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Vertical fatty acid composition in the blubber of leopard seals and the implications for dietary analysis

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ABSTRACT

The analysis of blubber fatty acids (FAs) is a useful tool to infer diet of mammals that live in remote regions where year-round studies are difficult. The FA may not be distributed uniformly within the blubber, which can have implications for dietary predictive studies. The aim of this study was to determine the FA composition in the blubber core of the Antarctic leopard seal, *Hydrurga leptonyx*, and evaluate the potential implications of FA stratification for dietary analysis. The blubber cores of 24 seals were sub-sectioned into outer, middle and inner layers and their FA were compared to those of their potential prey species. A vertical variation in FA composition was found across the whole blubber core of the leopard seal. 17 FAs were found at greater than trace amounts (>0.5%) across all samples and the most abundant were: C18:1ω9, C16:1, C22:6ω3, C16:0 and C18:1ω7, which accounted for approximately 70% of the total FA. Almost all FAs had a continuous gradient through the blubber. Principal Component Analysis confirmed separation between inner and outer layers while the middle layer was a transition. The stratification of the leopard seal blubber was similar to the general pattern observed in a variety of marine species: monounsaturated FA (MUFA) dominated the three layers being more abundant in the outer layer, polyunsaturated (PUFA) and saturated FA (SFA) were more abundant in the inner layer. Polyunsaturated FAs are of dietary origin and SFAs are chemically inert so they can be used as a long-term reserve, which suggest that the inner layer is the site of deposition of the FA obtained from diet. The influence of prey on the composition of the leopard seals' blubber was clearer in the inner layer, although neither outer nor inner layers exactly matched the FA of the potential prey. This suggests that there are other components influencing the FA composition of this predator; therefore, in order to carry out dietary analysis it is important to consider the stratification of blubber and to use the inner layer, where the influence of diet is more evident. This has significant implications for sampling methods in the field.

1. Introduction

The blubber of a marine mammal plays an important role in a variety of functions such as energy reserve, buoyancy control, streamlining and thermal insulation (Castellini et al., 2009; Samuel and Worthy, 2004). The fatty acids (FAs) making up this adipose tissue are mainly from dietary origin (Grahl-Nielsen et al., 2010); for this reason the FA composition of blubber has attracted considerable scientific interest, because of its potential applicability for studies of foraging ecology. Fatty acid analysis can provide a long-term indication of diet history (Bradshaw et al., 2003; Dahl et al., 2000); therefore it can be used to obtain more complete data on diet composition than traditional methods, such as scat and stomach analyses. Although these methods are useful to determine overall trends (Hoberecht, 2006), they have known biases

and limitations associated with incomplete consumption of prey items, gut passage rate and differential degradation of prey remains (Arnould et al., 2005).

Fatty acid analysis is based on the principle that particular FAs are present in prey and can be transferred mainly unmodified into the blubber of the predator (Iverson, 1993). This occurs because while animals produce a limited number of FA, most FAs are synthesised by phytoplankton at low trophic levels, so that the presence of specific compounds can be attributed only to diet (Bromaghin et al., 2012; Budge et al., 2008).

The FA composition of blubber, however, is not exactly identical to that of the diet (Cooper et al., 2005; Grahl-Nielsen, 2009) since it may be regulated by other factors (Grahl-Nielsen and Mjaavatten, 1991). Researches have described that many marine mammals exhibit at least two distinct blubber layers: the outer layer, located under the epidermis; and the inner layer, located just above the muscle (Best et al., 2003; Lambert et al., 2013; Samuel and Worthy, 2004). They

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may also exhibit a third layer which is usually a transition between inner and outer blubber layers (Aguilar and Borrell, 1990). This variation, or stratification, in the composition of blubber indicates that some components of blubber are synthesised independently of diet and may have different functions (Wheatley et al., 2007), which complicates the investigation of foraging ecology through this method.

The inner blubber layer is thought to be metabolically more active and dietary FAs are most likely deposited in this layer rather than in the outer layer (Best et al., 2003; Budge et al., 2008; Herman et al., 2005). On the other hand, it has been suggested that the outer layer may have another physiological role (Grahl-Nielsen et al., 2011) and differs from the diet (Grahl-Nielsen, 2009). Therefore, before using the blubber for obtaining dietary information, it is important to determine the species-specific FA composition through the blubber.

Different studies have taken different approaches to infer diet; some use either the outer component only (Herman et al., 2005; Waugh et al., 2012), or a section across the whole blubber (Bradshaw et al., 2003; Meynier et al., 2008; Newland et al., 2009) while other studies recommend the use of the inner component (Grahl-Nielsen et al., 2005; Olsen and Grahl-Nielsen, 2003; Skoglund et al., 2010). This has implications for sampling as collection of the inner blubber core requires the chemical sedation or physical restraint of research animals whereas collection of the outer blubber samples (just under the skin) can be done remotely by biopsy darting of free-ranging animals.

The leopard seal, *Hydrurga leptonyx* (Blainville, 1820), is an ideal model to study their foraging ecology via FA analysis, as they live in the remote Antarctic pack ice where long-term dietary studies are very difficult to carry out. They have a dispersed distribution (Forcada et al., 2012; Rogers et al., 2013; Southwell et al., 2008), individuals travel widely (Meade et al., 2015; Rogers et al., 2005) and haul out on the drifting ice floes (Rogers and Bryden, 1997; Southwell et al., 2003; Rogers et al., 2013) making year-round studies difficult. Their feeding ecology has been determined by different means including direct hunting observations (Ainley et al., 2005; Hiruki et al., 1999; Penney and Lowry, 1967; Rogers and Bryden, 1995), stomach contents (Siniff and Stone, 1985), scats (Casaux et al., 2009; Green and William, 1986; Hall-Aspland and Rogers, 2004, 2007; Rogers and Bryden, 1995; Walker et al., 1998) and stable isotope (Hall-Aspland et al., 2005a,b) analyses. This study will identify initially whether the blubber of the leopard seal is stratified by establishing the vertical variation in FA composition across whole blubber cores. The influence of stratification of FAs on dietary studies will be tested by comparing how FA composition of the inner and outer blubber layers perform in inferring diet and trophic level from the same individuals.

2. Materials and methods

2.1. Sample collection

In total, 24 leopard seals, 9 females and 15 males, were sampled off the Danco Coast, Western Antarctic Peninsula (64°09′ S 60°57′ W) during the austral summer (February) of 2008 and 2009. Seals hauled out on sea ice were immobilised using a Tele-inject air gun darting system using tiletamine/zolazepam (Higgins et al., 2002). Following immobilisation, sex, standard length (SL straight line nose to tail) and pectoral girth (PG) were recorded and 8 mm diameter biopsies containing whole cores of blubber (from the skin to the muscle layer) were collected from the mid-dorsal surface. All individuals were adults and the body length averaged 280.8 ± 18.3 mm in females and 269.3 ± 19.1 mm in males. In the laboratory, the blubber cores were separated into three sections (inner, middle and outer layers) as shown in Fig. 1. Each sample was stored in airtight vials and frozen at −20 °C for further analyses. The immobilisation and sampling of leopard seals in the Antarctic Specially Protected Area No. 134 was approved by the Dirección Nacional del Antártico, Buenos Aires, Argentina, and performed according to the SCAR Code of Conduct for Animal Experiments
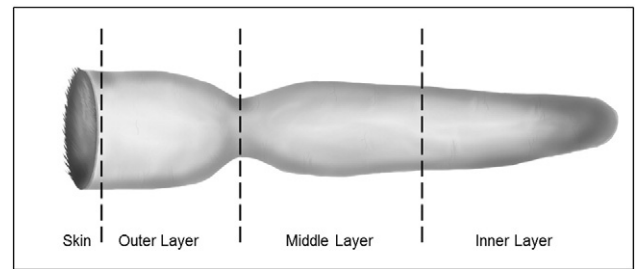

Fig. 1. Sectioning of the blubber core. The outer layer was taken between the skin and the narrowest part of the blubber column. The remaining sample was divided into two sections: the middle layer (the closest to the outer layer) and the inner layer (the closest to the muscle).

under UNSW Animal Care and Ethics Committee (Protocols 08/103B and 11/112 A).

2.2. Fatty acid analysis

Total lipid was extracted following a modified Folch et al. (1957) method. Approximately 0.3 g–0.5 g of blubber was weighed and placed in 9 ml of 2:1 chloroform: methanol with 50 mg/L of butylated hydroxytoluene in test tubes with Teflon caps. Samples were mashed manually with a glass homogeniser until thin and transparent, then vortexed for 20s, and allowed to soak overnight at 4 °C. To remove protein precipitates the homogenate was filtered into Teflon screw cap glass tubes, 2 ml of water were added and the whole mixture was agitated and then allowed to separate into two phases. The lower phase containing lipids was carefully collected by siphoning/pasture pipette and placed in Teflon lined screw cap tubes. The solvent (chloroform) was evaporated from lipid under nitrogen stream to avoid lipid oxidation.

Fatty acid methyl esters (FAMEs) were prepared directly from the extracted lipid, which was dissolved in 1.5 ml of boron trifluoride (10% in methanol) and 1.5 ml of toluene. The solution was capped under nitrogen and heated at 50 °C overnight. Esters were extracted into hexane and stored in vial tubes for gas chromatography analysis.

Gas chromatography analyses were performed with Agilent 6850 Series GC System (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionisation detector. FAMEs were analysed using a 100 m long fused carbon-silica capillary column (SP 2560 Column). The flow rate of the hydrogen carrier gas was set at an initial flow rate of 2.7 ml/min, at a pressure of 200 kPa. The injector and detector port temperatures were set at 260 °C. The column oven was initially held at 140 °C for 5 min, then increased 4 °C/min and then maintained at a temperature of 235 °C. The total run time per cycle was 35 min. Peak areas and retention times were calculated (ChemStation Software, Rev. B.03.01; Agilent Technologies), and FAME were identified by comparison of retention times with a range of standards. The concentrations of individual FA in each sample were converted to percentage contributions of the total FA.

2.3. Data analyses

Fatty acids present in trace amounts (<0.5%) were excluded from statistical analyses. Thus, the number of FA was reduced to 17. The largest FA proportion was more than 2000 times greater than the smallest FA proportion, so all values (x) were arcsin-transformed ($x' = \arcsin\sqrt{x}$) in order to reduce the heterogeneity of variance among groups. Normality and homogeneity of variance were checked with normality plots and with plots of residuals versus fitted values, respectively.

Due to the multivariate nature of FA profile data, Principal Component Analysis (PCA) was used to investigate patterns in FA association among the different individuals. In this manner the 17 variables could be described in two dimensions. Multivariate analysis of variance

(MANOVA) was then carried out on the PC scores with gender, year, and blubber layers as factors.

A stratification index was calculated by subtracting the percentage in the inner layer from percentage in the outer layer and dividing the difference by mean of totals for outer and inner layers (Olsen and Grahl-Nielsen, 2003). This index was calculated in order to determine the degree of stratification in the blubber.

A correlation between body condition and inner layer FA was tested. Body condition was assessed using two condition indices: Smirnov index and fineness ratio (Van der Hoff et al., 2005) were derived from standard body length (*SL*) and girth (*PG*) measurements.

Fineness ratio (*FR*) is an index to measure streamlining and hydro-dynamic performance. Assuming that, when supported by water the seals body would have a roughly circular cross-section it is calculated as the standard body length (*SL*) divided by its maximum diameter (D_{ma}):

$$FR = SL \div D_{ma}$$

where maximum diameter was calculated from the girth (*PG*) measurement using the equation:

$$D_{max} = PG_{max} \div \pi$$

All statistical tests have an α level of statistical significance of 0.05 (SPSS Release 17.0, SPSS 2008). Where not identified variability around the mean is standard deviation.

2.4. Potential prey fatty acids

In order to test whether the use of inner or outer layers would lead to the same predictions of diet, a PCA for leopard seal blubber layers and potential prey FA composition was plotted. Additionally, their trophic level was investigated by using FA ratios of vaccenic acid/oleic acid (C18:1ω7/C18:1ω9) and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) (C20:5ω3/C22:6ω3).

Data of prey species was collated from published literature, which included species of fish (Connan et al., 2010; Lea et al., 2002; Mayzaud et al., 2011; Raclot et al., 1998), krill (Alonzo et al., 2005; Cripps et al., 1999; Phleger et al., 2002; Polito et al., 2012) and penguins (Speake et al., 1999; Tierney et al., 2008).

3. Results

3.1. Fatty acid stratification

Although 46 FAs were originally identified, only 17 (comprising 89.34% of total) were found consistently in all blubber layer samples and in proportions greater than 0.5%. The most abundant FAs,
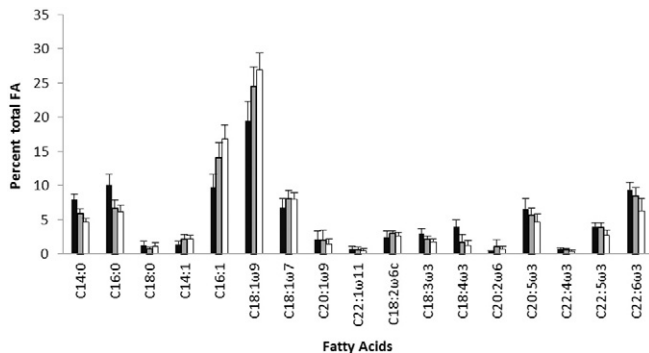

Fig. 2. Proportion of fatty acids across blubber layers. Relative amounts (percent total) of 17 fatty acids (FAs) in the inner (black), middle (grey) and outer (white) blubber layers of leopard seals, *H. leptonyx* (n = 24).

accounting for approximately 70% of total FA, were: C18:1ω9 (19–27%), C16:1 (10–17%), C22:6ω3 (6–9%), C16:0 (6–10%), and C18:1ω7 (7–8%) (Fig. 2). The FA profiles of the three layers were dominated by monounsaturated FAs (MUFAs) (40–55%), followed by polyunsaturated FAs (PUFAs) (20–30%) and with smaller proportions of saturated FAs (SFAs) (12–19%) (Table 1, Fig. 3).

Monounsaturated FAs were present at higher proportions in the outer blubber layer (55.7 ± 4.2%) decreasing to 40.1 ± 5.3% in the inner layer. On the other hand, SFAs and PUFAs are present at higher proportions in the inner layer (19.3 ± 2.7% and 30.4 ± 3.8%, respectively) rather than in the outer layer (11.9 ± 1.6% and 20.1 ± 5.0%, respectively).

The first three components derived from the PCA accounted for 67% of the variation (PC1 43%, PC2 13%, PC3 11%) in FA composition among the samples. The bivariate plot (Fig. 4) shows inner and outer layers as separated groups, while the middle layer sits between them. The princi-pal FA driving the segregation of the PC1 were the positive Eigen values for C18:4ω3, C18:3ω3, C14:0 and C22:6ω3 (more abundant in the inner layer) and the negative Eigen values for C16:1, C18:1ω9 and C18:1ω7 (more abundant in the outer layer). A MANOVA was carried out on the 3 PC scores and it confirmed the significant differences between blubber layers (Wilks' λ = 0.154, P < 0.001). Fig. 4 also shows a group of seals, whose inner layer was separated from the others and was rath-er sitting with the middle layer group. This segregation of leopard seals inner blubber was not caused by differences in body condition of the seals, since there was no correlation between the fineness ratio (t_{18} = 0.42, P = 0.67) or Smirnov index (t_{18} = 1.23, P = 0.23), and the first PC for inner layer FA across the 24 seals.

The inner-outer difference per individual FA is represented by the stratification index (Fig. 5). The mean overall FA stratification index was 0.44 ± 0.22 and the maximum reached an absolute value of 1.11 for stearidonic acid (C18:4ω3). While SFAs were more abundant in the inner layer, MUFAs were enriched in the outer layer. Most PUFAs showed an inner-layer trend, except C18:2ω6c and C20:2ω6, which were in greater amounts in the outer layer.

There were no significant inter-sexual (Wilks' λ = 0.961, P = 0.482) or inter-yearly (Wilks' λ = 0.955, P = 0.413) differences.

3.2. Fatty acids and implications for dietary analysis

From the principal component analysis comparing leopard seals to their potential prey species (Fig. 6), the PC1 values indicate segregation between prey and predator, which is influenced by higher amounts of C16:0 and C20:5ω3 in prey species and higher amounts of C18:1ω9 and C16:1 in leopard seals. The inner layer aligned more closely with prey species, compared to the outer layer. The second PC reveals similarity between fish and penguins, with higher amounts of C18:0 and C20:1ω9, and between leopard seals and krill species, due to the influence of positive Eigen values for C18:4ω3 and C14:0.

Fig. 7 reveals that leopard seals, penguins and most fish species have low vaccenic acid/oleic acid ratios whereas most krill species have higher ratios. For leopard seals, penguins and most fish species, EPA/DHA ratios are low; but krill species show a wide range of values.

4. Discussion

4.1. Stratification

Significant variations were observed in the FA composition between the outer and inner blubber layers, however rather than being discrete layers the FA composition changed as a gradient from the outer, middle to the inner layer through the blubber core. Blubber stratification has been reported for several phocid species including: harbour seals, *Phoca vitulina* (Andersen et al., 2004); southern elephant seals, *Mirounga leonina* (Best et al., 2003); Baikal seals, *Phoca sibirica*; ringed seals, *Pusa hispida* (Grahl-Nielsen et al., 2005; Strandberg et al., 2008);

Table 1
Fatty acid profiles[*] (per cent total FA) of inner, middle and outer blubber layers from leopard seals (n = 24).

	2008						2009					
	Female			Male			Female			Male		
	I L	M L	O L	I L	M L	O L	I L	M L	O L	I L	M L	O L
C14:0	8.21	6.12	4.99	7.41	5.67	4.77	7.97	5.98	4.49	8.25	5.75	4.61
C16:0	10.42	6.78	6.88	9.38	6.24	4.93	10.40	7.07	6.46	10.33	6.60	6.86
C18:0	1.22	0.89	1.41	1.12	0.88	0.62	1.58	0.67	0.96	1.38	0.73	1.49
Σ SFA	19.85	10.34	13.28	17.91	7.99	10.32	19.95	10.98	11.91	19.96	11.21	12.96
C14:1	1.50	2.04	2.17	1.42	2.39	2.63	1.31	1.76	1.73	1.34	2.28	1.97
C16:1	10.00	13.50	15.91	10.62	14.36	17.90	9.58	14.55	16.77	8.70	13.91	15.99
C18:1ω9	20.44	23.35	27.10	19.96	24.67	27.17	18.55	22.83	25.66	18.84	25.90	27.36
C18:1ω7	6.73	8.69	8.20	7.05	7.92	7.72	6.29	8.58	7.86	6.64	7.42	8.13
C20:1ω9	1.60	1.16	1.39	2.77	2.91	1.79	1.59	2.00	1.32	2.04	1.79	1.17
C22:1ω11	0.47	0.66	0.32	0.68	0.75	0.61	0.69	0.30	0.26	0.82	0.57	0.35
Σ MUFA	40.74	37.06	55.08	42.50	33.13	57.83	38.01	40.01	53.60	38.37	44.47	54.96
C18:2ω6	2.04	3.05	2.69	2.21	2.92	2.30	2.94	2.80	2.86	2.68	3.11	2.66
C18:3ω3	3.18	2.22	1.66	2.89	2.22	2.01	2.71	1.99	1.55	3.03	2.09	1.48
C18:4ω3	3.38	2.10	1.26	3.82	1.84	1.79	4.30	1.08	0.84	4.06	1.75	0.77
C20:2ω6	0.27	0.67	0.34	0.37	0.73	0.66	0.44	1.74	0.83	0.39	1.00	0.66
C20:5ω3	5.93	5.52	4.00	6.97	5.35	5.05	7.13	5.99	5.09	6.04	5.72	4.14
C22:4ω3	0.55	0.75	0.31	0.77	0.65	0.53	0.86	0.53	0.38	0.76	0.62	0.25
C22:5ω3	3.99	4.00	2.42	3.75	3.62	3.02	4.34	4.33	2.97	3.97	3.63	2.40
C22:6ω3	9.30	8.94	5.62	8.74	8.11	6.90	9.99	9.02	6.75	9.69	8.29	5.50
Σ PUFA	28.66	20.43	18.30	29.52	15.90	22.27	32.71	21.99	21.29	30.61	22.47	17.87

[*] Only those FAs contributing >0.5% of the total are shown.

Weddell seals, *Leptonychotes weddellii* (Wheatley et al., 2007); and harp seals, *Pagophilus groenlandicus* (Grahl-Nielsen et al., 2011). Winter and Nunn (1950) reported FA composition of the whole blubber core from three sites (the belly, back and neck) on the body of a single leopard seal. Similar FA compositions were reported between these three sites, but only four individual FAs were identified and stratification of the blubber core was not considered.

The stratification of blubber is attributed to the different roles that the layers provide, either related with thermoregulation for the outer layer and dietary processes for the inner layer (Arnould et al., 2005; Best et al., 2003; Koopman et al., 1996).

Saturated FAs were more abundant in the leopard seals' inner blubber layers than in the outer layers. In otariids there is little stratification of SFA across the blubber (Arnould et al., 2005; Lambert et al., 2013) but SFA stratification is pronounced in other phocids (Grahl-Nielsen et al., 2005; Strandberg et al., 2008), in the walrus (Skoglund et al., 2010) and in cetaceans (Budge et al., 2008; Olsen and Grahl-Nielsen, 2003). Saturated FAs are thought to be important for long-term energy storage (Arriola et al., 2013) since they are comparatively chemically inert (Christie, 2003). In addition, having the highest melting point compared to the other groups of FA (Christie, 2003), the decreasing amount of SFAs towards the outer layer is reasonable as greater amounts of SFAs in the outer layer would make it very rigid, particularly at low temperatures, which would be unfavourable for movement and streamlining.
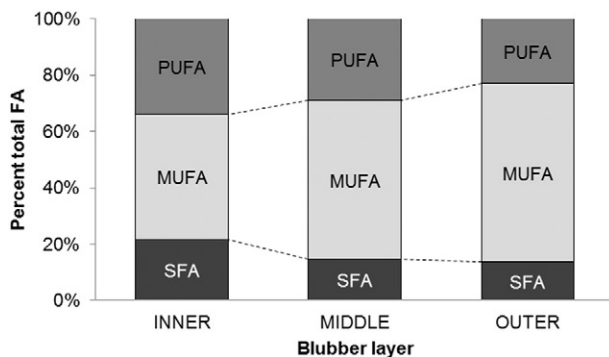
Leopard seals have higher quantities of MUFAs across all layers, but there are significantly more MUFAs in the outer blubber layer. This is similar to the MUFA distribution patterns across the blubber of other phocid seals, the harp (Grahl-Nielsen et al., 2011), ringed (Strandberg et al., 2008) and southern elephant (Best et al., 2003) seals as well as the otariid: the New Zealand sea lion, *Phocarctos hookeri* (Lambert et al., 2013). It has been suggested that because MUFAs have a lower melting point, relative to their saturated counterparts (Christie, 2003; Rustan and Drevon, 2005), their presence in the outer layer is important to maintain softness and fluidity under the skin and to help reduce heat loss from the body (Best et al., 2003). Moreover, in the case of cold-water inhabiting species, the temperature of the outer layer could be extremely low, in ringed seals, for example, while the outer layer temperature is 5 °C, the muscle temperature is 36 °C (Käkelä and Hyvärinen, 1996). Low temperatures may limit enzymatic reactions (Koopman, 2007) which could affect dietary processes ion the outer layer.

Polyunsaturated FAs are more abundant in the leopard seals' inner blubber layer, which is not common across other seals, but has been reported for several cetacean species. They are in higher abundance in the
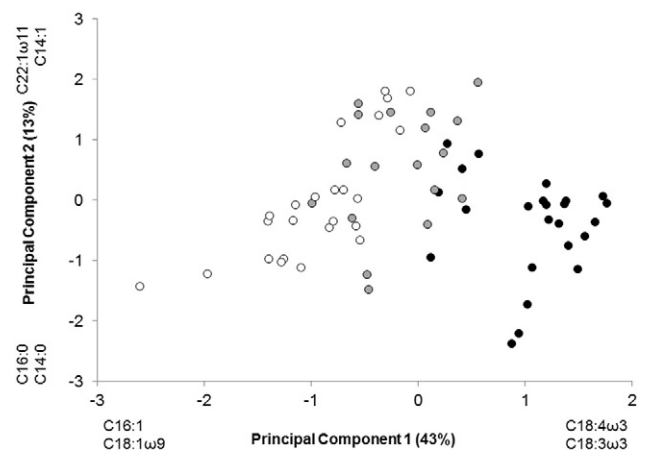

Fig. 3. Fatty acid groups across blubber layers. Mean proportion of polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids from the inner, middle and outer blubber layers for leopard seals, *H. leptonyx* (n = 24).

Fig. 4. Vertical variation of fatty acids across leopard seal blubber. Principal component plot for the inner (black circles), middle (grey circles) and outer (white circles) blubber layers of leopard seals, *H. leptonyx* (n = 24).
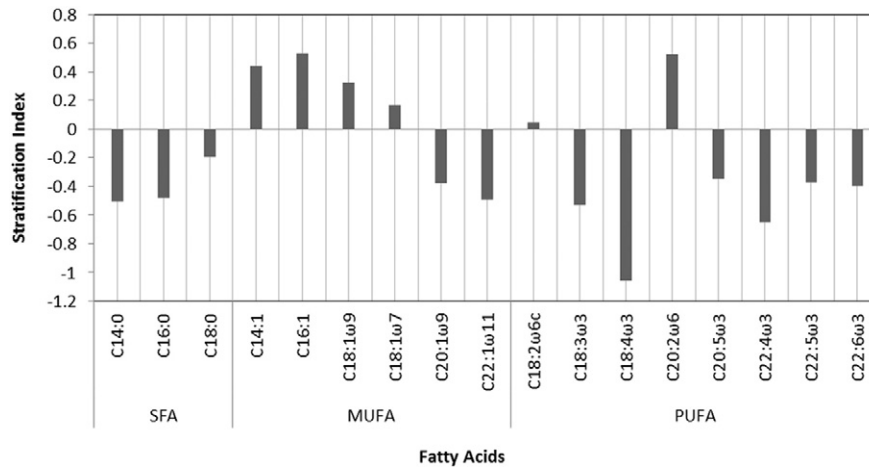
Fig. 5. Stratification Index for fatty acids of leopard seal blubber (n = 24). Positive values represent a greater amount of the fatty acid in the outer layer and negative values indicate a greater presence in the inner layer.

inner blubber layer of dusky dolphins, *Lagenorhynchus obscurus* (Grahl-Nielsen et al., 2010), short-beaked common dolphins (Smith and Worthy, 2006), harbour porpoises, *Phocoena phocoena* (Koopman et al., 1996), minke (Olsen and Grahl-Nielsen, 2003) and fin whales (Ruchonnet et al., 2006). Polyunsaturated FAs are similar in quantities across the inner and outer layer of the phocids: Weddell seals (Wheatley et al., 2007), ringed seals (Grahl-Nielsen et al., 2005; Strandberg et al., 2008); and the otariids: the cape fur seal, *Arctocephalus pusillus* (Arnould et al., 2005) and New Zealand sea lion (Lambert et al., 2013); the Atlantic walrus, *Odobenus rosmarus* (Skoglund et al., 2010); and the cetacean: the bowhead whale, *Balaena mysticetus* (Budge et al., 2008).

Higher presence of PUFAs in the inner blubber layer of the leopard seal may be due to their prevalence in the marine environment and the fact that they are usually of a dietary origin (Hoberecht, 2006; Samuel and Worthy, 2004). Several PUFAs are essential FAs, which must be acquired through the diet (Arriola et al., 2013; Liwanag et al., 2012). They are produced by phytoplankton and are tightly conserved with little catabolism in predators (Dahl et al., 2000; Hoberecht, 2006). As PUFAs are susceptible to oxidative deterioration or autoxidation (Christie, 2003), they are rapidly available from the inner layer as a fuel source (Arriola et al., 2013). Despite this, the differences in PUFA distribution between marine mammals can be explained by distinct

deposition and mobilisation of FAs through the depth of the blubber, which will also depend on their nutritional state.

Overall, the stratification index of the leopard seals' FA shows similar values to those found on other phocids (Grahl-Nielsen et al., 2005, 2011; Strandberg et al., 2008, 2011) but larger than those reported for otariids (Arnould et al., 2005; Lambert et al., 2013). This means that phocids have blubber with higher vertical variation than otariids. Pinnipeds in general, use a combination of blubber and fur as a mechanism of insulation (Liwanag et al., 2012), but phocids blubber as thermal insulator, since their fur has poor insulating properties when wet (Kvadsheim and Aarseth, 2002). Therefore, the higher level of blubber stratification in phocids could be a consequence of its better specialisation as an insulator: the outer layer may have differentiated to play an exclusive thermoregulatory role while the inner layer is the energy reserve ready to be utilised when the animal needs it.

4.2. Fatty acids and implications for dietary analyses

The two most abundant FAs (C18:1ω9 and C16:1) present in leopard seal's blubber are ubiquitous to marine mammals. They have been recorded in the highest proportions for phocids (Andersen et al., 2004; Grahl-Nielsen et al., 2005; Strandberg et al., 2008; Wheatley et al.,
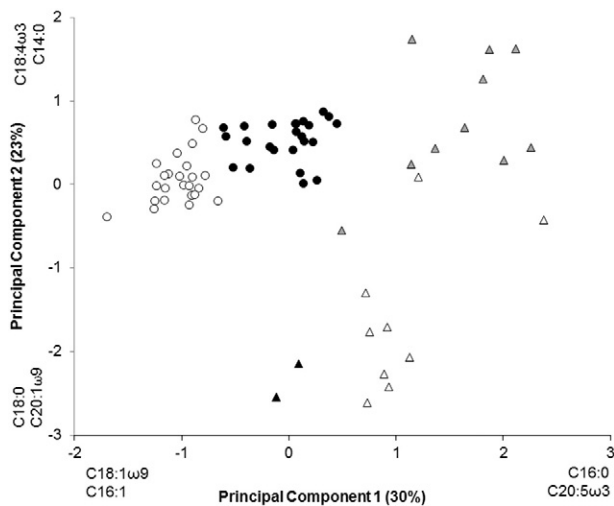

Fig. 6. Dietary inference from fatty acids. Principal Component plot of leopard seals inner (black circles) and outer (white circles) blubber layers (n = 24) and their potential prey species: fish (white triangles), krill (grey triangles) and penguins (black triangles).
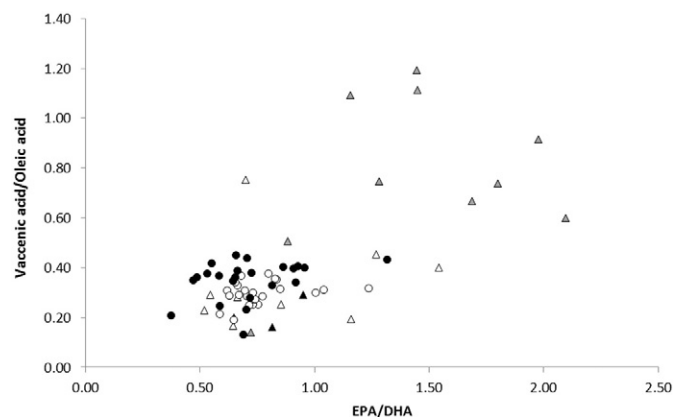

Fig. 7. Trophic level analysis. Vaccenic acid/oleic acid and eicosapentaenoic acid (EPA)/docosahexaenoic acid (DHA) ratios of leopard seals' inner (black circles) and outer (white circles) blubber layers, krill (grey triangles; Alonzo et al., 2005; Cripps et al., 1999; Phleger et al., 2002; Polito et al., 2012), fish (white triangles; Connan et al., 2010; Lea et al., 2002; Mayzaud et al., 2011; Raclot et al., 1998) and penguins (black triangles; Speake et al., 1999; Tierney et al., 2008).

2007), otariids (Beck et al., 2007; Iverson et al., 1997; Lambert et al., 2013; Rosen and Tollit, 2012) and cetaceans (Budge et al., 2008; Quérouil et al., 2013; Samuel and Worthy, 2004; Waugh et al., 2012). They are considered endogenous FAs (Herman et al., 2005) which do not have a dietary origin (Raclot et al., 1998), although it has also been suggested that high levels of these FAs may be consistent with a carnivorous diet (Phleger et al., 2002).

Docosahexaenoic acid (C22:6ω3) was the third most abundant FA present in leopard seal's blubber, which is similar to other phocids (Arriola et al., 2013; Grahl-Nielsen et al., 2005; West et al., 1979), otariids (Arnould et al., 2005; Beck et al., 2007; Iverson et al., 1997), Atlantic walrus (Skoglund et al., 2010), and some cetaceans (Budge et al., 2008; Quérouil et al., 2013; Ruchonnet et al., 2006; Smith and Worthy, 2006). This is an exogenous dietary FA (Herman et al., 2005) and hence a likely biomarker for the assessment of long-term dietary intakes (Raclot et al., 1998). Moreover, the higher presence of DHA in the inner stratus supports the thesis of a dietary role of this layer. This FA is characteristic of krill species (Tierney et al., 2008); therefore, the higher presence of DHA in leopard seals compared to other Antarctic seals such as southern elephant (Best et al., 2003) and Weddell (Wheatley et al., 2007) seals suggests that leopard seals are incorporating higher levels of krill in its diet. The other FA linked to krill intake is docosapentaenoic acid (C22:5ω3) (Tierney et al., 2008) which is also more abundant in the leopard seals than reported in the other Antarctic seals (Best et al., 2003; Wheatley et al., 2007).

Gondoic acid (C20:1ω9) was in low (~2%) quantities in the leopard seal inner blubber compared to quantities in other phocid seals; such as the southern elephant (Best et al., 2003), harbour (Andersen et al., 2004) and ringed (Grahl-Nielsen et al., 2005; Strandberg et al., 2008) seals; where it represents from 10 to 15% of the dietary FA in the blubber. This FA has a dietary origin and is characteristic of many teleost species but it is found in very low levels in krill (Iverson et al., 1997). This may indicate a low intake of fish in leopard seals, unlike the other seals that may be preying on fish more heavily.

When the FAs of the leopard seals were compared with those of their potential prey species (from the literature), there was no definitive indication that the seals were feeding on any of the prey items included in this study, irrespective of the blubber sampling site (inner or outer blubber layer). This study supports others (Best et al., 2003; Grahl-Nielsen et al., 2011; Waugh et al., 2012) where FAs of the predator have not matched exactly with those of their prey. A close alignment between predator and prey FAs was not anticipated here as the FA results from prey were not collected at the same sampling site but prey FAs were obtained from the literature, an approach typical of other FA dietary studies (Bradshaw et al., 2003; Newland et al., 2009). The aim of this study was to identify how the inner and outer layers performed, relative to one another, in predicting dietary items. Whereas the FAs of the outer layer were clustered together and were not closely aligned with the FA of any of the potential prey species, the FA of the inner blubber layer aligned more closely with FA of krill, but only on the PC2 axis (Fig. 6), which was driven by higher amounts of C18:4ω3 and C14:0, which are FA from dietary origin (Raclot et al., 1998). Leopard seals in this region are known to feed on krill, from scat analysis (Casaux et al., 2009), conferring with the prediction from the FA values of the innermost layer.

FAs of either the inner or outer blubber layer were equally valid trophic predictors for the leopard seal. The ratio of vaccenic acid to oleic acid is used frequently to estimate the degree of carnivory (lower values) versus herbivory (Stubing and Hagen, 2003). The analysis of vaccenic acid/oleic acid and EPA/DHA ratios shows that the leopard seals inner and outer blubber layers align together along with other Antarctic predators: most fish species and penguins (Fig. 7). The wide range of krill EPA/DHA ratios indicates that different krill species derive energy from either a diet of diatom (high ratio) or flagellate (low ratio) origin (Phleger et al., 2002). The similar trophic level of leopard seals, fish and penguins suggests that there is no a predator–prey relationship.

Krill species are in lower trophic levels, which can be indicative of leopard seals preying on them. Identifying that the ratios of vaccenic acid/ oleic acid and EPA/DHA were similar between the outer and inner leopard seal blubber layers has significant implications for sampling methods. To collect samples from the inner blubber core (closest to the muscle) requires the chemical sedation or physical restraint of a research animal in order to collect a total blubber core whereas the outer blubber layer (just under the skin) can be collected remotely by biopsy dart from free-ranging animals.

5. Conclusions

This study provides the first description of FA composition of the leopard seal. The study of feeding ecology through FA analysis has become a valuable tool to study feeding behaviour of animals living in remote regions, like the Antarctic, where year-round studies are difficult. As shown in this study, the considerable variation in FA composition across the blubber structure of the leopard seal needs to be taken into account. The ability to use samples from the outer blubber layer for FA analysis has significant implications for sampling methods. The outer blubber layer can be collected remotely by biopsy dart from free-ranging animals and it is commonly the most feasible way of sampling, especially in animals that cannot be sedated, such as cetaceans. To collect samples from the inner blubber core requires the chemical sedation or physical restraint of the research animal in order to collect a total blubber core. For the leopard seal we recommend the use of the innermost blubber layer for diet prediction, as the influence of diet in the outer layer is significantly reduced. However, even though the inner layer will provide more accurate results on diet inference, this may not exactly indicate the prey items consumed by the predator, as predator's metabolism will influence their FA composition. This biochemical technique should be used in complement with others, such as stable isotopes analysis, to minimise the biases produced by the animal's metabolism.

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