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Food and feeding ecology of the Tasmanian short-tailed shearwater (*Puffinus tenuirostris*, Temminck): insights from three complementary methods

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ABSTRACT: The diet of seabirds is usually studied by the identification of prey items recovered from their stomachs. This method is however limited to recently ingested prey and to non-digestible hard parts, precluding the determination of marine resources consumed by birds during long foraging trips. Thus, alternative indirect approaches are necessary to assess the potential importance of digested prey from long-term foraging activity. In this study, we present three complementary techniques to determine the prey of breeding short-tailed shearwaters (*Puffinus tenuirostris*) when they feed for themselves during long foraging trips: (1) conventional food analysis, (2) stable isotope analysis of carbon and nitrogen signatures ($\delta^{13}C$ and $\delta^{15}N$) of plasma, and (3) lipid analysis of stomach oil and the use of fatty acids and fatty alcohols as trophic markers (stomach oil is of dietary origin).

Dietary analysis showed that fish dominated by mass over crustaceans (82 and 18%, respectively). Two euphausiids *Euphausia vallentini* (a sub-Antarctic species) and *Nyctiphanes australis* (a Tasmanian species), and fish postlarvae represented more than 94% of the total number of food items, with myctophid fish of larger size dominating by mass. Plasma isotopic signature of birds suggested that shearwaters foraged mainly in Antarctic waters ($\delta^{13}C$ = -23.8‰), and fed at a trophic level close to that of a myctophid-eater, the king penguin ($\delta^{15}N_{\text{short-tailed shearwater}}$ = 8.7‰, $\delta^{15}N_{\text{king penguin}}$ = 9.8‰). Comparisons between fatty -acid and -alcohol patterns of stomach oil wax esters with those of potential prey also suggested a food based on myctophids (*Electrona antarctica*, *Krefftichthys anderssoni* and *Gymnoscopelus braueri*).

To conclude, both lipid and stable isotope methods emphasized the importance of myctophids in the nutrition of short-tailed shearwaters during the chick-rearing period

when adult birds feed for themselves. This study illustrates the interest of using both direct and indirect methods to determine trophic relationships between marine organisms.

KEY WORDS: Top predator, Myctophids, Southern Ocean, Stable isotopes, Stomach content, Lipid analyses.

Introduction

The structure and dynamics of predator-prey relationships are a key component in the knowledge of ecosystem functioning. Defining the marine resources consumed by marine predators is the first step in the study of their trophic ecology. Trophic relationships of seabirds have often been studied using conventional analysis (i.e. direct analysis of stomach contents). This direct method allows both the identification of prey items at the species level (freshly ingested prey and non-digestible hard parts: crustacean exoskeleton, fish otoliths and jaws, cephalopod beaks) and an estimation of their size. However, several drawbacks limit the method, such as difficult identifications of digested items, differential digestion according to prey types, and a small temporal integration (last meals of the bird). Hence, alternatively, indirect approaches have been developed to assess the potential importance of already digested prey from long-term foraging activity (stable isotope analysis: e.g. Hobson et al., 1994; Cherel & Hobson, 2007; lipids, fatty acids and fatty alcohols as trophic markers: e.g Horgan & Barrett, 1985; Connan et al., 2007). These approaches are based on the concept that 'you are what you eat'. The utilisation of stable isotope ratios ($^{13}C/^{12}C$ or $\delta^{13}C$, and $^{15}N/^{14}N$ or $\delta^{15}N$) as trophic markers is based on the assumption that the isotopic signature of a consumer reflects that of its food in a predictable manner (DeNiro & Epstein, 1978; Peterson & Fry, 1987). In marine ecosystems, stable isotope ratios of carbon and nitrogen are indicators of foraging areas and trophic levels, respectively. Regarding the lipid method, fatty acid and fatty alcohol signatures have been used to investigate dietary interactions at different trophic levels of the oceanic food web, ranging from primary producers to top predators, such as seabirds and marine mammals (review in Daalsgard et al., 2003).

In the Southern Ocean, the importance of seabirds, especially the Procellariiformes (albatrosses, petrels, shearwaters), as top predators has been illustrated in several studies (e.g. Croxall et al., 1984; Warham, 1990; Cherel & Weimerskirch, 1995). During the chick-rearing period, the adults of several species of Procellariiformes use a two-fold foraging strategy: they alternate short foraging trips to feed their chicks with a high frequency, with long foraging trips during which they feed for themselves and build up energy reserves (Weimerskirch et al., 1994, 2003). Interestingly, almost all stomach contents collected after long foraging trips contain significant amounts of oils (Weimerskirch & Cherel, 1998; Cherel et al., 2002). 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 by adults (Warham, 1977). An energetic study showed that adult self-feeding during long trips is crucial for the success of short trips because most of the energy used during short trips is likely to derive from the energy stored during long trips (Weimerskirch et al., 2003). Hence, investigating on the prey species ingested by adults during long foraging trips is essential to really determine the relationships between Procellariiformes and the marine environment.

The food and feeding ecology of the short-tailed shearwater (*Puffinus tennuirostris* Temminck) and its dual foraging strategy during chick rearing have already been

described (Weimerskirch & Cherel, 1998; Klomp & Schultz, 2000). Birds alternate an average of two short trips (1 to 4 days) with one long trip (8 to 19 days). While suggesting that adults fed in cold waters during long foraging trips, those studies gave no clear indication on the foraging areas or the prey ingested when adult birds were self-feeding. Our study aimed to ascertain the food and feeding ecology of breeding short-tailed shearwaters by determining the marine species eaten during their long foraging trips when birds feed for themselves far away their breeding grounds. Three complementary techniques were used: the direct identification of prey remains collected from the stomach contents, and two indirect methods: stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid trophic markers.

This paper is a synthesis of three studies investigating the trophic ecology of short-tailed shearwater during the chick rearing period with each of them using a different method (Weimerskirch & Cherel, 1998; Cherel et al., 2005b; Connan et al., 2005). We here demonstrate the usefulness of using those three complementary methods together to determine the diet of top predators.

Material and methods

Sampling

Fieldwork was carried out in March 1997 at The Neck Game Reserve, Bruny Island (43°18'S, 143°18'E, south-east of Tasmania; fig. 1). All sampled short-tailed shearwaters were breeding adults coming back to the colony after a long foraging trip at sea (8 to 19 days; Weimerskirch & Cherel, 1998). Duration of foraging trips was defined as the time elapsed between two recoveries of the same bird. Visits by adults were detected when sticks, placed at the mouth of burrows, were displaced. Burrows were checked daily at 17.00 and then every 30 min from dusk until midnight. Food samples were obtained either through spontaneous regurgitation of birds or using the water off-loading technique (Wilson, 1984). Blood samples (1-2 mL) were taken from a wing vein, and kept on ice until centrifugation. Stomach contents and plasmas were then stored at -20°C until laboratory analysis.

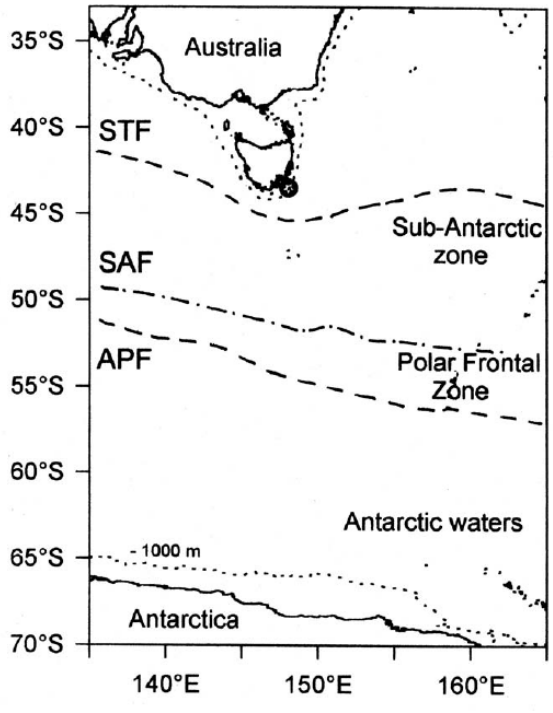

Figure 1: Map showing the location of the breeding colony of short tailed-shearwaters and oceanic fronts south of Tasmania (✪: Bruny Island; STF: Sub-Tropical Front; SAF: Sub-Antarctic Front; APF: Antarctic Polar Front).

Stomach content analysis

Each food sample was thawed and drained by gravity in a graduate cylinder to separate the solid fraction (more or less digested prey items) from the liquid fraction (stomach oil and water). Only fresh items were numbered and identified to the lowest possible taxon using published keys (Baker et al., 1990; Smale et al., 1995; Vinogradov et al., 1996; Xavier & Cherel, 2009) and our own reference collection.

Stable isotope analysis of plasma

Plasma samples were freeze-dried, and lipids were then removed using a Soxhlet apparatus (chloroform for 4 to 6 h). Lipids need to be removed as they are depleted in ^{13}C relative to whole tissues (Tieszen et al., 1983). Stable isotope analyses of carbon and nitrogen were performed by combusting 1 mg of homogenized plasma at 1800°C (Robo-Prep elemental analyzer). Resultant gases (CO_2 and N_2) were then analyzed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS). Stable isotope abundances were expressed in δ notation (in parts per thousand, ‰) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard})-1]*1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. $R_{standard}$ values were based on the PeeDee Belemnite for ^{13}C and atmospheric N_2 for ^{15}N. Stable isotope analyses of carbon and nitrogen were performed in parallel for the most numerous prey species recovered from the stomach contents of birds.

Consumer tissues are 2 to 5‰ enriched in ^{15}N relative to their food and consequently $\delta^{15}N$ measurements serve as indicators of a consumer trophic position. In contrast, $\delta^{13}C$ values vary little along the food web and are mainly used to determine primary sources in a trophic network. In the marine environment, $\delta^{13}C$ values indicate consumer foraging areas, i.e. inshore versus offshore or pelagic versus benthic contribution to food intake. In the Southern Ocean, lower-latitude plankton food bases are enriched in ^{13}C relative to higher-latitude waters. Hence, geographical $\delta^{13}C$ gradients have been used as an effective way for investigating the foraging areas of seabirds (Cherel et al., 2000; Cherel & Hobson, 2007). The turnover of carbon in plasma is high with a half-live of about 3-4 days (Hobson & Clark, 1993); the two ratios $\delta^{13}C$ and $\delta^{15}N$ thus give information about the adult diet over the last 1-2 weeks before blood sampling.

Lipid analysis of stomach oils

Total lipids were extracted from stomach oils according to the method of Bligh & Dyer (1959). Individual lipid classes were quantified using an Iatroscan MKV TH10 (see details in Connan et al., 2005). Wax esters clearly dominated the lipid class composition (72 ± 14% of total lipids; Connan et al., 2005). Fatty acid and fatty alcohol compositions of this lipid class were then analysed. Briefly, lipid classes were first isolated by thin-layer chromatography. Fatty acids and fatty alcohols of wax esters were then methylated (Morrison & Smith, 1964) and acetylated (Ackman et al., 1972), respectively, before being analysed by gas-liquid chromatography (polar column Famewax – Restek 30 m x0.32 mm internal diameter). The column was operated isothermally at 190°C during 120 min for the fatty acid methyl esters and 200°C during 100 min for the fatty alcohol acetates. Individual components were identified by reference to authentic standards and well-characterized fish oils (Capelin/Menhadden 50:50). Fatty acids and fatty alcohols are reported in the form x:an-b where x is the number of carbon atoms in the acyl chain, a is

the total number of double bonds, and b is the position of the first double bond from the methyl end of the molecule.

In the past few decades this method has often been used to delineate trophic relationships low in the food web, and is nowadays more and more used to investigate trophic relationship of top predators (review in Dalsgaard et al., 2003). To determine the prey from which the stomach oils originate, comparisons between fatty alcohol and fatty acid patterns from potential prey species and stomach oils were performed. Two datasets (fatty alcohols and fatty acids) were built by gathering information from published studies on potential prey (i.e. zooplankton, micronekton and nekton) from Tasmanian, sub-Antarctic and Antarctic waters. Fatty alcohol dataset grouped 86 profiles of 10 crustacean and fish species, while fatty acid dataset provided 64 profiles of 9 crustaceans and fish species (detailed references in Connan et al., 2005). Homogeneity of fatty alcohol and fatty acid patterns within a given species or group of species was checked using preliminary principal component analyses. Discriminant analyses were then used to classify prey species based on either fatty alcohol or fatty acid patterns (8 fatty alcohol descriptors and 17 fatty acid descriptors). Stomach oils were finally attributed to a pre-existing prey group; stomach oils were integrated as supplementary observations and were not used in the definition of the discriminant functions. Normality being presupposed for most of these analyses, percentages were normalized using the arcsine transformation. All the statistical analysed were conducted using Statgraphics Plus 5.0.

Results

Stomach content analysis

Conventional analysis of stomach contents of short-tailed shearwaters showed that fish dominated by mass over crustaceans (82 and 18%, respectively). Nineteen different taxa were identified, including not only crustaceans and fish, but also squids (table 1). By number, the diet was dominated by the sub-Antarctic krill *Euphausia vallentini*, followed by fish postlarvae and the Australian krill *Nyctiphanes australis*; those three taxa represented more than 94% of the total number of prey. With the exception of *N. australis*, prey items were generally almost completely digested. The fish portion was very digested and it was mainly composed of lanternfish (Myctophidae), with *Krefftichthys anderssoni* as the main species. However, due to their larger size compared to crustaceans and fish postlarvae, the relatively low number of myctophids was dominant by mass.

Stable isotope analyses of plasma

The stable isotope signature of plasma was 8.7 ± 0.5‰ for $\delta^{15}N$ and -23.8 ± 0.5‰ for $\delta^{13}C$. $\delta^{15}N$ signatures of prey ranged from -0.7 ± 1.0‰ for *Euphausia vallentini* to 5.6 ± 0.8‰ for fish postlarvae. Regarding $\delta^{13}C$, the euphausiid *Euphausia vallentini* and the amphipod *Themisto gaudichaudii* showed the lowest values (-23.4 ± 0.3‰ and -23.2 ± 0.6‰, respectively) and the fish postlarvae the highest (-20.5 ± 0.7‰).

Table 1: Composition of the food delivered to the chicks after long foraging trips of adult short-tailed shearwaters (n = 14) (adapted from Weimerskirch & Cherel, 1998).

	Occurrence in stomachs		Numbers	
	n	%	n	%
Squids	**3**	**21.4**	**3**	**<0.1**
Onychoteuthidae				
Kondakovia longimana	1	7.1	1	<0.1
Moroteuthis ingens	1	7.1	1	<0.1
Unidentified squids	1	7.1	1	<0.1
Crustaceans	**14**	**100.0**	**4752**	**67.7**
Amphipoda				
Themisto australis	1	7.1	1	<0.1
Themisto gaudichaudii	12	85.7	298	4.2
Euphausiacea				
Euphausia sp.	2	14.3	2	<0.1
Euphausia vallentini	5	35.7	3242	46.2
Nematoscelis megalops	1	7.1	3	<0.1
Nyctiphanes australis	3	21.4	1181	16.8
Thysanoessa gregaria	6	42.9	21	0.3
Reptantia				
Brachyura. Megalopa larvae	2	14.3	3	<0.1
Thoracica				
Cyprid stage	1	7.1	1	<0.1
Fish	**14**	**100.0**	**2261**	**32.2**
Myctophidae				
Krefftichthys anderssoni	3	21.4	24	0.3
?*Lampadena notialis*	1	7.1	1	<0.1
Lampanyctus intricarius	1	7.1	1	<0.1
Unidentified myctophid	1	7.1	1	<0.1
Photichthyidae				
Photichthys argenteus	1	7.1	1	<0.1
Unidentified fish postlarvae	12	85.7	2202	31.4
Unidentified fish	6	42.9	31	0.4

Comparison between $\delta^{13}C$ values of short-tailed shearwaters with those of their prey showed that birds foraged mainly in Antarctic waters (low value of $\delta^{13}C$; fig. 2). $\delta^{15}N$ showed a difference of 7-9‰ between the birds and the two crustacean species from cold waters (*T. gaudichaudii* and *E. vallentini*), hence a difference of two trophic levels. Finally, shearwater isotopic signature was close to that of the king penguin sampled in Crozet Islands ($\delta^{15}N = 9.8\pm0.2$‰, $\delta^{13}C = -22.5\pm0.3$‰; Cherel et al., 2005a).

Lipid analysis of stomach oils

Stomach oil was recovered in 94% of stomach contents collected from adults returning from a long foraging trip. Regarding the fatty alcohol fraction of wax esters, 12 fatty alcohols were present at levels > 1% of total fatty alcohols with a dominance of saturated and monounsaturated ones (58% and 41% of total fatty alcohols, respectively; table 2). Palmitol clearly dominated the profiles of fatty alcohols (16:0 – 42%), followed by 20:1n-9 (10%), 14:0 (9%) and the group 22:1n-13+11 (8%). Considering the fatty acid fraction of wax esters, more than half of components were monounsaturated (72% of total fatty acids) (table II). Major fatty acids were oleic (18:1n-9 – 39%), eicosapentaenoic (20:5n-3 – 9%) and docosahexaenoic (22:6n-3 – 5%) acids.

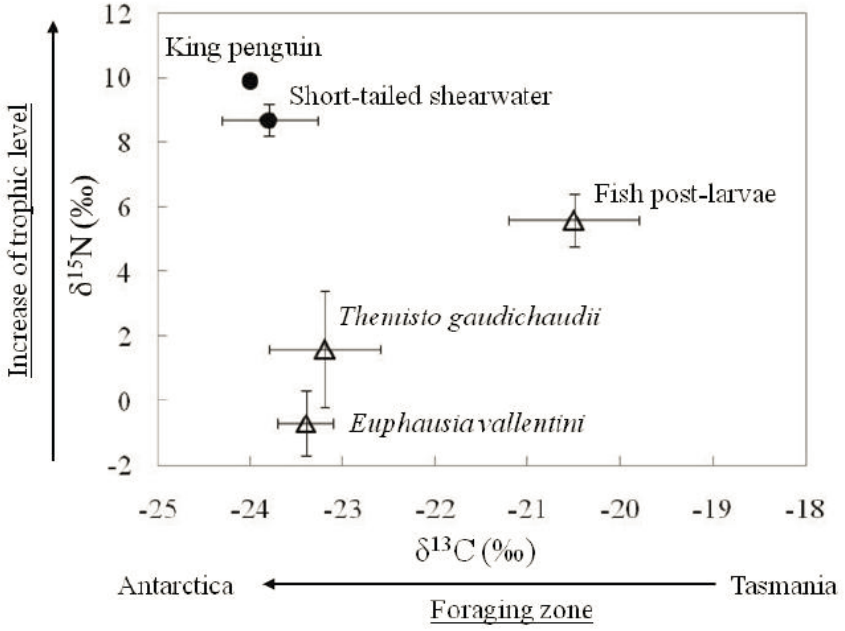

Figure 2: Isotopic signatures of short-tailed shearwaters, king penguins and prey recovered from shearwater stomach contents (adapted from Cherel *et al.*, 2005*b*).

Regarding the predator-prey analysis, classes have been defined for each prey species except for the two myctophids *Gymnoscopelus braueri* and *Krefftichthys anderssoni* that grouped in a single class (Gb/Ka) due to the similarity of their fatty alcohol and fatty acid profiles. Discrimant analysis performed on fatty alcohol signatures of potential prey species defined four discriminant functions representing 99% of the total inertia. The first two discriminant functions separated single species groups (*Calanoides acutus* [Caa], *Thysanoessa macrura* [Thm], *Electrona carlsbergi* [Ec]) from two 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). When stomach oils were integrated as supplementary individuals within the same model, they showed the highest probability of grouping with the fish *G. braueri* / *K. anderssoni*, and then the species *E. antarctica.* Hence, stomach oils had fatty alcohol compositions close to those of myctophid fishes.

The second discriminant analysis, conducted on the fatty acid patterns, confirmed the grouping of stomach oils with the myctophid species (fig. 3B). The first three discriminant functions accounted for 91% of the total inertia. The two euphausiids *E. crystallorophias* and *T. macrura*, and the copepod *C. acutus* were clearly separated from the other potential prey (*R. gigas*, *P. antarctica*, *E. antarctica*, *G. braueri* / *K. anderssoni*) with the two first discriminant functions. Based on this model, comparisons of oils with the prey data showed that stomach oils presented the highest probability of association with the patterns of *E. antarctica*, and *G. braueri* / *K. anderssoni*. These results are illustrated on fig. 4, with the fatty acid signatures of stomach oils compared to those of the copepod C. acutus (fig. 4A) and of the myctophid *E. antarctica* (fig. 4B).

Table 2: Wax ester fatty alcohol and fatty acid composition of the 14 stomach oils (% of total fatty alcohols [or fatty acids]; mean ± standard deviation; *SFAl*: saturated fatty alcohols; *MUFAl*: monounsaturated fatty alcohols; *SFA*: saturated fatty acids; *PUFAl*: Polyunsaturated fatty alcohols; *MUFA*: monounsaturated fatty acids; *PUFA*: polyunsaturated fatty acids; ∑ *Others*: < 1%) (adapted from Connan *et al.,* 2005).

Fatty alcohols		Fatty acids	
14:0	8.5 ± 0.6	14:0	1.1 ± 0.4
16:0	42.3 ± 2.5	16:0	1.0 ± 0.3
18:0	3.2 ± 0.2	16:1n-7	14.4 ± 0.9
16:1n-7	4.5 ± 0.2	18:1n-9	39.3 ± 1.8
18:1n-9	3.8 ± 1.1	18:1n-7	4.3 ± 0.6
18:1n-7	3.5 ± 1.2	20:1n-9	4.1 ± 0.4
18:1n-5	1.1 ± 0.2	22:1n-13+11	4.6 ± 1.1
20:1n-9	10.1 ± 1.4	22:1n-9	1.4 ± 0.3
22:1n-13+11	7.5 ± 1.4	18:2n-6	1.9 ± 0.2
22:1n-9	4.6 ± 0.8	18:4n-3	1.8 ± 0.5
24:1n-11	1.0 ± 0.3	20:5n-3	8.8 ± 1.8
24:1n-9	2.8 ± 0.3	22:6n-3	5.4 ± 1.0
∑ *Others*	*7.0 ± 0.4*	∑ *Others*	*10.7 ± 0.7*
Total SFAl	*57.9 ± 3.2*	*Total SFA*	*3.4 ± 1.0*
Total MUFAl	*40.8 ± 3.1*	*Total MUFA*	*72.1 ± 3.5*
Total PUFAl	*1.3 ± 0.2*	*Total PUFA*	*24.5 ± 3.5*

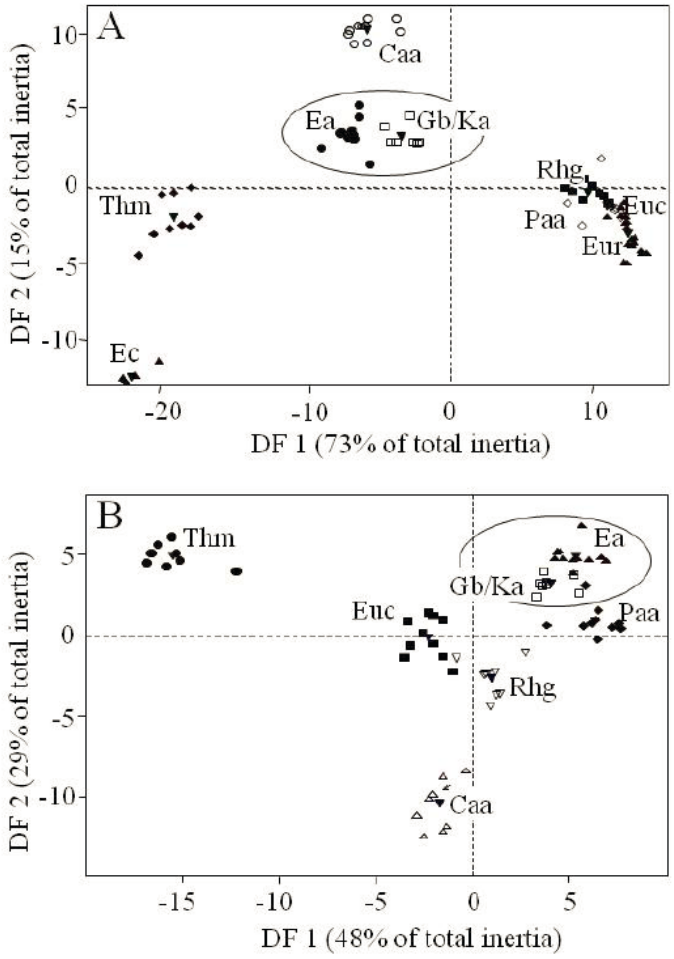

Figure 3: Discriminant analyses of fatty alcohol (A) and fatty acid (B) patterns of prey species wax esters (DF: discriminant function; Crustaceans: *Calanoides acutus* [Caa], *Euchirella rostromagna* [Eur], *Euphausia crystallorophias* [Euc], *Paraeuchaeta antarctica* [Paa], *Rhincalanus gigas* [Rhg], *Thysanoessa macrura* [Thm]; Fish: *Electrona antarctica* [Ea], *Electrona carlsbergi* [Ec], *Gymnoscopelus braueri/Krefftichthys anderssoni* [Gb/Ka]; ellipses highlight the most probable prey species at the origin of the stomach oils of short-tailed shearwaters; from Connan *et al.*, 2005).

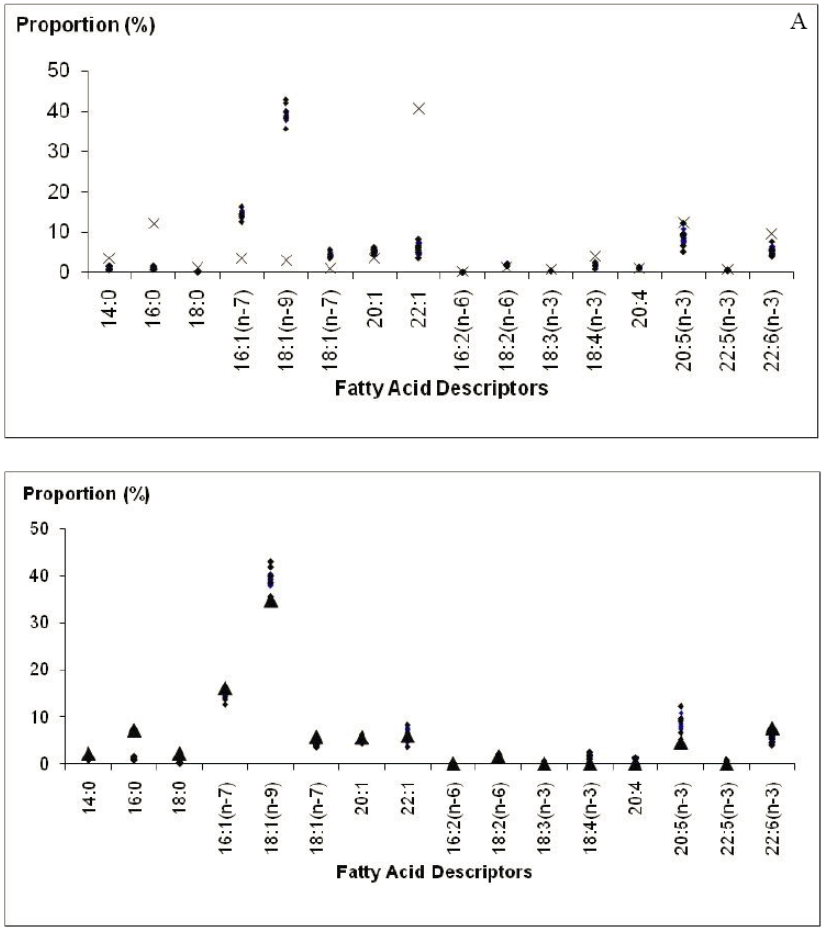

Figure 4: Comparison of the wax esters' fatty acid pattern of lipids' stomach oil (◆) with those of the copepod *Calanoides acutus* (A; ×; data from Kattner *et al*., 1994) and of the myctophid *Electrona antarctica* (B; ▲; data from Phleger *et al*., 1997).

Discussion

With more than 23 million breeding birds along the Australian coasts (Skira et al., 1985), short-tailed shearwaters are likely to have a significant impact on the pelagic ecosystem. Previous studies on the diet of breeding short-tailed shearwaters, based on data on average feeding frequency and/or identification of prey remains from stomach contents, concluded that adults mainly relied on neritic prey caught in the vicinity of the colonies, especially the Australian krill *Nyctiphanes australis* (Bishop et al., 1983; Montague et al., 1986; Skira, 1986). The numerous short-tailed shearwaters observed in Antarctic waters south of Tasmania (Woehler et al., 1990) were previously thought to be non-breeding or immature birds, or failed breeders (Kerry et al., 1983). However, Klomp & Schultz (2000) published the first satellite tracking of breeding short-tailed shearwaters showing that they were able to fly up to 15,000 km to the ice-shelf off Antarctica in a single long foraging trip. This tracking investigation does not give any information on the prey targeted by the adults in Antarctic waters.

Weimerskirch & Cherel (1998) were the first to study the food and feeding ecology of short-tailed shearwaters during short and long trips. Conventional dietary analysis (i.e.

identification and quantification of prey remains from stomach contents) found a mixture of thoroughly and partially digested prey items, suggesting that prey have been taken at different times during long trips and from different water masses (the neritic and less digested prey items having been caught near the colony).

At the species level, high numbers of Tasmanian (*Nyctiphanes australis*, fish post-larvae) or sub-Antarctic (*Euphausia vallentini*) euphausiids clearly indicated that adults foraged in both neritic waters and in the Polar Frontal Zone (between the sub-Antarctic Front and the Antarctic Polar Front) on their way back to the colony (Lomakina, 1966; Blackburn, 1980). The known biogeography of rarer species recovered from stomach contents, such as the squid *Kondakovia longimana* or the myctophid fish *Krefftichthys anderssoni*, suggested that birds also foraged in the vicinity and south of the Antarctic Polar Front (Lubimova, 1985; Sabourenkov, 1991). Importantly, stomach contents represent the food aimed to the chick, with the adult food (when they feed for themselves) still remaining unknown. Plasma isotope ratios and lipids from stomach oils were therefore used as trophic markers of the adult food.

Stable isotope ratios of a consumer give information in two dimensions, with $\delta^{13}C$ and $\delta^{15}N$ values reflecting the foraging habitat and trophic position of the consumer, respectively. Plasma $\delta^{13}C$ values measured in breeding short-tailed shearwaters were very negative (-24‰) indicating that they fed for themselves in Antarctic waters during the previous long foraging trip (Cherel & Hobson, 2007). Regarding $\delta^{15}N$, data ranged from -1‰ (*Euphausia vallentini*) to 9‰ (shearwaters), thus encompassing slightly less than three trophic levels. Crustacean species, *Euphausia vallentin*i and *Themisto gaudichaudii* (Cherel et al. 2005b) and *Euphausia superba*, *Thysanoessa macrura* and *Thysanoessa vicina* (Wada et al., 1987; Rau et al., 1991; Schmidt et al., 2004) showed very low $\delta^{15}N$ signatures compared to the breeding birds. This result indicates that crustaceans did not form the staple food of breeding short-tailed shearwaters, but, instead, that organisms feeding on crustaceans were the main prey of the birds. In the Southern Ocean, mesopelagic fish of the family Myctophidae represent the main biomass of macrozooplankton feeders in oceanic waters (Kock, 1992; Pakhomov et al., 1996). Myctophids may therefore be a major prey of breeding short-tailed shearwaters. Interestingly, the nitrogen isotopic signature of adult short-tailed shearwaters (9‰) is close to that of the myctophid-eater king penguin (10‰) reinforcing the hypothesis that myctophids would be the main, but not exclusive, prey for breeding short-tailed shearwaters.

A complementary method has been used to verify this hypothesis: fatty acids and fatty alcohols of lipids used as trophic markers. Oil was only recovered in stomach contents after long foraging trips, and it results from physical and chemical processes concentrating the lipids of ingested prey (Clarke, 1989). This oil is then stored for delivery to the chick. Fatty acid and alcohol signatures of stomach oil wax esters differed strongly from the signatures of crustaceans (*Thysanoessa macrura, Rhincalanus gigas, Euphausia crystallorophias, Euchirella rostromagna, Paraeuchaeta antarctica, Calanoides acutus*) or fish (*Electrona carlsbergi*) species. Thus, breeding short-tailed shearwaters did not forage extensively neither on those species during their long foraging trips, nor on the Antarctic krill Euphausia superba (Connan et al., 2005). By contrast, the resemblance between stomach oil lipids and those of three myctophid species (*Krefftichthys anderssoni, Gymnoscopelus braueri and Electrona antarctica*) highlights the role of myctophid lipids at the origin of the oils, and hence as prey for breeding short-tailed shearwaters. Importantly, the three species are rich in wax esters (Phleger et al., 1997; Lea et al., 2002; Connan et al., unpubl. data), and, together with *Electrona*

carlsbergi and *Gymnoscopelus nicholsi*, they contribute to the bulk of the myctophid biomass in the Southern Ocean (Sabourenkov, 1991; Kozlov, 1995).

Conclusion

The present study used the combination of three complementary methods and showed that the feeding ecology of the short-tailed shearwater is complex, with birds foraging in different water masses where they feed on different prey. No information about the adult diet could be determined from the prey remains in stomach contents, because they corresponded to prey taken on the way back to the colony, and not to the prey caught earlier during the long trips. The stable isotope technique indicated that when the adults foraged for themselves they do so in cold waters where they feed upon organisms which are feeding on crustaceans, such as myctophid fish. This hypothesis was verified with the lipid technique which highlighted the strong resemblances between the myctophid lipids and bird oil signatures. This paper emphasized the importance of myctophid fish in the nutrition of top predators from the Southern Ocean and further north (i.e. Tasmania). We can conclude that while the chicks relied on prey caught by the adults in different water masses, adults closely depended on marine resources (and especially myctophid fish) taken in Antarctic waters far away their breeding grounds. This study also illustrates the interest of a multi-disciplinary approach, here the use of both direct and indirect methods, to determine trophic relationships between organisms (table 3).

Table 3 Summary of how various diet assessment techniques can be integrated to investigate diet of top predators, especially short-tailed shearwater.

Type of analysis	Stomach content	Stable isotope ratios of plasma	Lipid classes, fatty acids and fatty alcohols of stomach oils
Temporal integration	Last meals (represent mainly chick food)	Up to the last 2 weeks	Last long foraging trips (8 to 19 days in the short-tailed shearwater)
Resolution	High – quantitative	Low ($\delta^{13}C$: geographic area; $\delta^{15}N$: trophic level)	Medium (to High with a complete dataset of potential prey species) - qualitative
Time of analysis - Cost	Long – Not expensive	Quick – Expensive	Long – Expensive
Short-tailed shearwater study	- Digested remains of fish => Myctophids?	- Sub-Antarctic and Antarctic waters - Staple food of birds = organisms feeding on crustaceans -=> Myctophids?	**Myctophids** and especially *Electrona antarctica, Krefftichthys anderssoni* & *Gymnoscopelus braueri*

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