Blubber fatty acid profiles reveal regional, seasonal, age-class and sex differences in the diet of young Steller sea lions in Alaska

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ABSTRACT: Blubber fatty acid (FA) profiles of young Steller sea lions *Eumetopias jubatus* (< 24 mo old; n = 477) were investigated to determine whether regional, seasonal, age-class and sex differences occur in the diets of these demographic groups. Blubber FA profiles of spring (March to May) pups differed significantly by region, probably reflecting regional differences in the diet of adult females, who provide their pups with milk. While there were statistically significant seasonal and age-class differences in both Prince William Sound (PWS) and SE Alaska (SEA), differences in FA profiles between sexes were only evident in the blubber of yearlings from PWS. Within SEA, blubber FA profiles of summer pups differed significantly from all other seasonal/age-class groups in that region. This is consistent with movements of female–pup pairs from rookeries to haul-outs and hence differences in female foraging. In SEA, seasonal differences in blubber FA composition were evident and similar between yearlings and pups. In contrast, there was a gradual change in the FA profile of pup blubber from summer to spring in PWS, which did not follow the same seasonal pattern of yearling profiles. These differences in FA profiles suggest either differences in the timing of weaning between the 2 areas or shifts in the diets of lactating females, or both.

KEY WORDS: Fatty acids · Dietary intake · *Eumetopias jubatus* · Independent foraging

INTRODUCTION

The transition to nutritional independence and subsequent juvenile survival is an important component of the population dynamics in many mammalian species (Blumstein & Foggin 1997, Beauplet et al. 2005). In pinnipeds, declines in population size of Hawaiian monk seals *Monachus schauinslandi* and Steller sea lions (SSL) *Eumetopias jubatus* have been theoretically (York 1994) and empirically (Craig & Ragen 1999, Pendleton et al. 2006) attributed to reduced juvenile survival. In the endangered western stock of SSL, the

causes of reduced juvenile survival are uncertain. Nevertheless, a change in quality and/or quantity of preferred prey species has been one of the leading hypotheses to explain reduced survival (Merrick et al. 1997, Trites & Donnelly 2003). Juveniles, specifically newly weaned individuals, may be particularly vulnerable to changes in prey quality or quantity given their limited foraging experience and range (Merrick & Loughlin 1997, Craig & Ragen 1999).

The western stock (west of 144° W; Prince William Sound to Russia) of SSLs declined by up to 85% between 1970 and 2000 (Merrick et al. 1987, Sease et

al. 2001), leading to the listing status 'endangered' under the US Endangered Species Act. In contrast, the genetically distinct (Bickham et al. 1996) eastern SSL stock (east of 144° W; SE Alaska, to California) increased by 150 % over the same time period (Calkins et al. 1999, Sease et al. 2001). The different population trends evident in the western and eastern stocks has led researchers to compare various aspects of SSL ecology (e.g. Brandon et al. 2005) and physiology (e.g. Zenteno-Savin et al. 1997) to elucidate the mechanism(s) that generated population decline in the west. To further investigate differences between the 2 stocks it is useful to gain a better understanding of juvenile foraging behavior and diet (Raum-Suryan et al. 2004).

Recent estimations of SSL diets have been based on the analysis of fecal samples collected at haul-outs and rookeries (e.g. Merrick et al. 1997, Sinclair & Zeppelin 2002). Although these studies have been informative, the diet of specific age groups cannot be determined as scats cannot be linked with individual SSLs. Given the known dietary and foraging differences between adults and juveniles in a variety of vertebrate species (e.g. harbor seals *Phoca vitulina* [see review by Hoover-Miller 1988], hoary bats *Lasiurus cinereus* [Rolseth et al. 1994] and birds [Marchetti & Price 1989]), it is critical to use a method which can match sample data to individuals. Using digestion-resistant hard parts found in the stomachs of adult and juvenile (≤4 yr old) SSLs, Merrick & Calkins (1996) found that juveniles around Kodiak Island (KOD) consumed small forage fish more frequently, and flatfish and cephalopods less frequently, than adults from the same area, suggesting that age differences in diet exist for this species. Nevertheless, the use of digestion-resistant prey hard parts collected from stomachs or scats can bias our understanding of diet (Jobling & Breiby 1986, Pierce & Boyle 1991, Bowen 2000). For example, if diagnostic hard parts are not consumed by the predator (e.g. crustacean shells) these prey items will not be identified in the diet. Additionally, differential rates of digestion of hard parts (Bowen 2000) may bias diet estimates in favor of prey species with large robust hard parts.

These difficulties and biases have lead to the development of alternative methods to understand foraging patterns and diet differences, including analysis of fatty acid (FA) profiles. Marine ecosystems contain a diverse array of FAs (Ackman 1980), which are consumed and deposited in a predator's adipose tissues with little or no modification (Ackman & Eaton 1966, Cook 1991) or in a predictable way (Lhuillery et al. 1988, Summers et al. 2000, Cooper 2004, Iverson et al. 2004). Predator FA profiles are also influenced by the biosynthesis of certain FAs and the reduced deposition of others. As a result, the predator's FA profile will never exactly match that of their prey. Nevertheless,

the FA profile of a predator's adipose tissue has been shown to reflect the FA profile of consumed prey in many marine taxa (e.g. Holland et al. 1990, Raclot et al. 1998, Herman et al. 2005). One of the advantages of FA analysis is that, due to the manner in which FAs are deposited in adipose tissue, FA profiles represent an integration of the predator's diet over several weeks to months and represents all species eaten, rather than the most recently consumed prey. In addition, because tissue samples can be collected directly from individuals, relationships between phenotypic and demographic characteristics of the animal can be tested.

The FA profile of a predator can be used to investigate diet in 2 ways. Quantitative estimation of the predator's diet is possible using the FA profile of the predator and that of its prey (Iverson et al. 2004). However, adequate data on the FA composition of all potential prey species and an understanding of how a predator metabolizes individual FAs (i.e. calibration coefficients, see Iverson et al. 2004) are required. In the absence of prey FA and calibration data, FA profiles of predators are useful to detect qualitative dietary changes and differences among demographic groups (e.g. Walton et al. 2000, Beck et al. 2005, Herman et al. 2005), based on the established understanding that FA structures are transferred across trophic levels largely unchanged (e.g. Ackman & Eaton 1966, Cook 1991). Despite some debate in recent years over the use of this method of dietary investigation in apex predators (Grahl-Nielsen et al. 2003, 2004, Thiemann et al. 2004), the qualitative use of FA has proven to be useful in elucidating geographical (e.g. Walton et al. 2000), ecotype (e.g. Herman et al. 2005) and sex (e.g. Beck et al. 2005) differences in diet, among a broad range of taxa, when appropriate sampling and chemical analysis of the predator's tissue is conducted (Thiemann et al. 2004).

In this study, we qualitatively investigated the blubber FA profiles of young SSLs (<24 mo old) and tested for regional, seasonal, age-class and sex differences in FA profiles. We hypothesized that the FA profile, and thus diet, of young SSLs would differ by region and season given the known temporal and spatial variation in prey assemblages throughout their Alaskan range. We also predicted that the FA profile of young SSLs would differ by age-class as individuals transition from maternal dependence on milk to independent foraging on prey. SSLs exhibit a long and variable period of lactation (Calkins & Pitcher 1982, Merrick & Loughlin 1997) and it has proven difficult to determine when the transition to independent foraging occurs in this species. Some SSL offspring are thought to wean at the end of their first year (11 to 12 mo old) just prior to the female giving birth to the next year's pup (Loughlin et al. 2003), although precise dates have not been determined. However, there is evidence of maternal depen-

dence continuing, in some cases, until offspring are 2 or 3 yr of age (Pitcher & Calkins 1981, Pitcher et al. 2004). By investigating the variation in FA profile by age and sex we further explored this diet transition at weaning in young SSLs.

MATERIALS AND METHODS

Sample collection. Between March 1998 and February 2004, blubber samples were collected from 477 individual SSLs (Table 1), ranging in age from 0.5 to 23.5 mo, in 3 regions of the western stock (PWS, KOD, and the Aleutian Islands, AI) and 1 region in the eastern stock (SEA; Fig. 1). Ages were estimated based on canine length, patterns of tooth eruption (King et al.

2007; a technique verified by a sample of 47 known-age individuals between the ages of 2 and 44 mo) and standard length (Alaska Department of Fish & Game unpubl. data) assuming a mean calendar birth date of June 15 for all individuals.

Individuals were captured underwater near haul-outs or rookeries (Raum-Suryan et al. 2004) or on land with hoop nets. Captured sea lions were transported to a research vessel, weighed to the nearest 0.1 kg and immobilized with isoflurane gas anesthesia (Heath et al. 1997). During anesthesia, a 3 cm^2 area on the dorsal right flank of the sea lion was shaved and washed with povidone-iodine (Betadine solution) and alcohol before making a 1 cm incision through the epidermis with a sterile scalpel blade. A full-depth blubber biopsy was collected from this site using a sterile 6 mm biopsy

Table 1. *Eumetopias jubatus*. Number of Steller sea lion blubber samples collected between March 1998 and February 2004 by region, season, year and age-class. P, Y: pups and yearlings, respectively; SEA: SE Alaska; PWS: Prince William Sound; KOD: Kodiak Island; AI: Aleutian Islands

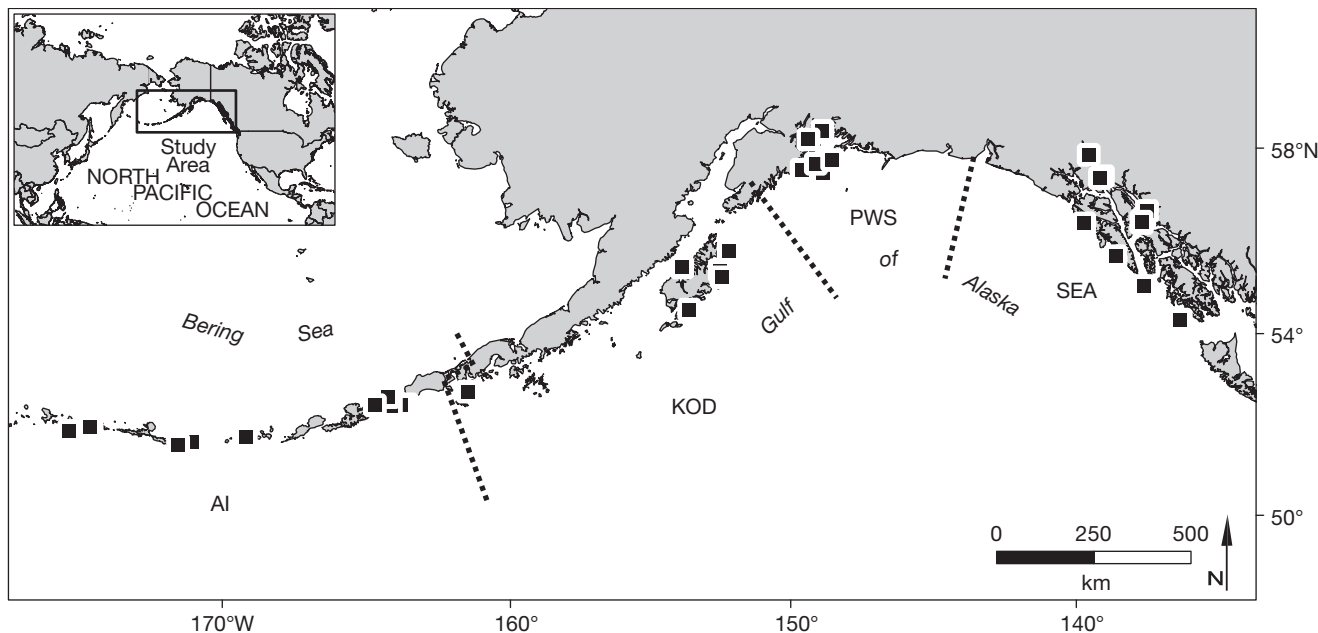| Year	SEA				PWS			KOD		AI	
	Summer	Fall	Winter	Spring	Summer	Fall	Spring	Summer	Spring	Fall	Spring
1998				10P/8Y							
1999	12P										
2000		13P/17Y	10P/4Y		13P/14Y		11P/5Y				
2001	10P/23Y			28P		21P/10Y			13P	17P/1Y	
2002	18Y	11P/12Y				6P/ 9Y	20P/9Y	7Y	15P		25P
2003	15P	8P/ 9Y				18P/12Y			6P		11P/4Y
2004			20P/5Y								
Total	37P/41Y	32P/38Y	30P/9Y	38P/8Y	13P/14Y	45P/31Y	31P/14Y	7Y	33P	17P/1Y	34P/4Y

Fig. 1. *Eumetopias jubatus*. Alaska Steller sea lion range. (■) Sampling locations in this study. Regions delineated by dotted lines based on Merrick et al. (1987). SEA: SE Alaska; PWS: Prince William Sound; KOD: Kodiak Island; AI: Aleutian Islands

punch. The incision was not sutured and subsequent recapture of 12 of these individuals showed good healing at this site. Blubber samples were wrapped in acetone-washed aluminum foil, placed in a polyethylene sample bag (Whirl-Pak©), and stored on dry ice while in the field. Once in the laboratory, samples were placed in a solution of 2:1 chloroform/methanol containing 0.01% 2, 6,-di-*tert*-butyl-4-methyl-phenol (BHT) and stored frozen (–80°C) until processed.

We chose to collect and analyze the full-depth blubber core in this study on young SSLs. Although previous studies have indicated that the blubber of cetaceans can be highly stratified (e.g. Koopman 2001), stratification of individual FAs, while present, is far less in pinniped species. Recent studies have shown that, in pinnipeds, the FAs in the inner portion of the blubber layer (i.e. closest to the muscle) turn over more quickly than the outer layer, such that the inner-half of the blubber layer may reflect recent (i.e. within the previous 2 wk) dietary FAs intake (Cooper 2004). However, whole, full-depth blubber cores, avoiding the near-skin FAs, provide the long-term (months) integration of dietary FAs (e.g. Cooper 2004, Iverson et al. 2004). Thus, our interest in the long-term integration of the diet in young SSLs was most appropriately addressed by analyzing the full-depth blubber core.

Laboratory procedures. Lipid was extracted from each full-depth blubber sample using a modified Folch method (Folch et al. 1957, Iverson et al. 2001). FA methyl esters (FAME) were prepared as described in Iverson et al. (1997). FAME from samples collected prior to 2000 were analyzed at Dalhousie University in Halifax, Nova Scotia as described in Budge et al. (2002). After 2000, FAME were analyzed at the Applied Science, Engineering and Technology laboratory at the University of Alaska Anchorage as described in Dodds et al. (2004). In both laboratories, specific FAs were identified using known standard mixtures (Sigma, Supelco, Matreya and/or Nu-check Prep), silver nitrate (argentation) chromatography, and gas chromatography/mass spectrometry. Individual FAs are reported as percent weight of total FAs and are designated using the shorthand nomenclature of carbon chain length: number of double bonds and location (n–x) of the double bond nearest the terminal methyl group.

A set of 12 SSL FAME samples were analyzed in both laboratories to assure compatibility of analyzed samples. There was no significant statistical difference between the 2 laboratories in the resulting FA composition of samples when compared using univariate *t*-tests with a Bonferroni correction factor for multiple comparisons. This analysis was conducted using all 66 FAs identified by both laboratories (statistically significant $p = 0.0007$; p-value range 0.9100 to 0.0009) and for the subset of 20 FAs (see below) used in the analysis (statistically significant $p = 0.0025$; p-value range 0.9280 to 0.0027). The average percent difference between labo-
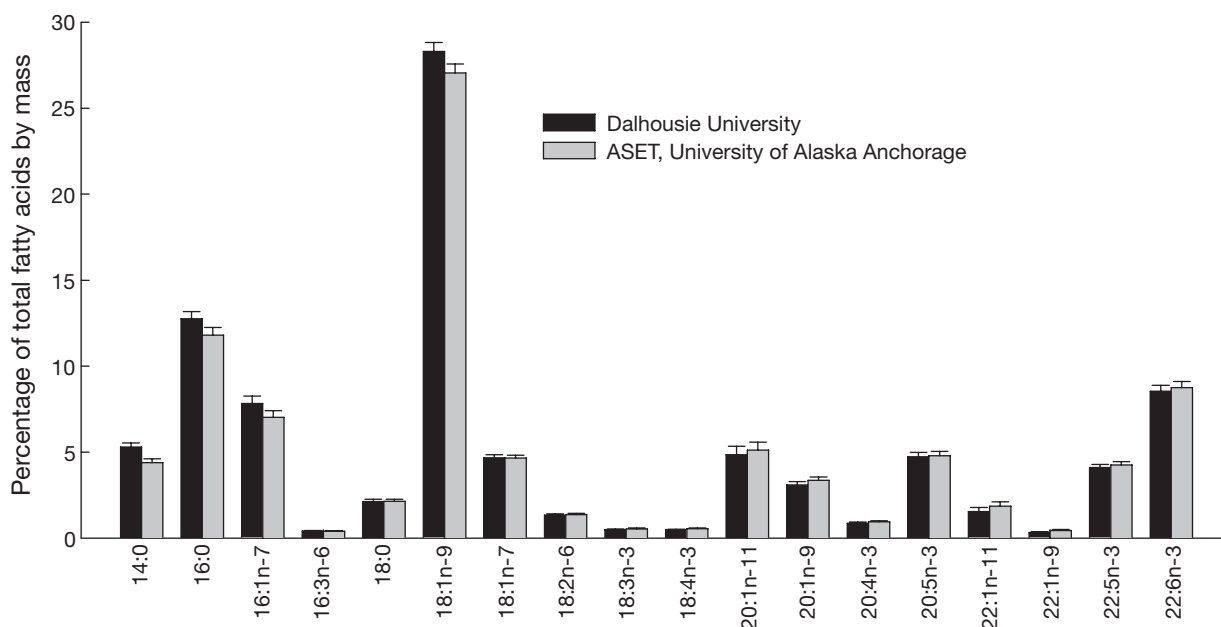

Fig. 2. *Eumetopias jubatus*. Mean (+1 SE) select abundant fatty acids quantified in blubber samples of 12 young (<24 mo) Steller sea lions analyzed at Dalhousie University in Halifax, Nova Scotia and in the Applied Science, Engineering, and Technology (ASET) laboratory at the University of Alaska Anchorage. There were no significant differences in FA profile composition between laboratories (univariate *t*-tests with Bonferroni correction factor [statistically significant $p = 0.001$]; all p-values < 0.001)

ratories for individual FAs ranged from 0 to 1.24 % (Fig. 2).

Statistical analysis of fatty acid profiles. General linear models (MANOVA) and discriminant function analysis (DFA) were used to examine regional, seasonal, age-class and sex differences in the FA profiles (and thus diet) of young SSLs. Samples collected between June 1 and August 31 were considered to be summer samples, while those collected between September 1 and November 30, December 1 and February 28, and March 1 and May 31, were considered to be fall, winter and spring samples, respectively. Individuals <12 mo old were classified as pups whereas those between 12 and 24 mo were considered yearlings. Because of the uneven yearly sampling of SSLs by regions and seasons (Table 1), samples were pooled among years for statistical analysis of regional and seasonal variability. Although inter-annual shifts in diet have been observed in a variety of pinniped species (Merrick et al. 1997, Beck et al. 2005), the sampling logistics of this study prevented an inter-annual analysis of this data.

MANOVA and DFA require that the number of samples in each group exceed the number of variables used in the analysis to avoid over-fitting of the data and to provide reasonable assurance that the covariance matrices are homogeneous (Stevens 1986). Thus, although 66 individual FAs were routinely identified and quantified, only a subset (n ≤ 20; Table 2) of FAs were used to examine the variability in SSL profiles due to limited and uneven sampling across regions, seasons and/or age-classes. The subsets of FAs used in each analysis were the most variable and abundant of the FAs that are dominantly or solely derived from dietary intake (see Iverson et al. 2004, their Appendix A).

Two separate analyses were conducted. The first examined regional and sex-related variation and included only blubber data collected from spring pups to control for age and seasonal effects while still providing the largest sample size across regions (Table 1). The second set of analyses examined seasonal, sex and age-class variation within region. Only SEA and PWS regions were investigated due to small seasonal sample sizes in other regions (Table 1). Regional analyses included the 20 most variable dietary FAs, which each contributed more than 0.2 % of the total FAs by weight in at least 1 region (Table 2). These 20 FAs accounted for 85.5 ± 0.17 % of total FAs by weight. For the within-region analyses, the largest subset of samples and FAs were used to answer specific questions (see 'Results' and Table 2). Prior to each analysis, FAs were transformed to normalize the data using a logarithmic transformation designed for compositional data (Budge et al. 2002).

RESULTS

Nine FAs (14:0, 16:0, 16:1n-7, 18:1n-9, 18:1n-7, 20:1n-11, 20:5n-3, 22:5n-3, and 22:6n-3) accounted for between 66.6 and 84.2 % of the total blubber FAs by weight for all individuals. Overall, monounsaturated FAs accounted for 55.0 ± 0.17 % whereas saturated FAs and polyunsaturated FAs accounted for 20.6 ± 0.09 and 24.0 ± 0.14 % of total blubber FA, respectively.

Regional analysis

FA profiles were highly variable between regions, with the isomers of 22:1 and 20:1 showing the greatest variability. Blubber FA profiles of spring pups differed significantly by region (MANOVA: $F_{60,333}$ = 11.0, p < 0.001) but there was no statistically significant sex effect ($F_{20,109}$ = 1.1, p = 0.354) and no statistically significant region × sex interaction ($F_{60,333}$ = 1.1, p = 0.267). Post-hoc univariate analysis indicated that 13 of the 20 FAs used differed significantly by region after applying a Bonferroni correction factor for multiple comparisons (statistically significant p-value = 0.003; Fig. 3).

DFA also indicated statistically significant differences in the blubber FA profiles of SSL pups by region (Wilks' λ = 0.017, χ^2 = 503.2, p < 0.001; Fig. 4). 96 % (130 of 136) blubber FA profiles were correctly classified to region with a cross-validation error rate of 11.0 %. Misclassifications occurred in all regions: 2 AI and 2 SEA individuals were misclassified as KOD, 1 KOD individual was misclassified as PWS, and 1 PWS individual was misclassified as SEA.

Seasonal, age-class and sex analysis

Southeast Alaska

SEA pups were sampled in large numbers across all seasons (Table 1), whereas sample sizes of SEA yearlings were sufficiently large (>20) only in summer and fall. Therefore, winter and spring yearlings were not included in the generalized linear model analysis. Blubber FA profiles from SEA individuals differed significantly by age-class (MANOVA: $F_{20,191}$ = 47.9, p < 0.001), season (MANOVA: $F_{60,579}$ = 19.1, p < 0.001) and seasonally within age-class (age-class × season interaction MANOVA: $F_{20,191}$ = 17.9, p < 0.001), when the same 20 FAs used in the regional analysis were included in the model (Table 2). In a DFA conducted to investigate these divergent seasonal patterns, winter and spring yearling samples were classified using the functions derived from all other samples. This analysis

Table 2. *Eumetopias jubatus*. Fatty acid composition of blubber tissue from young Steller sea lions in 4 regions of their Alaskan range. Values are mean ± 1 SE percent of total fatty acid by weight for all fatty acids that averaged ≥0.2% in any age-class/region group. Boldface indicates 20 fatty acids used in regional and SEA analyses; *: 9 variables used in PWS analysis

Fatty acids (FAs)	SEA Pups (n = 137)	SEA Yearlings (n = 96)	PWS Pups (n = 89)	PWS Yearlings (n = 59)	KOD Pups (n = 33)	KOD Yearlings (n = 7)	AI Pups (n = 51)	AI Yearlings (n = 5)
Saturated								
14:0*	4.96 ± 0.07	5.06 ± 0.09	5.36 ± 0.11	5.19 ± 0.12	4.57 ± 0.09	4.55 ± 0.18	4.96 ± 0.09	4.33 ± 0.23
15:0	0.36 ± 0.01	0.32 ± 0.01	0.35 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.30 ± 0.02	0.33 ± 0.01	0.31 ± 0.02
16:0*	13.71 ± 0.12	12.22 ± 0.13	12.55 ± 0.13	10.65 ± 0.14	12.24 ± 0.21	12.10 ± 0.64	11.35 ± 0.23	10.93 ± 0.59
7methyl 16:0	0.26 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.01	0.24 ± 0.01	0.19 ± 0.01	0.25 ± 0.01	0.24 ± 0.01
17:0	0.24 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.24 ± 0.01	0.31 ± 0.02	0.22 ± 0.01	0.22 ± 0.02
18:0	1.89 ± 0.03	1.82 ± 0.05	1.67 ± 0.04	1.80 ± 0.08	1.52 ± 0.05	2.13 ± 0.11	1.43 ± 0.05	1.44 ± 0.15
Monounsaturated								
14:1n-5	0.38 ± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.41 ± 0.02	0.42 ± 0.02	0.31 ± 0.02	0.43 ± 0.01	0.40 ± 0.03
16:1n-11	0.45 ± 0.01	0.43 ± 0.01	0.47 ± 0.01	0.39 ± 0.01	0.46 ± 0.01	0.37 ± 0.04	0.54 ± 0.02	0.45 ± 0.05
16:1n-9	0.44 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.34 ± 0.01	0.38 ± 0.01	0.40 ± 0.02	0.37 ± 0.01	0.38 ± 0.02
16:1n-7*	8.59 ± 0.13	8.49 ± 0.14	8.48 ± 0.13	7.67 ± 0.24	10.14 ± 0.22	7.01 ± 0.55	8.89 ± 0.29	8.65 ± 0.52
17:1	0.44 ± 0.01	0.38 ± 0.01	0.41 ± 0.01	0.35 ± 0.01	0.45 ± 0.03	0.41 ± 0.04	0.41 ± 0.01	0.40 ± 0.03
18:1n-13	0.24 ± 0.01	0.20 ± 0.01	0.32 ± 0.01	0.26 ± 0.01	0.27 ± 0.01	0.24 ± 0.02	0.38 ± 0.02	0.25 ± 0.02
18:1n-11	2.22 ± 0.08	2.79 ± 0.11	2.84 ± 0.10	2.94 ± 0.11	2.26 ± 0.13	2.27 ± 0.44	3.74 ± 0.24	3.44 ± 0.74
18:1n-9*	26.41 ± 0.24	26.86 ± 0.28	24.26 ± 0.22	23.66 ± 0.28	26.10 ± 0.34	27.13 ± 0.63	22.39 ± 0.73	23.47 ± 1.41
18:1n-7*	4.84 ± 0.10	4.91 ± 0.10	4.26 ± 0.08	4.21 ± 0.10	5.55 ± 0.19	5.06 ± 0.19	4.68 ± 0.14	5.33 ± 0.50
18:1n-5	0.50 ± 0.01	0.45 ± 0.01	0.54 ± 0.01	0.50 ± 0.01	0.41 ± 0.01	0.43 ± 0.02	0.48 ± 0.01	0.44 ± 0.03
20:1n-11*	3.95 ± 0.08	4.93 ± 0.15	6.74 ± 0.27	8.62 ± 0.15	4.39 ± 0.28	6.28 ± 0.85	7.59 ± 0.59	6.59 ± 1.65
20:1n-9	3.02 ± 0.08	3.72 ± 0.10	2.64 ± 0.06	3.10 ± 0.10	2.54 ± 0.15	3.27 ± 0.27	3.16 ± 0.11	3.42 ± 0.52
20:1n-7	0.35 ± 0.01	0.34 ± 0.01	0.31 ± 0.01	0.35 ± 0.01	0.33 ± 0.07	0.44 ± 0.02	0.36 ± 0.02	0.37 ± 0.02
22:1n-11*	1.36 ± 0.06	1.61 ± 0.08	2.03 ± 0.10	2.88 ± 0.08	1.16 ± 0.10	1.67 ± 0.29	2.39 ± 0.20	1.95 ± 0.54
22:1n-9	0.28 ± 0.01	0.33 ± 0.01	0.29 ± 0.01	0.38 ± 0.01	0.24 ± 0.01	0.43 ± 0.04	0.40 ± 0.02	0.35 ± 0.07
Polyunsaturated								
16:2n-4	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.27 ± 0.02	0.22 ± 0.01	0.29 ± 0.02
16:3n-6	0.38 ± 0.01	0.41 ± 0.01	0.33 ± 0.01	0.30 ± 0.01	0.43 ± 0.02	0.28 ± 0.03	0.42 ± 0.02	0.38 ± 0.03
18:2n-6	1.45 ± 0.03	1.32 ± 0.02	1.47 ± 0.02	1.41 ± 0.02	1.32 ± 0.04	1.51 ± 0.10	1.51 ± 0.02	1.45 ± 0.06
18:3n-4	0.22 ± 0.01	0.27 ± 0.01	0.17 ± 0.01	0.18 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.10 ± 0.01	0.08 ± 0.01
18:3n-3	0.62 ± 0.02	0.49 ± 0.01	0.65 ± 0.01	0.58 ± 0.01	0.54 ± 0.02	0.58 ± 0.05	0.58 ± 0.02	0.55 ± 0.04
18:4n-3	0.68 ± 0.02	0.50 ± 0.01	0.68 ± 0.02	0.58 ± 0.02	0.61 ± 0.03	0.65 ± 0.08	0.79 ± 0.03	0.66 ± 0.11
18:4n-1	0.31 ± 0.01	0.29 ± 0.01	0.26 ± 0.01	0.21 ± 0.01	0.35 ± 0.02	0.20 ± 0.02	0.38 ± 0.02	0.29 ± 0.05
20:2n-6	0.31 ± 0.01	0.24 ± 0.01	0.30 ± 0.01	0.29 ± 0.01	0.25 ± 0.01	0.18 ± 0.06	0.30 ± 0.01	0.26 ± 0.01
20:3n-6	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.25 ± 0.04	0.12 ± 0.01	0.12 ± 0.01
20:4n-6	0.69 ± 0.03	0.63 ± 0.01	0.63 ± 0.01	0.55 ± 0.01	0.68 ± 0.02	0.33 ± 0.11	0.59 ± 0.01	0.63 ± 0.05
20:3n-3	0.11 ± 0.01	0.10 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.22 ± 0.01	0.38 ± 0.10	0.11 ± 0.01	0.11 ± 0.01
20:4n-3	1.09 ± 0.04	0.89 ± 0.02	1.07 ± 0.02	1.01 ± 0.02	0.95 ± 0.04	1.02 ± 0.11	1.05 ± 0.03	0.98 ± 0.04
20:5n-3*	4.61 ± 0.08	4.13 ± 0.08	4.14 ± 0.10	3.82 ± 0.13	5.90 ± 0.34	3.63 ± 0.40	4.85 ± 0.17	4.91 ± 0.64
21:5n-3	0.34 ± 0.01	0.35 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	0.41 ± 0.01	0.37 ± 0.03	0.42 ± 0.01	0.43 ± 0.04
22:5n-3	3.48 ± 0.05	3.88 ± 0.07	3.66 ± 0.07	4.29 ± 0.11	3.80 ± 0.09	4.22 ± 0.13	3.62 ± 0.09	4.36 ± 0.28
22:6n-3*	8.13 ± 0.03	7.78 ± 0.11	8.97 ± 0.20	9.48 ± 0.26	7.80 ± 0.22	8.52 ± 0.32	7.69 ± 0.17	8.68 ± 0.85
% total FA								
Saturated	22.1 ± 0.15	20.5 ± 0.19	21.0 ± 0.17	19.0 ± 0.21	19.7 ± 0.24	20.1 ± 0.77	19.2 ± 0.24	18.1 ± 0.50
Monounsaturated	53.8 ± 0.31	56.6 ± 0.24	54.7 ± 0.46	56.4 ± 0.51	55.5 ± 0.52	56.1 ± 1.10	56.5 ± 0.49	56.4 ± 2.01
Polyunsaturated	24.1 ± 0.27	22.9 ± 0.22	24.3 ± 0.42	24.6 ± 0.43	24.8 ± 0.83	23.8 ± 0.83	24.2 ± 0.33	25.6 ± 1.84

also indicated significant seasonal and age-class differences (Wilks' λ = 0.005, χ^2 = 1075.6, p < 0.001; Fig. 5), correctly classified 90.6% of all samples (n = 233) to season and 90.1% of samples to the correct age-class (Fig. 5). Misclassifications by age-class were due to winter and spring yearling samples being assigned to winter and spring pup categories, respectively. Seasonal misclassifications were the result of

overlap between fall and winter samples (Fig. 5). Post-hoc analysis indicated that the significant interaction between age and season resulted from differences between pups and yearlings in summer, whereas there was no statistically significant difference in the FA profiles of pups and yearlings in other seasons.

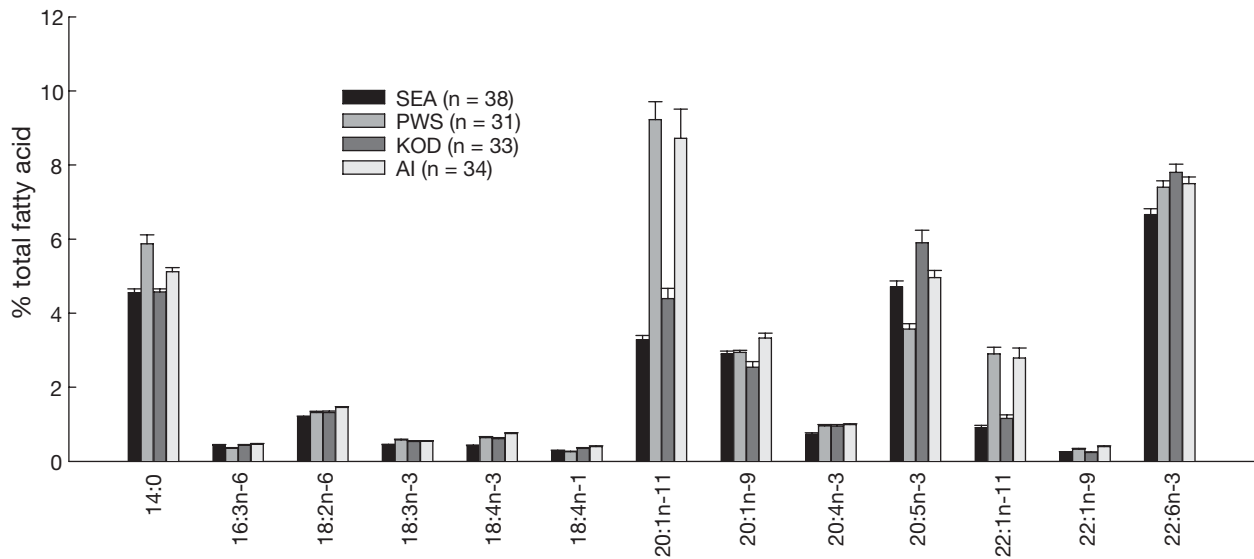Sex and age-class differences were examined separately from seasonal differences in order to

Fig. 3. *Eumetopias jubatus*. Percent composition by weight of the 13 fatty acids which differed significantly by region in blubber of spring pups (9 to 12 mo old). Region abbreviations as in Fig. 1 legend
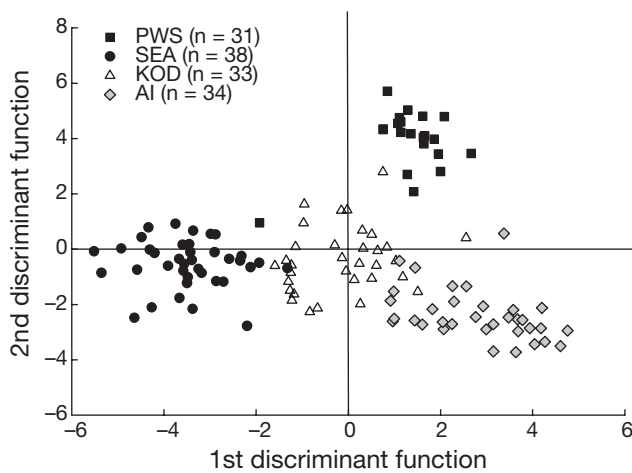

Fig. 4. *Eumetopias jubatus*. Discriminant functions plot of blubber fatty acid profiles from spring (March 1 to May 31) pups (9 to 12 mo old) in SE Alaska (SEA), Prince William Sound (PWS), Kodiak (KOD) and Aleutian Islands (AI). First discriminant function explained 52.9% of variation between samples and was most influenced by 20:1n-11, 18:4n-3, 20:4n-3; second discriminant function explained an additional 42.5% of variation and was most influenced by 22:1n-11 and 20:5n-3
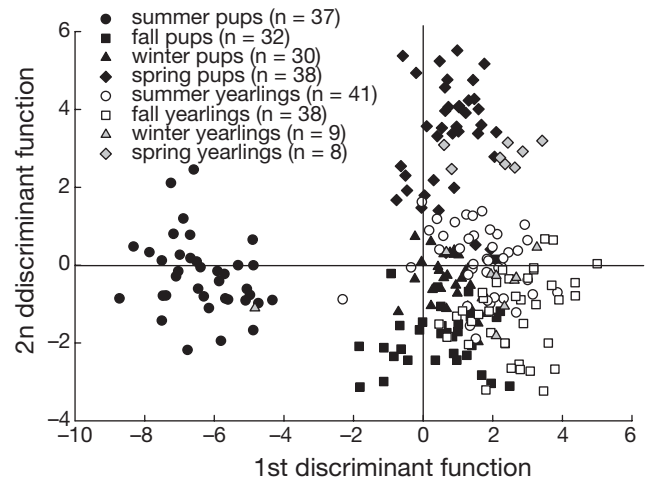
Fig. 5. *Eumetopias jubatus*. Discriminant function plot of blubber fatty acid profiles of young (<24 mo) Steller sea lions by season and age-class in SE Alaska. First discriminant function explained 63.7% of variation among samples and was most influenced by 16:3n-6 and 20:1n-9; second discriminant function explained an additional 19.7% of variation and was most influenced by 18:3n-4, 18:1n-7 and 22:1n-11

maximize the number of samples available for analysis. Using the same set of variables as in the previous analysis, blubber FA profiles from SEA still differed significantly between age-classes (MANOVA: $F_{20,210}$ = 18.1, p < 0.001), but were similar between sexes ($F_{20,210}$ = 1.4, p = 0.146) and between sexes within age-class (interaction term: $F_{20,210}$ = 1.4, p = 0.107).

Prince William Sound

Due to the small number of PWS pups sampled in summer and PWS yearlings sampled in summer and spring (Table 1), only 9 FAs (Table 2) were included as variables in the seasonal and age-class analyses. These 9 FAs were the most variable and accounted for 79.4 ± 0.12% of the total FA by weight. As in SEA, blubber FA profiles of sea lions from PWS differed significantly

between age-class (MANOVA: $F_{9,134}$ = 39.8, p < 0.001) and season ($F_{18,270}$ = 14.7, p < 0.001). The interaction between age-class and season was also statistically significant ($F_{18,270}$ = 8.7, p < 0.001) suggesting that seasonal changes in diet differed between pups and yearlings in PWS. A DFA (conducted using the same 9 variables) also indicated statistically significant differences by season and age-class (Wilks' λ = 0.036, χ^2 = 465.0, p < 0.001; Fig. 6). This analysis correctly classified 83.8% of blubber samples (n = 148) to season and 87.2% to age-class (Fig. 6). Most seasonal misclassifications occurred among yearlings while most age-class misclassifications involved spring samples.

Pooling data over season and including sex in the model indicated that blubber FA profiles from PWS differed significantly between age-classes (MANOVA: $F_{9,136}$ = 16.6, p < 0.001) and sexes ($F_{9,136}$ = 3.3, p = 0.001). A statistically significant age × sex interaction was also evident ($F_{9,136}$ = 3.3, p = 0.001), indicating that sex differences in blubber FA profiles were age-dependent. A DFA (Wilks' λ = 0.325, χ^2 = 158.0, p < 0.001; Fig. 7) indicated that blubber FA profiles of pups were very similar between sexes, with 32.9% misclassified by sex. Among yearlings, males and females had more divergent blubber FA profiles, with only 19.1% of samples being classified to the wrong sex.

DISCUSSION

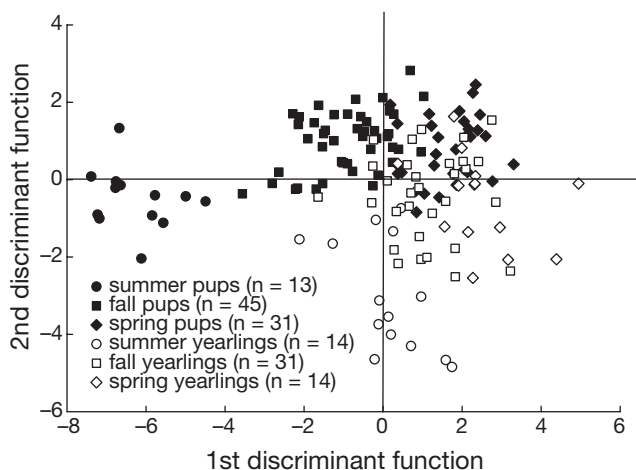We found statistically significant differences in the blubber FA profiles, and thus diet, of young SSLs by re-
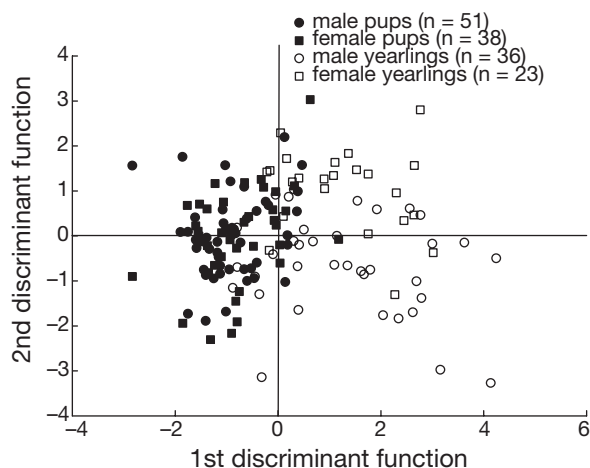

Fig. 7. *Eumetopias jubatus*. Discriminant function plot of blubber fatty acid profiles of young (<24 mo) Steller sea lions by sex and age-class in Prince William Sound. First discriminant function explained 74.3% of variation and was most influenced by 22:1n-11, 20:1n-11 and 20:5n-3; second function accounted for an additional 17.9% of variation and was most influenced by 16:1n-7, 18:1n-9 and 22:6n-3

gion, season and age-class. Yearling blubber FA profiles in PWS also differed by sex. Although regional and seasonal differences in SSL diet have been known for some time (Merrick et al. 1997, Sinclair & Zeppelin 2002), this study provides greater insight into how those factors, sex and age may influence the diet of juvenile SSL.

Differences in FA profiles by region

Only spring pups were sampled sufficiently (n > 20 from all regions; Table 1) to investigate regional differences in FA profiles. We observed statistically significant regional variation in the blubber FA, and thus dietary intake, of spring pups (Figs. 3 & 4), which is consistent with previous studies based on analysis of stomach contents and scats from SSLs of both known (stomach contents; juveniles vs. adults) and unknown (scat) ages (Merrick & Calkins 1996, Merrick et al. 1997, Sinclair & Zeppelin 2002). Spring pups range in age from 9 to 12 mo and may have been nutritionally dependent, at least in part, on their mothers' milk. Assuming complete dependence, regional differences in pup blubber FA profiles would be a reflection of regional differences in the FA composition of adult female diets that were passed on to offspring through milk (Puppione et al. 1996, Iverson et al. 2001). However, recent studies of maternal attendance patterns (Trites & Porter 2002) and the dispersal and movement patterns of young SSLs (Loughlin et al. 2003, Raum-Suryan et al. 2004, Fadely et al. 2005) have suggested that some individuals may wean during the spring of

Fig. 6. *Eumetopias jubatus*. Discriminant function plot of blubber fatty acid profiles of young (<24 mo) Steller sea lions by season and age-class in Prince William Sound. First discriminant function explained 71.3% of variation among samples and was most influenced by 22:1n-11 and 20:1n-11; second discriminant function explained an additional 19.4% of variation and was most influenced by 18:1n-7 and 16:0

their first year of life. If this is true, regional differences in the blubber FA profile of pups may also result from regional differences in prey consumption or in the timing of when pups begin to forage independently.

Seasonal, age-class and sex variation in FA profiles

Within regions, statistically significant seasonal and age-class differences in blubber FA profiles were evident in young SSLs from both SEA and PWS (Figs. 5 & 6, respectively) indicating significant changes in dietary intake across temporal scales. FA blubber profiles collected from pups during the summer in SEA were particularly distinct from other season/age-class groups within SEA and differed primarily in the relative levels of 20:1n-9 and 16:3n-6 (Fig. 5). SSL pups are <3 mo old in the summer and are completely dependent on maternal milk due to their limited swimming and diving capability. As a result, females with young pups are limited to foraging areas near the parturition rookery. As pups grow and swimming skills mature, mother–pup pairs begin to disperse from the rookeries to winter haul-outs in the fall, where females remain until the following breeding season (Calkins & Pitcher 1982, Raum-Suryan et al. 2004). In SEA, many haul-outs are located within the calmer inside waters compared to the rookeries, located on the outer coast (Fig. 1). Analysis of scat samples indicated statistically significant differences in diet composition between individuals at rookeries and haul-outs in SEA (Trites et al. in press). As the sea lions moved to inside waters, the proportions of gadids (particularly walleye pollock), forage fish (e.g. sand lance and herring) and cephalopods increased while salmon and rockfish proportions decreased. Currently there are no data available on the FA composition of SSL prey in SEA, however the FA composition of 26 prey species in PWS has been examined (Iverson et al. 2002) and indicates that the relative amount of 20:1n-9 is higher in walleye pollock, herring, and cephalopods than in salmon species or rockfish. A similar trend in 16:3n-6 is also present among these prey types sampled from PWS. Assuming similar trends in the FA composition of prey in SEA, the distinct change in blubber FA profiles between summer and fall pups would appear to reflect this change in the diet of adult females, who passed this change on to their pups through milk production (Puppione et al. 1996, Iverson et al. 2001). However, it should be noted that this assumption is somewhat limited, since several studies (Budge et al. 2002, Iverson et al. 2002) have shown regional and geographical differences in prey FA composition.

Blubber FA profiles of SSL pups in SEA also varied between the fall, winter and spring (Fig. 5), such that

movement of pairs to haul-outs could not account for all seasonal variation. Seasonal changes in the diet of SSL have previously been described from scat (Merrick et al. 1997, Sinclair & Zeppelin 2002) and stomach content (Merrick & Calkins 1996) analysis. Sinclair & Zeppelin (2002) showed that the seasonal frequency of major prey species in the scats of SSL from the western stock paralleled seasonal patterns in the nearshore spawning and migratory movements of these prey, suggesting that SSL target dense prey aggregations which result from annual life cycle events of the prey. If similar foraging behavior strategies are employed among individuals in the eastern population, the seasonal changes evident in the blubber FA profiles of SEA pups are likely to represent seasonal shifts in mothers' diets, and thus the FA composition of their milk, as they forage on seasonally aggregated prey. Due to the lack of detailed data on the FA composition of prey in the SEA region, it is unclear from the predator data which prey species are responsible for generating these differences.

Seasonal changes in the blubber FA profiles of yearling SSLs from SEA were also evident and, with the exception of the summer season, mirrored the seasonal trends of pup blubber FA profiles in this region. If SEA yearlings were nutritionally independent at the time of sampling this would reflect seasonal changes in prey selected by foraging yearlings. However, it is also possible that some of these individuals were not yet weaned and these seasonal changes in FA profiles of the blubber reflect seasonal prey preferences of lactating females similar to that seen in pups from this area. There is behavioral evidence that a high proportion (~80%) of SSL in SEA continue to be nursed through their second year (Pitcher et al. 2004).

In PWS, young SSLs also showed statistically significant seasonal and age-class differences in their blubber FA profile (Fig. 6), with summer pups again being the most distinct from all other groups. The FA profile of summer and fall pups contained a lower proportion of 22:1n-11 and 20:1n-11 relative to spring pups and all yearling samples. These 2 dietary FAs tend to be quite high in some marine fishes (e.g. up to 9–12 and 7–10% respectively in herring, cephalopods, and walleye pollock; Iverson et al. 2002) compared to milk samples collected via gastric intubation of young SSLs from this region (22:1n-11 = 1.0 to 3.0%, 20:1n-11 = 2 to 5%; Alaska Department of Fish & Game unpubl. data). Thus, spring PWS pups with relatively higher levels of 20:1n-11 and 22:1n-11 could either be directly ingesting some marine prey high in these FAs, or the diets of mothers could have shifted from fall to spring. However, as stated previously, we do not yet know what proportion of spring pup or yearling diet is derived from maternal support. Similar to SEA, seasonal varia-

tion in blubber FA profiles was also significant among PWS yearlings; however, yearlings' seasonal patterns did not mirror the seasonal changes of PWS pup blubber. These seasonal differences among PWS yearlings probably represent changes in the prey species seasonally available (Merrick & Calkins 1996, Sinclair & Zeppelin 2002) to these young foragers.

Significant sex differences were found in the blubber FA profiles of PWS yearlings, but not among the profiles of SEA yearlings. In this size-dimorphic species, males are significantly larger than females at 2 to 4 wk of median age (Merrick et al. 1995), with differences in growth rate continuing through maturity (Winship et al. 2001). In many marine mammal species, body size is directly correlated with diving and hence foraging ability due to the relationships between body size, oxygen storage capacity and mass-specific metabolic rate (Castellini & Kooyman 1989). Sex differences in the diving behavior and movement pattern of young SSLs have been documented (Raum-Suryan et al. 2004, Rehberg 2005) and are consistent with sex differences in other size-dimorphic marine mammal species (Le Boeuf et al. 2000). Juvenile (>12 mo) male SSLs make significantly longer and deeper dives than females (Rehberg 2005). In contrast, time-at-sea, trip distance and trip duration are significantly greater among female SSL juveniles (Raum-Suryan et al. 2004). Thus, the larger size of male SSL yearlings compared to females appears to result in differences in the way each sex utilizes its habitat and resources. These sex differences in diving characteristics may allow independently foraging males and females in PWS access to different prey resources which are reflected in their blubber FA profiles. Although this would explain the sex differences in blubber FA profiles of PWS yearlings, it is not known whether they were independently foraging or still suckling. The lack of sex differences in SEA yearlings could result from either continued suckling, or from male and female yearlings foraging similarly in the environment.

Until it is possible to identify the source of dietary FA (maternal attendance or independent foraging), the questions regarding when individuals transition to independent foraging and what prey species are important to juvenile SSLs remain. Quantitative estimation of diet composition for individual juvenile SSLs using the method of quantitative fatty acid signature analysis (Iverson et al. 2004) will be the next important step. With appropriate sampling of prey species, together with SSL milk, from all regions and an understanding of how young sea lions metabolize the meals they ingest, a quantitative estimate of diet composition and, in turn, a better understanding of the variability in weaning time can be gained.

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