Vertical stratification of fatty acids in the blubber of southern elephant seals (*Mirounga leonina*): implications for diet analysis

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Abstract

Fatty acid signature analysis (FASA) is a powerful ecological tool that uses essential fatty acids (FA) from the tissues of animals to indicate aspects of diet. However, the presence of vertical stratification in FA distribution throughout blubber complicates the application of FASA to marine mammals. Blubber biopsy samples were collected from adult female southern elephant seals (*Mirounga leonina*) from Macquarie Island ($n=11$), and blubber cores were divided into inner and outer sections to determine the degree to which the blubber layer was stratified in FA composition, we found 19 FA from both blubber layers in greater than trace amounts ($>0.5\%$). The inner and outer blubber layers could be separated using principal components analysis based on the relative proportion of FA in each layer. Dietary polyunsaturated fatty acids (PUFA) were also observed in significantly higher proportions in the inner blubber layer. Due to the degree of FA stratification in southern elephant seals, we concur with other marine mammal studies that sampling only the outer blubber layer will result in a loss of recently accumulated information regarding diet structure (as indicated by 'surplus' PUFA from the diet). This finding suggests that differential mobilization/deposition of certain FA may result in a modified signature from prey to predator. Thus, sampling animals to recover the inner blubber layer is important for studies attempting to describe aspects of marine mammal diet. This can be achieved in animals such as pinnipeds where the whole blubber layer can be readily sampled.
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1. Introduction

Knowledge of diet, and how it varies in time and space, are fundamental to studies of the ecology of marine mammals, and especially in assessing their role as consumers in marine eco-systems (Laws, 1993; Knox, 1994; Santos et al., 2001). Dietary information forms the basis of our understanding of trophic interactions, foraging ecology and life-history patterns. The interpretation of predator–prey interactions also governs our ability to apply predictive models for understanding possible effects of environmental change and commercial fishing on predator and prey populations. By virtue of a marine existence, however, the study of marine mammal diet is one of the most challenging of any vertebrate taxon (Iverson et al., 1997a).

The application of traditional methods of diet analysis to marine predators has well-established biases and limitations (Dellinger and Trillmich, 1988; Pierce and Boyle, 1991; Fea et al., 1999).

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Fatty acid signature analysis (FASA) is a technique that can indicate aspects of diet and may potentially overcome some of the difficulties that arise using traditional methods such as stomach content and scat analyses (Borobia et al., 1995; Iverson et al., 1997a,b; Brown et al., 1999; Dahl et al., 2000; Walton et al., 2000; Hooker et al., 2001—although see Grahl-Nielsen, 1999; Smith et al., 1999 for comments concerning the limitations of the technique). In marine mammals, FASA is typically done with samples of subcutaneous blubber or milk. Blubber is defined as the layer of fatty tissue between the epidermis and the fascia of the underlying muscle (Laws, 1993), and in pinnipeds, virtually all dissectible lipid is contained in the blubber (Bryden, 1964). However, the use of FASA is not without its own unique set of difficulties. For example, blubber stratification (previously observed mainly in cetaceans—Ackman et al., 1965, 1975a,b; Lockyer et al., 1984; Koopman et al., 1996; Dahl et al., 2000; Hooker et al., 2001) is the occurrence of vertical stratification in fatty acid (FA) distribution throughout the depth of blubber. A limited number of studies have examined blubber stratification in pinnipeds, but these have been restricted to Northern Hemisphere species (Kakela et al., 1993; Fredheim et al., 1995). When arbitrarily divided sections of blubber are compared in those species where blubber stratification has been identified, the inner blubber layer (closest to muscle) is more active metabolically than the outer layer (closest to the skin) in terms of lipid deposition and mobilization (Koopman et al., 1996). Only one study (Hooker et al., 2001) has linked the need to determine the degree and influence of stratification of blubber FA with the sampling protocol for blubber that will give the best representation of diet with FASA. Consequently, dietary interpretations may vary depending on which area (layer) of the blubber is analyzed (Hooker et al., 2001). While the magnitude of the stratification is unclear, it is apparent that analyses of the whole blubber layer may give different FA profiles than the inner or outer sections alone (Hooker et al., 2001).

Interpretation of diet data for pinniped species that spend most of their annual life cycle at sea and travel considerable distances from their haulout sites (e.g. southern elephant seals—*Mirounga leonina*) present particular challenges for dietary analysis. FASA is a potential solution to this problem because seals accumulate blubber at sea for use in the subsequent breeding or moulting fasts. Elephant seals have an estimated rate of passage of food of only 8 h (Krockenberger and Bryden, 1994), so samples collected from stomachs reveal little of the diet during the majority of the time spent at sea. FASA has the potential to provide insight into the diet structure during this time. However, the validity of FASA depends on the degree of modification of the ingested FA from the prey species when being processed and stored in the blubber. Further, there is little information on the structure and composition of the blubber of southern elephant seals available. The likely presence of stratification of FA in the blubber may suggest that differential mobilization or selective deposition of certain FA could result in a modified signature from prey to predator. Thus, any preliminary attempts to interpret FA composition of the blubber in this species should be accompanied by some understanding of the structure of the blubber layer itself.

To examine these issues, we investigated the FA composition of blubber from adult female southern elephant seals. *M. leonina* is a major predator in the Southern Ocean (Knox, 1994; Santos et al., 2001); however, the diet of this species is still poorly understood because all difficulties encountered when using traditional methods of diet analysis are exemplified in this species. In this paper, we analyzed the inner and outer vertical sections of blubber to determine the degree to which the blubber is stratified in FA composition, and to assess which part of the blubber layer will give the best representation of long-term diet structure. We also discuss the implication of FA stratification in elephant seal blubber in terms of insulation and metabolic processes.

2. Materials and methods

2.1. Samples

Blubber samples were obtained from adult female southern elephant seals from Macquarie Island (54°30′S, 158°50′E). The sample consisted of known-age animals born in 1993 (branded after weaning; McMahon et al., 2000) to minimize the confounding effects of age and cohort in the analyses. Animals sampled were 8 years old when captured in 2001. Further, all of the study animals

were known to be multiparous at the time of sampling, thus any reproductive-related variation was also reduced. Blubber samples were obtained in October 2001 immediately post-parturition ($n = 11$). Each animal was captured, sedated and measured following the procedures outlined by McMahon et al. (2000) and Field et al. (2002).

The biopsy site was located by measuring 5–7 cm laterally from a site on the posterior dorsal surface of the seal (known as 'G5'—Field et al., 2002). The G5 site is directly above the midline approximately one third of the distance between the shoulder and hips (Field et al., 2002). A 2×2 cm^2 area was shaved and disinfected with an alcohol swab. A 1-cm anterior–posterior line was cut through the skin, and the biopsy corer (6 mm diameter) was inserted into this incision, until the muscle layer was reached, as evident by a notable increase in resistance. In the laboratory, the core was extended to its full length (mean length ≈ 50 mm), and cut into two equal pieces. No visible differences were apparent along the core. The 'outer' portion (the half closest to the skin) and the 'inner' portion (the half closest to the muscle) were placed in pre-weighed glass vials, containing a solvent mixture comprised of 2:1 v/v chloroform and methanol, and 0.05% by weight of the anti-oxidizing agent, butylated hydroxy toluene (BHT). Samples were maintained at -20 °C until lipid analyses.

2.2. Lipid extraction and analyses

A modified version of the Bligh and Dyer (1959) one-phase methanol/chloroform/water extraction (2:1:0.6, by volume) was used to extract lipids from blubber samples quantitatively (Phleger et al., 2001); this modification (ratio is generally 2:1:0.8, by volume) was made to allow for the relative amounts of water already present in the blubber samples. Each blubber core was squeezed during the transfer from the vial to the separating funnel to aid the extraction of the lipid. Following overnight extraction, chloroform and saline water were added to separate the phases (final solvent ratio, 1:1:0.9, by volume, methanol/chloroform/water). The solvents were removed using rotary evaporation at 40 °C and the total lipid (TL) concentrated. TL (10–20 mg) was dissolved in chloroform, and an aliquot treated with methanol/hydrochloric acid/chloroform (10:1:1, v/v/v; 80 °C; 2 h). Each sample was vortexed 2–4 times

during that period to maximize the conversion to fatty acid methyl esters (FAME). After the addition of 1 ml of water, the FAME were extracted into hexane/chloroform (4:1, v/v, 3×1.6 ml). Gas chromatographic (GC) analyses of FAME were done with a Hewlett Packard 5890A GC (Avondale, PA) equipped with an HP-5 cross-linked methyl silicone-fused silica capillary column (50 m, 0.32 mm i.d.), an FID, a split/splitless injector, and an HP 7673A auto-sampler. Helium was the carrier gas. The samples were injected in splitless mode at an oven temperature of 50 °C. After 1 min, the oven temperature was raised to 150 °C at 30 °C min^{-1}, then to 250 °C at 2 °C min^{-1}, and finally to 300 °C at 5 °C min^{-1}. Peaks were quantified with Waters Millenium software (Milford, MA). Individual components were identified by comparison of retention time data to authentic and laboratory standards. The use of polyunsaturated fatty acids (PUFA)-containing standards also served to ascertain whether loss of these components occurred during analysis. When losses with PUFA standards occurred, the GC injector was cleaned. Components were also verified using mass spectral data. GC results are subject to an error of $\pm 5\%$ of individual component abundance. GC–mass spectrometric (GC–MS) analyses were done on a Finnigan Thermoquest GCQ GC–MS spectrometer (Austin, TX) fitted with an on-column injector. This instrument was fitted with capillary column similar to that described above.

Integrated chromatograms were normalized by expressing the FA components as a relative amount of total FA (percentage of the total). FA components that occurred at less than 0.5% of total FA in either layer were not included in the statistical analyses, as the precision of their determination is low (Dahl et al., 2000; Walton and Pomeroy, 2003). If only one sample (inner or outer for all sampled seals) exceeded the 0.5% threshold, the FA was still excluded from analyses. Thus, the resultant number of variables was reduced to 19 individual FA. Total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), short-chain MUFA (SC-MUFA), long-chain MUFA (LC-MUFA), and PUFA were also calculated (as mean percentages of total FA $\pm$ their standard deviations, S.D.). LC-MUFA have a chain length of >18 carbons; SC-MUFA have a chain length of $\leqslant 18$ carbons. Pair-wise t-tests were used to determine if differences in the combined proportions of SFA,
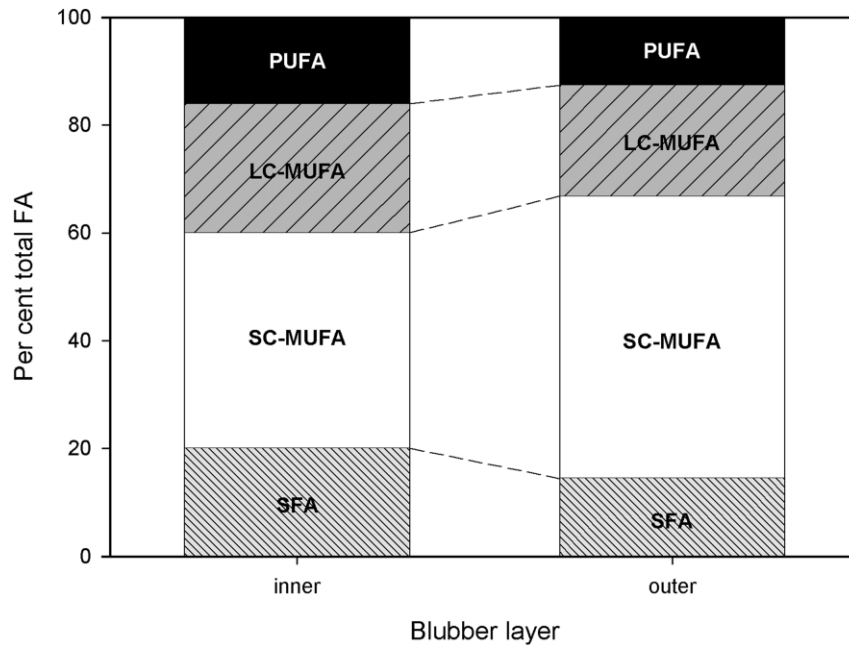
Fig. 1. Mean proportion ($\pm$S.D.) of the FA groups from the inner and outer blubber layers for southern elephant seals, *M. leonina* ($n=11$). Saturated fatty acid, SFA; monounsaturated fatty acid, MUFA; Polyunsaturated fatty acid, PUFA; LC-MUFA have a chain length of greater than 18 carbons; SC-MUFA have a chain length of 18 or less carbons.

PUFA, SC-MUFA and LC-MUFA were significant between layers.

The largest FA proportion was approximately 1000 times greater than the smallest FA proportion, thus all FA proportions (x) were treated using the angular transformation ($x'=\arcsin\sqrt{x}$) to reduce the heterogeneity of variances among test groups (Sokal and Rohlf, 1981). Principal components analysis reduces the dimensionality of the data that is produced by the large number of variables. This method uses linear correlations (principal components or PC) that summarize the data while losing as little information as possible. Using SPSS 10.0 (SPSS Inc., Chicago, IL), three PC were derived to describe the observed variance, and the Eigen values of the first three PC are presented. A biplot of PC1 and PC2 was produced describing largest and second largest variances among the samples.

3. Results

3.1. Blubber fatty acid composition

Nineteen FA were consistently observed in greater-than-trace amounts (0.5% of total FA) in the inner and outer blubber layer samples ($n_{\text{inner}}=$ 11, $n_{\text{outer}}=11$). These FA generally accounted for 94–96% of total FA (Fig. 1). The configuration of all double bonds for the FA in greater-than-trace amounts was *cis*. Of the trace FA, three isomers of 19:1 and C_{16} PUFA, and 2 isomers of C_{21} PUFA and C_{24} PUFA were identified. The position of the double bond/s was not determined.

3.2. Broad-scale differences between inner and outer blubber layers

The FA profiles of both the inner and outer parts of the blubber layer were dominated by MUFA, with smaller proportions of SFA and PUFA (Table 1; Fig. 1). There were greater proportions of SFA and PUFA in the inner compared to the outer blubber layer (SFA: $t_{10}=12.30$; $P<0.001$; PUFA: $t_{10}=5.05$; $P<0.001$; Table 1; Fig. 1). Finer-scale differences were also discernible between the relative proportions of LC- and SC-MUFA (LC includes C_{20}, C_{22} and C_{24} MUFA; SC includes C_{14}, C_{16} and C_{18} MUFA). There was a smaller proportions of LC-MUFA in the outer blubber layer ($t_{10}=4.37$; $P=0.001$), but a greater proportion of SC-MUFA in the outer layer ($t_{10}=22.36$; $P<0.001$). On the basis of the ratio of total

Table 1
FA profiles from 11 female southern elephant seals from Macquarie Island at the beginning of the 2001 breeding season (October)

FA	Inner blubber layer (mean %)	Inner S.D.	Outer blubber layer (mean %)	Inner S.D.
14:0	4.0	1.8	2.8	0.7
16:1ω7	5.2	1.3	7.6	1.5
16:0	11.1	1.0	7.9	0.9
18:2ω6	1.5	0.3	1.8	0.2
18:1ω9	25.3	1.2	34.9	1.7
18:1ω7	7.1	0.6	7.2	0.4
18:1ω5	0.6	0.1	0.5	0.0
18:0	3.3	0.4	2.2	0.2
20:4ω6 AA	0.4	0.1	0.4	0.1
20:5ω3 EPA	4.6	0.9	2.2	0.5
20:4ω3	0.7	0.1	0.5	0.0
20:1ω9[a]	15.2	3.2	14.8	2.7
20:1ω7	0.8	0.1	0.6	0.1
22:6ω3 DHA	5.5	0.6	4.6	0.9
22:5ω3 DPA	1.7	0.2	1.7	0.3
22:1ω11	3.8	0.9	2.3	0.5
22:1ω9	2.0	0.3	1.3	0.2
24:1ω11	0.6	0.1	0.3	0.1
24:1ω9	0.8	0.1	0.4	0.1
Trace (<0.5%)	5.3	0.4	5.0	0.3
Sum SFA	20	2	15	1
Sum SC-MUFA	40	2	52	2
Sum LC-MUFA	24	5	21	4
Sum PUFA	16	2	13	2

Only those FA contributing >0.5% of the total are shown. Values are means and S.D.

[a] Includes 20:1ω11.

MUFA to total PUFA, the inner and outer layers fall into distinguishable groups (Fig. 2). Two points fell outside the main grouping of the ratio of MUFA to PUFA for each blubber layer, and these points belong to one individual seal—C699. The FA composition of this animal displayed notable differences, having a higher or higher-than-average value of many FA including 14:0, 16:1ω7, 20:1ω9, 20:5ω3 and 22:6ω3 (Table 1).

3.3. Differences in individual fatty acids between blubber layers

The most abundant FA within the inner blubber layer (Table 1) were 16:1ω7, 18:1ω9, 18:1ω7, 20:1ω9 and 22:1ω11 (≈89% of total MUFA, ≈57% of total FA), 14:0, 16:0 and 18:0 (≈91% of total SFA, ≈18% of total FA), and 20:5ω3 and 22:6ω3 (≈47% of total PUFA, ≈10% of total

FA). In total, these 9 components represented approximately 62% of total FA. Greater proportions of ω3 PUFA were present than ω6 PUFA (≈79% compared to ≈17% total PUFA).

3.4. Multivariate comparisons of fatty acid composition

A plot of the first two PC divided the inner and outer blubber samples into distinct groups (Fig. 3). PC1 accounted for 52% of the variation of FA composition among individuals, and the addition of PC2 accounted for 79% of the total sample variation. The cumulative variation explained with the addition of the third PC was 89%. As in Fig. 2, the two outlying points corresponded to the individual C699 (Fig. 3). The observed variation between layers could be explained largely by PC1; positive values were indicative of a FA combination for the outer blubber layer, while negative values of PC1 were indicative of the inner blubber. The 3 most positive values driving the layer separations were LC-MUFA 22:1ω11, 24:1ω11, and the SFA18:0 (Table 2; Fig. 3).

Conversely, the three most negative values (indicating outer blubber) were the SC-MUFA 16:1ω7 and 18:1ω9, and the PUFA 18:2ω6 (Table 2; Fig. 3). These trends reflect the relative differences in abundances of shorter-chain and long-chain FA in the inner and outer layers. The inner blubber layers contained a mean value of 3±0.4% (inner) and 2±0.2% (outer) of the SFA 18:0. The most abundant FA in both layers was 18:1ω9 (25±1%, inner; 35±2% outer).

4. Discussion

4.1. Fatty acid composition

The range of FA components found in the blubber of *M. leonina* was identical to those reported in other pinnipeds (e.g. West et al., 1979; Kakela et al., 1993; Iverson et al., 1995, 1997a; Walton et al., 2000). Similarly, the range of FA contained in *M. leonina* was also consistent with those observed in cetaceans (e.g. Lockyer et al., 1984; Borobia et al., 1995; Dahl et al., 2000; Hooker et al., 2001). The most abundant FA in *M. leonina* (16:0, 16:1ω7, 18:1ω9, 20:1ω9 and 22:6ω3) were also present in the greatest amounts in the blubber of grey (*Halichoerus grypus*; Walton et al., 2000), harbour (*Phoca vitulina*; Iverson et
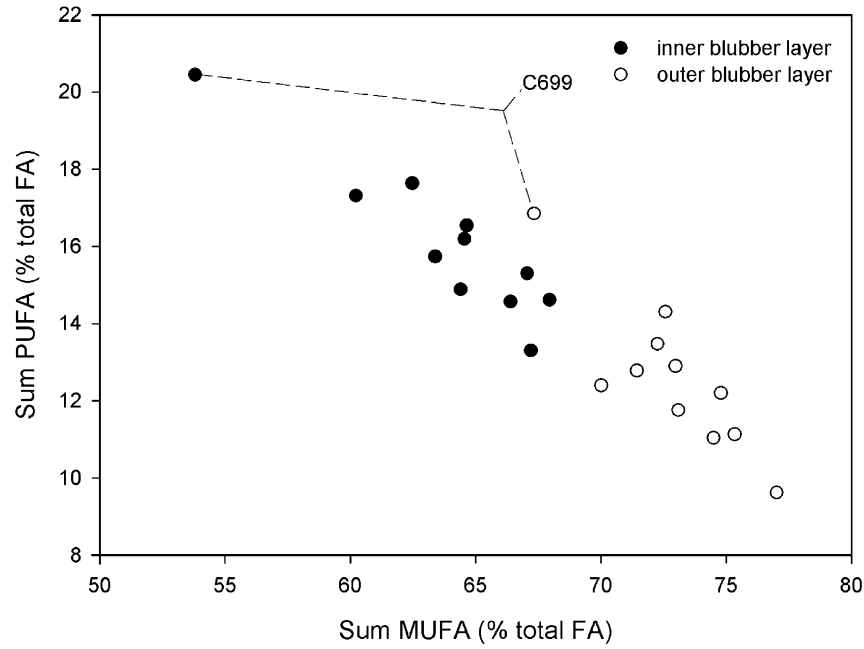
Fig. 2. Sum of the monounsaturated fatty acids (sum MUFA) versus the sum of the polyunsaturated fatty acids (sum PUFA) as a percentage of total FA, for the inner (filled circles) and outer (open circles) blubber layers of southern elephant seals *M. leonina* ($n =$ 11). Shown also are the extreme values for elephant seal number C699.
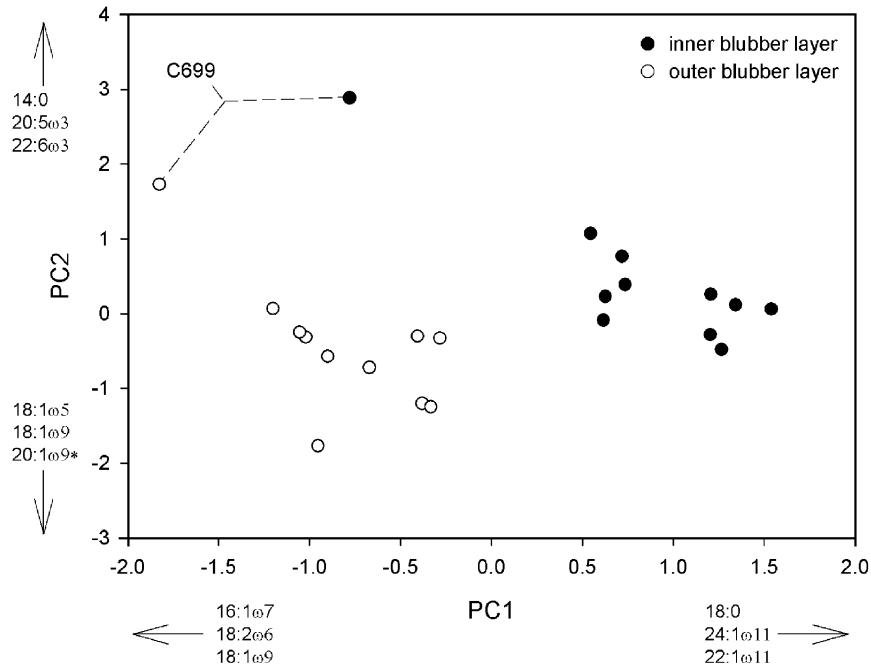

Fig. 3. Biplot of first and second PC derived from the FA composition of inner (filled circles) and outer (open circles) blubber layers from southern elephant seals, *M. leonina* ($n = 11$). PC1 explained 52% of the total variation and the addition of PC2 explained 79% of the total variation between blubber layers. The three FA with the most extreme positive and negative loadings (Eigen values) for each PC are shown for each axis. Shown also are the extreme values for elephant seal number C699.

Table 2
Eigen values from PC analysis of the inner and outer blubber layers of the southern elephant seal (*M. leonina*) ($n = 11$)

FA	PC1	PC2	PC3
16:1ω7	*−0.92*	0.26	0.14
18:2ω6	*−0.86*	0.20	−0.31
18:1ω9	*−0.81*	*−0.55*	0.19
22:5ω3	−0.43	*0.68*	0.28
18:1ω7	−0.12	−0.30	−0.78
20:4ω6	−0.07	0.60	0.71
14:0	−0.03	*0.82*	−0.47
22:6ω3	0.22	*0.87*	0.29
20:1ω9[a]	0.51	*−0.82*	0.04
18:1ω5	0.56	*−0.60*	0.43
20:5ω3	0.58	0.78	−0.14
16:0	0.64	0.63	−0.26
20:4ω3	0.84	0.40	0.05
20:1ω7	0.89	−0.21	0.08
22:1ω9	0.95	0.04	0.08
24:1ω9	0.95	0.05	−0.09
18:0	*0.96*	0.05	−0.16
24:1ω11	*0.97*	−0.13	0.03
22:1ω11	*0.97*	−0.17	0.04

Data are sorted by Eigen values for PC1; FA with the three most extreme positive and negative loadings on PC1 and PC2 are given in italic type.

[a] Includes 20:1ω11.

al., 1997b), spotted (*Phoca largha*; West et al., 1979), ribbon (*Phoca facitata*; West et al., 1979) and Antarctic fur seals (*Arctocephalus gazella*; Iverson et al., 1997a).

The FA composition of milk from *M. leonina* (Brown et al., 1999) showed slight differences to the blubber samples presented in this study. These differences were the presence of 16:1ω11 in milk and absence of the FA 15:0, 17:0, 19:1, C_{21} PUFA, C_{24} PUFA and 24:1 in milk. Preliminary observations of blubber FA by Bryden and Stokes (1969) for *M. leonina* did not present data for those components. In our study, only the 24:1 FA of those mentioned above were present in the blubber in greater than trace (0.5%) amounts. These FA were also important variables in explaining the variance between inner and outer blubber layers, and were in highest concentrations in the inner layer. The C_{24} FA were also found in *M. leonina* in a previous investigation of blubber (Wilson, 1996). Therefore, it is possible that the C_{24} components were not identified in previous studies due to differences in methodology (Bryden and Stokes, 1969), or possibly because C_{24} FA did not occur in detectable amounts in milk samples (Brown et al., 1999).

Of interest was the greater relative proportion of PUFA, and the lower proportion of total MUFA in both layers of the blubber sample derived from seal C699 (Figs. 2 and 3). This individual was the heaviest (691 kg) of the 11 females weighed upon return to Macquarie Island (mean of others = $512 \pm$ S.D. = 59 kg; C.J.A. Bradshaw and M.A. Hindell, unpublished data). Further, location data from this individual indicated that she spent much of the post-moult trip foraging directly over the relatively shallow waters of the Antarctic continental shelf between 130 and 150° east longitude and $\sim 60°$ south latitude (C.J.A. Bradshaw and M.A. Hindell, unpublished data). We hypothesize that her foraging pattern may have resulted in a diet dominated by more benthic, rather than pelagic prey species, thus modifying her FASA signature relative to the others in the sample. More research is required to test this hypothesis.

4.2. Role of stratified blubber

Blubber has three primary functions in marine mammals: energy storage, insulation and buoyancy (Iverson, 2002). Blubber depot fats are the principal source of metabolic energy; *M. leonina* fast twice annually and rely heavily on the lipid stores within their blubber layer for energy during this time (Le Boeuf and Laws, 1994). *M. leonina* has only sparse guard hairs (relative to many other species of pinniped) that do not contribute greatly to heat retention. Therefore, blubber fats provide the majority of insulation (Ling, 1965). The lipid content of the blubber layer also affects buoyancy; 'fat' seals have been shown to sink more slowly than 'lean' seals (Crocker et al., 1997; Webb et al., 1998; Beck et al., 2000).

Blubber FA originate from two processes; either by direct deposition from dietary lipids, or by biosynthesis within the layer (Allen, 1976; Iverson, 1993; Koopman et al., 1996). FA will be retained and accumulate in the blubber when the animal is in a state of net energy gain (Young, 1976). If the animal is in a state of net energy loss (e.g. during the breeding and or moulting fast), blubber FA will be mobilized and used to provide energy (Young, 1976). The observed differences in FA composition between inner and outer blubber sections have two likely determinants: (1) blubber may be stratified biochemically due to differential rates of component turnover within the layers (Koopman et al., 1996); and alternatively (2)

structural demands could potentially impact the FA distribution within blubber (Fredheim et al., 1995).

Fredheim et al. (1995) proposed that in terms of FA composition, layering of blubber in pinnipeds is due mainly to temperature regulation. Grey, harbour, ringed (*Phoca hispida*) and harp seals (*Pagophilus groenlandica*) were identified as having much higher proportions of SC-MUFA in the outer layer compared to the inner blubber layer (Fredheim et al., 1995), and *M. leonina* in our study exhibited a similar trend in distribution of SC-MUFA. Unsaturated FA have lower melting points (mp) than SFA of the same chain length, and FA with lower chain lengths have lower mp (Stryer, 1988). A lower mp is advantageous in maintaining membrane fluidity, and also reducing the potential for heat loss across the body surface. This may account for the extreme stratification between inner and outer regions of blubber for SC-MUFA (Fredheim et al., 1995).

It is well accepted that PUFA, much more than MUFA, have an important role in combating temperature gradients between body and ambient temperature, at least in fish (Sigurgisladottir and Palmadottir, 1993; Delgado et al., 1994; Dunstan et al., 1999; Kamler et al., 2001). The proportion of PUFA in fish is inversely related to the temperature of water to which fish are exposed. In relatively cold waters, fish have higher relative levels of PUFA than fish that inhabit warmer waters. *M. leonina* is a good test of the hypothesis that temperature affects the FA composition of blubber for sea-going mammals. The variation in latitudinal distribution of *M. leonina* (Bradshaw et al., 2002), ranging from 40 to 73° S, is comparable to that demonstrated by Northern Hemisphere pinnipeds (e.g. Gentry, 1998; Le Boeuf et al., 2000). Thus, the range in ambient temperatures that these species experience is similar (Bradshaw et al., 2002; Hall, 2002). If PUFA (especially short-chain PUFA) in the blubber are important for conserving heat, a higher relative proportion of PUFA would be expected in the outer portion of blubber, which was not the case with *M. leonina* in our study.

Membrane fluidity is also influenced by PUFA that are in the form of phospholipids (PL—Delgado et al., 1994). The adipose tissue of *M. leonina* contains approximately 99% triacylglycerol and only minor levels of PL (Wilson, 1996; N. Best, unpublished data). We hypothesize that either (a) storing PUFA in the blubber of *M. leonina* provides little benefit regarding insulation, or (b) relatively greater proportions of PUFA in the inner blubber layer may be used to regulate the higher temperature of the body interior.

The inner blubber is the region where FA are initially deposited and subsequently mobilized (Ackman et al., 1975b; Lockyer et al., 1984; Koopman et al., 1996). The FA composition in this layer is therefore in a constant state of flux and has the highest turnover rates. The outer blubber region is considered more stable (Koopman et al., 1996; Hooker et al., 2001). Dietary FA are most likely deposited initially in the inner region. 'Surplus' FA from the diet may accumulate first in the inner region during periods of 'fattening' or net weight gain (Ackman et al., 1975b). In our study, blubber of *M. leonina* was sampled close to the time when the animals first return to land after the winter foraging trip, so the blubber layer was potentially close to maximum lipid content. There was a significantly greater proportion of PUFA in the inner part of the blubber layer immediately after this time. A higher proportion of 'surplus' dietary PUFA in the inner blubber layer is evidence that this is the area of greatest metabolic activity; surplus dietary FA are rapidly deposited (although not mobilized) in the inner blubber layer of a rapidly fattening seal.

M. leonina has been shown to mobilize long-chain PUFA preferentially during the lactation period (Bryden and Stokes, 1969); however, that study examined the blubber layer as a whole. To determine whether the inner blubber layer is the site of FA mobilization, blubber samples should be obtained from individual animals at the beginning and at the end of the lactation period. Because the inner blubber layer would be expected to exhibit a greater turnover in FA relative to the outer layer, then PUFA should be depleted in the inner blubber layer during the fast. Similarly, investigating male animals, or investigating this process over the moulting period, would determine the degree of FA metabolism from different regions of blubber. Studying both periods (lactation and non-lactation) would be more conclusive than only using one period of the annual cycle. The mobilization of specific FA preferentially for milk production as opposed to the need to simply produce energy for the mother could be assessed by making a comparison between the two periods.

4.3. Comparison among pinniped species

Trends in FA distributions of *M. leonina* follow broadly those observed in northern pinniped species (Kakela et al., 1993; Fredheim et al., 1995). Here, the blubber closest to the muscle layer is less unsaturated than the superficial layer that is closest to the epidermis. This stratification was also observed in another investigation of the ringed seal and the freshwater saimaa ringed seal (*Phoca hispida saimensis*), but on a much-reduced scale (Kakela et al., 1993). Additionally, the difference between the two layers in SFA (≈ 15–20%; outer to inner) was similar in four other species of marine mammal (Fredheim et al., 1995). No significant differences in the proportion of PUFA in the inner blubber layer compared to the outer layer were observed in ringed, harp, harbour or grey seals (Fredheim et al., 1995).

Body condition at the time of sampling could have a strong influence on the magnitude of stratification; animals that do not have the stress of starvation during their breeding cycle may not need to accumulate as many reserves as fasting species like *M. leonina*. The observed differences between different pinniped species could be explained by the consumption of more lipid-rich prey by *M. leonina*.

4.4. Implications of blubber stratification for the application of fatty acids as dietary indicators

FA have been used increasingly in recent years as tools to investigate aspects of diet in top-level marine predators (Iverson et al., 1997b; Dahl et al., 2000; Walton et al., 2000; Hooker et al., 2001). In those studies employing blubber FA as dietary indicators, the implication of blubber stratification has largely been ignored. The theory behind the use of FA as dietary tracers relies on the integration of dietary FA from the prey, with minimal modification of the FA components in the process (Iverson, 1993). Stratification of blubber gives strong evidence that the blubber layer as a whole is not necessarily a direct reflection of the diet, and that selective processes bias the FA information that is incorporated from the diet. The magnitude and direction of this bias is unknown, and the present study did not address this particular question specifically; rather, the aim was to investigate the degree to which FA composition varied within two, coarse-scale strata of the blubber. As

the inner blubber region is the site for greatest FA turnover, it has been proposed that the outer blubber region is more representative of longer-term diet (Borobia et al., 1995; Hooker et al., 2001). Notwithstanding, our results confirm that the inner blubber layer is an important source of dietary FA for southern elephant seals.

Sampling free-ranging whales often does not allow the collection of full blubber cores, and the outer blubber region is perhaps all that can be obtained in any biopsy procedure of a free-ranging cetacean. Obtaining biopsies of live cetaceans also typically yields only part of the outer blubber layer, which may be less than 25% of the blubber thickness (Hooker et al., 2001). However, obtaining full blubber cores is achievable for pinnipeds that are ashore for breeding, resting or moulting using the outlined and validated biopsy procedure.

The degree of stratification should be determined in a greater range of other species. It may be that biochemical stratification is less in other animals, and thus sampling the outer blubber region may not achieve a significantly different FA profile compared to analysis of the whole blubber layer. This study has highlighted that due to the presence of biochemical stratification in the blubber of *M. leonina*, the use of FASA requires obtaining samples of, or including, the inner blubber layer to maximize the potential identification of dietary shifts in time and space. Thus, standardization of the sampling method by taking at least the whole blubber core is recommended for future studies.

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