Vol. 366: 271–279, 2008
doi: 10.3354/meps07617

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published August 29

Temporal and sex differences in the blubber fatty acid profiles of the New Zealand sea lion *Phocarctos hookeri*

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ABSTRACT: We determined the fatty acid (FA) composition of the blubber of 82 New Zealand (NZ) sea lions caught as squid fishery by-catch between the years 2000 and 2006 on the Auckland Islands (51° S, 166° E) shelf. A combination of univariate and multivariate analyses showed significant variation in the FA composition between sexes and years. Blubber FA compositions of some males differed significantly from that of females, whereas blubber FA compositions of lactating (LF) and non-lactating females (NLF) were similar. Significant annual FA variation was revealed between the pooled years 2005/2006 and the previous years and between 2000 and 2004. Part of these differences can be attributed to different diets. Indeed, FA variation between the sexes suggests that males feed on deeper species than females, which is consistent with the current knowledge on the different diving behaviours between male and female otariids. Concerning annual variation, NZ sea lions are generalist predators, thus their diet is expected to follow the trends of prey stock availability. Nonetheless, FA metabolism is likely to cause some of the FA variation seen between sexes and years, because the deposition and mobilization of FAs would vary according to the nutritional and reproductive states of the individuals.

KEY WORDS: Fatty acid analysis · *Phocarctos hookeri* · Sex variation · Temporal variation · Fatty acid metabolism · Diet

INTRODUCTION

Pinnipeds are amphibious mammals balancing their time between land, for breeding, nursing, resting and moulting, and sea, where they search for food. However, different species use different strategies for breeding and foraging. Otariids are 'income breeders', with lactation periods of several months to several years during which lactating females (LF) alternate between nursing pups on land and foraging at sea (Costa 1991). They are considered 'central place foragers', optimising the time and energy costs of foraging with the need to return frequently to the colony to feed their dependent pups (Orians & Pearson 1979). Thus LF would be expected to forage as close to the rookery as possible provided that enough energy can be obtained from prey to compensate for the energetic cost of the return trip (Orians & Pearson 1979). In contrast, male otariids and non-lactating females (NLF), without the constraints of a dependent offspring, would be expected to forage in the most productive regions, which may be further from the colonies than the foraging grounds of LF. Further-

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more, male otariids are often twice the mass of females, giving them the ability to dive longer (Costa 1991), but have higher metabolic requirements, which they may satisfy by consuming more of the same food eaten by females or eating a different diet with a higher calorific content. It is possible that these differences in reproduction investment and body size between sexes could give rise to sex-specific foraging strategies and consequently differences in the composition of the diet between the sexes and between females of different reproductive status.

Little is known about how gender influences foraging behaviour or diet of otariids. Indeed, most foraging studies on otariids focused on LF because they are a critical component of the population and easily accessible while lactating. For 6 species where male foraging patterns have been studied, they tend to have longer foraging trips and deeper dives than the female counterparts (*Arctocephalus gazella,* Green et al. 1997, Boyd et al. 1998, Staniland & Robinson 2008; *Otaria flavescens,* Campagna et al. 2001; *Eumetopias jubatus,* Raum-Suryan et al. 2004; *Arctocephalus forsteri,* Page et al. 2005a; *Arctocephalus pusillus doriferus,* Kirkwood et al. 2006; *Zalophus californianus,* Weise et al. 2006). Dietary studies comparing gender are even more scarce (Koen Alonso et al. 2000, Page et al. 2005b, Beck et al. 2007), probably because the most common method for studying the diet of pinnipeds is the analysis of faeces collected on haul-outs or rookeries, where the identity of the animal or its gender are generally unknown.

New Zealand (NZ) sea lions *Phocarctos hookeri* have a restricted breeding range, with 86% of the pups being born at the Auckland Islands (51° S, 166° E) (Chilvers et al. 2007). To date, LF are the only segment of the population for which foraging and diving behaviours are known and this only over the summer (Chilvers et al. 2005, 2006). Stomach contents of NZ sea lions bycaught by the squid fishery at the edge of the Auckland Islands shelf indicate that females and males feed on the same prey with minor differences in the proportions (L. Meynier unpubl. data), but stomach data provides only a snapshot of the most recent meals, which may underestimate sex differences in the diet, if they exist.

In contrast to analyses of stomach contents and faeces, fatty acids (FAs) of adipose tissue have the potential to reflect the dietary intake over ecologically significant periods, i.e. several weeks to months depending on the tissue turnover (e.g. Kirsch et al. 1998, 2000). The underlying principle is the assumption that long-chain FAs in prey species are conservatively deposited into the adipose tissue of the predator, thereby providing biochemical signatures with which prey species can be identified. Although advantages over traditional methods have been pointed out, the inference of diet from FA profiles of an animal is not straightforward. Indeed, the FA composition in the blubber is the result of complicated processes of deposition from dietary lipids, differential metabolism and biosynthesis de novo. Moreover, stratification of FAs has been observed (e.g. Arnould et al. 2005, Montie et al. 2008), and rates of mobilisation can vary according to the nutritional and reproductive states of the animal (e.g. Andersen et al. 2004, Wheatley et al. 2007, Montie et al. 2008). However, despite the multiple origins of FA variation in adipose tissue, FA analysis has been used extensively to investigate the diets of pinniped species (e.g. Käkelä & Hyvärinen 1998, Brown et al. 1999, Walton et al. 2000, Lea et al. 2002, Staniland & Pond 2005, Beck et al. 2007).

The aim of the present study was to investigate the potential of analysing the blubber FA profiles of NZ sea lions by-caught by the squid fishery during the beginning of the lactation period (February to May) as a means of assessing dietary differences between sexes and years. It was hypothesized that the post-breeding diet of LF would differ from the other groups, given that foraging trips of LF are restricted in time and distance by the need to nurse their pups.

MATERIALS AND METHODS

Tissue collection. Since 1997, NZ sea lions captured accidentally in the squid fishery (operating from February to May each year) have been frozen onboard and sent frozen to Massey University, NZ, for necropsy. During each necropsy, a full-depth 60 mm^2 piece of blubber (including skin and some muscle) is cut from the pectoral area and stored in a plastic bag in the freezer (–20°C). All blubber samples in the present study were taken from the mid-sternal region to be comparable, as FA profiles can vary with the location around the body (Arnould et al. 2005). Moreover, stratification can occur in the blubber of pinnipeds (e.g. Best et al. 2003, Arnould et al. 2005, Wheatley et al. 2007), thus the complete blubber core was analysed. During necropsy, females were categorised as either NLF or LF by the examination of the mammary gland for development and presence of secretion. The individuals included in this study were all mature as determined by visual examination of the ovaries and histological examination of the testes (details in Duignan et al. 2003).

Laboratory methods. Lipids from blubber were extracted following Folch et al. (1957), using a chloroform:methanol:water mixture. Ca. 0.5 g of blubber (whole core) was sub-sampled from the bulk sample and homogenised in 15 ml of chloroform:methanol (2:1, vol:vol) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. The extract was fil-

tered and washed with 1% sodium chloride to a final ratio of 8:4:3 chloroform:methanol:saline water (v:v:v). The organic phase was then dehydrated over anhydrous sodium sulphate. Finally, the lipid extract was dried in a rotary evaporator at 38°C and weighed.

FA methyl esters (FAMEs) were prepared directly from 30 mg of the pure extracted lipid using 1.5 ml of toluene and 1.5 ml of 10% boron trifluoride in methanol (methylating reagent). Each extract was capped under nitrogen and heated at 50°C for 14 to 19 h (overnight). Esters were then extracted into hexane and stored over anhydrous sodium sulphate at –20°C before chromatographic analysis.

Analysis of FAMEs was carried out using temperature-programmed gas–liquid chromatography performed with a Shimadzu Gas Chromatograph GC-17A (Shimadzu Scientific Instruments) equipped with a flame ionisation detector and fitted with a 30 m × 0.25 mm i.d. column (50% cyanopropyl polysiloxane, 0.25 µm film thickness; J&W DB-23). Helium was the carrier gas. FAMEs (1 µl) were injected manually in split mode (1:50) at an injection port temperature of 250°C. The detector temperature was set at 270°C. The temperature of the oven was programmed to stay at 140°C for 4 min, rise to 190°C at 25°C min^{-1}, held for 5 min, then to 236°C at 2°C min^{-1}.

FA components were identified by comparison of retention time data to authentic (Nu-Chek GLC standard 68D, Supelco 37 FAME mix, Matreya menhaden oil) and laboratory standards (cod liver oil). Cod liver oil was used in every series of runs to determine accurate retention times. Nu-Check 68D was injected regularly to check the quantitation of each FA. Peak areas were measured by a computerized integration system attached to the gas chromatograph (CLASS-VP version 7.3, Shimadzu Scientific Instruments). Each chromatogram was checked to ensure correct identification. The identification of some minor peaks was uncertain, and these were not included in the final normalisation. FAs were designated by the shorthand notation of carbon chain length:number of double bonds and location (n-x) of the double bond nearest to the terminal methyl group. Theoretical response factors calculated according to Ackman & Sipos (1964) were used for the quantitation of FAs expressed in mass percentages.

Statistical methods. FAs were expressed as a percentage mass of total FAs and they were ln-transformed as advised by Budge et al. (2006) for parametric statistical analyses. A transformed FA *i* equalled ln (x_i $18{:}0^{-1}$) where x_i is the FA, *i* expressed as percent of total FAs, and 18:0 is the percentage of stearic acid of total FAs, used as a reference FA. A combination of principal component analysis (PCA), discriminant function analysis (DFA) and general linear model (GLM) was used to examine inter-annual and sex differences in the FA profiles of NZ sea lions (MINITAB Release 14.1, MINITAB 2003 and SPSS for Windows Release 15.0, SPSS 2006). These multivariate methods have been commonly used in numerous papers on FA analysis (e.g. Grahl-Nielsen & Mjaavatten 1991, Walton et al. 2000, Staniland & Pond 2005) and give complementary results. A first GLM was applied to the transformed FA compositions with year and sex as independent variables. A second GLM was used on the principal components (PCs). The Tukey test was used for post-hoc multiple comparisons. Finally, the DFA requires that the number of samples (sea lion blubber) per group exceeds the number of variables (FAs) to minimize the heterogeneity of covariance matrices and to avoid overfitting (Budge et al. 2006). Because the number of samples per year was too small to run a DFA, we limited this analysis to the sex category, and the number of FAs was reduced to satisfy the requirement stated above: the smallest group among sex categories was NLF with 23 ind., therefore a set of 20 FAs was selected from the original set of 30 FAs, with the highest absolute PC loadings. After re-normalisation of the FA percentages, a linear DFA with cross-validation was performed.

All statistical tests have an α level of statistical significance of 0.05, and all averages were followed by the standard deviation (SD). Since the blubber thickness may have an influence on FA profiles, the average blubber thickness was calculated and tested for differences between each category by an ANOVA on log-transformed data.

RESULTS

Composition of the sample set

In total, 82 blubber samples were analysed from 51 female and 31 male sea lions by-caught between February and May every year from 2000 to 2006 (Table 1). Although mature, the majority of the males caught were not over 200 kg (average 167 ± 60 kg) and were considered to have been non-territorial animals during the breeding period (mid-December to mid-January). The sex categories were not all represented within the last 2 yr, thus 2005 and 2006 were pooled for univariate and multivariate analyses.

Overall thickness of sea lion blubber averaged 28 ± 11 mm and was similar among sex categories (Table 1). Between years, the blubber of sea lions caught in 2000 was significantly thicker than the blubber of animals caught in the combined years 2005/2006 (ANOVA, $F_{5,75}$ = 3.80, p = 0.004; Tukey test between 2000 and 2005/2006, p = 0.027).

Table 1. *Phocarctos hookeri.* Number of lactating female (LF), non-lactating female (NLF) and male (M) sea lions analysed per year. The blubber thickness (mm) is expressed as the average ± SD. Significant differences between categories were ANOVA-tested on log-transformed data (log values ± SD in parentheses). The years 2005 and 2006 were pooled for the ANOVA because of the limiting number of individuals. *Significant difference between some years at α = 0.05

Year	LF	NLF	M	**Total**	Blubber thickness x (log x)
2000	6	2	8	**16**	32 ± 5 (1.50 ± 0.07)*
2001	6	2	8	**16**	24 ± 5 (1.37 ± 0.10)
2002	8	1	5	**14**	32 ± 10 (1.49 ± 0.14)
2003	2	4	5	**11**	34 ± 20 (1.47 ± 0.28)
2004	2	6	4	**12**	24 ± 6 (1.37 ± 0.10)
2005	3		1	**4**	22 ± 6
2006	1	8		**9**	(1.33 ± 0.10)*
Total	**28**	**23**	**31**	**82**	28 ± 11 (1.42 ± 0.15)
Blubber thickness x (log x)	29 ± 11 (1.43 ± 0.17)	25 ± 12 (1.37 ± 0.15)	30 ± 10 (1.45 ± 0.13)	28 ± 11 (1.42 ± 0.15)	

Overall blubber FA composition

Although 38 FAs were originally identified, only 30 FAs ranging from 14:0 to 22:6n-3 were used (Table 2), representing 99.4 ± 0.2% of the total. The 8 FAs removed were either short-chain FAs (<14 carbons), known to come primarily from endogenous biosynthesis (Budge et al. 2006), or FAs for which the identification was not certain.

Average FA composition of sea lion blubber is shown in Table 2. The FAs in greatest concentration in order of importance were 18:1n-9, 16:0, 22:6n-3, 20:1n-9, 16:1n-7, 14:0, 18:1n-7, 22:5n-3 and 20:5n-3, accounting for ca. 85% of the total FAs in the blubber. Monounsaturated and polyunsaturated FAs accounted for ca. ½ and ¼ of the total, respectively.

A PCA using a correlation matrix was run on ln-transformed data to assess the most important FAs explaining the variance between FA profiles. The first 3 PCs accounted for 65% of the total variation in blubber FAs. The FAs with the greatest influence on PC1 were 18:1n-5, 16:1n-7, 20:4n-3, 18:2n-6 and 22:6n3, with the last 3 coming primarily from the diet. High loadings in the other PCs were attributed to 22:1n-9, 22:1n-11, 15:0, 22:5n-6 and 16:3n-4 on PC2 and 18:4n-3, 14:0, 20:4n-6, 22:5n-6 and 17:0 on PC3, all of which are derived primarily from the diet with the exception of the 3 saturated FAs.

Temporal and sex differences in FA profiles

The GLM on FA percentages showed significant differences in the percentages of 11 FAs between sex categories and 17 FAs between years ($p < 0.05$, Table 2). There was an interaction between the 2 factors for only 2 FAs, 20:5n-3 and 22:5n-6, which involved the only male in years 2005/2006 being segregated from the other groups. The percentages of 14:0, 18:1n-5, 20:1s, 22:1n-11, 18:4n-3, 20:4n-3, 20:5n-3, 21:5n-3 and 22:5s in males differed significantly from those of LF and NLF. Most of the significant inter-annual variation involved a difference between the combined years 2005/2006 and some or all of the previous years (9 FAs) and a difference between the years 2000 and 2004 (9 FAs).

The GLM on PCs confirmed these results on a general scale (FA profile instead of individual FA, Table 3): Year and sex had a significant effect on each of the first 3 components and on the overall model, but the interactions between the 2 factors were not significant. Post-hoc Tukey tests on PCs showed that the combined years 2005/2006 were significantly different from the other years on PC2 and PC3 ($p < 0.05$), and the year 2000 was different from 2004 on PC1. Furthermore, males were different from all females for each PC.

A DFA was run on the 20 FAs with the highest PC loadings (noted in bold in Table 2). The classic analysis gave an overall percentage of correct classifications of 76%, while with cross-validation this result was lowered to 54%. This sizeable difference between the percentages was interpreted as too many predictors (FAs) in the analysis (Walton et al. 2000). Thus, we lowered the number of variables for a second DFA by choosing 10 FAs only (noted by an asterisk in Table 2), with the highest absolute coefficients on the discriminant functions generated by the first DFA. The new DFA gave an overall percentage of correct classification of 66% with the classification matrix and 55% with cross-validation. The discriminant function plot showed that some male FA profiles differed from female profiles along the first discriminant function (Fig. 1) mainly because of differences in the proportions of 20:1n-9, 22:1n-11 and 16:3n-4. The percentage of correct classification for males was 68%. No difference was apparent between the 2 groups of females.

Table 2. Fatty acid (FA) composition by sex category from 82 New Zealand sea lion blubbers in mean mass percent ± SD. The values in parenthesis are the ln-transformations of the FA percentages [ln (FA% 18:0%$^{-1}$)] used in the general linear model (GLM). For the GLM, post-hoc comparisons were indicated when significant differences ($p \leq 0.05$, p values in bold) were present among groups: M = males, LF = lactating females, NLF = non-lactating females, 00 = year 2000 etc. The years 2005 and 2006 were pooled (coded 056). SAFAs are saturated FAs, MUFAs monounsaturated FAs and PUFAs polyunsaturated FAs. Origin represents the predominant origin of a FA: 'diet' when a FA came entirely or primarily from diet, 'both' when large contributions come from both endogenous biosynthesis and diet (Iverson et al. 2004). Boldface type FAs indicate the 20 FAs used in the first discriminant function analysis (DFA).*: 10 FAs used in the second DFA

FAs	FA composition mean % ± SD (ln transformed % ± SD)			p-values of GLM (on ln transformed %)			Origin
	LF	NLF	M	Sex	Year	Sex × year	
SAFAs	21.7 ± 3.1	22.0 ± 3.0	20.7 ± 2.9				
14:0	5.1 ± 1.3 (0.6 ± 0.3)	5.4 ± 1.5 (0.7 ± 0.4)	3.8 ± 0.9 (0.2 ± 0.4)	**<0.001**; M ≠ LF, NLF	**0.009**; 056 ≠ others	0.515	both
15:0*	0.4 ± 0.1 (–2.0 ± 0.3)	0.4 ± 0.1 (–1.9 ± 0.2)	0.5 ± 0.1 (–1.9 ± 0.2)	0.364	0.115	0.504	both
16:0*	12.7 ± 1.8 (1.5 ± 0.2)	13.0 ± 1.7 (1.6 ± 0.2)	12.8 ± 1.9 (1.5 ± 0.2)	0.119	**0.024**; 056 ≠ others	0.772	both
17:0	0.5 ± 0.2 (–1.7 ± 0.4)	0.4 ± 0.2 (–2.0 ± 0.7)	0.6 ± 0.1 (–1.7 ± 0.3)	0.660	**<0.001**; 056 ≠ others	0.965	diet
18:0	2.8 ± 0.6	2.7 ± 0.5	3.0 ± 0.8	–	–	–	
MUFAs	52.9 ± 4.1	53.1 ± 2.6	54.8 ± 5.2				
14:1	0.3 ± 0.1 (–2.4 ± 0.6)	0.3 ± 0.1 (–2.5 ± 0.5)	0.3 ± 0.1 (–2.6 ± 0.8)	0.080	**0.026**; 00 ≠ 04	0.656	both
15:1	0.1 ± 0.0 (–3.0 ± 1.7)	0.2 ± 0.5 (–3.2 ± 1.2)	0.1 ± 0.0 (–3.4 ± 1.2)	0.873	**0.008**; 04 ≠ 056	0.835	both
16:1n–7*	6.0 ± 1.5 (0.7 ± 0.4)	7.0 ± 2.0 (1.0 ± 0.4)	6.0 ± 1.7 (0.7 ± 0.5)	0.085	**0.031**; 056 ≠ 01,03,04	0.586	both
18:1n–9*	28.1 ± 3.8 (2.3 ± 0.3)	28.6 ± 2.4 (2.4 ± 0.2)	32.0 ± 4.7 (2.4 ± 0.3)	0.740	**0.003**; 00 ≠ 01,04	0.330	both
18:1n–7	3.9 ± 0.4 (0.4 ± 0.3)	4.0 ± 0.4 (0.4 ± 0.2)	4.4 ± 0.5 (0.4 ± 0.3)	0.716	**0.014**; 04 ≠ 00,03	0.402	both
18:1n–5	0.4 ± 0.1 (–2.1 ± 0.3)	0.4 ± 0.1 (–2.0 ± 0.3)	0.3 ± 0.1 (–2.3 ± 0.5)	**0.004**; M ≠ NLF	**0.012**; 04 ≠ 00,056	0.583	both
20:1n–11	1.4 ± 0.3 (–0.7 ± 0.3)	1.3 ± 0.3 (–0.7 ± 0.3)	1.2 ± 0.3 (–0.9 ± 0.4)	**0.007**; M ≠ LF	**0.051**; 00 ≠ 04	0.751	diet
20:1n–9*	10.9 ± 2.4 (1.3 ± 0.3)	9.9 ± 2.4 (1.3 ± 0.2)	9.0 ± 1.8 (1.1 ± 0.3)	**<0.001**; M ≠ LF, NLF	**0.001**; 00 ≠ 04,056	0.392	diet
22:1n–11*	1.2 ± 0.6 (–0.9 ± 0.5)	1.0 ± 0.5 (–1.0 ± 0.4)	0.9 ± 0.4 (–1.3 ± 0.5)	**<0.001**; M ≠ LF	**<0.001**; 056 ≠ 00,01	0.668	diet
22:1n–9	0.6 ± 0.3 (–1.5 ± 0.3)	0.5 ± 0.2 (–1.7 ± 0.3)	0.6 ± 0.3 (–1.7 ± 0.4)	0.088	0.259	0.999	diet
PUFAs	25.5 ± 3.4	24.9 ± 3.3	24.5 ± 4.5				
18:2n–6	1.5 ± 0.2 (–0.6 ± 0.3)	1.6 ± 0.2 (–0.5 ± 0.2)	1.6 ± 0.2 (–0.6 ± 0.3)	0.169	**0.020**; 00 ≠ 04	0.180	diet
20:2n–6*	0.4 ± 0.1 (–2.0 ± 0.3)	0.4 ± 0.1 (–1.9 ± 0.2)	0.4 ± 0.1 (–2.1 ± 0.4)	0.102	0.361	0.941	diet
16:3n–4*	0.3 ± 0.1 (–2.1 ± 0.4)	0.4 ± 0.1 (–2.0 ± 0.4)	0.5 ± 0.1 (–1.9 ± 0.4)	0.106	**0.010**; 00 ≠ 04	0.286	diet
18:3n–3	0.5 ± 0.2 (–1.7 ± 0.8)	0.6 ± 0.2 (–1.5 ± 0.6)	0.5 ± 0.2 (–1.9 ± 0.7)	0.216	0.299	0.567	diet
20:3n–6	0.1 ± 0.0 (–2.9 ± 0.9)	0.1 ± 0.0 (–2.9 ± 0.7)	0.1 ± 0.0 (–2.8 ± 1.2)	0.834	0.161	0.674	diet
20:3n–3	0.2 ± 0.0 (–2.6 ± 0.3)	0.2 ± 0.0 (–2.5 ± 0.3)	0.2 ± 0.1 (–2.8 ± 0.8)	0.094	0.327	0.973	diet
18:4n–3	0.5 ± 0.2 (–1.8 ± 0.6)	0.5 ± 0.2 (–1.7 ± 0.5)	0.4 ± 0.3 (–2.1 ± 0.7)	**0.002**; M ≠ NLF	0.069	0.378	diet
20:4n–6	0.7 ± 0.2 (–1.4 ± 0.3)	0.7 ± 0.2 (–1.4 ± 0.3)	0.9 ± 0.2 (–1.2 ± 0.3)	0.378	**0.005**; 01 ≠ 02,03	0.083	diet
20:4n–3	1.2 ± 0.2 (–0.8 ± 0.3)	1.3 ± 0.2 (–0.7 ± 0.3)	1.0 ± 0.3 (–1.1 ± 0.5)	**<0.001**; M ≠ LF, NLF	**0.012**; 00 ≠ 04,056	0.083	diet
22:4n–6	0.1 ± 0.1 (–2.6 ± 1.0)	0.1 ± 0.1 (–2.8 ± 0.7)	0.2 ± 0.1 (–2.4 ± 0.9)				diet
20:5n–3	3.2 ± 1.0 (0.1 ± 0.4)	3.3 ± 0.6 (0.2 ± 0.4)	2.8 ± 1.4 (–0.2 ± 0.6)	**<0.001**; M ≠ LF, NLF	0.084	**0.013**; M056 ≠ others	diet
21:5n–3	0.3 ± 0.1 (–2.3 ± 0.4)	0.3 ± 0.1 (–2.2 ± 0.4)	0.2 ± 0.1 (–2.6 ± 0.6)	**0.002**; M ≠ LF, NLF	0.312	0.690	diet
22:5n–6	0.2 ± 0.1 (–2.5 ± 0.4)	0.2 ± 0.1 (–2.7 ± 0.6)	0.2 ± 0.1 (–2.6 ± 0.7)	**<0.001**; M ≠ LF, NLF	**<0.001**; 056 ≠ others	**0.001**; M056 ≠ others	diet
22:5n–3*	3.7 ± 0.7 (0.3 ± 0.3)	3.3 ± 0.8 (0.2 ± 0.3)	3.2 ± 0.6 (0.1 ± 0.4)	**0.014**; M ≠ LF	0.204	0.452	both
22:6n–3*	12.3 ± 2.0 (1.5 ± 0.3)	11.9 ± 2.3 (1.5 ± 0.3)	12.3 ± 2.5 (1.4 ± 0.3)	0.121	0.115	0.199	diet
n–3	22.0 ± 3.4	21.5 ± 3.2	20.6 ± 4.4				
n–6	3.1 ± 0.4	3.1 ± 0.3	3.4 ± 0.4				

Table 3. General linear model (GLM) testing the influence of Year and Sex on the first 3 principal components (PC) representing the fatty acid (FA) composition of NZ sea lion blubber. Significant values are in bold for each component and the overall model

Factor	df	Principal components						Overall model		
		PC1 (42%)		PC2 (14%)		PC3 (9%)		MANOVA (Pillai's trace)		
		F	p	*F*	p	*F*	p	df	*F*	p
Year	5	2.71	**0.028**	3.91	**0.004**	7.15	**<0.001**	15/192	4.52	**<0.001**
Sex	2	4.63	**0.013**	7.90	**0.001**	6.28	**0.003**	6/126	7.14	**<0.001**
Year × Sex	10	1.28	0.261	0.19	0.997	0.90	0.539	30/192	1.17	0.884

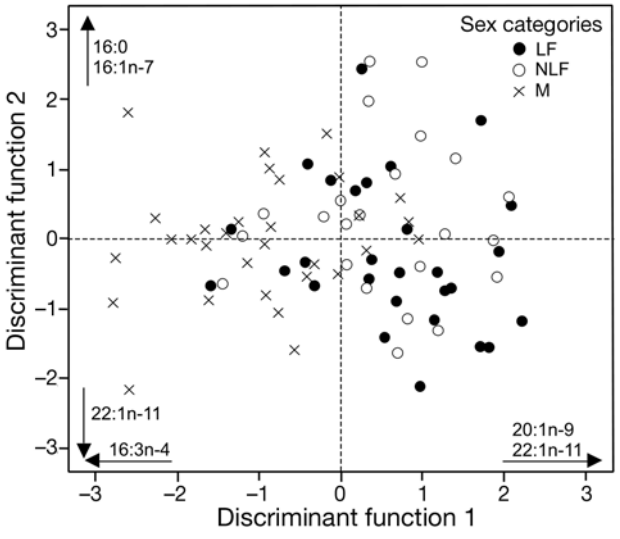

Fig. 1. Plot of canonical discriminant functions. LF: lactating females; NLF: non-lactating females; M: males. This analysis included 10 FAs only (see details in 'Results'). The first and the second functions explained 93.8% and 6.2% of the variation among samples, respectively. The FAs with the most important positive or negative loadings on functions 1 and 2 are displayed along the axes

DISCUSSION

The diet of the NZ sea lion and its variation has been studied through the analysis of stomach contents and faeces (Lalas 1997, McMahon et al. 1999, Childerhouse et al. 2001, L. Meynier unpubl. data), but these methods are subject to biases encountered with the recovery of prey hard parts. Since the 1960s, FAs have been used as food tracers in marine trophic webs, allowing dietary variation to be examined among marine individuals (e.g. Ackman & Eaton 1966, Kirsch et al. 1998, Staniland & Pond 2005, Beck et al. 2007). The present study is the first report of the FA composition of the NZ sea lion's blubber. FA compositions revealed some significant differences between females and males, and from the years 2000 to 2006, which may be attributed, at least in part, to differences in their diets. However, FA deposition and mobilisation in the blubber, and hence its FA composition, can vary according to the nutritional and reproductive states of the animal (Andersen et al. 2004, Wheatley et al. 2007, Montie et al. 2008). Thus, part of the FA differences between the sex categories is likely to be the result of differential metabolism.

Limitations of the study

Blubber samples were stored at −20°C tightly wrapped but nonetheless under air for up to 5 yr, which may have resulted in some oxidation of unsaturated FAs on the outside of the sample (Whiteley et al. 1992). Consequently lipids for analyses were extracted from a core taken from the centre of the original sample as outlined by Learmonth (2006) who reported no evidence of FA oxidation in the core of blubber of harbour porpoises stored at −20°C for 566 d.

Deposition of dietary lipids in the blubber will depend on the nutritional status of the animal: it is expected that a substantial amount of the FAs ingested above the metabolic requirements will be deposited in the blubber, whereas the extent to which deposition of dietary FAs occurs during periods of negative energy balance is unclear. Thus, the blubber FA signatures are more likely to reflect dietary FAs in animals in positive energy balance (e.g. Kirsch et al. 2000). LF are thought to be the segment of the population with the highest metabolic constraints, and the lactating NZ sea lions, captured and weighed on land, had stabilized or were gaining mass at a 1 mo interval during the first months of the lactation period (B.L. Chilvers unpubl. data). Furthermore, the blubber thickness of the bycaught sea lions studied (Table 1) was comparable to captive adult female Steller sea lions kept on a maintenance diet (Mellish et al. 2007). Thus, we believed that sea lions caught were in positive energy balance and depositing dietary FAs.

Moreover, deposition and mobilisation of blubber lipids have been shown to vary with the body region in otariid seals (Arnould et al. 2005, Mellish et al. 2007), while phocids present a more uniform fat distribution (e.g. Ryg et al. 1988, Mellish et al. 2007). The uniformity of fat distribution is believed to minimize heat loss

to the environment by optimising insulation efficiency (Ryg et al. 1988). Otariids do not have extended periods of fasting like phocids, thus blubber fat may have a less important role in term of insulation (Mellish et al. 2007), resulting in heterogeneity of fat distribution with preferred depot sites along the body. Blubber samples were taken from the sternum region because the thickness of the blubber over the sternum is positively correlated with body mass (Massey University unpubl. data), suggesting that this region is a fat depot when sea lions are in positive energy balance.

Variation in FA profiles among sex categories

The interactions between sex and year were limited to 2 dietary FAs (20:5n-3 and 22:5n-6). Thus, the variation in FA profiles between the sex categories was consistent within each year, and each factor has been interpreted separately. LF and NLF did not show any significant difference in their FA composition (Table 2, Fig. 1). However, lactation is the most energetically demanding period of mammalian reproduction (Oftedal, 1984), and marine mammals are no exception (Costa et al. 1986, Williams et al. 2007). Lactating marine mammals are thought to mobilize substantial quantities of lipids into milk, affecting their blubber FA profiles (e.g. Wheatley et al. 2007, Montie et al. 2008). Similar FA profiles between LF and NLF show that blubber FAs in the sternum region were not mobilized to produce milk. Instead, sternal blubber is likely to represent dietary lipids, and in this case, NLF displayed the same diet as lactating conspecifics. Indeed, LF are seen to have high site fidelity to foraging areas, which is thought to represent long-term learnt foraging behaviour (Chilvers in press); therefore, it is expected that this forage fidelity would continue even when not rearing a pup.

Concerning variation between sexes, the GLMs (Table 2, Table 3) and the DFA (Fig. 1) demonstrated significant differences between FA profiles of females and males by-caught by the squid fishery at the Auckland Islands: percentages of individual FA in males were significantly different ($p < 0.05$) from those in LF and NLF in 11 out of 30 FAs (Table 2). Although some caution is necessary as the percentages are not independent values, these results were confirmed by a second GLM on the PCs (Table 3) and the DFA (Fig. 1). These variations result from the combination of different diet and different metabolism. Indeed, males caught by the fishery were generally heavier than females, which give them the ability to dive deeper at the edge or to stay longer at the bottom of the shelf than females, exploiting different resources or similar food in different proportions. The main FAs causing the separation between females and males in the DFA were 20:1n-9 and 22:1n-11 in higher proportions in females and 16:3n-4 in higher proportions in males (Fig. 1). FA compositions of several species of fish, cephalopods and crustaceans from the Auckland Islands shelf have been examined (L. Meynier unpubl. data) and indicate that the relative amount of 16:3n-4 is higher in deep-benthic species, while the reverse trend occurs for 20:1n-9 and 22:1n-11 in demersal fish. Therefore, if most of the FA variation is influenced by the diet, males would feed on more deep-benthic prey than the females do. This is consistent with foraging studies comparing female and male otariids, for which males displayed deeper dives than females (e.g. Page et al. 2005a, Staniland & Robinson 2008). However, a previous study on the stomach contents of the same individuals studied here did not show such a trend: dietary differences between males and females were limited to the proportions of opalfish, a benthic prey living on the shelf, which was in higher proportion in the stomach contents of females (L. Meynier unpubl. data). But these differences may have been underestimated because stomach contents give a limited picture of the diet over several days only, which will not reflect long-term dietary differences between female and male NZ sea lions. Furthermore, part of the FA variation between sexes is likely to originate from differential metabolism. Indeed, a larger mass for males implies a higher maintenance metabolism (Costa 1991), which can result in a greater FA mobilization in the sternal blubber than in females. To date, there is no information on the differential use of fat depots between male and female otariids. Thus, it is difficult to draw any conclusion on the dietary differences between NZ sea lion males and females inferred from blubber FA profiles as long as no foraging and diving data are available for males. So far, LF are the only segment of the population for which foraging and diving behaviours are known (Chilvers et al. 2005, 2006). It must be pointed out that males analysed here were mainly non-territorial during breeding and were caught in the same area as females, thus they were not representative of territorial males. Territorial males tend to disperse to distant regions after breeding (Robertson et al. 2006) and are rarely caught by the squid fishery around the Auckland islands.

Year variation in FA profiles

Although the DFA was not performed on years due to a small sampling size, both GLMs showed differences in the FA profiles of by-caught sea lions between years (Tables 2 & 3), especially between 2005/2006 and the previous years and between 2000 and 2004. Indi-

vidual FA variation concerned 17 out of 30 FAs (Table 2). As discussed above, the variation in blubber FA profiles between years may be caused by both differential metabolism and different diet. Differential FA metabolism between years could arise from animals in different body condition. The blubber thickness was significantly lower in the combined years 2005/2006 than in 2000 (Table 1); thus, the differences in FA profiles noticed between these years can be the result of different FA mobilization in the sternal blubber. However, significant variation in FA profiles was not limited to the differences between 2000 and the years 2005/2006; therefore, diet must play a significant role in the FA variation reported between 2005/2006 and the previous years and between 2000 and 2004. Inter-annual and seasonal variation in the diet of the NZ sea lion males has already been investigated through the analysis of faeces (Lalas 1997, McMahon et al. 1999, Childerhouse et al. 2001). Seasonal differences in the diet were found only at Otago Peninsula, South Island, NZ (Lalas 1997), and were attributed to changes in prey availability. Indeed, NZ sea lions are considered generalist predators, and the changes noticed in the present study between the combined years 2005/2006 and the previous years, and also between 2000 and 2004, may be interpreted by a variation in prey stocks availability on the Auckland Islands shelf. However, information on fish and squid populations and their variation around the Auckland Islands are non-existent. Therefore, it is currently not possible to validate the hypothesis of a change in prey availability between 2000 and 2006.

CONCLUSIONS

In the present study, significant differences in the blubber FA compositions between male and female NZ sea lions, and between years from 2000 to 2006, were detected. These differences are likely to be the result of both metabolism and diet. Because of different metabolic requirements, an ability to dive deeper and a lack of investment in pup rearing, male NZ sea lions would utilise food resources differently, explaining some of the differences in FA profiles between the sexes. However, these differences were probably underestimated, as territorial males, with a significant higher mass than females, were not represented in the present study. FA metabolism in the blubber is still poorly understood, thus limiting the potential of FA signature analysis to infer diets of animals in different nutritional or reproductive states (e.g. females versus males, LF versus NLF). However, this method can overcome some biases encountered in traditional dietary techniques. Thus, FA analysis must be seen as a complementary tool to stomach and faeces analyses, along with foraging telemetry studies to assess the feeding ecology of marine mammals.

Acknowledgements. NZ sea lions were necropsied at Massey University under contracts with the Conservation Service Levies Providers, administrated by the Department of Conservation and the Ministry of Fisheries, NZ. We thank observers who collected the carcasses as well as people who assisted with the necropsies including M. Stratton, N. Gibbs, M. Bando, F. Riet-Sapriza and G. Jones. Thanks to R. Galois, M. Walton and M. Reynolds for their advice on fatty acid analysis and to 3 anonymous reviewers who provided comments. This project was sponsored by Lewis Fitch Research Fund, Massey University Research Fund, the Whale and Dolphin Adoption Project, the Department of Conservation and the NZ Ministry of Fisheries.

LITERATURE CITED

Ackman RG, Eaton CA (1966) Lipids of the fin whale (*Balaenoptera physalus*) from north Atlantic waters. III. Occurrence of eicosenoic and docosenoic fatty acids in the zooplankter *Meganyctiphanes norvegica* (M. Sars) and their effect on whale oil composition. Can J Biochem 44: 1561–1566

Ackman RG, Sipos JC (1964) Application of specific response factors in the fast chromatographic analysis of methyl esters of fatty acids with flame ionization detectors. J Am Oil Chem Soc 41:377–378

Andersen SM, Lydersen C, Grahl-Nielsen O, Kovacs KM (2004) Autumn diet of harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Can J Zool 82:1230–1245

Arnould JPY, Nelson MM, Nichols PD, Oosthuizen WH (2005) Variation in the fatty acid composition of blubber in Cape fur seals (*Arctocephalus pusillus pusillus*) and the implications for dietary interpretation. J Comp Physiol [B] 175: 285–295

Beck CA, Rea LD, Iverson SJ, Kennish JM, Pitcher KW, Fadely BS (2007) Blubber fatty acid profiles reveal regional, seasonal, age-class and sex differences in the diet of young Steller sea lions in Alaska. Mar Ecol Prog Ser 338:269–280

Best NJ, Bradshaw CJA, Hindell MA, Nichols PD (2003) Vertical stratification of fatty acids in the blubber of southern elephant seals (*Mirounga leonina*): implications for diet analysis. Comp Biochem Physiol (B) 134:253–263

Boyd IL, McCafferty DJ, Reid K, Taylor R, Walker TR (1998) Dispersal of male and female Antarctic fur seals (*Arctocephalus gazella*). Can J Fish Aquat Sci 55:845–852

Brown DJ, Boyd IL, Cripps GC, Butler PJ (1999) Fatty acid signature analysis from the milk of Antarctic fur seals and southern elephant seals from South Georgia: implications for diet determination. Mar Ecol Prog Ser 187:251–263

Budge SM, Iverson SJ, Koopman HN (2006) Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Mar Mamm Sci 22:759–801

Campagna C, Werner R, Karesh W, Marín MR, Koontz F, Cook R, Koontz C (2001) Movements and location at sea of South American sea lions (*Otaria flavescens*). J Zool (Lond) 255:205–220

Childerhouse SJ, Dix B, Gales NJ (2001) Diet of New Zealand sea lions (*Phocarctos hookeri*) at the Auckland Islands. Wildl Res 28:291–298

Chilvers BL (in press) Foraging site fidelity of lactating New

Zealand sea lions, *Phocarctos hookeri.* J Zool Lond

Chilvers BL, Wilkinson IS, Duignan PJ, Gemmell NJ (2005) Summer foraging areas for lactating New Zealand sea lions *Phocarctos hookeri.* Mar Ecol Prog Ser 304:235–247

Chilvers BL, Wilkinson IS, Duignan PJ, Gemmell NJ (2006) Diving to extremes: Are New Zealand sea lions (*Phocarctos hookeri*) pushing their limits in a marginal habitat? J Zool (Lond) 269:233–240

Chilvers BL, Wilkinson IS, Childerhouse SJ (2007) New Zealand sea lion, *Phocarctos hookeri*, pup production — 1995 to 2006. N Z J Mar Freshw Res 41:205–213

Costa DP (1991) Reproductive and foraging energetics of pinnipeds: implications for life history patterns. In: Renouf D (ed) Behavior of pinnipeds. Chapman & Hall, London, p 300–344

Costa DP, Le Boeuf BJ, Huntley AC, Ortiz CL (1986) The energetics of lactation in the Northern elephant seal, *Mirounga angustirostris.* J Zool 209:21–33

Duignan PJ, Gibbs NJ, Jones GW (2003) Autopsy of pinnipeds incidentally caught in fishing operations 1997/98, 1999/2000, and 2000/01. DOC Science Internal Series 118, Department of Conservation, Te Papa Atawhai, Wellington

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Grahl-Nielsen O, Mjaavatten O (1991) Dietary influence on fatty acid composition of blubber fat of seals as determined by biopsy: a multivariate approach. PSZN I: Mar Ecol 110:59–64

Green K, Williams R, Burton HR (1997) Foraging ecology of Antarctic fur seals *Arctocephalus gazella* around Heard Island. In: Hindell MA, Kemper C (eds) Marine mammal research in the Southern Hemisphere, Vol 1: status, ecology and medicine. Surrey Beatty & Sons, Sydney, p 105–113

Iverson SJ, Field C, Bowen WD, Blanchard W (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. Ecol Monogr 74:211–235

Käkelä R, Hyvärinen H (1998) Composition of polyunsaturated fatty acids in the liver of freshwater and marine ringed seals (*Phoca hispida* spp.) differs largely due to the diet of the seals. Comp Biochem Physiol B 120:231–237

Kirkwood R, Lynch M, Gales NJ, Dann P, Sumner MD (2006) At-sea movements and habitat use of adult male Australian fur seals (*Arctocephalus pusillus doriferus*). Can J Zool 84:1781–1788

Kirsch PE, Iverson SJ, Bowen WD, Kerr SR, Ackman RG (1998) Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 55:1378–1386

Kirsch PE, Iverson SJ, Bowen WD (2000) Effect of a low-fat diet on body composition and blubber fatty acids of captive juvenile harp seals (*Phoca groenlandica*). Physiol Biochem Zool 73:45–59

Koen Alonso M, Crespo EA, Pedraza SN, García NA, Coscarella MA (2000) Food habits of the South American sea lion, *Otaria flavescens*, off Patagonia, Argentina. Fish Bull 98:250–263

Lalas C (1997) Prey of Hooker's sea lions *Phocarctos hookeri* based at Otago Pensinsula New Zealand. In: Hindell MA, Kemper C (eds) Marine mammal research in the Southern Hemisphere, Vol 1: status, ecology and medicine. Surrey Beatty & Sons, Sydney, p 130–136

Lea MA, Cherel Y, Guinet C, Nichols PD (2002) Antarctic fur seals foraging in the Polar Frontal Zone: inter-annual shifts in diet as shown from fecal and fatty acid analyses. Mar Ecol Prog Ser 245:281–297

Learmonth JA (2006) Life history and fatty acid analysis of harbour porpoises (*Phocoena phocoena*) from Scottish waters. PhD dissertation. University of Aberdeen

McMahon CR, Holley D, Robinson S (1999) The diet of itinerant male Hooker's sea lions, *Phocarctos hookeri*, at sub-Antarctic Macquarie Island. Wildl Res 26:839–846

Mellish JA, Horning M, York AE (2007) Seasonal and spatial blubber depth changes in captive harbor seals (*Phoca vitulina*) and Steller's sea lions (*Eumetopias jubatus*). J Mammal 88:408–414

Montie EW, Garvin SR, Fair PA, Bossart GD and others (2008) Blubber morphology in wild bottlenose dolphins (*Tursiops truncatus*) from the Southeastern United States: influence of geographic location, age class, and reproductive state. J Morphol 269:496–511

Oftedal OT (1984) Milk composition, milk yield and energy output at peak lactation: a comparative review. Symp Zool Soc Lond 51:33–85

Orians GH, Pearson NE (1979) On the theory of central place foraging. In: Horn DJ, Stairs BR, Mitchell RD (eds) Analysis of ecological systems. Ohio State University Press, Columbus, p 155–177

Page B, McKenzie J, Goldsworthy SD (2005a) Inter-sexual differences in New Zealand fur seal diving behaviour. Mar Ecol Prog Ser 304:249–264

Page B, McKenzie J, Goldsworthy SD (2005b) Dietary resource partitioning among sympatric New Zealand and Australian fur seals. Mar Ecol Prog Ser 293:283–302

Raum-Suryan KL, Rehberg MJ, Pendleton GW, Pitcher KW, Gelatt TS (2004) Development of dispersal, movements patterns, and haul-out use by pup and juvenile Steller sea lion (*Eumetopias jubatus*) in Alaska. Mar Mamm Sci 20: 823–850

Robertson BC, Chilvers BL, Duignan PJ, Wilkinson IS, Gemmell NJ (2006) Dispersal of breeding, adult male *Phocarctos hookeri*: implications for disease transmission, population management and species recovery. Biol Conserv 127: 227–236

Ryg M, Smith TG, Øritsland NA (1988) Thermal significance of the topographical distribution of blubber in ringed seals (*Phoca hispida*). Can J Fish Aquat Sci 45:985–992

Staniland IJ, Pond DW (2005) Investigating the use of milk fatty acids to detect dietary changes: a comparison with faecal analysis in Antarctic fur seals. Mar Ecol Prog Ser 294:283–294

Staniland IJ, Robinson SL (2008) Segregation between the sexes: Antarctic fur seals, *Arctocephalus gazella*, foraging at South Georgia. Anim Behav 75:1581–1590

Walton MJ, Henderson RJ, Pomeroy PP (2000) Use of blubber fatty acid profiles to distinguish dietary differences between grey seals *Halichoerus grypus* from 2 UK breeding colonies. Mar Ecol Prog Ser 193:201–208

Weise MJ, Costa DP, Kudela RM (2006) Movement and diving behavior of male California sea lion (*Zalophus californianus*) during anomalous oceanographic conditions to those of 2004. Geophys Res Lett 33(22):L22S10

Wheatley KE, Nichols PD, Hindell MA, Harcourt RG, Bradshaw CJA (2007) Temporal variation in the vertical stratification of blubber fatty acids alters diet predictions for lactating Weddell seals. J Exp Mar Biol Ecol 352:103–113

Whiteley GSW, Fuller BJ, Hobbs KEF (1992) Deterioration of cold-stored tissue specimens due to lipid peroxidation: modulation by antioxidants at high subzero temperatures. Cryobiology 29:668–673

Williams TM, Rutishauser M, Long B, Fink T, Gafney J, Mostman-Liwanagm H, Casper D (2007) Seasonal variability in otariid energetics: implications for the effects of predators on localized prey resources. Physiol Biochem Zool 80: 433–443

Editorial responsibility: Yves Cherel, Villiers-en-Bois, France

Submitted: January 21, 2008; Accepted: June 18, 2008
Proofs received from author(s): August 22, 2008