Use of stable-carbon and -nitrogen isotopes to assess weaning and fasting in female polar bears and their cubs

S.C. Polischuk, K.A. Hobson, and M.A. Ramsay

Abstract: In some species, stable-isotope techniques can provide insights into dietary regimens where there are temporal shifts in trophic level or feeding frequency. We determined stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values for plasma and milk proteins and $\delta^{13}C$ values for milk lipids from female polar bears (*Ursus maritimus*) and cubs to (*i*) ascertain whether cubs are at a higher trophic level than their mothers as a result of nursing and whether we can determine when weaning occurs, and (*ii*) determine the impact of seasonal fasting on $\delta^{13}C$ and $\delta^{15}N$ values. The plasma $\delta^{13}C$ values for mothers and cubs were similar to milk-protein $\delta^{13}C$ values and were significantly enriched in ^{13}C compared with those for milk lipid. Plasma from cubs of the year (COYs) in spring, when milk was their only diet, was isotopically enriched in ^{15}N by 1.0‰ over that of their mothers ($\delta^{15}N$ = 21.5 ± 0.8‰ (mean ± SD) for cubs and 20.5 ± 0.5‰ for mothers) and depleted in ^{13}C by 0.8 ‰ ($\delta^{13}C$ = –19.6 ± 0.5‰ for cubs and –18.8 ± 0.8‰ for mothers). For bears who fasted between summer and fall (3–4 months), plasma became depleted in ^{13}C by 0.5‰ and in ^{15}N by 1‰. Plasma from females, who had fasted from summer to spring (7–8 months) and given birth to cubs, became enriched in ^{13}C by 0.7‰ and in ^{15}N by 2‰. By using stable-isotope analyses we were able to show that (*i*) young cubs were at a higher trophic level than their mother when milk was their only food source, and (*ii*) seasonal fasting influenced $\delta^{13}C$ and $\delta^{15}N$ values. However, we were not able to use stable-isotope analyses to determine the exact time of weaning.

Résumé : Chez certaines espèces, les techniques basées sur les isotopes stables peuvent renseigner sur les régimes alimentaires là où il y a des variations temporelles du niveau trophique ou de la fréquence des repas. Nous avons déterminé la valeur des isotopes de carbone ($\delta^{13}C$) et d'azote ($\delta^{15}N$) stables dans le plasma et les protéines du lait et les valeurs de $\delta^{13}C$ dans les lipides du lait de femelles de l'Ours blanc (*Ursus maritimus*) et chez leurs petits dans le but (*i*) de vérifier si les oursons sont à un niveau trophique plus élevé que leurs mères à cause de l'allaitement et s'il est possible de déterminer à quel moment a lieu le sevrage et (*ii*) d'évaluer l'impact d'un jeûne saisonnier sur les mesures de $\delta^{13}C$ et de $\delta^{15}N$. Les valeurs de $\delta^{13}C$ du plasma chez les mères et leurs petits étaient semblables aux valeurs de $\delta^{13}C$ des protéines du lait et significativement plus riches en ^{13}C que les lipides du lait. Le plasma des oursons de l'année (COYs) au printemps, moment où ils ne consomment que du lait, était plus riche de 1,0 ‰ en isotopes ^{15}N que celui de leurs mères ($\delta^{15}N$ = 21,5 ± 0,8 ‰ (moyenne ± écart type) chez les oursons vs. 20,5 ± 0,5 ‰ chez les mères) et moins riches de 0,8 ‰ en ^{13}C ($\delta^{13}C$ = –19,6 ± 0,5 ‰ chez les oursons vs. –18,8 ± 0,8 ‰ chez les mères). Chez les ours qui ont jeûné de l'été à l'automne (3–4 mois), le plasma était appauvri de 0,5 ‰ en ^{13}C et de 1 ‰ en ^{15}N. Chez les femelles qui ont jeûné de l'été au printemps (7–8 mois) et donné naissance à des petits, le plasma était enrichi de 0,7 ‰ en ^{13}C et de 2 ‰ en ^{15}N. Les analyses des isotopes stable nous ont permis de démontrer (*i*) que les oursons sont d'un niveau trophique supérieur à celui de leur mère quand ils ne consomment que du lait et (*ii*) qu'un jeûne saisonnier influence les valeurs de $\delta^{13}C$ et de $\delta^{15}N$. Cependant, les analyses des isotopes ne nous ont pas permis de déterminer le moment exact du sevrage.

[Traduit par la Rédaction]

Introduction

Polar bears (*Ursus maritimus*) are at the top of a five-level Arctic marine food chain (Hobson and Welch 1992) and feed primarily on ringed seals (*Phoca hispida*). Accessibility of their prey varies throughout the year, therefore many polar bears go through cycles of feasting and fasting (Ramsay and Stirling 1988). Peak feeding for many polar bears occurs

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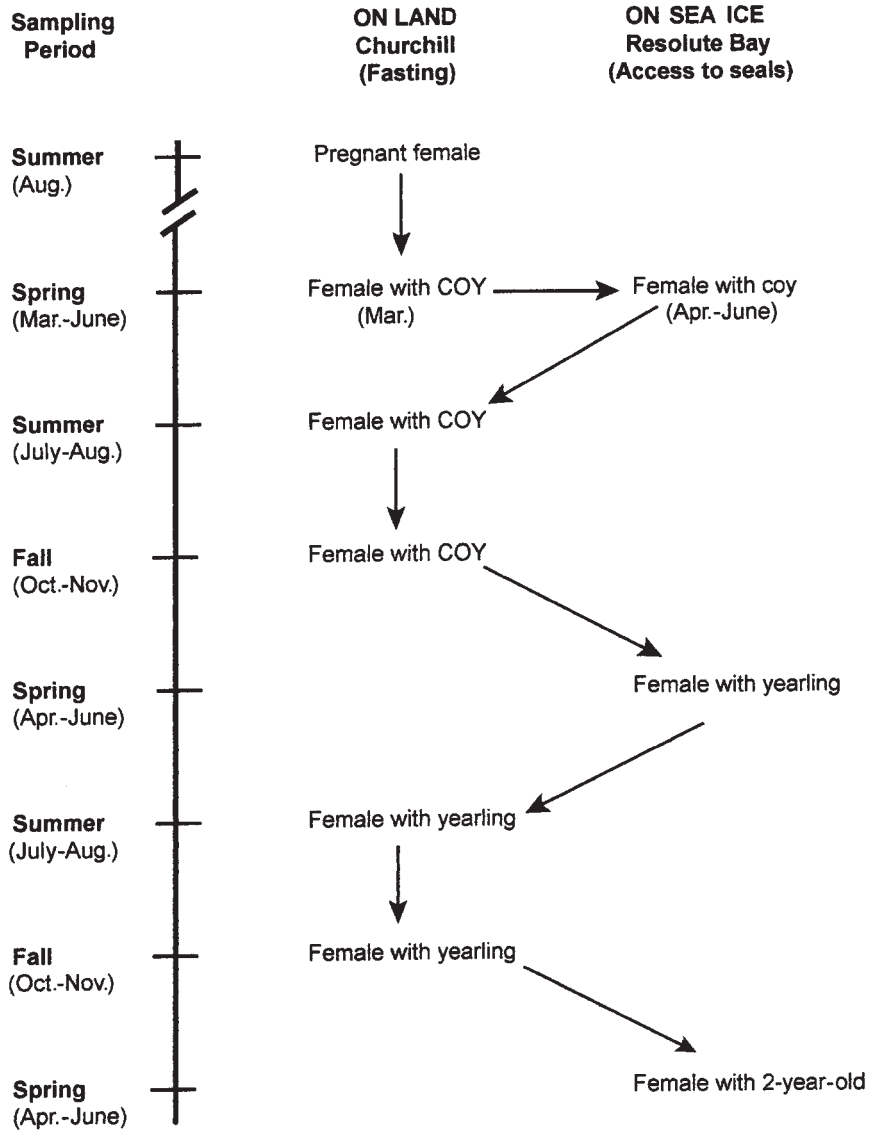
Fig. 1. Analyses of $\delta^{13}C$ and $\delta^{15}N$ for adult female polar bears captured at Churchill, Manitoba, and Resolute Bay, N.W.T. (now called Nunavut), illustrating the feeding and fasting regime, reproductive chronology, and habitat use.



in spring, when seal pups are weaned. Polar bears can then efficiently hunt naive seals on the sea ice and rapidly accumulate massive fat depots.

Polar bears inhabiting western Hudson Bay, Canada, experience one of the longest fasts because they are forced on land during the ice-melt in summer and, thus, no longer have access to seals (Derocher and Stirling 1990). When the bears are forced on shore, they fast and do not rely significantly on terrestrial foods as a source of energy (Ramsay and Hobson 1991; Hobson and Stirling 1997). Instead, they rely on their fat depots, which were accumulated in late spring and early summer on the sea ice.

Adult males, females with cubs, and subadults may fast for 4 months or more (July–November) while they wait until Hudson Bay refreezes. In contrast, pregnant females find dens and stay on land until spring (Fig. 1). Pregnant bears, therefore, can fast for more than 8 months during which they undertake gestation and the first 3 months of lactation. Polar bears are not nutritionally stressed when fasting if they have accumulated adequate fat stores for the duration of their fast (Atkinson et al. 1996). Like other ursid species, polar bears have evolved physiological pathways to use their fat depots efficiently and conserve their lean body mass (Nelson 1980).

When cubs of the year (COYs) emerge from their birth dens between March and April, they are completely dependent on their mother's milk for nutrition. As well as her milk, the mother has supplied all nutrients for the fetuses from her own lipid and protein reserves while fasting (Ramsay and Dunbrack 1986). Milk from polar bears has a high lipid content (approximately 30%) compared with that of most terrestrial species (Jenness et al. 1972). After emerging from their dens in spring, when the cubs are about 3 months old, families travel to the sea ice, where they hunt for seals. Cubs on the sea ice continue to nurse, but the extent to which they also feed on seals is not known. Females continue to lactate until the time of weaning, but the lipid content and volume of milk decrease during the lactation period (Derocher et al. 1993; Arnould and Ramsay 1994). Cubs stay with their mothers for 1–2 years depending on location: within Canada, bears living on Hudson Bay tend to be weaned at an earlier

age than bears from more northerly populations (Ramsay and Stirling 1988).

Conventional biological field techniques provide limited information on the kinetics of milk yield in free-ranging bears, and the timing of the functional onset of weaning (e.g., when milk constitutes a small part of the diet; Martin 1984) remains poorly understood. For much of the year, polar bears cannot be observed directly, owing to harsh environmental conditions and their wide-ranging movements (Messier et al. 1992). Hence, our knowledge of the feeding ecology and weaning of cubs is necessarily limited. Stable carbon and nitrogen isotope analyses, however, have proved to be useful for delineating the trophic position of organisms and the source of primary production in marine and terrestrial ecosystems (Peterson and Fry 1987; Michener and Schell 1994). By analyzing tissues with different turnover rates, it may be possible to determine diet even at times when the animals cannot be sampled (e.g., Hobson and Clark 1992). Mother's milk represents a higher trophic level for polar bear cubs than seals, therefore we felt that the stable-isotope approach might allow us to trace cubs' dependence on milk and the time of functional weaning.

Polar bears typically accumulate sufficient fat stores in late spring and early summer to sustain themselves during lengthy fasting periods. For adult females, stored body fat is the most important nutrient affecting reproductive performance during a fast (Atkinson et al. 1996). Protein also plays an important role, however (Atkinson and Ramsay 1995). The efficiency of protein-sparing in polar bears is not fixed but varies according to the relative size of body fat and protein pools (Atkinson et al. 1996). Although little is known about the effects of fasting on plasma isotope patterns, initial studies with quail (*Coturnix japonica*) suggest that muscle, liver, bone, and blood become enriched in ^{15}N during fasting (Hobson et al. 1993). The stable-isotope composition of blood could therefore provide insight into the extent of endogenous body stores used during fasting in polar bears.

We used stable-isotope measurements of polar bear plasma and milk to address two objectives. First, we hypothesized that mother's milk and seal tissues would represent two discrete isotopically labelled diets to cubs, and that we could therefore describe differences in feeding sources between mother and young during the lactation period. Second, we wanted to determine the effects of fasting on $\delta^{15}N$ and $\delta^{13}C$ values for bear plasma so that we might better use tissue isotope patterns to interpret the feeding status of free-ranging bears.

Methods

Plasma and milk samples were collected from polar bears captured in the vicinity of Churchill, Manitoba (57°00′–58°50′N, 92°25′–94°15′W), and Resolute Bay, Northwest Territories (N.W.T., now called Nunavut) (74°00′–76°50′N, 88°00′–101°00′W), from 1992 to 1997. Logistical constraints due to sea-ice conditions required us to collect samples from two different regions. Sampling in the Resolute Bay region occurred between mid-April and beginning of June, during which period bears were on the sea ice and had access to seals. Samples were obtained from females with COYs who had recently emerged from dens, females with yearling cubs, and females with 2-year-old cubs. Bears from the Churchill area were captured on land and did not have access to seals during our three sampling periods: February–March (spring), July–August (summer), and October–November (fall). Samples were collected from females with COYs in spring at the time of den emergence, females with COYs in summer and fall, and females with yearlings in summer and fall. Some family groups were captured sequentially, the first in summer as they came on land from Hudson Bay and the second in fall after a 3- to 4-month interval and before they returned to Hudson Bay. Pregnant females were sampled in summer and then with their cubs the following spring, 7–8 months later. Thus, our sampling periods covered a broad spectrum of female and cub feeding regimens.

Polar bears were captured using standard immobilization techniques (Stirling et al. 1989). A vestigial premolar tooth was extracted from bears older than 1 year for age determination (Calvert and Ramsay 1998). Body composition (fat and lean body mass) was determined by the isotope-dilution method (Farley and Robbins 1994). Standard body measurements were taken and all bears were weighed by using a tripod and scale to determine total body mass to the nearest 0.5 kg. Blood samples were collected in heparinized vacutainer tubes via jugular catheterization. Blood was kept cool until centrifugation, when plasma was removed and frozen immediately at –20°C. Milk samples were collected from some females by administering oxytocin via the jugular catheter and palpating the teats. Milk samples were either frozen immediately or kept cool until they could be frozen at –20°C within 8 h.

Plasma and milk samples were subsequently freeze-dried. Plasma samples were then powdered and loaded directly into tin boats for stable carbon and nitrogen isotope analyses. Milk samples were rinsed three times with a 2:1 chloroform:methanol solution to remove lipids. The solvent and lipid mixture was set in a fume hood for 48 h until the solvent had completely evaporated. Both the lipid and protein portions of the milk samples were loaded independently into aluminum boats. Milk protein was analyzed for both stable carbon and nitrogen isotope values, whereas milk lipid, with its low N content, was analyzed only for stable carbon isotope values. All stable-isotope samples were combusted on-line at 1800°C in a Robo Prep elemental analyzer interfaced with a Europa 20:20 continuous-flow isotope-ratio mass spectrometer at the Department of Soil Science, University of Saskatchewan. Stable-isotope composition is expressed in δ notation as the proportional deviation in parts per thousand (‰) of the isotope ratio in the sample from that of appropriate standards according to

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000$$

where X is ^{15}N or ^{13}C and R_{sample} and $R_{standard}$ are the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios in the sample and standard, respectively. Standards were atmospheric air for nitrogen and PeeDee Belemnite for carbon. Based on replicate measurements of an organic (egg albumen) laboratory standard, measurement precision was estimated to be ±0.3‰ for $\delta^{15}N$ and ±0.1‰ for $\delta^{13}C$ measurements. Twelve polar bear plasma samples were chosen randomly and reassayed for stable-carbon and -nitrogen composition.

We analyzed 131 plasma samples for both $\delta^{13}C$ and $\delta^{15}N$; 90 samples representing 43 adult females and 47 cubs from Churchill and 41 samples representing 16 adult females and 25 cubs from Resolute Bay. Twenty-eight milk samples were similarly analyzed, 23 from Churchill and 5 from Resolute Bay. Thirty-one of the bears were sampled sequentially, once in summer near the start of fasting and once in fall after 3–4 months of fasting. Milk was also collected sequentially from 5 of these 31 individuals. Plasma was also collected sequentially from 4 females handled as pregnant and again in spring with young cubs. The adult polar bears sampled were assigned to four different groups: pregnant females, females with COYs, females with yearlings, and females with 2-year-olds. Each reproductive category was also divided according to the time of year when sampling was done: spring, summer, and fall (Fig. 1).

Table 1. Stable carbon and nitrogen isotope values (‰) for plasma and milk from female polar bears (*Ursus maritimus*) and cubs, Churchill, Manitoba, and Resolute Bay, N.W.T. (now called Nunavut), 1992–1997.

			Plasma			Milk protein		Milk lipid
Reproductive status	Season	Location	*n*	$\delta^{15}N$	$\delta^{13}C$	*n*	$\delta^{15}N$	$\delta^{13}C$	$\delta^{13}C$
Pregnant female	Summer	Churchill	5	18.5±1.2	–19.8±0.2	—	—	—	—
Female with cub of the year (COY)	Spring	Churchill	8	20.3±0.6	–19.0±1.0	5	20.8±0.5	–19.4±0.8	–25.0±0.1
Female with COY	Spring	Resolute Bay	7	20.7±0.6	–19.3±0.4	5	20.8±1.0	–17.8±0.8	–25.3±0.9
Female with COY	Summer	Churchill	7	16.6±2.5	–20.9±0.9	5	18.1±2.9	–19.2±1.4	–25.7±0.3
Female with COY	Fall	Churchill	8	16.7±1.5	–20.9±0.4	5	18.1±1.7	–19.0±0.5	–25.3±0.2
Female with yearling	Spring	Resolute Bay	7	21.3±0.5	–19.3±0.6	—	—	—	—
Female with yearling	Summer	Churchill	5	19.2±0.6	–19.9±0.4	5	19.5±0.4	–19.1±0.2	–25.7±0.2
Female with yearling	Fall	Churchill	7	18.2±2.3	–20.6±0.9	3	19.7±0.2	–19.1±0.3	–25.2±0.3
Female with 2-year-old	Spring	Resolute Bay	2	20.9±0.4	–19.1±0.04	—	—	—	—
COY	Spring	Churchill	9	21.4±0.6	–19.7±0.2	—	—	—	—
COY	Spring	Resolute Bay	11	21.5±0.9	–19.6±0.7	—	—	—	—
COY	Summer	Churchill	11	16.8±2.8	–21.1±0.8	—	—	—	—
COY	Fall	Churchill	11	15.7±2.0	–21.3±0.8	—	—	—	—
Yearling	Spring	Resolute Bay	11	21.4±0.6	–19.9±0.3	—	—	—	—
Yearling	Summer	Churchill	12	19.5±0.7	–19.8±0.4	—	—	—	—
Yearling	Fall	Churchill	7	17.8±2.0	–20.9±0.9	—	—	—	—
2-year-old	Spring	Resolute Bay	3	20.9±0.4	–19.2±0.2	—	—	—	—

Note: Values are given as the mean ± SD.

Results

Plasma $\delta^{15}N$ and $\delta^{13}C$ values in mothers and cubs

At the time of cubs' emergence from the den in spring, when milk was their only diet, their plasma was enriched in ^{15}N and depleted in ^{13}C relative to their mother's plasma (Wilcoxon's matched pairs, $n = 20$, $p < 0.0005$; Figs. 2*a* and 3*a*). Plasma from COYs in spring was enriched isotopically in ^{15}N by 1.0‰ over their mother's ($\delta^{15}N = 21.5 \pm 0.8$‰ (mean ± SD) for cubs and 20.5 ± 0.5‰ for mothers) and depleted in $\delta^{13}C$ by 0.8 ‰ ($\delta^{13}C = –19.6 \pm 0.5$‰ for cubs and –18.8 ± 0.8‰ for mothers). In summer, after family groups had recently come ashore in Churchill, $\delta^{15}N$ and $\delta^{13}C$ values for mother and COY plasma were similar (Wilcoxon's matched pairs, $n = 11$, $p > 0.05$; Table 1, Figs. 2*b* and 3*b*). By fall, the plasma from COYs was depleted in ^{15}N and ^{13}C relative to their mothers' plasma (Wilcoxon's matched pairs, $n = 11$, $p < 0.05$; Table 1, Figs. 2*b* and 3*b*). Even though these differences were statistically significant, there was a large variation among individual family groups (Figs. 2*b* and 3*b*). The range of $\delta^{15}N$ values for plasma from both mothers and their COYs was considerably greater in summer and fall than in spring (Fig. 2*b*). Members of a family tended to display similar degrees of ^{15}N enrichment in their plasma in summer and fall. The degree of ^{15}N enrichment in plasma of a female was related to her body condition (Fig. 4).

In spring, plasma $\delta^{15}N$ values for females with yearlings and their cubs were similar (Wilcoxon's matched pairs, $n = 11$, $p > 0.05$; Fig. 2*c*) while the $\delta^{13}C$ signature of the yearlings was depleted relative to their mothers (Wilcoxon's matched pairs, $n = 11$, $p < 0.005$; Fig. 3*c*). In both summer and fall, $\delta^{15}N$ and $\delta^{13}C$ values for plasma from females with yearlings and their cubs were similar (Wilcoxon's matched pairs, summer: $n = 11$, $p = 0.18$; fall: $n = 7$, $p = 0.45$; Figs. 2*c* and 3*c*); however, the range of $\delta^{15}N$ and $\delta^{13}C$ values for plasma from mothers and cubs was significantly greater in fall than in summer. It should be noted that, once again, there was a large variation in isotope values among individual family groups. Plasma from females with 2-year-old cubs and the cubs also had similar $\delta^{15}N$ and $\delta^{13}C$ values (Wilcoxon's matched pairs, $n = 3$, $p < 0.05$; Figs. 2*c* and 3*c*).

The relative enrichment and depletion of ^{15}N and ^{13}C in plasma of cubs relative to that of their mothers during the maternal care period is summarized in Table 2. The availability of food to mothers and cubs during the same period is also listed. Plasma from mothers and cubs was generally depleted in ^{15}N and ^{13}C compared with milk protein and enriched in ^{13}C compared with milk lipid (Figs. 2*a*–2*c* and 3*a*–3*c*).

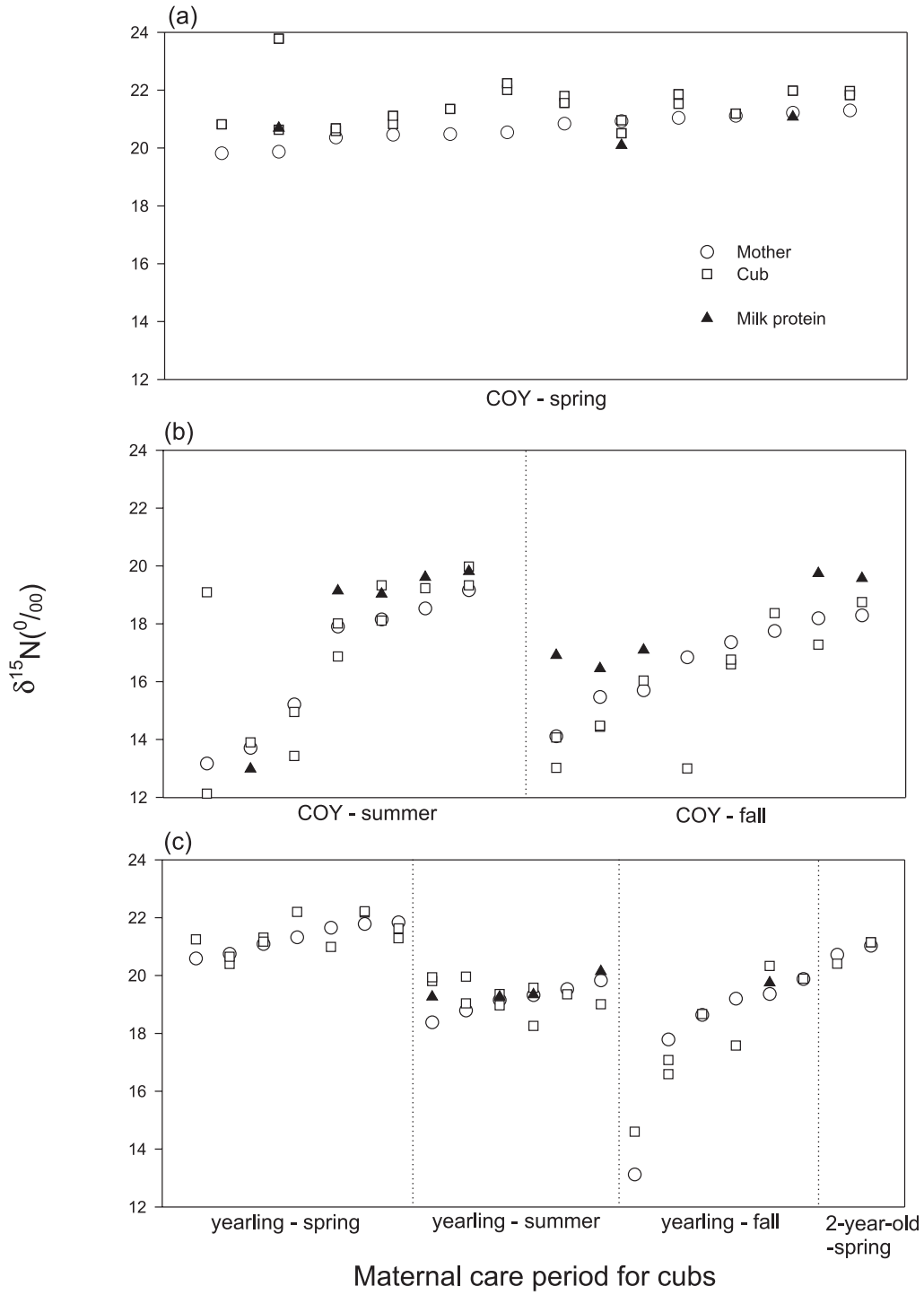
Dietary $\delta^{15}N$ and $\delta^{13}C$ values

The $\delta^{15}N$ values for plasma from females and cubs (COYs, yearlings, and 2-year-olds) in spring were similar to those in polar bear muscle (Fig. 5). In contrast, plasma $\delta^{15}N$ values from females with COYs and from COYs captured in summer and fall (Fig. 5*a*) overlapped $\delta^{15}N$ values for ringed seal muscle (Fig. 5*b*). The $\delta^{13}C$ values for milk lipid, seal fat, and polar bear fat were lower than those for milk protein, seal muscle, and polar bear muscle (Fig. 6*b*). Stable carbon isotope values for plasma in spring were more similar to $\delta^{13}C$ values for polar bear muscle than to plasma $\delta^{13}C$ values for bears in summer and fall. Polar bear plasma was depleted in ^{13}C during summer and fall relative to spring (Fig. 6).

Fasting and plasma $\delta^{15}N$ and $\delta^{13}C$ values

Plasma from females with COYs in spring was enriched in ^{15}N by 2‰ over plasma from pregnant females in summer (females in spring: $\delta^{15}N = 20.5$‰, $n = 5$; pregnant females: $\delta^{15}N = 18.5$‰, $n = 15$; $t = –4.83$, df = 18, $p < 0.0005$; Fig. 5*a*). Plasma from bears captured in summer, before a

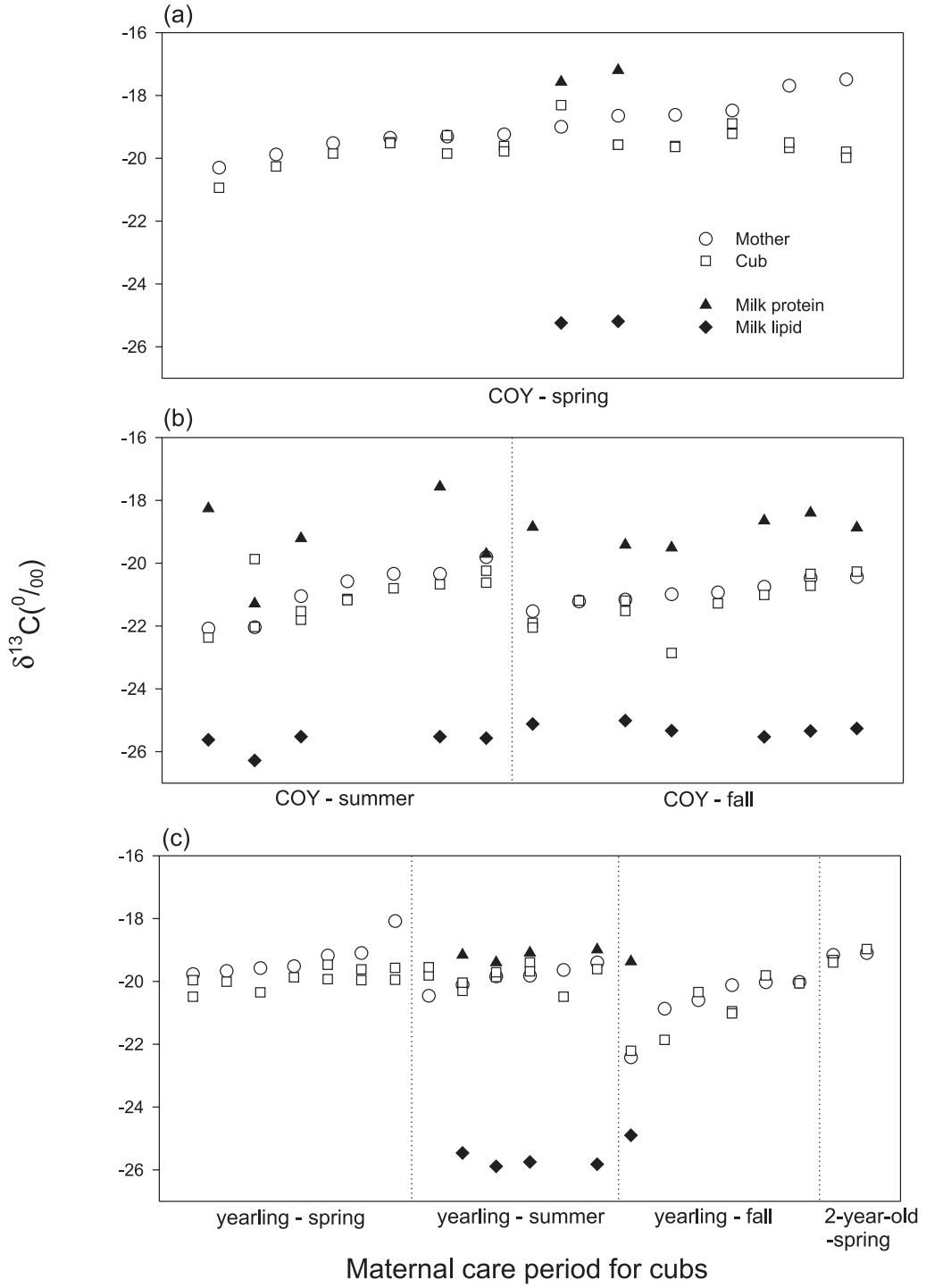
Fig. 2. Stable nitrogen isotope values in plasma from female polar bears and their cubs during the 2-year maternal care period (according to age of the cub (cub of the year (COY), yearling, or 2-year-old) and season (spring (March–June), summer (July–August), or fall (October–November)) at Churchill and Resolute Bay (ranked according to $\delta^{15}N$ values for the plasma of mothers). For some mothers, $\delta^{15}N$ values for milk protein are given. (*a*) Females and COYs in spring. (*b*) Females and COYs in summer and fall. (*c*) Females and yearlings in spring, summer, and fall, and females with 2-year-olds in spring.



fasting period, was enriched in ^{15}N by 1.1‰ over plasma from bears captured after a 2- to 3-month fasting period (summer: $\delta^{15}N$ = 18.0‰, n = 35; fall: $\delta^{15}N$ = 16.9‰, n = 33; t = 2.02, df = 66, $p < 0.05$; Fig. 5*a*).

Plasma from females with COYs in spring was similar in ^{13}C to that of pregnant females in summer (females in spring: $\delta^{13}C$ = –19.1‰, n = 15; pregnant females: $\delta^{13}C$ = –19.8‰, n = 5: t = –1.9, df = 18, p > 0.05; Fig. 6*a*). Plasma from females with yearlings and their cubs captured after a 3- to 4-month fasting period in fall was depleted in ^{13}C by

Fig. 3. Stable carbon isotope values for plasma from female polar bears and their cubs during the 2-year maternal care period at Churchill and Resolute Bay (ranked according to $\delta^{13}C$ values in plasma of mothers). For some mothers, $\delta^{15}N$ values for milk protein are given. For other details see Fig. 2. (*a*) Females and COYs in spring. (*b*) Females and COYs in summer and fall. (*c*) Females and yearlings in spring, summer, and fall and females with 2-year-olds in spring.



0.5‰ compared with plasma from the same reproductive-status groups captured before fasting in summer (fall: $\delta^{13}C$ = –21.0‰, n = 33; summer: $\delta^{13}C$ = –20.5‰, n = 35; t = 2.61, df = 66, $p < 0.05$; Fig. 6*a*).

Longitudinal sampling

Polar bears that were captured sequentially near the beginning and end of a fasting period did not show consistent trends in $\delta^{15}N$ and $\delta^{13}C$ values in plasma; however, mothers and their cubs followed the same pattern (Figs. 7 and 8). The plasma of three family groups consisting of females with COYs (group A) became depleted in ^{15}N and ^{13}C during the summer-to-fall fasting period (Fig. 7). In contrast, the plasma of two family groups consisting of females with COYs

Fig. 4. Stable nitrogen isotope values for plasma and percent body fat of adult female polar bears in summer and fall at Churchill. These data are cross-sectional. One of the summer and fall samplings of bears captured sequentially was randomly selected as one data point.

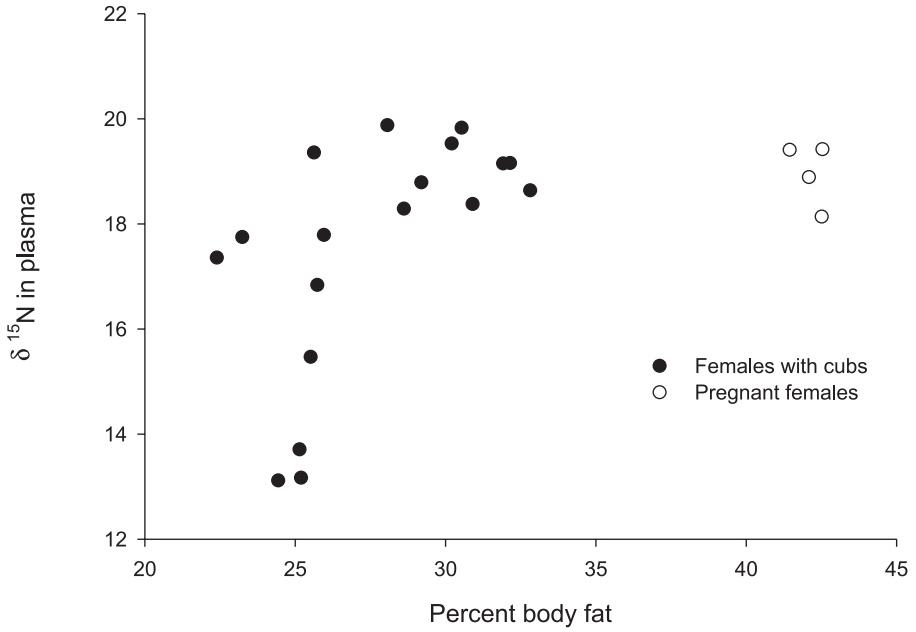


Table 2. Relative enrichment and depletion of ^{15}N and ^{13}C in cubs' plasma relative to their mother's plasma during the maternal care period, Churchill and Resolute Bay, 1992–1997; food sources available to cubs, and their mother's nutritional state (fasting versus feeding), are also given.

	Season	^{15}N	^{13}C	Cub food source/access	Mother's nutritional status
Cub of the year	Spring	+	–	Milk only	Fasting (~8 months)
	Summer	nd	nd	Milk, recent access to seals	Recent feeding
	Fall	–	–	Milk, lipid reserves	Fasting (~3 months)
Yearling	Spring	nd	–	Milk, access to seals	Feeding
	Summer	nd	nd	Milk, recent access to seals	Recent feeding
	Fall	nd	nd	Milk,* lipid reserves	Fasting (~3 months)
2-year-old	Spring	nd	nd	Females not lactating, access to seals	Feeding

Note: + denotes enriched cub plasma and – denotes depleted cub plasma (Wilcoxon's matched pairs, $p < 0.05$); nd, no difference in isotope values for mothers and cubs (Wilcoxon's matched pairs, $p > 0.05$).
*Most females were not lactating.

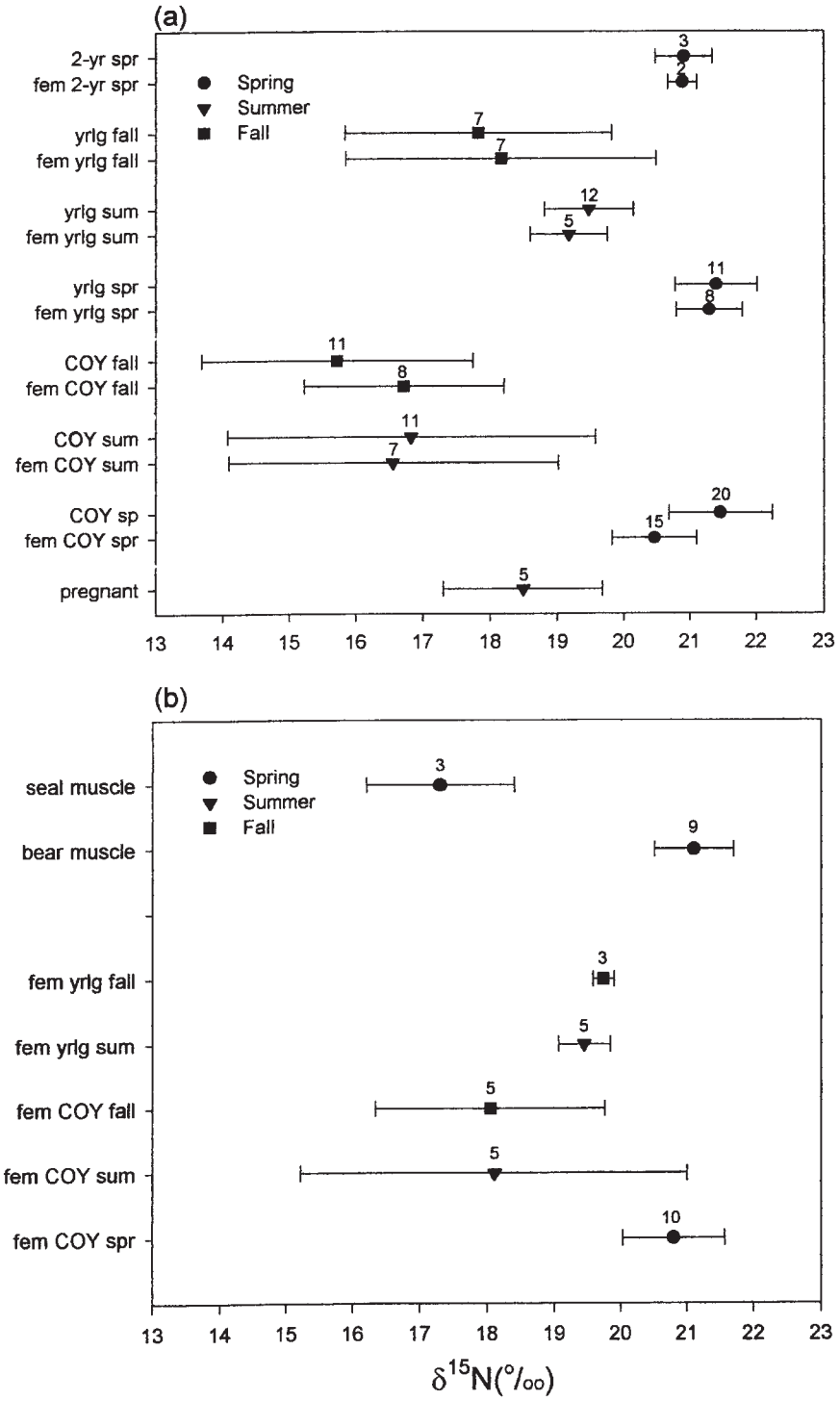
(group B) became enriched in ^{15}N and ^{13}C during the same period (Fig. 8). There were two notable differences between these groups. First, the percentage of body fat for group A at initial sampling was 26 ± 5% (mean ± SD) compared with 20 ± 7% for group B ($t = 1.97$, df = 10, $p = 0.08$). Although both groups of bears were fasting during the period of sampling, bears in group A had a higher percentage of body fat at the start of the on-land period. Second, the ages of mothers in group A ranged from 13 to 16 years, whereas mothers in group B were younger (7 and 11 years).

Discussion

The relative importance of mother's milk and seals to the diet of polar bear cubs during the maternal care period has not been well established. We were able to document temporal variation in $\delta^{15}N$ and $\delta^{13}C$ values for plasma from mother and cubs during the 2-year maternal care period. Since plasma has a short turnover rate, any recent changes in diet or metabolism should be reflected in its isotope value. In particular, the mobilization of endogenous substrates, such as stored lipids and muscle proteins, should be particularly significant to carbon and nitrogen pools in polar bear plasma during their on-land fast.

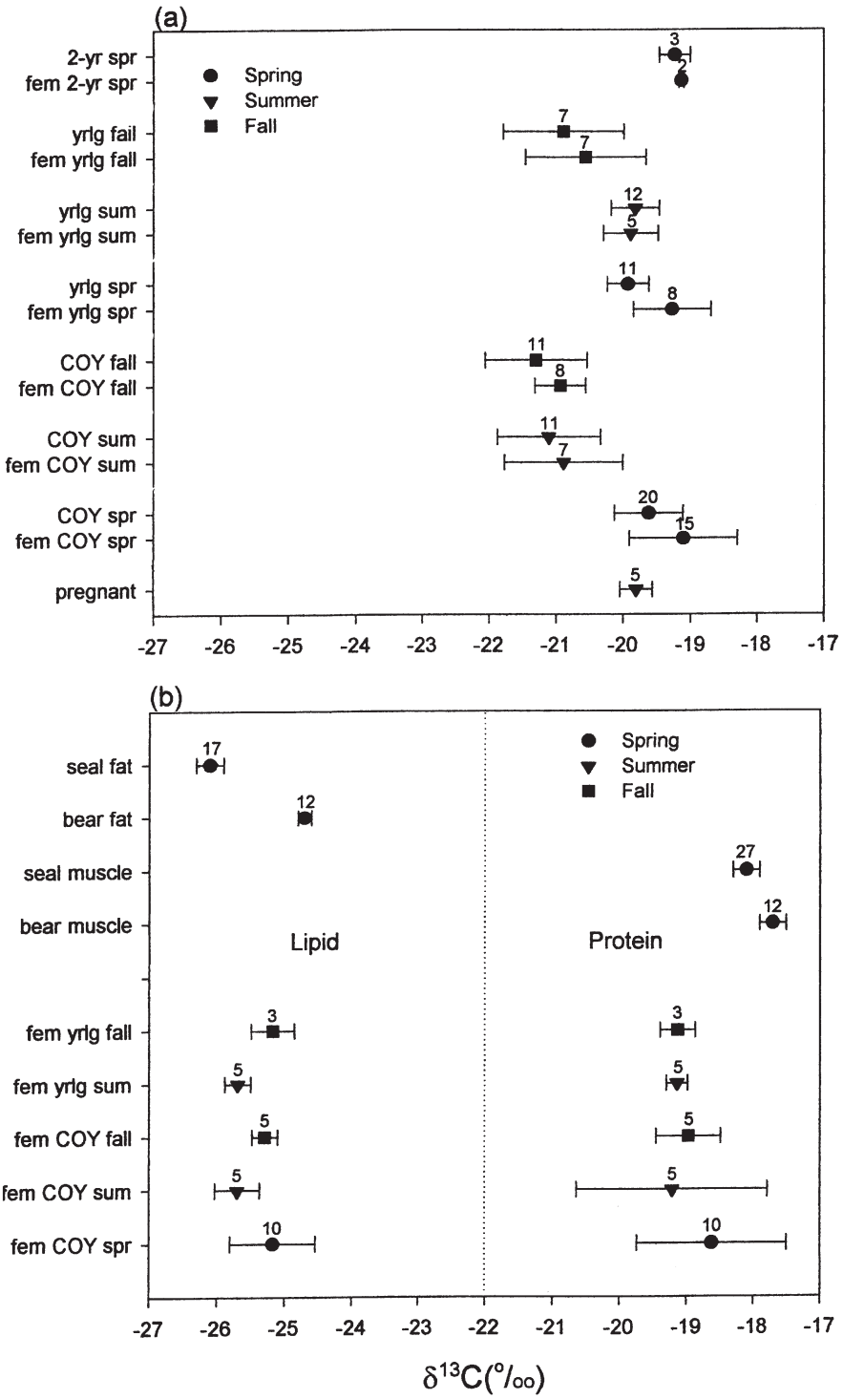
We showed that in spring the plasma of COYs was enriched in ^{15}N by 1.0‰ relative to their mothers' plasma, which is likely due to their reliance on mother's milk, since alternative food sources were not available at the den site. Enrichment in ^{15}N as isotopic evidence for trophic enrichment in nursing young compared with their parents has been found in humans and Stellar sea lions, *Eumetopias jubatus* (Fogel et al. 1989; Hobson and Sease 1998). Other studies have similarly shown enrichment of ^{15}N in tissues of neonates compared with those of their mothers. For example, the $\delta^{15}N$ values for muscle tissue of nursing northern fur seal (*Callorhinus ursinus*) pups were 1.9‰ higher than in their mothers (Hobson et al. 1997), and $\delta^{15}N$ values in tooth annuli from Steller's sea lions were higher during their

Fig. 5. The $\delta^{15}N$ values (mean ± SD) for female and cub plasma during the maternal care period (*a*) and milk protein for females who were lactating while fasting (*b*). Periods are divided according to reproductive status and season as follows: females with COYs (fem COY), females with yearlings (fem yrlg), females with 2-year-olds (fem 2-yr), COYs, yearlings (yrlg), and 2-year-olds (2-yr) in spring (spr); summer (sum); and fall. Values for polar bear and ringed seal muscle from Hobson and Welch (1992) are also shown (*b*). Numbers above the symbols are sample sizes.



first year of life than later (Hobson and Sease 1998). Similarly, fingernails from human infants were enriched in ^{15}N by 2.4‰ over those of their mothers (Fogel et al. 1989). Bocherens et al. (1994) showed the role played by suckling in the ^{15}N enrichment measured in deciduous teeth of European cave bears (*Ursus spelaeus*). Recently, Nelson (1998) speculated that enriched $\delta^{15}N$ and depleted $\delta^{13}C$ in bone collagen of young versus adult extinct European cave bears

Fig. 6. The $\delta^{13}C$ values (mean ± SD) for female and cub plasma during the maternal care period (*a*) and milk protein and lipid for females who were lactating while fasting (*b*). For details see Fig. 5. Values for polar bear and ringed seal muscle from Ramsay and Hobson (1991) are also given (*b*). Numbers above the symbols are sample sizes.



were related in part to the dependence of the young on mother's milk.

An implicit assumption in our isotope-analysis approach was that stable-isotope signatures in the food webs used by Churchill and Resolute Bay bears did not differ. Females with COYs in spring and COYs in spring were the only two status groups that could be sampled at both Churchill and Resolute Bay under similar seasons and circumstances. The $\delta^{15}N$ and $\delta^{13}C$ values for plasma were similar for the two groups at both locations. Thus, we concluded that there was no significant geographical variation in the food-web stable-isotope values that would complicate our interpretations.

Fig. 7. Changes in $\delta^{15}N$ and $\delta^{13}C$ values for polar bear plasma from three family groups of mothers and COYs during a period of fasting (August–October) at Churchill. Different lines (solid, broken, or dotted) designate different family groups. The plasma of these bears was depleted in ^{15}N and ^{13}C during fasting.

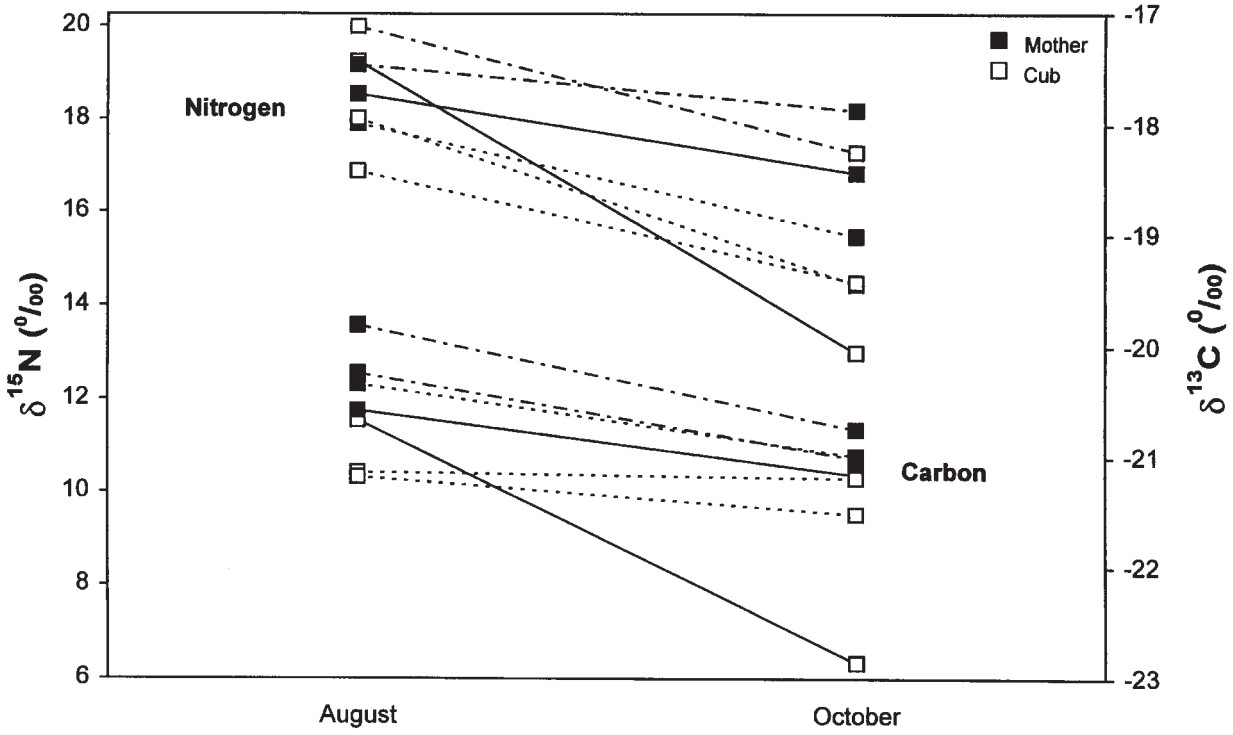
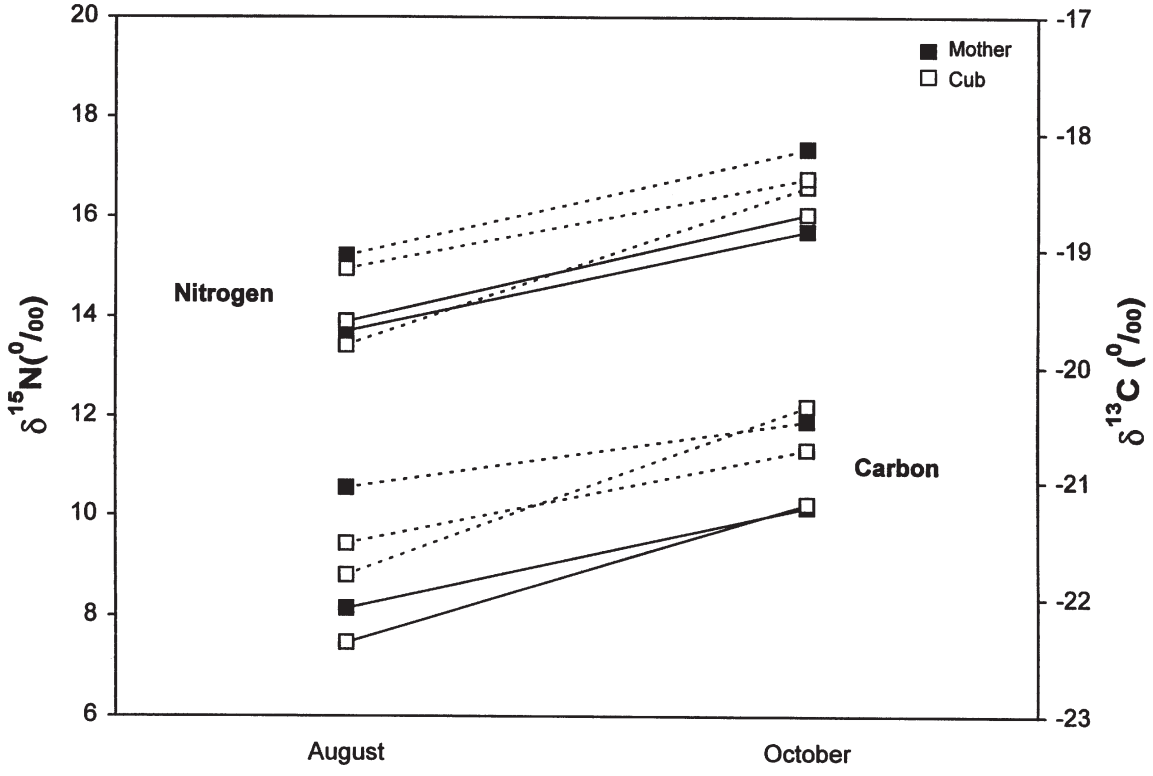


Fig. 8. Changes in $\delta^{15}N$ and $\delta^{13}C$ values for plasma from two polar bear family groups of mothers and COYs during a period of fasting (August–October) at Churchill. Different lines (solid or dotted) designate different family groups. The plasma of these bears was enriched in ^{15}N and ^{13}C during fasting.



Variation in stable-isotope values between mothers and cubs

We documented temporal variation in $\delta^{15}N$ and $\delta^{13}C$ values for mother and cub plasma during the 2-year maternal care period; however, we could not clearly determine the relative importance of mother's milk and seals to cubs. Since plasma has a relatively short turnover rate, any changes in diet or metabolism were probably reflected in the isotope values for mother and cub plasma. Dramatic variation in isotope values for plasma from bears sampled in summer and fall was evident.

In spring, when COYs and their mothers had recently emerged from the maternity den, the plasma of COYs was enriched in ^{15}N by 1‰ and depleted in ^{13}C by 0.8‰ relative to their mother's plasma (see Figs. 2*a* and 3*a*). COYs are completely dependent on mother's milk for their nutrition at this time in their life, and this was the only one of our sampling periods during which their plasma was enriched in ^{15}N compared with their mother's plasma (Table 2). Statistically, mean $\delta^{15}N$ and $\delta^{13}C$ values in plasma were similar for both mothers and COYs in summer after they had spent 3–4 months on the sea ice, where they had access to seals. We interpret these values to mean that by then, cubs had been feeding on seals, and milk was no longer their primary food source as it was in spring. By fall, the plasma of COYs was depleted in ^{15}N and ^{13}C relative to their mother's plasma. COYs have a high energy demand in fall, since they are still growing (Arnould and Ramsay 1994). Even though COYs are still suckling in fall, mother's milk does not provide them with enough nourishment to meet their energy requirements. They must also access their own lipid depots to meet their growth and maintenance demands. Although this is our explanation of the data, we do acknowledge that further research to explain the variability is warranted.

Plasma from yearlings in spring was also depleted in ^{13}C relative to their mother's plasma. This may also be a period in the life of growing cubs when they have been in a negative energy balance over winter but are still undertaking growth in linear dimensions. At this time, yearlings may rely on their mother's milk, which is depleted in ^{13}C (Fig. 6*b*). In summer and fall, there was no significant difference in plasma $\delta^{15}N$ and $\delta^{13}C$ values for mother and cub, indicating that they had similar feeding sources or metabolism.

Use of $\delta^{15}N$ and $\delta^{13}C$ values to determine food sources

The similarity of mean $\delta^{15}N$ values for milk protein (20.8‰) and adult bear muscle (21.1‰) in spring suggests that the cub's nitrogen source (milk protein) is derived from the female's endogenous lean body mass. In summer and fall, mean $\delta^{15}N$ values in milk protein (18.1‰) and seal muscle (17.3‰) were significantly depleted compared with adult polar bear muscle (21.1‰). The wide range of $\delta^{15}N$ values for milk in summer and fall compared with spring suggests that nitrogen sources for milk production varied among bears, a factor that probably depends on adult female body condition (see Fig. 4) and previous hunting success.

The large variance in plasma $\delta^{15}N$ values in summer may be due to differences in the bears' feeding ecology. Polar bears feed primarily on ringed seals, but we have also observed them feeding on bearded seals (*Erignathus barbatus*), belugas (*Delphinapterus leucas*), and arctic char (*Salvelinus alpinus*). Each of these species might have different isotope signatures. Similarly, bears from the same family group may have different plasma $\delta^{15}N$ values in summer and fall, owing to differences in body composition. In Fig. 2*b*, for example, the first family group shows one male cub with a much higher plasma $\delta^{15}N$ value than his mother and sister. He was in poorer body condition and had 7% less body fat than the mother and sister. Since this male cub had a lower percentage of body fat than expected, he might have been using primarily lean body mass for energy. This cub's plasma was significantly enriched in ^{15}N and depleted in ^{13}C, which is consistent with the metabolism of lean body tissue and not adipose tissue.

The variation in plasma $\delta^{15}N$ values for mothers and cubs in summer suggests that cubs were not completely dependent on milk as they were in spring, and that they were consuming seals. Young COYs on the sea ice do consume seals (personal observation). Mean $\delta^{15}N$ values in milk (20.8‰) differed from those in seal muscle (17.3‰) in spring, although values were similar in summer. We were therefore unable to use $\delta^{15}N$ values for milk and seal to determine their relative contribution to values in the bear's plasma at that time.

Plasma from COYs in spring was depleted in ^{13}C compared with that of their mothers, probably because only lipid-rich milk was consumed. In spring, COYs are completely dependent on their mother's milk, which, at this time, has the highest lipid content during the whole of the maternal care period (Derocher et al. 1993). Plasma of females with COYs and their cubs had similar $\delta^{13}C$ values in summer, but was depleted in ^{13}C relative to $\delta^{13}C$ values in spring. Since $\delta^{13}C$ values for milk lipid (–25.4‰) and seal fat (–26.1‰), as well as milk protein (–18.9‰) and seal muscle (–18.1‰), were similar in summer, $\delta^{13}C$ values for milk and seal were similar enough to prevent their use for determining relative inputs to the cubs' diet. In fall, plasma $\delta^{13}C$ values for females with COYs and their cubs were further depleted, probably because of bears mobilizing their own adipose-tissue reserves. Stable carbon isotope values were therefore useful for tracking seasonal changes in consumption or metabolism of lipid versus protein tissue, since $\delta^{13}C$ values for lipid were substantially depleted compared with those for protein.

Effect of fasting on plasma $\delta^{15}N$ and $\delta^{13}C$ values

In addition to metabolism of fat stores, both male and female polar bears also catabolize lean body mass during their on-land fast in summer and fall (Atkinson and Ramsay 1995; Atkinson et al. 1996). The mobilization of these substrates should influence the carbon and nitrogen pools in polar bear plasma. Pregnant females undertake the longest fast, which is 8 months long. During gestation and fasting, the plasma of females became enriched in ^{13}C, suggesting that muscle protein contributed significantly to the carbon pool. Had only adipose tissue been used during fasting, we would have expected the plasma to become depleted in ^{13}C. Plasma from adult females with COYs in spring was enriched in^{15}N by 2‰ relative to plasma from pregnant females in summer, suggesting that as they fast during pregnancy and initial stages of lactation, females likely catabolize muscle tissue. Atkinson and Ramsay (1995) found that pregnant females lost significant quantities of lean body mass while fasting

over an interval that included the predenning period lasting up to 2 months, gestation, and at least 2 months of lactation. During the seasonal fast (summer to fall), plasma from some adult females became depleted in ^{13}C, suggesting that adipose tissue rather than muscle protein was used primarily as a carbon source. Stable nitrogen isotope analysis further suggests that these bears did not primarily use their protein reserves between summer and fall because their plasma did not become enriched in ^{15}N, which would be expected to occur if they were catabolizing muscle.

We found considerable individual variation in isotope signatures among bears sampled sequentially. The initial body condition of bears seemed to influence how they used their body stores. Atkinson et al. (1996) found that bears who were relatively fat in summer subsequently lost a higher percentage of body energy in the form of fat than did leaner bears, and that fat male polar bears were able to achieve and maintain a lower rate of protein catabolism than leaner males. Our data show that $\delta^{15}N$ values in plasma in adult female polar bears that are more than 25% body fat are relatively similar (see Fig. 4). Once the bear reaches 25% body fat or lower, there is a large variation in $\delta^{15}N$ values, which is most likely attributable to differences in metabolic pathways undertaken during nutrient use or mobilization.

In our subjects, depletion of ^{15}N and ^{13}C in plasma occurred during fasting in females that had a higher percentage of body fat. During fasting, fatter females seemed to benefit from their excess adipose depots by preferentially using this reserve as an energy source rather than their protein mass. Plasma of bears with a relatively higher percentage of body fat initially in summer became depleted in ^{13}C during fasting, which suggests that they used their adipose reserves as the primary carbon source. We do not know why ^{15}N in plasma was also depleted in these bears during fasting. However, if polar bear adipose tissue was depleted in ^{15}N relative to muscle, mobilization of this minor protein source might account for the depleted plasma $\delta^{15}N$ values. Plasma that became enriched in ^{15}N over the fasting period was obtained from bears who had a relatively lower percentage of body fat initially in summer and who presumably catabolized a relatively higher proportion of body proteins for energy. The plasma from this group also became enriched in ^{13}C, suggesting a greater carbon contribution from muscle than from adipose tissue. The females with a relatively lower percentage of body fat in summer were also younger than females who had a greater percentage of body fat. Atkinson and Ramsay (1995) found a positive correlation of total body fat stores with age in pregnant female polar bears.

Summary

Our study demonstrates that stable carbon and nitrogen isotope measurements can be used to provide insights into the nutrition of polar bear cubs and adult females during the maternal care period. We found a large variation in isotope values within and among family groups. We have suggested plausible explanations for our results, but we encourage further research to determine the functional significance of this variation. Our isotope measurements are consistent with individuals varying their use of body energy stores during fasting, a process that seems to depend on body condition. We assumed that isotope signatures in seals, the only exogenous source of food for these bears, did not change between locations or periods of capture. In general, $\delta^{13}C$ values in marine systems in the northern hemisphere show increasing depletion with latitude (Rau et al. 1982; Hobson et al. 1997), but this variation was not obvious in polar bears captured from Churchill and Resolute Bay (Table 2). Future work could include testing for variation in $\delta^{15}N$ and $\delta^{13}C$ values in seals with location and time of capture. Sampling polar bears from Resolute Bay later in the season, when they would have fed on seals for a longer period, could also indicate how $\delta^{15}N$ and $\delta^{13}C$ signatures change during active feeding. Finally, since plasma isotope values represent dietary integrations over about 4 days (Hilderbrand et al. 1996), other tissues such as claws, hair, and muscle that reflect diet over longer periods might provide further insights to patterns of weaning and fasting in polar bears.

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