies of top predators (Jackson et al. 2007). When whole squid and/or digestive gland are used to represent potential prey items (e.g. Bradshaw et al. 2003), the squid lipid profile may be very similar to other prey such as myctophids, thus constraining the use of fatty acids to assess the importance of squid in the diet of predators (Jackson et al. 2007). Within that context, it is remarkable that lipid analysis classified elephant seals with squid known to be primarily myctophid eaters (Phillips et al. 2002, 2003), and that seals had a $\delta^{15}\text{N}$ value not statistically different from that of the mesopelagic fish-eating warty squid (Cherel & Duhamel 2003).

In summary, a thorough comparison of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of predators, prey and elephant seals supports the hypothesis that the main prey of female elephant seals are myctophid fishes, not squid. We therefore suggest that they occupy a unique ecological niche amongst the guild of subantarctic and Antarctic air-breathing diving vertebrates by feeding on mesopelagic fish at great depths. Elephant seals routinely dive much deeper than penguins and other seals (Hindell et al. 1991), making them, together with the squid-eating toothed whales, the only warm-blooded consumers physiologically capable of foraging in the deep. At depths > 300 m, they may target both migrating myctophids and the non-migrating

deepest living mesopelagic fish. Noticeably, myctophids descend to greater depths (350 to 500 m) in winter (Kozlov 1995), and thus are largely unavailable to king penguins at that time, but not to female elephant seals. At depth, the most significant competitors of seals for food resources are probably oceanic squid; the little information available indicates that these major consumers target primarily mesopelagic fish (Lubimova 1985). Foraging in the same habitat and on the same prey is therefore the likely explanation why female elephant seals opportunistically include some squid in their diet.

In the present study, a small variance in $\delta^{15}\text{N}$ values of female elephant seals was observed over a larger range of $\delta^{13}\text{C}$ values, suggesting that all female seals fed at the same TL and on the same prey whatever their foraging zones. Within that context it is noticeable that the $\delta^{15}\text{N}$ value of the main Antarctic myctophid *Electrona antarctica* was almost identical in both Kerguelen (the present study) and high-Antarctic waters (Rau et al. 1992), suggesting little latitudinal variation in myctophid $\delta^{15}\text{N}$ value. However, more information is needed on the $\delta^{15}\text{N}$ values of other potential prey in the Southern Ocean to better define the isotopic niche of elephant seals. The present study focused on adult female elephant seals at Kerguelen Islands, but elsewhere in the Southern Ocean, females also forage in oceanic waters (McConnell & Fedak 1996, Campagna et al. 1998, Jonker & Bester 1998, Hindell et al. 2003) where myctophids dominate the mesopelagic fish assemblage (Kozlov 1995). Therefore, female southern elephant seals may target myctophid fish, and to a lesser extent squid, across their wide geographic distribution. Elephant seals are highly sexually dimorphic as adults and pronounced sexual and ontogenetic differences were found in the feeding ecology of the species (Slip et al. 1994, Field et al. 2007). Further investigation on males, immature animals and females at other locations are required for better overall knowledge of the trophic relationships of southern elephant seals. Nevertheless, the present study adds new information on the poorly documented composition of southern elephant seal food, which can be used to improve prey-consumption models and, thus, our understanding of the functioning of the pelagic ecosystem (Hindell et al. 2003). It also emphasizes the role of mesopelagic fish in the Southern Ocean as a link between meso- and macrozooplankton and large predators foraging in oceanic waters (Kozlov 1995, Connan et al. 2007b).

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