Limnol. Oceanogr., 55(1), 2010, 324–332

Isotopic niches and trophic levels of myctophid fishes and their predators in the Southern Ocean

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Abstract

We report the trophic structure of a myctophid assemblage by measuring the isotopic niches of 14 species living in Kerguelen waters, southern Indian Ocean. Most of the species show distinct isotopic niches that differ by at least one of the two niche axes (δ^{13}C habitat and δ^{15}N trophic position), indicating trophic partitioning within the assemblage. Strong niche segregation occurs within each of the three most common genera of myctophids (*Electrona*, *Gymnoscopelus*, and *Protomyctophum*), illustrating the different mechanisms (habitat and dietary segregation) that allow coexistence of closely related species. Calculated trophic levels (TLs) of myctophids ranged from 3.3 to 4.2, showing that they are secondary and tertiary consumers in the pelagic ecosystem. The positive relationship between TL and standard length of fish points out a structuring effect of size, with larger species (*Gymnoscopelus* spp.) occupying a higher trophic position than smaller species (*Krefftichthys anderssoni* and *Protomyctophum* spp.). Myctophids occupy an intermediate trophic position between macrozooplanktonic crustaceans and seabirds and marine mammals within the pelagic ecosystem. However, the TLs of large myctophids overlap those of crustacean-eating seabirds [e.g., *Eudyptes* spp. (crested penguins) and *Pachyptila belcheri*]. The isotopic niche of myctophids indicates that *Aptenodytes patagonicus* (king penguin) adults prey upon *K. anderssoni* when they feed for themselves, thus exemplifying the usefulness of isotopic datasets on potential prey of predators to depict trophic relationships.

Myctophid (and gonostomatid) fishes are the dominant mesopelagic fish of the oceanic ecosystem worldwide, playing a key role in pelagic food webs. They mainly feed on planktonic crustaceans and are therefore considered to be an essential component of the tertiary level of the pelagic ecosystem (Pakhomov et al. 1996; Pusch et al. 2004). In turn, they are consumed by a range of predators, including fish, squids, seabirds, and marine mammals (Rodhouse and Nigmatullin 1996; Connan et al. 2007). Myctophids thus play a pivotal role in the transfer of energy from zooplankton to higher trophic levels (TLs). Determining their feeding habits and trophic positions are thus essential for a better understanding of the functioning of the pelagic ecosystem. In the Southern Ocean (south of the Subtropical Front), myctophids form by far the bulk of the mesopelagic fish biomass; their standing stock is estimated to be 70–200 10^9 kg (Sabourenkov 1992; Kozlov 1995). Hulley (1990) lists ~ 43 species in the area, of which 11 are abundant in the epipelagic and mesopelagic layers, where their role as prey is demonstrated by their importance in the diet of top predators (Sabourenkov 1992; Kozlov 1995; Guinet et al. 1996). Despite the biological significance of Southern Ocean myctophids, data on their ecology is remarkably sparse. Dietary information is restricted to the most common species and to a few assemblages with limited spatio-temporal scales and, in some cases, with very small sample sizes (Pakhomov et al. 1996; Gaskett et al. 2001; Schreeve et al. 2009).

Measuring the isotopic niche of animals is a powerful alternative to the conventional means of investigating various dimensions of their ecological niche (Newsome et al. 2007). The basic isotopic concept is that an animal's chemical composition is directly influenced by what it consumes. Consumers are enriched in ^{15}N relative to their food, and consequently, stable-nitrogen-isotope measurements (^{15}N : ^{14}N, δ^{15}N) serve as indicators of a consumer trophic position. By contrast, stable-carbon signatures (^{13}C : ^{12}C, δ^{13}C) vary little along the food chain and, in the marine environment, δ^{13}C values are mainly used to indicate the foraging habitats of predators, including fish. The stable isotope method is based on time-integrated assimilated food. Hence, the isotopic signature of white muscle was considered to be representative of the isotopic niche of fish during the months preceding sampling (Herzka 2005), thus contrasting with the snapshot method of analyzing stomach contents and avoiding biases induced by feeding in nets. The stable isotope method also allows quantifying trophic levels of consumers and thus comparing taxonomically related and unrelated species within a given ecosystem (Hobson et al. 1994; Pinnegar et al. 2002; Cherel et al. 2008).

To date, no isotopic investigations of myctophid assemblages are available in the scientific literature, and isotopic information on myctophids from the Southern Ocean is restricted to a few species and individuals (Rau et al. 1992; Pakhomov et al. 2006). The primary objective of this study was to define and compare the isotopic niches and trophic levels of coexisting myctophid fishes living in the Southern Ocean. We focused on resource partitioning within the fish assemblage occurring in the vicinity of the Polar Front, where myctophids concentrate and are targeted by top consumers (Bost et al. 2009). Stable isotope analyses were

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Table 1. Sizes, isotopic niches, and estimated trophic levels of myctophid fishes from Kerguelen waters. Age was estimated using standard length (Hulley 1990). Values are mean ± SD.

Species	Age	*n*	SL (mm)	Mass (g)	δ^{13}C (‰)	δ^{15}N (‰)	C : N	Trophic level
Electrona antarctica	Adults and subadults	12	71±8	4.6±1.5	−21.4±0.5	8.9±0.3	3.26±0.04	3.8±0.1
E. carlsbergi	Adults	12	90±4	11.4±2.2	−21.6±0.4	9.5±0.2	3.31±0.03	3.9±0.1
E. subaspera	Mostly subadults	14	99±14	16.9±7.5	−20.2±0.4	7.3±0.3	3.31±0.04	3.3±0.1
Gymnoscopelus bolini	Subadults	12	205±9	97±14	−20.5±0.4	9.9±0.5	3.24±0.04	4.1±0.2
G. braueri	Adults and subadults	12	107±7	11.5±2.5	−22.3±0.7	9.8±0.3	3.26±0.03	4.0±0.1
G. fraseri	Adults	12	83±4	6.4±0.9	−21.1±0.4	9.0±0.4	3.26±0.03	3.8±0.1
G. nicholsi	Subadults	12	126±3	20.5±1.6	−21.1±0.3	10.2±0.5	3.33±0.04	4.2±0.2
G. piabilis	Adults	12	137±6	30.0±4.6	−19.8±0.3	8.8±0.2	3.24±0.02	3.7±0.1
Krefftichthys anderssoni	Adults and subadults	12	51±4	1.5±0.4	−22.3±0.2	7.6±0.2	3.41±0.05	3.3±0.1
Protomyctophum andriashevi	Mostly adults	7	51±4	1.9±0.4	−20.9±0.3	8.7±0.4	3.30±0.05	3.7±0.1
P. bolini	Adults	12	56±4	2.2±0.3	−22.4±0.6	9.2±0.4	3.26±0.03	3.9±0.1
P. choriodon	Subadults	12	62±4	3.7±0.7	−20.0±0.5	7.8±0.3	3.46±0.22	3.4±0.1
P. gemmatum	Adults	4	77±4	6.5±1.4	−22.1±0.1	8.7±0.4	3.20±0.02	3.7±0.1
P. tenisoni	Adults	11	52±1	1.7±0.2	−22.1±0.3	8.1±0.3	3.28±0.04	3.5±0.1

also performed on potential prey (macrozooplankton) and predators (seabirds) to create a basis for the interpretation of the isotopic signatures and trophic levels of myctophids within the oceanic pelagic ecosystem.

The present work is a part of a larger program examining isotopically the trophic web at Kerguelen Islands to better interpret the isotopic niches, and thus the feeding ecology of seabirds and marine mammals in an area where *Euphausia superba* (Antarctic krill) do not occur. This makes the archipelago an ideal location to investigate interspecies resource partitioning in the absence of the masking effect of the superabundance of a single prey on segregating mechanisms. The selected sampling sites for myctophids were included within the foraging areas of *Aptenodytes patagonicus* (king penguins) and female *Arctocephalus gazella* (Antarctic fur seals) at a time of the year (summer) when both species are known to prey on them (Guinet et al. 2001; Bost et al. 2009). The usefulness of the approach was tested first by comparing the isotopic signature of myctophids with those of the food of *A. patagonicus* chicks and of the chicks themselves. Second, the comparison was extended to breeding and molting adult *A. patagonicus*, since their feeding habits cannot be directly investigated. In most seabirds, the conventional method of dietary analysis is temporally limited to the chick-rearing period, when parents feed their chicks in the colonies; therefore, no indications are given with regard to diet of breeding adults when they feed for themselves and with regard to dietary variations over the annual cycle (Cherel et al. 2007; Connan et al. 2007).

Methods

Fieldwork was carried out at Kerguelen Islands (southern Indian Ocean), which is located in the southern part of the Polar Frontal Zone, in the immediate vicinity of the Polar Front. The purpose of the cruise was neither to quantify the myctophid diversity and biomass nor to detail their vertical distribution and diet, but, instead, to collect the main mesopelagic fish and macrozooplankton species occurring in Kerguelen waters in order to build biochemical reference datasets (i.e., stable isotopes and lipids) to better investigate trophic relationships within the pelagic ecosystem. Following Duhamel et al. (2000), the study area (49°05′–49°20′S, 71°15′–72°15′E) was located in slope waters, where the Polar Front runs northward along and off the eastern shelf of the archipelago. Biological data were collected on board the 25-m-long trawler *La Curieuse* during two consecutive nights (from 22 to 24 January 2005). Fish and invertebrates were sampled using an International Young Gadoid Pelagic Trawl (IYGPT; opening: 12 m × 7 m) with a 10-mm cod-end mesh (Duhamel et al. 2000). Fishing depth was estimated during trawling operations using relationship between meters-wire-out (the number of meters the wire of the collection gear was out from the collecting vessel) and depth, and it was logged using a time-depths recorder (Mk7, Wildlife Computers). Eight trawls (four per night) were performed at different depths ranging from 50 m to 425 m. The net was deployed using standardized 30-min duration of trawling at a given depth with a constant speed (5.6 km h^{-1}). The catches from each haul were sorted onboard into four categories: myctophids, other mesopelagic fish, macrozooplankton, and squid. Only myctophids and macrozooplankton species are considered in the present work. Fish identification relied on their external features and on otolith morphology, using published guides (Hulley 1981, 1990) and our own reference collection. When available, 12 individuals from each myctophid species were selected for isotopic analyses (Table 1). Each individual fish was weighed and measured (standard length, SL).

To help investigate trophic relationships of myctophids within the pelagic ecosystem, the isotopic signatures of some additional species were determined. They were the

euphausiid *Thysanoessa* spp., and some diving and flying air-breathing vertebrates. *Thysanoessa* spp. was collected from stomach contents ($n = 9$) of *Pelecanoides georgicus* (South Georgian diving petrels). Three groups of seabird and pinniped species were selected according to the importance of myctophids in their diet. In group 1, myctophids form the bulk of their food (*A. patagonicus* and *A. gazella*); in group 2, they are an important component of their diet [*Eudyptes chrysolophus* (macaroni penguin) and *Halobaena caerulea* (blue petrel)], and in group 3, they only constitute a minor dietary component [*Eudyptes chrysocome* (rockhopper penguin), *Pachyptila belcheri* (thin-billed prion), and *Diomedea melanophrys* (black-browed albatross)] (Table 2). Blood samples were taken from seabird chicks, *A. patagonicus* adults, and from lactating female *A. gazella* in summer. Additional groups of *A. patagonicus* were blood sampled in spring, including *A. patagonicus* chicks, breeding *A. patagonicus* adults rearing chicks, and molting *A. patagonicus* adults. Food samples were collected from the breeding *A. patagonicus* adults using the stomach lavage method, and food analysis followed Cherel et al. (2002*c*).

Blood was kept in 70% ethanol, and food samples were kept at −20°C until analysis. Once specimens of invertebrates and fish were identified, whole crustaceans and pieces of fish white muscle were collected for isotopic analysis and were kept in 70% ethanol. After being dried in an oven at +60°C, crustaceans, fish muscles, and subsamples of *A. patagonicus* food were ground to a fine powder, and lipids were extracted using cyclohexane (C_6H_{12}). The extent of lipid extraction was subsequently checked through the C : N mass ratio of the samples, because C : N in soft tissues is positively related to their lipid content. The low C : N ratio of the delipidated samples allowed the comparison of the carbon isotopic signature of the fatty myctophids without any deleterious effect due to different lipid contents among individuals and species (Table 1). The low blood-lipid content does not typically necessitate lipid extraction. Carbonates were removed prior to lipids from crustaceans using 1 mol L^{-1} HCl. Relative abundance of ^{13}C and ^{15}N were determined using an Isoprime (Micromass) continuous-flow isotope-ratio mass spectrometer. Results are presented in the usual δ notation relative to PeeDee belemnite and atmospheric N_2 (Air) for $\delta^{13}C$ and $\delta^{15}N$, respectively. Replicate measurements of internal laboratory standards (acetanilide) indicate measurement errors < 0.15‰ and < 0.20‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

To compare TLs 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 $\delta^{15}N_x$ is the nitrogen isotope signature of animal "x"; 3.2‰ is the average trophic enrichment factor between muscle of fish and their food (Sweeting et al. 2007); and 3.4‰ is the average value for *Salpa thompsoni*, a dominant herbivorous salp in the Southern Ocean with an assumed TL of 2.0. Concurrent measurements of $\delta^{15}N$ values of particulate organic matter (POM) in summer 2005 in Kerguelen oceanic waters were accordingly $\sim$ 0.0‰ (Trull et al. 2008), i.e., approximately one trophic level below salps. Since enrichment factors vary with prey and predator tissue types, we used 1.7‰ as the enrichment factor between prey muscle and penguin and fur seal blood (references in Cherel et al. 2008), and the following equation for estimated TL of mammals and birds: $TL_x = [(\delta^{15}N_x - 3.4 - 1.7) : 3.2] + 3.0$. Values are means ± SD. Data were statistically analysed using SYSTAT 12 (Systat).

Results

Myctophid assemblage: Species and genera—According to their size (Hulley 1990), all the myctophids investigated in the present study were subadult and adult specimens (Table 1). At Kerguelen, the 14 species of coexisting myctophids were segregated by their overall isotopic signatures [multivariate analysis of variance (MANOVA), Wilk's λ, $F_{26,282} = 58.56$, $p < 0.0001$] and, in univariate analysis, both $\delta^{13}C$ [analysis of variance (ANOVA), $F_{13,142} = 48.88$, $p < 0.0001$] and $\delta^{15}N$ muscle values ($F_{13,142} = 72.44$, $p < 0.0001$). Species were deliberately placed in trophic sequence, and not in taxonomic order, according to their nitrogen signatures to illustrate the trophic structure of the assemblage (Fig. 1). As indicated by their $\delta^{15}N$ values, myctophids from Kerguelen waters fed along a continuum amounting to 2.9‰, from 7.3 ± 0.3‰ (*Electrona subaspera*) to 10.2 ± 0.5‰ (*Gymnoscopelus nicholsi*). The myctophids showed a 2.6‰ range in $\delta^{13}C$ values, with all the 14 species, when arranged in order of increasing values, showing a gradual enrichment in ^{13}C from −22.4 ± 0.6‰ (*Protomyctophum bolini*) to −19.8 ± 0.3‰ (*Gymnoscopelus piabilis*) (Fig. 1). Pairwise intergenus species comparisons indicated that most species had distinct isotopic niches that differed by at least one of the two niche axes ($\delta^{13}C$ and $\delta^{15}N$ values). Two relevant exceptions were first *Krefftichthys anderssoni* and *Protomyctophum tenisoni*, and second *Electrona antarctica*, *Gymnoscopelus fraseri*, and *Protomyctophum andriashevi* (post-hoc Tukey's honestly-significant-difference tests, $p > 0.05$).

Resource partitioning of closely related species belonging to the same genus was also investigated (Fig. 2). Species of *Electrona*, *Gymnoscopelus*, and *Protomyctophum* were segregated by their overall isotopic signatures (MANOVA, Wilk's λ, $F_{4,68} = 46.65$, $F_{8,108} = 35.27$, and $F_{8,80} = 30.14$, respectively, all $p < 0.0001$). The three *Electrona* species had distinct isotopic niches, with only the $\delta^{13}C$ values of *E. antarctica* and *E. carlsbergi* being not significantly different (post-hoc Tukey's honestly-significant-difference test, $p = 0.578$). Each species of *Gymnoscopelus* also had its own isotopic niche, with *G. fraseri* and *G. nicholsi* overlapping in their $\delta^{13}C$ values ($p = 0.994$). Their $\delta^{15}N$ values grouped the five species into two different trophic levels: *G. fraseri* and *G. piabilis* ($p = 0.774$), and *G. bolini*, *G. braueri*, and *G. nicholsi* ($p = 0.100$–0.989). Finally, the $\delta^{13}C$ and $\delta^{15}N$ values of the five species of the genus *Protomyctophum* defined four distinct isotopic niches, with a complete overlap in the $\delta^{13}C$ and $\delta^{15}N$ values of *P. bolini* and *P. gemmatum* ($p = 0.826$ and $p = 0.163$, respectively).

Trophic levels within the pelagic ecosystem—Estimated TLs of myctophids ranged from 3.3 to 4.2, thus encom-

Table 2. Blood $\delta^{15}N$ values, estimated trophic levels, and dietary information on fur seals and seabirds from Kerguelen Islands. Values are mean ± SD.

Groups	Season	*n*	$\delta^{15}N$ (‰)	Trophic level	Diet		
					Main prey groups	Main myctophid prey	References
Aptenodytes patagonicus							
Chick food	Spring	10	7.5±0.3	3.3±0.1	Fish	*K. anderssoni*	This study
Chicks	Spring	10	10.0±0.2	4.6±0.1			
Adults rearing chicks	Spring	12	9.8±0.2	4.5±0.1			
Molting adults	Spring	10	9.8±0.3	4.5±0.1			
Chick food	Summer	6	7.8±0.4	3.4±0.1	Fish (cephalopods)	*K. anderssoni*	This study
Chicks	Summer	8	10.1±0.1	4.6±0.04			
Adults rearing chicks	Summer	10	10.0±0.1	4.6±0.04			
Eudyptes chrysolophus							
Chicks	Summer	9	8.6±0.1	4.1±0.04	Crustaceans and fish	*K. anderssoni*	Unpubl. data
Eudyptes chrysocome							
Chicks	Summer	11	8.1±0.3	4.0±0.1	Crustaceans and fish	Postlarvae	Unpubl. data
Halobaena caerulea							
Chicks	Summer	13	9.2±0.4	4.3±0.1	Crustaceans and fish	*P. bolini*, *K. anderssoni*, *E. antarctica*, *E. carlsbergi*	Cherel et al. (2002*b*)
Pachyptila belcheri							
Chicks	Summer	9	8.4±0.4	4.1±0.1	Crustaceans	*K. anderssoni*	Cherel et al. (2002*a*)
Diomedea melanophrys							
Chicks	Summer	18	12.4±0.4	5.3±0.1	Fish (cephalopods, penguins)	None	Cherel et al. (2000)
Arctocephalus gazella							
Lactating females	Summer	10	10.7±0.2	4.8±0.1	Fish	*G. piabilis*, *E. subaspera*, *G. nicholsi*	Lea et al. (2002*a*)

passing slightly less than one full trophic level (Table 1). Since size is an important factor structuring trophic links within communities and ecosystems, we looked at the potential relationships between SL of myctophids and their TLs. Overall, SLs were statistically different between fish species (ANOVA, $F_{13,141} = 458.73$, $p < 0.0001$) and encompassed a large range within the assemblage, from the small *K. anderssoni* (51 mm ± 4 mm) to the largest species of the family *G. bolini* (205 mm ± 9 mm). Values of TL correlated linearly with myctophid size ($F_{1,12} = 5.24$, $p = 0.041$), with *E. subaspera* being an outlier (Fig. 3). Hence, removing the latter species increased the statistical power of the relationship ($F_{1,11} = 8.74$, $p = 0.013$).

There was no TL overlap between the seven myctophid species with the lowest TLs and the main macrozooplanktonic crustaceans from Kerguelen waters (the euphausiids *Euphausia vallentini*, *E. triacantha*, and *Thysanoessa* spp., and the hyperiid amphipod *Themisto gaudichaudii*), whose TLs ranged between 2.5 and 3.0 (ANOVA, $F_{10,100} = 57.10$, $p < 0.0001$; post-hoc Tukey's honestly-significant-difference tests between myctophids and crustaceans, all $p < 0.05$) (Fig. 4). In contrast, TLs of some so-called "higher" predators (seabirds and fur seals) overlapped with TL of the seven myctophid species with the highest TLs ($F_{13,148} = 196.26$, $p < 0.0001$). For example, *G. nicholsi* had a higher TL than *E. chrysocome* ($p = 0.001$), and its TL is not significantly different from that of *E. chrysolophus*, *P. belcheri*, and *H. caerulea* ($p = 1.000$, 0.624, and 0.138, respectively). Otherwise, *A. patagonicus*, *A. gazella*, and *D. melanophrys* had higher TLs than myctophids (all $p < 0.0001$) (Table 2).

A. patagonicus*: A case study of a myctophid specialist predator—K. anderssoni*, *A. patagonicus* chick food, and *A. patagonicus* blood differed by their isotopic nitrogen signatures (ANOVA, $F_{7,70} = 248.81$, $p < 0.0001$). Overall, *K. anderssoni* and *A. patagonicus* chick food were significantly ~ 2.3‰ depleted in ^{15}N when compared to *A. patagonicus* blood (post-hoc Tukey's honestly-significant-difference tests, all $p < 0.0001$) (Table 2). The five groups of birds (summer *A. patagonicus* chicks, spring *A. patagonicus* chicks, summer *A. patagonicus* adults, spring *A. patagonicus* adults, and spring-molting *A. patagonicus* adults) showed no differences in their nitrogen isotopic signatures (all $p > 0.394$). Similarly, the $\delta^{15}N$ values of *K. anderssoni* and of *A. patagonicus* chick food in summer and spring were not significantly different (all $p > 0.526$) (Fig. 5). Accordingly, dietary analysis showed that the main food item of *A. patagonicus* chicks by far was *K. anderssoni*, which accounted for 98.0% and 96.4% of the total number of prey in summer and spring, respectively.

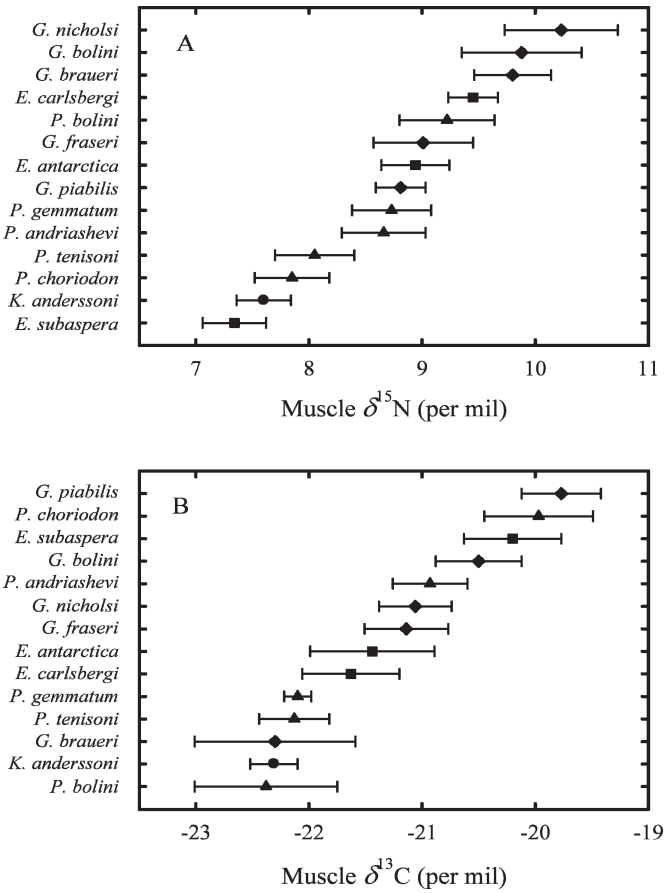

Fig. 1. (A) Stable-nitrogen and (B) stable-carbon isotope values in white muscle of myctophid fishes from Kerguelen waters. Circle: *K. anderssoni*; diamonds: *Gymnoscopelus* spp.; squares: *Electrona* spp.; triangles: *Protomyctophum* spp. Values are mean ± SD.

Discussion

Myctophid assemblage and isotopic niches—This study is the first, to our knowledge, that investigates the isotopic niches and TLs of an assemblage of myctophid fishes. The assemblage is representative of the community of myctophids living in the Southern Ocean (Hulley 1990; Duhamel et al. 2005). It includes the four most abundant species (*E. antarctica*, *E. carlsbergi*, *K. anderssoni*, and *G. nicholsi*) that form the bulk of mesopelagic fish biomass in the Southern Ocean and all the most important species eaten by predators (Sabourenkov 1992; Kozlov 1995; Cherel et al. 2002*c*). The large diversity of myctophids occurring in Kerguelen waters can be related to the location of the archipelago within the Polar Frontal Zone that explains the mixture of species with various water affinities (Cherel et al. 1996; Duhamel et al. 2000). This explanation was also proposed for copepods (Errhif et al. 1997) and cephalopods (Cherel et al. 2004). All of the 14 myctophids belong to the cool-water group, including species with the semi-subantarctic (two species out of five species in the pattern), holosubantarctic (six out of nine), broadly Antarctic (five out of five), and Antarctic (one out of one) distribution patterns (Hulley 1981).

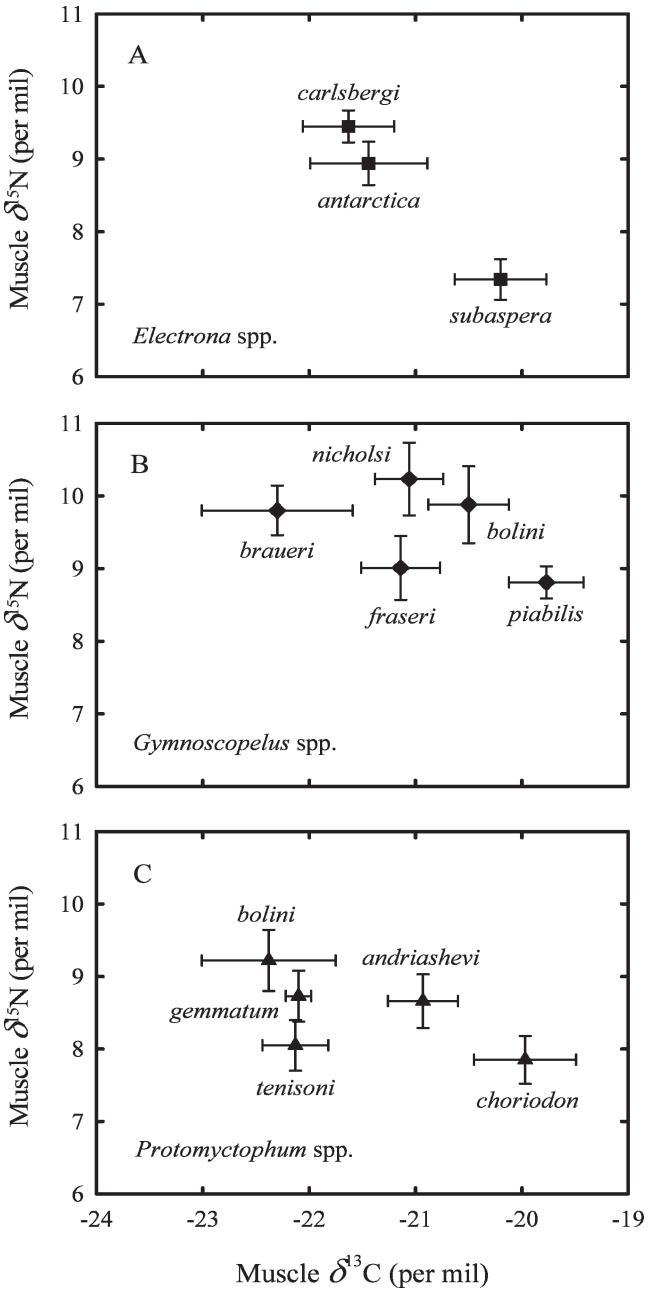

Fig. 2. Stable-carbon and stable-nitrogen isotope values of myctophid fishes from the genera (A) *Electrona* (squares), (B) *Gymnoscopelus* (diamonds), and (C) *Protomyctophum* (triangles) in Kerguelen waters. Values are mean ± SD.

Within that biogeographical context, it is noticeable that the $\delta^{13}C$ values encompassed a 2.6‰ range within the myctophid assemblage. The stable-carbon isotopic signature indicates the consumers' foraging habitat. In oceanic waters of the Southern Ocean, particulate organic matter (POM) $\delta^{13}C$ values decrease with increasing latitudes, and these latitudinal changes are also reflected in organisms at higher trophic levels (Cherel and Hobson 2007). The myctophid $\delta^{13}C$ values are in general agreement with the distribution patterns (Hulley 1981), with species known to occur in colder waters (e.g., *G. braueri*, *K. anderssoni*, and

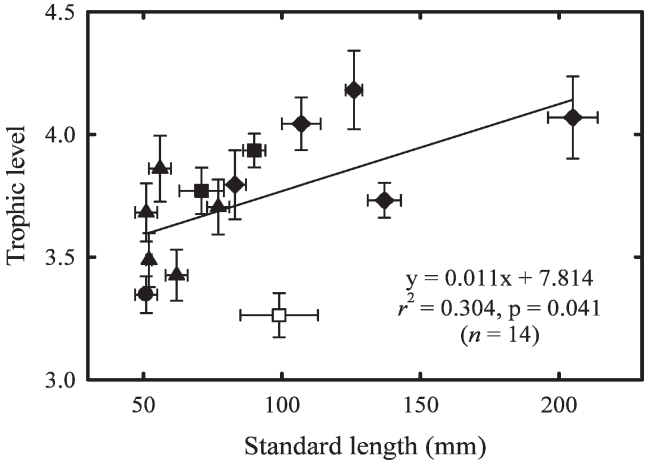

Fig. 3. Estimated trophic levels vs. standard length (SL) of myctophid fishes in Kerguelen waters. Circle: *K. anderssoni*; diamonds: *Gymnoscopelus* spp.; squares: *Electrona* spp. (empty square: *E. subaspera*, *see* text); triangles: *Protomyctophum* spp. Values are mean ± SD.

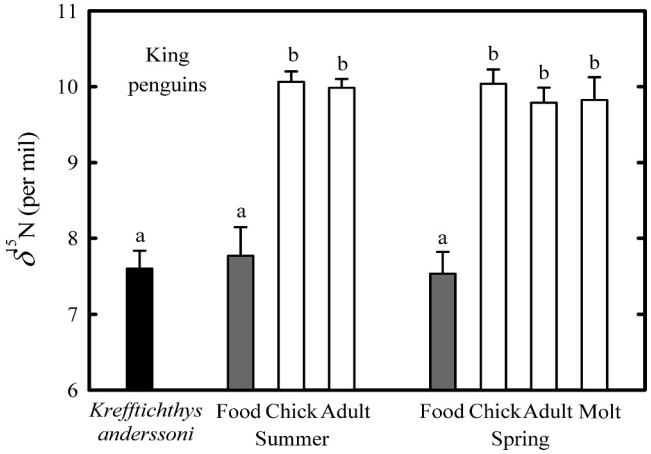

Fig. 5. Stable nitrogen isotope values of the myctophid *K. anderssoni* (black bar); and of food (gray bars); and *A. patagonicus* chicks, breeding adults, and molting adults (white bars) in summer and spring at Kerguelen Islands. Values not sharing the same superscript letter are significantly different ($p < 0.0001$; post-hoc Tukey's honestly-significant-difference tests). Values are mean ± SD.

P. bolini) and warmer waters (e.g., *G. piabilis* and *Protomyctophum choriodon*) showing the lowest and highest stable-carbon isotopic signatures, respectively. A notable exception is the high δ^{13}C value of *E. antarctica* (−21.4‰) that does not fit with its Antarctic habitat (Hulley 1981, 1990). Indeed, *E. antarctica* caught in more southern waters present a typical high-Antarctic δ^{13}C signature (< −24‰; Rau et al. 1992). At Kerguelen, the species exhibits a large range in size (Duhamel et al. 2005), which suggests that its whole life cycle takes place in oceanic waters near the archipelago, with individuals retaining the isotopic signature of the area.

How can the broad range in δ^{13}C values for myctophid species caught in the same trawls, thus theoretically minimizing spatio-temporal variations be explained? The stable isotope method is based on time-integrated assimilated food, and white muscle of subadult and adult fish integrates a period of several months (Herzka 2005). Hence, isotopic niches correspond here to the food consumed by myctophids over the long term. Carbon isotopic signatures therefore suggest latitudinal movements, with some species migrating from colder waters and others from warmer waters to the Kerguelen slope, where they mixed with more resident myctophids. Since POM δ^{13}C values vary with water depth (Lourey et al. 2004), the vertical distribution of myctophids can potentially influence their isotopic signatures. However, most Kerguelen species are vertical migrants with overlapping distribution (Duhamel et al. 2000, 2005), thus suggesting a minimal effect of depth on their δ^{13}C values. The isotopic signature of myctophids nevertheless underlines how poorly known is their basic biology, including reproductive cycle and ontogenic migratory patterns.

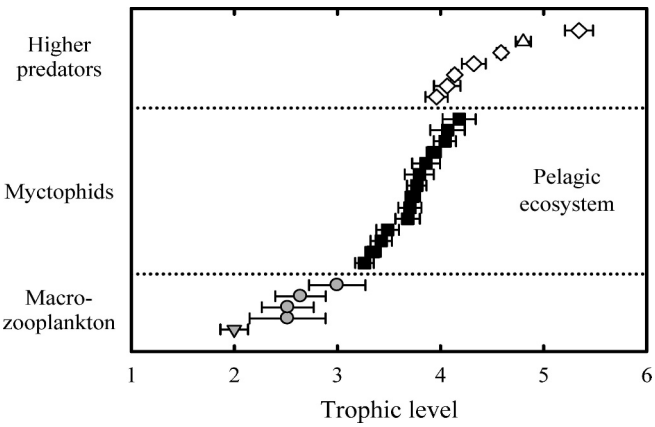

Fig. 4. Trophic levels of the main macrozooplanktonic species, myctophid fish, and of some higher predators (seabirds and fur seals) from Kerguelen waters (for details of species, *see* text). Circles: crustaceans; diamonds: seabirds; squares: myctophids; gray triangle: *Salpa thompsoni*; open triangle: *A. gazella*. Values are mean ± SD.

Most of the species showed distinct isotopic niches that differ by at least one of the two niche axes (δ^{13}C values for habitat and δ^{15}N values for trophic position), indicating a high level of trophic segregation within the assemblage. Habitat and resource partitionings are keys to coexistence of species that would otherwise be potential competitors (Hopkins and Gartner 1992). Segregation is likely to operate through a combination of different factors, including morphological, physiological, and behavioral adaptations. Trophic ecology of myctophids was recently related to two ecomorphological types, the so-called active and inactive species, with the former being diel vertical migrants that store lipids as triacylglycerols and the latter being nonmigratory and partly migratory species containing wax esters (*see* details in Suntsov and Brodeur 2008). Such a classification is not supported by myctophids from Kerguelen since most species are active migrants (Duhamel et al. 2000, 2005), which, depending on species, store either triacylglycerols or wax esters

(Phleger et al. 1999; Lea et al. 2002*b*). No relationship was found between the myctophid $\delta^{13}C$ and $\delta^{15}N$ values and their lipid metabolism.

Strong niche segregation occurs within each of the three most common genera of myctophids from the Southern Ocean, thus illustrating the different mechanisms that allow coexistence of closely related species living within the same ecosystem. For example, the five *Gymnoscopelus* species first differ by their habitats, from the colder-water species *G. braueri* to the warmer-water species *G. piabilis*. When living in the same habitat, segregation operates through different feeding habits, with *G. nicholsi* occupying a higher trophic position than *G. fraseri* (TL = 4.2 and TL = 3.8, respectively). Traditional stomach-content analyses also showed dietary segregation within the same genera (Gaskett et al. 2001; Pusch et al. 2004; Shreeve et al. 2009), with substantial trophic overlaps in some cases, however (Pakhomov et al. 1996; Gaskett et al. 2001). The only complete isotopic niche overlap within a given genus occurs for *P. bolini* and *P. gemmatum*. This does not preclude segregation of the two species through different dimensions of their ecological niches that cannot be depicted by the stable isotope method. *P. bolini* is a common and small myctophid that feed mainly on copepods (Hulley 1990; Pusch et al. 2004), while *P. gemmatum* is a rarer and larger species whose dietary habits are still unknown (and thus need further biological investigations).

Trophic levels within the pelagic ecosystem—It is generally assumed that myctophids constitute the tertiary level of the pelagic ecosystem worldwide, including that of the Southern Ocean (Kozlov 1995; Pakhomov et al. 1996; Pusch et al. 2004). In Kerguelen waters, estimated TLs of myctophids ranged from 3.3 to 4.2, thus showing that they occupy a higher trophic position, being not only secondary but also tertiary consumers of the pelagic ecosystem. The mismatch between conventional and isotopic investigations is likely to result from the coarse estimation of trophic position from prey analysis, while $\delta^{15}N$ values allow more precise quantification of TL from food assimilated over the long-term by consumers (Pinnegar et al. 2002). TL of myctophids were higher and did not overlap with those (TL = 2.5–3.0) of the main macrozooplanktonic swarming crustaceans from Kerguelen waters, which is in agreement with myctophids feeding mainly on meso- and macrozooplankton (Pakhomov et al. 1996; Pusch et al. 2004; Schreeve et al. 2009). Overall, the continuum of $\delta^{15}N$ values (and hence TL) within the assemblage also agrees with a high degree of overlap in the food spectrum of temperate and high-latitude myctophids (Pakhomov et al. 1996). The positive relationship between TL and SL nevertheless pointed out a structuring effect of size, with larger species fishing up the food web (macrozooplankton) when comparing to smaller species (mesozooplankton). Indeed, among the four species occupying the lowest trophic level (TL = 3.3–3.5), two (*K. anderssoni* and *P. tenisoni*) are small species that share the same isotopic niche and prey mainly upon herbivorous and omnivorous copepods (Williams 1985; Gaskett et al. 2001). In contrast, the three species occupying the highest trophic level (TL = 4.0–4.2) belong to the large-sized genus *Gymnoscopelus*. *G. bolini* is known to feed on fish, and *G. nicholsi* is known to feed on euphausiids and fish (Gaskett et al. 2001; Pusch et al. 2004), and the high TL of *G. braueri* fits well with a diet mainly based on euphausiids and amphipods (Pusch et al. 2004; Schreeve et al. 2009). Isotopic niches also shed a new light on the feeding ecology of some poorly known myctophids. Almost no information is available on the food of *E. subaspera* (copepods: Pakhomov et al. 1996; amphipods: Gaskett et al. 2001). Its low TL, however, indicates that the species preys mainly upon primary consumers, and not higher in the food web, as its medium size could erroneously suggest (outlier on Fig. 3).

A major result of the present work is the significant overlap between the highest TL values of myctophids (*G. braueri*, *G. bolini*, and *G. nicholsi*) and the lowest TL values of seabirds (*E. chrysocome*, *E. chrysolophus*, *P. belcheri*, and *H. caerulea*). The main dietary characteristic of all these tertiary consumers is that they prey mainly upon swarming macrozooplanktonic crustaceans in Kerguelen waters (Table 2). The highest TLs within this group correspond to species (*G. bolini*, *G. nicholsi*, and *H. caerulea*) that complement their crustacean diet with significant amounts of fish, mainly small myctophids (Gaskett et al. 2001; Cherel et al. 2002*b*; Pusch et al. 2004). Accordingly, the myctophid-eaters *A. patagonicus* and *A. gazella* occupy higher and intermediate positions between TL 4 and TL 5 (*see* below), and, finally, *D. melanophris* exemplifies organisms from the fifth trophic level of the pelagic ecosystem. The species does not feed on myctophids but instead feeds on larger prey items, namely fishes, penguins, and cephalopods (Cherel et al. 2000).

When compared to other marine organisms from other marine ecosystems, TL of myctophids was either in the lower range (Sydeman et al. 1997) or was similar (Hobson and Welch 1992; Pinnegar et al. 2002) to the isotopically calculated TL of forage fish (e.g., clupeids, sandeels, Arctic cod). Accordingly, their TLs were generally in the lower range or lower than those of top predators living in oceanic and neritic waters (Sydeman et al. 1997; Pinnegar et al. 2002). However, myctophids also occupy the same trophic position as many large predatory fishes, seabirds, and marine mammals (Hobson et al. 1994; Estrada et al. 2005; Sara and Sara 2007). At least three nonexclusive explanations may account for this surprising result. First, some so-called top predators are indeed mainly crustacean feeders (e.g., some seabirds, juvenile tunas). Second, a methodological bias in isotopic TL calculation (e.g., a too low isotopic baseline level) in some studies may induce an underestimation of TL throughout the whole ecosystem. Third, large myctophids occupy a higher trophic position in the Southern Ocean when compared with other ecosystems. Unfortunately, however, no isotopically calculated TL of myctophids is available, thus precluding a direct comparison among different pelagic food webs.

K. anderssoni*, A. patagonicus, and stable isotopes*—In agreement with a stepwise ^{15}N-enrichment between prey and predators, TLs of myctophid fishes were lower than

those of the two known myctophid predators, namely *A. patagonicus* chicks and female *A. gazella* in summer. *A. gazella* had a higher TLs than *A. patagonicus*, which can be explained by overall predation of *A. gazella* upon myctophids with a higher trophic position (e.g., *G. piabilis*, and *G. nicholsi*; Lea et al. 2002*a*) than those eaten by *A. patagonicus* (e.g., *K. anderssoni*, this study).

The isotopic signature of *K. anderssoni* helps to interpret the $\delta^{13}C$ and $\delta^{15}N$ values of the food of king penguin chicks and, subsequently, the poorly known isotopic niches of adult *A. patagonicus*. First, dietary analysis indicated that chick food was mainly composed of *K. anderssoni* in both summer and spring. Accordingly, the $\delta^{15}N$ values of *A. patagonicus* chick food and of *K. anderssoni* were not statistically different, thus validating the use of food samples collected on breeding adults to investigate the isotopic signature of predators' diet (Cherel et al. 2007). This suggests that digestion processes within the birds' proventriculus had little influence on fish isotopic signature. Second, $\delta^{15}N$ values in the blood of breeding *A. patagonicus* adults were not significantly different from those of their chicks, both in summer and the following spring. This strongly suggests that *A. patagonicus* adults feed for themselves on the same prey as those given to their chicks, most likely *K. anderssoni*, during the two periods of chick growth. Mean $\delta^{15}N$ value of molting *A. patagonicus* was also identical to the chicks' (and the breeding adults') values. Since penguins fast during moult, their nitrogen signature reflects the dietary origin of energy reserves built up during the premolt period of hyperphagia at sea. The most parsimonious explanation of their $\delta^{15}N$ value is that their main prey was again myctophids, most likely *K. anderssoni*. Interestingly, a previous investigation showed that the fatty acid profiles of penguin adipose tissue and chick food were quite similar, also indicating a myctophid diet during the premolt period (Raclot et al. 1998). Hence, the two complementary indirect methods of investigating consumers' feeding habits that are either based on protein (stable isotopes) or lipid (fatty acids as trophic markers) metabolisms concurred to indicate a myctophid diet of *A. patagonicus* during most of their annual cycle. The results for *A. patagonicus* thus exemplify the usefulness of constituting datasets on the isotopic and lipid signatures of potential prey of predators to better depict their trophic relationships. These methods are most powerful when combined with conventional approaches and when applied to species or age and seasonal groups for which almost nothing is known (e.g., juveniles and nonbreeding birds). For example, recent investigations have revealed the importance of Southern Ocean myctophids in the nutrition of adult petrels (lipids; Connan et al. 2007) and female elephant seals (stable isotopes; Cherel et al. 2008).

Acknowledgments

We thank G. Duhamel for his advice, Captain Jean-Marc Guédé and crew from *La Curieuse* who helped collect samples, and G. Guillou for stable isotope analysis. The work was supported financially and logistically by the Institut Polaire Français Paul Emile Victor (IPEV, Programme N°109, H. Weimerskirch) and the Terres Australes et Antarctiques Françaises.

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Associate editor: Michael R. Landry

Received: 01 July 2009
Accepted: 24 September 2009
Amended: 05 October 2009