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# Lipid composition of stomach oil in a procellariiform seabird *Puffinus tenuirostris*: implications for food web studies

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**ABSTRACT:** Some procellariiform seabirds use a dual strategy for provisioning their chicks by alternating between short and long foraging trips (LT). Trophic relationships of adult birds are unknown when they feed for themselves during LT because digestion processes preclude direct prey determination. Since stomach contents collected after LT contain oil of dietary origin, we tested the use of oil lipids as prey trophic markers using the Tasmanian short-tailed shearwater *Puffinus tenuirostris* as a model seabird. The intra-specific variability of stomach oils was investigated through lipid class composition, and their fatty acid and fatty alcohol profiles. Oils mainly consisted of wax esters (WE) and triacylglycerols (TAG) (49 to 86 and 7 to 41%, respectively). Major fatty acids of TAG were in a decreasing order 18:1n-9, 16:0, 16:1n-7, 14:0, 20:5n-3 and 22:6n-3. The WE-fatty acid profiles were dominated by 18:1n-9 and 16:1n-7 while fatty alcohol profiles were dominated by 16:0. Fatty alcohol and fatty acid patterns were tested as possible descriptors of ingested prey (derived from literature data) through multivariate discriminant analyses. Comparisons of the WE fatty alcohol patterns showed a close association with the alcohol structure of 3 myctophid fish species namely *Krefflichthys anderssoni*, *Gymnoscopelus braueri* and *Electrona antarctica*; these results were corroborated by WE fatty acid analysis. Comparison of TAG fatty acid patterns showed the highest similarity between oils and the digestive gland of the myctophid-eater squid *Moroteuthis ingens* in association with the myctophid *Electrona carlsbergi*. Hence, biochemical analysis of both WE and TAG strongly suggested that adult short-tailed shearwaters mainly prey upon Antarctic/sub-Antarctic myctophids when they feed for themselves, thus emphasizing the role of these oceanic mesopelagic fish in the marine ecosystem of the Southern Ocean.

**KEY WORDS:** Antarctica · Short-tailed shearwater · Fatty alcohols · Fatty acids · Trophic interactions · Myctophids · Tasmania

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## INTRODUCTION

In the Austral Ocean, Procellariiformes (albatrosses and petrels) dominate the seabird community in terms of specific diversity (Marchant & Higgins 1990). They spend the majority of their life at sea, only coming on land to breed and rear a single chick (Warham 1990). With the exception of diving petrels, all procellariiform species are characterized by the presence of oil in their

proventriculus (Warham 1977). The dietary origin of this stomach oil is now accepted (Lewis 1969, Cheah & Hansen 1970a,b, Clarke & Prince 1976, Warham et al. 1976, Place et al. 1989). It is likely produced by mechanical rupture of lipid-rich food organisms (crustacean, fish and squid) followed by differential digestion of proteins and lipids (Clarke 1989). By concentrating dietary lipids in their stomach, adult petrels and albatrosses reduce the mass and frequency of meal

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delivery to their chicks, thus decreasing the time and energy costs involved in transporting food from patchily distributed pelagic food sources. Stomach oil also plays other significant roles, such as an energy reserve for adult birds and as an offensive/defensive weapon (Warham et al. 1976, Warham 1977).

During the breeding season, some Procellariiformes use a 2-fold foraging strategy to feed their chick: they alternately perform short trips (ST) where adults forage near the colony, and long trips (LT) where they reach more distant feeding areas (Weimerskirch et al. 1994). Parent birds gain mass during LT and lose mass during ST, indicating that LT are profitable for adults probably through a build up of energy reserves and ST are profitable for chicks through an increase in their feeding frequency. A recent energetic study showed that adult self-feeding during LT is crucial for the success of ST because most of the energy used during ST is likely to derive from the energy stored during LT (Weimerskirch et al. 2003). However, the self-feeding behaviour of adult seabirds during ST cannot be investigated through the direct method of food analysis because the available information is restricted to their stomach contents that correspond to the chick—not the adult—diet. Consequently, the trophic relationships of oceanic seabirds when they feed for themselves are essentially unknown.

Unlike stomach contents collected after ST, LT-food samples contain significant amounts of stomach oil (Weimerskirch & Cherel 1998, Weimerskirch et al. 1999, Cherel et al. 2002), whose biochemical composition could include the lipid signature of the prey caught far away the breeding colony. Studies on fatty acid and fatty alcohol signatures to investigate trophic interactions have been proposed for many levels of the oceanic food web, ranging from the initial planktonic stages to the higher predators such as birds and marine mammals (e.g. Sargent & Falk-Petersen 1981, Iverson 1993, Raclot et al. 1998, Best et al. 2003). Lipids in marine organisms are characterized by their great diversity and high levels of long chain polyunsaturated fatty acids (PUFA), which originate from the various primary producers: phytoplankton and seaweeds (Ackman 1980). Unlike other nutrients, dietary fatty acids of carbon chain length >14 can be deposited in animal tissue with little or no modification (Ackman et al. 1971). Since stomach oil is derived from mechanical rupture of fat-rich prey items, its biochemical composition should be little affected by digestive processes, and it should be therefore possible to consider the patterns of fatty alcohols and fatty acids as linked to the lipid signature of the prey ingested.

The food and feeding ecology of the short-tailed shearwater *Puffinus tenuirostris* and its dual foraging

strategy during chick rearing were recently described (Weimerskirch & Cherel 1998, Klomp & Schultz 2000, Schultz & Klomp 2000). Birds alternate an average of 2 ST lasting 1 to 4 d before departing for a LT lasting 8 to 19 d. They forage from Australian to Antarctic oceanic waters during LT and remain over neritic waters close to their breeding colony during ST. While suggesting birds fed in cold waters during LT, the afore mentioned studies give no indication on the prey ingested when adult birds are self-feeding at that time. This is a crucial issue first to fully understand the adult foraging strategy, and second to determine the key species of the pelagic ecosystem because the huge population of short-tailed shearwaters (about 23 million breeding birds; Skira et al. 1985) is likely to have significant interactions in food webs.

Using the Tasmanian short-tailed shearwater as a model, the aims of this investigation were (1) to study the intra-specific variability of stomach oil composition, and (2) to test the use of the lipid-signature of stomach oil to elucidate trophic relationships of procellariiform seabirds when they feed far away their breeding grounds.

## MATERIALS AND METHODS

**Field study and sample collection.** Field work was carried out during austral summer 1997 (between 1 and 27 March) at The Neck Game Reserve (43° 18' S, 147° 18' E), Bruny Island, located in the south-eastern of Tasmania (Fig. 1). The methodology used is detailed in Weimerskirch & Cherel (1998). Briefly, sticks were placed at the mouth of the burrows, so that a visit by breeding *Puffinus tenuirostris* could be detected by displacement of the sticks. Burrows were inspected every half-hour from dusk to midnight, and 1 h before dawn. The duration of individual foraging trips was defined as the time elapsed between 2 successive recoveries of the same bird. Food samples were obtained either by the 'water off-loading technique' (Wilson 1984) or through spontaneous regurgitation of adult short-tailed shearwaters on arrival back at the colony after a foraging trip, before they fed their chick. Fourteen stomach contents were collected after LT lasting from 9 to 17 d. Diet samples were frozen and returned within 2 wk to the laboratory in France (Centre d'Etudes Biologiques de Chizé) for subsequent analyses. Stomach contents were composed of a liquid fraction made of oil and water, and a solid fraction made of more or less digested prey. In the laboratory, each sample was thawed and drained by gravity overnight to separate the liquid fraction from the solid items. These 2 fractions were then measured in a graduated tube.

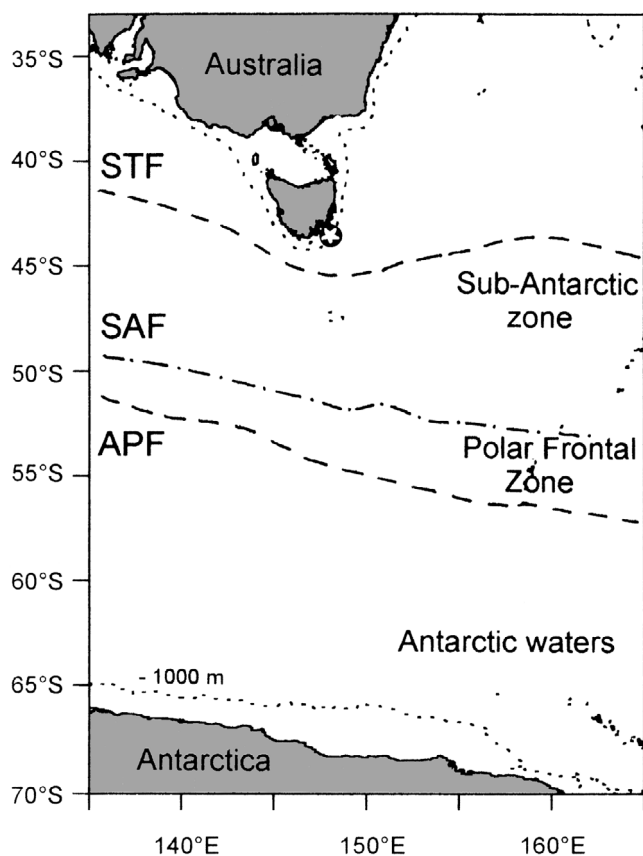


Fig. 1. Location of Bruny Island (star) off south east Tasmania in the Southern Ocean. STF: sub-tropical front; SAF: sub-antarctic front; APF: antarctic polar front

Prey analysis of the 14 solid fractions, i.e. identification and counting of fresh and accumulated items found in stomach contents, has been reported earlier by Weimerskirch & Cherel (1998).

**Lipid analysis.** Total lipids were quantitatively extracted from each of the 14 liquid fractions according to the method of Bligh & Dyer (1959). Crude extracts were placed in chloroform, concentrated under vacuum and stored at  $-80^{\circ}\text{C}$ . To avoid lipid auto-oxidation, a nitrogen atmosphere was maintained at all times.

**Lipid classes:** Individual lipid classes were quantified using an 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 microcapillaries (1  $\mu\text{l}$ ) and analysed in duplicate. Neutral lipids were separated using a double development procedure with the following solvent systems: n-hexane: benzene: formic acid 80:20:1 (v/v/v) followed by n-hexane: diethyl ether: formic acid 97:3:1.5 (v/v/v). After development, the chromarods were oven dried and analysed immediately to minimise adsorption of atmospheric contaminants. Calibration of the rods was

achieved using analytical grade commercial standards (Sigma—cholesterol [Chol], diacylglycerols (DG), free fatty acids (FFA), polar lipids (PL), triacylglycerols [TAG] and sterol esters). Peaks were quantified using ChromStar software version 4.14. Iatroscan results are generally reproducible to  $\pm 10\%$  of individual component abundance (Volkman & Nichols 1991).

**Fatty acids and fatty alcohols:** Lipid classes were further isolated by preparative TLC using n-hexane: diethyl ether: acetic acid (85:15:1 v/v/v). After development, the plates were dried and sprayed with a solution of 0.2% 2,7-dichlorofluorescein in ethanol. The lipids were visualised under UV light and identified by comparisons with co-chromatographed standard mixtures (Sigma). The bands of wax esters (WE) and TAG were scraped off and eluted with diethyl ether: chloroform (1:1 v/v).

Before gas-liquid chromatography (GLC), fatty acids from both WE and TAG, and fatty alcohols from WE were converted into methyl esters and acetates, respectively. Fatty acid methyl esters were prepared using 7% boron trifluoride in methanol (Morrison & Smith 1964). Fatty alcohols were acetylated using acetic anhydride (Ackman et al. 1972). GLC analyses were performed with an Autosystem XL gas chromatograph (Perkin Elmer) equipped with a polar column Fawmax (Restek), 30 m  $\times$  0.32 mm internal diameter) and a FID detector. Helium was used as carrier gas at 7 psig. The column was operated isothermally at  $190^{\circ}\text{C}$  during 120 min for fatty acid methyl esters and  $200^{\circ}\text{C}$  during 100 min for fatty alcohol acetates. Injector and detector were maintained at 225 and  $250^{\circ}\text{C}$ , respectively. Peaks were quantified with Turbochrom Navigator software version 4.1. Individual components were tentatively identified by reference to authentic standards and well-characterized fish oils (Capelin: Menhaden 1:1). In addition to the examination of esters and acetates recovered, 1 part of fatty acid methyl ester or fatty alcohol acetate samples was completely hydrogenated and the products examined qualitatively and quantitatively by GLC. Corrections were made on the results according to the differential FID response to fatty acids or fatty alcohols depending on their chain-length.

**Statistical analyses.** The intra-specific variability of stomach oil was evaluated by cluster analyses using Euclidean distance and average linkage on lipid class composition, fatty acid and fatty alcohol profiles using Systat 9.0.

To achieve meaningful comparisons between fatty alcohol and fatty acid patterns from potential prey species and stomach oils, 3 databases were built using the majority of published studies on Tasmanian, sub-Antarctic and Antarctic potential prey, i.e. zooplankton, micronekton and nekton (Table 1). The first database

regrouped 86 fatty alcohol profiles of 10 species of crustacean and fish (database WEAL). The second one regrouped the WE fatty acid profiles (database WEAC), and the third one regrouped the TAG fatty acid profiles of potential prey (database TAG). Unfortunately, most of studies about sub-Antarctic and Antarctic species only presented the fatty acid composition of total lipids. In this case, we assigned the fatty acid data to either the second or the third database, based on the percent composition of the prey lipids (more than 60% of WE in database WEAC and more than 60% of TAG in database TAG). Hence, in the second database, 64 profiles belonging to 9 species of crustacean and fish were compiled, while in the third one 71 profiles belonging to 19 species of crustacean, fish and squid were grouped. The data in each database were validated by performing a preliminary principal component analysis to check the homogeneity of the fatty alcohol and fatty

acid patterns within a given species or group of species. The outliers were removed from the groupings used in the further discriminant analyses. Similarly, when the literature indicated conflicting results that could not be easily resolved, the corresponding species was not considered in the analyses.

Linear and Stepwise linear discriminant analyses (LDA and SLDA) were used to classify prey species based on either fatty alcohol or fatty acid patterns. The number of descriptors considered in the analyses was limited to the data available from the literature. For the WEAL and WEAC databases, LDA were performed using 8 fatty alcohols and 17 fatty acids, respectively. Then, stomach oil samples were attributed to pre-existing prey group. Stomach oils were used as supplementary observations and were not used in the definition of the discriminant functions. For the TAG database, fatty acid data were analysed using the same

Table 1. Prey species gathered from the literature and included in the 3 databases. WEAL: fatty alcohol profiles of wax esters; WEAC: fatty acid profiles of wax esters; TAG: fatty acid profiles of triacylglycerols; Code: acronyms used in the statistical analyses

Species	Code	Database			Source
		WEAL	WEAC	TAG	
<b>Crustacean</b>					
<i>Calanoides acutus</i>	Caa	X	X		Albers et al. (1996), Falk-Petersen et al. (1999), Hagen et al. (1993), Kattner et al. (1994)
<i>Calanus propinquus</i>	Cap			X	Albers et al. (1996), Falk-Petersen et al. (1999), Hagen et al. (1993), Kattner et al. (1994)
<i>Euchirella rostromagna</i>	Eur	X	X	X	Albers et al. (1996), Hagen et al. (1995)
<i>Euphausia crystallorophias</i>	Euc	X	X	X	Bottino (1975), Falk-Petersen et al. (1999), Kattner & Hagen (1998)
<i>Euphausia superba</i>	Eus			X	Mayzaud (pers. comm.)
<i>Euphausia vallentini</i>	Euv			X	Mayzaud et al. (2003)
<i>Paraeuchaeta antarctica</i>	Paa	X	X		Albers et al. (1996), Hagen et al. (1995), Mayzaud (pers. comm.)
<i>Rhincalanus gigas</i>	Rhg	X	X		Kattner et al. (1994), Reinhardt & Van Vleet (1986)
<i>Themisto gaudichaudii</i>	Thg			X	Fricke & Oehlenschläger (1988), Nelson et al. (2001), Phleger et al. (1998)
<i>Thysanoessa macrura</i>	Thm	X	X		Falk-Petersen et al. (1999), Mayzaud et al. (2003)
<b>Fish</b>					
<i>Electrona antarctica</i>	Ea	X	X		Lea et al. (2002), Phleger et al. (1997)
<i>Electrona carlsbergi</i>	Ec	X		X	Lea et al. (2002), Phleger et al. (1999), Phleger et al. (1997)
<i>Gymnoscopelus braueri</i>	Gb	X	X		Phleger et al. (1999)
<i>Gymnoscopelus fraseri</i>	Gf			X	Lea et al. (2002)
<i>Gymnoscopelus nicholsi</i>	Gn			X	Lea et al. (2002), Phleger et al. (1999)
<i>Gymnoscopelus opisthopterus</i>	Go			X	Phleger et al. (1999)
<i>Gymnoscopelus piabilis</i>	Gp			X	Lea et al. (2002)
<i>Krefflichthys anderssoni</i>	Ka	X	X		Phleger et al. (1999), Nelson (pers. comm.)
<i>Pagothenia borchgrevinki</i>	Pb			X	Phleger et al. (1999)
<i>Protomyctophum tenisoni</i>	Pt			X	Lea et al. (2002)
<i>Trematomus bernacchii</i>	Tb			X	Phleger et al. (1999)
<i>Trematomus hansonii</i>	Th			X	Phleger et al. (1999)
<i>Trematomus newnesi</i>	Tn			X	Phleger et al. (1999)
<i>Trematomus pennellii</i>	Tp			X	Phleger et al. (1999)
<b>Cephalopod</b>					
<i>Moroteuthis ingens</i>	Mi			X	Phillips et al. (2001)
<i>Moroteuthis robsoni</i>	Mr			X	Phillips et al. (2002)

procedure but with SLDA to optimise classification. All discriminant analyses were conducted using Statgraphics Plus 5.0. Normality being presupposed for most of these analyses, percentages were normalized using the arcsine transformation (Zar 1984).

## RESULTS

Stomach contents collected after a LT showed accumulated solid components and variable amounts of orange-coloured oil (mean value  $14.6 \pm 12.7$  ml, range: 3.5 to 55.0 ml). This oily fraction represented 44 % of the total volume of the liquid fraction, the remaining being water.

### Lipid composition

The 14 stomach oils were mostly composed of WE and TAG. Other lipid classes (Chol, PL, FFA and DG) were present, but in small amounts (Table 2). Nine oils were WE-rich (76 to 86 % of total lipids), whereas 5 oils were characterized by a mean composition in WE and TAG (49 to 65 % and 28 to 41 % of total lipids, respectively).

### Fatty acid and fatty alcohol profiles

#### Triacylglycerols

Twenty-one different fatty acids were found at levels exceeding traces (>0.5 %) representing 90 to 94 % of the total fatty acids (Table 3). Monounsaturated fatty acids (MUFA; 46 to 56 %) were more prevalent than both saturated fatty acids (SFA; 22 to 31 %) and PUFA (11 to 20 %). Two fatty acids dominated by mass (>10 %): the oleic (18:1n-9; 19 to 28 %) and palmitic acids (16:0; 14 to 17 %). Main fatty acids (>5 %) included the palmitoleic acid (16:1n-7; 6 to 10 %), the myristic acid (14:0; 3 to 11 %), the eicosapentaenoic acid (EPA, 20:5n-3; 3 to 8 %), the docosahexaenoic acid (DHA, 22:6n-3; 4 to 8 %) and

the 18:1n-7 (4 to 8 %) in a decreasing order of importance (Table 3). Together, these 7 fatty acids accounted for 63 to 79 % of the total fatty acids. PUFA of the n-3 series were 3 to 6 times more abundant (10 to 19 versus 3 %) than those of the n-6 series.

Two main groups of oils could be discriminated on the basis of their percentages in 22:1, 24:1 and EPA/DHA ratio. This partition of oils, confirmed by cluster analysis (Fig. 2), resembles that previously observed in lipid class composition (see above).

#### Wax esters

Twenty-two different fatty acids were present at levels >0.5 % in all stomach oils (Table 4). The MUFA clearly dominated the profiles (66 to 79 % of the total fatty acids). The major fatty acid was 18:1n-9 (36 to 43 %) and there were substantial amounts of 16:1n-7 (13 to 16 %), 20:5n-3 (5 to 12 %) and 22:6n-3 (4 to 8 %). PUFA of the n-3 series were 3 to 7 more abundant than those of the n-6 series.

Eighteen different fatty alcohols were identified (>0.5 %, Table 5). More than half of components were saturated (52 to 62 %), while monounsaturated fatty alcohols represented 34 to 45 % of the total. Very few polyunsaturated components were identified. The principal fatty alcohols included palmitol (16:0; 38 to 47 %), gadoleol (20:1n-9; 8 to 12 %), 14:0 (7 to 9 %) and 22:1n-13+11 (5 to 9 %).

### Dietary implications of stomach oil analyses

#### Fatty alcohol descriptors of wax esters

To investigate species variation in the potential prey database, we conducted a LDA of the 9 major groups of prey species. LDA was performed using the 8 major fatty alcohol descriptors. Classes have been defined for each prey species except for the 2 myctophid fish *Gymnoscopelus braueri* and *Krefftichthys anderssoni*,

Table 2. *Puffinus tenuirostris*. Lipid composition (% of total lipids) of the 14 stomach oils. WE: wax esters; TAG: triacylglycerols; FFA: free fatty acids; Chol: cholesterol; DG: diacylglycerols; PL: polar lipids; -: not detected

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean $\pm$ SD
WE	75.7	84.1	51.7	65.0	80.5	76.1	84.3	54.7	48.9	81.6	83.2	58.4	82.8	85.6	72.3 $\pm$ 13.6
TAG	9.0	7.0	38.4	28.3	13.9	18.8	10.9	38.7	40.9	13.8	12.3	31.7	11.5	10.3	20.4 $\pm$ 12.4
FFA	3.7	4.0	1.9	0.9	–	–	–	1.0	2.0	–	–	4.0	1.9	1.6	1.5 $\pm$ 1.5
Chol <sup>a</sup>	9.3	4.2	5.6	5.0	4.4	3.9	4.0	3.2	4.8	3.2	2.9	4.7	2.3	1.1	4.2 $\pm$ 1.9
DG	0.7	–	–	–	0.4	0.5	0.2	1.1	1.7	0.4	–	–	0.5	–	0.4 $\pm$ 0.5
PL	1.6	0.7	2.5	0.8	0.8	0.7	0.6	1.2	1.7	1.0	1.7	1.1	1.0	1.3	1.2 $\pm$ 0.5

<sup>a</sup>Could include other sterols



Table 3. *Puffinus tenuirostris*. Triacylglycerol fatty acid compositions (% of triacylglycerol fatty acids) of the 14 stomach oils SD: standard deviation; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: 20:5n-3; DHA: 22:6n-3; –: not detected; 'Others' includes all fatty acids present at <0.5%

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean ± SD
14:0	6.6	4.1	10.6	9.9	3.7	7.7	4.4	11.0	9.5	3.0	4.8	11.4	5.5	4.1	6.9 ± 3.0
16:0	16.6	16.8	14.7	15.5	16.4	15.5	15.2	16.6	14.3	16.8	17.5	16.2	15.4	15.7	15.9 ± 0.9
18:0	1.7	1.9	2.2	2.6	1.5	2.0	1.4	1.8	2.3	2.8	2.0	2.4	1.9	1.8	2.0 ± 0.4
Phytanate	0.6	1.1	0.1	–	0.6	0.4	0.6	0.4	0.4	0.6	0.9	0.5	0.6	0.6	0.6 ± 0.2
Total SFA	25.5	23.9	27.5	28.0	22.3	25.6	21.7	29.8	26.5	23.2	25.2	30.5	23.3	22.2	25.4 ± 2.8
16:1n-7	10.0	8.2	9.5	9.8	10.1	10.1	10.3	10.2	9.4	6.4	9.0	9.9	10.1	9.3	9.4 ± 1.1
18:1n-9	22.2	22.3	26.0	25.5	21.4	23.0	19.1	21.2	27.8	21.3	23.8	27.2	22.3	21.9	23.2 ± 2.5
18:1n-7	5.5	4.4	7.6	7.4	3.8	5.6	3.8	6.6	7.7	3.7	4.2	7.1	5.1	4.2	5.5 ± 1.5
18:1n-5	0.7	0.6	0.4	0.4	0.8	0.6	0.7	0.6	0.4	0.7	0.7	0.4	0.7	0.8	0.6 ± 0.2
20:1n-9	2.4	2.6	3.0	2.2	2.4	2.7	1.9	2.1	3.6	6.0	2.1	2.9	1.9	2.5	2.7 ± 1.0
22:1n-13+11	3.4	7.7	0.7	1.1	7.1	3.5	5.8	2.1	1.4	6.8	6.1	0.9	5.0	7.1	4.2 ± 2.6
22:1n-9	1.7	3.5	0.9	1.2	3.0	2.0	2.4	1.9	1.5	2.4	2.4	1.1	2.5	2.9	2.1 ± 0.8
24:1n-13	0.4	0.8	–	–	0.7	0.3	0.7	0.2	0.2	0.6	0.7	0.1	0.4	0.6	0.5 ± 0.2
24:1n-11	1.3	3.4	0.2	0.8	3.0	1.4	2.3	0.6	0.5	1.9	2.6	0.2	2.1	3.0	1.7 ± 1.1
24:1n-9	1.4	2.8	0.5	0.8	2.3	1.2	2.2	0.9	1.1	2.3	2.3	0.6	1.8	2.1	1.6 ± 0.8
Total MUFA	49.1	56.1	48.7	49.1	54.6	50.5	49.1	46.4	53.5	51.9	53.9	50.5	51.9	54.4	51.4 ± 2.8
18:2n-6	1.5	1.0	1.8	1.9	1.1	1.5	1.4	1.7	1.7	1.2	1.2	1.5	1.5	1.3	1.4 ± 0.3
16:3n-6	0.5	0.6	0.5	0.9	0.7	0.4	0.7	0.2	0.4	0.8	0.6	0.2	0.6	0.8	0.6 ± 0.2
18:4n-3	1.7	0.6	1.2	1.2	1.6	1.6	2.1	1.4	0.9	0.8	1.0	0.8	1.5	1.1	1.2 ± 0.4
20:4n-3	0.6	0.3	0.7	0.4	0.5	0.6	0.6	0.5	0.5	0.5	0.4	0.5	0.6	0.5	0.5 ± 0.1
20:5n-3	6.6	2.6	8.3	5.8	4.7	6.5	6.6	7.7	6.0	3.9	3.6	6.0	5.7	4.5	5.6 ± 1.6
22:5n-3	0.5	0.4	0.6	0.4	0.5	0.5	0.6	0.5	0.5	0.7	0.4	0.5	0.5	0.5	0.5 ± 0.1
22:6n-3	6.4	5.2	4.8	3.8	5.8	5.7	7.7	4.5	4.2	7.7	5.7	4.2	6.2	6.8	5.6 ± 1.3
Total PUFA	17.8	10.7	17.7	14.4	14.9	16.8	19.7	16.4	14.2	15.6	12.9	13.6	16.5	15.5	15.5 ± 2.3
Others	7.6	9.4	6.0	8.6	8.2	7.1	9.6	7.4	5.7	9.2	8.0	5.5	8.2	8.0	7.8 ± 1.3
Total n-3	16.7	9.6	16.4	12.2	14.0	15.7	18.8	15.4	12.8	14.4	11.7	12.5	15.3	14.3	14.3 ± 2.4
Total n-6	3.0	2.4	3.4	3.7	2.8	2.9	3.3	2.9	2.9	3.2	2.5	2.6	3.1	2.9	3.0 ± 0.3
Ratio n-3/n-6	5.6	4.0	4.9	3.3	5.0	5.4	5.7	5.3	4.4	4.6	4.6	4.7	5.0	4.9	4.8 ± 0.6
EPA/DHA	1.0	0.5	1.7	1.5	0.8	1.1	0.8	1.7	1.4	0.5	0.6	1.4	0.9	0.7	1.1 ± 0.4

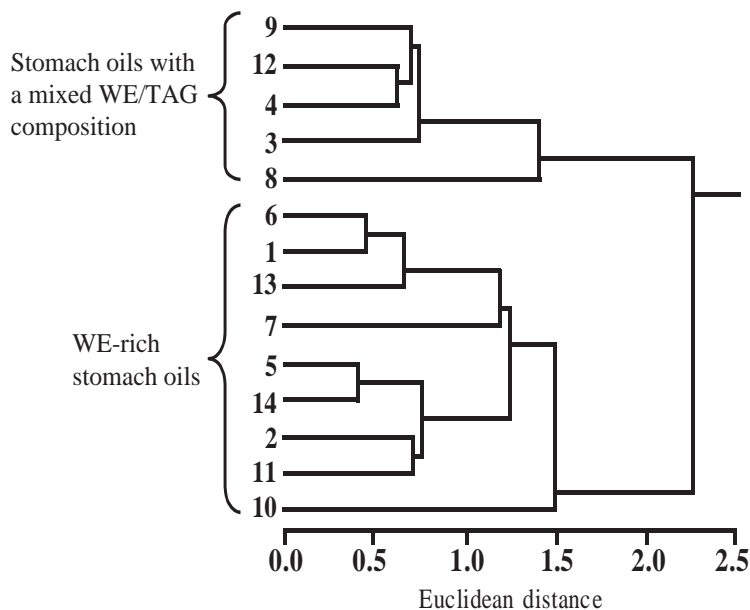


Fig. 2. *Puffinus tenuirostris*. Classification by clustering of the 14 stomach oils (1 to 14) based on the fatty acid profiles of triacylglycerols (TAG). WE: wax esters

which were regrouped in a single set (Gb/Ka). Four discriminant functions accounted for 99% of the total inertia. A plot of the scores of the first 2 discriminant functions (representing 88% of the variance) showed single species groups (*Calanoides acutus* [Caa], *Thysanoessa macrura* [Thm], *Electrona carlsbergi* [Ec]) and 2 groups of species with relatively similar fatty alcohol profiles, i.e. the fish species *Electrona antarctica* (Ea) together with the fish group Gb/Ka, and the copepod-euphausiid group: *Rhincalanus gigas* (Rhg), *Euphausia crystallorophias* (Euc), *Paraeuchaeta antarctica* (Paa) and *Euchirella rostromagna* (Eur) (Fig. 3A). The first function, which explained 73% of the inertia, is linked to 2 groups, the fish species Ec and the euphausiid Thm in opposition to other zooplankton species Euc, Eur, Paa, and Rhg. This function contrasted between species that showed very low levels of 14:0 and high percentages of 18:1n-9, and other zooplankton species having significantly higher levels of 14:0. The second function, which accounted for 14.5% of the inertia, was related to Ec, lacking 20:1 alcohol, in opposition to the copepod Caa. Using this model of discriminant functions, 100% of the cases were correctly assigned.

Table 4. *Puffinus tenuirostris*. Wax ester fatty acid compositions (% of wax ester fatty acids) of the 14 stomach oils. SD: standard deviation; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: 20:5n-3; DHA: 22:6n-3; 'Others' includes all fatty acids present at <0.5%

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean ± SD
14:0	1.6	0.8	1.7	1.6	0.8	1.1	0.8	0.8	1.8	0.7	0.6	1.7	0.9	0.9	1.1 ± 0.4
16:0	1.1	0.9	1.2	1.4	0.7	0.9	0.9	0.9	1.8	0.8	0.7	1.5	0.9	0.8	1.0 ± 0.3
Total SFA	2.6	1.7	2.9	3.0	1.5	2.0	1.7	1.7	3.6	1.5	1.4	3.2	1.7	1.6	2.1 ± 0.7
16:1n-7	14.2	13.7	15.2	13.8	14.9	15.4	14.9	14.0	14.8	14.1	12.6	13.6	14.5	16.3	14.4 ± 0.9
17:1	0.8	0.8	0.7	0.7	0.8	0.8	0.7	0.8	0.8	0.7	0.7	0.7	0.8	0.8	0.8 ± 0.1
18:1n-9	40.0	37.8	39.9	38.6	40.2	39.3	39.2	38.3	43.0	38.3	35.7	39.9	38.6	42.0	39.3 ± 1.8
18:1n-7	4.5	3.8	5.0	4.3	4.3	4.4	4.1	3.9	4.6	3.8	3.4	5.7	4.0	4.0	4.3 ± 0.6
18:1n-5	0.7	0.8	0.8	0.6	0.8	0.8	0.7	0.7	0.8	0.7	0.8	0.7	0.8	0.6	0.7 ± 0.1
20:1n-11	0.7	0.7	0.7	0.6	0.9	0.8	0.9	0.7	0.7	0.7	0.7	0.4	0.8	1.1	0.7 ± 0.2
20:1n-9	4.0	4.0	4.2	3.7	4.3	4.2	4.4	3.1	3.4	4.6	4.4	4.2	3.8	4.6	4.1 ± 0.4
22:1n-13+11	3.7	5.5	3.5	3.5	5.8	5.4	5.7	3.8	3.9	4.6	4.6	2.6	5.0	6.5	4.6 ± 1.1
22:1n-9	1.1	1.8	1.1	1.0	1.8	1.6	1.7	1.2	1.3	1.2	1.5	1.0	1.6	1.8	1.4 ± 0.3
24:1n-13	0.5	0.7	0.4	0.5	0.9	0.7	0.7	0.5	0.4	0.6	0.6	0.2	0.7	0.7	0.6 ± 0.2
24:1n-11	0.5	1.1	0.4	0.4	1.0	0.9	0.9	0.6	0.7	0.7	0.7	0.2	0.8	1.0	0.7 ± 0.3
Total MUFA	70.6	70.7	71.8	67.8	75.7	74.2	74.0	67.4	74.3	70.0	65.8	69.4	71.4	79.4	71.6 ± 3.6
18:2n-6	2.1	1.7	2.1	2.1	1.8	1.8	1.7	2.0	2.1	1.9	1.8	2.1	1.9	1.8	1.9 ± 0.2
16:3n-6	0.7	0.6	0.6	0.6	0.6	0.6	0.7	0.6	0.7	0.7	0.5	0.6	0.6	0.7	0.6 ± 0.1
18:4n-3	2.0	1.9	1.7	1.8	1.4	1.6	2.0	2.4	1.1	2.4	2.5	1.8	2.0	0.9	1.8 ± 0.5
20:4n-6	0.5	0.4	0.5	0.6	0.4	0.5	0.4	0.6	0.7	0.4	0.5	0.5	0.5	0.4	0.5 ± 0.1
20:4n-3	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.8	0.7	0.8	0.8	0.7	0.8	0.6	0.7 ± 0.1
20:5n-3	9.3	9.6	8.6	9.5	7.3	8.1	7.9	10.8	6.7	9.6	12.2	9.6	9.2	5.1	8.8 ± 1.8
21:5n-3	1.1	0.6	0.5	0.7	0.4	0.5	0.6	0.6	0.4	0.6	0.7	0.6	0.6	0.3	0.6 ± 0.2
22:5n-3	0.6	0.6	0.5	0.5	0.5	0.5	0.6	0.7	0.4	0.7	0.7	0.6	0.6	0.5	0.6 ± 0.1
22:6n-3	5.3	6.3	4.6	5.4	4.8	4.7	4.9	6.4	4.4	5.9	7.6	5.4	5.8	3.8	5.4 ± 1.0
Total PUFA	22.1	22.5	19.7	22.0	17.9	18.8	19.3	24.9	17.1	23.0	27.2	21.8	21.9	14.1	20.9 ± 3.3
Others	4.8	5.1	5.6	7.3	4.9	4.5	5.1	6.0	5.0	5.5	5.6	5.6	5.0	4.8	5.3 ± 0.7
Total n-3	19.4	20.4	16.9	19.3	15.6	16.4	17.0	22.2	14.1	20.5	25.9	19.1	19.4	11.7	18.4 ± 3.5
Total n-6	4.1	3.7	4.4	4.2	3.7	3.8	3.7	4.4	4.5	4.0	3.6	4.2	3.9	3.8	4.0 ± 0.3
Ratio n-3/n-6	4.8	5.6	3.8	4.6	4.2	4.3	4.6	5.1	3.2	5.1	7.2	4.5	5.0	3.1	4.6 ± 1.0
EPA/DHA	1.8	1.5	1.9	1.8	1.5	1.7	1.6	1.7	1.5	1.6	1.6	1.8	1.6	1.3	1.6 ± 0.1

Table 5. *Puffinus tenuirostris*. Wax ester fatty alcohol compositions (% of wax ester fatty alcohols) of the 14 stomach oils. SD: standard deviation; SFAl: saturated fatty alcohols; MUFAI: monounsaturated fatty alcohols; 'Others' includes all fatty alcohols present at <0.5%

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean ± SD
Iso 17:0	0.6	0.7	0.6	0.8	0.6	0.6	0.6	0.8	0.8	0.8	0.7	0.7	0.8	0.8	0.7 ± 0.1
Iso 18:0	0.6	0.5	0.5	0.6	0.5	0.5	0.5	0.6	0.5	0.6	0.5	0.6	0.6	0.5	0.5 ± 0.0
14:0	8.6	9.0	8.1	8.9	8.6	9.0	7.7	8.7	9.2	7.4	8.6	9.4	8.6	7.8	8.5 ± 0.6
15:0	0.7	0.8	0.6	0.7	0.7	0.7	0.7	0.7	0.8	0.6	0.7	0.6	0.7	0.7	0.7 ± 0.1
16:0	42.3	45.2	38.3	41.0	43.2	41.5	41.0	45.1	47.2	39.9	43.7	41.4	43.4	39.4	42.3 ± 2.5
18:0	3.0	3.4	3.3	2.9	3.4	3.4	3.3	3.3	3.5	3.0	3.3	3.2	3.3	3.0	3.2 ± 0.2
20:0	0.4	0.6	0.3	0.3	0.6	0.6	0.5	0.4	0.5	0.4	0.5	0.3	0.5	0.5	0.5 ± 0.1
Total SFAl	56.2	60.2	51.6	55.2	57.6	56.3	54.3	59.6	62.4	52.7	58.0	56.2	57.8	52.7	56.5 ± 3.1
16:1n-7	4.7	4.3	4.5	4.9	4.3	4.3	4.3	4.9	4.2	4.6	4.4	4.5	4.6	4.2	4.5 ± 0.2
18:1n-9	4.3	2.8	5.1	4.8	3.0	3.3	3.0	3.8	4.0	3.6	3.0	6.9	2.9	3.0	3.8 ± 1.1
18:1n-7	4.2	2.4	5.3	4.5	2.6	3.2	2.5	3.1	3.9	2.9	2.6	6.3	2.6	2.8	3.5 ± 1.2
18:1n-5	1.1	0.8	1.2	1.2	1.0	1.1	0.9	1.2	1.0	1.3	1.1	1.1	0.8	1.2	1.1 ± 0.2
20:1n-9	10.6	8.8	12.3	10.4	9.8	10.1	10.9	8.0	7.6	12.2	10.1	10.1	9.8	11.2	10.1 ± 1.4
20:1n-7	0.5	0.4	0.6	0.6	0.5	0.5	1.1	0.7	0.7	0.8	0.6	0.8	0.5	0.5	0.6 ± 0.2
22:1n-13+11	6.7	7.6	7.5	6.6	7.9	8.0	9.6	6.9	5.4	9.1	7.4	4.7	7.8	9.4	7.5 ± 1.4
22:1n-9	4.6	4.8	4.5	4.1	5.2	5.1	5.2	3.5	3.3	4.7	4.9	3.6	5.2	6.2	4.6 ± 0.8
22:1n-7	0.4	0.5	0.4	0.4	0.5	0.6	0.6	0.4	0.4	0.6	0.4	0.3	0.5	0.6	0.5 ± 0.1
24:1n-11	0.8	1.2	0.8	0.7	1.4	1.1	1.2	0.9	0.6	1.1	1.2	0.3	1.0	1.4	1.0 ± 0.3
24:1n-9	2.7	2.9	2.9	2.8	3.0	2.8	3.2	3.0	2.6	2.9	2.7	2.1	3.1	3.2	2.8 ± 0.3
Total MUFAI	40.5	36.4	45.0	40.9	39.1	40.0	42.4	36.5	33.6	43.7	38.3	40.5	38.7	43.6	39.9 ± 3.2
Others	3.4	3.4	3.3	3.9	3.4	3.7	3.3	3.8	3.9	3.7	3.8	3.3	3.5	3.7	3.6 ± 0.2



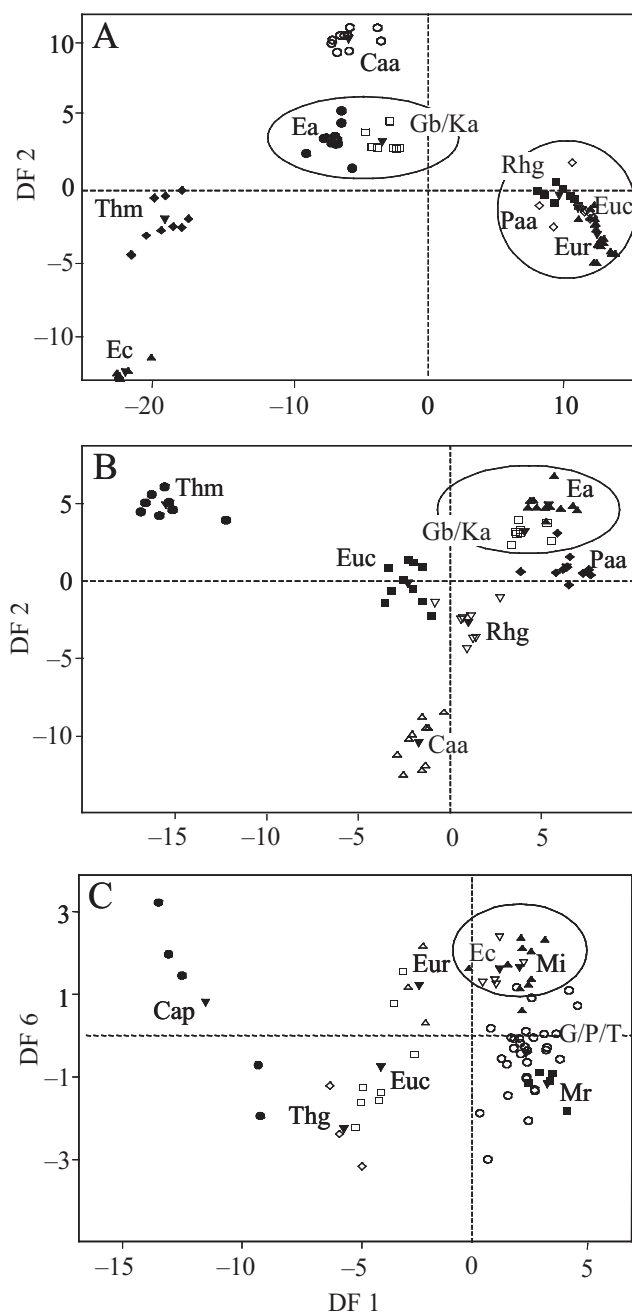


Fig. 3. Discriminant scores of (A) fatty alcohol, (B) wax ester fatty acid and (C) triacylglycerol fatty acid patterns of prey species. DF: discriminant function; see Table 1 for acronyms. G/P/T: *Gymnoscopelus* spp., *Pagothenia borchgrevinki*, *Protomyctophum tenisoni*, *Trematomus* spp.

The 14 patterns of stomach oil alcohols were then used as test samples and prediction of group allocation achieved with the same model. The results indicated highest probability of grouping with the fish group Gb/Ka. The second highest probability grouped oils with the myctophid species Ea (Table 6).

#### Fatty acid descriptors of wax esters and triacylglycerols

The same procedure was used with the fatty acid fraction of WE and TAG. However, the data on fatty acids from potential prey corresponded mainly to total lipids rather than specific class (WE or TAG), so the fatty acid patterns could be partially modified in relation with the proportions in the polar lipid content. Thus, these comparisons should be considered as a confirmation of the conclusion derived from the fatty alcohol patterns.

For the WE fatty acids, the score plot of the first 2 functions confirmed the grouping of Ea with Gb/Ka (Fig. 3B), a grouping still valid on the score plots with function 3 (not shown). The first 3 discriminant functions accounted for 91% of total variance. The first discriminant function represented 48% of the inertia and clearly separated the euphausiid Thm from the 8 other species (zooplankton species: Caa, Eur, Euc, Paa, Rhg; fish species: Ea, Gb and Ka). This function can be interpreted as a contrast between proportions of 14:0 and 16:0 and proportion of 22:6n-3. The second discriminant function accounted for 29% of the inertia. It illustrated the contrast between the proportions of 18:1n-9 and 22:6n-3, and was associated with the copepod species Caa rich in 22:6n-3 and poor in 18:1n-9, in opposition with the myctophid fish Ea rich in 18:1n-9. The third discriminant function represented 14% of the inertia and was linked with the fish species Ea. It corresponded to the presence of 24:1 associated with very low levels of 18:3n-3. With this second model of discriminant functions, 100% of the cases were correctly assigned. Based on this model, comparison of oil WE samples with the prey data showed that stomach oils presented the highest probability of association with the patterns of Ea, and Gb/Ka (Table 6) in agreement with the earlier findings.

Comparison of the TAG fatty acid patterns of stomach oils with the literature data was performed using 71 profiles from 11 fish species, 6 crustacean species and 2 cephalopod species (digestive gland data). When actual TAG profiles were not available, total fatty acid profiles were used but the very low levels of WE (< 2%) suggested that the total fatty acid patterns reflected mostly the TAG variability and to a minor extent that of the PL fraction. A SLDA was performed with the 16 major fatty acid descriptors. Classes have been defined for each prey species except for 1 set of fish species (*Gymnoscopelus* spp., *Pagothenia borchgrevinki*, *Protomyctophum tenisoni*, *Trematomus* spp.) grouped as 'G/P/T', because of their strong similarity in fatty acid patterns. Both ascending and descending deletion resulted in a set of 10 fatty acid descriptors and 6 significant discriminant functions ( $p < 0.01$ ), which accounted for 99% of the total inertia. The first 2 discriminant functions represented 46 and 28% of the

Table 6. Discriminant analyses of prey database comparing wax ester fatty alcohol, wax ester fatty acid or triacylglycerol fatty acid signatures of prey species. WEAL: wax ester fatty alcohols; WEAC: wax ester fatty acids; TAG: triacylglycerol fatty acids; for species acronyms see Table 1. G/P/T: *Gymnoscopelus* spp., *Pagothenia borchgrevinki*, *Protomyctophum tenisoni*, *Trematomus* spp.

Species	Allocated group			First (and second) highest probability group ( $p < 0.01$ )			Correct classification (%)		
	Database			Database			Database		
	WEAL	WEAC	TAG	WEAL	WEAC	TAG	WEAL	WEAC	TAG
<b>Crustacean</b>									
Caa	Caa	Caa	–	Caa	Caa	–	100	100	–
Cap	–	–	Cap	–	–	Cap	–	–	100
Eur	Eur	Euc	Eur	Eur	Euc	Eur	100	100	100
Euc	Euc	Euc	Euc	Euc	Euc	Euc	100	100	100
Eus	–	–	Euc	–	–	Euc	–	–	100
Euv	–	–	Euc	–	–	Euc	–	–	100
Paa	Paa	Paa	–	Paa	Paa	–	100	100	–
Rhg	Rhg	Rhg	–	Rhg	Rhg	–	100	100	–
Thg	–	–	Thg	–	–	Thg	–	–	100
Thm	Thm	Thm	–	Thm	Thm	–	100	100	–
<b>Fish</b>									
Ea	Ea	Ea	–	Ea	Ea	–	100	100	–
Ec	Ec	–	Ec	Ec	–	Ec	100	–	100
Gb	Gb/Ka	Gb/Ka	–	Gb/Ka	Gb/Ka	–	100	100	–
Gf	–	–	G/P/T	–	–	G/P/T	–	–	94
Gn	–	–	G/P/T	–	–	G/P/T	–	–	94
Go	–	–	G/P/T	–	–	G/P/T	–	–	94
Gp	–	–	G/P/T	–	–	G/P/T	–	–	94
Ka	Gb/Ka	Gb/Ka	–	Gb/Ka	Gb/Ka	–	100	100	–
Pb	–	–	G/P/T	–	–	G/P/T	–	–	94
Pt	–	–	G/P/T	–	–	G/P/T	–	–	94
Tb	–	–	G/P/T	–	–	G/P/T	–	–	94
Th	–	–	G/P/T	–	–	G/P/T	–	–	94
Tn	–	–	G/P/T	–	–	G/P/T	–	–	94
Tp	–	–	G/P/T	–	–	G/P/T	–	–	94
<b>Squid</b>									
Mi	–	–	Mi	–	–	Mi	–	–	100
Mr	–	–	Mr	–	–	Mr	–	–	100
Stomach oils	–	–	–	Gb/Ka (Ea)	Ea (Gb/Ka)	Mi	100	93	100

total variance, respectively. The first function is linked to the copepod *Calanus propinquus* (Cap) in relation to the changes in 16:1 and 24:1. The second function is related to an opposition between the euphausiid species Euc and the copepod Cap with variations in 24:1, 16:0 and 18:1. Of interest in the present study is the sixth function that accounted for 3% of the inertia. It contrasts the 2 cephalopod species *Moroteuthis ingens* and *Moroteuthis rosoni* (Mi and Mr, respectively) in relation with changes in 24:1 and 22:6n-3, and it singles out 1 group comprising Mi and Ec with very similar fatty acid patterns. This proximity of Mi and Ec fatty acid patterns suggested by the discriminant functions 1 and 6 (Fig. 3C) is confirmed by the first 2 discriminant functions. With this last model of 6 discriminant functions, 94% of the cases were correctly assigned and the prediction of stomach oil triacylglycerol allocation indicated highest probabilities of grouping with the pattern recorded in the cephalopod Mi (Table 6).

## DISCUSSION

Most previous studies on procellariiform stomach oils were carried out in the 1970s. Until now, oils from 25 bird species have been analysed in terms of lipid classes, and only 12, 7 and 4 in terms of TAG fatty acid profiles, WE fatty acid and fatty alcohol profiles, respectively (Lewis 1966, 1969, Cheah & Hansen 1970a,b, Clarke & Prince 1976, Warham et al. 1976). Moreover, these published data are far from detailed: they presented groups of fatty acids or fatty alcohols (as 16:1, 18:2, etc.) and not the proportion of each compound. To our knowledge, the present study is the first to consider a significant number of stomach oils from adult Procellariiformes for which the duration of foraging trip was known. This greatly reduces the risk of mixing oils from different foraging trips and from different birds (males and females), as it is the case for oils from chicks. Unlike previous studies, each oil

sample was also analysed separately to assess the intra-specific variability of lipid oil composition.

### Stomach oil biochemical composition

Two lipid classes dominated in the stomach oils of *Puffinus tenuirostris*: WE and TAG. Our values are comparable to those reported by Woodward et al. (1995) in adult *P. tenuirostris* stomach oil and Cheah & Hansen (1970a), Warham et al. (1976) and Bishop et al. (1983) in chick oil of the same bird species. Oils from the 25 species previously investigated also contained these main components, i.e. WE and TAG (76 and 92 % of bird species, respectively; review by Warham 1996). PL showed only very low concentrations (e.g. 0.6 to 2.5 % in our study) compared with the actual percentages recorded in most of marine organisms listed in our prey species databases. This difference is probably due to the structural nature of the PL, which cannot diffuse easily following mechanical rupture and are rapidly transferred with the aqueous phase of the prey. Traces of FFA (<4 %) and absence of free fatty alcohols indicated a good degree of oil conservation.

The detailed profiles of fatty acids, from both WE and TAG and fatty alcohols, obtained in this work are consistent with earlier studies in chicks of the same bird species, where a dominance of MUFA in fatty acid profiles (WE and TAG) and SFA in fatty alcohol profiles were reported. Similarly, the major compounds recorded in our study agreed with those described in the 3 other studies (Cheah & Hansen 1970a, Warham et al. 1976, Bishop et al. 1983). However, when considering the overall pattern of fatty acids or fatty alcohols, differences in proportions can be detected. For example, we found in our study fewer long carbon chain PUFA (20:5, 22:5 and 22:6) in TAG profiles than Cheah & Hansen (1970a) and Warham et al. (1976) (12, 20 and 18 %, respectively).

The inter-individual degree of heterogeneity of stomach oil composition varied with the lipid class considered. Because stomach oil originated from mechanical disruption of the prey (Clarke 1989), the structure of the neutral lipids accumulated is likely to be directly linked to the oil composition of the prey ingested without the possible modifications observed when assimilation processes are involved (see Dahl et al. 2000). We have seen earlier that 2 bird groups were detected considering the lipid class composition (1 had 9 oils rich in WE and the other was composed of the remaining 5 oils with a mean composition in WE and TAG). Furthermore, if the WE were homogeneous for all 14 stomach fatty acid and fatty alcohol profiles, then the TAG structure suggested 2 groups of individuals with different fatty acid compositions corresponding to the 2 groups obtained by lipid classes. The most conser-

vative hypothesis to explain this classification is that all birds would have eaten a single WE-rich prey species, and the 5 birds whose stomach oils have a mean composition WE/TAG would have preyed, in addition, on TAG-rich prey species. Indeed, as shown in the references from Table 1, most marine pelagic species displayed a single dominance of 1 neutral lipid class with either WE or TAG.

### Use of fatty alcohol and fatty acid patterns in trophic studies

Different approaches using biochemical characteristics of prey have been used to describe trophic interactions. Horgan & Barrett (1985) attempted to use lipid class composition to compare the diet of seabirds. However, 2 different prey species may present almost identical lipid class profiles due to similarities in their feeding pattern and/or life cycle. This limitation has been overcome with the use of fatty acid descriptors, as shown for myctophids by Lea et al. (2002). Specific biomarkers are in limited number and associated to the lower part of the food chain. For instance, 16:4n-1, 18:5n-3 or 20:1n-9 and 22:1n-13+11 fatty acids are characteristics of plankton assemblages dominated by diatoms, dinoflagellates (some Haptophyceae) and *Calanus* spp. type copepods, respectively (Ackman et al. 1964, Joseph 1975, Mayzaud et al. 1976, Pascal & Ackman 1976). Ratios among fatty acids have also been used to clarify the dominant trophic interactions between zooplankton and phytoplankton or zooplankton and higher trophic levels. The ratios EPA/DHA, PUFA/SFA, 18:1n-9/18:1n-7 or 18:1n-9/16:1n-7 have often been used to discriminate between herbivores, omnivores and carnivores, for example (Cripps & Atkinson 2000, Auel et al. 2002, Phleger et al. 2002). However, the concept of specific markers or ratios is of limited value to a study dealing with trophic interactions of higher marine predators or large-scale comparisons. Consequently, various statistical multivariate approaches have been proposed (Grahl-Nielsen & Mjaavatten 1991, Smith et al. 1997, Mayzaud et al. 1999) to account for the covariation of entire profiles of fatty alcohols and/or fatty acids from both potential prey and predator.

Ideally, to reduce variability, the data on prey and predator should be collected simultaneously to avoid possible changes associated with season and/or location (e.g. Hagen et al. 1993, Phleger et al. 1997, Kattner & Hagen 1998, Lea et al. 2002). Multivariate analyses of fatty acid descriptors should also be based on the same lipid classes to reduce the influence of components not directly involved in the feeding processes. In the specific case of Antarctic seabirds, the geographi-

cal area covered during foraging trips precludes the possibility of simultaneous sampling of plankton, fish and other potential prey (mainly for logistic reasons). As a result, literature data have to be considered and sources of variability minimized as far as possible. Lipids, as trophic markers, can be used to assess diet composition in a qualitative manner. For seabirds and their prey, this method has been used in very few studies (Horgan & Barrett 1985, Raclot et al. 1998, Dahl et al. 2003). In our work, care was taken to consider separately the WE and TAG compositions of *Puffinus tenuirostris* stomach oils to avoid confusion in the trophic signals from different lipid sources. Unfortunately, data available in the literature are, to a large extent, limited to total fatty alcohol and fatty acid patterns. If this has no consequence with the fatty alcohols, which can only be attributed to the WE fraction, it increases the noise to the signal ratio of the fatty acid information. To overcome this difficulty, we chose to consider only those prey species with more than 60% of either WE or TAG for integration in the prey databases. Hence, the fatty acid patterns can be related to 1 group of neutral lipids, with limited influence of the PL fraction. As shown by Mayzaud et al. (2000, 2003) from field data and by Jobling & Bendiksen (2003) from experiments on fish, a large percentage of the variability in total fatty acid composition can usually be assigned to that of neutral lipid classes

#### **On which prey species did short-tailed shearwaters feed during long trips?**

Our results on lipid class composition of stomach oils suggested that all birds of our study had preyed on WE-rich prey species and 5 of these birds had probably preyed, in addition, on TAG-rich prey species (see above). Comparisons of WE patterns of fatty alcohols and fatty acids with LDA yielded some interesting features. The patterns of fatty alcohols and fatty acids of stomach WE from *Puffinus tenuirostris* differed highly from corresponding patterns in various zooplankton species (*Calanoides acutus*, *Euchirella rostromagna*, *Euphausia crystallorophias*, *Paraeuchaeta antarctica*, *Rhincalanus gigas* and *Thysanoessa macrura*). Hence, none of these species extensively contributed to the formation of stomach oils. On the contrary, the fatty alcohol of 3 myctophid fish species (*Gymnoscopelus braueri*, *Krefflichthys anderssoni* and *Electrona antarctica*) showed a high probability of resemblance with signatures recorded in stomach oils. This conclusion is moreover supported by the fatty acid signature of oil WE.

Comparisons of TAG fatty acid patterns have shown a strong similarity between the squid *Moroteuthis*

*ingens* and the myctophid *Electrona carlsbergi*, suggesting a potential trophic link. The profile of *M. ingens* is that of its digestive gland, which is rich in lipids of dietary origin (Phillips et al. 2002). In that context, it is important to emphasize that *M. ingens* mainly prey upon myctophids (Cherel & Duhamel 2003), and that its digestive gland lipid has a myctophid signature (Phillips et al. 2001, 2003). In addition, the biological cycle of *M. ingens* makes it largely unavailable to shearwaters (it was rarely eaten; Weimerskirch & Cherel 1998), the species being mainly targeted as juveniles by penguins and as adults by large Procellariiformes and marine mammals (Cherel & Weimerskirch 1999). Hence, the similarity between bird stomach oils and digestive gland of *M. ingens* can be seen either as an indication that shearwaters fed on squids or on prey of squids, i.e. myctophid fish (including *E. carlsbergi*). Comparisons of TAG fatty acid patterns also indicated that the ecologically important Antarctic *Euphausia superba* was not the major prey species participating to the formation of shearwater stomach oils contrary to the hypothesis developed by Kerry et al. (1983).

The main limitation in the use of literature data is that it is difficult to assess the validity of published results. Within a given prey species, homogeneity can be verified using multivariate analyses but little can be achieved when conflicting results are available. An example is provided with the data for the euphausiid *Nyctiphanes australis*, which has been reported as a major prey item of *Puffinus tenuirostris* (Bishop et al. 1983). Indeed, high levels of WE were reported by Bishop et al. (1983), while Cheah & Hansen (1970a) and Virtue et al. (1995) reported a dominance of TAG without any WE. At this stage, it is impossible to ascertain whether samples were contaminated as suggested by Virtue et al. (1995) or if other reasons may explain the discrepancy. What seems unlikely however is that the same species sampled in a similar area (Tasmanian waters) would show such differences in lipid accumulation processes over years. Our dietary analysis showed that *N. australis* was a major prey during ST (during which there was no storage of stomach oil) but it was much less important in LT samples (Weimerskirch & Cherel 1998). This, together with the fact that Tasmanian krill were barely digested in stomach contents, strongly suggests that the species was not involved in the storage of oil during LT in adult birds.

One of the major findings of our study is that biochemical analysis of lipids from stomach oil indicate that adult short-tailed shearwaters mainly prey upon sub-Antarctic and Antarctic myctophid fish during LT performed far away their breeding colony. Indeed, the patterns of fatty alcohols and fatty acids of WE both suggest that the main prey species were *Krefflichthys*

*anderssoni*, *Gymnoscopelus braueri* and *Electrona antarctica*, and the fatty acid patterns of TAG add another myctophid species: *Electrona carlsbergi*. These results are in agreement with direct and indirect evidences of the feeding ecology of shearwaters during LT. First, the main component by mass of LT-food was fish, including very digested remains of various myctophids with *K. anderssoni* being the commonest (Weimerskirch & Cherel 1998). Second, the nitrogen stable isotopic signature of shearwater plasma after a LT suggests the staple food of birds was not crustaceans but organisms feeding on crustaceans (like myctophids), and its signature is close to that of a specialist myctophid-eater, the king penguin (Y. Cherel, K. A. Hobson & H. Weimerskirch unpubl. data).

Four species of myctophids contribute to the bulk of the myctophid biomass in the Southern Ocean (Sabourenkov 1991, Kozlov 1995), they are the WE-rich species *Krefflichthys anderssoni* and *Electrona antarctica* and the TAG-rich species *Electrona carlsbergi* and *Gymnoscopelus nicholsi* (Phleger et al. 1997, 1999, Lea et al. 2002). Feeding on these species indicates that short-tailed shearwaters feed for themselves in the Antarctic zone (south of the Polar Front) and the Polar Frontal Zone (between the Sub-Antarctic and the Antarctic Polar Fronts) where the species are known to occur (Hulley 1981). This is in agreement with the few satellite-tracked birds that foraged far away in the south of Australia and reached Antarctic waters during LT. Birds from Montague Island (New South Wales, Australia), took only 3 to 4 d to get south of 60° S, then spent differing amounts of time at specific locations in the Southern Ocean before flying directly back to their colony (Klomp & Schultz 2000).

## CONCLUSION

Our investigations underline, for the first time, the importance of sub-Antarctic and Antarctic myctophid fish in the nutrition of adult short-tailed shearwaters during LT. They further emphasize the trophic role of myctophids in the pelagic ecosystem of the Southern Ocean, where they account for the main available biomass of macrozooplankton-eating animals for top predators (Sabourenkov 1991, Kozlov 1995). The study also stresses the need to have a reference database of fatty acid and fatty alcohol patterns of potential prey. It demonstrates the usefulness of the lipids of stomach oils of Procellariiformes as trophic markers to shed new light on predator-prey relationships and on resource acquisition and allocation processes, which are key issues in both ecosystemic and evolutionary ecology.

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