Milk fatty acid signatures indicate both major and minor shifts in the diet of lactating Antarctic fur seals

S.J. Iverson, J.P.Y. Arnould, and I.L. Boyd

Abstract: Fatty acid signature analysis is based on the principle that unique arrays of fatty acids within groups of organisms can be transferred, largely unaltered, up the marine food chain and thus may be an indicator of diet composition. We applied fatty acid signature analysis to milks collected from Antarctic fur seals (*Arctocephalus gazella*) at South Georgia in 1990–1991, during the perinatal period (N = 19) and subsequently during early (N = 11), mid (N = 11), and late (N = 8) foraging trips. In lactating otariid females, milk fatty acids secreted during the perinatal fast are derived largely from blubber mobilization and thus are influenced by dietary history prior to parturition. Conversely, milk fatty acids secreted during foraging trips are derived primarily from immediate dietary intake. The fatty acid signature of perinatal milks was significantly different from that of all other milks, suggesting differences in the prepartum diet when females are away from the breeding grounds. At the onset of foraging periods, the fatty acid composition of milks changed dramatically to reflect a diet composed mainly of krill. However, during late foraging periods, milk fatty acids again changed from those of early and mid foraging, and suggested a predominance of teleost fish in the diet. These findings were consistent with independent assessments of diet by faecal analysis and indicate the potential value of fatty acid signature analysis in studying foraging ecology in free-ranging pinnipeds.

Résumé : L'analyse des signatures d'acides gras est basée sur le principe selon lequel des regroupements spécifiques d'acides gras retrouvés chez des groupes d'organismes peuvent être transférés, presque intacts, dans la chaine alimentaire marine et donc servir d'indices de la composition du régime alimentaire. Nous avons utilisé l'analyse des pools d'acides gras dans des laits recueillis chez des Otaries de Kerguelen en Georgie du Sud, en 1990–1991, pendant la période entourant la naissance (N = 19) et, par la suite, au cours d'excursions précoces (N = 11), intermédiaires (N = 11) et tardives (N = 8) de recherche de nourriture. Chez les femelles nourricières, les acides gras lactiques sécrétés durant le jeûne entourant la naissance proviennent en grande partie de l'utilisation du lard et donc sont influencés par l'alimentation avant la parturition. En contre-partie, les acides gras lactiques sécrétés pendant les excursions proviennent principalement de la consommation immédiate d'aliments. Le pool des acides gras dans les laits périnataux était significativement différent de celui trouvé dans tous les autres laits, ce qui semble indiquer des variations dans la régime alimentaire prépartum lorsque les femelles sont loin des aires de reproduction. Au début des périodes d'excursion, la composition en acides gras du lait a changé abruptement et reflétait la consommation prédominante de krill. En revanche, au cours des excursions alimentaires plus tardives, le pool des acides gras s'est de nouveau modifié par rapport aux pools reconnus au cours des excursions plus précoces et reflétait la prédominance des poissons téléostéens dans le régime. Ces résultats correspondent à ceux de l'évaluation des régimes alimentaires à partir d'analyses fécales et confirment le potentiel des analyses des pools d'acides gras dans l'étude de l'écologie de la recherche de nourriture chez les pinnipèdes en nature.
[Traduit par la Rédaction]

Introduction

Understanding the foraging ecology and diets of free-ranging marine mammals is critical to evaluating predator–prey relationships within marine ecosystems, as well as life-history patterns and the impact of ecological variation on these patterns (e.g., Gentry and Kooyman 1986; Trillmich and Ono 1991). Unfortunately, our knowledge of the diets of marine mammals is limited. In pinnipeds (seals, sea lions, and fur seals), current methods used to determine diets (by sampling stomach contents collected from killed animals, lavage sampling, or recovery of hard parts from faeces) yield valuable but potentially biased results, or relatively little information, owing to rapid passage from the gut, selective regurgitation, and differential rates of digestion of various hard and soft parts (Jobling and Brieby 1986; Harwood and Croxall 1988; Bowen et al. 1993). Conclusions about diet using these methods are also generally restricted to the last meal from a limited area around the haulout site.

Recently, "fatty acid signature analysis" (Iverson 1993) has been proposed as a method that could potentially overcome many of the difficulties inherent in existing methods based on the recovery of hard parts. Fatty acids are essentially the largest constituent of common lipids, and those with a carbon chain length of 14 or greater are often deposited in animal tissue from the diet with minimal modification. Owing to various restrictions and specificities in the biosyn-

Received May 29, 1996. Accepted September 13, 1996.

S.J. Iverson.[1] Department of Biology, Dalhousie University, Halifax, NS B3H 4J1, Canada.
J.P.Y. Arnould and I.L. Boyd. British Antarctic Survey, Natural Environmental Research Council, High Cross, Madingley Road, Cambridge CB3 OET, United Kingdom.

[1] Author to whom all correspondence should be addressed (e-mail: siverson@is.dal.ca).

thesis and modification of fatty acids among different taxonomic groups (e.g., Paradis and Ackman 1976; Ackman 1980; Cook 1985), many components appear in animal tissues which have a specific ecological origin that may be traced. Lipids in the marine food chain are characterized by an exceptionally complex and unusual array of fatty acids, which have been found to occur in specific relationships depending upon the prey species and their geographical location (e.g., Ackman and Eaton 1966; Iverson 1993), and have direct effects on the fatty acid patterns of their predators (Kirsch et al. 1995; Iverson and Frost 1996). In rapidly fattening seals, ingested fatty acids appear to be deposited in adipose tissue in proportion to their intake, so blubber tissue may be a mirror of the diet (Iverson et al. 1995*b*). In lactating females, milk fatty acids are derived largely from blubber mobilization during fasting, but are likely derived primarily from the diet when the animal is feeding (Iverson and Oftedal 1992; Iverson 1993; Iverson et al. 1995*b*; Iverson et al. 1995*a*). Hence, in lactating otariids, milk secreted during the perinatal fast should resemble blubber and thus represent an integration of the diet during the fattening period prior to parturition, whereas milk secreted during foraging trip intervals should resemble the dietary intake during the lactation period.

The Antarctic fur seal (*Arctocephalus gazella*) is considered a specialist feeder during lactation, consuming a diet of mainly krill, *Euphausia superba* (Croxall and Pilcher 1984; Boyd et al. 1991; Reid and Arnould 1996). However, there is no information on their distribution or feeding habits during the winter, when adult female Antarctic fur seals are away from their breeding grounds. Adult females return to the breeding grounds mainly during December and give birth to a single pup, which is then suckled for approximately 117 days. After the initial perinatal fast of about 7 days, females typically alternate cyclic foraging trips lasting 3–6 days with 1- to 3-day suckling periods on shore (Lunn and Boyd 1993*a*). This behavior is altered radically during years for which commercial harvest and research data show low krill availability, and which are associated with poor reproductive performance in female fur seals and krill-eating penguins and albatrosses (Croxall et al. 1988). Recent studies of Antarctic fur seal diets, based on analysis of faeces or stomach contents, have indicated a shift to include pelagic lantern fish (Myctophidae) in the diet during the final one-third of lactation in 3 of 4 years studied; 2 of these 3 years were considered to be poor krill years (Reid and Arnould 1996). However, it is not known how much these conclusions are affected by the biases of faecal analysis, nor was it possible to assess the overall importance of fish versus krill in the diet.

In the present study we used milks collected from lactating Antarctic fur seals during the perinatal period and subsequent foraging cycles to investigate aspects of diet and foraging ecology. We also illustrate the use of classification and regression tree analysis (CART) in the statistical interpretation of complicated fatty acid patterns containing more than 60 variables per observation. Our specific aims were (*i*) to use milk fatty acid signatures to compare the prepartum diet with the postpartum diet, (*ii*) to examine whether changes in diet occurred throughout a breeding season, and (*iii*) to compare the changes and possible trophic levels of the diet estimated using fatty acid signatures with those determined from faecal analysis in the same breeding season.

Materials and methods

Sample collection and laboratory analysis

Milk samples were collected between December and March of the austral summer of 1990–1991 from fur seals south of the Antarctic Convergence at South Georgia (Bird Island, 54°S, 38°W), where an estimated 96% of the world population breeds (Boyd 1993). A large number of mother–pup pairs were marked at parturition between 1 and 7 December with commercially available hair bleach, so that known-age pairs were available throughout the study period. All seals were captured using standard noose-pole and restraint-board techniques (Gentry and Holt 1982). Milk was collected from non-sedated animals by manual expression after injection of oxytocin. In total, 49 milk samples were obtained during four periods of lactation, designated as perinatal (the period from parturition until first foraging trip; $N = 19$) and early ($N = 11$), mid ($N = 11$), and late ($N = 8$) foraging (attendance intervals between foraging trips). Early, mid, and late foraging milks were collected at random from mothers of known-age pups as they arrived at the breeding colony during late December, early February, and mid-March, respectively. During this study we meticulously followed the humane standards of both the United Kingdom Animal Scientific Procedures Act and the Falkland Islands legislation.

Milk samples were stored frozen in airtight vials after collection. Samples were then extracted into chloroform according to a modification (Arnould et al. 1995) of the Bligh and Dyer method (Bligh and Dyer 1959). Fatty acid methyl esters were prepared from 100 mg of the pure extracted lipid (filtered and dried over anhydrous sodium sulfate), using 1.5 mL 8% boron trifluoride in methanol (w/w) and 1.5 mL hexane, capped under nitrogen, and heated at 100°C for 1 h. Fatty acid methyl esters were extracted into hexane, concentrated, and brought up to volume (50 mg/mL) with high-purity hexane. This method produced results identical with those obtained using 0.25 M H_2SO_4 in methanol as methylating reagent.

Fatty acid methyl esters were analyzed and identified using temperature-programmed gas liquid chromatography according to Iverson (1988) and Iverson et al. (1992) on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30 m × 0.25 mm i.d. column coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; J&W DB-23; Folsom, Calif.) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson). Individual fatty acids are expressed as mass percent of total fatty acids and designated according to the shorthand nomenclature of the International Union of Pure and Applied Chemistry for carbon chain length : number of double bonds and location ($n - x$) of the double bond nearest the terminal methyl group.

Data analysis and interpretation

Milk fatty acid data were grouped according to lactation stage and analyzed using CART in S-plus (Version 3.5 for Windows). CART is a nonparametric multivariate technique for classifying data and provides an alternative and often preferable method of analyzing complex data sets to the more traditional linear regression, generalized linear models, or other multivariate methods such as discriminant analysis and principal components analysis (Clark and Pregibon 1992). CART methods have recently been applied to and modified for use specifically in fatty acid signature analysis (Smith et al. 1997). The initial goals of our analysis were (*i*) to determine whether there were statistical differences between the fatty acid composition of fur seal milk during the four lactation stages (perinatal and early, mid, and late foraging) and (*ii*) to formulate classifi-

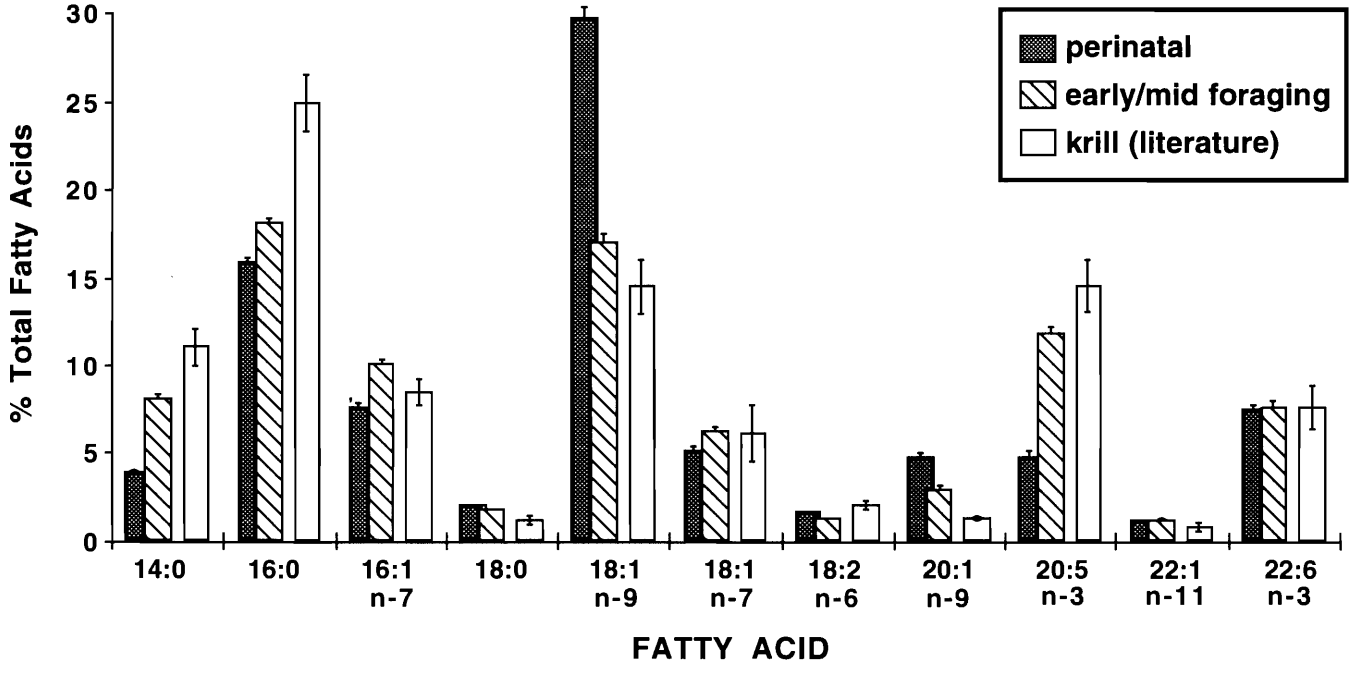
Table 1. Fatty acid composition (mass %) of Antarctic fur seal milk during four stages of lactation from the austral summer of 1990–1991.

Fatty acid	Perinatal ($N = 19$)	Early foraging ($N = 11$)	Mid foraging ($N = 11$)	Late foraging ($N = 8$)
Percent fat*	40.8±1.56*a*	44.0±3.12*a*	42.1±1.92*a*	55.1±2.41*b*
12:0	0.15±0.011	0.16±0.009	0.17±0.007	0.16±0.009
14:0	3.94±0.165	7.65±0.422	8.74±0.347	6.94±0.222
14:1*n* − 9,7	0.12±0.014	0.11±0.019	0.13±0.015	0.14±0.008
14:1*n* − 5	0.28±0.024	0.30±0.019	0.30±0.011	0.21±0.012
Iso15	0.16±0.003	0.19±0.009	0.19±0.012	0.20±0.005
15:0	0.36±0.010	0.31±0.008	0.30±0.007	0.32±0.014
Iso16	0.15±0.005	0.11±0.011	0.11±0.006	0.13±0.004
16:0	15.91±0.306	17.99±0.352	18.49±0.276	16.50±0.216
16:1*n* − 11	0.49±0.021	0.36±0.016	0.35±0.012	0.39±0.005
16:1*n* − 9	0.62±0.022	0.29±0.021	0.26±0.010	0.25±0.013
16:1*n* − 7	6.57±0.262	9.56±0.393	9.44±0.401	7.10±0.213
7Me16:0	0.23±0.008	0.22±0.011	0.25±0.005	0.26±0.004
16:1*n* − 5	0.33±0.006	0.27±0.015	0.26±0.008	0.29±0.004
Iso17	0.18±0.003	0.16±0.008	0.17±0.007	0.20±0.011
16:2*n* − 4	0.32±0.015	0.18±0.030	0.12±0.019	0.20±0.008
16:3*n* − 6	0.22±0.018	0.62±0.049	0.69±0.033	0.49±0.020
17:0	0.40±0.016	0.36±0.017	0.35±0.012	0.35±0.010
17:1	0.56±0.017	0.33±0.020	0.28±0.008	0.29±0.021
16:3*n* − 1	0.31±0.008	0.25±0.012	0.26±0.008	0.26±0.012
16:4*n* − 1	0.09±0.016	0.32±0.034	0.34±0.018	0.42±0.030
18:0	2.12±0.061	1.87±0.083	1.76±0.066	2.10±0.026
18:1*n* − 11	1.93±0.087	0.54±0.116	0.35±0.113	0.62±0.160
18:1*n* − 9	29.86±0.615	18.23±0.977	15.87±0.214	15.57±0.771
18:1*n* − 7	5.23±0.204	6.21±0.392	6.33±0.193	4.83±0.132
18:1*n* − 5	0.48±0.015	0.34±0.041	0.29±0.026	0.50±0.019
18:2*n* − 6	1.69±0.056	1.35±0.040	1.36±0.031	1.31±0.028
18:3*n* − 6	0.08±0.005	0.13±0.008	0.15±0.003	0.16±0.004
18:3*n* − 4	0.22±0.011	0.18±0.010	0.16±0.018	0.14±0.008
18:3*n* − 3	0.46±0.015	0.43±0.012	0.50±0.017	0.57±0.015
18:4*n* − 3	0.38±0.029	0.81±0.051	0.91±0.038	1.06±0.041
18:4*n* − 1	0.23±0.022	0.39±0.022	0.44±0.034	0.57±0.030
20:1*n* − 11	0.91±0.065	0.38±0.056	0.25±0.059	0.08±0.078
20:1*n* − 9	4.79±0.241	2.90±0.381	3.03±0.473	7.38±0.461
20:1*n* − 7	0.31±0.012	0.33±0.014	0.32±0.014	0.40±0.013
20:2*n* − 6	0.22±0.011	0.13±0.019	0.11±0.013	0.18±0.011
20:3*n* − 6	0.16±0.006	0.15±0.008	0.14±0.008	0.17±0.008
20:4*n* − 6	0.72±0.025	0.46±0.027	0.40±0.014	0.46±0.015
20:4*n* − 3	0.92±0.041	0.81±0.038	0.92±0.048	1.24±0.044
20:5*n* − 3	4.77±0.379	11.16±0.573	12.53±0.339	10.37±0.176
22:1*n* − 11	0.76±0.060	0.68±0.186	0.81±0.209	2.65±0.282
22:1*n* − 9	0.51±0.030	0.46±0.068	0.49±0.074	1.09±0.087
22:2*n* − 6	0.13±0.023	0.19±0.036	0.15±0.033	0.22±0.037
21:5*n* − 3	0.24±0.009	0.46±0.020	0.47±0.015	0.46±0.015
22:4*n* − 6	0.07±0.008	0.13±0.060	0.03±0.007	0.03±0.013
22:5*n* − 3	2.20±0.073	2.43±0.100	2.36±0.120	2.04±0.042
22:6*n* − 3	7.58±0.236	8.04±0.518	7.37±0.419	8.18±0.315
24:1	0.23±0.018	0.27±0.049	0.26±0.032	0.60±0.060

Note: Values are given as means ± SEM of all fatty acids present in greater than trace amounts (>0.05%).

*Approximate percent fat in samples, estimated using a stoichiometric method (Arnould et al. 1995). Values were tested by ANOVA on arcsine-transformed data; values followed by a different letter were significantly different ($P = 0.0007$, Fisher's PLSD). ANOVA was performed only on major fatty acids (see Fig. 1), since some differences might arise from chance alone, given the large number of individual comparisons.

Fig. 1. Mass percent composition of major fatty acids in Antarctic fur seal milk during the perinatal fast (N = 19) and early to mid foraging trip intervals (N = 22) in comparison with that expected in Antarctic krill (*Euphausia superba*). Values are given as means ± SEM. Significant differences ($P < 0.001$, ANOVA on arcsine-transformed data) were found between perinatal and foraging milks for all components except 22:1$n-11$ and 22:6$n-3$. Data for krill represent an average of 7 values obtained from the literature for whole krill (Tsuyuki and Itoh 1976; Lee et al. 1981; Fricke et al. 1984; Virtue et al. 1993).



cation rules for estimating the stage of lactation in the 1990–1991 summer season, given the milk fatty acid composition.

In overview, CART uses an algorithm that automatically selects the "best" variable for splitting data into two groups ("nodes") that are as different as possible (Clarke and Pregibon 1992). The deviance of a node is then a measure of the homogeneity of the observations which fall into that node. The CART algorithm begins at the root node by considering all possible ways to split the data, i.e., all variables (fatty acids) and all possible splitting points within each variable, and chooses the split that maximizes the difference in deviance at that node. The observations (milk samples) in that split are then sent down one of two branches. This splitting is continued in a tree-like form and occurs until one of two stopping criteria (based on a minimum number of observations in a node or minimum deviance of a node relative to the root node) is met. Tree growth (splitting) ends at a terminal node where a classification is made and the associated misclassification rate (number of observations not correctly classified in the node) is given. The distribution of the data at each nonterminal node can then be viewed in the form of tree box plots, which successively remove the effect of the previous split(s) and allow examination of the splitting point at each node.

The stopping rules in CART are conservative, causing an initial completed tree to be "overfitted" (analogous to using too many parameters to fit a regression) and hence it needs to be "pruned." We did this by successively removing the "least important" splits from the tree, based on a cost complexity function in S-plus that yielded a sequence of pruned subtrees. We used cross-validation to test the efficiency (residual mean deviance and misclassification rate) of each size subtree and select the best subtree. This is typically performed by randomly dividing the data into N (using a default value of 10) subsets of equal sample sizes. A tree is then grown from (N − 1) of the subsets and the remaining subset is used to validate the performance of the tree by dropping it through the tree and calculating the deviances for the terminal nodes. Since the random allocation of data into the 10 subsets could potentially change the results, we performed 25 separate cross-validations on the data.

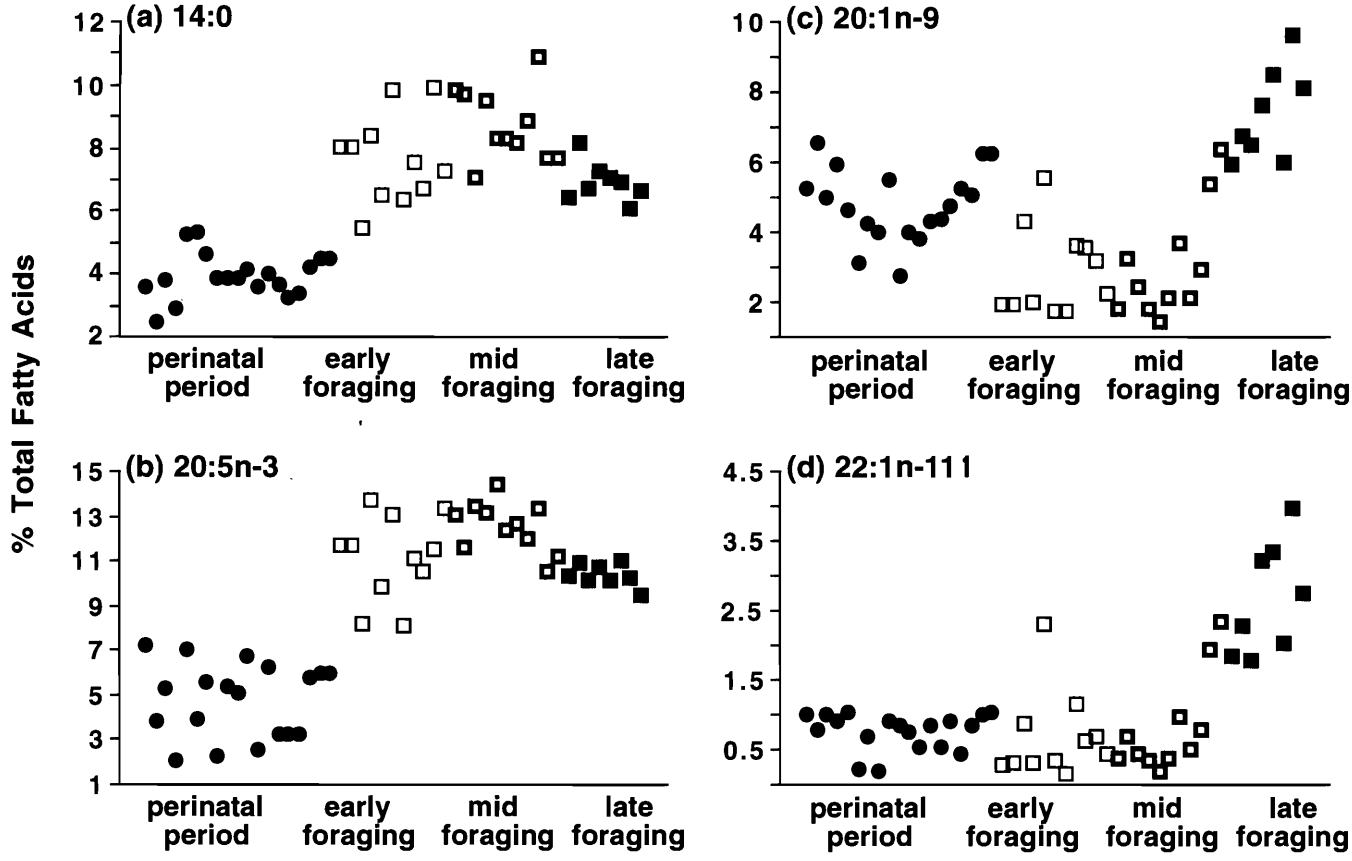
Finally, since the fatty acids and splitting points in the tree are selected algorithmically by maximizing the change in deviance between the root (or parent) node and subsequent (or child) nodes, we also examined which, if any, other fatty acids might have been nearly as close to being selected. Additionally, we forced the algorithm to select only the major (most plentiful) fatty acids for the first and second node splits, since as large percentages, these would be least influenced by changes in other fatty acids, and some are considered to be important dietary indicators. The efficiencies of the corresponding trees and competing fatty acids were then compared with the initial tree. Application of the S-Plus software is described in Clark and Pregibon (1992) and Venables and Ripley (1994).

Results

Fur seal milk contained about 41–44% fat throughout most of lactation, but increased significantly to 55% fat during late foraging (Table 1). Approximately 70 different fatty acids were identified and quantified in fur seal milk lipid, and of these, 47 were most consistently found in greater than trace amounts (>0.05%) among all samples and accounted for 98–99% of total fatty acids (Table 1). Of the major components (>1% of total fatty acids), levels of 16:0, 18:0, 18:2$n-6$, 22:5$n-3$, and 22:6$n-3$ were relatively constant across lactation stages, while the greatest variability was found in 14:0, 18:1$n-9$, 20:1$n-9$, 20:5$n-3$, and 22:1$n-11$; variability in 16:1$n-7$ and 18:1$n-7$ was intermediate (Table 1).

Some of the most dramatic changes in fatty acid patterns occurred between milks collected during the perinatal period

Fig. 2. Scattergram showing changes in selected Antarctic fur seal milk fatty acids (mass % composition) over the course of lactation ($N = 49$). Data points are ordered by group (perinatal and early, mid, and late foraging); however, within each group the order of points is random. Significant differences were found between perinatal and early, mid, and late foraging milks for all components ($P < 0.001$, ANOVA on arcsine-transformed data).

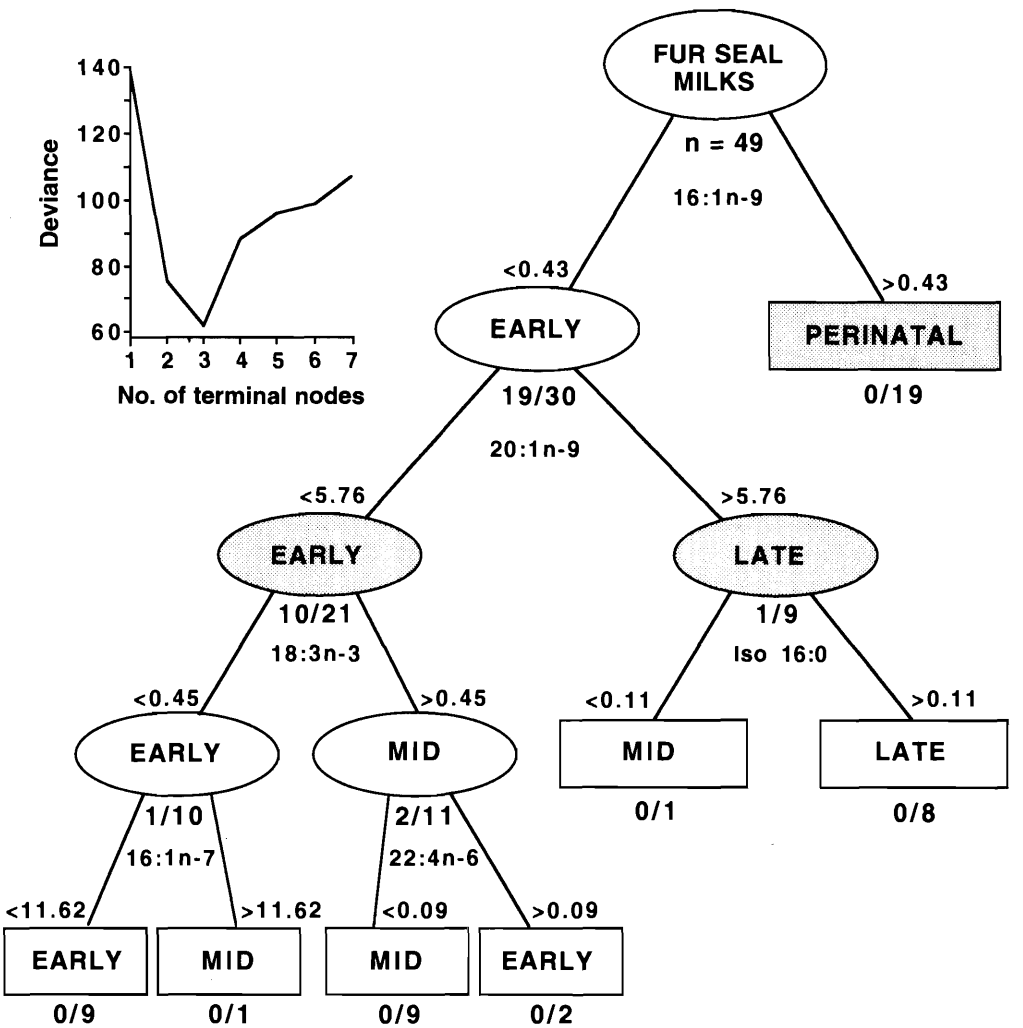


and those collected during early to mid foraging (Table 1). These changes are illustrated by 11 of the major fatty acids, which comprised between 85 and 87% of total fatty acids throughout lactation (Fig. 1). Most notable were increases of up to 2.6-fold in levels of 14:0 and 20:5$n-3$ and decreases of up to 1.9-fold in 18:1$n-9$ and 20:1$n-9$ between the perinatal period and early to mid foraging trip intervals. Although samples of krill were not available from this study, the fatty acid patterns of fur seal milks during early and mid foraging exhibited marked similarities to that of krill analyzed in previous studies, whereas patterns in milks secreted during the perinatal period differed greatly from both foraging-interval milks and krill (Fig. 1).

The composition of milk fatty acids not only differed between the perinatal period and foraging periods, but also changed over the period of foraging trips (Table 1). Scatter plots of selected fatty acids illustrate several types of patterns observed in milk fatty acids over lactation stages (Figs. 2*a*–2*d*). For example, levels of 14:0 and 20:5$n-3$ were comparatively low during the perinatal period, increased substantially during early and mid foraging, but declined again during late foraging (Figs. 2*a*, 2*b*). In contrast, levels of 20:1$n-9$ were relatively high during the perinatal period, decreased at the onset of foraging trips, and then increased substantially during late foraging (Fig. 2*c*). Levels of 22:1$n-11$ were relatively constant and low until late foraging, when percentages increased dramatically (Fig. 2*d*). In the case of 22:1, the other major isomer ($n-9$) followed the same pattern as the $n-11$ isomer (Table 1), but in 20:1 the other major isomer ($n-11$) exhibited the reverse pattern to the $n-9$ isomer (Fig. 2*c*), with a continual decrease over lactation (Table 1). In comparison with the above changes, the variation in components such as 16:0 and 18:0 was small and did not change systematically through lactation, while levels of 16:1$n-9$ and 18:1$n-9$ declined sharply between the perinatal period and later, but did not appear to vary substantially during subsequent foraging trips (Table 1). In summary, changes in the percentages of individual fatty acids were most apparent between the perinatal period and the onset of foraging trips and then again between late foraging and early to mid foraging.

The results of CART analysis confirmed these general observations. The initial (unpruned) tree grown by the algorithm produced six splits, resulting in seven terminal nodes (Fig. 3). This initial tree was "fully fitted" in the sense that none of the observations (milks) would be misclassified when "dropped" through the tree; that is, each of the 7 terminal nodes had a misclassification rate of zero. The fatty acid 16:1$n-9$ was automatically selected for splitting data at the root node, resulting in all perinatal observations being correctly classified as one group. A box plot of the data at the first split illustrates the clear separation of perinatal from

Fig. 3. Initial unpruned classification tree of Antarctic fur seal milks determined by CART. Ellipses represent intermediate nodes and rectangles represent terminal nodes; labels within an ellipse or rectangle indicate the classification at that node as represented by the largest number of observations with that label in that node. The fatty acid listed at each split is the variable chosen by the algorithm to create the split; > and < indicate the optimal splitting level (mass %) of that fatty acid. Fractions under each intermediate and terminal node indicate the number of misclassifications over the total number of observations in that node. Shaded ellipses and rectangles represent the final optimal 3-terminal-node subtree. Inset: Average of 25 cross-validations performed to determine the optimal tree size or number of terminal nodes (see the text).



foraging milks (Fig. 4). The second split was based on $20{:}1n-9$, which essentially classified late foraging separately from early and mid foraging. All late foraging milks fell in this node, but one mid foraging milk was misclassified by CART as a late milk. All early and mid foraging milks (except one) split correctly into the opposite node (Figs. 3, 4). Early and mid foraging appeared to be somewhat different from one another (e.g., Fig. 4), but obviously shared some similarities as indicated by errors in classifications at the third split based on $18{:}3n-3$ (Fig. 3).

As stated previously, the initial tree in CART is likely to be overfitted. This is most apparent by the less "meaningful" splits of early and mid foraging, where observations from each group appear on opposite sides at the last 4 terminal nodes (Fig. 3). The results of the 25 cross-validations performed on the data were consistent with this observation. Cross-validation almost always calculated the lowest terminal node deviances with a three-node subtree and the average of all 25 cross-validations indicated a three-terminal node tree (Fig. 3, inset), which classified the perinatal period separately from all other observations and distinguished late foraging from early and mid foraging.

The variables and splitting points used in Figs. 3 and 4 were determined algorithmically by maximizing the deviance between the parent node and child nodes. However, there were other choices for splitting variables, some of which were abundant fatty acids, that could easily have given the same results (Table 2). This is potentially valuable information, given that some of the initial variables selected were minor components. Nine other trees, including four using only plentiful fatty acids, were then created by changing the root node and second node variable to a major component from Table 2. These additional trees provided essentially the same conclusions as the initial tree: a 3-node subtree was the

Fig. 4. Box plots of the algorithmically selected variables, showing the distribution of the data at the first three nonterminal nodes from the initial tree (see Fig. 3). The box plot for the root node variable contains data from all observations. The box plots for subsequent variables have removed the effects (observations) of the nodes higher up in the tree, i.e., once an observation has been classified, it is removed from any of the plots that follow, since it may mask the effects that should be observed. The notched area of each box represents the 95% confidence interval on the means; dots indicate outliers. The horizontal line represents the splitting value used by the CART algorithm.

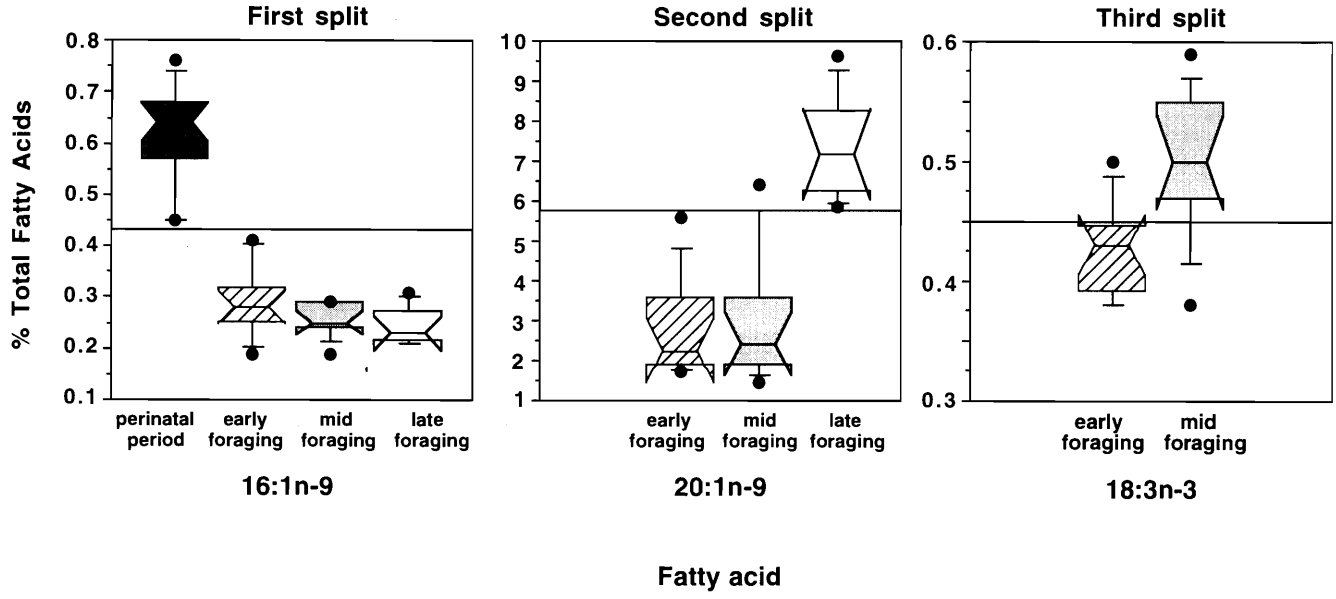


Table 2. Competition among variables at splits in the pruned (3-terminal-node) subtree (from Fig. 1).

Change in deviance	Fatty acids
Node 1 splits (perinatal period vs. foraging)	
65.437	16:1*n* − 9, 14:0, 18:1*n* − 11, 18:1*n* − 9, 18:4*n* − 3, 20:5*n* − 3, 21:5*n* − 3
59.564	16:3*n* − 6
Node 2 splits (late vs. early and mid foraging)	
29.950	20:1*n* − 9, 20:4*n* − 3
26.539	18:1*n* − 5
26.297	18:3*n* − 3
24.787	16:1*n* − 7
22.296	14:1*n* − 5, 20:1*n* − 5, 22:1*n* − 11, 22:1*n* − 9, 24:1

Note: At each split, the underlined fatty acid is the variable determined algorithmically by maximizing the change in deviance between the root (or parent) node and subsequent (or child) nodes; the other fatty acids are those that were equally likely, or almost as likely, to have been chosen on the basis of the same (or a similar) change in deviance. For absolute levels of fatty acids see Table 1.

preferred one based on cross-validations, with the perinatal period differing from foraging, and late foraging differing from early and mid foraging. Differences between early and mid foraging remained apparent, but somewhat less convincing.

Discussion

A general feature of maternal strategies in otariids is the deposition of moderate-sized energy stores as blubber prior to parturition in order to support milk secretion during the first week post partum. Subsequently, females make intermittent foraging trips to sea during which body stores are replenished to support continued milk secretion. Antarctic fur seals inhabit productive environments characterized by predictable but highly seasonal food resources. Hence, migrating fur seals return to traditional breeding sites to give birth and lactate at the time when conditions are most favorable, namely in the austral summer, when there is a large influx of krill biomass (e.g., Gentry et al. 1986; Duck 1990).

Evidence for the predominant consumption of krill by Antarctic fur seals during lactation comes from faecal analyses conducted over a number of years (Croxall and Pilcher 1984; Doidge and Croxall 1985; Boyd et al. 1991; Reid and Arnould 1996). Additional support for this reliance on krill comes from a number of studies that have investigated the diving behavior of lactating Antarctic fur seals by means of time–depth recorders (TDRs). These studies have shown that the foraging behavior of fur seals is closely linked with krill movements, specifically with the depth distribution, swarming behavior, and diel vertical migration of krill to and from the surface (Croxall et al. 1985; Boyd and Croxall 1992). Nevertheless, despite advances in understanding the aquatic behavior of fur seals, accurate knowledge of their diet throughout lactation has remained limited. Potential limitations and biases of faecal analysis are well known, and may be exaggerated in the case of fur seals (Reid and Arnould 1996). The slow rate of digestion of the hard chitinous skeleton of krill (Jackson and Ryan 1986; Martensson et al. 1994), and the possible voiding of less eroded otoliths prior to coming ashore (Daneri and Coria 1993), given the long transit times of females from feeding sites, are both likely to bias any faecal analysis towards an overestimation of the krill content of the diet.

There are several general advantages to the use of fatty acid signature analysis as an indicator of diet. First, it does not depend upon the recovery of hard parts of prey, so prey without hard parts can also be identified. Second, information can potentially be obtained on both recent and past diets (e.g., this study) and on offshore as well as near-shore diets (J.G. Lassner, S.J. Iverson, G.B. Stenson, and W.D. Bowen, unpublished data). When a female fur seal comes ashore to give birth, her blubber stores will represent an integration of dietary history, but will probably be most influenced by the previous months of fattening in advance of parturition (e.g., Iverson 1993; Iverson et al. 1995*b*). During the initial perinatal fasting period, these blubber lipids will be mobilized to largely support milk fat secretion; hence, this milk is likely to resemble blubber and thus reflect the previous diet (Iverson 1993; Iverson et al. 1995*b*). However, when the female begins foraging trips, dietary fatty acids will probably be directed first to the mammary gland by lipoprotein lipase (Iverson et al. 1995*a*), second to blubber stores that are being replenished, and ultimately again to the milk during several-day suckling bouts, when blubber is again mobilized. Hence, by sampling milk at various times during lactation we can study changes in foraging and diet.

In the breeding season of 1990–1991, significant changes in the fatty acid composition of Antarctic fur seal milks indicated changes in diet across lactation stages. Milk fatty acids secreted during initial foraging were notably different from those secreted during the perinatal fast and suggested a large influence of krill in the diet of females once foraging began (Table 1, Fig. 1). Although we were unable to directly analyze the fatty acid composition of krill, the pattern of milk fatty acids secreted during initial foraging was remarkably similar to, or changed in the direction of, krill as estimated from previous studies (Fig. 1). Krill is generally notable for its high levels of 14:0 and 20:5$n-3$ and very low levels of both 20:1$n-9$ and 22:1$n-11$, patterns that appear to be reflected in early and mid foraging milks. These fatty acids are useful dietary indicators in marine mammals, since the latter three, in particular, typically come only from the diet (Iverson 1993; Iverson et al. 1995*b*); however, many of the other fatty acids also changed in milk in the direction of krill during initial foraging (Fig. 1).

It is also clear that females had a distinctly different diet prior to parturition than during lactation. Even without data on the fatty acid compositions of an array of potential fur seal prey, high levels of 18:1$n-9$, 20:1$n-9$, and 22:1$n-11$ tend to be characteristic of many teleost fish, not of krill (Fig. 1; Ackman 1980; Reinhardt and Van Vleet 1986; Iverson and Frost 1996), therefore high levels of these components in perinatal milks suggest the importance of fish in the prepartum diet. This difference may reflect different diet preferences at different times of the annual cycle, as well as foraging in a different location before the return to the breeding grounds, which is consistent with hypotheses that females are migratory and winter away from the breeding grounds (e.g., Duck 1990).

The finding that females probably feed upon krill at the onset of foraging trips was not surprising. However, contrary to previous findings, there appeared to be a significant change in diet during mid to late foraging trips, as evidenced by changes in the composition of milk fatty acids (Fig. 2). In particular, the very large increases of 20:1$n-9$ and 22:1$n-11$ to 9.7 and 4.0%, respectively, indicated diets low in krill and high in teleost fish, which are likely to have high levels of these components. In fact, these findings were supported by several types of independent evidence. In the austral summer of 1990–1991, krill availability was reported to be greatly reduced, and this was associated with poor reproductive performance of female Antarctic fur seals and poor growth rate and milk stealing in pups (Lunn 1992; Lunn and Boyd 1993*b*). Although, in general, female fur seals make 4-day foraging trips to feed at sea, this may vary, since females will remain at sea long enough to sufficiently replenish body stores (Costa et al. 1989). Consistent with this, in the summer of 1990–1991, females were in poorer condition, made significantly longer foraging trips, and invested significantly greater effort in foraging (Boyd et al. 1994). This implies that they were searching farther and longer for food and possibly consumed alternative prey to krill. A recent 4-year study using faecal analysis has since demonstrated a significant switch to myctophid fish late in lactation in 3 of 4 years studied, and indeed, a higher occurrence of fish otoliths in scats in 1990–1991 than in all other years (Reid and Arnould 1996). However, in that study it was only possible to assess higher frequencies of occurrence of fish prey in faeces, but not the proportion of the overall diet represented by fish, owing to prolonged retention of krill carapaces. Fatty acid signatures, which are not dependent upon hard parts, independently confirmed the finding of increased amounts of fish in the diet, but were also able to establish that fish indeed dominated the diet.

The analysis of fatty acid signatures using CART appears to be an effective way to detect differences in complicated data sets containing up to 70 fatty acids per sample. Milks could be reliably classified into groups by fatty acid composition using CART (Fig. 3), and these groups most likely represented dietary changes over the lactation period. Although CART compares favorably with discriminant analysis in fatty acid signature analysis (Smith et al. 1997), among its advantages over other multivariate methods are that it is nonparametric and eliminates a subjective variable-selection process. In the present study, many of the fatty acids chosen for constructing the classification tree were in fact quite minor components, but their use resulted in the most accurate groupings, although the tree was nevertheless robust to the use of other candidate fatty acids. These advantages and other procedures involved in the use of fatty acid signatures are discussed thoroughly in Smith et al. (1997).

Although studying the diet or foraging patterns of any free-ranging animal may pose difficulties, these are probably most exaggerated in the study of marine mammals, because these animals spend most or all of their year at sea, where direct observation is not possible. Until relatively recently, nearly all of our knowledge about pinnipeds came from the limited times they come ashore to breed or molt. With the advent of instrumentation such as TDRs and satellite transmitters, as well as isotope techniques for studying at-sea physiology, information on the behavior and foraging ecology of these animals has broadened substantially (e.g., Gentry and Kooyman 1986). For instance, results from such studies suggest that within otariid species, foraging trip duration increases with distance to feeding sites when the species is a specialist feeder and (or) if there is a catastrophic reduction in prey stocks (Gentry et al. 1986). However, changes

in trip duration alone are not a reliable indicator of the local resource level, since foraging characteristics (e.g., dive depth, frequency, location) or the prey species eaten on trips can change without changing the trip duration (Gentry et al. 1986). In the present study we demonstrated the use of fatty acid signature analysis for detecting shifts in prey intake that correspond to changes observed in foraging behavior and faecal analysis during a poor food year. Milk fatty acid patterns revealed that Antarctic fur seals probably have a different diet during the winter away from the breeding grounds and, at least in a poor year, do not feed exclusively on krill throughout lactation. Our results suggest that fatty acid signature analysis could be a valuable tool in studying the foraging ecology of other free-ranging pinnipeds.

Acknowledgments

We thank W.D. Bowen and S.J. Smith for valuable contributions to data analysis and for ongoing collaboration and support in the application of CART to fatty acid signature analysis. B. Field and especially J. Smith provided help in developing and interpreting CART analyses using this data set and W.D. Bowen provided comments on an earlier version of the manuscript. We thank T. Barton for assistance in collecting milk samples. We also thank R.G. Ackman for general laboratory support. This study was supported by an International Fellowship to S.J.I. from the Natural Sciences and Engineering Research Council of Canada (NSERC) and by NSERC Strategic Grant No. STRO133825.

References

Ackman, R.G. 1980. Fish lipids. Part 1. *In* Advances in fish science and technology. *Edited by* J.J. Connell. Fishing News Books Ltd., Farnham, Surrey, England. pp. 86–103.

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

Arnould, J.P.Y., Boyd, I.L., and Clarke, A. 1995. A simplified method for determining the gross chemical composition of pinniped milk samples. Can. J. Zool. **73**: 404–410.

Bligh, E.G., and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. **37**: 911–917.

Bowen, W.D., Lawson, J.W., and Beck, B. 1993. Seasonal and geographic variation in the species composition and size of prey consumed by grey seals (*Halichoerus grypus*) on the Scotian shelf. Can. J. Fish. Aquatic Sci. **50**: 1768–1778.

Boyd, I.L. 1993. Pup production and distribution of breeding Antarctic fur seals (*Arctocephalus gazella*) at South Georgia. Antarct. Sci. **5**: 17–24.

Boyd, I.L., and Croxall, J.P. 1992. Diving behaviour of lactating Antarctic fur seals. Can. J. Zool. **70**: 919–928.

Boyd, I.L., Lunn, N.J., and Barton, T. 1991. Time budgets and foraging characterisitics of lactating Antarctic fur seals. J. Anim. Ecol. **60**: 577–592.

Boyd, I.L., Arnould, J.P.Y., Barton, T., and Croxall, J.P. 1994. Foraging behaviour of Antarctic fur seals during periods of contrasting prey abundance. J. Anim. Ecol. **63**: 703–713.

Clark, L.A., and Pregibon, D. 1992. Tree-based models. *In* Statistical models in S. *Edited by* J.M. Chambers and T.J. Hastie. Wadsworth and Brooks/Cole Advanced Books and Software, Chapman and Hall, New York. pp. 377–419.

Cook, H.W. 1985. Fatty acid desaturation and chain elongation in eucaryotes. *In* Biochemistry of lipids and membranes. *Edited by* D.E. Vance and J.E. Vance. Benjamin-Cummings Publishing Co., Inc., Menlo Park, Calif. pp. 181–211.

Costa, D.P., Croxall, J.P., and Duck, C.D. 1989. Foraging energetics of Antarctic fur seals in relation to changes in prey availability. Ecology, **70**: 596–606.

Croxall, J.P., and Pilcher, M.N. 1984. Characteristics of krill *Euphausia superba* eaten by Antarctic fur seals *Arctocephalus gazella* at South Georgia. Br. Antarct. Surv. Bull. No. 63. pp. 117–125.

Croxall, J.P., Everson, I., Kooyman, G.L., Ricketts, C., and Davis, R.W. 1985. Fur seal diving behaviour in relation to the vertical distribution of krill. J. Anim. Ecol. **54**: 1–8.

Croxall, J.P., McCann, T.S., Prince, P.A., and Rothery, P. 1988. Reproductive performance of seabirds and seals at South Georgia and Signy Island, South Orkney Islands, 1976–1987: implications for Southern Ocean Monitoring Studies. *In* Antarctic Ocean and resources variability. *Edited by* D. Sahrhage. Springer-Verlag, Berlin. pp. 261–285.

Daneri, G.A., and Coria, N.R. 1993. Fish prey of Antarctic fur seals, *Arctocephalus gazella*, during the summer–autumn period at Laurie Island, South Orkney Islands. Polar Biol. **13**: 287–289.

Doidge, D.W., and Croxall, J.P. 1985. Diet and energy budget of the Antarctic fur seal, *Arctocephalus gazella*, at South Georgia. *In* Antarctic nutrient cycles and food webs. *Edited by* W.R. Siegfried, P.R. Condy, and R.M. Laws. Springer-Verlag, Berlin. pp. 543–550.

Duck, C.D. 1990. Annual variation in the timing of reproduction in Antarctic fur seals, *Arctophephalus gazella*, at Bird Island, South Georgia. J. Zool. (Lond.), **222**: 103–116.

Fricke, H., Gercken, G., Schreiber, W., and Oehlenschlager, J. 1984. Lipid, sterol and fatty acid composition of Antarctic krill (*Euphausia superba* Dana). Lipids, **19**: 821–827.

Gentry, R.L., and Holt, J.R. 1982. Equipment and techniques for handling northern fur seals. NOAA Tech. Rep. NMFS SSRF No. 758.

Gentry, R.L., and Kooyman, G.L. 1986. Introduction. *In* Fur seals: maternal strategies on land and at sea. *Edited by* R.L. Gentry and G.L. Kooyman. Princeton University Press, Princeton, N.J. pp. 3–27.

Gentry, R.L., Costa, D.P., Croxall, J.P., David, J.H.M., Davis, R.W., Kooyman, G.L., Majluf, P., McCann, T.S., and Trillmich, F. 1986. Synthesis and conclusions. *In* Fur seals: maternal strategies on land and at sea. *Edited by* R.L. Gentry and G.L. Kooyman. Princeton University Press, Princeton, N.J. pp. 41–60.

Harwood, J., and Croxall, J.P. 1988. The assessment of competition between seals and commercial fisheries in the North Sea and the Antarctic. Mar. Mammal Sci. **4**: 13–33.

Iverson, S.J. 1988. Composition, intake and gastric digestion of milk lipids in pinnipeds. Ph.D. thesis, University of Maryland, College Park.

Iverson, S.J. 1993. Milk secretion in marine mammals in relation to foraging: Can milk fatty acids predict diet? Symp. Zool. Soc. Lond. No. 66. pp. 263–291.

Iverson, S.J., and Frost, K.J. 1996. The use of fatty acid signature analysis to investigate foraging ecology and food webs in Prince William Sound, Alaska: harbor seals and their prey. State–Federal Natural Resource Restoration Study No. 95064, Alaska Department of Fish and Game, Fairbanks.

Iverson, S.J., and Oftedal, O.T. 1992. Fatty acid composition of black bear (*Ursus americanus*) milk during and after the period of winter dormancy. Lipids, **27**: 940–943.

Iverson, S.J., Sampugna, J., and Oftedal, O.T. 1992. Positional specificity of gastric hydrolysis of long-chain $n-3$ polyunsatu-

rated fatty acids of seal milk triglycerides. Lipids, **27**: 870–878.

Iverson, S.J., Hamosh, M., and Bowen, W.D. 1995*a*. Lipoprotein lipase and its relationship to high milk fat transfer during lactation in grey seals. J. Comp. Physiol. B, **165**: 384–395.

Iverson, S.J., Oftedal, O.T., Bowen, W.D., Boness, D.J., and Sampugna, J. 1995*b*. Prenatal and postnatal transfer of fatty acids from mother to pup in the hooded seal (*Cystophora cristata*). J. Comp. Physiol. B, **165**: 1–12.

Jackson, S., and Ryan, P.G. 1986. Differential digestion rates of prey by White-chinned Petrel *Procellaria aequinoctialis*. Auk, **102**: 617–619.

Jobling, M., and Brieby, A. 1986. The use and abuse of fish otoliths in studies of feeding habits of marine piscivores. Sarsia, **71**: 265–274.

Kirsch, P.E., Iverson, S.J., and Bowen, W.D. 1995. Diet composition based on fatty acid signatures: captive feeding experiments on harp seals and grey seals. Abstracts of the Eleventh Biennial Conference on the Biology of Marine Mammals, Orlando, Fla., December 1996. p. 62. [Abstr.]

Lee, J.-H., Fujimoto, K., and Kaneda, T. 1981. Antioxygenic and peroxide decomposition properties of Antarctic krill lipids. Bull. Jpn. Soc. Sci. Fish. **47**: 881–888.

Lunn, N.J. 1992. Fostering behaviour and milk stealing in Antarctic fur seals. Can. J. Zool. **70**: 837–839.

Lunn, N.J., and Boyd, I.L. 1993*a*. Influence of maternal characteristics and environmental variation on reproduction in Antarctic fur seals. Symp. Zool. Soc. Lond. No. 66. pp. 115–129.

Lunn, N.J., and Boyd, I.L. 1993*b*. Effects of maternal age and condition on parturition and the perinatal period of Antarctic fur seals. J. Zool. (Lond.), **229**: 55–67.

Martensson, P.-E., Nordoy, E.S., and Blix, A.S. 1994. Digestibility of krill *Euphausia superba* and *Thysanoessa* sp. in minke whales *Balenoptera acutorostra* and crabeater seals *Lobodon carcinophagus*. Br. J. Nutr. **72**: 713–716.

Paradis, M., and Ackman, R.G. 1976. Localization of a source of marine odd chain length fatty acids. I. The amphipod *Pontoporeia femorata*, Kroyer. Lipids, **11**: 863–870.

Reid, K., and Arnould, J.P.Y. 1996. The diet of Antarctic fur seals *Arctocephalus gazella* during the breeding season at South Georgia. Polar Biol. **16**: 105–114.

Reinhardt, S.B., and Van Vleet, E.S. 1986. Lipid composition of twenty-two species of Antarctic midwater zooplankton and fish. Mar. Biol. (Berl.), **91**: 149–159.

Smith, S.J., Iverson, S.J., and Bowen, W.D. 1997. Fatty acid signatures and classification trees: new tools for investigating the foraging ecology of seals. Can. J. Fish. Aquat. Sci. **54**(6). In press.

Trillmich, F., and Ono, K.A. (*Editors*). 1991. Pinnipeds and El Nino: responses to environmental stress. Springer-Verlag, Berlin.

Tsuyuki, H., and Itoh, S. 1976. Fatty acid component of lipid of *Euphausia superba*. Sci. Rep. Whales Res. Inst. **28**: 167–174.

Venables, W., and Ripley, B. 1994. Modern applied statistics with S-Plus. Springer-Verlag, Heidelberg.

Virtue, P., Nichols, P.D., Nicol, S., McMinn, A., and Sikes, E.L. 1993. The lipid composition of *Euphausia superba* Dana in relation to the nutritional value of *Phaeocystis pouchetii* (Hariot) Lagerheim. Antarct. Sci. **5**: 169–177.