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Fatty acid signature analysis from the milk of Antarctic fur seals and Southern elephant seals from South Georgia: implications for diet determination

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ABSTRACT: Fatty acid signature analysis (FASA) makes use of specific fatty acids, as well as entire profiles, to study dietary relationships at different trophic levels. Previously, FASA has been used in marine ecosystems in which diet determination by more direct methods is difficult and sometimes misleading. This study examined fatty acid profiles in milk from 2 species of pinniped from the Southern Ocean that were expected to have highly contrasting diets. Milk samples were collected from Antarctic fur seals *Arctocephalus gazella* in 3 consecutive years, from 1991 to 1993 (n = 72), and from Southern elephant seals *Mirounga leonina* in 1988 (n = 53) at South Georgia. Lipids were extracted and fatty acid profiles determined by temperature-programmed gas chromatography. Possible prey species collected from waters around South Georgia were also analysed. Cluster analysis as well as classification and regression trees (CART) indicated that profiles from fur seals and elephant seals were significantly different. Southern elephant seal data could be distinguished from Antarctic fur seals by lower levels of the fatty acids 16:4 n1, 18:2 n6, 18:4 n3, 18:4 n1 and 20:5 n3 and by higher levels of 18:0, 18:1 n9/ n11 (i.e. 18:1 n9 co-eluting with 18:1 n11) and 20:1 n9. Fatty acid signatures from the milk of Antarctic fur seals were closest to krill and fish species that were also known to feed on krill. Southern elephant seal fatty acid profiles were closest to species that are not known as krill predators such as larger notothenids and myctophids. The fatty acid profiles of Antarctic fur seals showed considerable inter- and intra-annual variability, which was congruent with diet variability detected using scat analyses. Southern elephant seals showed little variation in profile through lactation. In contrast to previous diet analyses based on examination of stomach contents, the results from FASA were consistent with a fish-based diet for Southern elephant seals.

KEY WORDS: Fur seal · Elephant seal · Diet · Foraging · Southern Ocean · Milk · Fatty acids · South Georgia

INTRODUCTION

Understanding trophic interactions requires detailed investigations of diet. This is particularly difficult when top predators in marine ecosystems are being studied. In the past, the diet of pinnipeds has usually been determined using scat (faecal) and stomach lavage analyses, relying on the recovery of hard part remains of prey (e.g. otoliths, krill carapaces, squid beaks and statoliths; see Clarke & Macleod 1982a,b, North et al. 1983, Green & Burton 1987, Plotz et al. 1991, Rodhouse et al. 1992, Reid & Arnould 1996, Walker et al. 1998). Depending on the species being studied, estimates of diet based on these approaches are often biased towards the moulting and breeding periods in the life cycle when pinnipeds come ashore and, therefore, when samples can be collected. Scat and stomach

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sampling tend to bias estimates of diet towards recent feeding and towards items most likely to resist digestion (Croxall 1993).

Fatty acid signature analysis (FASA) overcomes some of the biases inherent in scat and stomach analyses. Dietary lipids are broken down into their constituent fatty acids during digestion and are incorporated, relatively unmodified, into the tissues of predators and may be traced through a number of trophic levels (Sargent 1976, Iverson 1993, Iverson et al. 1997b). Studies using both terrestrial and marine animals have shown that dietary fatty acid profiles were reflected in adipose deposits (Ackman & Eaton 1966, Hooper et al. 1973, Paradis & Ackman 1976, 1977, Falk-Petersen 1988, Rouvinen & Kiiskinen 1989, Rouvinen et al. 1992, Graeve et al. 1994, 1997, Castell et al. 1995, Pond et al. 1995).

Fatty acid profiles in diet are also reflected in the adipose tissue of pinnipeds (Smith et al. 1996, Iverson et al. 1997b). During lactation in pinnipeds, fatty acids derived from the diet and blubber are monopolised by the mammary gland (Iverson et al. 1995a). This suggests that fatty acids from milk may indicate the content of the diet (Green et al. 1993, Iverson et al. 1995b, 1997a).

Antarctic fur seals *Arctocephalus gazella* and Southern elephant seals *Mirounga leonina* are top marine predators in the Southern Ocean. Although the diet of Antarctic fur seals is relatively well understood using data from scats (e.g. Croxall & Pilcher 1984, Green et al. 1989, 1991, Reid 1995b, North 1996, Reid & Arnould 1996), knowledge of Southern elephant seal diet is limited to observations of the hard part remains of prey in the stomach and sightings from ships (Laws 1956, Clarke & Macleod 1982a, Rodhouse et al. 1992, Reid & Nevitt 1998). In general, these indicate a cephalopod-based diet for elephant seals at South Georgia.

Pregnant Antarctic fur seal females haul out from late November to late December (Duck 1990), 24 to 48 h prior to parturition (Doidge et al. 1986). They remain ashore to suckle their young for 5 to 7 d (perinatal period). Subsequently, mothers will come ashore for approximately 2 d to suckle their young, between 3 to 7 d foraging trips at sea (Doidge et al. 1986, Kooyman et al. 1986, Boyd et al. 1991, Arnould & Boyd 1995) throughout a lactation period of approximately 120 d (Doidge et al. 1986, Lunn et al. 1993). In contrast, Southern elephant seal females come ashore to give birth from late September to late October, up to 8 d prior to parturition (Laws 1960, McCann 1980), but the pups are suckled for 23 d, during which time the mothers remain on land and do not feed (McCann 1980).

This study used FASA of milk samples from lactating Antarctic fur seals and Southern elephant seals at South Georgia to examine the diet of these sympatric seal species. The aims were to: (1) compare fatty acids in the diet in female Antarctic fur seals and Southern elephant seals, (2) determine trends, within and between years, of dietary variability in Antarctic fur seals using FASA and (3) compare the results obtained from FASA and concurrent scat analysis documented by Reid & Arnould (1996).

Table 1. Fatty acid composition (mass %) of (A) antarctic fur seal (AFS) milk samples from 1991 to 1993 and Southern elephant seal (SES) milk samples from 1988. Values are given as means ±SEM of all named fatty acids. (B) prey samples

A	AFS 1991 mean	SEM	AFS 1992 mean	SEM	AFS 1993 mean	SEM	SES 1988 mean	SEM
14:0	2.47	±0.19	2.39	±0.35	4.55	±0.27	2.70	±0.12
14:1 n9	0.01	±0.00	0.00	±0.00	0.01	±0.00	0.03	±0.00
14:1 n7	0.03	±0.01	0.02	±0.00	0.03	±0.00	0.02	±0.00
14:1 n5/ iso 15	0.23	±0.03	0.19	±0.02	0.30	±0.01	0.18	±0.01
15:1 n5	0.03	±0.01	0.02	±0.01	0.04	±0.00	0.06	±0.00
16:0	8.02	±0.42	7.61	±0.72	10.95	±0.42	10.40	±0.29
16:1 n11	0.30	±0.02	0.27	±0.02	0.26	±0.01	0.46	±0.01
16:1 n9	0.23	±0.02	0.24	±0.03	0.20	±0.02	0.47	±0.03
16:1 n7	6.22	±0.24	5.71	±0.48	5.56	±0.18	4.10	±0.13
7Me16:0/ 16:1 n5	0.21	±0.01	0.19	±0.01	0.31	±0.02	0.30	±0.01
17:1	0.19	±0.03	0.22	±0.03	0.31	±0.01	0.27	±0.01
16:4 n1	1.77	±0.19	1.97	±0.19	0.91	±0.11	0.29	±0.02
18:0	1.08	±0.07	0.82	±0.05	1.00	±0.07	2.77	±0.16
18:1 n13	0.20	±0.03	0.15	±0.02	0.17	±0.01	0.17	±0.02
18:1 n9/ 18:1 n11	15.76	±0.87	13.23	±0.90	13.09	±1.20	25.00	±0.45
18:1 n7	4.03	±0.18	3.31	±0.20	4.39	±0.17	5.00	±0.10
18:1 n5	0.22	±0.02	0.15	±0.02	0.21	±0.01	0.34	±0.01
18:2 n6	3.89	±0.16	4.09	±0.19	1.53	±0.07	1.34	±0.02
18:2 n4	0.12	±0.01	0.07	±0.01	0.09	±0.01	0.09	±0.00
18:3 n6	0.16	±0.01	0.22	±0.02	0.13	±0.01	0.22	±0.04
18:3 n4	0.10	±0.02	0.07	±0.03	0.08	±0.01	0.03	±0.00
18:3 n3	0.69	±0.03	0.87	±0.05	0.65	±0.03	0.38	±0.02
18:4 n3	5.19	±0.32	6.76	±0.50	3.90	±0.34	1.69	±0.13
18:4 n1	3.00	±0.20	2.58	±0.17	1.58	±0.12	0.67	±0.03
20:0	0.01	±0.01	0.18	±0.06	0.11	±0.03	0.01	±0.00
20:1 n11	0.16	±0.04	0.11	±0.04	0.20	±0 05	0.81	±0.05
20:1 n9	2.60	±0.27	1.28	±0.20	1.56	±0.19	7.09	±0.28
20:1 n7	0.04	±0.01	0.00	±0.00	0.23	±0.02	0.31	±0.02
20:2 n6	0.01	±0.01	0.02	±0.01	0.03	±0.01	0.28	±0.02
20:3 n6	0.05	±0.02	0.06	±0.03	0.18	±0.01	0.14	±0.01
20:4 n6	0.72	±0.06	0.77	±0.10	0.53	±0.03	0.86	±0.05
20:3 n3	0.00	±0.00	0.00	±0.00	0.01	±0.01	0.07	±0.01
20:4 n3	2.13	±0.26	2.14	±0.18	2.61	±0.12	1.73	±0.06
20:5 n3	14.92	±0.83	16.78	±0.97	14.54	±0.83	8.40	±0.37
22:1 n11	0.54	±0.12	0.04	±0.02	0.27	±0.06	0.86	±0.09
22:1 n9	0.18	±0.05	0.00	±0.00	0.27	±0.05	0.61	±0.06
22:2 n6	0.00	±0.03	0.00	±0.00	0.00	±0.01	0.00	±0.00
22:5 n3	6.06	±0.35	6.28	±0.38	7.82	±0.25	5.25	±0.17
22:6 n3	18.31	±0.70	20.99	±1.29	19.81	±0.68	15.87	±0.63

MATERIALS AND METHODS

Sample collection. Antarctic fur seal milk samples (n = 72) were collected at Bird Island, South Georgia (54° 00' S, 38° 03' W), during the breeding seasons 1991 (n = 31), 1992 (n = 25) and 1993 (n = 16). (Breeding seasons are referred to by the year they ended, so 1990/91 season will be 1991, etc.) The Antarctic fur seal lactation period was divided into 3 stages, according to Iverson et al. (1997a): early (the perinatal and early attendance periods, including December and early January); middle (the mid-attendance period from late January to late February); and late (late attendance, the whole of March). The early period in this study included the perinatal and early attendance periods as referred to by Iverson et al. (1997a), as no indication of the stage of lactation was given when the milk samples were collected. Fur seals were captured using standard noose-pole and restraint board techniques (Gentry & Holt 1982). Milk samples were then expressed manually from fur seals, aided by repeated intramuscular injection of oxytocin (InterVet Ltd, Cambridge, UK) (0.5 to 1.0 ml, 10 IU ml^{-1}) (Arnould & Boyd 1995).

Southern elephant seal samples (n = 53) were collected at Husvik, South Georgia (54° 12' S, 36° 43' W), between September and October 1988. The Southern elephant seal season was not subdivided, as fatty acid profiles were similar in all samples taken throughout lactation (see Table 1A). Each seal was anaesthetised to allow handling. The anaesthetic was a tiletamine/zolazepam mixture (Zoletil 100, Reading, UK), administered at 1 mg per 100 kg body mass by a dart delivered by blowpipe (Fedak et al. 1996). Milk was then expressed, aided by 40–60 IU Oxytocin (Leo Labs Ltd)

B	*Gobionotothen gibberifrons*	*Dissostichus eleginoides*	*Chaenocephalus aceratus*	*Pagothenia hansoni*	*Gymnoscopelus nicholsi*	*Martialia hyadesi*	*Champsocephalus gunnari*	*Euphausia superba* K168	K257	K000
14:0	0.58	3.65	2.91	2.12	2.59	0.53	3.88	5.05	1.81	5.76
14:1 n9	0.04	0.04	0.12	0.07	0.02	0.04	0.04	0.06	0.04	0.06
14:1 n7	0.16	0.01	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.02
14:1 n5/ iso 15	0.11	0.28	0.30	0.12	0.12	0.03	0.22	0.11	0.08	0.15
15:1 n5	0.08	0.02	0.00	0.02	0.04	0.02	0.03	0.02	0.01	0.02
16:0	11.23	9.17	10.89	12.78	8.98	8.21	13.25	17.37	3.03	17.59
16:1 n11	0.35	0.18	0.22	0.22	0.25	0.08	0.26	0.26	0.14	0.28
16:1 n9	0.35	0.13	0.00	0.54	0.05	0.03	0.00	0.00	0.02	0.08
16:1 n7	2.76	7.00	11.04	4.99	2.91	0.30	6.82	3.89	2.33	6.95
7Me16:0/ 16:1 n5	0.62	0.22	0.20	0.25	0.23	0.16	0.30	0.25	0.08	0.31
17:1	0.90	0.29	0.39	0.33	0.24	0.08	0.38	0.39	0.56	0.46
16:4 n1	0.08	0.48	0.75	0.43	0.45	0.05	0.78	2.43	4.35	1.78
18:0	4.99	4.88	2.45	3.85	3.24	3.00	1.66	0.81	0.16	0.97
18:1 n13	0.41	0.00	0.00	0.14	0.09	0.15	0.20	0.16	0.18	0.24
18:1n9/ 18:1n11	7.15	37.72	17.92	18.56	15.41	4.51	14.35	3.92	2.61	7.56
18:1 n7	7.49	5.24	6.08	5.81	4.99	1.56	7.33	3.56	1.53	6.40
18:1 n5	0.62	0.35	0.35	0.55	0.39	0.25	0.27	0.13	0.00	0.18
18:2 n6	0.58	1.44	1.05	0.84	1.24	0.34	1.64	1.68	0.71	2.16
18:2 n4	0.18	0.06	0.00	0.18	0.20	0.06	0.07	0.06	0.29	0.17
18:3 n6	0.00	0.16	0.09	0.07	0.09	0.01	0.17	0.15	0.17	0.17
18:3 n4	0.11	0.00	0.00	0.03	0.08	0.01	0.00	0.00	0.00	0.00
18:3 n3	0.22	0.51	0.34	0.32	0.67	0.33	0.42	0.70	0.42	0.66
18:4 n3	0.71	4.91	3.70	2.31	4.69	1.07	4.21	6.81	8.96	4.48
18:4 n1	0.00	0.23	0.00	0.21	1.36	0.17	0.25	0.00	0.12	0.00
20:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:1 n11	0.33	0.00	0.00	0.19	0.24	1.74	0.00	0.00	0.00	0.00
20:1 n9	1.78	3.94	4.75	5.07	13.50	5.75	2.59	0.00	0.00	0.36
20:1 n7	1.51	0.32	0.81	3.14	0.46	0.51	0.33	0.00	0.00	0.00
20:2 n6	0.34	0.00	0.00	0.13	0.53	0.67	0.00	0.00	0.43	0.00
20:3 n6	0.00	0.00	0.00	0.00	0.23	0.11	0.00	0.00	0.17	0.00
20:4 n6	8.56	0.48	1.09	1.13	0.64	1.20	0.88	0.27	0.35	0.34
20:3 n3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:4 n3	0.00	0.64	1.07	0.26	3.76	1.54	0.64	0.53	0.80	0.44
20:5 n3	19.89	9.80	17.41	15.67	12.59	17.92	20.27	22.89	34.97	17.78
22:1 n11	0.00	0.55	0.00	0.89	3.12	1.58	0.21	0.00	0.00	0.00
22:1 n9	0.92	0.52	0.74	2.70	1.16	1.02	0.30	0.00	2.17	0.60
22:2 n6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.84	5.26	1.71
22:5 n3	2.30	0.49	0.00	0.63	2.50	1.34	0.96	0.00	0.72	0.00
22:6 n3	23.55	5.03	14.15	14.06	12.26	44.90	15.00	25.83	25.65	19.99

injected intravenously. All milk samples were stored at –20°C prior to analysis.

Although the study did not set out to identify the specific prey groups taken by fur seals and elephant seals, potential seal prey species were collected during marine biology research cruises around the waters of South Georgia. No indication of age, gender or size was available for prey samples, except mass (g). One of each of *Martialia hyadesi* (Ommastrephid squid) (493.5 g), *Gobionotothen gibberifrons* (Humphead notothen) (215.6 g), *Champsocephalus gunnari* (Mackerel icefish) (304.8 g), *Chaenocephalus aceratus* (Blackfin icefish) (890.7 g), *Pagothenia hansoni* (Striped notothen) (312.5 g), *Gymnoscopelus nicholsi* (Myctophid) (17.1 g), *Dissostichus eleginoides* (Patagonian toothfish) (1189.0 g), and 3 samples of *Euphausia superba* (Antarctic krill) (weights not taken) were homogenised in a high-speed blender (Robot Coupe [UK] Ltd, Middlesex, UK) for FASA. All prey samples were stored at –20°C until lipid extraction. Despite the small sample sizes of the prey that were available, we used the fatty acid profiles of the prey to provide a broad indication of diet. We justified this based upon the assumption that variability among individual prey species was likely to be no greater than the variability observed among the fatty acid profiles from individual predators.

Table 2. Summary of 10 and 15 cluster analyses of all Antarctic fur seals (1991 to 1993) and prey samples

Cluster no.	Frequency[a] Total predators	Season 1991	1992	1993	Type of prey[b]	Closest cluster and distance of nearest cluster centroid
10 cluster analysis r^2 = 0.91						
1	3	3	–	–	–	3 (0.51)
2	16	6	6	4	D.e, C.a, P.h, G.n	9 (0.13)
3	4	4	–	–	–	5 (0.29)
4	4	1	3	–	–	7 (0.15)
5	2	2	–	–	–	3 (0.29)
6	2	–	2	–	M.h	9 (0.17)
7	4	4	–	–	–	4 (0.15)
8	1	1	–	–	–	1 (0.79)
9	35	9	14	12	G.g, C.g, K (all 3)	2 (0.13)
10	1	1	–	–	–	5 (0.44)
15 cluster analysis r^2 = 0.94						
1	1	1	–	–	–	11 (0.26)
2	4	3	–	1	D.e	5 (0.15)
3	2	–	2	–	–	10 (0.12)
4	3	3	–	–	–	7 (0.40)
5	21	7	5	9	C.a, P.h, G.n, C.g	12 (0.10)
6	–	–	–	–	M.h	12 (0.27)
7	2	2	–	–	–	11 (0.28)
8	2	–	2	–	–	5 (0.17)
9	–	–	–	–	K257	12 (0.21)
10	4	4	–	–	–	3 (0.12)
11	3	3	–	–	–	1 (0.26)
12	26	5	15	6	G.g, K168, K000	5 (0.10)
13	2	1	1	–	–	3 (0.15)
14	1	1	–	–	–	4 (0.79)
15	1	1	–	–	–	1 (0.42)

[a]Clusters contain a finite number of observations (total) and this is separated into 3 seasons of Antarctic fur seals (1991, n = 31; 1992, n = 25; 1993, n = 16) and type of prey

[b]C.a = *Chaenocephalus aceratus*; G.n = *Gymnoscopelus nicholsi*; G.g = *Gobionotothen gibberifrons*; D.e = *Dissostichus eleginoides*; P.h = *Pagothenia hansoni*; C.g = *Champsocephalus gunnari*; M.h = *Martialia hyadesi*; K000; K168; K257; K (all 3) = *Euphausia superba* from 3 different locations around South Georgia. See text for further explanation of table

Laboratory protocol. Milk samples (~0.1 g) were defrosted at room temperature overnight and then homogenised for 20 min in a high-speed blender. The lipid fraction was extracted from milk using a method modified from that of Bligh & Dyer (1959). Greater volumes of methanol, chloroform and water were needed because of the high lipid concentration of pinniped milk. The final ratio of methanol:chloroform:water (2:2:1.8) was maintained. Homogenised tissue was refluxed overnight at 100°C with 10% potassium hydroxide in methanol (150 ml). After cooling, the sample was acidified to pH 1 with 2 M hydrochloric acid (100 ml). Saponified lipids were extracted into hexane, washed with distilled water and then dried over anhydrous sodium sulphate. All lipid samples were stored in chloroform at –20°C under nitrogen in a glass vial secured with an aluminium-lined screw cap.

The lipid extracts were transesterified to produce fatty acid methyl esters (FAMEs) using a method modified from that of Iverson et al. (1997a). Hexane (0.5 ml) and 7% boron trifluoride in methanol (0.5 ml) were added to 30 mg of pure lipid. The mixture was capped under nitrogen and heated at 90°C for 1 h. FAMEs were extracted into hexane and stored under nitrogen in high purity hexane at a concentration of approximately 50 mg ml^{-1} at –20°C.

Analysis of the FAMEs was by capillary gas chromatography (GC) using a Hewlett Packard 5890A fitted with a 60 m × 0.25 mm i.d. column coated with 50% cyanopropyl polysiloxane (0.25 µm film thickness; JW DB-23). The column temperature was programmed from 50°C, held for 2 min, then ramped at

Table 3. Summary of 10 and 15 cluster analyses of all Southern elephant seals, all Antarctic fur seals (1991 to 1993) and all prey samples

Cluster no.	Frequency[a]			Type of prey	Closest cluster and distance of nearest cluster centroid
	Total predators	Elephant seals	Antarctic fur seals		
10 cluster analysis $r^2 = 0.87$					
1	3	–	3	–	3 (0.51)
2	57	51	6	D.e, P.h, G.n	9 (0.17)
3	4	–	4	–	5 (0.29)
4	4	–	4	–	7 (0.15)
5	2	–	2	–	3 (0.29)
6	6	–	6	M.h, K168, K257	9 (0.13)
7	4	–	4	–	4 (0.15)
8	1	–	1	–	1 (0.79)
9	43	2	41	C.a, C.g, G.g, K000	6 (0.13)
10	1	–	1	–	5 (0.44)
15 cluster analysis $r^2 = 0.90$					
1	1	–	1	–	11 (0.26)
2	30	28	2	D.e	5 (0.10)
3	2	–	2	–	10 (0.12)
4	3	–	3	–	7 (0.40)
5	27	22	5	G.n	2 (0.10)
6	–	–	–	M.h	12 (0.27)
7	2	–	2	–	11 (0.28)
8	17	2	15	C.a, P.h, C.g, K000	12 (0.11)
9	–	–	–	K257	12 (0.22)
10	4	–	4	–	3 (0.12)
11	3	–	3	–	1 (0.26)
12	32	1	31	G.g, K168	8 (0.11)
13	2	–	2	–	3 (0.15)
14	1	–	1	–	4 (0.79)
15	1	–	1	–	1 (0.42)

[a]Clusters contain a finite number of observations (total) and this is separated into Southern elephant seals (n = 53), Antarctic fur seals (n = 72) and type of prey. Abbreviations for prey samples as in Table 2. See text for further explanation of table

25°C min^{-1} to 180°C and held for 5 min, then ramped at 2°C min^{-1} to 200°C, then at 2.1°C min^{-1} to 240°C and finally held at 240°C for 8 min. Total running time was 49.24 min. Samples (run in duplicate or triplicate) were introduced onto the column by manual splitless injection of 1 µl of high purity hexane, with helium as the carrier gas.

The GC was linked to a computerised integration system (Unicam 4880 software) to identify the peaks by comparison with absolute retention times (RT) from a standard mixture (Supelco UK, Poole, Dorset, UK). The standard was run daily to determine accurate RTs. Individual fatty acids were named according to the IUPAC shorthand nomenclature of carbon chain length: number of double bonds and location ($n - x$) of the double bond nearest to the terminal methyl group.

Data analysis. Only fatty acids common to all the samples were used in data analysis (Table 1). Cluster analysis was performed using PROC FASTCLUS, SAS Version 6.11 (SAS Institute Inc., Cary, USA). PROC FASTCLUS is an iterative process for clustering of large data sets and identifies disjoint clusters from coordinate data. Classification and regression trees (CART) in S-plus (Version 3.5 for Windows) is a non-parametric multivariate technique for classifying data (Clark & Pregibon 1992, Venables & Ripley 1994). The data were analysed using CART according to Iverson et al. (1997a,b) and Smith et al. (1997).

Prior to cluster analysis, the data were arcsine transformed. This allowed the data, originally presented as mass percent composition, to be treated with parametric statistics. PROC FASTCLUS allocated points in space for each observation (i.e. fatty acid profile), such that profiles from samples in the same cluster were more similar than profiles in different clusters. By using the whole profile, PROC FASTCLUS used all fatty acids to place samples in a particular cluster. PROC FASTCLUS also indicated the closest cluster to each cluster and the distance between them (Tables 2 & 3). The number of clusters was chosen by a stepwise approach to observe the structural progression of the clusters and to examine how individuals were segregated. Ultimately, all profiles were different. In analyses using 135 samples, for example, 1 cluster would place all samples in a single cluster ($r^2 = 0$), and 135 clusters would place each sample in an individual cluster ($r^2 = 1$). It was found that analyses using 10 and 15 clusters suggested the clearest biological insight (Tables 2 & 3). Each FASTCLUS procedure produced a summary table, with information about each cluster. Relevant details are shown in Tables 2 & 3. Each analysis was given an r^2 statistic, which represented the proportion of variation accounted for by that number of clusters in the analysis. Each fatty acid was given a mean using the values from the samples in the cluster, which could be used to determine differences between clusters.

CART split data into 2 or more groups (like branches of a tree) and looked through all the fatty acids to find the subset that partitioned the data into relatively homologous groups. The fatty acid and cutoff value used at each node were determined by calculating the maximum change in deviance between the root node

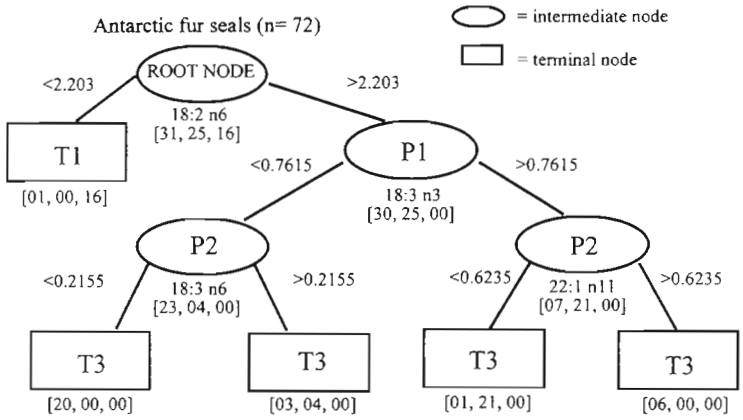

Fig. 1. Classification tree produced by CART: all Antarctic fur seals, classified by year. Labels inside ellipses and rectangles are as follows: intermediate nodes (P) are sequentially numbered as the tree splits, so that the first nodes from the root node are P1, and the nodes splitting from them P2; terminal nodes (T) are numbered according to the number of splits it takes to get to that node from the root. The fatty acid at each branch fork is the variable chosen by the CART algorithm to create the fork. < and > values indicate the level (% composition) of the fatty acid at which the fork is made. Bracketed numbers under the nodes indicate the number of samples from each year that appear at that node, in the order [1991, 1992, 1993]. 1991 (n = 31), 1992 (n = 25), 1993 (n = 16), total Antarctic fur seals (N = 72). Residual mean deviance = 0.30

and subsequent nodes (Smith et al. 1997). Observations then went down 1 branch or the other, depending on whether the value for that fatty acid was less than, or more than, this mathematically derived threshold. By using the '1 step look-ahead' method of tree construction, the CART algorithm chose the optimal split at each node without attempting to optimise the performance of the entire tree. Fatty acids could be picked manually to improve the performance of the tree. Tree branching ceased when 1 of 2 stopping criteria was satisfied (see Smith et al. 1997). Tree growth ended at a terminal node.

The seal data were classified for CART in the following ways: Antarctic fur seals by season (Fig. 1); Antarctic fur seals by early and middle and late season (Fig. 2); Antarctic fur seals by season, using attendance data only—i.e. ensuring that samples taken during the perinatal period (according to Iverson et al. 1997a) were excluded (Fig. 3); all seals by species (Fig. 4). Trees were not constructed using prey species data, as CART could not use data from single observations. However, prey data were 'dropped' through trees, already constructed from seal data, at the root node, to see in which part of the tree they terminated.

Previous studies using CART for pinniped diet determination (Iverson et al. 1997a,b, Smith et al. 1997) used a misclassification error rate (MER) to indicate the number of samples classified into the 'wrong' category at the terminal node. This has been used as a measure of the tree's 'success'. Given that these categories were created on an ad hoc basis, the term 'misclassification' may be misleading. A sample's final place in a tree is based on its fatty acid composition, and will therefore always appear in the 'correct' terminal node. The nodes in the trees published here do not contain the categories that might have been used to explain the nodes in previous work. Instead, intermediate nodes (P) are sequentially numbered as the tree splits, so that the first nodes from the root node are P1, and the nodes splitting from them, P2 etc. Terminal nodes (T) are numbered according to the number of splits it takes to get to that node from the root (see Figs. 1 to 4). In this study, it is the residual mean deviance (i.e. the deviance not explained by the tree), not the misclassification error rate, that indicates the tree's 'success'.

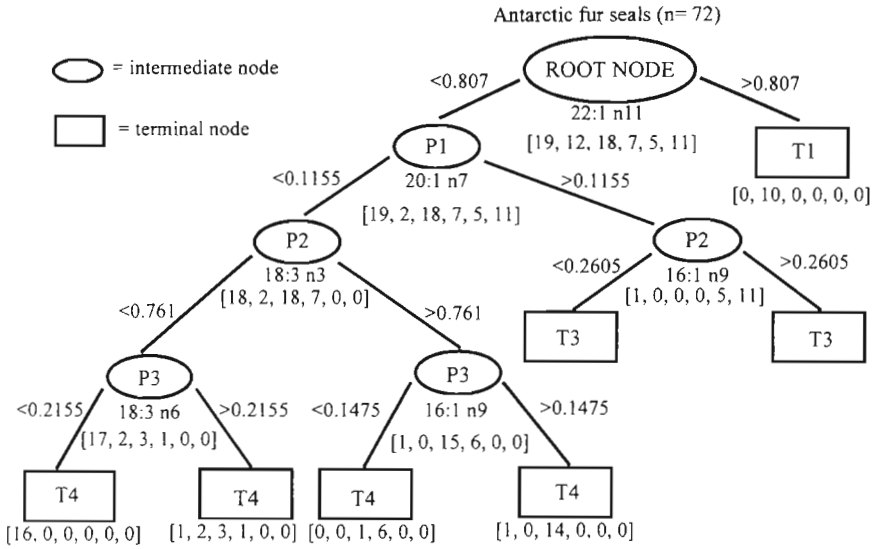

Fig. 2. Classification tree produced by CART: all Antarctic fur seals, classified by early (E) or middle and late (L) season. Labelling of the tree as in Fig. 1. Bracketed numbers under the nodes indicate the number of samples from each year, and the phase from that year, that appear at that node, in the order [1991E, 1991L, 1992E, 1992L, 1993E, 1993L]. 1991E (n = 19), 1991L (n = 12), 1992E (n = 18), 1992L (n = 7), 1993E (n = 5), 1993L (n = 11), total Antarctic fur seals (N = 72). Residual mean deviance = 0.61

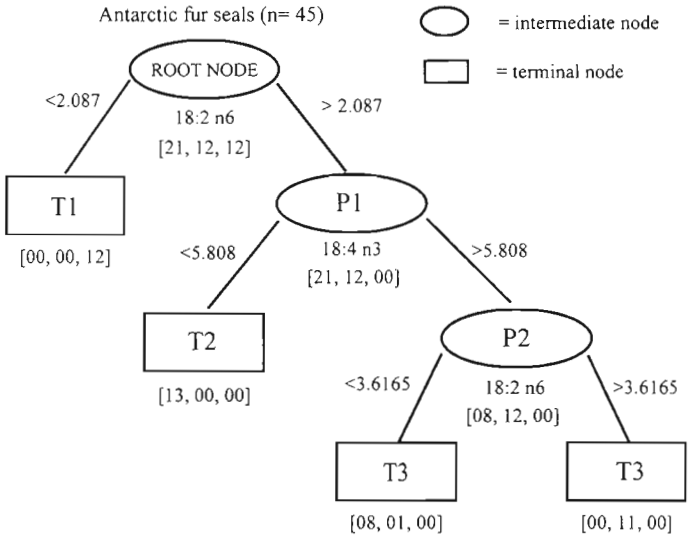

Fig. 3. Classification tree produced by CART: Antarctic fur seals, classified by year, using attendance data only. Labelling of the tree as in Fig. 1. Data are from Antarctic fur seal samples taken after December in any season (1991, 1992, 1993). Bracketed numbers under the nodes indicate the number of samples taken during the attendance period of each year that appear at that node, in the order [1991, 1992, 1993]. 1991 (n = 21), 1992 (n = 12), 1993 (n = 12), total Antarctic fur seal samples from attending seals (N = 45). Residual mean deviance = 0.15

RESULTS

The results from fatty acid signature analysis of milk and prey samples are given in Table 1.

Analysis of Antarctic fur seal and prey species data

Antarctic fur seal milk samples from early 1991 were approximately equally divided between Clusters 2 and 9 (Table 2), in a 10 cluster analysis (r^2 = 0.91). Of the 10 prey samples, Cluster 2 contained *Dissostichus eleginoides*, *Chaenocephalus aceratus*, *Pagothenia hansoni* and *Gymnoscopelus nicholsi*. Cluster 9 contained *Champsocephalus gunnari*, *Gobionotothen gibberifrons* and the 3 samples of Antarctic krill. Generally, Antarctic fur seal milk samples from the latter half of the 1991 season appeared in clusters not associated with the prey examined in this study. Seals from the 1992 season were mostly found in Cluster 9 (see Table 2), clustering with Antarctic krill, *G. gibberifrons* and *C. gunnari*, but some seal samples taken in the early and middle season were clustered with fish only (Cluster 2). From the 1993 season, 12 out of 16 seals were in Cluster 9. The remaining 4 from 1993, in Cluster 2, came from samples taken at various time points through the season. In summary, fur seal samples from 1991 were scattered across 9 of 10 clusters. In 1992, 20 of 25 milk samples were found in clusters associated with krill and fish (Clusters 2 and 9). In 1993, samples from throughout the season associated with krill and fish.

These results were supported by the 15 cluster analysis (r^2 = 0.94). The 1991 season still appeared to be divided into early and latter stages. Samples taken early in the season clustered with krill and fish, and clusters containing seals from the latter half of the season were not associated with any of the current prey examples. In 1992, most milk samples clustered with krill. However, in 1993 there was evidence that fur seals clustered with fish samples (Cluster 5) more than with krill samples (Cluster 12) (Table 2).

The differences between the results from 10 and 15 cluster analyses may be explained by the clustering of *Champsocephalus gunnari* (Table 2). In the 10 cluster analysis, *C. gunnari* clustered with *Gobionotothen gibberifrons* and the 3 krill samples. When analysed with 15 clusters, *C. gunnari* became associated with *Chaenocephalus aceratus*, *Pagothenia hansoni* and *Gymnoscopelus nicholsi*, shifting some fur seals that were originally in a cluster associated with 'krill' to a cluster associated with 'fish' (Table 2).

CART supported the description of inter-annual variability found in cluster analysis (Figs. 1 to 3). Classification of Antarctic fur seal milk samples by year required 4 fatty acids, giving 5 terminal nodes (Fig. 1). The residual mean deviance (RMD) for this tree was 0.30. Fatty acid 18:2 n6 was chosen by the tree algorithm to split the first node, based on the maximum

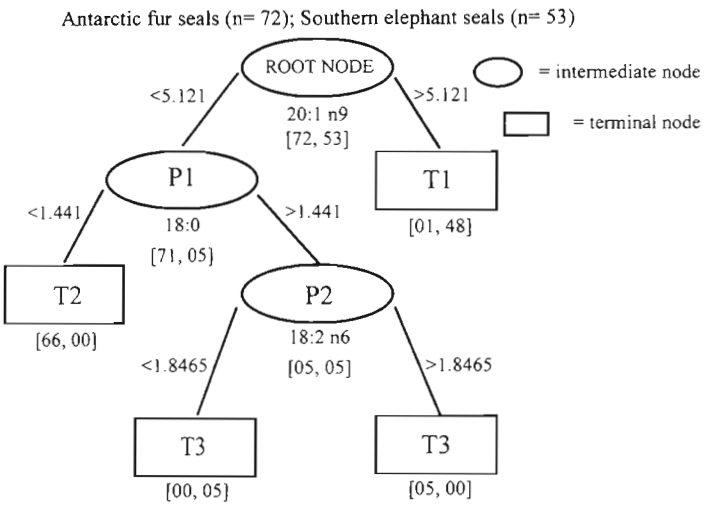

Fig. 4. Classification tree produced by CART: all seals, classified by species. Labelling of the tree as in Fig. 1. Bracketed numbers under the nodes indicate the number of samples from each species that appear at that node, in the order [AFS, SES]. Antarctic fur seals (AFS) (n = 72); Southern elephant seals (SES) (n = 53). Residual mean deviance = 0.04

change in deviance (69.87). All milk samples from 1993 were separated from 1991 and 1992 samples. Fatty acid 18:3 n3 differentiated between 1991 and 1992 seasons. However, this was not a completely clear split. Both 18:3 n6 and 22:1 n11 were used to separate these P2 nodes.

CART created a tree from 6 fatty acids giving 7 terminal nodes when classifying Antarctic fur seal samples into early and middle and late phases in each year (Fig. 2). Fatty acid 22:1 n11 was picked to split the root node (deviance = 47.21) as it gave the tree with the lowest RMD (0.61). The automatic choice of CART was to use 18:2 n6 to split the first node, based on the maximum change in deviance (70.87). However, this created a tree with an RMD of 0.67 (tree not shown). Values of 22:1 n11 >0.81 split middle and late 1991 season seals from the remainder. Then 20:1 n7 split 1993 samples from 1991 and 1992 samples. Early and middle and late 1993 could be differentiated by 16:1 n9. CART split 1991 and 1992 seasons with 18:3 n3 (P2), and split 1992 into early and middle and late phases with 16:1 n9 (P3). Fatty acid 18:3 n6 split 16 of 19 early season 1991 samples.

Classification by year of Antarctic fur seal milk samples taken from mothers during the attendance period used 3 fatty acids, giving 4 terminal nodes (Fig. 3). The final tree chosen gave an RMD of 0.15. Fatty acid 18:2 n6 was chosen by the algorithm to split the root node based on the maximum change in deviance (52.19). All 1993 samples separated from the others by 18:2 n6. Both 18:4 n3 and 18:2 n6 split 1991 samples from 1992.

CART was able to differentiate between samples taken from Antarctic fur seals from different seasons. Classification by year using all Antarctic fur seal milk samples (Fig. 1) and Antarctic fur seal milk samples taken during attendance (Fig. 3) highlighted that fatty acid 18:2 n6 separated all 1993 milk samples from other years. Samples from 1993 contained 1.53% ± 0.07 18:2 n6, which was closer to levels of this fatty acid in krill and *Champsocephalus gunnari* samples than levels found in Antarctic fur seal samples from 1991 and 1992 (Table 1). Otherwise, Antarctic fur seal data could not be separated on a temporal basis using single fatty acids chosen by the CART algorithm.

Analysis of Southern elephant seal, Antarctic fur seal and prey species data

Both cluster analysis and CART supported inter-specific variation in seal milk fatty acid profiles. In a 10 cluster analysis (Table 3), Clusters 2 and 9 accounted for 107 of 135 observations. Cluster 2 contained 51 out of 53 Southern elephant seals (i.e. 96% of all Southern elephant seal samples) and only 6 Antarctic fur seals. The prey *Dissostichus eleginoides*, *Pagothenia hansoni* and *Gymnoscopelus nicholsi* were also in Cluster 2. Cluster 9 contained 41 out of 71 Antarctic fur seals (i.e. 58% of all Antarctic fur seal samples), 2 Southern elephant seals and 4 prey species (*Chaenocephalus aceratus*, *Champsocephalus gunnari*, *Gobionotothen gibberifrons* and Antarctic krill [K000]). The remaining 8 clusters contained small numbers of Antarctic fur seals (a total of 25 seals), and the remaining prey were assigned to Cluster 6 (*Martialia hyadesi*, and Antarctic krill [K168, K257]).

In 15 cluster analysis, 4 clusters contained 114 of the 135 observations (Table 3). The 19 remaining Antarctic fur seal samples were found in 9 of the remaining 11 outlying clusters. *Martialia hyadesi* and Antarctic krill (K257) were assigned a lone cluster each. Cluster 2 contained 28 Southern elephant seals, *Dissostichus eleginoides* and 2 Antarctic fur seals. The nearest cluster to it, Cluster 5, was assigned 22 Southern elephant seals, *Gymnoscopelus nicholsi* and 5 Antarctic fur seals. In total, 31 Antarctic fur seals were found in Cluster 12 with *Gobionotothen gibberifrons*, Antarctic krill (K168) and a single Southern elephant seal. Cluster 8 contained 15 Antarctic fur seals, 2 Southern elephant seals, *Chaenocephalus aceratus*, *Pagothenia hansoni*, *Champsocephalus gunnari* and Antarctic krill (K000).

The 15 cluster analysis divided the data into clusters dominated by samples from Southern elephant seals (Clusters 2 and 5) and clusters dominated by samples from Antarctic fur seals (Clusters 8 and 12). Comparing the mean values of fatty acids from each cluster, fatty acids 16:4 n1; 18:0; 18:1 n9/ n11; 18:2 n6; 18:4 n3; 18:4 n1; 20:1 n9; and 20:5 n3 showed the greatest differences in mean values between species (i.e. Clusters 2 and 5 compared to Clusters 8 and 12). Percentage composition of each of these fatty acids, in prey, was plotted with the mean values from Southern elephant seals and Antarctic fur seals (Fig. 5A to H). These fatty acids highlighted the differences between the profiles of the 2 seal species in different clusters, as well as similarities between predator and prey species found in the same clusters. For example, relatively high levels of 16:4 n1 (A), 18:4 n3 (E) and 20:5 n3 (H), found in Antarctic fur seals, were similar to levels in krill, *Champsocephalus gunnari*, *Chaenocephalus aceratus* and *Pagothenia hansoni*, as were relatively low levels of 18:0 (B), 18:1 n9/ n11 (C) and 20:1 n9 (G) in Antarctic fur seals, krill and *C. gunnari*. In Southern elephant seals, relatively low levels of 16:4 n1 (A), 18:2 n6 (D), 18:4 n1 (F) and 20:5 n3 (H), as well as relatively high amounts of 18:0 (B), 18:1 n9/ n11 (C) and 20:1 n9 (G), had similarities to those prey with which they clustered (*Dissostichus eleginoides* and *Gymnoscopelus nicholsi*).

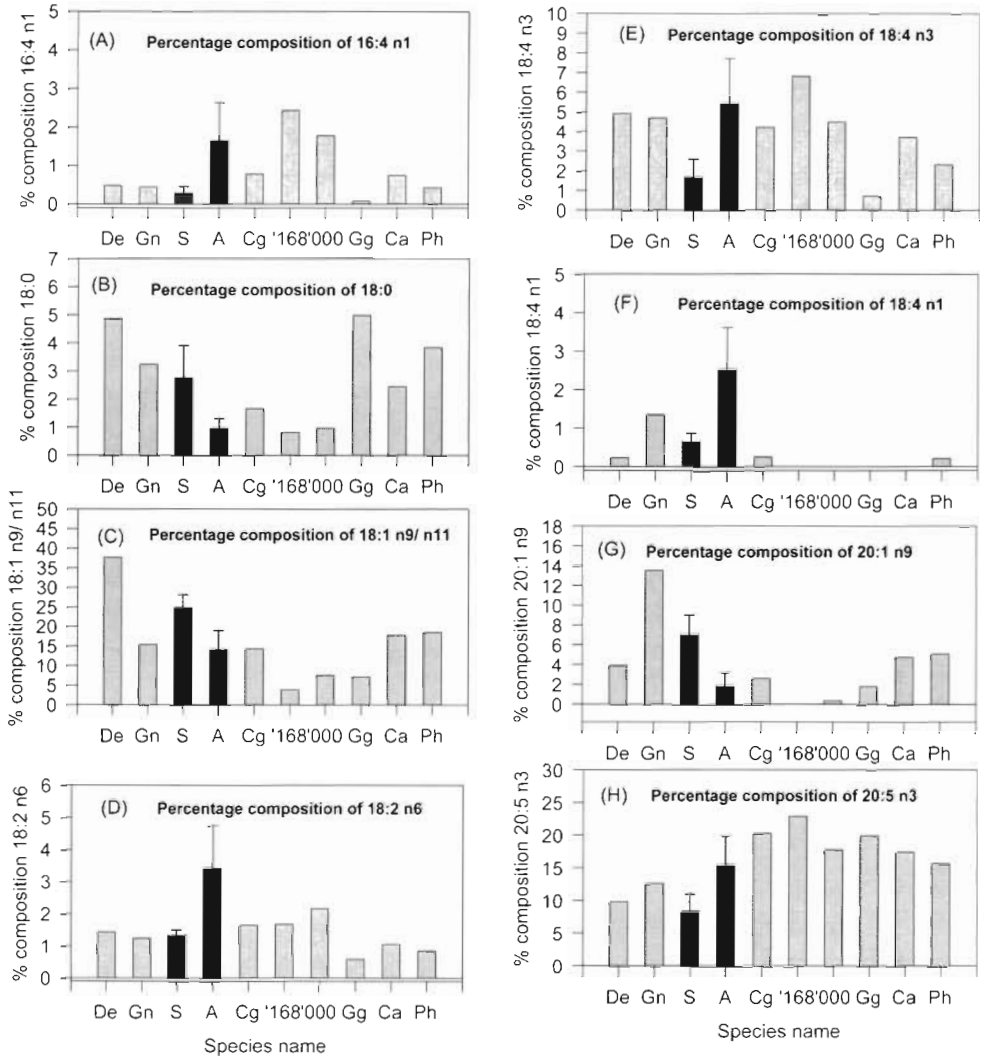

Fig. 5. (A to H) Percentage composition of major fatty acids found by cluster analysis to describe differences in fatty acid profiles between seal species. Results from 15 cluster analysis of Antarctic fur seal data and prey species data (Table 3) indicated clustering of specific prey species samples with particular seal species. Values for Antarctic fur seals and Southern elephant seals are given as means ± 1 standard deviation. Values for single samples of prey species are plotted next to the seal species with which they clustered in 15 cluster analysis (see Table 3). De: *Dissostichus eleginoides*; Gn: *Gymnoscopelus nicholsi*; S: Southern elephant seal; A: Antarctic fur seal; Cg: *Champsocephalus gunnari*; '168: krill (no. 168); '000: krill (no. 000); Gg: *Gobionotothen gibberifrons*; Ca: *Chaenocephalus aceratus*; Ph: *Pagothenia hansoni*

CART separated Antarctic fur seals from Southern elephant seals well. Classification by seal species required 3 fatty acids, giving 4 terminal nodes (Fig. 4). CART's automatic choice was 18:0, based on the maximum change in deviance (142.17). However, 20:1 n9 (deviance = 123.75) gave the better residual mean deviance value (0.04). The tree separated 48 out of 53 Southern elephant seals from Antarctic fur seals, using 20:1 n9 (T1). Fatty acid 18:0 split 66 of 72 Antarctic fur seals (T2). Five of each of the Antarctic fur seals and Southern elephant seals split from each other with 18:2 n6. Dropping prey sample profiles through the tree classified by seal species (Fig. 4) at the root node split *Gymnoscopelus nicholsi* and *Martialia hyadesi* into T1, with levels of 20:1 n9 greater than 5.12%. At P1, the only prey with levels of 18:0 < 1.44% were the 3 krill samples, placing them in T2 with 66 of 72 Antarctic fur seals. The remaining prey were found in T3 with the remaining elephant seal samples.

DISCUSSION

The purpose of this study was to examine fatty acid signatures in the milk of Antarctic fur seals and Southern elephant seals with a view to comparing diet in these species. Using 2 different statistical methods, we found significant inter- and intra-annual differences in the fatty acid profiles of these 2 species, as well as systematic variation in the fatty acid signatures of Antarctic fur seals. Although we also examined the fatty acid signatures of several potential prey species, because of the small sample sizes involved, these are unlikely to reflect the full range of variation in these species. However, our purpose was not to provide a definitive view of the diet at the prey species' level using fatty acid profiles from milk. Instead it was to make an initial attempt to relate variation in predator fatty acid signatures to the general features of fatty acid signatures derived from

some potential dietary species. Likewise, we wished to examine the variation in predator fatty acid signatures in relation to concurrent diet analysis using scats.

Antarctic fur seal diet

Cluster analysis, using samples from Antarctic fur seals and probable prey species, suggested that diet changed through the season (Table 2). CART confirmed these results using fatty acid signatures from milk samples only, indicating shifts in fatty acid profiles within and between years (Figs. 1 to 3).

Iverson et al. (1997a) suggested that fur seals lactating in the 1991 season exhibited a shift from krill, in early lactation, to fish late in lactation. Using scat analysis, Reid & Arnould (1996) observed larger numbers of myctophids in the diet of fur seals late in lactation, which coincided with the timing of the changes observed by Iverson et al. (1997a). The results of this study broadly confirmed the conclusions of Iverson et al. (1997a). CART indicated differences in fatty acid signatures between milk samples taken in the early and latter parts of the 1991 season (Fig. 2). Cluster analysis suggested a diet mainly composed of krill and fish early in 1991, followed by a late season shift towards profiles different from those of krill (see Table 2). However, this study was unable to establish the nature of the diet in the latter half of the 1991 season, as milk samples from that period did not cluster with the prey species examined in the study. Although the myctophid *Gymnoscopelus nicholsi* was analysed in this study, it was not possible to obtain samples of *Protomyctophum choriodon*, which is the most abundant myctophid in the diet of fur seals (Reid & Arnould 1996). Therefore, based on this analysis, we cannot be certain that the fatty acid signatures observed at the end of the 1991 lactation period were caused by a higher proportion of myctophids. In 1992, 20 of 25 Antarctic fur seal profiles clustered with krill and fish (Table 2). In CART, generally, samples from 1992 terminated on the same side of the tree (Figs. 1 to 3), indicating similar fatty acid profiles, and thus, similar diets. However, in that season, milk samples were analysed up to the end of January only. Any change in diet late in the season would have been missed. Seals from 1993 showed clustering associated with krill and fish throughout the season. Results from CART (Figs. 1 to 3) indicated that samples from 1993 had similar fatty acid profiles throughout the season, either all clustering at the same terminal node (Figs. 1 & 3), or on the same side of the tree (Fig. 2), implying a consistent diet throughout the seals sampled.

All fish species that clustered with the Antarctic fur seals (*Gobionotothen gibberifrons*, *Chaenocephalus aceratus*, *Champsocephalus gunnari*, *Pagothenia hansoni* and *Gymnoscopelus nicholsi*) (Table 2) are thought to feed on krill (Gon & Heemstra 1990, McKenna 1991, Kock et al. 1994). Therefore, it was expected that all krill feeders would have had a similar fatty acid profile and would probably cluster together. Consequently, it may not be possible to distinguish between those fur seals that fed on krill and those that fed on other krill predators using FASA alone. However, data from scat analysis confirmed that fur seals took both krill and other krill predators (in particular *C. gunnari*) during the years of this study (Reid & Arnould 1996). Generally, Antarctic fur seal milk samples clustered with samples of krill and *C. gunnari* (Tables 2 & 3), confirmed by specific fatty acids (Fig. 5A to H) highlighted as important by cluster analysis.

Southern elephant seal diet

The lack of change in the fatty acid profiles of milk throughout lactation in Southern elephant seals implied that diet prior to lactation was similar in individuals (Tables 1A & 3). When clustering of the Southern elephant seal data alone was carried out, the distances between cluster centroids was so low that only one of the 53 seals could be identified as an outlier (data not shown). Most Southern elephant seal samples aggregated in clusters containing *Dissostichus eleginoides* and to a lesser extent *Gymnoscopelus nicholsi*, and remained distant from clusters containing krill and fish that predate on krill. This was observed with specific fatty acids (Fig. 5A to H) and suggested that Southern elephant seals fed on *D. eleginoides* and *G. nicholsi* (or fish species that forage at the same trophic level) but that krill, smaller notothenids and possibly also squid were not as important in the diet.

Rodhouse et al. (1992) indicated that Southern elephant seals fed on *Martialia hyadesi* around South Georgia, prior to the moulting fast (November to February). However, the presence of squid beaks in the stomach may not have provided an accurate indication of their true frequency in the diet, due to accumulation and resistance to digestion. Due to the biases in stomach lavage analysis (Croxall 1993), the importance of *M. hyadesi* in the diet of elephant seals may have been overestimated. Although only 1 squid sample was examined in this study and is possibly unrepresentative, these results support the view that stomach lavage samples may have misrepresented the importance of squid in the diet of Southern elephant seals.

The Patagonian toothfish *Dissostichus eleginoides* has been reported around South Georgia and other

sub-Antarctic Islands, off the South American coast and the Falkland Islands (Gon & Heemstra 1990) at depths between 70 and 1500 m, a maximum depth range similar to that of the Southern elephant seal (Hindell et al. 1991, McConnell & Fedak 1996). There has also been an observation of a Southern elephant seal feeding on what appeared to be *D. eleginoides* at South Georgia (Reid & Nevitt 1998).

Cluster analysis showed that *Gymnoscopelus nicholsi* was a possible prey species of elephant seals (Table 3). The distribution of *G. nicholsi* is circumpolar, extending northward from the sea ice edge, including South Georgia, and it is typically caught in trawls at depths between 50 and 700 m (Gon & Heemstra 1990), making it a potential prey item of elephant seals. *G. nicholsi* has been found in the stomachs of Southern elephant seals at Heard Island, though it is thought the prey was taken infrequently and opportunistically (Green & Burton 1993).

Comparison of Antarctic fur seal and Southern elephant seal diets

Antarctic fur seal and Southern elephant seal milk samples exhibited very different fatty acid profiles as distinguished by cluster analysis (Table 3) and CART (Fig. 4). Interspecific differences and intraspecific similarities were reflected in CART by the low residual mean deviance (Fig. 4) and by the mean values of particular fatty acids highlighted by cluster analysis (Fig. 5A to H). Working on the premise that profiles in the milk were derived predominantly from the diet (Iverson 1993), the implication is that the diets of the 2 species were very different. Given the differences in the species' vertical and horizontal foraging ranges (Kooyman et al. 1986, Boyd & Arnbom 1991, Hindell et al. 1991, Boyd & Croxall 1992, McConnell & Fedak 1996, Boyd et al. 1999), such differences in diet and thus in fatty acid profile might be expected.

In CART, dropping prey species through the pre-constructed tree (Fig. 4) placed *Gymnoscopelus nicholsi* and *Martialia hyadesi* in the same terminal node as 91% of Southern elephant seal samples (T1), and all 3 krill samples with 92% of Antarctic fur seal milk samples (T2). Generally, this concurred with the results from cluster analysis. However, CART implied that *M. hyadesi* was a component of Southern elephant seal diet on the strength of a single fatty acid (20:1 n9), whereas cluster analysis indicated *M. hyadesi* was not, using a suite of fatty acids (see Table 3). This particular example indicates that care must be taken if a single fatty acid is used to determine dietary links. Perhaps if CART and cluster analysis are used in tandem, cluster analysis may be used to determine differences within data sets and CART employed to highlight the important fatty acids involved to describe the differences.

Cluster analysis and CART effectively described differences in fatty acid profiles derived from milk samples taken from Antarctic fur seals and Southern elephant seals and, using fatty acid signatures from potential prey species, provided an indication of their dietary content. This confirmed the significance of krill and *Champsocephalus gunnari* in the diet of lactating Antarctic fur seals using fatty acid signature analysis and demonstrated variation in diet within and between seasons. Fatty acid profiles indicated that Southern elephant seals may feed on large fish that do not normally eat krill. Further analyses need to be carried out to determine the relative importance of squid in the diet of Southern elephant seals.

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