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Fatty acid signature analysis documents the diet of five myctophid fish from the Southern Ocean

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Abstract Fatty acid (FA) and fatty alcohol (FAlc) compositions of both total lipid and neutral lipid fractions were studied for five myctophid species sampled in Kerguelen waters. Both qualitative and quantitative FA signature analyses were then performed to investigate their diet over longer time scales than the conventional stomach content analysis. Regarding their lipid class, FA and FAlc compositions, the five species could be discriminated into two groups: wax-ester-rich species (*Electrona antarctica*, *Krefftichthys anderssoni*) characterised by large amounts of monounsaturated FAs (>73% of total FAs) and triacylglycerol-rich species (*Electrona carlsbergi*, *Gymnoscopelus nicholsi*, *Protomyctophum bolini*) with major amounts of saturated and monounsaturated FAs (>29 and >46% of total FAs, respectively). Qualitative and quantitative FA analyses showed that *K. anderssoni* mainly preyed upon copepods, *E. antarctica* upon copepods and more euphausiids and *P. bolini* and *E. carlsbergi* mainly upon euphausiids with some copepods, while *G. nicholsi* had a more diverse diet. This study shows the usefulness of quantitative statistical analysis to determine the diet of Antarctic and sub-Antarctic predators and stresses the need of increasing the lipid and FA analyses of more zooplanktonic and micro-nektonic marine species.

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Introduction

In the Southern Ocean, several studies have highlighted that lanternfish (Myctophidae) are a key component in the sub-Antarctic and Antarctic food webs, firstly because they are major predators of zooplankton (Pakhomov et al. 1996) and secondly as they constitute a significant part of the diet of pinnipeds, penguins and other marine birds (Cherel et al. 1993, 2008; Connan et al. 2007). In this area, myctophids dominate the micro-nektonic fish community of the mesopelagic zone with more than 35 species occurring in oceanic waters from south of the subtropical front to the edge of the Antarctic continent (Gon and Heemstra 1990). Most of myctophid species have a circumpolar distribution and are the second major component after Antarctic krill in terms of biomass, representing between 70 and 200 million tonnes (Lubimova et al. 1987). More than 80% of the

myctophid biomass is composed by four species: *Krefftichthys anderssoni*, *Electrona antarctica*, *Electrona carlsbergi* and *Gymnoscopelus nicholsi* (Sabourenkov 1991; Collins et al. 2008).

Most myctophid species migrate upwards every night to feed on zooplankton in the productive epipelagic zone (Perissinotto and McQuaid 1992; Duhamel et al. 2000). Therefore, ecological studies of myctophids are required to understand energy flow from lower to higher trophic levels and the vertical transport of organic materials from epipelagic to mesopelagic layers. Several methods can be used to investigate predator–prey interactions within an ecosystem. Investigations using classic methods such as the identification of prey within stomach contents showed high spatial variability of the myctophid diet during summer time (Kozlov and Tarverdiyeva 1989; Pakhomov et al. 1996; Gaskett et al. 2001; Push et al. 2004; Flores et al. 2008; Shreeve et al. 2009); however, very little is known about their diet during winter time. This stomach content method is only a snapshot of the diet and therefore provides no information about the assimilation of food or long-term dietary trends. To investigate the diet over longer time scales, indirect methods have been proposed such as stable isotopic signature of tissue protein and the use of lipids, particularly fatty acids (FAs) and fatty alcohols (FAlcs), as dietary tracers (stable isotopes: Hobson et al. 1994; Cherel et al. 2005, 2010; lipids: Horgan and Barrett 1985; Connan et al. 2005, 2007; Tierney et al. 2008). In comparison to stable isotope analyses, which allow mainly a trophic level approach, FA and FAlc analyses give an insight into the prey species level.

In the past few decades, the application of FA signatures has been used as a potential tool for delineating marine food webs and a powerful technique for qualitative and quantitative diet assessment of predators (review in Dalsgaard et al. 2003). As many FAs are readily transferred from prey to predators with little or no modification (Kirsch et al. 1998), the lipid composition of a predator is therefore assumed to reflect, to some extent, a temporal integration of its diet over a much longer time frame than stomach contents. Marine organisms have diverse FA compositions, containing up to 70 different FAs of varying amounts, characterised by several long-chain polyunsaturated fatty acids (PUFAs) originating from phytoplankton, and several double-bond positional isomers of long-chain monounsaturated fatty acids (MUFAs) originating from zooplankton (review in Dalsgaard et al. 2003). However, the lipid method infers to use FA compositions of all potential prey species, and an understanding of how a predator metabolises individual FAs is preferably required for quantitative analysis (Iverson et al. 2004).

The majority of previous investigations into lipids of sub-Antarctic and Antarctic myctophids have, so far, only been carried out on a few samples from different tissues and/or on total lipid fractions (Phleger et al. 1997, 1999; Lea et al. 2002; Stowasser et al. 2009). The aims of our study were (1) to detail FA and FAlc compositions for whole specimens of five myctophid species, which represent an important biomass in Kerguelen waters (Duhamel et al. 2005) and in the diet of predators (Cherel et al. 1997, 2010), (2) to compare their neutral and total lipid compositions to ascertain the best protocol in future trophic studies and (3) to discuss the implications of their lipid biochemistry with respect to feeding using both qualitative and quantitative FA signature analyses and thus to investigate the myctophid diet over longer time scales.

Materials and methods

Collection of samples

Specimens from five myctophid species *Electrona antarctica*, *Electrona carlsbergi*, *Gymnoscopelus nicholsi*, *Krefftichthys anderssoni* and *Protomyctophum bolini* ($n = 31$; Table 1) were collected offshore in the Indian sector of the Southern Ocean off the Kerguelen Plateau during cruises devoted to the studies of relationships between marine top predators and prey. Midwater trawls (International Young Gadoid Pelagic Trawl with a 10-mm mesh size in the codend) were deployed during both day and night onboard the scientific vessel 'La Curieuse' during cruises IPEKER (1995) and ICHTYOKER (1998–2000) at depths ranging from subsurface to about 500 m (epipelagic and upper mesopelagic layers) (see Duhamel et al. 2000, 2005). Onboard, fish were quickly identified to the lowest possible taxonomic level and stored at −20°C and then at −80°C prior to lipid analyses. Identification was checked afterwards from the otoliths, which were removed just before lipid extraction (Hecht and Hecht 1987; Williams and McEldowney 1990; Duhamel et al. 2005). Measurements of standard length and weight were taken in the laboratory.

Lipid analyses

Lipid extraction

Fish were weighed (ww; in g) and measured (standard length, SL; in mm) just before lipid extraction (Bligh and Dyer 1959). The method of Bligh and Dyer is a rapid extraction (total 10 min), which is known to show incomplete recovery at high lipid concentration (Iverson et al. 2001) when used as described originally. This problem is classically overcome by extending the extraction time in the one-phase solvent ratio and by repeating the extraction

Table 1 General information on the analysed myctophids

Species	*n*	Date	Location	Depth	Standard length (mm)	Wet weight (g)	Lipid content (% ww)
Electrona antarctica	8	3/3/1995	49°00′S 72°22′E	400	49.9 ± 14.9	2.3 ± 1.7	18.8 ± 3.1
					31–80	0.4–6.2	14.9–24.1
Electrona carlsbergi	3	17/4/2000	47°04′S 74°44′E	350	89.3 ± 2.1	12.9 ± 0.9	17.0 ± 3.2
					87–91	11.9–13.7	13.8–20.3
Gymnoscopelus nicholsi	5	6/4/2000	48°49′S 71°22′E	250	108.0 ± 15.0	18.5 ± 4.9	18.7 ± 2.9
					84–122	11.1–24.3	14.8–22.6
Krefftichthys anderssoni	5	9/10/2000	49°08′S 71°49′E	150	50 ± 5.5	1.4 ± 0.7	17.9 ± 4.3
					45–59	1.0–2.7	11.6–22.4
Protomyctophum bolini	10	12/3/1999	49°10′S 71°35′E	350	51.8 ± 5.2	2.1 ± 0.5	10.2 ± 2.2
					43–57	1.1–2.9	7.4–14.6

n number of specimens

procedure until complete lipid removal. Whole fish were analysed without removing stomach contents. Individual fish were crushed in one-phase chloroform/methanol/water + NaCl 0.7% (1:2:0.8; by volume) solution. The procedure was repeated twice on the solid phase. All one-phase solutions were combined, and the phases were then separated by the addition of chloroform and water + NaCl 0.7% (final solvent ratio, 2:2:1.8 by volume). After solvent evaporation at high vacuum, the extracted lipids were weighed in tarred vials and the lipid extracts were placed in nitrogen at −80°C until analysis.

Lipid classes

Individual lipid classes were quantified using a Iatroscan MK V TH10 thin-layer chromatography–flame-ionization detector analyser (TLC–FID; Iatron Laboratories; Ackman 1981). Aliquots of total extracts were applied to chromarods SIII using an SES A 4100 Autospotter (SES Analyse System) and analysed in triplicate. Neutral lipids were separated using a double development procedure with the following solvent systems: n-hexane/benzene/formic acid 80:20:1 (by volume) followed by n-hexane/diethyl ether/formic acid 97:3:1.5 (by volume). After development, the chromarods were oven-dried and analysed immediately to minimise the adsorption of atmospheric contaminants. Calibration of the rods was achieved using analytical-grade commercial standards (Sigma—cholesterol [Chol], diacylglycerols [DGs], free fatty acids [FFAs], phosphatidyl choline, triacylglycerols [TAGs], and sterol esters and diacylglycerol ethers [DAGEs] purified from shark oil). Peaks were quantified using ChromStar software version 4.14.

Fatty acids and fatty alcohols of total lipids

FAs of total lipids were directly converted into their methyl esters using 7% boron trifluoride in methanol (Morrison and Smith 1964). Then, FA methyl esters were separated from the FAlcs by a TLC using the system n-hexane/diethyl ether/acetic acid (85:15:1 by volume). After development, the plates were dried and sprayed with a solution of 0.2% 2,7-dichlorofluorescein in ethanol. The FA methyl esters and the FAlcs were visualised under UV light and identified by comparisons with co-chromatographed standards, then scraped off and eluted with diethyl ether/chloroform (1:1 by volume) and chloroform. FAlcs were acetylated using acetic anhydride (Ackman et al. 1972).

Fatty acids and fatty alcohols of neutral lipids

Lipid classes of total extracts were first isolated by preparative TLC using the same system described above (n-hexane/diethyl ether/acetic acid 85:15:1 by volume, visualised with 2,7-dichlorofluorescein in ethanol under UV). Lipid classes were identified by comparisons with co-chromatographed standard mixtures (Sigma). The bands of wax esters (WE) or TAG were scraped off and eluted with diethyl ether/chloroform (1:1 v:v) and chloroform. FAs from both WE and TAG, and FAlcs from WE were converted into methyl esters and acetates, respectively, as described above.

Gas–liquid chromatography analyses

FA methyl esters and FAlc acetates were then identified by a gas–liquid chromatography (GLC). GLC analyses were performed with an Autosystem XL gas chromatograph (Perkin Elmer) equipped with a polar column Famewax (Restek), (30 m × 0.32 mm internal diameter) and a FID detector. Helium was used as carrier gas at 7 psig. The column was operated isothermally at 190°C during 120 min for FA methyl esters (WE, TAG and total lipids) and 200°C during 100 min for FAlc acetates (WE and total lipids). Injector and detector were maintained at 225 and

250°C, respectively. Peaks were quantified with Turbochrom Navigator software version 4.1. Individual components were tentatively identified with reference to authentic standards and well-characterised fish oils (capelin:menhaden 1:1). In addition to the examination of esters and acetates recovered, one part of FA methyl ester or FAlc acetate samples was completely hydrogenated, and the products were examined qualitatively and quantitatively by GLC. Corrections were made on the results according to the differential FID response to FAs or FAlcs depending on their chain length.

Statistical analysis

Lipid contents of the five myctophid species were compared using the non-parametric Kruskal–Wallis test. FA and FAlc profiles of total and neutral lipids were compared using the non-parametric Mann–Whitney test at $P = 0.05$ significance level unless otherwise stated. Then, FA (and FAlc) signatures of both total and neutral lipids of the five myctophid species were compared using correspondence analyses (CA; Benzecri 1973) preferred to the principal component analysis for frequencies data (Greenacre 1984). The FAs (and FAlcs) responsible for the variability between species were identified using these CAs. Briefly, the distance between FA (or FAlc) profiles was computed with χ^2 metrics. The χ^2 metrics established symmetry between the variables and the observations, rendering a simultaneous representation of both variables and observations. Interpretation requires both (i) that the proximity between variables and observations is monitored and (ii) that the quality of the representation in the factorial plan considered is correct. Such quality is generally expressed by the $\cos^2$ index, which illustrates the distance to the axes. FAs (or FAlcs) that contributed a mean of less than 1% of the total fatty acids (TFAs; or total fatty alcohols TFAlcs) to the profile were not included in statistical analyses as the precision of their determination is low and as a result they introduce more noise than real information to the results. Univariate and multivariate analyses were conducted on non-transformed and arcsine-transformed data, respectively, using Statistica 8.0.

Then, the myctophid diet was inferred only from their FA compositions using both qualitative and quantitative analyses. Several studies have shown that many species of marine fish possess the capacity to digest WE followed by the oxidation of the released FAlc to the corresponding acid and then treated as would be a dietary acid (Sand et al. 1971; Patton et al. 1975; Bauermeister and Sargent 1979). Due to those physiological processes, the dietary signal in FAlc fraction would not be as clear as it is with the FA fraction and more research would be necessary in WE-rich myctophid species. As a result in this study, only the FA fraction was used to investigate the diet of the five myctophid fish. To achieve meaningful comparisons between FA signatures from potential prey species and myctophids, a data set was built using published neutral and total FA signatures of sub-Antarctic species. Regarding the five myctophid species analysed in our study, Koubbi et al. (1991) and Duhamel et al. (2005) found reproductively mature adults, sub-adults and eggs/larvae of various species in waters surrounding Kerguelen, which tend to suggest that the whole cycle of those species might occur in Kerguelen waters. We therefore gathered the FA signature of 12 zooplanktonic and micro-nektonic species commonly found in Kerguelen and sub-Antarctic waters (Errhif et al. 1997; Bernard 2002) from 15 publications (*Calanoides acutus*, *Euphausia frigida*, *Euphausia triacantha*, *Euphausia vallentini*, *Oithona similis*, *Oncaea antarctica*, *Paraeuchaeta antarctica, Rhincalanus gigas*, *Sagitta gazellae*, *Salpa thompsoni*, *Themisto gaudichaudii* and *Thysanoessa macrura*; Online resource). The FA data set consisted of 12 species (80 individual signatures) described by 23 FAs.

The predator–prey relationships were first studied with CAs. Myctophid signatures were added as supplementary individuals, so they have not been used in the definition of the axes. A first attempt of using a quantitative analysis (Iverson et al. 2004) was pursued even though no calibration coefficients were available for myctophid species. The quantitative FA signature analysis estimates the proportional contribution of a prey species to the fish diet by taking a weighted mixture of the FA signatures of potential prey species and then choosing weighing that minimises a statistical distance from that of myctophids (Iverson et al. 2004). The statistical distance used was the Kulback–Liebler distance (Iverson et al. 2004), and the best fit of the solution was evaluated with 'unknown species' and referred to the part of the diet that could not be explained by any potential prey of our data sets. The optimisation model (Gay 1983) and relevant calculations were carried out in R software version 2.9.2.

Results

Lipid content and lipid class composition

Lipid content of *Protomyctophum bolini* was significantly lower than that of the four other myctophid species (10% ww and >17% ww, respectively; $H = 19$, $P < 0.001$; Table 1). Regarding their lipid class composition, two groups of myctophids were characterised (Fig. 1): one WE-rich myctophid group (WE > 65% of total lipids; *Electrona antarctica, Krefftichthys anderssoni*) and one TAG-rich myctophid group (TAG > 55% of total lipids;

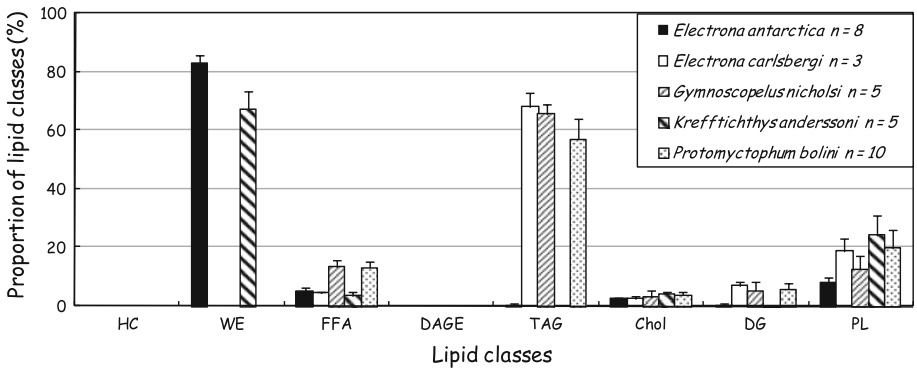

Fig. 1 Lipid class composition of the five myctophid species (*HC* Hydrocarbons; *WE* Wax esters; *FFA* Free fatty acids; *DAGE* Diacylglycerol ethers; *TAG* Triacylglycerols; *Chol* Cholesterol, could include other sterols; *DG* Diacylglycerols; *PL* Polar lipids)

Electrona carlsbergi, *Gymnoscopelus nicholsi*, *Protomyctophum bolini*). The second major lipid class was polar lipids (PLs) in the five species (from 8 to 24% of total lipids), and FFAs represented 4–13% of total lipids depending on myctophid species.

Fatty acid and alcohol compositions of total lipids versus fatty acid and alcohol compositions of neutral lipids

Myctophids rich in triacylglycerols

Eighteen FAs were detected at more than 0.5% of the TFAs in *Electrona carlsbergi* lipids (Table 2). There was no significant difference between FA compositions of total lipids and of TAGs, with MUFAs clearly dominating the compositions (50 and 52% in total lipids and TAGs, respectively), followed by saturated fatty acids (SFAs) and PUFAs. Palmitic acid was dominant (16:0; 19% TFAs) followed by oleic (18:1ω9; 18% TFAs), palmitoleic (16:1ω7; 8–9% TFAs), eicosapentaenoic (20:5ω3; 8% TFAs) and docosahexaenoic (22:6ω3; 5–7% TFAs) acids.

Nineteen FAs were identified at more than 0.5% TFAs in *Gymnoscopelus nicholsi* (Table 2). Major FAs (>10% TFAs) included 16:0 (19% TFAs), 18:1ω9 (16% TFAs) and 20:1ω9 (11–12% TFAs), and there were substantial amounts of 14:0, 16:1ω7, 18:1ω7 and 20:5ω3 (>5% TFAs). The total lipid fraction was significantly poorer in 20:1ω9 ($U = 24.0$, $P < 0.05$) and richer in 22:6ω3 ($U = 2.0$, $P < 0.05$) and in PUFAs ($U = 2.0$, $P < 0.05$) compared to the TAG fraction.

Nineteen FAs were identified at more than 0.5% TFAs in *Protomyctophum bolini* (Table 2). The 16:0 and 18:1ω9 acids occurred at high levels accounting for 23–24% TFAs and 22% TFAs, respectively, followed by 16:1ω7, 14:0, 22:6ω3 and 20:5ω3 (Table 2). Total lipid composition was significantly poorer in SFAs (SFA: $U = 86$, $P < 0.01$; 14:0: $U = 83.5$, $P < 0.01$; 15:0: $U = 100$, $P < 0.01$) and richer in PUFAs (PUFA: $U = 4$, $P < 0.01$; 20:5ω3: $U = 11$, $P < 0.01$; 22:5ω3: $P = 10.5$, $P < 0.01$; 22:6ω3: $U = 0$, $P < 0.01$).

Myctophids rich in wax esters

The FA fraction of WEs of *Electrona antarctica* was characterised by large amounts of MUFAs (>73% TFAs) with oleic acid (35–38% TFAs), 16:1ω7 (20–22% TFAs) and 18:1ω7 (5% TFAs) dominating the profiles (Table 3). WE composition was poorer in PUFAs ($U = 58.0$, $P < 0.01$) and SFAs ($U = 64$, $P < 0.01$) than the total lipid composition and richer in MUFAs ($U = 0$, $P < 0.01$). The most striking difference between total lipid and WE compositions was for the 16:0 almost twice more abundant in total lipid fraction than in WE fraction (5% and 3% of TFAs, respectively; $U = 64.0$, $P < 0.01$). The FAlc fraction was characterised by a dominance of saturated fatty alcohols (SFAlcs; 57–58% of TFAlcs), large amounts of monounsaturated fatty alcohols (MUFAlcs; 40–41% TFAlcs) and few polyunsaturated components (2% TFAlcs). Although several significant differences were found between total lipid and WE compositions (Table 4), the patterns were similar with a dominance of 16:0 (39–40% TFAlcs), followed by 18:1ω9 (17–19% TFAlcs), 14:0 (13% TFAlcs), 18:1ω7 (7–8% TFAlcs) and 16:1ω7 (5% TFAlcs).

As for *Electrona antarctica*, FA profiles of WE of *Krefftichthys anderssoni* were characterised by significantly higher amounts of MUFAs (87 and 77% TFAs, respectively; $U = 0$, $P < 0.05$) and lesser amounts of PUFAs (9 and 13% TFAs, respectively; $U = 23$, $P < 0.05$) and SFAs (4 and 10% TFAs, respectively; $U = 25$, $P < 0.05$) (Table 3). Oleic (41–47% TFAs) and palmitoleic (17–20% TFAs) acids dominated the profiles. Total lipids were significantly richer in 16:0 (6 and 1% TFAs, respectively; $U = 25$, $P < 0.05$) and in 22:6ω3 (4 and 1% TFAs, respectively; $U = 25$, $P < 0.05$). FAlc compositions of total lipids and WE were similar with a prevalence of SFAlcs (65–66% TFAlcs) on MUFAlcs (33–34% TFAlcs)

Table 2 Fatty acid composition of total lipids and of triacylglycerols in *Electrona carlsbergi* ($n = 3$), *Gymnoscopelus nicholsi* ($n = 5$) and *Protomyctophum bolini* ($n = 10$)

Fatty acids	*Electrona carlsbergi*		*Gymnoscopelus nicholsi*		*Protomyctophum bolini*	
	Total lipids	Triacylglycerols	Total lipids	Triacylglycerols	Total lipids	Triacylglycerols
14:0	5.4 ± 0.5	5.6 ± 0.5	7.4 ± 1.0	7.7 ± 0.8	6.3 ± 0.6[a]	7.1 ± 0.7
15:0	0.3 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.0[b]	0.5 ± 0.0
16:0	19.1 ± 1.7	19.0 ± 1.3	18.6 ± 3.3	18.7 ± 2.8	22.8 ± 1.7	24.1 ± 1.7
i17:0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
17:0	0.4 ± 0.0	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
18:0	2.9 ± 0.4	2.7 ± 0.2	3.7 ± 1.2	3.9 ± 1.3	2.9 ± 0.4	2.8 ± 0.3
16:1ω7	8.3 ± 0.3	9.1 ± 0.5	6.5 ± 0.8	6.4 ± 0.8	9.8 ± 1.1	10.0 ± 1.0
18:1ω9	18.1 ± 3.6	18.6 ± 4.1	16.3 ± 1.4	16.2 ± 1.3	21.5 ± 1.5	22.3 ± 1.4
18:1ω7	3.5 ± 0.4	3.3 ± 0.3	7.4 ± 0.5	7.9 ± 0.4	5.4 ± 0.7	5.2 ± 0.9
18:1ω5	0.9 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
20:1ω9	5.3 ± 1.7	5.6 ± 1.9	10.8 ± 0.5[a]	12.2 ± 0.9	3.3 ± 0.6	3.6 ± 0.7
20:1ω7	0.6 ± 0.2	0.7 ± 0.3	0.8 ± 0.1	1.0 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
22:1ω13 + 11	4.8 ± 1.4	5.3 ± 1.6	2.7 ± 0.9	3.0 ± 1.0	1.2 ± 0.4	1.4 ± 0.4
22:1ω9	3.7 ± 1.1	3.9 ± 1.2	2.2 ± 0.5	2.5 ± 0.6	1.2 ± 0.3	1.3 ± 0.3
24:1ω13 + 11	1.5 ± 0.1	1.6 ± 0.1	1.0 ± 0.4	1.1 ± 0.4	0.4 ± 0.2	0.5 ± 0.2
24:1ω9	1.8 ± 0.3	1.7 ± 0.3	1.4 ± 0.3	1.4 ± 0.3	1.7 ± 0.2	1.8 ± 0.3
18:2ω6	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	1.0 ± 0.1
18:4ω3	0.8 ± 0.0	0.8 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.5 ± 0.1
20:4ω3	0.4 ± 0.1	0.4 ± 0.0	0.7 ± 0.2	0.6 ± 0.2	0.3 ± 0.0	0.3 ± 0.0
20:5ω3	8.1 ± 0.9	7.5 ± 0.7	5.7 ± 0.7	4.7 ± 0.9	6.3 ± 0.7[b]	5.1 ± 0.8
22:5ω3	0.5 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.3 ± 0.0[b]	0.2 ± 0.0
22:6ω3	7.0 ± 0.4	5.4 ± 0.6	4.5 ± 0.9[a]	2.9 ± 0.9	7.4 ± 1.1[b]	4.4 ± 0.9
Others	5.3 ± 0.5	5.5 ± 0.4	6.1 ± 0.8	6.0 ± 0.5	5.0 ± 0.4	5.6 ± 0.4
SFAs	29.7 ± 1.8	29.6 ± 1.2	32.7 ± 4.4	33.4 ± 3.7	34.6 ± 2.2[b]	37.6 ± 2.4
MUFAs	49.5 ± 0.9	51.9 ± 0.8	51.2 ± 2.6	53.7 ± 2.6	47.0 ± 2.6	48.4 ± 2.6
PUFAs	20.8 ± 1.3	18.5 ± 0.9	16.1 ± 2.3[a]	12.9 ± 2.2	18.4 ± 1.8[b]	14.0 ± 1.9

Mean ± SD; Mann–Whitney test, [a] $P < 0.05$; [b] $P < 0.01$

and PUFAlcs (1% TFAlcs) (Table 4). Two FAlcs dominated the profiles: palmitol (16:0; 48–49% TFAlcs) and myristol (14:0; 11% TFAlcs).

Interspecific comparison of fatty acid and alcohol compositions of both total and neutral lipids

Factorial axes 1 and 2 resulting from CA of FA data explained 70 and 14%, respectively, of the total inertia in the data set. Hierarchical cluster analysis of Factor-1 and Factor-2 scores identified four groupings. TAG-rich myctophids (*Electrona carlsbergi*, *Gymnoscopelus nicholsi*, *Protomyctophum bolini*) were associated with high levels of myristic and palmitic acids, whereas WE-rich myctophids (*Electrona antarctica*, *Krefftichthys anderssoni*) were two times richer in palmitoleic acid (Tables 2, 3; Fig. 2a). The TAG-rich species *G. nicholsi* and *P. bolini* were mainly segregated according to the factorial axis 2 by their amounts in 20:1ω9 and 22:6ω3 (Table 2; Fig. 2a). The neutral lipids of the WE-rich myctophids could be separated by amounts in 18:1ω9 (higher in *K. anderssoni*), while no distinction could be made between the two species taking into account their total lipid compositions (Table 3; Fig. 2a).

The FAlc segregation of the two WE-rich myctophids is illustrated by a second CA, where the factorial axis 1 explained 78% of the total inertia (Fig. 2b). The two species were mainly separated by their contents in 18:1 (four times higher in *E. antarctica* than in *K. anderssoni* 26 and 7% TFAlcs, respectively) and 22:1 (four times lower in *E. antarctica* than in *K. anderssoni* 2 and 9% TFAlcs, respectively) (Table 4; Fig. 2b).

Table 3 Fatty acid composition of total lipids and of wax esters in *Electrona antarctica* ($n = 8$) and *Krefftichthys anderssoni* ($n = 5$)

Fatty acids	*Electrona antarctica*		*Krefftichthys anderssoni*	
	Total lipids	Wax esters	Total lipids	Wax esters
14:0	2.4 ± 0.3	2.5 ± 0.4	1.5 ± 0.2	1.2 ± 0.2
16:0	5.3 ± 0.4[b]	2.9 ± 0.3	6.1 ± 0.3[a]	1.4 ± 0.1
18:0	0.9 ± 0.2[b]	0.3 ± 0.1	0.8 ± 0.0[a]	0.2 ± 0.0
14:1ω5	0.4 ± 0.1	0.5 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:1ω7	19.7 ± 1.2[b]	22.3 ± 1.3	17.0 ± 1.6	19.5 ± 1.8
17:1	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
18:1ω9	35.0 ± 2.1[a]	37.6 ± 1.6	40.7 ± 3.6	46.9 ± 4.9
18:1ω7	5.1 ± 0.5	5.0 ± 0.5	5.0 ± 0.4[a]	5.8 ± 0.4
18:1ω5	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.0	0.8 ± 0.1
20:1ω9	3.7 ± 0.6	4.2 ± 1.0	4.2 ± 0.9	4.5 ± 1.1
22:1ω13 + 11	2.8 ± 0.8	2.9 ± 0.9	3.8 ± 1.5[a]	4.4 ± 1.9
22:1ω9	2.4 ± 0.6	2.7 ± 0.8	1.8 ± 0.7	2.1 ± 1.0
24:1ω13 + 11	0.9 ± 0.4	0.8 ± 0.4	1.0 ± 0.2	0.8 ± 0.3
24:1ω9	1.1 ± 0.2	1.0 ± 0.2	0.6 ± 0.1[a]	0.2 ± 0.1
18:2ω6	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.3	1.3 ± 0.4
16:3ω6	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
18:4ω3	1.3 ± 0.3	1.1 ± 0.2	0.9 ± 0.3	0.6 ± 0.3
20:5ω3	4.6 ± 0.8	4.0 ± 0.9	3.8 ± 1.0	2.3 ± 1.1
22:6ω3	5.5 ± 0.7[b]	3.3 ± 0.5	3.9 ± 0.8[a]	1.3 ± 0.7
Others	6.2 ± 0.9	5.7 ± 0.5	5.6 ± 0.7	5.3 ± 0.9
SFAs	10.2 ± 0.8[b]	7.1 ± 0.7	9.9 ± 0.4[a]	4.2 ± 0.3
MUFAs	73.5 ± 2.4[b]	79.7 ± 1.6	76.8 ± 2.9[a]	87.1 ± 3.1
PUFAs	16.3 ± 1.9[b]	13.2 ± 1.7	13.4 ± 2.8[a]	8.7 ± 3.1

Mean ± SD; Mann–Whitney test, [a] $P < 0.05$; [b] $P < 0.01$

Table 4 Fatty alcohol composition of total lipids and of wax esters in *Electrona antarctica* ($n = 8$) and *Krefftichthys anderssoni* ($n = 5$)

Fatty alcohols	*Electrona antarctica*		*Krefftichthys anderssoni*	
	Total lipids	Wax esters	Total lipids	Wax esters
14:0	12.5 ± 1.1	12.8 ± 1.2	10.9 ± 3.0	11.0 ± 3.0
15:0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
i17:0	0.7 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.6 ± 0.1
16:0	39.4 ± 0.6[b]	40.9 ± 1.1	47.7 ± 6.2	48.7 ± 5.7
18:0	1.8 ± 0.2	2.0 ± 0.3	3.0 ± 0.3	3.2 ± 0.3
16:1ω7	5.2 ± 0.2[a]	4.9 ± 0.3	5.0 ± 0.7	4.8 ± 0.8
16:1ω5	0.7 ± 0.4[a]	0.4 ± 0.2	1.0 ± 00.4[a]	0.5 ± 0.1
18:1ω9	16.8 ± 1.1[b]	18.7 ± 0.9	2.5 ± 0.3	2.6 ± 0.5
18:1ω7	8.3 ± 0.3[b]	6.6 ± 0.5	3.6 ± 0.5	3.3 ± 0.4
18:1ω5	1.7 ± 0.5[a]	1.1 ± 0.2	1.2 ± 0.2[a]	1.0 ± 0.1
20:1ω9	3.1 ± 1.1	3.5 ± 1.0	5.8 ± 2.5	6.4 ± 2.9
20:1ω7	1.2 ± 0.3[a]	0.8 ± 0.2	2.0 ± 1.1	1.6 ± 0.7
22:1ω13 + 11	0.6 ± 0.1[a]	0.7 ± 0.1	3.4 ± 2.1	3.9 ± 2.0
22:1ω9	1.2 ± 0.1	1.2 ± 0.1	4.2 ± 1.9	4.4 ± 2.2
22:1ω7	0.4 ± 0.1[b]	0.3 ± 0.0	1.9 ± 0.8	1.2 ± 0.4
24:1ω9	0.8 ± 0.5	0.9 ± 0.2	2.2 ± 0.4	2.5 ± 0.4
18:2ω9	0.6 ± 0.1[b]	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
18:2ω6	0.6 ± 0.1[b]	0.5 ± 0.0	0.3 ± 0.1	0.2 ± 0.1
Others	3.6 ± 0.4	3.3 ± 0.4	3.7 ± 0.2	3.4 ± 0.5
SFAlcs	56.7 ± 1.2[a]	58.5 ± 1.0	64.7 ± 7.0	65.8 ± 6.6
MUFAlcs	41.0 ± 1.2[a]	39.8 ± 0.9	33.9 ± 7.1	33.0 ± 6.6
PUFAlcs	2.3 ± 0.2[b]	1.8 ± 0.2	1.4 ± 0.3	1.1 ± 0.3

Mean ± SD; Mann–Whitney test, [a] $P < 0.05$; [b] $P < 0.01$

Prey species of myctophids inferred from fatty acid analyses

Qualitative analyses

A comparison of the FA signatures of 12 potential prey species of myctophid fish was achieved in the first CA where the two-first axes explained 52% of the total inertia (Fig. 3). The first axis clearly separated the copepod *Calanoides acutus* (rich in 20:1 and 22:1) from the euphausiid *Thysanoessa macrura* (rich in SFAs). The second axis separated those two latter species from the copepods *Rhincalanus gigas* and *Paraeuchaeta antarctica* (richer in PUFAs). The myctophid species, added as supplementary individuals, were placed between the euphausiid species and the copepod *C. acutus*. In accordance with results above, the three TAG-rich species were separated one from each other. *Protomyctophum bolini* samples were close to the euphausiid *T. macrura*, when *G. nicholsi* and *E. carlsbergi* would have a diet richer in *E. triacantha* and *C. acutus* than *P. bolini*. The two WE-rich species were undistinguishable one from the other and were situated in the middle of *C. acutus* and the euphausiid species but were segregated from the TAG-rich species by the copepod influence (*R. gigas* or *P. antarctica*).

Quantitative analyses

Regarding the comparison of total and neutral FA fractions, the model estimations showed overall similar patterns for each myctophid species (Fig. 4). The mixture of prey species for the TAG-rich myctophid *Electrona carlsbergi* was dominated by the euphausiid *Euphausia vallentini* (>30% of prey mixture), followed by the copepod *Calanoides acutus*, and the other euphausiid species *E. triacantha* and *E. frigida* (>20, >15 and >13% of prey mixture, respectively). The euphausiid *E. vallentini* also represented a major proportion in prey mixture estimated for *Protomyctophum bolini* (>40%) in addition with *E. frigida* (>17%) and *E. triacantha* (>8%). The estimated prey mixture for the last TAG-rich myctophid *Gymnoscopelus nicholsi* was more diverse than the two previous myctophid species. It was also dominated by a euphausiid, *E. triacantha* (>29%), with significant proportions of the

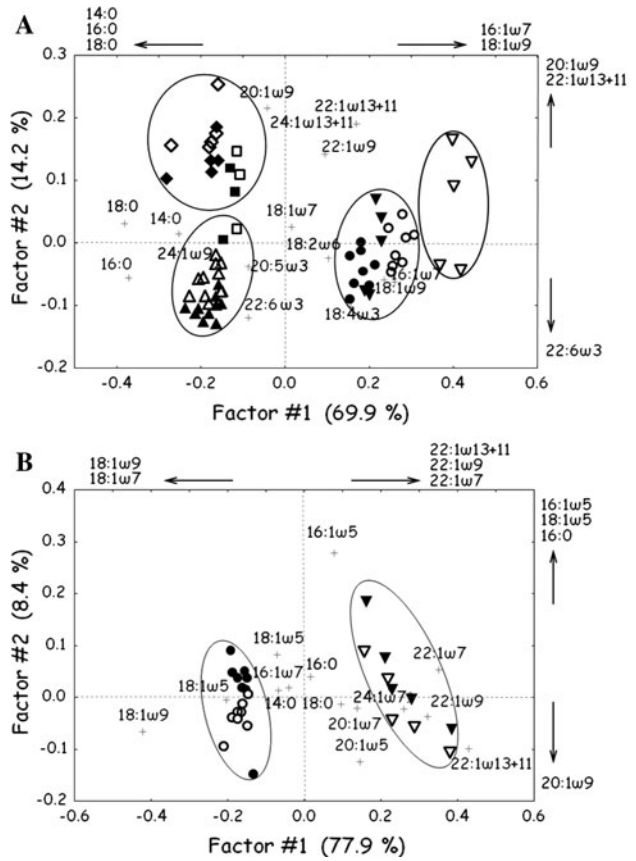

Fig. 2 Correspondence analysis of fatty acid (**a**) and fatty alcohol (**b**) compositions of myctophid fish. *Arrows* indicate FA contributing most to the distribution of species along each axis, i.e. higher value of $\cos^2$, and ellipses denote the groupings separated by hierarchical cluster analysis on the first two axes (*open circle*, *filled circle*—*Electrona antarctica*; *open square*, *filled square*—*Electrona carlsbergi*; *open diamond*, *filled diamond*—*Gymnoscopelus nicholsi*; *open downward triangle*, *filled downward triangle*—*Krefftichthys anderssoni*; *open triangle*, *filled triangle*—*Protomyctophum bolini*; *open symbols* neutral FA compositions; *filled symbols* total FA compositions)

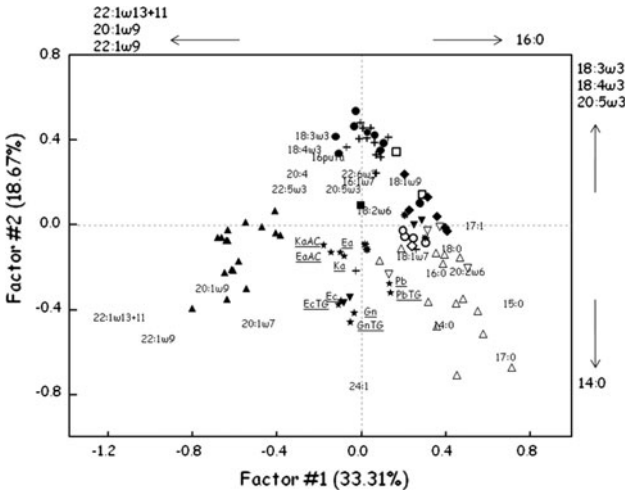

Fig. 3 Correspondence analysis of fatty acid data sets of potential prey species and myctophid fish as supplementary individuals. *Arrows* indicate FA contributing most to the distribution of prey species along each axis, i.e. higher value of $\cos^2$ (*filled triangle*—*Calanoides acutus*; *open downward triangle*—*Euphausia frigida*; *filled downward triangle*—*Euphausia triacantha*; *open circle*—*Euphausia vallentini*; *open square*—*Oithona similis*; *filled square*—*Oncaea antarctica*; *plus symbol*—*Paraeuchaeta antarctica*; *filled circle*—*Rhincalanus gigas*; *open diamond*—*Sagitta gazellae*; *filled diamond*—*Salpa thompsoni*; *times symbol*—*Themisto gaudichaudii*; *open triangle*—*Thysanoessa macrura*; *open star*—myctophid fish; see legend Fig. 4 for definition of Ea, EaAC, Ec, EcTG, GN, GnTG, Ka, KaAC, Pb, PbTG)

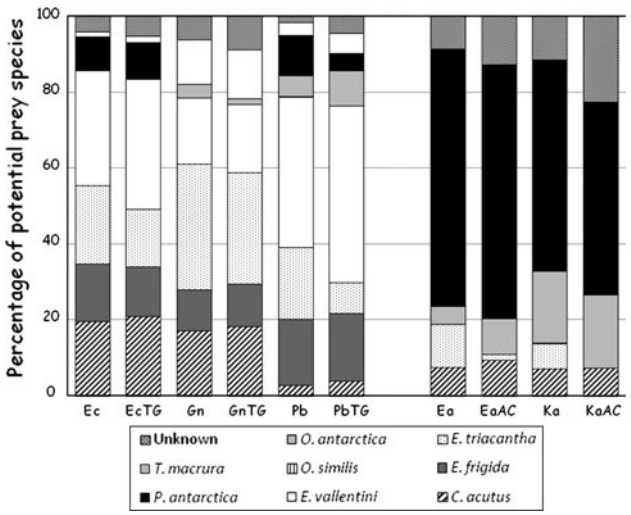

Fig. 4 Model estimates of the contribution of prey species to the fatty acid fraction of both total and neutral lipids of myctophids *Electrona antarctica* (Ea and EaAC); *Electrona carlsbergi* (Ec and EcTG); *Gymnoscopelus nicholsi* (Gn and GnTG); *Krefftichthys anderssoni* (Ka and KaAC); and *Protomyctophum bolini* (Pb and PbTG)

euphausiids *E. vallentini* (>17%), *Thysanoessa macrura* (>12%), *E. frigida* (>11%) and the copepod *C. acutus* (>17%). The 'unknown species' were between 2 and 9% depending on fish species and lipid fractions; meaning that more than 91% of the FA signatures measured in the fish could be explained by the mix of prey obtained.

The optimisation model estimated that FA fractions of WE-rich myctophid species *Electrona antarctica* and *Krefftichthys anderssoni* mainly originated from one WE-rich copepod species *Paraeuchaeta antarctica* (>67 and >51% of prey mixture, respectively; Fig. 4). The small copepod *Oncaea antarctica* would be part of *K. anderssoni* diet at more than 19%. The 'unknown species' category accounted for 9–23%.

Discussion

As vertically migratory species, myctophids are a key component to the active transport of food energy to depth

and play a significant role as consumers of zooplankton in the food web of the Southern Ocean (Lancraft et al. 1989). As prey, they constitute a significant part of the diet of penguins, flying seabirds, as well as seals (Sabourenkov 1991). In the Kerguelen Islands, Guinet et al. (1996) estimated that the breeding seabirds and the breeding seal community consumed annually 1.6 million tonnes of marine resources, including 463,000 tonnes of myctophids, hence showing the importance of those fish in Southern Ocean ecosystems. Despite their enormous biomass in the Southern Ocean (70–200 million tonnes; Lubimova et al. 1987), myctophids are still little harvested commercially with 70 tonnes fished between 1999/2000 and 2008/2009 (68 tonnes in 1999/2000 and a total of only 2 tonnes during the 9 following years; CCAMLR 2010). Although the myctophid diet during the austral summer has already been studied using the stomach content analysis method, this work is the first to investigate the diet of myctophids over a longer time scale using a prey data set and FA signature analysis.

Lipid biochemistry

The high lipid contents found in the five myctophid species (from 10 to 19% ww) are comparable to those obtained in myctophids from the Southern Ocean (Lea et al. 2002) and from the sub-Arctic Pacific (Saito and Murata 1998). Stowasser et al. (2009) have detected higher lipid contents in myctophids from South Georgia waters (up to 36% dry weight in *Electrona antarctica*); the difference might result from geographical variations. FFA amounts detected up to 13% in *Gymnoscopelus nicholsi* and *Protomyctophum bolini* might result from the storage period at −20°C, as storage at −80°C is usually recommended for tissue samples before FA analysis. Depending on myctophid species, fat is deposited as WEs (*Electrona antarctica*, *Krefftichthys anderssoni*) or TAGs (*Electrona carlsbergi*, *Gymnoscopelus nicholsi*, *Protomyctophum bolini*); both TAG-rich and WE-rich species are successful in all regions of the Southern Ocean (Phleger 1998; Phleger et al. 1999). WEs are less dense than TAGs (0.86 vs. 0.93 g ml^{-1}, respectively), and their buoyancy in sea water is higher (0.165 g ml^{-1} lipid vs. 0.095 g ml^{-1} lipid, respectively; Sargent 1976); however, they are hydrolysed and re-esterified four times slower than TAGs (Patton et al. 1975) and thus are not as efficient as TAGs as a source of energy. WEs have been regarded as long-term energy storage, playing a role in buoyancy, while TAGs are often regarded as short-term energy reserves (Neighbors and Nafpaktitis 1982). Interestingly, TAGs have been recorded in high proportions in whole *E. antarctica* by Reinhardt and Van Vleet (1986) and in viscera by Phleger et al. (1997). However, Reinhardt and Van Vleet (1986) used an open-column chromatography separation, which may provide an incomplete separation and/or overlapping between TAG and WE fractions, the high TAG amounts being thus a methodological artefact. Moreover, the TAGs recovered by Phleger et al. (1997) originated most likely from prey species in viscera as evidenced by the large variability between the two analysed specimens (TAG: 29 ± 40% of total lipids; Phleger et al. 1997). In our study, the small amounts of TAG detected in *E. antarctica* (0.3 ± 0.8% of total lipids) might also have originated from prey species in the stomach contents since whole fish were analysed without removing stomach contents. Thus, lipid class, FA and FAlc signatures reflect not only the composition of the fish but might also include the composition of prey within stomachs. The reason why two species of the same genus (*E. carlsbergi* and *E. antarctica*, present study; *G. nicholsi* and *G. braueri*, present study and Phleger et al. 1999) store their energy in different ways is still unclear. As observed in other areas (Suntsov and Brodeur 2008), vertical diel migratory patterns (Duhamel et al. 2005) mostly due to the availability of their preferred prey species might explain this difference, but other factors such as various reproductive patterns or different lifestyles should not be excluded.

To our knowledge, this study is the first to separately evaluate the FA and FAlc fractions of both total and neutral lipids on several specimens of five myctophid species. As expected with the high contents of neutral lipids (>65% of total lipids in WE-rich species and >55% of total lipids in TAG-rich species), FA and FAlc compositions of total lipids showed strong similarities with those of neutral lipids, even if some statistical differences have been highlighted for particular FAs and FAlcs. Regarding the FAlc fraction, the majority of FAlcs originated from WEs explaining the resemblance between neutral and total lipid fractions, and only a tiny part might have originated from plasmalogens (Ackman 1989). A common feature within the FA fraction of the five species was a total lipid fraction richer in PUFAs than the neutral lipid fraction. This can be explained by the influence of PLs on the total lipid fraction; PLs are characteristically rich in the long-chain n-3 PUFAs 20:5ω3 and 22:6ω3, as is true of marine animals in general, reflecting their basic roles in cell membrane structure and function (Bell et al. 1986; Gurr and Harwood 1991).

FA and FAlc profiles of total lipid fractions are consistent with previous studies conducted on a few individuals and/or on body parts (Phleger et al. 1997, 1999; Lea et al. 2002; Stowasser et al. 2009). SFAs and MUFAs dominated the profiles of TAG-rich species, with 16:0, 18:1ω9 and 16:1ω7 being the most abundant FAs. WE-rich species showed large amounts of MUFAs, especially 18:1ω9 and 16:1ω7, and for the FAlc fractions, SFAlcs (16:0, 14:0) and MUFAlcs (18:1ω9, sum 22:1) were dominant. Regarding the interspecific variability of the FA profiles, the five species were easily distinguished

according to their neutral lipid signatures. TAG-rich myctophids (*E. carlsbergi*, *G. nicholsi*, *P. bolini*) were distinguished from WE-rich myctophids (*E. antarctica*, *K. anderssoni*) by higher levels of SFAs (14:0, 16:0), whereas WE-rich species were richer in 16:1ω7. The three TAG-rich species were segregated according to their amounts in 16:1ω7, 18:1ω7 and 22:6ω3. The neutral lipids of the WE-rich myctophids were mainly separated by their contents in 14:0 and 16:1ω7. The FAlc signatures of *E. antarctica* could be distinguished from the ones of *K. anderssoni* by higher contents in sum 18:1 and lower contents in sum 22:1. These differences in the FA (and FAlc) signatures of the five myctophid species might be due to specific metabolisms (TAG- vs. WE-rich species) or differences in their diet.

Diet information inferred from fatty acid analyses

In the marine environment, the lipid profiles of most animal species are characterised by complex and unique arrays of FAs. Combined with the deposition in predator tissue with little modifications, FA profiles represent an integration of its diet over several weeks to months (Fraser et al. 1989) and of all species eaten, rather than the most recently consumed prey as for the stomach content analysis. Working on sub-Arctic myctophid species, Saito and Murata (1996; 1998) were the first to suggest that lanternfish may simply incorporate directly prey lipids into their tissue as FAs and FAlcs. In fact, WE-rich myctophid fish have very high capability of biosynthesis of WEs including incorporation of dietary-derived FAs, especially in the liver (Lee and Patton 1989; Seo 2001).

According to the literature, different approaches have been used for detecting dietary relationships. Either the total lipid composition has been analysed or individual lipid classes have been evaluated separately. Neutral lipids are however preferred for resolving dietary contribution in 'end' predators, since the FA composition of those lipid classes usually reflects trophic influences much better than total lipids (Stubbs and Smith 1990; Parrish et al. 1995). In the present study, trophic investigations into neutral or total lipid fractions both showed similar diets (Figs. 3, 4), as the interspecific differences in diet were higher than the differences between both lipid fractions. The FA method requires a prey data set as complete as possible to compare the FA signature of a predator with those of its potential prey species. In an ideal case and if the predator's movements are known, the potential prey species and the predator should be caught in the same geographical area. Only species living in waters surrounding Kerguelen were, hence, selected in the prey data set. Moreover, the utilisation of quantitative analyses should normally require a calibration coefficient for every FA considered (Iverson et al. 2004). However, to our knowledge, no study has been published dealing with the selective deposition or metabolism of specific FAs by myctophids. Neither calibration coefficient nor lipid content has hence been integrated in this initial work. To take into account those limitations, we used a combination of both qualitative and quantitative analyses to estimate the composition of the myctophids' diet.

The qualitative CA conducted on FA signatures showed that myctophid signatures were between those of euphausiid and copepod species (*Calanoides acutus*; Fig. 3). *Protomyctophum bolini* was separated from the two other TAG-rich species by the axis 1 inferring its diet might be richer in euphausiids. *Electrona antarctica* and *K. anderssoni* were distinguished from the three other myctophid species according to axis 2 implying a diet richer in copepods (especially *Paraeuchaeta antarctica* and/or *Rhincalanus gigas*). In accordance with the qualitative results, the mixture of prey species obtained after quantitative FA analyses highlighted the importance of copepods in the nutrition of both WE-rich myctophids *E. antarctica* and *K. anderssoni*. The copepod *P. antarctica* would be the main prey species of both myctophids throughout the year, added with *Oncaea antarctica* in *K. anderssoni*. *Paraeuchaeta antarctica* is one of the most commonly recorded species of the copepod family Euchaetidae throughout the Antarctic, usually occurring in smaller numbers in sub-Antarctic waters (Park 1994), while it is abundant in Kerguelen waters including coastal ones (Bocher et al. 2002). In accordance with our results, previous works on stomach content analysis have shown the importance of copepods in *K. anderssoni* diet (Table 5; Kozlov and Tarverdiyeva 1989; Pakhomov et al. 1996; Gaskett et al. 2001). Based on FA analyses, the prey mixture of *E. antarctica* would be richer in euphausiid species than the *K. anderssoni* one, with *Euphausia triacantha* represented 11% of the prey (total lipid fraction). Previous stomach content analyses have shown that *E. antarctica* could rely on diversity of resources with copepod, euphausiid or amphipod remains dominating the diet depending on studies (Table 5; Pakhomov et al. 1996; Gaskett et al. 2001; Push et al. 2004; Flores et al. 2008; Shreeve et al. 2009). Regarding the TAG-rich myctophid species, copepods would also be a large part of their diet (up to 30% in *E. carlsbergi*; Fig. 4), but the euphausiid species would be their main prey species (up to 79% in *P. bolini*). The sub-Antarctic species *Euphausia vallentini* would represent more than 40 and 30% of the diet of *P. bolini* and of *E. carlsbergi*, respectively. The last myctophid *G. nicholsi* would have the most diverse diet with the euphausiids *E. triacantha*, *E. vallentini*, *T. macrura*, *E. frigida* and the copepod *Calanoides acutus* representing the bulk of its diet. More than 91% of the FA signatures

Table 5 Diet of myctophid species inferred from stomach content analysis

Species	Locality	Date	Diet						Reference
			Amphipods	Copepods	Euphausiids	Ostracods	Fish	Others	
Electrona antarctica	Prydz Bay & Lazarev sea	March 1985 & Dec. 94–Jan. 95	5	75	6	1	1	12[a]	Pakhomov et al. (1996)*
	Macquarie Island	Jan. 1999	8	50	20	–	–	22[b]	Gaskett et al. (2001)**
	King George Island	Nov.–Dec. 1996	14	<1	85	<1	–	<1	Push et al. (2004)***
	King George Island	Nov.–Dec. 1996	2	27	48	16	–	7[a]	Push et al. (2004)*
	Lazarev Sea	April 2004	6	53	28	7	–	7	Flores et al. (2008)*
	Scotia sea	Autumn	71	4	19	–	–	6	Shreeve et al. (2009)*
Electrona carlsbergi	40–60°S, 0–10°E	Jan.–Feb. 1993	–	67	–	–	–	33[a]	Pakhomov et al. (1996)*
	Macquarie Island	Jan. 1999	1	4	58	–	12	24[c]	Gaskett et al. (2001)**
	Scotia sea	Autumn	10	47	10	–	–	24[d]	Shreeve et al. (2009)*
Gymnoscopelus nicholsi	Prydz Bay & South Georgia	March 1985 & Feb.–March 94	2	66	27	1	–	4[a]	Pakhomov et al. (1996)*
	Macquarie Island	Jan. 1999	–	25	19	–	–	56[b]	Gaskett et al. (2001)**
	King George Island	Nov.–Dec. 1996	1	4	78	<1	14	3[a]	Push et al. (2004)***
	King George Island	Nov.–Dec. 1996	<1	64	28	5	1	2[a]	Push et al. (2004)*
	Scotia sea	Autumn	4	59	33	<1	–	3	Shreeve et al. (2009)*
Krefftichthys anderssoni	40–60°S, 0–10°E	Jan.–Feb. 1993	–	96	4	–	–	–	Pakhomov et al. (1996)*
	Macquarie Island	Jan. 1999	3	58	13	1	–	25[b]	Gaskett et al. (2001)**
	Continental shelf Antarctic	Jan–March 1978, 1981, 1984 & Feb. 1983	5	96	20	–	–	–	Kozlov and Tarverdiyeva (1989)****
	Scotia sea	Autumn	1	33	56	–	–	10[b]	Shreeve et al. (2009)*
Protomyctophum bolini	40–60°S, 0–10°E	Jan.–Feb. 1993	13	80	7	–	–	–	Pakhomov et al. (1996)*
	Macquarie Island	Jan. 1999	8	32	57	<1	–	3[b]	Gaskett et al. (2001)**
	King George Island	Nov.–Dec. 1996	–	84	13	2	–	1	Push et al. (2004)***
	King George Island	Nov.–Dec. 1996	–	97	<1	3	–	<1	Push et al. (2004)*
	Scotia sea	Autumn	–	57	25	3	–	15[b]	Shreeve et al. (2009)*

* % Total number

** % Wet weight

*** % Dry weight

**** Encounter frequency

[a] Mostly chaetognaths

[b] Mostly crustaceans too digested to be identified

[c] Crustaceans too digested to be identified + chaetognaths

[d] Mainly salps

of those three TAG-rich myctophid species could be explained by those prey mixtures.

Recently, Cherel et al. (2010) investigated the trophic structure of a myctophid assemblage by measuring the isotopic signatures of 14 species living in Kerguelen waters. Their stable isotope analyses showed that small myctophid species such as *Krefftichthys anderssoni* occupy a third trophic level and are secondary consumers, when larger species such as *Gymnoscopelus* spp. would be tertiary consumers. Interestingly, *K. anderssoni* and *E. antarctica* showed the lowest δ^{15}N values (ratio mainly related to the trophic level of the organism) of the five species studied in our work, with *K. anderssoni* signature being significantly lower than that of *E. antarctica* (mean ± SD

($n = 12$): 7.6 ± 0.2 vs. 8.9 ± 0.3‰, respectively; two-sample t test, $t = 12.19$, $P < 0.0001$). Those isotopic signatures and the estimated corresponding trophic levels (3.3 for *K. anderssoni* and 3.8 for *E. antarctica*; Cherel et al. 2010) are in accordance with FA signatures that suggested a diet based on copepods (especially more *O. antarctica*) for *K. anderssoni*, added with some euphausiids for *E. antarctica*. The δ^{15}N signatures and estimated trophic levels were 9.2 ± 0.4‰ and 3.9 for *P. bolini*, 9.5 ± 0.2‰ and 3.9 for *E. carlsbergi*, and 10.2 ± 0.5‰ and 4.2 for *G. nicholsi* (Cherel et al. 2010). Hence, δ^{15}N signatures indicate a higher trophic level for *G. nicholsi*, which might be explained by a diet composed by zooplankton along with small fish (Push et al. 2004). Finally, those results are in accordance with the morphological characteristics of the five myctophid species. The species with the smallest mouth, and thus restricted to smallest prey species, is *K. anderssoni*, followed by *E. antarctica*, *E. carlsbergi* and *P. bolini*. All the *Gymnoscopelus* spp. have a larger and extensible mouth, which allow them to feed on larger prey species (Duhamel et al. 2005).

Use of quantitative FA signature analysis in the Southern Ocean

FA (and FAlc) signatures have been used in numbered ecosystems (review in Dalsgaard et al. 2003) from zooplankton to top predator studies (Phleger et al. 1998; Mayzaud et al. 2007; Connan et al. 2007). Those components would thus constitute a valuable tool for investigating trophic interactions of myctophid species. The FA method requires comparing FA signatures of both predator and potential prey species. In an ideal case, the prey have to be sampled in the predator's foraging area; some FA (and FAlc) geographical variations can occur as they depend on the diet of those potential prey species (Kattner and Hagen 1995). This could be possible at a Bay scale with a well-known and definite foraging area for the predator; however, it is more problematic for predators with a huge foraging area (e.g. sub-Antarctic seabirds) or in the open ocean. Regarding the myctophid species analysed in our study, reproductively mature adults, sub-adults and eggs/larvae of most of them can be found in waters surrounding Kerguelen (Koubbi et al. 1991; Duhamel et al. 2005), which tend to suggest that the whole cycle of those species might occur in Kerguelen waters. Our FA data set was thus restricted to zooplanktonic and micro-nektonic species, which are known to occur in Kerguelen waters.

Despite no calibration coefficients being used in the quantitative analysis, both qualitative and quantitative analyses showed complementary results. Laboratory studies on myctophid fish are, however, necessary to apprehend the selective deposition of particular FAs in fish of this family and to validate the present results. If calibration coefficients have been published for marine mammals (Iverson et al. 2004), none have been published for the fish species. Regarding the FA data set, FA signatures of 12 sub-Antarctic micro-nektonic and zooplanktonic species have been gathered, among those the most common species in waters surrounding Kerguelen (like the euphausiids *Euphausia vallentini*, *Thysanoessa macrura*, the amphipod *Themisto gaudichaudii*, the copepods *Paraeuchaeta antarctica*, *Calanoides acutus*). A major source of the variability in the results comes from variation in FA signatures among individuals of a particular prey type. Several FA signatures were available for most species; however, only one signature has been published for the chaetognath *Sagitta gazellae* (Phleger et al. 1998). Moreover, no lipid data have been published for the copepod *Calanus simillimus* (copepod that is abundant in sub-Antarctic waters; Errhif et al. 1997) or for any ostracod species in the Southern Ocean. This stresses the need to increase the lipid, FA (and FAlc) analyses of marine species in order to take into account the spatial and seasonal variability and to apply the quantitative FA signature analyses to more predator species.

Finally, stable isotope and stomach content analyses showed that *G. nicholsi* could prey on smaller fish species (Push et al. 2004; Cherel et al. 2010). Some fish remains have also been recovered in *E. carlsbergi* stomach contents in specimens from Macquarie Island (Gaskett et al. 2001). An attempt was thus made by integrating the small fish species (*K. anderssoni*, *P. bolini* and *E. antarctica*) as potential prey species in the FA data set. Not surprisingly, considering the similarity in their FA signatures, the model converged to a high percentage of *P. bolini* in the diet for both species (>55% for *G. nicholsi* and >65% for *E. carlsbergi*). However, whether these results just reflected the similarity in their FA signatures as TAG-rich myctophid species or a real trophic relationship could not be distinguished. Fish were not the biggest portion of the diet found in those two species (Push et al. 2004; Gaskett et al. 2001; Cherel et al. 2010); combination of FA/FAlc signatures, stable isotope signatures and conventional stomach content analyses would be necessary to fully determine the portion of fish to the diet of more piscivorous species.

Conclusion

This first attempt of using quantitative FA signature analysis to investigate the diet over a longer time scale of several myctophid species has highlighted the usefulness of this method despite several approximations made beforehand. With time, it has become increasingly clear that the Antarctic food web is more complex than the simplistic

krill-focused food web, with numerous studies demonstrating the important role of myctophids in ecosystems especially where Antarctic krill is not abundant or does not occur (Kozlov 1995; Rodhouse and White 1995; Connan et al. 2007). Understanding the complexity of sub-Antarctic and Antarctic food webs will require finding methods to fully determine both its spatial and temporal variability of which quantitative FA signature analysis might be one.

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