Journal of Zoology. Print ISSN 0952-8369

Seasonal, sexual and anatomical variability in the adipose tissue of polar bears (*Ursus maritimus*)

G. W. Thiemann[1], S. J. Iverson[1] & I. Stirling[2]

1 Department of Biology, Dalhousie University, Halifax, NS, Canada
2 Canadian Wildlife Service, Edmonton, AB, Canada

Keywords
polar bear; adipose tissue; lipid content; stratification; fatty acid.

Correspondence
Gregory W. Thiemann, Department of Biology, Dalhousie University, Halifax, NS, Canada B3H 4J1
Email: thiemann@dal.ca

Received 23 March 2005; accepted 7 September 2005

doi:10.1111/j.1469-7998.2006.00084.x

Abstract

We collected 245 adipose tissue biopsies from adult polar bears *Ursus maritimus* in north-eastern Manitoba during the course of long-term population studies between fall 2001 and spring 2004. In summer, the sea ice of Hudson Bay melts completely and the entire polar bear population is forced to fast on land for *c*. 4 months. During this period, the adipose tissue of females contained significantly more lipid than that of males, consistent with preparation for pregnancy and lactation. The adipose tissue of females with cubs contained less lipid than that of solitary females, likely reflecting greater mobilization of lipid during lactation. Although most of the population returns to the sea ice to hunt after freeze-up in mid-November, pregnant females enter maternity dens where they continue to fast for an additional 4 months. As a result, the adipose tissue of females emerging from dens at the end of this 8-month 'reproductive fast' contained significantly less lipid than females in the fall. There was also evidence of a decline in the adipose tissue lipid content of females emerging from dens over the course of the study. Although this trend was based on limited sample sizes, it suggests that the overall condition of new mothers may be declining. Fat biopsies collected from 20 adult polar bears during a mark–recapture survey on the winter sea ice of south-eastern Beaufort Sea showed that the fatty acid (FA) composition of the superficial adipose layer was largely uniform with depth; however, lipid content significantly increased from skin to muscle. Finally, adipose tissue collected from the belly, rump and baculum depots of bears killed by native subsistence hunters showed no site-specific differences in either FA composition or lipid content. These data suggest that a single sample from any large superficial depot will accurately reflect a polar bear's total superficial adipose store. We suggest that lipid content of adipose tissue may provide valuable information on changes in polar bear condition in response to changes in arctic climate and prey distribution.

Introduction

Adipose tissue is the primary site of fat storage in vertebrates and is composed of numerous adipocytes (fat cells) that shrink or swell with lipid mobilization and storage, respectively (Schemmel, 1976). Depending on the species, many of the fatty acids (FAs, the primary components of lipids) contained in adipose tissue are derived directly from diet (e.g. Ackman & Eaton, 1966; Rouvinen & Kiiskinen, 1989; Iverson *et al*., 1995). Therefore, the analysis of adipose tissue can provide important physiological and ecological information at several observational scales. At the individual level, lipid content and composition can reflect nutritional or physiological status (e.g. Lockyer, 1986; Moreau-Hamsany *et al*., 1988; Aguilar & Borrell, 1990; Bowen, Oftedal & Boness, 1992; Martin *et al*., 1994), contaminant burdens (e.g. Polischuk, Norstrom & Ramsay, 2002) and foraging patterns (e.g. Pond *et al*., 1995; Iverson, Frost & Lowry, 1997; Raclot, Groscolas & Cherel, 1998; Iverson *et al*., 2004). At the population or species level, the way in which lipids are stored in adipose tissue (e.g. as triacylglycerol vs. wax esters) can reflect phylogenetic or ecological relationships (Koopman, 2001; Iverson, 2002). Collecting superficial adipose tissue samples is relatively non-invasive and, in most cases, can be performed using minimal or no anaesthetic. This provides valuable opportunities for the study of large numbers of free-ranging animals.

As in other marine mammal species, polar bears *Ursus maritimus* store most of their adipose tissue in a subcutaneous layer, representing roughly 82% of total body fat stores (Pond *et al*., 1992), and it is composed of a diverse array of FAs acquired from their marine diets (Colby, Mattacks & Pond, 1993; Iverson, Stirling & Lang, in press). Unlike other marine mammals that possess a specialized

form of adipose tissue – blubber – the subcutaneous fat of polar bears appears to be similar to most other forms of adipose tissue; it functions primarily as the main site of energy storage, rather than a thermal insulator (Pond *et al*., 1992), and is heavily relied upon during the annual fasting periods that characterize their life history.

In western Hudson Bay, the entire polar bear population is forced to come ashore in north-eastern Manitoba when the sea ice breaks up and melts in late summer (see Stirling *et al*., 1977). Without access to their primary prey – ringed seals *Phoca hispida* and bearded seals *Erignathus barbatus* – these bears rely almost entirely on stored fat for metabolic energy, losing up to 1 kg body mass per day (Lunn & Stirling, 1985; Ramsay & Hobson, 1991; Atkinson, Nelson & Ramsay, 1996; Polischuk *et al*., 2002). While most bears return to the sea ice after freeze-up in mid-November, pregnant females retreat into maternity dens (Ramsay & Stirling, 1986) where they must rely on stored resources for an additional 4 months, during which time they give birth to, and nurse, one to three cubs. Therefore, the amount of stored fat is especially critical for pregnant females, and maternal mass at denning affects cub mass at den emergence and ultimately cub survival (Derocher & Stirling, 1996, 1998).

Perhaps the most ecologically significant aspect of fasting in polar bears is their ability to change their metabolism from a feeding to a fasting state at any time of year after not feeding for *c*. 10 days (Derocher *et al*., 1990). Although this fasting state is physiologically and biochemically similar to hibernation (Nelson *et al*., 1983), the bear is capable of re-feeding at any time and returning to a normal physiological state again soon after. Furthermore, when feeding on ringed seals, polar bears have a digestive efficiency of 97% for fat and almost 92% assimilation for dietary energy (Best, 1984). Thus, polar bears feed hyperphagically whenever possible, such as in late spring before breakup when young seals are most abundant, assimilate the energy extremely efficiently and shift into fasting mode whenever food is unavailable. This facultative ability to switch back and forth at need between feeding hyperphagically and fasting at any time of year is probably the polar bear's most important adaptation to survival in the Arctic.

Like other animals adapted to fasting, polar bears undergo dramatic seasonal changes in body composition and their adipose tissue may be specially adapted to deal with extreme fattening and prolonged seasonal fasts (Watts & Hansen, 1987; Derocher *et al*., 1990; Ramsay, Mattacks & Pond, 1992; Atkinson & Ramsay, 1995). Considering their life history in western Hudson Bay and the different metabolic demands placed on male and female polar bears, one may expect the adipose tissue of females in the fall to contain proportionally more lipid than that of males. Similarly, given the extensive depletion of fat stores during the 8-month reproductive fast, the adipose lipid content of females may be expected to be lower in spring than in fall, at the beginning of the onshore period. However, the only available data on seasonal and sex differences in lipid content do not agree with these expectations (Ramsay *et al*., 1992), and this aspect of polar bear biology requires further examination.

The hyperphagic feeding of polar bears and their ability to digest and assimilate fat efficiently makes them excellent candidates for ecological studies using fatty acid (FA) signature analysis (Iverson *et al*., 2004), and recent studies have utilized adipose tissue biopsies to examine polar bear foraging (e.g. Iverson *et al*., in press). However, relatively little is known about the distribution of lipid through the superficial adipose layer and whether a single biopsy is representative of a polar bear's entire FA energy store. Therefore, we undertook the current study (1) to evaluate the degree to which superficial adipose tissue could be used as an indicator of overall body condition and physiological state in male and female polar bears, and (2) to examine within-bear variation in lipid content and FA composition, both with depth in the superficial layer and across three large subcutaneous depots.

Methods

Capture of free-ranging animals

Free-ranging polar bears were located from a Bell 206B JetRanger helicopter and immobilized with Telazol (Fort Dodge Laboratories, Fort Dodge, IA, USA) following standard chemical immobilization protocols (Stirling, Spencer & Andriashek, 1989). At first capture, each animal was assigned a unique identification number that was permanently tattooed to the inside of each upper lip and engraved on plastic tags attached to each ear. If the bear was first captured as a cub, its age at subsequent captures could be derived; otherwise, ages of bears were determined by counting growth-layer groups in the cementum of a vestigial premolar tooth (Calvert & Ramsay, 1998). The body mass of captured bears was estimated using a regression equation based on axillary girth (Kolenosky *et al*., 1989), and all immobilization and live-capture procedures were annually reviewed and approved by the Animal Care Committee of the Canadian Wildlife Service, Prairie and Northern Region, Edmonton, Alberta.

Seasonal, interannual and sex differences in adipose tissue lipid content

To examine variability in the lipid content of superficial adipose tissue, we collected 245 tissue samples from 213 adult polar bears (5 years+) on land in north-eastern Manitoba (Fig. 1, Table 1) during the course of long-term population studies. Adipose tissue samples were collected using a 6 mm biopsy punch and consisted of a full-layer core (mean ± SEM: 0.027 ± 0.01 g; Fig. 2) from skin to muscle, taken *c*. 15 cm lateral to the base of the tail (Ramsay *et al*., 1992). This fat depot is large and easily accessed on an immobilized bear in sternal recumbency. In our experience, superficial biopsies taken using these methods from free-ranging bears, pinnipeds and seabirds heal within a very short time (days). Samples were collected during two

seasons each year beginning August 2001 until March 2004: (1) fall (late August to September), when the entire population was on shore fasting following annual break-up of the sea ice; and (2) spring (late February to mid-March), when adult females were emerging from maternity dens with cubs-of-the-year (COY) after fasting for 8 months. In total, 222 samples were collected in the fall (109 males, 113 females) and 23 samples were collected in the spring.

All samples were stored frozen in airtight containers until analysis (<6 months). In the lab, skin and muscle were removed and the whole biopsy sample was weighed. Lipid was then quantitatively extracted from each whole sample and total lipid content was determined according to Iverson, Lang & Cooper (2001). Lipid content was expressed as the per cent of total sample wet weight ± SEM.

Lipid and FA stratification in polar bear adipose tissue

To examine potential stratification of lipid and FA composition in superficial adipose tissue, we non-selectively sampled 20 adult polar bears (10 males, 10 females) on the winter sea ice of south-eastern Beaufort Sea (Fig. 1) in April 2004 and collected biopsy samples as described above. These polar bears were immobilized and tagged as part of a 4-year mark–recapture population survey, and adipose tissue samples were collected as part of a long-term foraging study (e.g. Iverson *et al*., in press). Extra care was taken to ensure that each sample represented the entire depth of the subcutaneous adipose layer and that it remained in a single piece until analysis in the lab. Following removal of the skin and muscle, the adipose tissue sample was divided into three

Figure 1 Three major locations of polar bears *Ursus maritimus* sampled between fall 2001 and spring 2004. See Table 1 for sample sizes and analyses.

Table 1 Summary of polar bear *Ursus maritimus* adipose tissue samples collected between fall 2001 and spring 2004

Analysis	Region	Season	Sex	Age (years) mean ± SEM (range)	Mass (kg) mean ± SEM	*n*	Samples
Lipid content – seasonal and sex differences	NE Manitoba	Fall	F	14.1 ± 0.7 (5–29)	206 ± 4	113	Live biopsy
			M	12.3 ± 0.5 (5–28)	390 ± 9	109	
		Spring[a]	F	15.4 ± 1.5 (5–27)	164 ± 6	23	
Lipid content/FA composition – stratification	Beaufort Sea	Spring[b]	F	10.9 ± 0.9 (7–14)	186 ± 6	10	Live biopsy
			M	8.1 ± 0.9 (5–11)	333 ± 30	10	
Lipid content/FA composition – across three body sites	Arviat, NU	Fall	Both	Independent (2 + years)	N/A	26	Harvested bears

See Fig. 1 for geographic locations. Specific age and weight data were not available for polar bears killed by native subsistence hunters near Arviat; however, these bears were all independent of their mothers and were therefore assumed to be at least 2.5 years old.

[a]Late February to mid-March.

[b]Early April to early May.

FA, fatty acid.

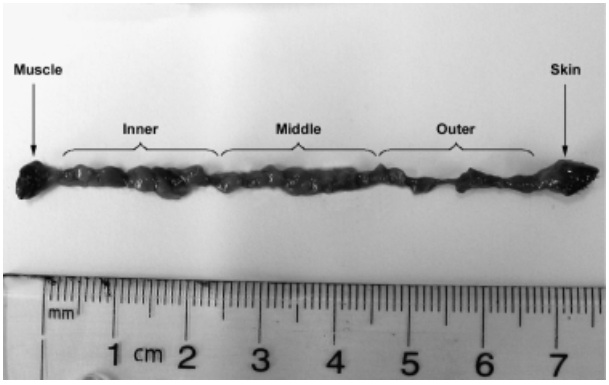

Figure 2 Example of an adipose tissue biopsy taken near the base of the tail of an adult male polar bear *Ursus maritimus* (captured on the sea ice of south-eastern Beaufort Sea in April 2004). Biopsies collected from bears in north-eastern Manitoba were analysed as a single sample (with skin and muscle removed) to examine seasonal and sex differences in overall lipid content. Biopsies from all Beaufort Sea bears were divided into equal-length inner, middle and outer segments (with skin and muscle removed) to examine changes in fatty acid composition and lipid content with depth.

segments of equal length: inner, middle and outer (Fig. 2). Each segment was then separately weighed and the lipid was quantitatively extracted as described above.

FA methyl esters (FAME) were prepared from each extracted lipid sample using an acidic catalyst (the Hilditch method; see Hilditch & Williams, 1964; Thiemann, Budge & Iverson, 2004*b*). Duplicate analyses and identification of FAME were performed using temperature-programmed gas–liquid chromatography (GLC) according to Iverson *et al*. (1997, 2004) and Budge *et al*. (2002), with each FA expressed as the weight per cent of total FAs ± SEM. Individual FAs were described by the standard nomenclature of carbon chain length:number of double bonds and location ($n-x$) of the double bond nearest the terminal methyl group.

Variability in adipose tissue composition across superficial fat depots

To examine how adipose tissue lipid content and FA composition varied among large superficial fat depots, we collected adipose tissue samples from 23 polar bears harvested by Inuit hunters near the community of Arviat, NU, during annual fall subsistence hunts in 2002 and 2003. Male bears ($n = 16$) were sampled near the base of the tail ('rump'), along the midline of the ventral side, approximately two-thirds of the distance between the fore- and hind-limbs ('belly'), and on the baculum. Females ($n = 7$) were sampled from the rump and belly. These locations were communicated to volunteer Inuit hunters through a written description and a basic diagram. Large samples (*c*. 8 cm × 8 cm) extending through the depth of the adipose layer and including a portion of attached muscle were collected at each site and then wrapped in foil and stored frozen until analysis. In the lab, a biopsy subsample was taken through the full depth of the adipose layer in the centre of each specimen. Per cent lipid and FA composition were determined for each subsample, as described above.

Statistical analyses

Seasonal, interannual and sex differences in the lipid content of adipose tissue were evaluated in Manitoba bears using analysis of variance (ANOVA) on arcsine-square-root-transformed proportional data (Zar, 1999). To examine differences in lipid content between sites within individual bears, both by depth (Beaufort Sea bears) and between depots (Arviat bears), repeated-measures ANOVA were performed on transformed data because each bear was sampled multiple times. To examine potential FA stratification, the values of the 19 most abundant FAs (i.e. sample size, n, minus 1) were transformed to improve normality by calculating the log of the ratio of each FA to 18:0 (Budge *et al*., 2002; Iverson, Frost & Lang, 2002). Variation across the three depths was then tested using repeated-measures multiple analysis of variance (MANOVA). FA data from the belly, rump and baculum of hunter-killed bears were similarly transformed and tested, but given sample sizes, statistical analyses were performed using the six and 15 most abundant FAs for females and males, respectively.

Results

Seasonal, interannual and sex differences in adipose tissue lipid content

Ages and body weights of all sampled bears are given in Table 1. In north-eastern Manitoba, 182 bears were captured and sampled once during the study, 29 were captured twice and two were captured thrice. Although some individual bears were sampled in more than one field season, the direction and significance of all trends were the same whether repeat-sample bears were included or excluded in analyses. As every sample collected contributed to the cross-sectional representation of the population at the time, all samples were included in the final analyses. Two adult females that were sampled in fall 2001 were sampled again the following spring. Although this small sample size precluded statistical analysis, the direction and general magnitude of the seasonal change in these longitudinal bears were the same as in the cross-sectional samples.

In the fall, the adipose tissue of female polar bears contained significantly higher per cent lipid than that of males ($P < 0.001$; Fig. 3a). Across all three years, adipose lipid content averaged 74.6 ± 0.6% in females and 70.9 ± 0.7% in males. Although per cent lipid content in males appeared to increase marginally over the course of the study (*c*. 2.3%), sampling year had no significant effect ($P = 0.359$) and there was no year–sex interaction ($P = 0.670$).

Across years, the adipose tissue of adult female polar bears contained 17.6% more relative lipid in the fall than in the spring ($P < 0.001$; Fig. 3b). Although the mean lipid

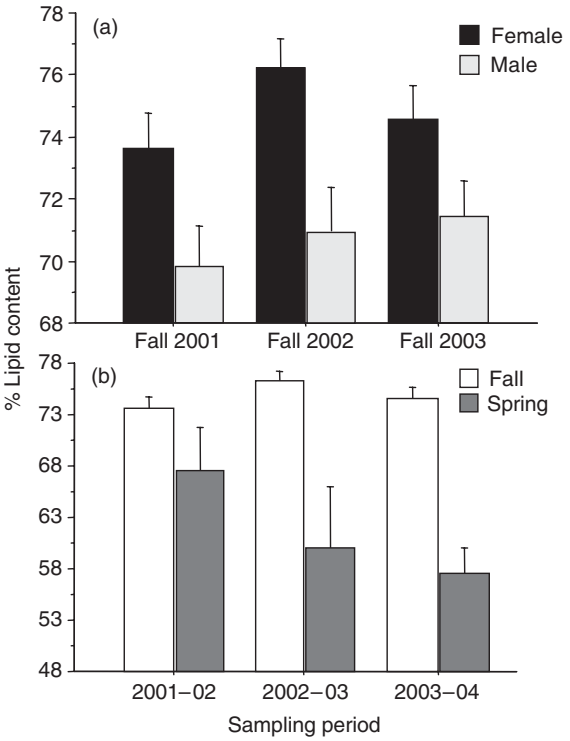

Figure 3 Mean lipid content (+1 SEM, per cent of wet sample weight) of adipose tissue biopsies taken in north-eastern Manitoba (Fig. 1) from (a) adult female (n=113) and adult male (n=109) polar bears *Ursus maritimus* in the fall, and (b) adult female polar bears in fall (n=113) and spring (n=23).

content did not vary significantly across the three sampling years ($P = 0.195$), a significant interaction between year and season ($P = 0.033$) was driven by an increasing seasonal difference over the course of the study. There was a relatively small decline (8.3%) in lipid content from fall 2001 to spring 2002. However, during the next two years, lipid content declined from fall to spring by 21.3 and 22.8%, respectively.

Unfortunately, the pregnancy status of solitary females in the fall was unknown and the potential effect of pregnancy on adipose lipid content could not be examined. However, the presence of cubs was associated with a significant difference in the adipose lipid content of female polar bears (Fig. 4). Across all fall seasons, the adipose tissue of solitary females had 5.0% greater lipid content than that of females with dependent cubs ($P = 0.021$). Additionally, the adipose tissue of mothers with cubs in the fall was 15.8% higher in lipid content than that of females with cubs in the spring ($P<0.001$). Although there was no difference in lipid content across sampling years ($P = 0.438$), a nearly significant interaction between year and reproductive status ($P = 0.059$) was driven by the apparent decline in adipose lipid content in spring females (see Fig. 3b). In contrast, the lipid content of fall females was relatively similar in the three study years (Fig. 3a and b).

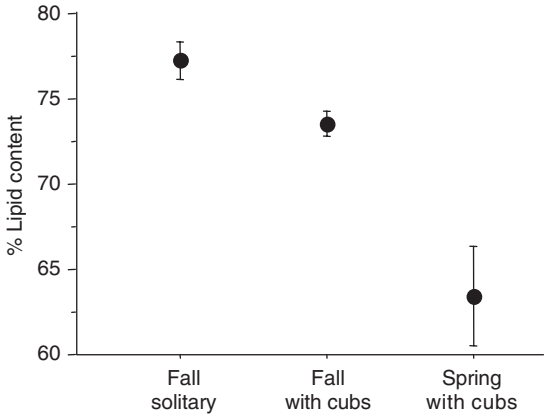

Figure 4 Mean lipid content (±1 SEM, per cent of wet sample weight) of adipose tissue biopsies taken across all three sampling years (fall 2001 to spring 2004) for solitary female polar bears *Ursus maritimus* in the fall (n=33), females with cubs in the fall (n=80) and females with cubs in the spring (n=23), in north-eastern Manitoba (Fig. 1).

Lipid and FA stratification in polar bear adipose tissue

The depth of the sample within the superficial fat layer had no effect on the relative levels of the 19 most abundant FAs ($P = 0.465$; Fig. 5). We sampled equal numbers of males and females (total $n = 20$), and because there was no reason to expect sex differences in FA metabolism or deposition, we pooled both sexes to increase the power of repeated-measures MANOVA.

In contrast to the uniformity of FA composition, the lipid content of adipose tissue did vary significantly with depth ($P<0.001$; Fig. 6). Per cent lipid content increased from the area nearest the skin to the muscle (i.e. body core) in both males and females. Across the entire tissue depth, the lipid content in males ($66.5 \pm 1.3\%$) did not differ significantly from females ($61.2 \pm 2.1\%$; $P = 0.204$).

Variability in adipose tissue composition across superficial fat depots

FA composition did not vary across the sampling sites of superficial adipose tissue in either males ($P = 0.296$) or females ($P = 0.283$; Fig. 7). Lipid content also did not vary between depots (males, $P = 0.299$; females, $P = 0.565$). Across both sexes, the average per cent lipid was 73.1 ± 1.9, 74.9 ± 1.7 and $75.6 \pm 2.2\%$ for the belly, rump and baculum, respectively.

Discussion

Overall, differences in the lipid content of adipose tissue were consistent with seasonal and sex differences in the physiological condition of polar bears. Although the chemical composition of superficial fat varied significantly between the sexes and through the year, adipose tissue

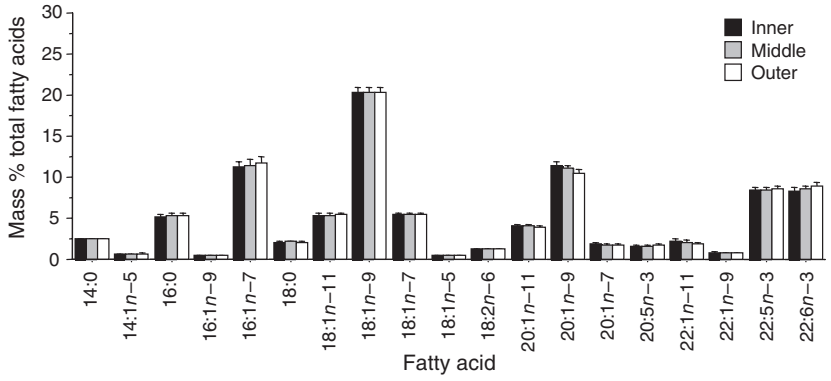

Figure 5 The 19 most abundant fatty acids (mass % of total + 1 SEM) in the inner, middle and outer segments (Fig. 2) of adipose tissue biopsies taken from 20 adult polar bears *Ursus maritimus* (10 males and 10 females, combined) on the winter sea ice of the south-eastern Beaufort Sea (Fig. 1).

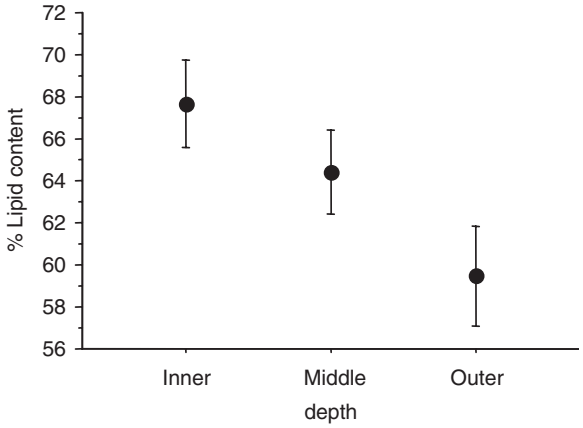

Figure 6 Mean lipid content (± 1 SEM, per cent of wet sample weight) of the inner, middle and outer segments (Fig. 2) of adipose tissue biopsies taken from adult male ($n = 10$) and female ($n = 10$) polar bears *Ursus maritimus* on the winter sea ice of the south-eastern Beaufort Sea (Fig. 1). All three depths differed significantly from each other (*post hoc* Bonferroni, $P < 0.05$).

appeared to be largely uniform within individual polar bears. This uniformity, along with the correlation between lipid content and physiological condition, has important implications for the use of adipose tissue samples in monitoring the long-term effects of environmental change on polar bear populations.

Temporal and demographic trends in lipid content of adipose tissue

Consistent with their life-history patterns of seasonal foraging and extensive annual fasts, polar bears in western Hudson Bay demonstrated substantial variability in the lipid content of their adipose tissue. In the fall in north-eastern Manitoba, lipid content was consistently higher in adult females than in adult males (Fig. 3a). This trend may reflect sex differences in the timing and magnitude of reproductive costs. Although all bears in the western Hudson Bay population fast on shore for *c*. 4 months during the annual ice-free period, pregnant females fast through an additional 4 months that includes all of gestation and *c*. 3 months of lactation. This extended 'reproductive fast' involves the mobilization of a substantial amount of stored energy, both for the physiological maintenance of the mother and for meeting the nutritional and energetic demands of milk production (Derocher, Andriashek & Arnould, 1993). Polar bear cubs are born in an altricial state and adequate milk production is critical to their survival. Consequently, pregnant polar bears must achieve a minimum physical condition in order to produce cubs successfully (Derocher, Stirling & Andriashek, 1992). Our data indicate that during the period of hyperphagia that precedes the fast, female polar bears produce adipose tissue that is higher in relative lipid content than that of males. This difference between the sexes may also be related to the sexual size dimorphism in polar bears. Adult females are roughly half the size of adult males and, consequently, have smaller absolute lipid stores. Females may therefore attempt to maximize their energy storage capacity by increasing the lipid content of their adipose tissue. Given that animals must balance both the costs and benefits of storing body energy, females may commit to storing more energy in preparation for reproduction than do males that face lower costs and uncertainty, as has been shown in other species that fast during lactation (Beck, Bowen & Iverson, 2003).

In contrast to the present study, Ramsay *et al*. (1992) found that the lipid content of adipose tissue was higher in males during the onshore period in north-eastern Manitoba ($65.1 \pm 0.9\%$) than in solitary females ($57.6 \pm 1.4\%$). We suggest that Ramsay *et al*.'s results may have been influenced by the collection of relatively few samples over a broad temporal range. Specifically, their fall samples (eight males, 14 females) were collected in north-eastern Manitoba from August through November. This period constitutes nearly the entire onshore fast for non-pregnant bears, and the mean physiological condition of bears at the beginning of the fast was likely very different from their condition at the end. The fact that the lipid content values reported by Ramsay *et al*. (1992) were 8.2 and 22.8% lower than our values for males and females, respectively, further suggests that these bears had been fasting for a longer period than the bears we sampled. The larger discrepancy between the two studies for females than for males may indicate that females

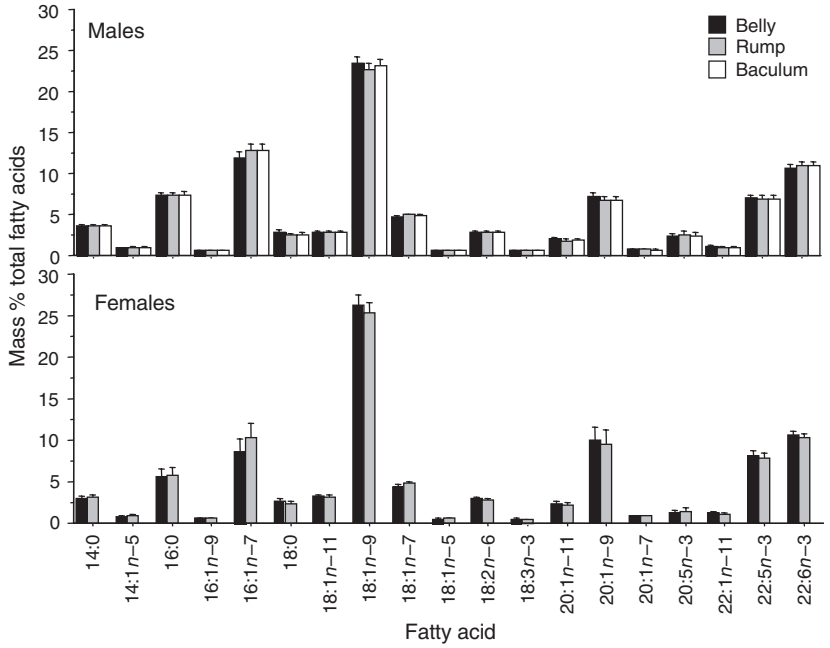

Figure 7 The 19 most abundant fatty acids (mass % of total + 1 SEM) of polar bears *Ursus maritimus* (> 2.5 years) killed by native subsistence hunters near Arviat, NU (Fig. 1). Fat was collected from the belly, rump and baculum fat depot of each male ($n = 16$), and the belly and rump of each female ($n = 7$).

lose lipid at a higher rate during the onshore fast or that females were sampled later than males in that study.

Although the adipose tissue of the females we sampled contained, on average, more lipid than that of males in the fall, females with cubs had significantly lower adipose lipid content than solitary females at this time (Fig. 4). These data are consistent with the substantial energetic cost of lactation. Female polar bears in western Hudson Bay nurse their cubs for up to 2.5 years (Derocher & Stirling, 1995) and the production of relatively high fat milk (*c.* 30%, Cook *et al.*, 1970; Derocher *et al.*, 1993) likely depletes adipose lipid stores during the onshore fast and limits the mother's ability to accumulate fat while feeding at sea. Although we did not know the pregnancy status of individual bears, the majority (*c.* 80%; Derocher *et al.*, 1992) of solitary females were likely pregnant, and our data indicate that these bears had the highest relative lipid content in the population. An increase in lipid stores in preparation for pregnancy is common among terrestrial mammals, including sheep (Vernon, Clegg & Flint, 1986), cattle (McNamara & Hillers, 1986), rats (Knopp *et al.*, 1973) and humans (Kalkhoff, Kissebah & Kim, 1978). Among marine mammals, pregnant females, or those near parturition, have among the highest blubber lipid content in populations of fin whales *Balaenoptera physalus* (Aguilar & Borrell, 1990), bottlenose dolphins *Tursiops truncatus* (Struntz *et al.*, 2004) and harbour seals *Phoca vitulina* (Bowen *et al.*, 1992). Aguilar & Borrell (1990) also found that solitary, non-pregnant female fin whales had greater lipid content than lactating mothers.

The simultaneous effects of fasting and lactation on adipose tissue composition are further illustrated by our data from female polar bears emerging from maternity dens in spring. These bears had significantly less lipid in their adipose tissue than fall females with or without dependent cubs (Figs 3b and 4). This is consistent with the loss of both adipose lipid content and of overall fat stores. Depletion of lipid stores during lactation is common even in feeding mammals (Grigor *et al.*, 1987; Dewey, Heinig & Nommsen, 1993; Laurien-Kehnen & Trillmich, 2003), but is especially pronounced in species that fast for part or all of the lactation period (Lockyer, 1986; Bowen *et al.*, 1992; Beck, Smith & Hammill, 1993; Atkinson & Ramsay, 1995; Farley & Robbins, 1995). The lipid content of adipose tissue also decreases in hibernating mammals during their dormant phase (Moreau-Hamsany *et al.*, 1988).

Although our female seasonal trend is consistent with the physiological effects of an extended fast coupled with the metabolic costs of reproduction, it conflicts with the results of Ramsay *et al.* (1992), who found that per cent lipid content in the adipose tissue of adult females actually increased from fall to spring. We suggest that their results may again have been affected by a small sample size ($n = 34$), spread over a large temporal and spatial range. In addition to sampling adult female polar bears emerging from dens in north-eastern Manitoba, the spring sample of Ramsay *et al.* included bears captured on the sea ice of the eastern Beaufort Sea, where many of the bears had resumed feeding. Consistent with this, Ramsay *et al.* (1992) reported spring lipid content values for females with cubs that were 11.3% higher than our data. Because the annual fast in western Hudson Bay was likely a driving factor behind the seasonal and sex differences we observed in lipid content, such differences may be less pronounced or absent in populations that experience a shorter onshore period (e.g. the Davis Strait population; Stirling, Calvert & Andriashek, 1980) or those that spend no time on land at all (e.g. High Arctic populations; Stirling, Calvert & Andriashek, 1984). For instance, the bears we sampled in the spring

in the Beaufort Sea region showed no sex differences in adipose lipid content ($P = 0.204$) and these bears may remain on the sea ice year-round (Lentfer, 1975). However, if we had sampled Beaufort bears in the fall, when pregnant females were preparing to den, we may have found sex differences associated with the differing costs of reproduction.

The significant interaction between sampling year and the spring-to-fall decline in the lipid content of female adipose tissue in north-eastern Manitoba appeared to be because of a relatively steady decline in spring lipid content over the course of our study (Fig. 3b). Each spring, we had more difficulty locating family groups and, consequently, our sample size declined each year: 12, seven and four adult females were captured in 2002, 2003 and 2004, respectively. In addition, a recent trend towards earlier summer break-up of the sea ice in Hudson Bay (see Gough, Cornwell & Tsuji, 2004) has resulted in polar bears having less time each year to hunt seals and has forced the population to fast on shore for *c*. 2.5 weeks longer than it did 30 years ago (Stirling, Lunn & Iacozza, 1999; Stirling *et al*., 2004). As a result, the body condition of adult polar bears in north-eastern Manitoba has declined significantly (Stirling *et al*., 1999; Derocher, Lunn & Stirling, 2004) and the drop in female condition appears to be the cause of a concomitant decrease in female natality (Stirling *et al*., 1999).

Our declining sampling success in spring over the 3 years of this study may indicate that some females were abandoning their dens (either with or without cubs) before the traditional time of emergence – one of the predicted effects of climate change on western Hudson Bay polar bears (Derocher *et al*., 2004). Additionally, the progressive decline in the lipid content of female adipose tissue in spring (Fig. 3b) is consistent with a large-scale decline in the overall body condition of new mothers. Although our current sample size of spring females is small, the clear relationship between lipid content and nutritional status suggests that adipose tissue samples may provide valuable insights into the effects of climate change on polar bear populations. Superficial fat that is easily collected from tagged or harvested bears could provide information on long-term changes in body condition that arise from changes in ice conditions and prey distribution.

Anatomical variation in the lipid content and FA composition of adipose tissue

The FA composition of adipose tissue that we sampled from polar bears in the Beaufort Sea (Fig. 5) and western Hudson Bay (Fig. 7) was generally similar to bears in other parts of the Arctic (e.g. Innis & Kuhnlein, 1987; Pond *et al*., 1992; Iverson *et al*., in press). Polar bears in the wild are characterized by high levels of the monounsaturated and polyunsaturated FAs that characterize their marine ecosystems and, significant regional differences arise from localized variability in prey sources and foraging patterns (e.g. Iverson *et al*., in press). Although the focus of this part of the study was on adipose tissue variability within individuals, polar bears in the Beaufort Sea (Fig. 5) and western Hudson Bay (Fig. 7) appeared to differ in their relative amounts of several FAs and isomers.

The FA composition of bears in the Beaufort Sea did not vary across the depth of superficial adipose tissue (Fig. 5). This is consistent with two previous studies, although these earlier reports were largely equivocal. Pond *et al*. (1992) compared enzymatic activity in the inner and outer portions of five superficial depots in 10 polar bears and found no evidence of stratification. Although these authors did find that long-chain FAs were more abundant in the outer portions of superficial fat, this finding was based on results from only two young bears (aged 2 and 4 years). Grahl-Nielsen *et al*. (2003) also reported some evidence of FA stratification in polar bear adipose tissue; however, their use of individual *t*-tests to examine 28 FAs was inappropriate (Grahl-Nielsen *et al*., 2004; Thiemann *et al*., 2004*a*) and the magnitude of the differences was generally very small. In contrast, marine mammal blubber is usually stratified in FA composition through its depth, sometimes substantially. This largely reflects differences in the primary role of blubber near the body core versus the body surface: energy storage versus structural functions, respectively (Koopman, Iverson & Gaskin, 1996; Mau & Castellini, 2001; Koopman *et al*., 2002; Struntz *et al*., 2004). The Beaufort bears were sampled at extremely cold temperatures (−5 to −30 °C), but clearly no temperature-related FA stratification was apparent. These results support the conclusion of Pond *et al*. (1992) that the superficial adipose tissue of polar bears is similar to typical forms of mammalian adipose tissue and acts almost exclusively as an energy storage depot.

In contrast to the uniformity of FA composition across the depth of superficial fat in polar bears, there was a significant gradient in mean lipid content, declining from the muscle to the skin (Fig. 6). Although it is possible that some outer samples included a small portion of the epidermis, thereby increasing the relative contribution of non-lipid components, this does not account for the significant difference between inner and middle sections. Although harp seals show no evidence of variation in lipid content with depth (Beck *et al*., 1993), cetaceans appear to preferentially mobilize and deposit lipid in the inner blubber layer (Aguilar & Borrell, 1990; Mau & Castellini, 2001; Koopman *et al*., 2002; Evans, Hindell & Thiele, 2003; Struntz *et al*., 2004). In mammals with typical adipose tissue, some metabolic differences have been shown to exist across the depth of subcutaneous fat (e.g. Hood & Allen, 1973; Enevoldsen *et al*., 2001), but little is known about how lipid content varies with depth. The polar bears that we sampled were actively hunting on the sea ice and therefore were likely accumulating fat stores. Our results suggest that in a thick layer of superficial adipose tissue, as is carried by polar bears, the inner adipocytes may fill more rapidly as lipid is deposited. However, these results may not apply to bears in every region at all times of year. For instance, it will be important to examine potential lipid content stratification in bears fasting during the fall in north-eastern Manitoba.

Within individual bears, both the lipid content and FA composition of adipose tissue did not differ across large superficial depots (Fig. 7). The layer of superficial adipose tissue in polar bears is formed by the lateral expansion of discrete fat depots into an apparently continuous mass (Pond *et al*., 1992). In most studies involving the collection of adipose tissue from live polar bears, a biopsy is taken through the skin near the base of the tail (e.g. Ramsay *et al*., 1992; Grahl-Nielsen *et al*., 2003; Iverson *et al*., in press; this study). In healthy polar bears, this rump depot tends to be the largest and is the fastest growing during fattening (Pond *et al*., 1992), thus making it an ideal sampling site for studies of the effects of diet on adipose composition. Our data indicate that other large superficial depots will just as accurately reflect recent dietary history.

Although the bears that we examined were all harvested in the fall and had recently finished their annual fast, there is no reason to suspect that polar bears in other populations or other times of the year would not yield similarly uniform results. Our findings are consistent with those of Ramsay *et al*. (1992), who examined nine females with cubs and found that the mean adipocyte volume did not vary between belly and rump depots. Although site-specific differences have been found in sheep *Ovis aries* (Banskalieva, 1996) and goats *Capra hircus* (Bas *et al*., 1987), studies of Svalbard reindeer *Rangifer tarandus platyrhynchus* (Pond *et al*., 1993), marmots *Marmota flaviventris* (Florant *et al*., 1990), mink *Mustela vison* (Layton, 1998) and seabirds (Iverson & Springer, 2002) all showed no differences in the FA composition of superficial adipose tissue sampled across sites on the body. Among marine mammals, blubber FA composition also remains consistent across sites on the main part of the body in harp and grey seals (Beck *et al*., 1993; Cooper, 2005; S. Tucker & S. Iverson, pers. comm.) and harbour porpoises (Koopman *et al*., 1996, 2002), and only differs in the structural extremities such as around the tailstock in cetaceans (Koopman *et al*., 2002). Although some small adipose depots may have specialized functions, such as supplying FAs to lymphoid cells (Pond & Mattacks, 2003), our results indicate that FA composition among large superficial depots is largely uniform and that a single sample will accurately reflect a polar bear's subcutaneous lipid energy store. In addition to providing important insights into polar bear lipid physiology, this finding may substantially increase the number of samples available for future analyses as the bacula from hunted bears are routinely submitted to resource managers for sex verification.

In summary, our results demonstrate that differences in the lipid content of superficial adipose tissue in polar bears can provide insights into temporal and demographic variations in physiological condition and status, which in turn may reflect large-scale ecological variability and sex differences in reproductive costs. We suggest that changes in the adipose lipid content of individual bears and populations may reflect changes in foraging patterns, food supply and the long-term effects of arctic warming. Thus, given that a sample from any large superficial depot will accurately reflect the overall FA composition and lipid content of a polar bear's adipose tissue, regardless of sample depth or orientation, single samples from captured or harvested bears can be used to answer important questions about polar bear ecology and the Arctic.

Acknowledgements

We are particularly grateful to Mitch Campbell of the Nunavut Department of Sustainable Development and to the Arviat hunters for collecting fat samples from bears harvested in their annual subsistence hunts. Dr N. J. Lunn and D. Andriashek, Canadian Wildlife Service, assisted with the collection of fat specimens from polar bears handled in mark–recapture studies in Manitoba and the Beaufort Sea. Two anonymous reviewers provided valuable comments on an earlier version of the manuscript. This study was primarily supported by Natural Sciences and Engineering Research Council (NSERC, Canada) and Killam Trust Scholarships to G. W. T., by NSERC operating and equipment grants to S. J. I. and by the Canadian Wildlife Service. We are also grateful to the following organizations for additional financial support: the Northern Scientific Training Program, Government of Northwest Territories, Government of Nunavut, National Fish and Wildlife Foundation, Nunavut Wildlife Management Board, World Wildlife Fund (Canada and International), Polar Continental Shelf Project, Fisheries and Oceans Canada and the Churchill Northern Studies Centre.

References

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

Aguilar, A. & Borrell, A. (1990). Patterns of lipid content and stratification in the blubber of fin whales (*Balaenoptera physalus*). *J. Mamm.* **71**, 544–554.

Atkinson, S.N., Nelson, R.A. & Ramsay, M.A. (1996). Changes in the body composition of fasting polar bears (*Ursus maritimus*): the effect of relative fatness on protein conservation. *Physiol. Zool.* **69**, 304–316.

Atkinson, S.N. & Ramsay, M.A. (1995). The effects of prolonged fasting of the body composition and reproductive success of female polar bears (*Ursus maritimus*). *Funct. Ecol.* **9**, 559–567.

Banskalieva, V. (1996). Effect of age, physiological state and nutrition on fatty acid composition in depot fat and ruminal volatile fatty acids in sheep. *Small Rumin. Res.* **24**, 37–42.

Bas, P., Chilliard, Y., Morand-Fehr, P., Rouzeau, A. & Mandran, N. (1987). Composition of adipose tissue in alpine goats during late lactation. *Ann. Zootech.* **36**, 361–374.

Beck, C.A., Bowen, W.D. & Iverson, S.J. (2003). Sex differences in the seasonal patterns of energy storage and expenditure in a phocid seal. *J. Anim. Ecol.* **72**, 280–291.

Beck, G.G., Smith, T.G. & Hammill, M.O. (1993). Evaluation of body condition in the northwest Atlantic harp seal (*Phoca groenlandica*). *Can. J. Fish. Aquat. Sci.* **50**, 1372–1381.

Best, R.C. (1984). Digestibility of ringed seals by the polar bear. *Can. J. Zool.* **63**, 1033–1036.

Bowen, W.D., Oftedal, O.T. & Boness, D.J. (1992). Mass and energy transfer during lactation in a small phocid, the harbour seal (*Phoca vitulina*). *Physiol. Zool.* **65**, 844–866.

Budge, S.M., Iverson, S.J., Bowen, W.D. & Ackman, R.G. (2002). Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. *Can. J. Fish. Aquat. Sci.* **59**, 886–898.

Calvert, W. & Ramsay, M.A. (1998). Evaluation of age determination of polar bears by counts of cementum growth layer groups. *Ursus* **10**, 449–453.

Colby, R.H., Mattacks, C.A. & Pond, C.M. (1993). The gross anatomy, cellular structure and fatty acid composition of adipose tissue in captive polar bears (*Ursus maritimus*). *Zoo Biol.* **12**, 267–275.

Cook, H.W., Lentfer, J.W., Pearson, A.M. & Baker, B.E. (1970). Polar bear milk. IV. Gross composition, fatty acid, and mineral constitution. *Can. J. Zool.* **48**, 217–219.

Cooper, M.H. (2005). *Fatty acid metabolism in marine carnivores: implications for quantitative estimation of predator diets*. PhD thesis, Dalhousie University.

Derocher, A.E., Andriashek, D. & Arnould, J.P.Y. (1993). Aspects of milk composition and lactation in polar bears. *Can. J. Zool.* **71**, 561–567.

Derocher, A.E., Lunn, N.J. & Stirling, I. (2004). Polar bears in a warming climate. *Integr. Comp. Biol.* **44**, 163–176.

Derocher, A.E., Nelson, R.A., Stirling, I. & Ramsay, M.A. (1990). Effects of fasting and feeding on serum urea and serum creatinine levels in polar bears. *Mar. Mamm. Sci.* **6**, 196–203.

Derocher, A.E. & Stirling, I. (1995). Temporal variation in reproduction and body mass of polar bears in western Hudson Bay. *Can. J. Zool.* **73**, 1657–1665.

Derocher, A.E. & Stirling, I. (1996). Aspects of survival in juvenile polar bears. *Can. J. Zool.* **74**, 1246–1252.

Derocher, A.E. & Stirling, I. (1998). Maternal investment and factors affecting offspring size in polar bears (*Ursus maritimus*). *J. Zool. (Lond.)* **245**, 253–260.

Derocher, A.E., Stirling, I. & Andriashek, D. (1992). Pregnancy rates and serum progesterone levels of polar bears in western Hudson Bay. *Can. J. Zool.* **70**, 561–566.

Dewey, K.G., Heinig, M.J. & Nommsen, L.A. (1993). Maternal weight-loss patterns during prolonged lactation. *Am. J. Clin. Nutr.* **58**, 162–166.

Enevoldsen, L.H., Simonsen, L., Stallknecht, B., Galbo, H. & Bulow, J. (2001). *In vivo* human lipolytic activity in preperitoneal and subdivisions of subcutaneous abdominal adipose tissue. *Am. J. Physiol. Endocrinol. Metab.* **281**, E1110–E1114.

Evans, K., Hindell, M.A. & Thiele, D. (2003). Body fat and condition in sperm whales, *Physeter macrocephalus*, from southern Australian waters. *Comp. Biochem. Physiol. A* **134**, 847–862.

Farley, S.D. & Robbins, C.T. (1995). Lactation, hibernation, and mass dynamics of American black bears and grizzly bears. *Can. J. Zool.* **73**, 2216–2222.

Florant, G.L., Nuttle, L.C., Mullinex, D.E. & Rintoul, D.A. (1990). Plasma and white adipose tissue lipid composition in marmots. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **258**, 1123–1131.

Gough, W.A., Cornwell, A.R. & Tsuji, L.J.S. (2004). Trends in seasonal sea ice duration in southwestern Hudson Bay. *Arctic* **57**, 299–305.

Grahl-Nielsen, O., Andersen, M., Derocher, A.E., Lydersen, C., Wiig, Ø. & Kovacs, K.M. (2003). Fatty acid composition of the adipose tissue of polar bears and their prey: ringed seals, bearded seals and harp seals. *Mar. Ecol. Prog. Ser.* **265**, 275–282.

Grahl-Nielsen, O., Andersen, M., Derocher, A.E., Lydersen, C., Wiig, Ø. & Kovacs, K.M. (2004). Reply to comment on Grahl-Nielsen *et al.* (2003): sampling, data treatment and predictions in investigations on fatty acids in marine mammals. *Mar. Ecol. Prog. Ser.* **281**, 303–306.

Grigor, M.R., Allan, J.E., Carrington, J.M., Carne, A., Geursen, A., Young, D., Thompson, M.P., Haynes, E.B. & Coleman, R.A. (1987). Effect of dietary protein and food restriction on milk production and composition, maternal tissues and enzymes in lactating rats. *J. Nutr.* **117**, 1247–1258.

Hilditch, T.P. & Williams, P.N. (1964). *The chemical constitution of natural fats*. London: Chapman & Hall.

Hood, R.L. & Allen, C.E. (1973). Lipogenic enzyme activity in adipose tissue during the growth of swine with different propensities to fatten. *J. Nutr.* **103**, 353–362.

Innis, S.M. & Kuhnlein, H.V. (1987). The fatty acid composition of Northern-Canadian marine and terrestrial mammals. *Acta Med. Scanda.* **222**, 105–109.

Iverson, S.J. (2002). Blubber. In *Encyclopedia of marine mammals*: 107–112. Perrin, W.F., Würsig, B. & Thewissen, J.G.M. (Eds). New York: Academic Press.

Iverson, S.J., Field, C., Bowen, W.D. & Blanchard, W. (2004). Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecol. Monogr.* **74**, 211–235.

Iverson, S.J., Frost, K.J. & Lang, S.L.C. (2002). Fat content and fatty acid composition of forage fish and invertebrates in Prince William Sound, Alaska: factors contributing to among and within species variability. *Mar. Ecol. Prog. Ser.* **241**, 161–181.

Iverson, S.J., Frost, K.J. & Lowry, L.F. (1997). Fatty acid signatures reveal fine scale structure of foraging

distribution of harbor seals and their prey in Prince William Sound, Alaska. *Mar. Ecol. Prog. Ser.* **151**, 255–271.

Iverson, S.J., Lang, S.L.C. & Cooper, M.H. (2001). Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids* **36**, 1283–1287.

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

Iverson, S.J. & Springer, A.M. (2002). *Estimating seabird diets using fatty acids: protocol development and testing of ReFER hypotheses*. Report to the National Pacific Marine Research Program, University of Alaska, Fairbanks.

Iverson, S.J., Stirling, I. & Lang, S. (in press). Spatial and temporal variation in the diets of polar bears across the Canadian arctic: indicators of changes in prey populations and environment. *Symp. Zool. Soc. Lond.*

Kalkhoff, R.K., Kissebah, A.H. & Kim, H.J. (1978). Carbohydrate and lipid metabolism during normal pregnancy: relationship to gestational hormone action. *Semin. Perinatol.* **2**, 291–307.

Knopp, R.H., Saudek, C.D., Arky, R.A. & O'Sullivan, J.B. (1973). Two phases of adipose tissue metabolism in pregnancy: maternal adaptations for fetal growth. *Endocrinology* **92**, 984–988.

Kolenosky, G.B., Lunn, N.J., Greenwood, C.J. & Abraham, K.F. (1989). Estimating the weight of polar bears from body measurements. *J. Wildl. Mgmt.* **53**, 188–190.

Koopman, H.N. (2001). *The structure and function of the blubber of odontocetes*. PhD thesis, Nicholas School of the Environment, Duke University.

Koopman, H.N., Iverson, S.J. & Gaskin, D.E. (1996). Stratification and age-related differences in blubber fatty acids of the male harbour porpoise (*Phocoena phocoena*). *J. Comp. Physiol. B* **165**, 628–639.

Koopman, H.N., Pabst, D.A., McLellan, W.A., Dillaman, R.M. & Read, A.J. (2002). Changes in blubber distribution and morphology associated with starvation in the harbor porpoise (*Phocoena phocoena*): evidence for regional differences in blubber structure and function. *Physiol. Biochem. Zool.* **75**, 498–512.

Laurien-Kehnen, C. & Trillmich, F. (2003). Lactation performance of guinea pigs (*Cavia porcellus*) does not respond to experimental manipulation of pup demands. *Behav. Ecol. Sociobiol.* **53**, 145–152.

Layton, H. (1998). *Development of digestive capabilities and improvement of diet utilization by mink pre- and post-weaning with emphasis on gastric lipase*. MSc thesis, Nova Scotia Agricultural College.

Lentfer, J.W. (1975). Polar bear denning on drifting sea ice. *J. Mammal.* **56**, 716–718.

Lockyer, C. (1986). Body fat condition in northeast Atlantic fin whales, *Balaenoptera physalus*, and its relationship with reproduction and food resource. *Can. J. Fish. Aquat. Sci.* **43**, 142–147.

Lunn, N.J. & Stirling, I. (1985). The significance of supplemental food to polar bears during the ice-free period of Hudson Bay. *Can. J. Zool.* **63**, 2291–2297.

Martin, A.D., Daniel, M.Z., Drinkwater, D.T. & Clarys, J.P. (1994). Adipose tissue density, estimated adipose tissue lipid fraction and whole body adiposity in male cadavers. *Int. J. Obes. Relat. Metab. Disord.* **18**, 79–83.

Mau, T.L. & Castellini, M.A. (2001). Control mechanisms of fattening in a large whale, *Balaena mysticetus*, and considerations in determining body condition. *FASEB J.* **15**, A90.

McNamara, J.P. & Hillers, J.K. (1986). Adaptations in lipid metabolism of bovine adipose tissue in lactogenesis and lactation. *J. Lipid Res.* **27**, 150–157.

Moreau-Hamsany, C., Castex, C., Hoo-Paris, R., Kacemi, N. & Sutter, B. (1988). Hormonal control of lipolysis from the white adipose tissue of hibernating jerboa (*Jaculus orientalis*). *Comp. Biochem. Physiol. A* **91**, 665–669.

Nelson, R.A., Folk, G.E. Jr, Pfeiffer, E.W., Craighead, J.J., Jonkel, C.J. & Steiger, D.L. (1983). Behavior, biochemistry, and hibernation in black, grizzly, and polar bears. *Int. Conf. Bear Res. Mgmt.* **5**, 284–290.

Polischuk, S.C., Norstrom, R.J. & Ramsay, M.A. (2002). Body burdens and tissue concentrations of organochlorines in polar bears (*Ursus maritimus*) vary during seasonal fasts. *Environ. Pollut.* **118**, 29–39.

Pond, C.M. & Mattacks, C.A. (2003). The source of fatty acids incorporated into proliferating lymphoid cells in immune-stimulated lymph nodes. *Br. J. Nutr.* **89**, 375–382.

Pond, C.M., Mattacks, C.A., Colby, R.H. & Ramsay, M.A. (1992). The anatomy, chemical composition, and metabolism of adipose tissue in wild polar bears (*Ursus maritimus*). *Can. J. Zool.* **70**, 326–341.

Pond, C.M., Mattacks, C.A., Colby, R.H. & Tyler, N.J.C. (1993). The anatomy, chemical composition and maximum glycolytic capacity of adipose tissue in wild Svalbard reindeer (*Rangifer tarandus platyrhynchus*) in winter. *J. Zool. (Lond.)* **229**, 17–40.

Pond, C.M., Mattacks, C.A., Gilmour, I., Johnston, M.A., Pillinger, C.T. & Prestrud, P. (1995). Chemical and carbon isotopic composition of fatty acids in adipose tissue as indicators of dietary history in wild arctic foxes (*Alopex lagopus*) on Svalbard. *J. Zool. (Lond.)* **236**, 611–623.

Raclot, T., Groscolas, R. & Cherel, Y. (1998). Fatty acid evidence for the importance of myctophid fishes in the diet of king penguins, *Aptenodytes patagonicus*. *Mar. Biol.* **132**, 523–533.

Ramsay, M.A. & Hobson, K.A. (1991). Polar bears make little use of terrestrial food webs: evidence from stable-carbon isotope analysis. *Oecologia* **86**, 598–600.

Ramsay, M.A., Mattacks, C.A. & Pond, C.M. (1992). Seasonal and sex differences in the structure and chemical composition of adipose tissue in wild polar bears (*Ursus maritimus*). *J. Zool. (Lond.)* **228**, 533–544.

Ramsay, M.A. & Stirling, I. (1986). On the mating system of polar bears. *Can. J. Zool.* **64**, 2142–2151.

Rouvinen, K. & Kiiskinen, T. (1989). Influence of dietary fat source on the body fat composition of mink (*Mustela vison*) and blue fox (*Alopex lagopus*). *Acta Agric. Scand.* **39**, 279–288.

Schemmel, R. (1976). Physiological considerations of lipid storage and utilization. *Am. Zool.* **16**, 661–670.

Stirling, I., Calvert, W. & Andriashek, D. (1980). Population ecology studies of the polar bear in the area of southeastern Baffin Island. Canadian Wildlife Service Occasional Paper No. 44.

Stirling, I., Calvert, W. & Andriashek, D. (1984). Polar bear (*Ursus maritimus*) ecology and environmental considerations in the Canadian High Arctic. In *Northern ecology and resource management*: 201–222. Olson, R., Hastings, R. & Geddes, F. (Eds). Edmonton: University of Alberta Press.

Stirling, I., Jonkel, C., Smith, P., Robertson, R. & Cross, D. (1977). The ecology of the polar bear (*Ursus maritimus*) along the western coast of Hudson Bay. Canadian Wildlife Service Occasional Paper No. 33.

Stirling, I., Lunn, N.J. & Iacozza, J. (1999). Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. *Arctic* **52**, 294–306.

Stirling, I., Lunn, N.J., Iacozza, J., Elliott, C. & Obbard, M. (2004). Polar bear distribution and abundance on the southwestern Hudson Bay coast during open water season, in relation to population trends and annual ice patterns. *Arctic* **57**, 15–26.

Stirling, I., Spencer, C. & Andriashek, D. (1989). Immobilization of polar bears (*Ursus maritimus*) with Telazol in the Canadian Arctic. *J. Wildl. Dis.* **25**, 159–168.

Struntz, D.J., McLellan, W.A., Dillaman, R.M., Blum, J.E., Kucklick, J.R. & Pabst, D.A. (2004). Blubber development in bottlenose dolphins (*Tursiops truncatus*). *J. Morphol.* **259**, 7–20.

Thiemann, G.W., Budge, S.M., Bowen, W.D. & Iverson, S.J. (2004*a*). Comment on Grahl-Nielsen *et al.* (2003) 'Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals'. *Mar. Ecol. Prog. Ser.* **281**, 297–301.

Thiemann, G.W., Budge, S.M. & Iverson, S.J. (2004*b*). Determining blubber fatty acid composition: a comparison of *in situ* direct and traditional methods. *Mar. Mamm. Sci.* **20**, 284–295.

Vernon, R.G., Clegg, R.A. & Flint, D.J. (1986). Adipose tissue metabolism in sheep: response to season and its modulation by reproductive state. *Horm. Metab. Res.* **18**, 308–312.

Watts, P.D. & Hansen, S.E. (1987). Cyclic starvation as a reproductive strategy in the polar bear. *Symp. Zool. Soc. Lond.* **57**, 305–318.

Zar, J.H. (1999). *Biostatistical analysis*. 4th edn. Upper Saddle River, NJ: Prentice-Hall Inc.