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Lipids from stomach oil of procellariiform seabirds document the importance of myctophid fish in the Southern Ocean

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Abstract

We investigated the relative importance of myctophid fish and Antarctic krill in the diet of adult flying seabirds of the Southern Ocean. The main prey of short-tailed shearwaters *Puffinus tenuirostris* (*P. ten.*), white-chinned petrels *Procellaria aequinoctialis* (*P. aeq.*), blue petrels *Halobaena caerulea* (*H. cae.*), thin-billed prions *Pachyptila belcheri* (*P. bel.*), and Antarctic prions *Pachyptila desolata* (*P. des.*) were mostly deduced from the lipid analysis of adult stomach oils. More than 97% of the 125 analyzed oils mainly consisted of wax esters (WEs) and triacylglycerols (TAGs) (>70% of total lipids). WE fatty alcohol (FAlc), WE fatty acid (FA), and TAG-FA profiles clearly segregated *P. aeq.* from *P. ten.*, with smaller, but still significant, differences among the three other petrel species. *P. aeq.* and *P. ten.* therefore preyed on distinct prey species, whereas *H. cae.*, *P. bel.*, and *P. des.* had a more similar diet, but still with some prey differences. Comparisons between FAlc and FA patterns of oils with those of potential prey species showed that >93% of FAlc and FA patterns of oil WEs had a high probability of resemblance with the myctophid signatures, and similar results were obtained with the TAG fractions. Almost no stomach oil fit the lipid patterns of subantarctic and Antarctic euphausiids, including those of the WE-rich *Thysanoessa macrura* and the TAG-rich Antarctic krill *Euphausia superba*. This study thus demonstrates for the first time the importance of myctophids in the nutrition of adult flying seabirds breeding in subantarctic islands and foraging in Antarctic waters during the austral summer.

Procellariiform seabirds (albatrosses, petrels, prions, shearwaters) exhibit exceptional life history traits with high and extended parental care while foraging on distant and unpredictable marine resources (Warham 1990, 1996). As a result of these constraints, several procellariiform species developed many adaptations, including a bimodal foraging strategy during the chick-rearing period. Adults alternately perform short trips (1–5 d) in the vicinity of the colony and long trips (6–29 d) in offshore waters (Chaurand and Weimerskirch 1994; Baduini and Hyrenbach 2003). An adaptation of these birds is the storage of oil in adult stomachs during long trips (Weimerskirch and Cherel 1998; Cherel et al. 2002*b*). Stomach oils result from the breakdown of ingested food and usually have been regarded as an adaptation for combining the provision of a concentrated high-energy food to chicks with a reduction in the costs of transport to adults (Warham 1977).

Investigations on seabird diets classically conducted by the identification of prey within stomach contents are subject to unavoidable biases (e.g., snapshot of diet, differential digestion, information restricted to the chick diet only). To overcome these disadvantages, indirect methods have been proposed for the stable isotopic signature of tissue protein and the use of lipids, particularly fatty acids (FAs) and fatty alcohols (FAlcs), as dietary tracers (e.g., isotopes: Hobson et al. 1994; Cherel et al. 2005; lipids: Horgan and Barrett 1985; Raclot et al. 1998; Dahl et al. 2003). The lipid method also has the potential to determine the energetically important key species ingested by the consumers. Many FAs are readily transferred from prey to predators with little or no modification (e.g., Kirsch et al. 1998). The lipid composition of a predator is therefore assumed to reflect, to some extent, a temporal integration of diet over a much longer time frame than stomach contents. With the use of neutral lipids from animal tissues, the method has already been applied to the study of trophic

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Table 1. List of seabird species whose stomach oils and food samples have been analyzed.

Species	Sample designation	Location	Date		No. of samples
			Months	Years	
Short-tailed shearwater *Puffinus tenuirostris*	*P. ten.*	Bruny Island (Tasmania)	Mar	1997	14
White-chinned petrel *Procellaria aequinoctialis*	*P. aeq.*	Possession Island (Crozet archipelago)	mid-Feb/mid-Mar	2000, 2001	13
Blue petrel *Halobaena caerulea*	*H. cae.*	Mayes Island (Kerguelen archipelago)	mid-Jan/end of Jan	1996, 2000–2002	51
Thin-billed prion *Pachyptila belcheri*	*P. bel.*	Mayes Island (Kerguelen archipelago)	end of Jan/beginning of Feb	1996, 1997, 2000–2002	29
Antarctic prion *Pachyptila desolata*	*P. des.*	Verte Island (Kerguelen archipelago)	beginning of Mar	1996, 1997, 2000, 2001	18

relationships of Southern Ocean zooplankton, fish, squid, seabirds, and seals (*see* review in Dalsgaard et al. 2003). We recently validated the method with the stomach oil of one procellariiform seabird species (Connan et al. 2005). Oil being only recovered in adults returning from long trips, its lipid signature reflects that of the prey ingested by the adults when they feed for themselves far away their breeding colony. The method has thus the potential to investigate the diet of adult procellariiforms, which is almost completely unknown up to now.

In this study, the main prey of adults of four burrowing procellariiforms (white-chinned petrel *Procellaria aequinoctialis* [*P. aeq.*], blue petrel *Halobaena caerulea* [*H. cae.*], thin-billed prion *Pachyptila belcheri* [*P. bel.*], and Antarctic prion *Pachyptila desolata* [*P. des.*]) were investigated with FA and FAlc tracers. The stomach oil composition of a fifth seabird species, the short-tailed shearwater (*P. ten*; Connan et al. 2005), were added and reanalyzed for comparison. The five species have large to huge populations (hundreds of thousands to millions of breeding pairs) in the Southern Hemisphere (Marchant and Higgins 1990), and all of them use a dual foraging strategy during the chick-rearing period. Both satellite tracking and conventional dietary approach showed that they feed in Antarctic waters during long trips, as pointed out by digested remains of Antarctic krill in food samples (Weimerskirch and Cherel 1998; Catard et al. 2000; Cherel et al. 2002*a*,*b*). Surprisingly, our validation study on stomach oil indicates more a myctophid diet than an Antarctic krill diet for one species of procellariiforms during long trips (Connan et al. 2005). The main goal of this work was thus to further investigate the relative importance of myctophid fish and Antarctic krill in the diet of flying seabirds of the Southern Ocean, focusing on the pelagic ecosystem in Antarctic waters.

Materials and methods

Field study and sample collection—Sources of stomach contents collected from five procellariiform species are summarized in Table 1. Fieldworks were carried out during five austral summers on Bruny Island (Tasmania: 43°18′S, 147°18′E; short-tailed shearwater; *see* Connan et al. 2005), Possession Island (Crozet Archipelago: 46°26′S, 51°45′E; white-chinned petrel), Mayes Island (Kerguelen Archipelago: 49°28′S, 69°57′E; blue petrel and thin-billed prion), and Verte Island (Kerguelen Archipelago: 49°31′S, 70°04′E; Antarctic prion) (Fig. 1). Stomach contents were collected from adults returning from foraging trips with the use of either the water off-loading method or spontaneous regurgitation during handling. Because the presence of stomach oil, Antarctic krill, or both indicates long trips (Weimerskirch and Cherel 1998; Cherel et al. 2002*b*), adult food samples were thus divided into two groups according to the presence (presumably long trips) or absence (presumably short trips) of oil or Antarctic krill. Long trips samples ($n = 125$) were drained by gravity to separate the liquid fraction, including oil, from the solid items. To prevent lipid auto-oxidation, an antioxidant (butylated hydroxy toluene) was added to the oily fraction. The solid and oily fractions were then frozen and stored at −20°C and −80°C, respectively, until subsequent analyses in France.

Analyses of lipid classes and FA and FAlc fractions of stomach oils—Total lipids were quantitatively extracted from each of the 125 oily fractions according to the method of Bligh and Dyer (1959). Crude extracts were placed in chloroform, concentrated under vacuum, and stored at −80°C. All samples were then analyzed according to the methods described by Connan et al. (2005). Briefly, the proportions of lipid classes were determined with an Iatroscan MKV TH10 thin-layer chromatography–flame-ionization detector (TLC-FID), and after methylation (FAs from both wax esters [WEs] and triacylglycerols [TAGs]) or acetylation (FAlcs from WE), FA and FAlc profiles were determined with an Autosystem XL gas chromatograph (Perkin-Elmer) equipped with a polar column Famewax (Restek, Bollende; 30 m length × 0.32 mm internal diameter) and an FID.

Analyses of food samples—Each of the 125 solid fractions was thawed and drained by gravity overnight to separate the solid items from the residual liquid fraction. Accumulated items were discarded (squid beaks, and squid and fish eye lenses), and fresh remains were divided into broad prey classes (fish, crustaceans, cephalopods, and other organ-

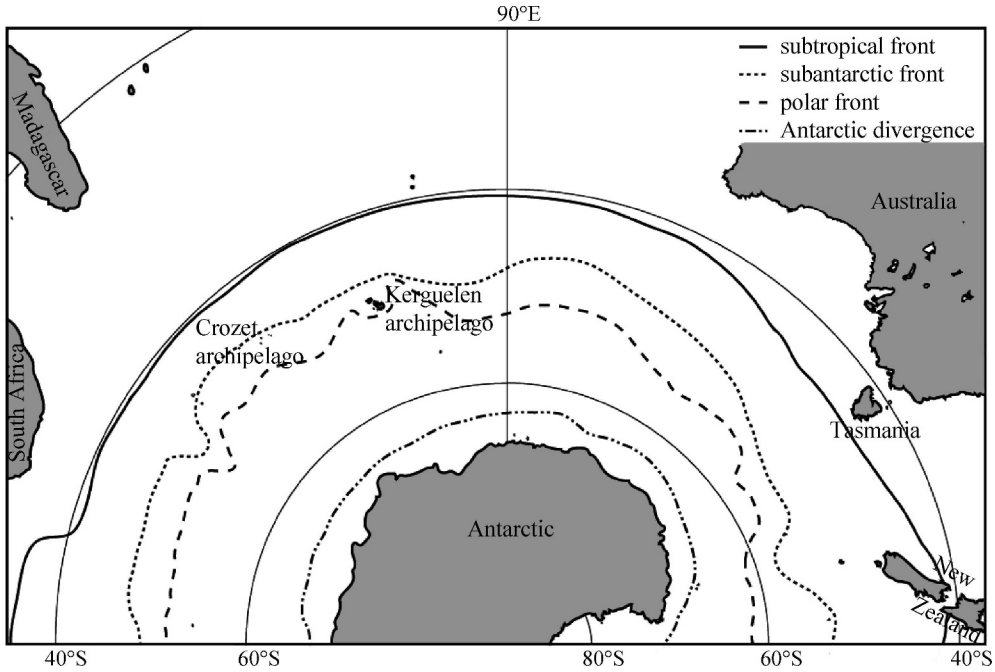

Fig. 1. Map of the Southern Ocean showing the main oceanic fronts and location of study colonies (Crozet Archipelago [white-chinned petrel], Kerguelen Archipelago [blue petrel, thin-billed prion, Antarctic prion], Tasmania [short-tailed shearwater]).

isms), which were weighed to estimate their proportions by fresh mass in the diet. Then, each prey item was numbered and identified to the lowest possible taxon with the use of published keys (Clarke 1986; Baker et al. 1990; Smale et al. 1995) and our own reference collection. The abundance of each prey taxon was described by its frequency of occurrence and numerical importance. Frequency of occurrence of a given taxon was calculated as the total number of food samples containing this taxon as a percentage of the total number of food samples. Numerical importance was calculated as the total number of individuals of a given taxon found in all the samples as a percentage of the total number of all prey items in all the food samples.

Statistical treatments—Interspecific variability of oil lipid composition was evaluated by a combination of univariate and multivariate analyses. First, normality (Shapiro–Wilk test) and homogeneity of variances (Fisher test) were verified to run analyses of variance followed by multiple range tests. When normality or homoscedasticity were not verified, the nonparametric Kruskal–Wallis test was used to evaluate differences between the five seabird species. Second, forward stepwise discriminant analyses (FSDA) were conducted to identify the main FAs and FAlcs that discriminate the oils of the five species. Wilk's λ and the number of observations correctly classified were used to test the significance of the FSDA (F to enter = 4, F to remove = 4) to separate seabird species and to evaluate the performance of the FSDA, respectively.

Then, the predator–prey relationship (dietary origin of oils) was approached by linear discriminant analyses (LDA). To achieve meaningful comparisons between FAlc and FA patterns from potential prey species and stomach oils, three databases were built using the majority of published studies on subtropical, subantarctic, and Antarctic potential prey (databases are available on request to the authors). They included the main species of meso- and macrozooplankton, micronekton and nekton of the Southern Ocean (*see* details in Connan et al. [2005] plus the notothenid *Dissostichus eleginoides* [Wilson 2004], and the myctophids *Electrona antarctica*, *Electrona carlsbergi*, *Gymnoscopelus nicholsi*, *Krefftichthys anderssoni*, and *Protomyctophum bolini* [Connan et al. unpubl.] patterns). To investigate the origin of the WE recovered in the stomach oils, two prey databases were constructed: A WE fatty alcohol database (WEAL database) regrouped 80 FAlc profiles of nine crustacean and fish species, and a WE fatty acid database (WEAC database) compiled 62 FA profiles belonging to eight potential prey species. The origin of TAG oils was approached through a third database (TAG database), where 17 crustacean, fish, and squid species were grouped. The prey species were then classified by LDA on the basis of either FAlc or FA patterns. The outliers were removed from each group defined a priori after verification of the homogeneity of FAlc or FA profiles with the use of Mahalanobis distance and chi-square test. The number of descriptors considered in the LDA was limited to the data available in the literature: 8, 17, and 16 descriptors for WEAL, WEAC, and TAG databases, respectively. Stomach oils, used as supplementary observations and thus not integrated in the definition of discriminant functions (DFs), were then attributed to a pre-existing prey group with a classification model built from prey patterns. Because

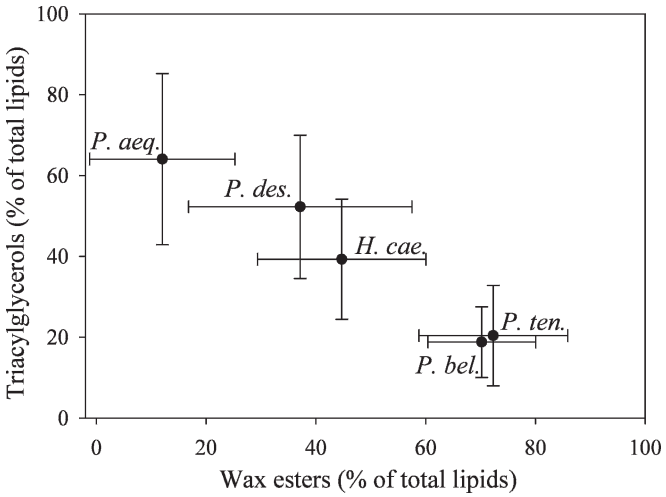

Fig. 2. Wax ester and triacylglycerol proportions in stomach oils of the five seabird species (*see Table 1* for bird species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*).

normality was presupposed for most of these analyses, percentages were normalized by arcsine transformation (Zar 1984).

Results

Biochemical stomach oil compositions—Two neutral lipid classes, WE and TAG, dominated in 118 of the 125 stomach oils (WE + TAG > 70% of total lipids). The seven remaining oils contained large proportions of a third neutral lipid class, diacylglycerol-ethers (DAGE; >30% of total lipids). This third class was particularly present in some oils of *P. aeq.* and *H. cae.* (18.5% ± 20.3% and 11.6% ± 12.7% of total lipids, respectively). The five other lipid classes (hydrocarbons [only present in *P. aeq.* oils], free FAs, cholesterol, diacylglycerols, and polar lipids) were detected in small amounts (<5% of total lipids). Taking into account these lipid class compositions, the oils of the five seabird species could be discriminated into three groups: rich-WE and poor-TAG oils (*Puffinus tenuirostris* [*P. ten.*] and *P. bel.* oils), rich-TAG and poor-WE oils (*P. aeq.* oils), and an intermediate group constituted by *P. des.* and *H. cae.* oils (WE : Kruskal-Wallis, $H = 76$, $p < 0.001$; TAG : Kruskal-Wallis, $H = 64$, $p < 0.001$; Fig. 2). Finally, WE (FA and FAlc patterns) of four *P. aeq.* oils have not been analyzed because of the very limited amount of WE in these samples (<4% of total lipids).

Thirteen different FAlcs were identified in the 120 oils analyzed (>1%; Table 2). Depending on bird species, saturated FAlcs (SFAlcs) or monounsaturated FAlcs (MUFAlcs) dominated the profiles, and represented together more than 97% of the total FAlcs. The principal FAlcs included 16:0 (27–42%), 20:1n-9 (5–22%), 18:1n-9 (4–22%), 14:0 (6–13%), and 18:1n-7 (3–11%). Interspecific variation was evaluated by discriminant analysis with a forward selection. Nine FAlcs (14:0, 16:0, 18:1n-9, 18:1n-7, 18:1n-5, 20:1n-9, 22:1n-9, 24:1n-11, and 24:1n-9) were selected to discriminate the five seabird species ($\lambda_{Wilks} = 0.030$, $F = 17.23$, $p < 0.001$; Fig. 3A). The first DF (48% of variability) separated *P. aeq.* oils (poorer in 20:1n-9 and 22:1n-9) from the others. The second DF (37% of variability) separated *P. ten.* oils (richer in 24:1n-9 and poorer in 18:1n-9) from those of *H. cae.*, *P. des.*, and *P. bel.* (statistics not shown). Finally, the *P. bel.* oils could be distinguished from *P. des.* oils considering mainly 20:1n-9, 22:1n-9 and 14:0 (Table 2). Taking into account this algorithm, more than 87% of profiles were assigned to the correct seabird species.

Sixteen different FAs were present at a level of >1% in all the 121 WE fractions of stomach oils (Table 3). The monounsaturated FAs (MUFAs) were clearly prevalent

Table 2. Mean values for fatty alcohol (FAlc) composition of stomach oil wax ester fractions for the five procellariiform species (*see* Table 1 for bird species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*; SFAlcs, saturated fatty alcohols; MUFAlcs, monounsaturated fatty alcohols; PUFAlcs, polyunsaturated fatty alcohols).

	FAlc composition of WE fraction (%)				
	P. ten. (n=14)	*P. aeq.* (n=9)	*H. cae.* (n=50)	*P. bel.* (n=29)	*P. des.* (n=18)
14:0	8.53±0.58	7.58±2.20	7.71±2.45	5.52±1.15	12.88±8.64
16:0	42.32±2.46	34.52±6.18	28.01±5.53	27.47±5.58	31.88±7.52
18:0	3.24±0.19	3.19±1.54	2.24±0.49	2.39±0.53	2.10±0.48
16:1n-7	4.48±0.24	4.09±0.60	3.63±0.80	3.22±0.67	3.67±0.85
18:1n-9	3.82±1.14	22.08±6.03	11.63±5.03	9.24±5.60	12.57±6.93
18:1n-7	3.47±1.19	8.92±2.74	10.07±2.71	11.11±2.93	8.36±3.89
18:1n-5	1.06±0.15	0.78±0.14	0.84±0.18	0.96±0.15	0.85±0.23
20:1n-9	10.12±1.36	5.19±1.88	19.42±9.40	22.31±7.63	11.21±6.54
20:1n-7	0.61±0.18	1.31±0.78	1.62±0.61	1.83±0.66	1.78±0.78
22:1n-13+11	7.46±1.38	1.28±0.76	4.13±2.52	4.84±1.58	4.06±3.75
22:1n-9	4.65±0.79	1.07±0.60	3.03±0.96	3.57±0.99	2.86±1.12
24:1n-11	0.97±0.32	0.04±0.05	0.32±0.19	0.45±0.33	0.14±0.14
24:1n-9	2.84±0.29	1.28±0.68	1.37±0.45	1.54±0.53	1.08±0.67
Others	6.43±0.39	8.68±1.32	5.99±1.23	5.53±0.69	6.55±1.84
Total SFAlcs	57.90±3.16	50.35±8.11	40.71±6.87	38.01±6.99	49.57±13.48
Total MUFAlcs	40.80±3.15	47.70±8.05	57.46±7.16	60.32±7.13	48.19±12.96
Total PUFAlcs	1.29±0.17	1.97±0.29	1.83±0.70	1.66±0.36	2.23±0.99

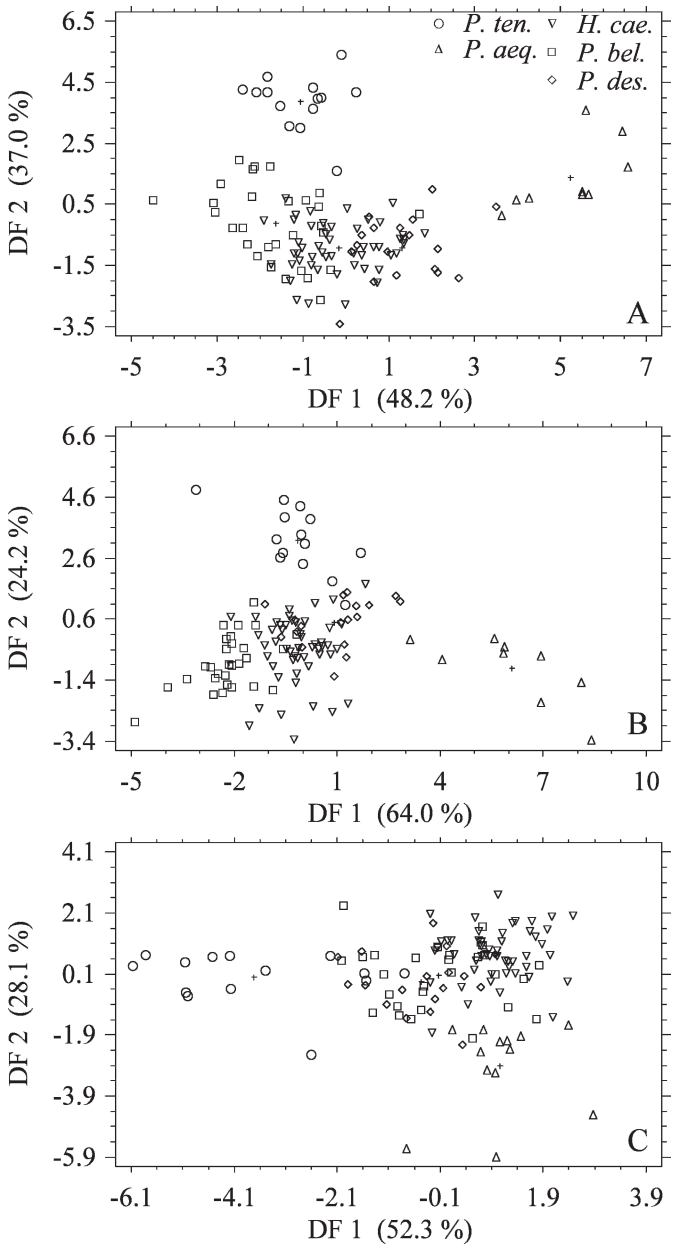

Fig. 3. Discriminant scores of (A) fatty alcohol and (B) fatty acid patterns of stomach oil wax esters, and (C) fatty acid patterns of stomach oil triglycerides. (DF: discriminant function; *see Table 1* for bird species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*).

(>68% of the total FAs). The major FA was 18:1n-9 (34–39%), with substantial amounts of 16:1n-7 (13–15%) and 20:5n-3 (3–10%). As for the FAlc fractions, interspecific variation was evaluated by FSDA. Eighty-three percent of oil profiles were correctly assigned by an algorithm built with eight FAs (14:0, 16:0, 18:0, 14:1n-5, 16:1n-7, 24:1n-11, 16:4n-1, and 22:6n-3; $\lambda_{Wilks} = 0.040$, $F = 17.56$, $p < 0.001$; Fig. 3B). *P. aeq.* and *P. ten.* oils were individualized and separated from *H. cae.*, *P. bel.*, and *P. des.* oils according to DF 1 (64% of variability) and DF 2 (24% of variability), respectively. *P. aeq.* oils were characterized by high levels of 18:0 and 14:1, and low levels of 20:5n-3, whereas *P. ten.* oils were particularly rich in 22:6n-3 and 24:1n-11 and poor in saturated FAs (SFAs) (statistics not shown). Finally, the three small bird species showed close FA profiles. However, *P. des.* oils presented lower amounts of 14:0 and 16:0 than *P. bel.* and *H. cae.* oils (Table 3).

Fifteen different FAs were found in the TAG fraction at levels exceeding 1%, representing together 90% of total FAs (Table 4). More than half of the FAs were monounsaturated (50–60%), whereas SFAs and polyunsaturated FAs (PUFAs) represented 24–30%, and 16–20% of total FAs, respectively. Two FAs dominated by mass (>10%), the oleic (18:1n-9; 20–26%) and palmitic (16:0; 14–16%) acids. Eight FAs (16:1n-7, 18:1n-7, 20:1n-9, 22:1n-9, 24:1n-11, 18:2n-6, 18:4n-3, and 20:5n-3) were selected by FSDA ($\lambda_{Wilks} = 0.074$, $F = 13.45$, $p < 0.001$; Fig. 3C). Seventy-seven percent of the 125 profiles were correctly assigned to the seabird class with this algorithm. The first DF (52% of variability) clearly isolated *P. ten.* oils (richer in 24:1n-11 and 24:1n-9) from the others, whereas DF 2 (28% of variability) separated *P. aeq.* oils (poorer in 16:1n-7) from those of *P. des.*, *P. bel.*, and *H. cae.* (statistics not shown). Oils of the last three species showed strong resemblance in their TAG-FA compositions.

Seabird diet inferred from stomach oil analyses—Dietary origins of WE and TAG oils were investigated by LDA. A first LDA was performed on the WEAL database. Nine classes were defined a priori, each corresponding to a potential pelagic prey: myctophid fish (*E. antarctica*, *E. carlsbergi*, *Gymnoscopelus braueri*, and *K. anderssoni*), euphausiids (*Euphausia crystallorophias* and *Thysanoessa macrura*), and copepods (*Calanoides acutus*, *Paraeuchaeta antarctica*, and *Rhincalanus gigas*). The first DF, representing 69% of total variance, contrasted between *T. macrura* and *E. carlsbergi* that have a high percentage of 18:1n-9, and *E. crystallorophias*, *P. antarctica*, and *R. gigas* that have very high levels of SFAlcs 14:0 and 16:0. The second DF segregated *C. acutus* from *E. crystallorophias* and *E. carlsbergi*, which almost lack 20:1 and 16:1 alcohols. With the classification model calculated from DF, 100% of prey profiles were correctly assigned. The 120 patterns of oil alcohols were then used as test samples, and prediction of class allocation was achieved with the same model. The results indicated the highest probability of grouping with the myctophid species for 90% of FAlc profiles (Table 5).

The same kind of analysis was applied to the WEAC. Eight similar classes have been defined including all of the above species except *E. carlsbergi.* The first DF (54% of total variance) clearly separated the euphausiid *T. macrura*, rich in 16:0, from the seven other classes. The second DF separated the fish *E. antarctica,* rich in 18:1 from *C. acutus*. One hundred percent of cases were correctly assigned with this second model, and the prediction of stomach oil WEAC allocation indicated highest probability of association of more than 97% FA profiles with myctophid fish (Table 5).

Comparison of our results for the TAG-FA patterns of stomach oils with the literature data was performed with 82 FA profiles from six crustacean, nine fish, and two squid

Table 3. Mean values for fatty acid composition of stomach oil wax ester fractions for the five procellariiform species (*see* Table 1 for bird species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids).

	FA composition of WE fraction (%)				
	P. ten. (n=14)	*P. aeq.* (n=9)	*H. cae.* (n=51)	*P. bel.* (n=29)	*P. des.* (n=18)
14:0	1.12±0.43	2.97±1.71	4.76±2.92	5.96±2.75	2.78±1.32
16:0	1.03±0.33	3.42±1.39	4.27±2.48	5.29±2.08	2.36±0.97
18:0	0.21±0.07	1.00±0.37	0.69±0.42	0.62±0.27	0.51±0.26
14:1n-5	0.19±0.09	1.13±0.52	0.45±0.16	0.36±0.11	0.39±0.14
16:1n-9+7	14.42±0.92	14.11±5.16	15.39±1.95	13.11±1.23	15.43±2.47
18:1n-9	39.34±1.77	38.75±7.31	35.76±6.86	33.97±4.81	35.00±9.08
18:1n-7	4.28±0.58	5.39±1.69	6.58±1.47	6.70±1.14	5.31±1.89
20:1n-11+9	4.82±0.53	8.69±6.30	6.92±1.58	6.57±1.46	6.07±1.96
22:1n-13+11	4.58±1.11	2.26±0.67	2.72±0.94	2.77±0.76	2.65±1.11
22:1n-9	1.41±0.30	1.57±0.45	1.58±0.40	1.36±0.31	1.60±0.53
24:1n-11	0.70±0.28	0.29±0.13	0.34±0.15	0.40±0.22	0.33±0.18
18:2n-6	1.93±0.17	1.56±0.41	1.91±0.51	1.80±0.25	1.89±0.44
16:4n-1	0.35±0.21	0.05±0.06	0.50±0.78	0.52±0.17	1.48±2.16
18:4n-3	1.80±0.48	0.46±0.25	1.06±0.94	1.34±0.51	1.78±1.15
20:5n-3	8.81±1.75	3.00±1.35	6.02±4.24	7.23±2.55	10.37±7.22
22:6n-3	5.37±0.96	3.65±2.11	2.53±1.25	3.14±1.15	3.19±1.07
Others	9.61±2.40	11.69±6.21	8.52±3.62	8.85±3.14	8.85±4.85
Total SFAs	3.41±0.96	9.76±3.39	11.30±6.00	13.53±5.07	7.11±2.49
Total MUFAs	72.96±3.61	77.15±5.41	72.82±8.27	68.67±5.70	69.44±11.17
Total PUFAs	23.60±3.63	13.09±3.78	15.88±7.99	17.80±4.85	23.45±11.99

species described by 16 FAs. Four classes of prey were defined in a first LDA: two crustacean classes (euphausiids: *E. crystallorophias*, *Euphausia superba*, and *Euphausia vallentini*; other species: *Calanus propinquus*, *Euchirella rostromagna*, and *Themisto gaudichaudii*), one fish class (*D. eleginoides*, *E. carlsbergi*, *G. nicholsi*, *Pagothenia borchgrevinki*, *P. bolini*, *Trematomus bernacchii*, *T. hansoni*, *T. newnesi*, and *T. pennellii*), and one squid class (*Moroteuthis ingens* and *M. robsoni*). The first DF, accounting for 69% of total variance, clearly separated the fish and squid classes from the crustacean classes. The second DF separated the squid class from the fish class, and the euphausiid class from the other crustacean class. With this last model of DF, 100% of the cases were correctly assigned, and comparison of TAG oil samples with the prey data showed that stomach oils presented the highest

Table 4. Mean values for fatty acid composition of stomach oil triacylglycerol fractions for the five procellariiform species (*see* Table 1 for bird species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids).

	FA composition of TAG fraction (%)				
	P. ten. (n=14)	*P. aeq.* (n=13)	*H. cae.* (n=51)	*P. bel.* (n=29)	*P. des.* (n=18)
14:0	6.87±3.04	6.86±2.96	5.73±1.73	5.74±2.32	8.45±1.71
16:0	15.94±0.89	16.22±1.77	13.53±1.96	15.86±2.01	16.31±3.05
18:0	2.02±0.40	2.84±0.62	2.12±0.46	2.13±0.52	2.02±0.34
16:1n-7	9.56±0.96	6.50±1.75	8.12±1.58	7.68±1.29	8.95±1.41
18:1n-9	23.21±2.52	23.59±3.44	25.93±4.45	22.27±3.73	20.04±5.23
18:1n-7	5.48±1.52	5.83±1.82	9.11±2.00	8.36±1.39	6.31±1.67
20:1n-9	2.97±1.03	8.24±2.43	8.37±2.36	8.66±3.43	5.04±2.37
22:1n-13+11	4.19±2.60	2.25±0.92	2.36±1.11	2.26±0.96	3.21±2.69
22:1n-9	2.09±0.76	1.60±0.54	1.94±0.66	1.73±0.63	2.55±1.89
24:1n-11	1.67±1.09	0.52±0.32	0.46±0.27	0.60±0.31	0.76±0.84
24:1n-9	1.60±0.75	0.77±0.34	0.81±0.27	0.98±0.33	0.84±0.40
18:2n-6	1.45±0.25	1.23±0.37	1.31±0.40	1.48±0.20	1.43±0.35
18:4n-3	1.24±0.41	0.50±0.16	0.57±0.20	0.81±0.27	1.10±0.41
20:5n-3	5.60±1.58	6.06±1.61	5.82±1.61	6.32±1.48	8.15±1.92
22:6n-3	5.63±1.26	5.97±3.47	4.25±1.30	4.95±1.17	4.87±0.85
Others	10.48±1.68	11.02±4.51	9.58±1.01	10.14±0.80	9.99±1.46
Total SFAs	28.07±2.37	29.68±5.62	24.04±3.60	26.81±3.81	29.56±3.60
Total MUFAs	53.61±3.13	52.03±5.64	60.14±4.56	55.79±5.25	50.34±4.78
Total PUFAs	18.31±2.70	18.29±4.96	15.81±3.22	17.37±3.12	20.10±3.57

probability of grouping with the fish class except five oils with the squid class and one *P. aeq.* oil with the euphausiid class. A second LDA was then conducted with only fish profiles (56 profiles described by 32 FAs). Nine classes were defined corresponding to the nine fish species. The first DF (74% of data variability) separated nototheniids (except *D. eleginoides*, which is poorer in PUFAs) from the four other species. This second model correctly classified 100% of fish profiles. Comparisons of TAG oil samples with prey data showed that stomach oils presented the highest probability of grouping with myctophids (>82% of total profiles) and nototheniids (12% of total profiles; Table 5).

Composition of food samples—Mass of food samples ranged from 16 g (*P. des.* samples) to 174 g (*P. ten.* samples). Samples of the five seabird species could be discriminated into three groups considering their fresh mass composition: *P. ten.* and *P. aeq.* samples contained mainly fish (>75% by fresh mass), *P. bel.* and *P. des.* samples were dominated by crustacean remains (>79% by fresh mass), and *H. cae.* samples contained both crustaceans (74%) and fish (25%; Fig. 4).

A total of 34,191 prey items belonging to 84 taxa were recovered from the 125 food samples. Crustaceans dominated the diet by number (50–99% of total items depending on seabird species) with four main species: three euphausiids (*E. superba*, *E. vallentini*, *T. macrura*/*vicina*) and one hyperiid amphipod (*T. gaudichaudii*; Table 6). Fish occurred in 100% of *P. ten.* samples, and in 77%, 71%, 55%, and 56% of *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.* samples, respectively (Table 6). Myctophids were the most numerous fish with 12 identified species (data not shown), *E. antarctica* and *K. anderssoni* being the commonest species (Table 6). Finally, most of the cephalopod remains were too digested to be identified at the species level.

Discussion

Using a new tool, lipid analysis of stomach oil, we demonstrate for the first time the importance of myctophid fish in the nutrition of flying seabirds breeding in subantarctic islands and foraging in Antarctic waters during the austral summer. The study points out the use of indirect methods, including lipids (Connan et al. 2005) and stable isotopes (Cherel et al. 2005), to investigate the feeding habits of adult procellariiforms, because parent birds feed for themselves in different areas and on prey different from that given to their chicks.

Interspecific variability of stomach oil lipid compositions—The original purpose of this work is to describe the lipid, FA, and FAlc compositions from a large number of stomach oils (n = 125) of breeding adults of five procellariiform species nesting in significant numbers in the Southern Ocean. Lipid class compositions and FA and FAlc signatures obtained in this study are consistent with the few works previously conducted on oils of *P. ten.*, *P. aeq.*, *H. cae.*, and *P. des.*, with no data available for *P. bel.* oils (review by Warham 1996). The comparison with this work, however, is difficult because most of these preliminary analyses were performed 30 yr ago with a less accurate methodology that did not discriminate isomers. Moreover, in most cases, the number of samples was small, oils were collected on birds with no well-defined status (adults vs. chicks and breeding vs. nonbreeding birds), and in some cases, oils from different individuals were pooled before analysis.

The two main forms of lipid storage in marine organisms are WE and TAG (Lee et al. 2006). Accordingly, WE and TAG dominated in more than 94% of the 125 stomach oils analyzed in this work (>70% of total lipids). This biochemical composition is also the consequence of differential digestion leading to oil formation. Briefly, oil lipid composition reflects not only the lipid composition of prey, but also the relative solubility of each class of lipids in previously accumulated stomach oils. For example, the low abundance of polar lipids (1% to 3% of total lipids) is due to their limited solubility in stomach oil and to rapid gastric emptying in the aqueous phase (Place et al. 1989).

The lipid composition obtained for the five seabird species classified stomach oils in three groups: those dominated by WE (*P. ten.* and *P. bel.*, indicating that seabirds have mainly preyed on WE-rich species), those dominated by TAG (*P. aeq.*, indicating that they primarily fed on TAG-rich species), and those containing high amounts of both WE and TAG (*P. des.* and *H. cae.*, indicating that birds preyed upon both WE- and TAG-rich species). Taking into account FA compositions of WE and TAG, and that of FAlcs of WE, differences have also been detected between the five seabird species. Oil FA and FAlc profiles clearly segregated *P. aeq.* from *P. ten.*, with smaller, but still significant, differences among the three other species of petrels (Fig. 3; Tables 2–4). For example, MUFAlcs clearly dominated in *H. cae.* and *P. bel.* oils, in contrast with *P. des.* oils, in which SFAlcs and MUFAlcs were equally important (Table 2). Also, *P. bel.* oils were richer in myristic (14:0) and palmitic (16:0) acids than *P. des.* oils in the WE fraction (Table 3). Taken together, these results suggested that *P. aeq.* and *P. ten.* have preyed on different species and that *H. cae.*, *P. bel.*, and *P. des.* had more similar, but still different, dietary habits.

The lipid method has thus the potential to give a new insight into trophic segregation between sympatric procellariiforms. *P. des.* and *P. bel.* are closely related species, and Kerguelen is the only place where the two species nest in high numbers (Weimerskirch et al. 1989). Analysis of chick food showed a large overlap in the feeding ecology of *P. des.* and *P. bel.* during the chick-rearing period, and accordingly, the stable carbon and nitrogen isotopic signatures of chick feathers indicate that *P. bel.* and *P. des.* fed at the same trophic level and in the same broad foraging areas (Cherel et al. 2002*a*; this study). Interestingly, however, analysis of stomach oils indicated that the diet of adult *P. des.* and *P. bel.* differed during long foraging trips, with *P. bel.* feeding more on WE-rich prey and *P. des.* more on TAG-rich prey.

Importance of myctophid fish for procellariiform seabirds—The major result of this work is the biochemical indication that most stomach oils mainly derived from

Table 5. Predicted group classification from linear discriminant analyses (*see* Table 1 for bird species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*).

Predicted group membership		Predicted classification					Total
		P. ten.	*P. aeq.*	*H. cae.*	*P. bel.*	*P. des.*	
Wax ester fatty alcohols*	Copepods	0	0	6	6	0	12
	Euphausiids	0	0	0	0	0	0
	Myctophids	14	9	44	23	18	108
	Total	14	9	50	29	18	120
Wax ester fatty acids†	Copepods	0	0	0	0	3	3
	Euphausiids	0	0	0	0	0	0
	Myctophids	14	9	51	29	15	118
	Total	14	9	51	29	18	121
Triglyceride fatty acids‡	Euphausiids	0	1	0	0	0	1
	Other Crustaceans	0	0	0	0	0	0
	Myctophids	9	9	44	26	15	103
	Nototheniids	4	0	7	2	3	16
	Squids	1	3	0	1	0	5
	Total	14	13	51	29	18	125

* Copepods (*C. acutus*, *P. antarctica*, *R. gigas*), euphausiids (*E. crystallorophias*, *T. macrura*), myctophids (*E. antarctica*, *E. carlsbergi*, *G. braueri*, *K. anderssoni*).
† Copepods (*C. acutus*, *P. antarctica*, *R. gigas*), euphausiids (*E. crystallorophias*, *T. macrura*), myctophids (*E. antarctica*, *G. braueri*, *K. anderssoni*).
‡ Euphausiids (*E. crystallorophias*, *E. superba*, *E. vallentini*), other crustaceans (*C. propinquus*, *E. rostromagna*, *T. gaudichaudii*), myctophids (*E. carlsbergi, G. nicholsi*, *P. bolini*), nototheniids (*D. eleginoides*, *P. borchgrevinki*, *T. bernacchii*, *T. hansoni*, *T. newnesi*, *T. pennellii*), squids (*M. ingens*, *M. robsoni*).

myctophid lipids in all the five species of seabirds investigated (Table 5). Surprisingly, almost no stomach oil fit the lipid patterns of subantarctic and Antarctic euphausiids, including those of the WE-rich *T. macrura* and of the TAG-rich subantarctic krill *E. vallentini* and Antarctic krill *E. superba*. Digested remains of Antarctic krill were previously found in food samples of four (*P. aeq.*, *H. cae.*, *P. des.*, and *P. bel.*) of the five species investigated and we therefore expected that the species was the main prey targeted by adult birds reaching Antarctic waters during long trips (Catard et al. 2000; Cherel et al. 2002*a*,*b*; this study). Instead, our study emphasized the role of mesopelagic fish in the area, whereas only a few previous works suggested their importance in the diet of flying seabirds (Ainley et al. 1991; Ferretti et al. 2001).

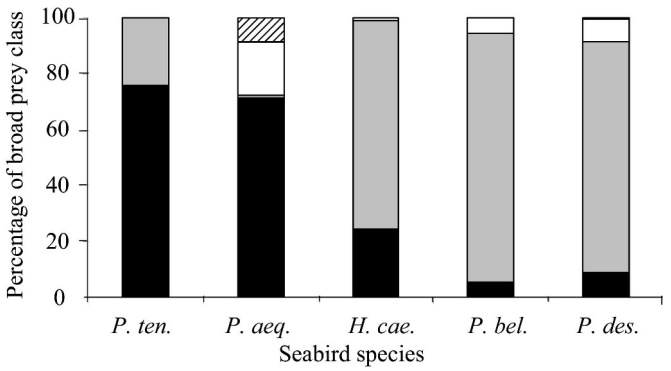

Fig. 4. Broad prey class composition (% by fresh mass; black, fish; gray, crustaceans; white, cephalopods; striped, others) of the chick diet after adult long trips for the five seabird species (*see Table 1* for species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*).

Overall, more than 93% of FA and FAlc patterns of oil WE showed a high probability of resemblance with the myctophid fish signatures, and similar results were obtained with the TAG fractions, whereas 82% of FA patterns showed strong similarities with myctophids. Some oil signatures were close to that of squids, including *M. ingens* (Connan et al. 2005; this study). The profiles of squids used in this study were of their digestive gland, which is rich in lipids of dietary origin (Phillips et al. 2002). Within that context, it is important to note that *M. ingens* mainly prey on mesopelagic fish, including myctophids (Cherel and Duhamel 2003), and that its digestive gland lipids have a myctophid signature (Phillips et al. 2001, 2003), thus further emphasizing the trophic relationships between myctophids and seabirds.

Oil analyses indicated that all five procellariiform species prey on myctophids but showed different prey signatures. This apparent discrepancy can be explained by the diversity of myctophids in their biochemical composition and number of species living in the Southern Ocean. In contrast with other oceans in which the Myctophidae and Gonostomatidae codominate, myctophids alone dominate the micronektonic fish community of the mesopelagic zone in the Southern Ocean, with more than 35 species occurring south of the subtropical front (Gon and Heemstra 1990). Most of the species have a circumpolar distribution and are widespread in oceanic waters from south of the subantarctic front to the edge of the Antarctic continent. The total biomass of mesopelagic myctophids there has been estimated to amount to 212–396 million tonnes, with four main species (i.e., *E. antarctica*, *E. carlsbergi*, *K. anderssoni*, and *G. nicholsi*) (Sabourenkov 1991). Myctophids are fatty fish (up 26% wet mass), and they deposit fat as WE or TAG, depending on the species (Saito and Murata 1998).

Table 6. Frequency of occurrence (FO; % of stomach contents) and number of prey items (Nb; % of total prey items) recovered in >20% of stomach contents of the five seabird species after adult long trips (*see* Table 1 for species acronyms *P. ten.*, *P. aeq.*, *H. cae.*, *P. bel.*, and *P. des.*).

	P. ten. (*n*=14)		*P. aeq.* (*n*=13)		*H. cae.* (*n*=51)		*P. bel.* (*n*=29)		*P. des.* (*n*=18)	
	FO	Nb	FO	Nb	FO	Nb	FO	Nb	FO	Nb
Crustaceans	100.0	67.7	46.2	50.0	98.0	99.2	100.0	99.1	100.0	98.9
Cirripedia										
Lepas australis (cypris larvae)	7.1	<0.1			2.0	<0.1	13.8	0.6	33.3	1.7
Amphipoda										
Vibiliidae										
Cyllopus magellanicus					37.3	0.4	31.0	0.2	66.7	3.5
Vibilia antarctica					27.5	0.3	6.9	<0.1	33.3	2.3
Hyperiidae										
Themisto gaudichaudii	85.7	4.2	7.7	1.1	88.2	14.3	86.2	15.1	100.0	82.5
Phrosinidae										
Primno macropa							34.5	0.2	22.2	0.2
Euphausiacea										
Euphausia superba			46.2	46.7	62.7	2.1	17.2	0.3	33.3	0.8
Euphausia vallentini	35.7	46.2			9.8	0.4	3.4	<0.1		
Nyctiphanes australis	21.4	16.8								
Thysanoessa gregaria	42.9	0.3								
Thysanoessa macrura/vicina					94.1	81.0	96.6	81.7	22.2	1.4
Decapoda										
Pasiphaea scotiae			7.7	1.1	49.0	0.2	27.6	0.1	22.2	0.1
Fish	100.0	32.2	76.9	28.9	70.6	0.6	55.2	0.3	55.6	0.4
Paralepididae										
Notolepis coatsi			23.1	5.6						
Myctophidae										
Electrona antarctica			15.4	3.3	21.6	0.1	3.4	<0.1		
Krefftichthys anderssoni	21.4	0.3			17.6	0.1	20.7	0.1	5.6	<0.1
Tasmanian unidentified postlarvae	85.7	31.4								
Unidentified fish	42.9	0.4	15.4	2.2	17.6	0.1	17.2	0.1	27.8	0.2
Cephalopods	21.4	<0.1	61.5	15.6	21.6	0.1	58.6	0.5	33.3	0.2
Brachioteuthidae										
Slosarczykovia circumantarctica			30.8	8.9			17.2	0.1	5.6	<0.1
Gonatidae										
Gonatus antarcticus			7.7	1.1	2.0	<0.1	27.6	0.3		
Oegopsida sp. A					5.9	<0.1	10.3	<0.1	22.2	0.1
Unidentified squid	7.1	<0.1	30.8	4.4	11.8	<0.1	10.3	<0.1		
Others			30.8	5.6	13.7	0.1	3.4	<0.1	5.6	0.6
Carrion			23.1	3.3						

Accordingly, Southern Ocean myctophids include both WE-rich (e.g., *E. antarctica, K. anderssoni*) and TAG-rich species (e.g., *E. carlbergi*, *G. nicholsi*, *P. bolini*) (Phleger et al. 1999; Lea et al. 2002; Connan et al. unpubl.). The little information available suggests that FA and FAlc patterns varied between species, and within species between different localities (Phleger et al. 1999; Lea et al. 2002; Connan et al. unpubl.). However, biochemical studies were performed on a small number of individuals, thus precluding the biochemical determination of myctophids at the species level. Interestingly, *E. antarctica*, *E. carlsbergi*, *K. anderssoni*, and *G. nicholsi* were identified, but in relatively low numbers, in food samples from the five seabirds investigated (Weimerskirch and Cherel 1998; Catard et al. 2000; this study). Noticeably the WE-rich *K. anderssoni* was the main myctophid prey of *P. bel.* (Cherel et al. 2002*a*; this study), whose oils mainly contained WE; both WE-rich (*E. antarctica*, *K. anderssoni*) and TAG-rich myctophids (*E. carlsbergi*, *P. bolini*) were important in the diet of *H. cae.* (Cherel et al. 2002*b*; this study), whose oils contained both WE and TAG (Fig. 2).

Analyses of chick food collected by adults during long trips showed that fish dominated by mass in *P. ten.* and *P. aeq.* stomach contents, and specific composition indicated that Antarctic and subantarctic mesopelagic myctophids were the most common fish prey (Weimerskirch and Cherel 1998; Catard et al. 2000; this study). By contrast, fish were not the main prey by mass of *H. cae.*, *P. bel.*, and *P. des*, whose chick diet was by far dominated by crustaceans (Fig. 4; Cherel et al. 2002*a*,*b*). The discrepancy between food and oil analyses can be explained by adult birds feeding on different prey when they forage for themselves

far away their breeding grounds and when they collect food for their chicks on the way back to the colony. In other words, food samples and stomach oil carry dietary information at different time scales, with oils retaining the lipid signature of prey ingested by adult birds before they collected food for their chicks. This has important implications at both the species and ecosystem levels. At the species level, the study highlights that adults can feed for themselves in areas and on prey different from those of their chicks. This behavior cannot be investigated by food analysis alone; instead, it necessitates the use of indirect methods like stable isotopes (Cherel et al. 2005) and lipids as trophic markers (Connan et al. 2005). At the ecosystem level, a common assumption in the quantification of the effect of seabirds on marine resources is to consider that all the individuals (chicks, immatures, breeding and nonbreeding adults) feed on the same prey all year long—those identified in chick food during the chick-rearing period. This assumption was not verified in this work, thus emphasizing that the feeding habits of different age classes at different spatiotemporal scales must be investigated to quantify trophic relationships of a given seabird species.

The five seabird species investigated (*Puffinus tenuirostris*, *Procellaria aequinoctialis*, *Halobaena caerulea*, *Pachyptila belcheri*, and *Pachyptila desolata*) exhibited a wide range of body size, from the largest burrowing petrel (*P. aeq.*, 1.2 kg and a wing span of 1.5 m) to much smaller species (prions, 150 g and a wing span of 60 cm). Moreover, birds originated from five colonies localized on three islands of the Southern Hemisphere (a subtropical island [Tasmania], and two subantarctic archipelagoes [Crozet and Kerguelen]). Hence, our results on the importance of myctophids in the nutrition of procellariiforms could probably be extrapolated to more species breeding in the Southern Ocean. More investigations are needed on other species from other areas, including, for example, south Georgia, where food analyses indicate that Antarctic krill form the staple food of the predator community during the summer months.

It has generally been assumed that Antarctic krill constitutes the major prey of Antarctic consumers. However, available evidence north of the area in which Antarctic krill occurs (and even within that area, *see* Ainley et al. 1991) suggests a major role for myctophid fish together with other euphausiid species (*E. vallentini* and *Thysanoessa* spp.) and the hyperiid *T. gaudichaudii* as prey of higher predators (Ridoux 1994; Bocher et al. 2001). The diet of myctophids comprises mesozooplankton and largely copepods, but also euphausiids, amphipods, ostracods, and pteropods (Kozlov and Tarverdiyeva 1989; Pakhomov et al. 1996; Gaskett et al. 2001). Interestingly, when present in the area, Antarctic krill was usually poorly represented in myctophid stomachs (Pakhomov et al. 1996). Our data are therefore in agreement with the importance of a recently recognized food chain excluding Antarctic krill (phytoplankton—copepod—myctophids—higher predators) of the pelagic ecosystem in both low and high latitudes of the Southern Ocean (Kozlov 1995; Rodhouse and White 1995).

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