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Journal of Experimental Marine Biology and Ecology 352 (2007) 103 – 113

Journal of EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY

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Temporal variation in the vertical stratification of blubber fatty acids alters diet predictions for lactating Weddell seals

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Received 1 July 2007; accepted 10 July 2007

Abstract

Fatty acid signature analysis of blubber has been used to study the foraging ecology of some marine mammals. However, species-specific information on fatty acid (FA) deposition, distribution and mobilization is required to develop further the application of FA as trophic markers within the marine environment. Blubber samples were collected from adult female Weddell seals post-parturition and end of lactation, and were divided into inner and outer half sections. We determined the degree to which there was vertical stratification in FA composition, and how this changed over the lactation period. Inner and outer layers of post-parturition blubber cores separated into two distinct groups. Sixty-two per cent of the dissimilarity between the two layers was accounted for by a higher abundance of monounsaturated fatty acids (18:1ω9c and 16:1ω7c) in the outer blubber layer, and more saturated fatty acids (16:0 and 14:0) in the inner layer. By end of lactation, the FA composition of the inner layer was different to post-parturition samples, and 20:5ω3 had the highest fractional mobilization of all FA. In contrast, the proportion of FA in the outer layer did not change, and there was more variability in the fractional mobilization of FA indicating mobilization was not uniform across the blubber layer. Dietary predictions changed considerably when highly mobilized FA were removed from analyses, and predictions were more consistent with previous dietary studies. The lack of uniformity in FA mobilization adds problems to the future use of FASA in dietary predictions, highlighting the need for more detailed information on FA mobilization.

Keywords: Blubber; Diet; Fatty acids; Lactation; Mobilization; Stratification

1. Introduction

Marine birds and mammals have been of increasing interest in ecosystem studies because of the premise that temporal shifts in their behaviour and physiology reflect the amplitude and timing of climate variability and change (Croxall, 1992; Hindell et al., 2003). In particular, variation in diet composition is expected to aid in the assessment of abundance and demographic shifts in lower trophic level taxa (*i.e.*, prey). A necessary precursor to this aim is an assessment of the accuracy and reliability of methods to measure diet variation (*e.g.*, Bradshaw et al., 2003) so that

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doi:10.1016/j.jembe.2007.07.005

they can be applied across different taxa and ecosystems. The diet of marine birds and mammals has been determined traditionally through the analysis of stomach contents and prey remains in faeces (Coria et al., 1995; Field et al., 2007; Lake et al., 2003). Several drawbacks occur with these approaches: (1) remains in stomachs and faeces only represent prey consumed over a short period of time (*i.e.*, days to weeks; Hammond and Rothery, 1996), (2) hard parts (*e.g.*, fish otoliths, cephalopod beaks) are more recognizable and therefore, possibly over-represented than partially digested soft tissue (Hyslop, 1980), (3) differential passage rates of different prey species bias estimates of frequency of occurrence (Harvey and Antonelis, 1994), and (4) taxonomic identification can be difficult and time consuming.

To alleviate problems associated with traditional diet analyses, biochemical approaches have been developed. Fatty acid signature analysis (FASA) has been of interest from both nutritional and tropho-dynamic perspectives, with the application of fatty acids (FA) as trophic markers to trace or confirm many different marine predator–prey relationships from secondary producers to upper trophic level predators (Ackman et al., 1970; Auel et al., 2002; Iverson et al., 1997; Lea et al., 2002; Nelson et al., 2001; Ruchonnet et al., 2006). In essence, FASA assumes that base lipid constituents, *i.e.*, fatty acids, are incorporated into the tissues of predators conservatively so that a predator's FA composition will reveal the dietary source of lipids. If the prey-to-predator lipid transfer is traceable, identification of ingested species can enable a description of trophic interactions and food webs (Bradshaw et al., 2003; Iverson et al., 1997).

Using FASA to determine diet composition is not straightforward, because (1) several FA are biosynthesized *de novo*, possibly altering the FA signature of the predator, (2) stratification of FA within the blubber has been observed in many species (Best et al., 2003; Birkeland et al., 2005; Grahl-Nielsen et al., 2003; Olsen and Grahl-Nielsen, 2003), indicating components of blubber are synthesized independently of diet, (3) rates of mobilization and breakdown of FA can vary according to life history stage and environmental context (Iverson et al., 1995; Pierce and McWilliams, 2005; Samuel and Worthy, 2004; Wheatley et al., in

Table 1
Average fatty acid composition (%) of the inner and outer blubber layer of Weddell seals at post-parturition and end-lactation

Fatty acid	Post-partum				End-lactation				Change			
	Inner	$n=19$	Outer	$n=19$	Inner	$n=10$	Outer	$n=10$	Inner	$n=10$	Outer	$n=10$
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:1ω5c	0.9	0.08	1.9	0.12	0.4	0.07	1.1	0.11	0.4	0.14	0.9	0.13
14:0	8.0	0.58	6.3	0.37	3.6	0.47	3.8	0.39	4.9	1.12	3.2	0.45
i15:0	0.3	0.02	0.3	0.01	0.2	0.02	0.2	0.02	0.1	0.04	0.1	0.02
16:1ω9c	0.3	0.01	0.3	0.01	0.1	0.02	0.2	0.02	0.1	0.02	0.1	0.02
16:1ω7c	10.1	0.52	13.0	0.49	3.3	0.54	8.0	1.02	7.2	0.92	5.9	0.95
16:1ω5c	0.3	0.01	0.3	0.01	0.1	0.02	0.2	0.03	0.2	0.03	0.2	0.03
16:0	8.5	0.46	5.7	0.28	3.2	0.44	3.6	0.47	6.0	0.75	2.5	0.45
i17:0	0.2	0.01	0.2	0.01	0.1	0.01	0.1	0.01	0.1	0.01	0.1	0.02
18:4ω3	0.9	0.03	0.9	0.04	0.3	0.05	0.5	0.07	0.6	0.06	0.4	0.06
18:2ω6	1.5	0.06	1.6	0.04	0.8	0.10	1.1	0.12	0.7	0.13	0.6	0.12
18:1ω9c	25.3	1.21	28.5	0.63	12.6	1.72	18.4	2.12	14.1	2.07	10.7	2.15
18:1ω7c	6.0	0.23	6.4	0.22	2.7	0.34	4.0	0.44	3.5	0.46	2.4	0.48
18:1ω5	0.5	0.02	0.5	0.02	0.2	0.03	0.3	0.04	0.3	0.03	0.2	0.04
18:0	1.1	0.04	0.7	0.03	0.6	0.08	0.4	0.05	0.5	0.08	0.3	0.06
20:4ω6	0.3	0.02	0.3	0.02	0.1	0.02	0.2	0.03	0.2	0.03	0.1	0.03
20:5ω3 EPA	3.2	0.18	2.8	0.18	0.6	0.13	1.6	0.26	2.6	0.25	1.3	0.22
20:4ω3	0.2	0.03	0.3	0.03	0.1	0.02	0.2	0.03	0.1	0.02	0.1	0.03
20:2ω6	4.0	0.16	4.1	0.19	1.1	0.04	0.6	0.07	0.5	0.07	0.0	0.07
20:1ω9c	4.5	0.22	3.7	0.14	3.4	0.43	2.5	0.29	1.4	0.37	1.3	0.32
20:1ω7c	0.5	0.02	0.4	0.01	0.4	0.04	0.3	0.02	0.2	0.04	0.1	0.03
22:6ω3 DHA	4.0	0.17	4.1	0.20	2.3	0.28	2.6	0.26	1.8	0.25	1.5	0.32
22:5ω3 DPA	1.2	0.15	1.4	0.14	0.8	0.11	0.8	0.10	0.3	0.07	0.5	0.13
22:1ω11c[a]	0.8	0.05	0.4	0.03	0.6	0.06	0.3	0.03	0.2	0.07	0.1	0.04
22:1ω9c	0.6	0.04	0.4	0.03	0.5	0.07	0.3	0.04	0.0	0.00	0.1	0.05
24:1	0.2	0.02	0.1	0.01	0.2	0.02	0.1	0.01	0.0	0.02	0.0	0.02

SEM = standard error of the mean.
[a] Includes 22:1ω13c.

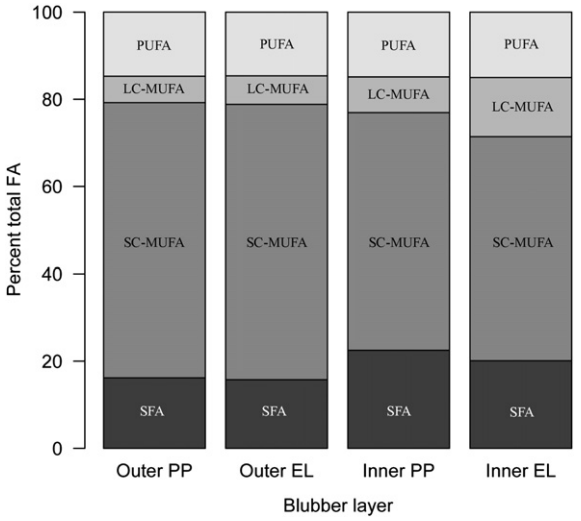

Fig. 1. Mean proportion of polyunsaturated (PUFA), long-chain monounsaturated (LC-MUFA), short-chain monounsaturated (SC-MUFA) and saturated (SFA) fatty acids in the inner and outer blubber layer at post-partum (PP) and end-lactation (EL).

press-b), and (4) molecular structure can alter FA mobilization patterns (Raclot, 2003; Raclot and Groscolas, 1993; Staniland and Pond, 2005). At higher trophic levels, markers may also become obscured because accumulated FA can originate from a variety of dietary sources and dietary FA signatures may be altered through *de novo* biosynthesis, metabolization and breakdown (Dalsgaard et al., 2003). Quantifying trophic relationships using FA therefore requires species-specific information on FA dynamics such as stratification in sampled tissues (Best et al., 2003), deposition rates and patterns (Iverson et al., 2004; Budge et al., 2004) and differential utilization patterns (Birkeland et al., 2005; Wheatley et al., in press-b).

Although some aspects of FASA have been applied successfully to phocid seals, their blubber composition is highly dynamic owing to their reliance on stored reserves for lactation. Further, highly stratified blubber (*e.g.*, Best et al., 2003) with differential mobilization or deposition rates among species has important repercussions for diet estimation. The diet itself may also play an important role in modifying energy expenditure because specific lipids may offer different characteristics in terms of energy density and oxidation rates (Maillet and Weber, 2006). Weddell seals (*Leptonychotes weddellii*) in particular are subject to high inter-annual variability in resource abundance ensuing from environmentally mediated prey availability (Pinaud and Weimerskirch, 2002). The resulting variability in diet composition affects reproductive performance and population size (Hindell et al., 2003; Le Boeuf and Crocker, 2005; Reid et al., 2005).

Being easily accessible for capture and measurement during breeding makes this species an ideal candidate to examine over-winter diet, lactational changes in fatty

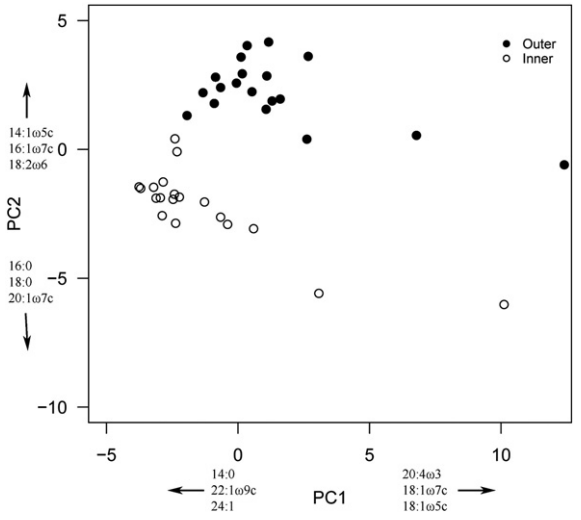

Fig. 2. Principal component plot for the inner and outer blubber layer of Weddell seals collected post-parturition. The first principal component (PC1) explained 49.0% of the total variation and the second principal component (PC2) explained 28.0% of the variation between the blubber layers. The three fatty acids with the most extreme positive and negative loadings (eigen values) for PC1 and PC2 are shown along the axes.

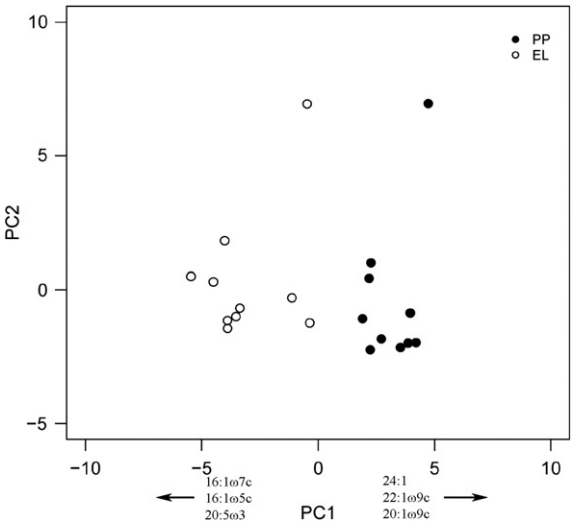

Fig. 3. Principal component plot for fatty acid changes in the inner blubber layer of Weddell seals between post-partum (PP) and end-lactation (EL). The three fatty acids with the most extreme positive and negative loadings (eigen values) for the first principal component (PC1) are shown along the axis.

Table 2
Fractional mobilization (%) of fatty acids (FA) from the inner and outer blubber layer during lactation

	Inner blubber layer								
Seal ID	Y536	W636	Y965	Pu194	Y4295	Pu114	P871	P130	Pu761
Fatty acid									
14:1ω5c	9.01	53.70	61.77	52.65	51.05	**78.63**	**77.93**	**62.90**	59.74
14:0	41.74	64.31	63.88	66.05	58.37	**75.96**	72.60	**59.52**	56.28
i15:0	30.19	53.24	58.33	55.84	47.00	62.43	55.66	39.64	44.57
16:1ω9c	42.18	64.95	70.57	60.42	52.61	63.26	62.65	41.99	56.04
16:1ω7c	54.34	**78.38**	**84.47**	71.97	**72.65**	75.75	74.28	57.40	**75.19**
16:1ω5c	**56.50**	**78.80**	**83.43**	**72.51**	**72.48**	73.90	**79.81**	57.20	**73.48**
16:0	**57.02**	75.26	78.95	**71.99**	68.68	69.68	67.29	50.11	67.58
i17:0	50.10	62.25	73.62	44.05	56.81	49.59	59.46	33.37	56.19
18:4ω3	49.95	72.10	80.05	69.40	66.23	68.71	65.99	47.40	67.06
18:2ω6	28.24	57.01	65.73	55.81	47.92	50.36	45.54	22.01	45.31
18:1ω9c	35.30	63.91	72.47	60.66	55.47	57.62	54.48	32.22	56.60
18:1ω7c	42.89	67.05	74.94	63.70	58.85	60.74	57.32	35.88	59.52
18:1ω5	39.04	61.73	71.84	60.08	56.91	57.80	56.78	37.54	55.47
18:0	39.53	59.35	65.48	58.19	51.25	51.21	47.62	25.55	43.44
20:4ω6	45.13	71.82	74.83	63.40	61.43	63.09	59.16	43.56	59.61
20:5ω3 EPA	**71.90**	**88.24**	**91.58**	**81.43**	**83.77**	**83.39**	**83.42**	**69.59**	**85.86**
20:4ω3	38.16	68.82	77.97	63.15	59.81	58.65	63.87	50.13	63.30
20:2ω6	−4.11	35.62	43.98	34.09	23.92	34.01	25.25	−1.97	16.91
20:1ω9c	30.77	35.55	52.13	46.30	29.86	38.00	29.06	3.03	26.21
20:1ω7c	19.55	42.55	50.06	45.82	27.20	37.00	26.02	0.83	25.25
22:6ω3 DHA	30.37	50.16	63.50	55.12	45.54	53.28	50.62	27.77	43.65
22:5ω3 DPA	24.22	36.98	53.93	43.13	33.39	42.89	40.15	13.38	27.72
22:1ω11c[a]	29.09	45.17	44.61	48.89	32.12	37.71	30.70	0.78	22.04
22:1ω9c	18.85	41.44	41.14	41.70	23.71	26.66	13.39	−17.49	15.61
24:1	−5.24	6.05	−4.57	32.71	10.19	13.30	17.57	−25.72	−10.00

Boldface designates the three FA with the highest fractional mobilization for each individual.
[a] Includes 22:1ω13c.

acid composition and feeding during lactation. We investigated the change in fatty acid composition of Weddell seal blubber during lactation specifically to assess characteristics of differential mobilization and its implications for diet interpretation. We aimed to determine (1) the extent of fatty acid stratification in the blubber of female Weddell seals; (2) if particular fatty acids were selectively mobilized from the inner compared to the outer blubber layer during lactation and; (3) how mobilization affected relative diet predictions.

2. Materials and methods

2.1. Sample collection

This study was conducted at Hutton Cliffs, Antarctica (77° 51′ S, 166° 45′ E) during the austral summer (October to December) of 2003. Blubber samples were collected from lactating female Weddell seals, captured 1 to 6 (mean 3.8±0.22) days post-parturition (n=19) and again near the end of lactation (n=10; 36 to 38 dpp; $\bar{x}$±SEM=36.9±0.26). Each animal was captured, immobilized and measured as described in Wheatley et al. (2006b).

Blubber biopsies were taken from the posterior flank of each animal by making a small (~1 cm) incision with a scalpel blade in an anterior–posterior direction. A 6-mm biopsy punch was inserted through the incision, and a core was taken from the whole blubber layer (*i.e.*, through until the muscle layer was reached). In the laboratory, the blubber core was extended to its full length without stretching and cut into two approximately equal pieces, assessed visually. There were no visible differences (*e.g.*, colour, opacity, texture) between the outer portion (closest to the skin) to the inner portion (closest to the muscle) of the cores. Each sample was stored in a pre-weighed glass vial (with a Teflon coated lid), containing a solution of 2:1 v/v chloroform and methanol, and 0.05% (by weight) butylated hydroxytoluene (BHT; Sigma, St. Louis, USA). Vials were reweighed and all samples were stored at −20 °C until laboratory analysis. We found no difference between the weight of the outer and inner portion (generalized linear mixed-effects model, information-theoretic evidence

Outer blubber layer								
Y536	W636	Y965	Pu194	Y4295	Pu114	P871	P130	Pu761
28.01	45.03	43.58	68.95	26.03	40.00	**51.96**	**74.72**	**61.17**
39.39	48.27	36.89	72.43	35.04	**43.67**	**42.44**	**69.23**	55.94
36.18	52.11	34.69	70.57	30.29	40.31	26.72	**57.85**	55.07
37.54	55.41	40.56	70.68	29.51	38.85	28.14	48.71	57.77
41.02	**60.67**	41.02	69.60	37.96	42.03	**32.87**	51.22	**62.28**
40.83	59.92	**44.67**	70.50	38.44	41.86	29.40	50.88	60.90
45.93	59.98	33.22	72.42	**41.16**	44.39	22.64	46.46	56.71
44.05	59.12	44.50	66.83	32.30	**55.93**	12.23	37.88	59.24
39.17	**61.22**	44.40	71.22	**38.88**	43.30	20.62	43.79	59.48
38.09	57.46	35.34	70.32	29.38	40.39	17.09	39.44	54.42
40.12	58.07	33.70	70.35	29.97	40.76	18.57	39.80	54.99
40.76	58.55	34.62	70.08	31.46	41.51	18.80	40.25	55.66
38.16	57.53	36.96	69.81	31.32	39.47	15.74	42.30	55.65
43.25	58.45	29.45	**73.52**	38.40	43.14	8.05	38.79	49.36
33.70	56.57	42.24	70.78	35.75	40.90	9.23	43.85	57.53
40.31	**66.78**	**46.80**	72.46	**49.83**	**46.52**	27.54	48.75	**65.74**
29.78	51.98	**46.05**	**74.23**	30.63	40.54	5.29	47.35	58.68
94.77	54.64	35.72	**72.73**	22.86	41.42	−2.00	32.80	46.14
40.97	48.26	27.09	72.00	25.92	43.17	2.44	35.36	46.15
37.97	55.10	30.93	71.52	25.93	42.42	4.27	37.03	46.55
35.04	57.78	38.55	73.10	31.71	42.01	9.69	39.13	53.20
33.63	55.40	41.25	**74.30**	24.77	40.16	6.60	35.90	51.83
39.46	56.67	22.03	**74.48**	35.06	**45.83**	−3.96	36.57	41.92
38.56	57.14	21.51	73.47	30.56	44.36	−2.55	4.66	39.02
33.03	52.78	**48.15**	51.72	34.00	26.08	7.81	34.59	53.23

ratio [see below] = 0.32); therefore, samples appeared to be separated evenly.

2.2. Laboratory and data analyses

Blubber lipids were extracted quantitatively using a modified overnight (Bligh and Dyer 1959) one-phase methanol/chloroform/water extraction. Following extraction, lipid was *trans*-methylated to produce fatty acid methyl esters (FAME) and analyzed using gas chromatographic (GC) and GC-mass spectrometric analyses (see Wheatley et al., in press-b). The concentration of individual FA was converted to a per cent of total FA mass, and FA present in trace amounts (<0.5%) were excluded from analyses. These proportions were used for analyses between the inner and outer blubber layer. However, the overall lipid content of the blubber samples changed (decreased) over lactation, so FA expressed as a percentage of mass composition did not accurately show the changes in FA during the two sampling periods. Lipid class results indicated that blubber was composed virtually entirely of triacylglycerol (TAG; 99.9%). TAG stored in the tissue consists of glycerol esterified with three FA molecules, and the FA moieties represent about 95% of the mass of TAG (Groscolas, 1990). Therefore, the lipid stores (kg) of each animal, as determined from hydrogen isotope dilution techniques (see Wheatley et al., 2006a), were multiplied by 95% to obtain the mass (kg) of FA in each individual, at each capture. All proportional values were arcsine-square-root transformed before analysis.

From evidence that females do not feed during the first 3 weeks of lactation (Eisert et al., 2005), we calculated a 'feeding index' for the second half of lactation based on differences in milk energy output values, which represented the number of times that milk energy (%) exceeded that of non-feeding individuals. This allowed us to rank the relative amount of feeding that occurred among individuals (see Wheatley et al., in press-a) and was used as a covariate in models constructed to explain variation in FA principal component scores (see below).

Principal Components Analysis (PCA) was used on proportional (% of total FA) and absolute (kg) values to investigate patterns of FA in the blubber layers and over

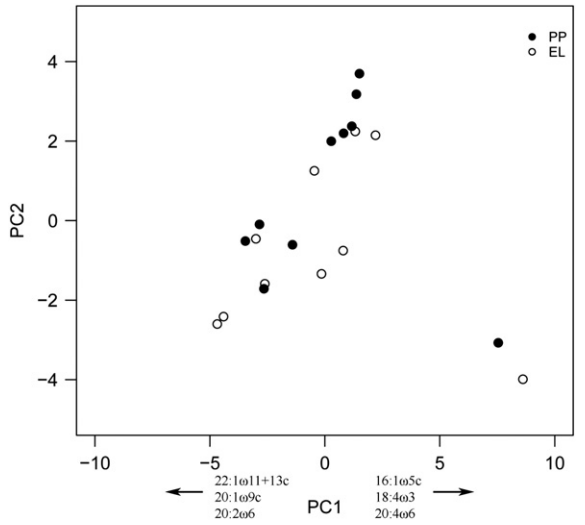

Fig. 4. Principal component plot for fatty acid changes in the outer blubber layer of Weddell seals between post-partum (PP) and end-lactation (EL). The three fatty acids with the most extreme positive and negative loadings (eigen values) for the first principal component (PC1) are shown along the axis.

time. Principal component scores were used in a series of generalized linear and mixed-effects models (GLM, GLMM) to examine differences in FA composition and mobilization. Examination of the residuals for all models determined the statistical error distribution and link function. Model ranking was based on Akaike's Information Criteria corrected for small samples (AIC_c, Burnham and Anderson, 2002). For the GLMMs, female identity was set as a random effect to account for repeated measurements of individuals. Specific model comparisons were based on the information-theoretic evidence ratio (ER) which is the AIC_c weight (w) of the full model divided by the w of another (in this case, the null) model (Burnham and Anderson, 2002). Higher ER values indicate higher likelihoods of the tested model relative to the null. We also calculated the per cent deviance explained (%DE) in the response as a measure of a model's goodness-of-fit.

The FA most responsible for the multivariate patterns were identified in SIMPER (similarity percentages) analysis (Clarke, 1993). The SIMPER procedure compares the average abundances and examines the contribution of each FA to the average Bray–Curtis dissimilarity between two defined groups of samples (*i.e.*, blubber layer).

To examine potential biases associated with the type of blubber sample taken (*i.e.*, whole, inner or outer portion) and the time of sampling (*i.e.*, post-parturition or end-lactation) we applied a linear discriminant function analysis (DFA) using cross-validation to identify distinct prey groups based on FA profiles (Bradshaw et al., 2003). We used this approach to examine differences in diet predictions, but not to construct a complete assessment of diet itself because we lacked FA data for many known and possible Weddell seal prey species (seeAppendix A). Our principal aim was to assess the degree to which FA mobilization affected DFA classification accuracy. However, we did believe it necessary to use prey species that closely reflected that of known diet (based on faecal and stomach contents), which consists mainly of notothenid fishes, cephalopods and crustaceans (Burns et al., 1998; Lake et al., 2003; Plötz, 1986). To do this we obtained FA profiles for 23 known and possible Weddell seal prey species, broadly classified as either notothenid fish (8 species) or cephalopods (15 species), from various published and unpublished sources (see Bradshaw et al., 2003). Many crustaceans were unavailable, and so were not included in analyses. Separate functions were estimated to predict the group membership of the seal blubber samples for each sampling time and region (*i.e.*, whole or partial blubber samples). We only used FA profiles for seals that had separate blubber samples from both sampling times ($n=10$).

3. Results

3.1. Fatty acid composition and vertical stratification

Twenty-four separate FA (comprising 94–98% of the total FA) were found in greater-than-trace amounts (>0.5%) in the inner and outer blubber samples (Table 1). Monounsaturated fatty acids (MUFA) dominated both layers (outer=69.1%, inner=62.6%; Fig. 1) consisting mostly of short-chain MUFA (≤18 carbons; SC-MUFA; outer=63.0%, inner=54.4%) with only a small proportion of long-chain MUFA (>18 carbons, LC-MUFA; outer=6.1%, inner=8.2%). There was no evidence for a

Table 3
Summary of per cent differences in prey group classifications between the full discriminant function (DF; $n=22$ fatty acids) and the DF with low mobilized fatty acids removed ($n=16$ fatty acids)

Sample	Whole PP_F	Inner PP_F	Outer PP_F	Whole EL_F	Inner EL_F	Outer EL_F
Whole PP_{-L}	**10%**					
Inner PP_{-L}	50%	**10%**				
Outer PP_{-L}	10%	50%	**10%**			
Whole EL_{-L}	20%	40%	20%	**10%**		
Inner EL_{-L}	10%	50%	10%	0%	**30%**	
Outer EL_{-L}	20%	40%	10%	10%	40%	**20%**

Subscript 'F' denotes results from the full classification, and subscript '-L' denotes low-excluded DF classification. Boldface designates comparisons between like samples.

Table 4
Summary of per cent differences in prey group classifications between the full discriminant function (DF; $n=22$ fatty acids) and the DF with low mobilized fatty acids removed ($n=16$ fatty acids)

Sample	Whole PP_F	Inner PP_F	Outer PP_F	Whole EL_F	Inner EL_F	Outer EL_F
Whole PP_{-H}	**80%**					
Inner PP_{-H}	70%	**30%**				
Outer PP_{-H}	70%	30%	**70%**			
Whole EL_{-H}	90%	30%	90%	**80%**		
Inner EL_{-H}	80%	40%	80%	90%	**50%**	
Outer EL_{-H}	100%	40%	100%	90%	60%	**100%**

Subscript 'F' denotes results from the full classification, and subscript '-H' denotes high-excluded DF classification. Boldface designates comparisons between like samples.

difference between layers using the first component (PC1) (GLMM; ER=0.44). Saturated fatty acids (SFA) were found in the next highest percentage in both layers, but in contrast to the MUFA, these FA were relatively more common in the inner (22.5%) than the outer (16.2%) layer, although there was still no strong evidence for a difference between the two (ER=1.92). Polyunsaturated fatty acids (PUFA) occurred in the lowest relative amounts and at similar percentages in both layers (outer=14.7%, inner=14.9%). Sixty-two per cent of the dissimilarity between the two layers post-partum was accounted for by four FA: 18:1ω9c (17.0%), 16:0 (16.1%), 16:1ω7c (15.6%), and 14:0 (13.6%), with MUFA (18:1ω9c and 16:1ω7c) more abundant in the outer blubber layer and SFA (16:0 and 14:0) more abundant in the inner layer.

A plot of the first two principal components divided the post-partum inner and outer blubber samples into two distinct groups (Fig. 2). PC1 accounted for 49.0% of the variation in FA composition among samples, and the second component (PC2) accounted for 28.0%. The GLMM used to examine only the influence of *layer* on PC2 scores revealed that this factor explained 77.9% of the variation in PC2, further supported by a high evidence ratio (1.7×10^{43}, $AIC_c=167.3$) when compared to the null model.

3.2. Layer variation in fatty acid composition

3.2.1. Inner layer

For the FA proportional changes within the inner blubber layer (comparing post-parturition to end-lactation cores), PC1 accounted for 50.2% of the variation in FA composition, and 27.5% in PC2. The FA driving the differences included 16:1ω7c, 16:1ω5c and 20:5ω3 with negative eigen values and 24:1, 22:1ω9c and 20:1ω9c with positive eigen values (Fig. 3). Testing only capture time, (*date*) explained 86.7% of the variation in PC1 (GLMM; ER=5.0×10^{42}, $AIC_c=75.3$). Polyunsaturated fatty acids remained relatively unchanged by the end of lactation, while the percentage of LC-MUFA increased, and SC-MUFA and SFA decreased over lactation (Fig. 1).

In absolute terms, 69.7% of the difference between the inner portion of the post-partum and end-lactation blubber layers was in SFA 14:0 and 16:0, and MUFA 18:1ω9c, 16:1ω7c. The most parsimonious model testing for the effect of total body lipid stores (kg) at post-parturition (TBL) and feeding index on the fractional mobilization (*i.e.*, the fraction of initial mass of the FA that was lost during lactation) of FA from the inner blubber layer between post-parturition and end of lactation included only the term *feeding* ($wAIC_c=0.739$, %DE=33.5%). Several FA were mobilized consistently more than others (Table 2), but in all females the essential fatty acid 20:5ω3 had the highest fractional mobilization (range: 69.6–91.6%).

3.2.2. Outer layer

There was little evidence for a difference in the proportional FA of the outer blubber layer between post-parturition and end-lactation cores according to the PCA (Fig. 4). The GLMM used to only examine the influence of *date* on PC1 scores revealed that *date* described only a small component of the variation in samples (%DE=0.48, $AIC_c=89.1$) and little evidence for a temporal change (Fig. 1). The most parsimonious model testing for the effect of *TBL* and *feeding* on the fractional mobilization of FA from the outer blubber layer included only the term *feeding* ($AIC_c=50.7$), although the %DE by this model was lower (%DE=14.6%). FA 20:5ω3 still had the highest fractional mobilization in some females, but the mobilization of other FA in the outer layer was much more variable compared to the inner layer (Table 2).

3.3. Prey and seal blubber classification

Discriminant function analysis using the two prey groups (nototheniids and cephalopods) correctly classified all (100%) prey species. We identified six FA with the highest mobilization properties (14:0, 16:0, 14:1ω5c, 16:1ω5c, 16:1ω7c, 20:5ω3) and six FA with low mobilization properties (20:1ω7c, 22:1ω9c, 22:1ω11c, 20:2ω6, 22:5ω3, 20:1 ω9; Table 2) during lactation. The DFA was re-run first with the highly mobilized FA excluded (referred to as the 'high-excluded' discriminant function) and then with the low mobilized FA excluded (referred to as the 'low-excluded' discriminant function). For each analysis prey remained 100% correctly classified.

Using the first discriminant function (DF) for the prey classification, we recalculated the DF for seal blubber by

inserting the proportions of each of the FA from blubber samples (*cf.* Bradshaw et al., 2003). This was done for whole samples and for layer samples (*i.e.*, inner or outer) taken at post-parturition and end-lactation. This produced a classification of a seal's blubber sample as being comprised of mainly 'notothenid' or 'cephalopod' FA for each sampling time and sample section. This was repeated for the high- and low-excluded DFs. On average, the full and low-excluded DFs categorized blubber samples as ≥50% cephalopod. The high-excluded DF classified samples as ≥50% notothenids. Using the number of samples classed into each prey group for each sample type, we calculated the per cent difference in diet classification predicted from the full DF and the high- and low-excluded DFs. The full and the low-excluded DF classified the seal blubber samples similarly (Table 3). However, there were considerable differences in classification with the high-excluded DF (Table 4).

4. Discussion

We have shown that some fatty acids were fractionally mobilized more than others during lactation in Weddell seals (*e.g.*, 20:5ω3), and that there are important fatty acid composition differences between the inner and outer blubber layers. Dietary predictions including the highly mobilized FA were questionable — they predicted a mostly cephalopod diet which is at odds with previous estimates of Weddell seal diet composition. However, the removal of highly mobilized FA from discriminant function analysis reversed predictions to a predominantly notothenid diet, consistent with most Weddell seal dietary studies (Burns et al., 1998; Lake et al., 2003; Plötz, 1986). This demonstrates that differences in FA mobilization rates affect dietary predictions, and that these need to be acknowledged and incorporated into dietary predictions based on FA. The FA used in our analyses were those identified as having either elevated or reduced mobilization rates during lactation. However, different FA may be of importance during other periods of fasting (*i.e.*, moult), so future research should examine selective FA use during this time.

The proportion of FA accumulated during foraging trips depends on energy expenditure while previously ashore. This may reduce the power to detect temporal differences in FA composition unless mobilization rates and selective use of FA are measured. We have shown that some FA are selectively mobilized to support both maintenance metabolism and milk production during lactation. These FA may be underestimated in the blubber if sampled throughout lactation, so failing to account for mobilization during periods of high turnover may seriously bias FASA diet estimates. We suggest that dietary predictions will be most reliable when full blubber core samples are taken at parturition (*cf.* Best et al., 2003).

Fatty acid mobilization and changes in composition during lactation occurred mainly in the inner blubber layer. We also found absolute (although not proportional) changes in the outer layer — an observation not previously reported. The main FA depleted in the inner layer were the same that dominated post-parturition (18:1ω9, 16:1ω7, 16:0 and 14:0) and overall SC-MUFA and SFA were used the most. Similar to the inner layer, 18:1ω9, 16:1ω7, 16:0 and 14:0 were the FA that caused the most (absolute) dissimilarity between sampling times in the outer layer. Conversely, all groups of FA (PUFA, LC-MUFA, SC-MUFA and SFA) were used in similar proportions in the outer layer, but not the inner layer. These observations indicate that although changes occurred within both halves of the blubber, they did not change uniformly.

Several previous studies have described stratification in the blubber of marine mammals (Arnould et al., 2005; Best et al., 2003; Olsen and Grahl-Nielsen, 2003); however, this is the first study that describes the changes in stratification over time. The strong vertical stratification in Weddell seal blubber was similar to that found in other species (Andersen et al., 2004; Arnould et al., 2005; Olsen and Grahl-Nielsen, 2003). Higher concentrations of SFA (particularly 16:0 and 14:0) were present in the inner compared to the outer layer, while MUFA (particularly 18:1ω9c and 16:1ω7c) were more prevalent in the outer layer. This may be due to a more metabolically active inner layer. SFA offer more chemical energy per unit mass (Maillet and Weber, 2006), while the outer layer is more structural and so requires more stable FA with lower melting points (Fredheim et al., 1995).

Blubber stratification in seals may arise because (1) fatty acids that enter the tissue first are more rapidly turned over and released compared to the cell's bulk lipid, *i.e.*, last in–first out (Ekstedt and Olivecrona, 1970); (2) some FA are differentially mobilized according to chain length, unsaturation, positional isomerism and melting point (Raclot and Groscolas, 1993); and (3) there may be a gradient of use across the entire blubber layer (Andersen et al., 2004) that might be related to the structural/physiological demands of both layers. Although all these may play a role in the differential mobilization of FA we observed, selective mobilization of FA should also arise in response to the energetic and specific nutritional demands of the mother and pup at different stages of development. For example, the essential fatty acid 20:5ω3 had the highest fractional mobilization from the blubber, specifically the inner layer, during lactation. It was also highest in the milk immediately post-parturition (Wheatley et al.,

in press-b), suggesting that mobilization of this FA occurs early in lactation when females are fasting. It also suggests that some fatty acids may be selectively mobilized at different times depending on energetic or growth requirements of mothers and pups.

Evidence from milk energy output and FA mobilization/transfer indicates and that some females fed during late lactation (Wheatley et al., in press-a,b), although not all females apparently fed, and FA trends were similar for feeders and non-feeders. Even though the feeding index was an important contributor to fractional mobilization of FA, our data suggest that some fractional mobilization of fat reserves occurs within Weddell seals during lactation regardless of feeding.

To account for differential metabolism, deposition and biosynthesis of FA by predators, Iverson et al. (2004) developed calibration coefficients for individual FA (Quantitative Fatty Acid Signature Analysis — QFASA) to compare the FA of predator lipid stores to FA of various prey types. This technique allows one to estimate the proportional contribution of each prey type in a predator's diet (Iverson et al., 2004). One of the fundamental requirements of the model is an understanding of, and accounting for, variable rates and patterns of lipid metabolism and deposition in the predator. The differential mobilization of particular FA we found highlights that it may be more applicable to use more 'inert' (low mobilized) FA for predictions. Furthermore, differences in the degree of differential mobilization may vary among species, and will need to be taken into consideration when estimating calibration coefficients for some FA. Accordingly, using FASA as a biomarker of dietary composition and change should be used cautiously because individual FA are mobilized differentially depending on the physiological state of the animal. This result emphasizes the need to understand species-specific FA mobilization and turnover to provide more accurate and robust quantitative estimates of diet and its variation.

Acknowledgements

We thank P. Brewin, M. Brock, W. Cook, L. Davis, D. Geddes, A. Hall, P. Isherwood, C. Littnan, B. Mooney and volunteers from Scott Base for assistance in the field and laboratory. C. Lydersen and K. Kovacs provided valuable comments on an earlier draft of this manuscript. This study was supported by the University of Tasmania Institutional Research Grants Scheme, the Australian Research Council Discovery Project grant (DP0342714) and the Seaworld Research & Rescue Foundation Inc. We thank Antarctica New Zealand for providing logistical support and the Australian Antarctic Division for providing some field clothing. Data was collected under permits from the University of Tasmania Animal Ethics Committee (A6790 & A6711), and the Department of Conservation of New Zealand (Per/22/2002/149 & Per/17/2003/188). **[SS]**

Appendix A. List of species of possible prey items of Weddell seals for which there were no fatty acid profiles

Species	
Fishes	Family
Aethotaxis mitopteryx	Nototheniidae
Artedidraco loennbergi	Artedidraconidae
Artedidraco orianae	Artedidraconidae
Artedidraco skottsbergi	Artedidraconidae
Bathydraco macrolepis	Bathydraconidae
Bathydraco marri	Bathydraconidae
Bathyraja eatoni	Rajidae
Bathyraja maccaini	Rajidae
Chaenodraco wilsoni	Channichthyidae
Chionodraco hamatus	Channichthyidae
Chionodraco myersi	Channichthyidae
Cryodraco antarcticus	Channichthyidae
Cygnodraco mawsoni	Bathydraconidae
Dolloidraco longedorsalis	Artedidraconidae
Gerlachea australis	Bathydraconidae
Gymnodraco acuticeps	Bathydraconidae
Gymnoscopelus opisthopterus	Myctophidae
Histiodraco velifer	Artedidraconidae
Lepidonotothen squamifrons	Nototheniidae
Lycodichthys dearborni	Zoarcidae
Mancopsetta maculata	Achiropsettidae
Neopagetopsis ionah	Channichthyidae
Notolepis coatsi	Paralepididae
Ophthalmolycus amberensis	Zoarcidae
Pachycara brachycephalum	Zoarcidae
Pagetopsis macropterus	Channichthyidae
Pagetopsis maculatus	Channichthyidae
Pagothenia brachysoma	Nototheniidae
Pogonophryne marmorata	Artedidraconidae
Pogonophryne scotti	Artedidraconidae
Prionodraco evansii	Bathydraconidae
Racovitzia glacialis	Bathydraconidae
Rhigophilia dearborni	Piscicolidae
Trematomus eulepidotus	Nototheniidae
Trematomus lepidorhinus	Nototheniidae
Trematomus loennbergii	Nototheniidae
Trematomus nicolai	Nototheniidae
Trematomus scotti	Nototheniidae
Trematomus tokarevi	Nototheniidae
Trematomus vicarius	Nototheniidae
Cephalopods	
Pareledone spp.	Octopodidae
Psychroteuthis glacialis	Psychroteuthidae

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