Changes in the diet of free-ranging black bears in years of contrasting food availability revealed through milk fatty acids

S.J. Iverson, J.E. McDonald, Jr., and L.K. Smith

Abstract: We studied patterns of fatty acid signatures in milks and major foods of free-ranging lactating black bears (*Ursus americanus*) in western Massachusetts to examine the degree to which changes in milk fatty acids could be related to measured changes in food availability and scat analysis, and to assess whether fatty acids could be used to infer aspects of the diets of individuals. Milk samples (n = 45) were obtained from 17 individual bears during years of contrasting hard-mast abundance. Paired winter-den and spring-foraging samples were obtained from females in 1994 (n = 10), 1995 (n = 2), and 1996 (n = 8). In seven of these females, paired den and foraging samples were collected in both 1994 and 1996, representing two consecutive lactation periods. Milk fatty acid patterns indicated that the diet of individuals responded strongly to food availability both prior to denning and during spring foraging. During spring foraging, although females likely continued to mobilize stored fat, the greatest contribution to milk fatty acids appeared to be from dietary fat intake. Hence, qualitative changes in spring diets of individual bears could be reasonably inferred from milk fatty acid signatures. During the year of lowest hard-mast abundance, milk fatty acid patterns suggested that females relied predominantly on a diet of skunk cabbage, although this varied among individuals. This study demonstrates that milk fatty acid signatures can be used to provide insight into the nutritional ecology of bears at the level of the individual.

Résumé : Nous avons étudié les variations de la composition des acides gras du lait et déterminé les habitudes alimentaires de mères nourricières chez l'Ours noir (*Ursus americanus*) dans l'ouest du Massachusetts, pour mesurer à quel point ces variations étaient reliées aux changements observés dans la disponibilité de la nourriture et l'analyse des fèces et pour déterminer si les acides gras pouvaient servir d'indicateurs des régimes alimentaires individuels. Des échantillons (n = 45) de lait ont été recueillis chez 17 femelles au cours d'années d'abondance inégale de la paisson. Des échantillons appariés ont été prélevés chez les mêmes femelles dans leurs terriers d'hiver et au printemps, alors qu'elles se nourrissaient, en 1994 (n = 10), en 1995 (n = 2) et en 1996 (n = 8). Chez sept de ces femelles, des échantillons appariés ont été obtenus en 1994 et en 1996, ce qui correspondait à deux périodes consécutives d'allaitement. Les variations des acides gras du lait indiquent que le régime alimentaire des femelles est fortement relié à la disponibilité de la nourriture aussi bien avant l'hibernation qu'au printemps. Avec le régime de printemps, bien que les femelles continuent probablement de mobiliser leurs réserves de graisses, la contribution principale aux acides gras du lait vient de l'ingestion directe de graisses dans la nourriture. La composition en graisses du lait peut donc servir à estimer assez bien les changements qualitatifs du contenu des régimes alimentaires individuels du printemps. Durant l'année où l'abondance de paisson a été minimale, les acides gras du lait ont indiqué que les femelles consommaient surtout du chou puant, bien qu'il y ait eu des variations d'un individu à un autre. Nos résultats démontrent que, chez l'Ours noir, la composition des acides gras du lait d'un individu peut servir à donner un aperçu de son écologie alimentaire.

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Introduction

The bears (family Ursidae) are a group known for their broad evolutionary and ecological plasticity (Stirling and Derocher 1990), exploiting a remarkable array of diets, habitats, and geographical ranges. However, the degree to which individuals respond to ecological variability is not well understood, principally because of the difficulties and constraints associated with studying large, wide-ranging animals. Black bears (*Ursus americanus*), like other bears, have secondarily adopted a largely herbivorous diet (Kurten 1976). Depending on the geographical region, they often rely primarily on a

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diet of vegetation; however, they lack a complex stomach (i.e., rumen or cecum) necessary for digesting and utilizing cellulose. As a consequence they must spend a major portion of their active time finding and consuming large amounts of food to obtain necessary quantities of digestible fats, proteins, and simple carbohydrates (Van Soest 1977; Dierenfeld et al. 1982; Schaller et al. 1985).

Nutritional factors can be important in limiting animal populations and determining rates of reproduction and survival (e.g., White and Luick 1984; Horton and Rowsemitt 1992; Thompson 1992). Pregnant black bears must enter winter dens with sufficient fat reserves to sustain both a 5- to 7-month period of winter dormancy and the initial 3 months of lactation. Hence, foods that are high in fat and protein (e.g., hard mast such as acorns and other nuts) and high in digestible carbohydrates (e.g., soft mast such as berries) are preferred foods when available. It is thought that abundance of such key foods plays a critical role in black bear foraging patterns, female condition, and reproductive success (Rogers 1976, 1987; Bunnell and Tait 1981; Elowe and Dodge 1989; Noyce and Garshelis 1994). To date, however, studies of the nutritional ecology of black bears have been based primarily upon assessment of the annual abundance of mast crops and (or) diet-content estimates from the analysis of scats collected from unknown individuals. Estimates of diet and foraging patterns based on scat analysis are likely biased (Hewitt 1989; DeBruyn 1992) and provide information only at the level of the population or subpopulation (e.g., Hildebrand et al. 1996). Yet nutritional influences act at the level of the individual, and bears can be highly individual in their feeding habits (Stirling and Derocher 1990). Thus, acquiring the ability to test hypotheses concerning diets at the level of individual females should lead to a better understanding of the nutritional ecology of bears.

Recently, fatty acid signatures in adipose tissue or milk have been used to study the foraging ecology of free-ranging mammals (Iverson 1993). Fatty acid signatures integrate the diet of an individual over longer periods of time than scats and thus can provide a better estimate of how individuals have met their energy requirements. Fatty acids are the largest constituent of lipids. Owing to constraints on their biosynthesis and modification among animals and plants, the ecological origin of individual fatty acids can be traced, sometimes to specific taxa (Cook 1985). In monogastric mammals, ingested lipids (primarily triglycerides) are hydrolyzed to free fatty acids and partial glycerides in the stomach and small intestine. These products are transported across the small intestine, and re-esterified into the circulating chylomicrons for transport to, and direct uptake by, tissues (Borgstrom 1977; Patton 1981). Thus, most dietary fatty acids remain intact through digestion and those found in tissues can reflect the original dietary intake (e.g., Iverson et al. 1995*b*).

Fatty acid signature analysis has been used both to identify the general trophic level of diets and to detect shifts in diet within populations of marine mammals (Iverson 1993; Iverson et al. 1997*a*; Smith et al. 1997). Fatty acid signatures have also indicated fine-scale spatial structure in the foraging distribution of harbour seals (*Phoca vitulina*) (Iverson et al. 1997*b*). Fatty acids of organisms in purely terrestrial environments tend to be less complex. Nevertheless, there are specific fatty acids that can be used to trace ecological origins. In particular, fatty acids with *n*–3 and *n*–6 double bonds (especially the essential fatty acids 18:2*n*–6 and 18:3*n*–3) cannot be synthesized by mammals and must be obtained from terrestrial plant sources.

Black bears undergo periods of both fasting and feeding during lactation. Black bears typically breed every second year, but all individuals feed and fatten intensively in the fall, prior to denning. Pregnant females generally give birth and begin lactating in mid-January, 2–4 months prior to emergence from the den with their cubs in late spring (Alt 1982). While in the den, females rely on body fat, stored during fall hyperphagia, for both maintenance metabolism and milk production (Nelson 1973, 1980; Nelson et al. 1983). While females may continue to mobilize some body fat to support milk production after emerging from the den, they forage heavily on spring vegetation and available hard mast from the previous fall (Rogers 1976; Elowe and Dodge 1989; McDonald and Fuller 1994). Thus, changes in milk fatty acid patterns should provide insight into the diet and feeding status of individual bears (Iverson and Oftedal 1992).

We studied black bears during years of contrasting hard-mast abundance and used milk fatty acid patterns to examine how individual bears responded to changes in food supply. Our objectives were to determine whether differences in fall and spring food availability were reflected by changes in milk fatty acid profiles of females sampled in the den and during spring foraging. Our second aim was to examine whether milk fatty acid profiles could be used to infer aspects of the diet of individual bears during spring foraging.

Materials and methods

Field sampling and analysis

Free-ranging black bears were studied during 1994–1996 in the 150-km^2 Conway–Williamsburg study area (CWSA; 42°27′N, 72°41′W) in western Massachusetts. The CWSA was 70% forested, 21% used agriculturally, and 3% used residentially, with elevations ranging from 30 to 450 m. We attempted to sample the same female during the winter-den and spring-foraging periods in both 1994 and 1996 (representing two consecutive lactation periods); however, this was not always possible because of a number of factors including litter loss and killing of animals by hunting. Additional female bears were also sampled in 1995 to examine interannual variability in diets. Each female had previously been fitted with a VHF radio collar. In February and March of each year, females with new cubs were tracked to their dens and chemically immobilized with ketamine HCL (10–17 mg/kg body mass) mixed with xylazine HCL (1–2 mg/kg body mass) via a dart or a jab stick. Milk (5–20 mL) was collected after an intramuscular injection of oxytocin (20–60 IU) to facilitate milk let-down. In late May or early June, after emergence from the den, females were again tracked and immobilized and milk samples were collected as described above. Immediately after collection all milk samples were stored frozen (–20°C) in airtight containers until analysis (<1 year). Approval for animal-handling procedures used in the collection of milk samples was provided through the Massachusetts Division of Fisheries and Wildlife and the University of Massachusetts, Amherst, and followed the guidelines of the Canadian Council on Animal Care.

During the same years, fall hard-mast abundance was estimated in CWSA and general food use by bears in the study area was estimated from scat collections. Annual mast abundance was estimated using line-transect surveys conducted by a single observer along twelve 0.5 km long transects with survey points at 100-m intervals

(McDonald et al. 1994). The nearest dominant or codominant tree of mast-producing species (oaks, *Quercus* spp., or American beech, *Fagus grandifolia*) in each of the cardinal directions was selected for ranking. Abundance was ranked as poor, good, or excellent according to Sharp (1958), based on visual estimates of the number of nuts observed (by counting acorns on the last 0.6 m of ≥10 branches per tree) in the late summer of each year preceding our study years (i.e., 1993, 1994, and 1995). To verify visual rankings, seed traps (each 0.5 m^2 in area) were also established along four transects at each survey point to capture mast falling from the canopy. Mast was collected throughout the fall until no additional mast was present. Mast was sorted according to species, assessed for soundness, and oven-dried to a constant mass. Mast production (kilograms dry mass per hectare) was calculated by pooling data for each survey point and transect. In each year, an annual fall red oak (*Quercus rubra*) index was then derived relative to the poorest year of acorn production (1995) as the annual total number of acorns collected in seed traps divided by the smallest total annual collection. Finally, spring mast availability was estimated as described above using the same transects and ground-searching of four 1-m^2 plots at each survey point soon after snowmelt.

We did not attempt to describe bears' food habits in detail using scats. However, we used scats to assess whether there were major shifts in general food use by the population and to compare these qualitatively with milk fatty acid patterns. Bear scats were collected opportunistically during the spring (April–June) foraging seasons of 1994 and 1996 by a combination of searching areas where females had been located via telemetry and searching during mast-collection and bear-capture periods. It was usually not possible to observe or know the creator of any sample because numerous bears (marked and unmarked) would use the same foraging areas. Scat samples were analyzed according to Hatler (1972) for major food types that included oak mast (acorns), skunk cabbage (*Symplocarpus foetidus*), grass/sedge, soft mast, other vegetation, and animal remains (McDonald 1998). We did not correct for digestibility, since no correction factor was available for skunk cabbage (the major spring food), and because it was fed on for over 2 months, its digestibility likely changed through time. Thus, we used our scat results as general indicators of food use. Since red oak mast and skunk cabbage were the major spring foods, samples of acorns (n = 5) and skunk cabbage (n = 18) were collected opportunistically from the CWSA in 1994–1996 for proximate and fatty acid analysis and stored frozen. Other potential diet items were not available for analysis.

Fatty acid analysis

Milk samples were thawed and homogenized prior to duplicate lipid extraction in chloroform–methanol using the Folch procedure (Folch et al. 1957), but increasing the solvent:sample ratio to 42:1 to account for the high lipid content of milk. Lipid was also quantitatively extracted from samples of acorns and skunk cabbage using a Folch extraction, but with some modifications for plant tissues (Christie 1982). Fatty acid methyl esters were prepared directly from 100 mg of the pure extracted lipid, using 8% boron trifluoride in methanol (w/w) (Iverson et al. 1997*b*). This method of transesterification, as employed in our laboratory with fresh reagents, produces results identical with those obtained using the Hilditch reagent (0.25 M H_2SO_4 in methanol). Duplicate analyses of fatty acid methyl esters were performed using temperature-programmed gas liquid chromatography according to Iverson et al. (1992) and Iverson et al. (1997*b*) on a Perkin Elmer Autosystem II Capillary FID gas chromatograph (GC) 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.). Fatty acids and isomers were identified from known standard mixtures (Nu Check Prep., Elysian, Minn.), silver-nitrate (argentation) chromatography, and GC mass spectrometry (Hewlett-Packard 6890 gas chromatograph, 1:20 split injection, Micromass Autospec oa-TOF mass spectrometer operated at 1000 resolution, scanning masses 120–450). Fatty acid levels are expressed as mass percent of total fatty acids and are designated by shorthand nomenclature of the International Union of Pure and Applied Chemistry (IUPAC) for carbon-chain length:number of double bonds and location (n–x) of the double bond nearest the terminal methyl group. All data are presented as the mean ± standard error (SEM) unless otherwise indicated.

Fatty acid data were analyzed using a combination of multivariate and univariate analysis of variance (MANOVA and ANOVA) on a subset of variables, using a Bonferroni correction for the number of tests performed where appropriate. Because of the small sample size, a subset of the 8 most abundant fatty acids (arcsine-transformed) was used in these tests. Data were also analyzed using classification and regression trees (CART) in S-plus as described in Iverson et al. (1997*a*) and Smith et al. (1997), since CART permits the use of all fatty acids without requiring a subjective or restricted variable selection. Briefly, CART is a supervised statistical method of classifying data that proceeds by recursively partitioning subjects into two or more groups based on a series of dichotomous splits of variables (fatty acids) (Clark and Pregibon 1992). The small number of milk fatty acid samples from 1995 were not included in statistical analyses.

Results

Milk composition

Milk samples (n = 45) were obtained from a total of 17 individual bears (Table 1). Paired winter-den ("den") and spring-foraging ("foraging") samples were obtained from 10 females in 1994, 2 females in 1995, and 8 females in 1996. In seven of these females, paired den and foraging samples were collected in both 1994 and 1996, representing two consecutive lactation periods. All females from which paired samples were obtained were known to have had at least one litter prior to 1994.

Milk samples collected from denned females averaged about 19% fat across years (Table 1), but ranged from 14.0 to 25.4% fat. Milk samples collected during spring foraging averaged about 22% fat, but ranged from 10.8 to 34.2% fat. In females from which paired samples were obtained, milk fat content tended to increase from den to spring foraging in 1994 (P = 0.057) but not in 1996 (P = 0.987, paired t test). Milk samples collected from three females in 1996 had exceptionally low fat content (11%) during the foraging period, resulting in a low mean value. Although these data may reflect the true values for milk secreted, it is not clear to what degree the results may have been influenced by the small volume of milk collected from the teats and the time elapsed since previous suckling, which was not known (differences may exist in the proximate composition of "fore" versus "hind" milk expressed). When we excluded these females, milk samples collected during spring foraging averaged 24–25% fat in 1994 and 1995 and 20% fat in 1996.

The fatty acid composition of milk varied across sample groups (Table 1). Although differences were apparent in many of the minor components, differences among groups were best illustrated by the most abundant fatty acids (Fig. 1*a*). Based on a subset of the 8 most abundant fatty acids, milk fatty acids differed significantly between years (1994 and 1996) and between den and foraging periods (P < 0.001, two-way MANOVA). Within each year, den and foraging samples differed significantly in all major components; how-

Table 1. Fatty acid composition of black bear (*Ursus americanus*) milk during the periods of winter dormancy ("den") and spring foraging ("foraging") for the years 1994–1996.

	1994		1995		1996	
	Den	Foraging	Den	Foraging	Den	Foraging
n	12	10	3	2	9	9
% fat	19.8 ± 0.90	25.5 ± 1.55	19.6 ± 1.26	24.0 ± 5.51	18.0 ± 0.74	17.4 ± 1.86
14:0	1.43 ± 0.10	0.85 ± 0.19	1.39 ± 0.12	2.78 ± 1.47	1.65 ± 0.08	3.95 ± 0.46
14:1*n*–5	0.13 ± 0.02	0.01 ± 0.01	0.11 ± 0.02	0.07 ± 0.02	0.16 ± 0.04	0.09 ± 0.02
Iso15	0.21 ± 0.01	0.03 ± 0.02	0.25 ± 0.01	0.13 ± 0.04	0.32 ± 0.04	0.14 ± 0.02
15:0	0.11 ± 0.01	0.09 ± 0.02	0.13 ± 0.02	0.22 ± 0.09	0.11 ± 0.01	0.34 ± 0.03
16:0	20.08 ± 1.14	12.22 ± 1.01	20.48 ± 1.21	21.50 ± 7.49	30.04 ± 0.85	30.72 ± 1.93
16:1*n*–9	0.60 ± 0.04	0.48 ± 0.04	0.56 ± 0.09	0.40 ± 0.09	0.42 ± 0.02	0.53 ± 0.04
16:1*n*–7	3.95 ± 0.44	0.74 ± 0.18	3.79 ± 0.34	1.80 ± 0.54	7.00 ± 0.30	3.00 ± 0.19
16:1*n*–5	0.29 ± 0.03	0.07 ± 0.03	0.45 ± 0.09	0.21 ± 0.08	0.96 ± 0.08	0.50 ± 0.09
17:0	0.12 ± 0.01	0.13 ± 0.01	0.16 ± 0.00	0.29 ± 0.10	0.13 ± 0.01	0.28 ± 0.05
17:1	0.20 ± 0.01	0.14 ± 0.01	0.20 ± 0.02	0.17 ± 0.08	0.21 ± 0.02	0.21 ± 0.05
18:0	1.54 ± 0.05	1.84 ± 0.18	2.00 ± 0.14	3.35 ± 0.26	1.99 ± 0.05	3.25 ± 0.27
18:1*n*–11	0.00 ± 0.00	0.07 ± 0.07	0.15 ± 0.15	0.07 ± 0.07	0.03 ± 0.02	0.04 ± 0.03
18:1*n*–9	45.02 ± 0.64	49.00 ± 1.38	44.29 ± 2.57	21.73 ± 4.68	41.20 ± 0.67	22.42 ± 3.42
18:1*n*–7	2.99 ± 0.13	2.31 ± 0.85	2.67 ± 0.26	1.29 ± 0.14	3.92 ± 0.14	1.72 ± 0.18
18:1*n*–5	0.06 ± 0.01	0.00 ± 0.00	0.13 ± 0.04	0.02 ± 0.02	0.15 ± 0.01	0.07 ± 0.01
18:2*n*–6	18.94 ± 1.77	25.25 ± 1.31	17.66 ± 2.45	31.18 ± 14.38	8.14 ± 0.99	11.70 ± 0.75
18:3*n*–6	0.40 ± 0.04	0.03 ± 0.01	0.37 ± 0.11	0.05 ± 0.00	0.13 ± 0.01	0.06 ± 0.01
18:3*n*–3	0.92 ± 0.05	5.41 ± 1.06	1.95 ± 0.78	12.49 ± 8.60	0.64 ± 0.08	18.21 ± 1.93
18:4*n*–3	0.03 ± 0.01	0.01 ± 0.01	0.06 ± 0.03	0.05 ± 0.05	0.02 ± 0.00	0.13 ± 0.05
20:0	0.01 ± 0.00	0.12 ± 0.01	0.03 ± 0.02	0.13 ± 0.01	0.03 ± 0.00	0.14 ± 0.01
20:1*n*–9	0.52 ± 0.03	0.37 ± 0.02	0.68 ± 0.12	0.27 ± 0.01	0.66 ± 0.03	0.41 ± 0.08
20:2*n*–6	0.47 ± 0.03	0.17 ± 0.01	0.44 ± 0.05	0.32 ± 0.04	0.32 ± 0.02	0.25 ± 0.02
20:3*n*–6	0.59 ± 0.04	0.08 ± 0.02	0.56 ± 0.11	0.16 ± 0.01	0.34 ± 0.03	0.15 ± 0.01
20:4*n*–6	0.95 ± 0.12	0.19 ± 0.03	0.82 ± 0.18	0.33 ± 0.06	0.57 ± 0.06	0.25 ± 0.02
20:3*n*–3	0.00 ± 0.00	0.03 ± 0.02	0.05 ± 0.02	0.29 ± 0.22	0.02 ± 0.01	0.35 ± 0.06
20:4*n*–3	0.06 ± 0.01	0.05 ± 0.01	0.09 ± 0.02	0.12 ± 0.12	0.05 ± 0.01	0.12 ± 0.03
20:5*n*–3	0.10 ± 0.02	0.02 ± 0.01	0.12 ± 0.05	0.16 ± 0.11	0.07 ± 0.01	0.17 ± 0.03
22:4*n*–6	0.10 ± 0.02	0.06 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.08 ± 0.02
22:5*n*–3	0.10 ± 0.02	0.08 ± 0.02	0.12 ± 0.02	0.19 ± 0.08	0.14 ± 0.03	0.20 ± 0.02
22:6*n*–3	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	0.10 ± 0.02
Total	99.98	99.92	99.88	99.95	99.62	99.60

Note: Fatty acids listed are those generally found to constitute ≥0.1% of total fatty acids. Values are given as the mean ± SEM. Numbers for paired samples (den and forage) obtained from the same female were as follows: 1994 (n = 10), 1995 (n = 2), 1996 (n = 8).

ever, the direction of differences was not consistent between years as indicated by significant interaction terms. For example, 14:0 and 16:0 decreased and 18:1*n*–9 increased from den to foraging in 1994, but the reverse pattern was seen in these fatty acids in 1996 (Fig. 1*a*). Within den samples, levels of 16:0, 16:1*n*–7, 18:0, and 18:1*n*–7 were significantly higher in 1996 than in 1994, while levels of 18:1*n*–9 and 18:2*n*–6 were lower ($P < 0.006$); levels of 14:0 and 18:3*n*–3 did not differ. Within foraging samples, these differences remained between years with the exception of 14:0 and especially 18:3*n*–3, levels of both of which were higher during 1996 foraging than during 1994 foraging ($P < 0.006$; Fig. 1*a*). In 1995, the direction and magnitude of changes in major milk fatty acid levels between den and foraging periods appeared to be intermediate between those observed in 1994 and 1996, although this was not tested, owing to the small sample size (Table 1).

To further examine the effects of year and foraging status on differences in milk fatty acid composition, we used CART analysis to examine how accurately we could classify samples using all fatty acids. CART initially chose 16:0 to separate 1994 milks from 1996 milks (Fig. 2*a*). Two other fatty acids were then used by CART to separate den from foraging samples within each year, resulting in four misclassifications. An alternative and better classification tree used 18:3*n*–3 to correctly distinguish all foraging samples from den samples, followed by further separation by year (Fig. 2*b*). The latter tree resulted in no misclassifications of the 40 samples, i.e., it was able to predict the sample group with 100% accuracy (Fig. 2*b*).

The above patterns were examined in a cross-sectional manner considering all animals sampled. Bears that were sampled repeatedly provided the opportunity to observe how milk fatty acid composition of individuals varied. This is illustrated using the 3 most abundant fatty acids (Fig. 3). Milk fatty acid patterns of individual bears changed within years as a function of foraging status, as well as between years (two-way repeated-measures ANOVA; Fig. 3). But patterns varied among individuals. In 1994 most bears showed little change in levels of 18:1*n*–9 between den and foraging, al-

Table 2. Summary of data on mast abundance and food use by the black bear population in the CWSA.

	Fall			Spring		
	1993	1994	1995	1994	1995	1996
Fall hard mast production (rating)[a]	Excellent	Good	Poor			
Available hard mast (kg dry mass/ha)[b]	520.6	48.3	7.9	415.8	1.1	1.0
Annual fall red oak index[c]	41.6	3.4	1.0			
Scat analysis of spring diet (% volume)[d]						
Oak mast				27.4		—
Skunk cabbage				52.2		99.3
Grass/sedge				7.6		—
Unidentified plant/fruit				11.4		<1.0
Corn				<1.0		—
Animal				<2.0		<1.0

[a] Visual estimates from transect surveys (see Methods).
[b] Measured using seed traps (see Methods).
[c] Total annual acorn production / 1995 CWSA acorn production.
[d] n = 102 scats in 1994 and n = 70 scats in 1996.

though levels did increase in some individuals. Den levels of 18:1*n*–9 in 1996 were fairly similar to those in 1994 (CV = 0.07), but there was a significantly large decline during foraging. In contrast, levels of 18:2*n*–6 were far more variable among individuals during both den (CV = 0.53) and foraging periods (CV = 0.43). Although levels of 18:2*n*–6 increased up to 2-fold from den to foraging in most individuals in both years, they were lower in all individuals during foraging in 1996 than in 1994. Levels of 18:3*n*–3 were uniformly low during the denning period in both years, and increased dramatically (up to 57-fold) in all bears during foraging (Fig. 3). In all individuals, levels of 18:3*n*–3 were higher during foraging in 1996 than in 1994 ($P < 0.001$).

General food use and fatty acid composition of plant foods

Based on fall hard-mast abundance and the red oak index in the CWSA prior to each year of study, 1993, 1994, and 1995 were ranked as excellent, good, and poor mast years, respectively, (Table 2; McDonald 1998). As production of hard mast was very high in the fall of 1993, availability of hard mast remained high in the spring of 1994. In contrast, the very low production in the fall of 1995 was followed by correspondingly low availability in the spring of 1996 (Table 2). Bear scats collected from unknown individuals in the CWSA population revealed that skunk cabbage was the predominant spring food of black bears in the CWSA in both years, estimated to compose about half of the diet in 1994 but almost the entire diet (>99%) in 1996 (Table 2). Overwintered oak mast composed a high proportion (27%) of the spring diet in 1994, following the very abundant fall 1993 mast crop. However, oak mast was absent in the spring 1996 diet following the exceptionally poor fall 1995 mast crop. Other, more minor food items found in scats included primarily grass/sedge and unidentified plants and fruit, but only in spring 1994.

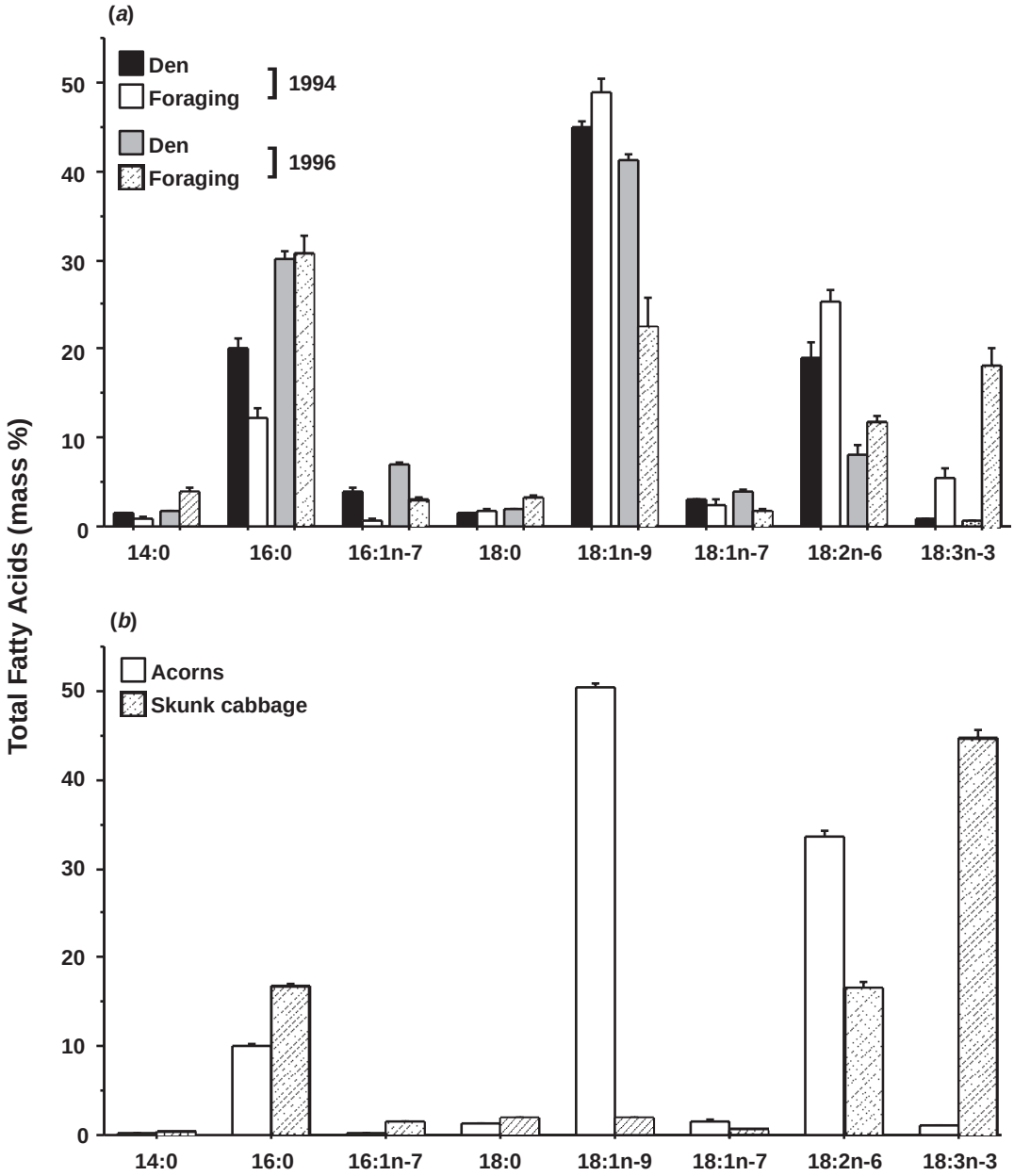
Analysis of CWSA samples of the two primary spring foods, skunk cabbage and acorns (oak mast), revealed large differences in both fat content and fatty acid composition (Table 3). Whole acorns contained up to 10% fat, while skunk cabbage contained only 0.2% fat. Although both plants contained the same array of fatty acids as found in bear milks, they differed greatly in the levels of specific fatty

Table 3. Fatty acid composition (mass %) of acorns and skunk cabbage from the CWSA.

	Acorns (nut)	Acorns (whole)	Skunk cabbage
n	4	5	18
% fat	9.0 ± 0.52	6.8 ± 0.94	0.2 ± 0.02
14:0	0.12 ± 0.00	0.13 ± 0.01	0.33 ± 0.03
14:1*n*–5	0.00 ± 0.00	0.00 ± 0.00	0.89 ± 0.06
Iso15	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
15:0	0.02 ± 0.00	0.02 ± 0.00	0.13 ± 0.01
16:0	10.02 ± 0.39	10.07 ± 0.31	16.76 ± 0.36
16:1*n*–9	0.14 ± 0.01	0.15 ± 0.01	3.01 ± 0.20
16:1*n*–7	0.18 ± 0.04	0.18 ± 0.03	1.50 ± 0.09
16:1*n*–5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
17:0	0.06 ± 0.00	0.06 ± 0.00	0.32 ± 0.03
17:1	0.10 ± 0.01	0.10 ± 0.01	0.02 ± 0.01
18:0	1.36 ± 0.03	1.36 ± 0.03	1.99 ± 0.08
18:1*n*–11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:1*n*–9	50.38 ± 0.78	50.23 ± 0.64	1.89 ± 0.11
18:1*n*–7	1.53 ± 0.22	1.53 ± 0.17	0.66 ± 0.03
18:1*n*–5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
18:2*n*–6	33.57 ± 0.74	33.55 ± 0.61	16.50 ± 0.79
18:3*n*–6	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
18:3*n*–3	1.02 ± 0.10	1.09 ± 0.10	44.85 ± 0.84
18:4*n*–3	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
20:0	0.38 ± 0.02	0.38 ± 0.02	1.08 ± 0.05
20:1*n*–9	0.47 ± 0.02	0.46 ± 0.02	0.23 ± 0.02
20:2*n*–6	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.03
20:3*n*–6	0.02 ± 0.02	0.02 ± 0.02	0.06 ± 0.02
20:4*n*–6	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
20:3*n*–3	0.00 ± 0.00	0.00 ± 0.00	0.23 ± 0.01
20:4*n*–3	0.06 ± 0.03	0.05 ± 0.03	0.08 ± 0.02
20:5*n*–3/22:0	0.17 ± 0.07	0.15 ± 0.06	0.10 ± 0.04
22:4*n*–6	0.05 ± 0.05	0.04 ± 0.04	0.53 ± 0.10
22:5*n*–3	0.03 ± 0.02	0.03 ± 0.02	0.15 ± 0.04
22:6*n*–3	tr	tr	tr
Total	99.67	99.61	91.59

Note: Fatty acids listed are those generally found to constitute ≥0.1% of total fatty acids in bear milks (Table 1) and (or) diet samples. Other major fatty acids in diet samples included 22:1 in skunk cabbage (1.06%) and 24:0 in acorns (0.12%) and skunk cabbage (3.67%). Values are given as the mean ± SEM; tr, trace.

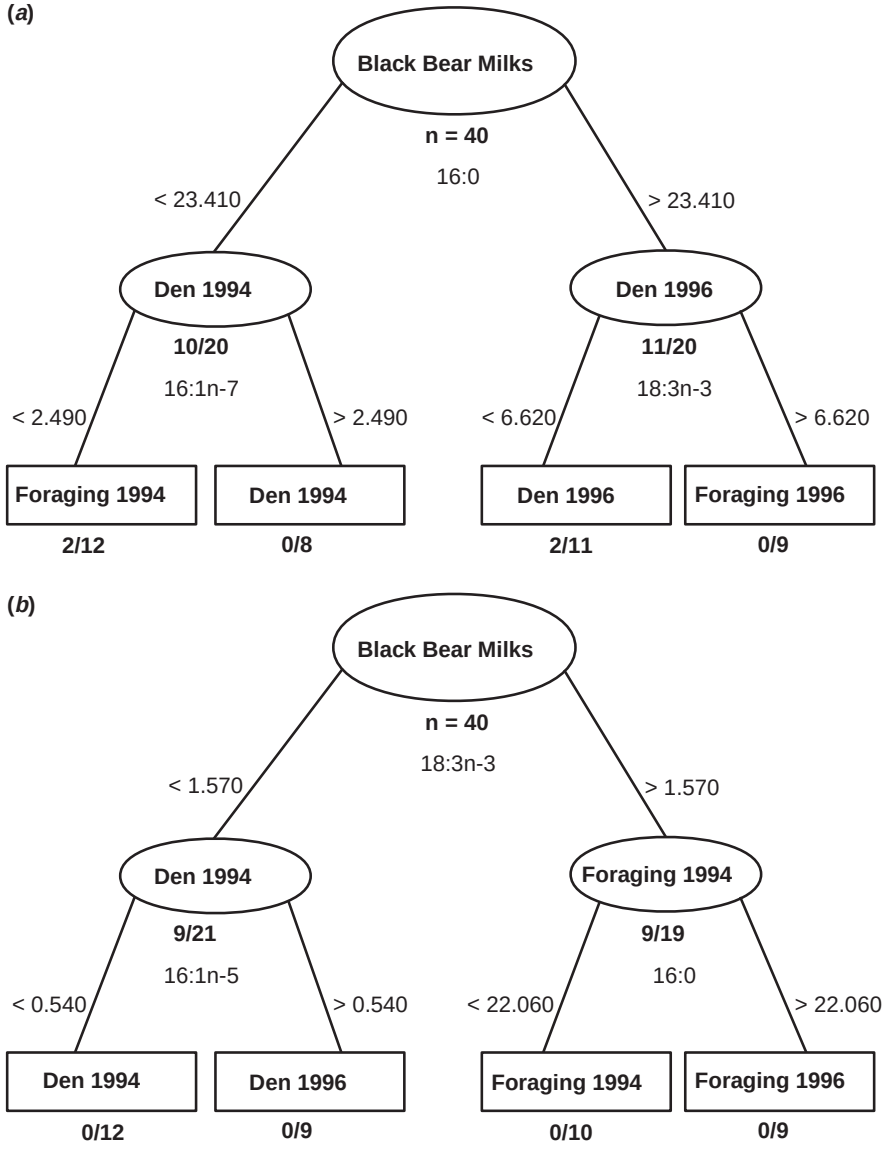
Fig. 1. Selected abundant fatty acids in milks collected from lactating black bears (*Ursus americanus*) during the periods of winter dormancy ("den") and spring foraging ("foraging") in 1994 and 1996 (*a*) and in major diet items from the same study area (CWSA) (*b*). For sample sizes see Tables 1 and 3. Bars show means and vertical lines represent 1 SEM. Significant differences were found for year ($P < 0.001$), foraging status ($P < 0.001$), and the interaction term ($P < 0.001$, two-way MANOVA on arcsine-transformed data). Significant differences were found between den and foraging for all components except 14:0 and between years for all components except 18:1*n*–7; interaction terms were significant for all components except 16:1*n*–7, 18:1*n*–7, and 18:2*n*–6 (two-way ANOVA with Bonferroni correction for number of tests, i.e., $P \leq 0.006$).



acids (Table 3). The most abundant fatty acids in both diet items were generally those that were most abundant in bear milks (Fig. 1*b*), but levels of 18:1*n*–9 and 18:2*n*–6 were 27- and 2-fold greater, respectively, in acorns than in skunk cabbage, whereas levels of 16:0, 16:1*n*–7, and 18:3*n*–3 were 2-, 8-, and 44-fold greater, respectively, in skunk cabbage than in acorns.

Given the large differences in fatty acid composition of acorns and skunk cabbage (Table 3) and the expected (based on scat analysis) differences in the spring diets of the bears between years (Table 2), we expected to see large changes in spring milk fatty acid composition. To illustrate this using a simple scenario, we estimated the potential average fatty acid composition of the diets of bears foraging in the CWSA in spring of 1994 and 1996, based on the results of scat analyses and the fat content and fatty acid composition of acorns and skunk cabbage. We then compared these coarse diet estimates with the fatty acid composition of milks of bears foraging during these periods (Fig. 4). Interannual differences in these estimated average spring fatty acid intakes were clearly reflected in the milks secreted by bears during the different spring foraging periods (Fig. 4). In the spring

Fig. 2. Classification trees for black bear milks using the fatty acid with the highest (*a*) and next highest deviance (*b*) chosen as variables for the root node. At each splitting point the fatty acid listed, and its level (mass %, > or <), is the variable and optimal value chosen for the split. Ellipses represent intermediate nodes and rectangles represent terminal nodes where a final classification is made; labels within them indicate the classification of samples at that node as represented by the largest number of observations in that node. The fraction under each node indicates the number of misclassifications at that node over the number of observations there. The total number of misclassifications for tree *a* was 4/40 (all were den 1994) and for tree *b* was 0/40 (i.e., 100% correctly classified).

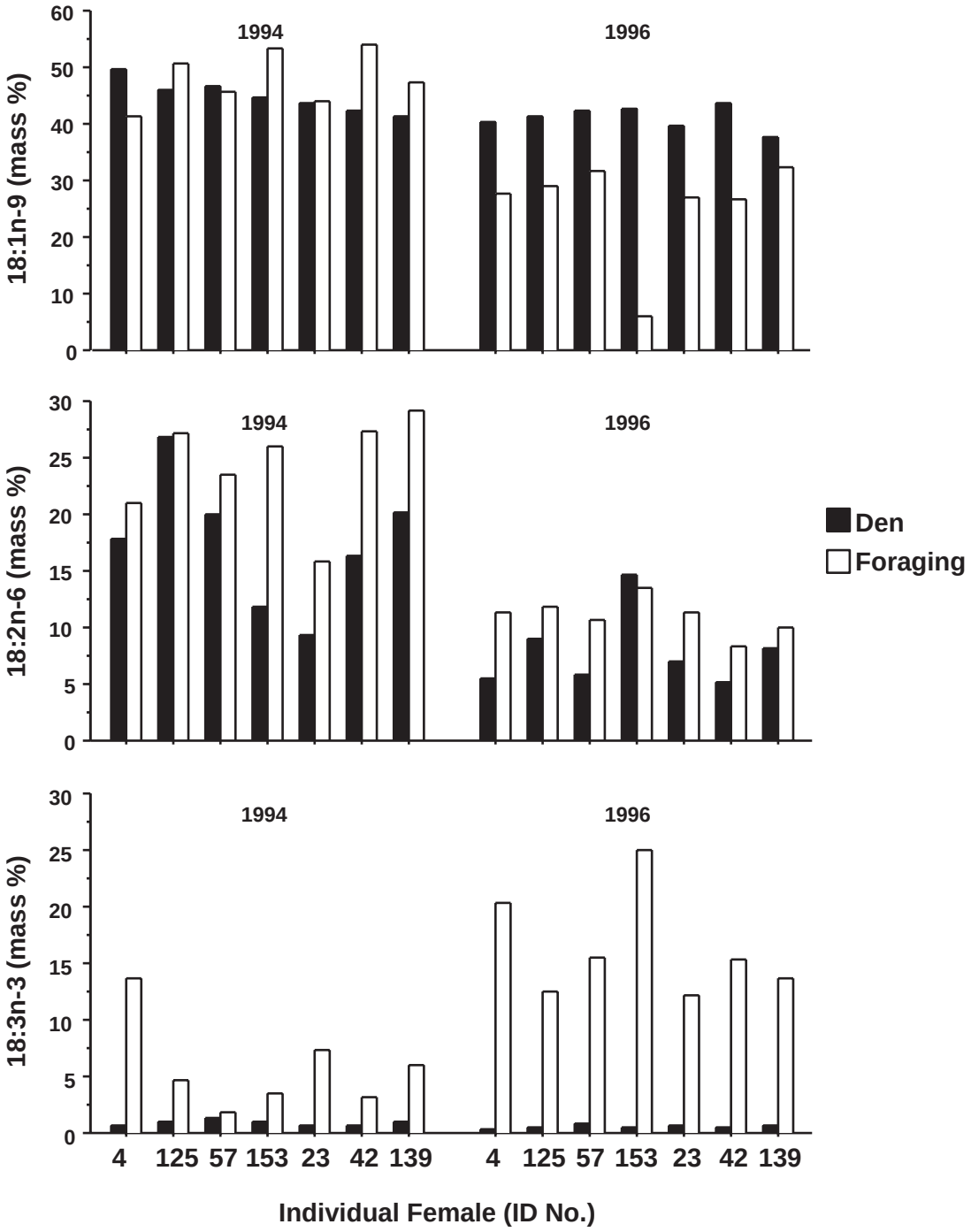


of 1994, even though acorns potentially accounted for only 27% (by volume) of the diet, this would account for 95% of estimated fat intake. Hence, the estimated average fatty acid intake in the spring of 1994 was very high in 18:1*n*–9 (48%) and 18:2*n*–6 (33%) and low in 16:0 (10%) and 18:3*n*–3 (3%). These percentages estimated in the diet of CWSA bears were generally within the observed ranges of 18:1*n*–9, 18:2*n*–6, 16:0, and 18:3*n*–3 found in bear milks sampled during this period: 42–54, 16–30, 9–20, and 2–14%, respectively. In contrast, during spring 1996 the estimated intake of fatty acids from a diet of primarily skunk cabbage was much lower in 18:1*n*–9 (2%) and 18:2*n*–6 (16%) and higher in 16:0 (17%) and especially 18:3*n*–3 (45%). These differences in estimated 1996 dietary fatty acid intake were associated with large changes in the percentages of 18:1*n*–9 (6–32%), 18:2*n*–6 (8–16%), 16:0 (23–40%), and 18:3*n*–3 (12–29%) in spring 1996 milks (Fig. 4). Other, less abundant fatty acids (e.g., 16:1*n*–7 and 18:0) also changed in a similar direction between 1994 and 1996 in both the estimated potential diet and in bear milks.

Discussion

Results from our study demonstrate that milk fatty acids can be used to understand how individuals as well as populations of bears respond to interannual variability in preferred foods. Our analysis of the milks, and primary foods, of black bears suggests that during spring foraging, the greatest contribution to milk fatty acids arises from dietary intake and that diets varied during years of contrasting food availability

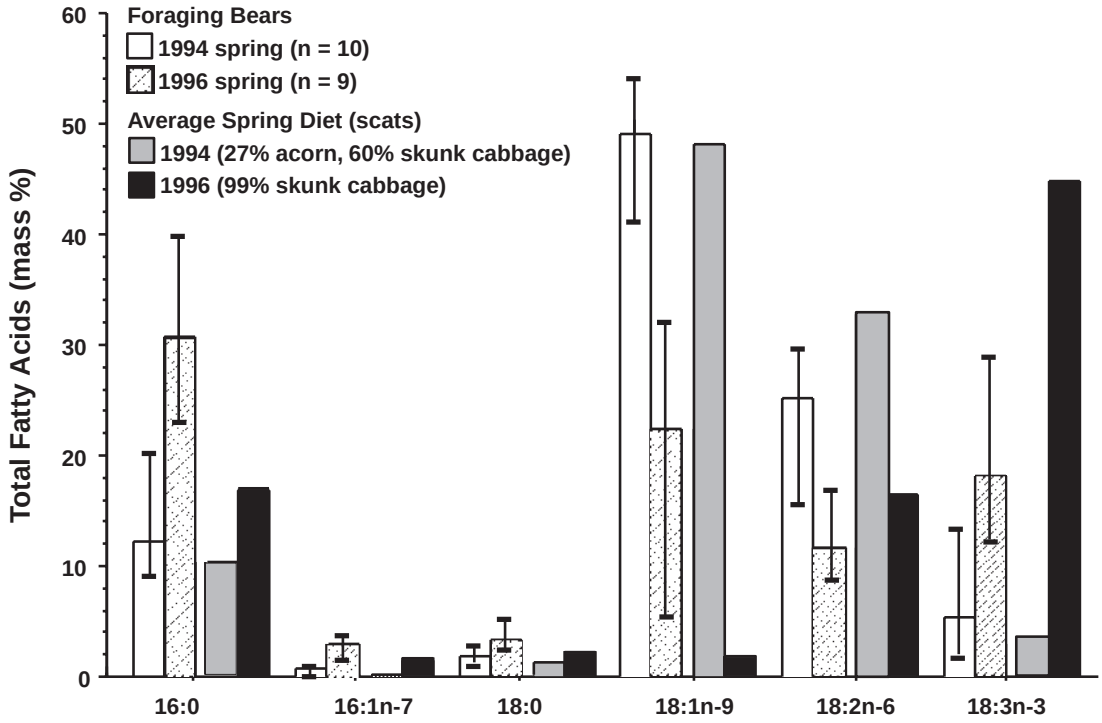
Fig. 3. Variability and changes in levels of the three most abundant fatty acids in milks of seven black bears studied longitudinally between den and foraging periods and across two consecutive lactation periods (1994 and 1996). Levels of 18:1*n*–9 differed between den and foraging in 1996 ($P = 0.015$) but not in 1994 ($P = 0.266$); den levels did not differ between years ($P = 0.020$) but foraging levels did ($P = 0.006$). Levels of 18:2*n*–6 and 18:3*n*–3 differed between den and foraging in both years ($P < 0.010$) and between years within den and foraging ($P < 0.010$, two-way repeated-measures ANOVA on arcsine-transformed data with Bonferroni correction for number of tests, i.e., $P \leq 0.017$).



(Figs. 1, 4). Our data also suggest that the spring diets of individuals could be reasonably inferred from milk fatty acid signatures (Fig. 4), although this remains a qualitative insight until more potential foods have been sampled.

The fatty acid composition of carnivore milk is primarily the result of the direct uptake of circulating fatty acids (Wamberg et al. 1992; Iverson and Oftedal 1995). Circulating fatty acids, in turn, arise in three potential ways: directly from dietary fatty acids, from biosynthesis primarily in the liver, or from mobilization of fatty acids stored in adipose tissue (originally from the diet or hepatic biosynthesis). Given the homeorhetic control of lactation, the mammary gland generally receives first priority for nutrients in general (Bauman and Currie 1980) and lipids in particular through differing

Fig. 4. Levels of the 6 most abundant fatty acids in milks of black bears during two spring foraging periods compared with levels of these fatty acids estimated to occur in average diets, based on scat analysis from bears in the same study area (CWSA). Bars represent means and vertical lines for bear milks represent ranges (minimum and maximum values) measured. Calculations of spring dietary fatty acid composition in 1994 were based on the estimate of bears consuming 27.4% oak mast (acorns) at 9.0% fat and 52.2% skunk cabbage at 0.2% fat (i.e., accounting for 79.6% of the diet) (from Tables 2 and 3). While we did not analyze other foods, most other diet items in 1994 were very low in fat (i.e., grass/sedge and fruit), so we likely accounted for much of the fat intake in 1994. Since the average spring 1996 diet estimated from scats was 99.3% skunk cabbage, we used the simplest coarse assumption that 100% of dietary fatty acids arose from skunk cabbage (Table 2).



tissue activities of lipoprotein lipase (LPL) (McBride and Korn 1963; Hamosh et al. 1970; Ramírez et al. 1983; Iverson et al. 1995*a*; Mellish et al. 1999), the principal enzyme responsible for the uptake and deposition of circulating fatty acids by tissues. Thus, in a fasting, lactating carnivore, fatty acids previously deposited in adipose tissue are mobilized and directed to the mammary gland (e.g., Iverson et al. 1995*b*). When a female is feeding, dietary fatty acids are directed to the mammary gland. If fat intake during feeding is insufficient to meet the demands of milk-fat secretion, then both sources of fatty acids will be used. These alternative scenarios provide the basis for interpreting milk fatty acid patterns observed in lactating black bears.

Milk fatty acid patterns during the denning period

In preparation for the winter-den period, black bears increase fattening capacity in the fall as shown by increases in the activity of adipose tissue LPL (Herminghuysen et al. 1995). Hence, fat, from both direct dietary intake and biosynthesis from ingested simple carbohydrates, will be readily deposited during fall hyperphagia. During the subsequent fasting period in the den, hepatic biosynthesis of fatty acids from glucose should be inhibited, as glucose is limited and thus spared to provide critical fuel for the central nervous system (Castellini and Rea 1992; Cherel et al. 1995). Hence, milk fatty acids at this time should largely reflect the composition of adipose stores, originally laid down during fall fattening.

Our data from fasting denned black bears suggest that females fattened on a diet that contained both hard mast and, probably, high amounts of carbohydrate. However, the fatty acid signatures of bears differed among years, indicating differences in diet. In all 3 years, milk secreted during the denning period had relatively high levels of 18:2*n*–6 (8–19%) and very low levels of 18:3*n*–3 (0.6–2.0%) (Table 1). These fatty acids cannot be biosynthesized and thus must come from dietary fat intake. High levels of 18:2*n*–6 (with low 18:3*n*–3) are characteristic of acorns (Fig. 1*b*). Consistent with this, levels of 18:2*n*–6 were highest in milks from denned bears in 1994 and 1995, after the excellent and good years, respectively, of acorn production, and lowest in 1996 after the poor year of acorn production (Tables 1, 2). Preliminary data from scat analysis also suggested that diets of bears in the CWSA likely consisted of about 64–82% hard mast in the fall of 1993 (i.e., immediately preceding sampling of 1994 den milks), whereas diets likely consisted of only about 5% hard mast in the fall of 1995 (i.e., preceding sampling of 1996 den milks; J.E. McDonald, Jr., unpublished data).

Den milks with lower amounts of 18:2*n*–6 and 18:3*n*–3 (i.e., in 1996, $P < 0.010$) also had significantly higher levels of 16:0 and 16:1*n*–7 (Fig. 1*a*; $P < 0.010$). These two fatty acids (16:0 and 16:1*n*–7), along with some 18:1*n*–9, are the

fatty acids most readily biosynthesized from carbohydrates consumed in excess of metabolic needs, especially in the presence of low dietary fat (Nelson 1992). Black bears are known to also feed heavily on soft mast (i.e., berries and fruit) and corn, which are high in simple carbohydrates (and low in fat), especially if fall hard mast is not abundant. Estimates of cornfield damage were higher during poor mast crop years (McDonald 1998) and diets of bears estimated from scat analyses were higher in carbohydrate (38–57% apple and 3–20% corn) in fall 1995 than in fall 1993 (2% apple and 0–16% corn; J.E. McDonald, Jr., unpublished data). This could have led to higher amounts of 16:0 and 16:1*n*–7 biosynthesized and stored in adipose tissue in the fall of 1995, which would then be observed in den milks in 1996. Greater biosynthesis of fatty acids from higher carbohydrate intake may also in part explain the high levels of 18:1*n*–9 in 1996 den milks (Fig. 1) even when the fall intake of acorns (high in 18:1*n*–9) was estimated to be low. Other minor foods were likely also consumed, and it would be important to analyze these, concurrently with samples of both bear adipose tissue and milk, to assess fall diets from fatty acids in more than a qualitative manner.

Milk fatty acid secretion during spring foraging

Across most regions of North America, the diet of black bears during spring foraging is heavily weighted towards leafy vegetation and grasses, which are high in protein but very low in fat, digestible carbohydrates, and energy (Rogers 1976; Elowe and Dodge 1989; McDonald and Fuller 1994). Hence, lactating females may often be in negative energy balance in spring. However, when available, overwintered high-fat hard mast is preferentially sought out. During this period, likely elevated mammary LPL and reduced adipose-tissue LPL (e.g., Iverson 1993) would ensure that dietary fatty acids are primarily directed to the mammary gland for milk-fat synthesis. Thus, the degree to which milk fatty acid composition will reflect the diet during spring foraging will depend upon how much fat continues to be mobilized from adipose stores, as well as how much biosynthesis of fatty acids (from ingested simple carbohydrates) occurs. Protein ingested would likely be devoted to females' nutrient needs and milk production. Although females in our study may have continued to mobilize previously stored fatty acids for milk synthesis, differences in expected spring dietary fatty acid composition between years (Table 2) were clearly reflected in milk fatty acid signatures (Fig. 4).

In 1994 and 1996, when bears emerged from the den, milk fatty acid composition increased dramatically in 18:2*n*–6 and 18:3*n*–3 (Figs. 1*a* and 3), indicating direct intake of these components and a diet high in terrestrial plants. Such extraordinarily high levels of 18:3*n*–3 are rare and characteristic of green leafy vegetation (e.g., Peng 1992). In spring 1994, when overwintered hard mast was available, milk fatty acid patterns suggested that bears fed preferentially on hard mast, but with some contribution from skunk cabbage. In 1996, following the poor mast crop year, the changes in milk fatty acid patterns were consistent with a high intake of skunk cabbage (Fig. 4).

The discrepancies between estimated fatty acid intakes from scats of CWSA bears and milk fatty acid patterns are probably due to the fact that we did not account for all foods eaten and to the likelihood that individual bears foraged differently (e.g., Fig. 3). Additionally, some continued mobilization from stores or biosynthesis from dietary carbohydrate could have influenced milk fatty acid composition as indicated by somewhat higher levels of 16:0, 16:1*n*–7, and 18:1*n*–9 in milks than were estimated in diets, especially in 1996 (Fig. 4). However, our results do suggest that relatively more of the fatty acids secreted in milk during the spring come from the diet than from body stores, even in a spring (1996) when mostly plants very low in fat were eaten.

Food availability and use are thought to significantly influence female black bear nutrition and reproductive success (e.g., Rogers 1976, 1987; Bunnell and Tait 1981; Eiler et al. 1989; Elowe and Dodge 1989; Noyce and Garshelis 1994). However, bears are also extremely flexible in their ability to exploit different types of foods (Stirling and Derocher 1990; Kasbohm et al. 1995). Despite their carnivorous type of digestive tract, it has been proposed that the large body size of bears allows them to compensate for a lack of food quality by consuming abundant poor-quality foods in quantity (Schaller et al. 1985; Stirling and Derocher 1990; Welch et al. 1997). Yet specific diet strategies of individuals may be more complex than was previously thought and may differ according to age or sex (Rode and Robbins 2000). To better understand the nutritional ecology of black bears, it is necessary to assess food habits at the level of individuals, which has not previously been done, owing to the difficulty of determining what individual bears eat (Rode and Robbins 2000). Results from our study demonstrate that fatty acid signature analysis may allow such insight and facilitate asking questions and testing ideas about the foraging ecology of individuals. Although our conclusions about the diets of bears in this study remain largely qualitative, fatty acids provide useful insight that may not suffer from the types of biases associated with scats, and that allow individuals to be studied over time. Our data also suggest that it may be possible to quantitatively assess diets of individuals from fatty acid signatures, with more rigorous sampling and analysis of all potential foods. While milk fatty acid composition may be most useful in estimating diets of individuals during spring foraging, analysis of adipose-tissue fatty acids near the time of den entrance would likely be most useful in evaluating fall diets. In either case it would be important to sample adipose tissue and milk simultaneously to more fully understand patterns of fatty acid deposition and milk-fat secretion.

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