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Original article

Fat dynamics and development of body condition indices for harvested populations of wolverine *Gulo gulo*

Jean-François Robitaille, Liane Villano, Thomas S. Jung, Helen P. Slama & Michelle P. Oakley

Sufficient energy reserves are crucial to the overwinter survival of northern non-hibernating mustelids. We sought a reliable index of body condition (fatness) in harvested populations of wolverine *Gulo gulo*, based on the relationship between fatness and the mass of distinct fat depots extractable by necropsy. Fatness did not differ significantly between genders or winter months, nor was it significantly related to body size or age. Using a first group of 18 males and 14 females, we developed predictive least-square linear regressions between fat depots (popliteal, sternal, omentum, mesenteric and perirenal) and fatness (g fat/100 g body mass) using skinned carcasses provided by fur trappers in the Yukon, Canada. Fatness was consistently better predicted in females than in males. Fatness was best predicted by the sternal fat depot ($R^2 = 0.73$) in males and by the omentum as well as sternal fat depots in females ($R^2 = 0.94$ and 0.87, respectively). We then compared known fatness and fatness predicted from regressions of the sternal fat depot using a second group of 14 males and nine females, and mean fatness did not differ significantly. We suggest that, due to its ease of extraction and predictive power, the sternal fat depot is a valid fat index with both sexes of wolverine, although it (or any other fat depot) should be used with caution with males, which seem more prone to obesity. This new index will help wildlife managers monitor changes in body condition of wolverines in response to changes in environmental conditions.

Key words: body condition, energy reserves, fat dynamics, fatness index, Gulo gulo, wolverine

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The body condition (or nutritional condition; *sensu* Patterson et al. 2000) of an animal refers to its energetic state, where an animal in good condition is assumed to have more energy reserves than an animal in poor condition. Individuals with larger energy reserves may thus have better fasting endurance and higher survival than individuals with smaller reserves (Schulte-Hostedde et al. 2005). Undernutrition is also a factor well known to affect reproduction in adult mammals (e.g. Guinet et al. 1998, Shine et al. 2001).

Especially in predators, fat content is a critical component of body condition because of its direct role as an energy source between meals (Lochmiller et al. 1985, Buskirk & Harlow 1989, Halfpenny & Ozanne 1989, Holand 1992, Pond & Ramsay 1992, Caughley & Sinclair 1994, Robitaille & Cobb 2003). Individuals better able to accumulate and store energy reserves are assumed to have a selective advantage, especially when energy demands are high and food intake does not meet short-term needs (Buskirk & Harlow 1989). From a wildlife manage-

ment perspective, the ability to detect trends in nutritional status will help monitor animal populations (Robitaille & Cobb 2003). If monitoring protocols remain simple, it should allow processing a large numbers of individuals, thus attaining the sample sizes necessary to detect and measure spatial or temporal trends at fine scales or where individual variability may be high.

The traditional study of body composition (i.e. water, protein and lipids) from carcasses allows assessing individual health and nutritional status (Winstanley et al. 1998, Garant & Crête 1999), and such a comprehensive, laboratory-based approach is most informative about the nutritional state of animals. However, its limited applicability at a large scale has stimulated interest in the development of condition indices based on fat content (Batzli & Esseks 1992, Virgl & Messier 1993, Matlack & Evans 1994, Winstanley et al. 1998, Garant & Crête 1999, Robitaille & Cobb 2003, Robitaille & Jensen 2005).

Following Brown & Lasiewski's (1972) landmark paper on metabolism and body shape in weasels *Mustela* sp., mustelids are known to carry relatively little fat. While this suggests that fat storage is a limiting factor of energy in weasels, it also means that assessing the nutritional condition in weasels requires whole body fat extraction. However, it has since been observed that some mustelids, such as fisher *Martes pennanti*, possess fat deposits that can be used as indices of fatness in the entire animal (Garant & Crête 1999, Robitaille & Jensen 2005). Some studies have used fat depots to analyze nutritional status in wild populations (Clem 1977, Rego 1984). Internal fat depots in American marten *Martes americana* were considered by Buskirk & Harlow (1989) and by Robitaille & Cobb (2003) as valid indices of fatness. In fishers from Maine, USA, Rego (1984) reported a correlation between a variety of body condition indices and mesenteric fat. In Ontario, Canada, temporal variation in mesenteric fat contents in fishers was also observed by Clem (1977) in a short time frame (November-December). Abdominal (Coulter 1966), renal and omental fat (Kuehn 1989) have also been proposed as indices of body fat in fishers. Garant & Crête (1999) developed regression models that estimated total body fat, water and protein levels of fishers from southern Québec, Canada. The accuracy of almost any of the five discernable fat depots appeared adequate to detect changes of fat levels in harvested Ontario fisher populations (Robitaille & Jensen 2005). Pond and collaborators provided detailed biochemical and body compositional data for a number of carnivores (Pond & Ramsay 1992). In wolverine *Gulo gulo*, Pond et al. (1994) provided detailed cytological as well as macroscopic analyses of fat distribution. Their analyses revealed that fat allocation to depots varied at different rates with fatness in a fashion similar to that described for other species (Pond & Ramsay 1992) including American marten (Robitaille & Cobb 2003) and fisher (Robitaille & Jensen 2005). Pond et al.'s (1994) study failed to detect significant differences in fatness between genders, possibly due to a small female sample size ($N = 5$). This also prevented further comparisons of fat dynamics between genders. The total storage lipid content of each carcass calculated from the chemical analyses correlated closely with fatness determined by gross dissection (Pond et al. 1994).

The objective of our study was to develop and validate fat indices in harvested populations of wolverine. On average, > 500 wolverines are legally harvested for fur each winter in Canada (Slough 2007). With the collaboration of trappers, relatively large numbers of carcasses could be analyzed to monitor spatial and temporal variation in wolverine body condition. Specifically, based on the relationship between selected macroscopic fat depots and body fat content, we built and evaluated regression-based predictive equations in order to identify the fat depot(s) that best predict(s) fatness in wolverine. Our study extends from previous work by addressing contrasts in fat dynamics between male and female wolverines. We further test the accuracy of predicted fatness against known fatness obtained by fat extraction on a separate sample. Our hypothesis is that the heavier fat depots will perform better at predicting fatness simply because they contribute more to it than smaller depots. Based on patterns known in wolverine and other carnivores (see above), the larger extractable depots would include (paired) perirenal, omentum and mesentery.

Methods

Specimen collection

We obtained skinned wolverine carcasses from fur trappers in the Yukon during the 2005/06 ($N = 68$) and 2006/07 ($N = 78$) trapping seasons, and stored them frozen until necropsy (< 6 months at -18°C). Carcasses were thawed at room temperature, sexed, weighed (using a Pesola scale $\pm$ 100 g) and retained for our study if the carcass condition was good (i.e.

no apparent freezer burn or damage from skinning or scavenging) and an ancillary data set (e.g. age and body mass) was complete. The heads were removed for separate studies. Because we received skinned carcasses and wished to use total body mass in the calculation of fatness, total body mass was estimated as 116.5% of the mass of the skinned carcass using Pond et al.'s (1994) measures of pelt masses in 23 wolverines. Those specimens were, as in our study, skinned by professional trappers. Thus, very little subcutaneous adipose tissue appeared to have been removed in the skinning process, and no correction for such losses was applied (Pond et al. 1994). In a few specimens, the head and/or the paws had been removed, so their mass was estimated from that of the appendages of other specimens of similar size.

We used 32 specimens (18 males and 14 females) from the 2005/06 harvest for the development of initial models, and 23 specimens (14 males and nine females) from the 2006/07 harvest as a test group to validate the models. Specimens were selected to obtain a wide range of fat condition, which was estimated at first examination by two independent observers using an ordinal scale (1-3: very poor-excellent condition) of subcutaneous fat levels. However, we excluded animals that displayed especially odd proportions in fat depot values. Other criteria were fairly balanced sex samples and accurate body masses. Wolverines were aged at a commercial lab (Matson's, Missoula, Montana, USA), by using counts of *cementum annuli* on a first premolar (PM1; Poole et al. 1994). Wolverines ranged between 0 (i.e. < 1 year) and eight years of age in the development group, and between 0 and 10 years of age in the test group. Harvest dates were between mid-December and early-March, and similarly distributed in both years. All wolverines were legally harvested by licensed fur trappers in accordance with regulations in the Yukon Wildlife Act.

Potential fat indices

We based our choice of test indices on the distribution of various fat depots in Carnivora (Pond & Ramsay 1992, Pond et al. 1992), particularly American marten (Robitaille & Cobb 2003), fisher (Robitaille & Jensen 2005) and wolverine (Pond et al. 1994), and on our preliminary observations of wolverine carcasses. We chose specific fat depots also based on their ease of access during necropsy and whether they were clearly discernable. The intramuscular paired popliteal fat depot, located posteriorly to the distal end of femurs, was the most superficial of the five selected fat depots. The intra-abdominal sternal fat depot was a relatively small v-shaped fat lump beneath the sternum and extending along abdominal muscles, corresponding to Pond et al.'s (1994) inner ventral wall of the abdomen. The greater omentum (hereafter omentum) was the mesentery attached to the greater curvature of the stomach and was much larger than the popliteal or sternal depots. The mesentery fat depot was the radial mesentery suspending the intestines from the dorsal wall of the abdominal cavity. This depot included a relatively large lymph node which was discarded prior to weighing due to its large vascular (i.e. non-fat) content. The paired perirenal fat depot, the most internal of the selected fat depots, surrounded each kidney and extended caudally in the pelvic area of the coelom. Coefficient of variation (CV) of depot fresh masses ranged between 41 and 53% in males and between 56 and 79% in females. Considering the fresh condition of carcasses (percent water in pre-drying carcasses ranged between 52 and 63) and the fact that all depots but the popliteal depot were internal (thus less exposed to dehydration), the variability in water content of fresh fat depots was deemed negligible.

In each carcass, the five depots were carefully dissected and their fresh mass measured using an electronic balance ($\pm$ 0.01 g; Sartorius, Goettingen, Germany). Some data were missing for some specimens (Table 1) due to obvious errors in data recording or entry. Considering the care taken to analyze only fresh specimens (see handling above), the fresh masses of fat depots were preferred to their dry mass to facilitate data collection in subsequent large-scale sampling. The issue of water content was tested by Cobb (2000) who concluded that in American marten, dehydration rates were variable, which was attributed to marten's small size and high surface-to-volume ratio, and lack of proper packaging of carcasses prior to necropsy. These issues were resolved with the wolverines as they are larger-sized (which decreases their relative surface area), and each carcass was carefully wrapped in plastic prior to necropsy and necropsied within half a day after they were thawed.

Fat extraction

Drying in preparation for grinding and fat extraction involved dismembering the carcass and sectioning the trunk to fit into two or three metal trays, and then dried to constant mass at 60-80°C for 4-6 days. The following structures were removed from each carcass

Table 1. Relative mass (in % of body mass) of fat depots in harvested male and female wolverines from the Yukon, 2005/06 and 2006/07. Sample sizes vary due to missing data for a particular variable. Total body mass was based on skinned carcass mass according to Pond et al. (1994).

	Skinned carcass mass (in kg)	Body mass (in kg)	Trunk length (in mm)	Tail length (in mm)	Percent tail (in %)	Fat depots (in % of body mass)				
						Popliteal	Sternal	Omentum	Mesentery	Perirenal
Males										
N	32	32	31	31	31	32	32	32	32	31
Range	6.4-13.3	7.5-15.5	631-850	165-274	18-30	0.02-0.31	0.03-0.42	0.17-1.12	0.17-1.35	0.13-1.15
Mean	9.8	11.3	738	207	22	0.15	0.20	0.61	0.58	0.54
SD	1.6	1.8	49.2	24	2	0.06	0.10	0.24	0.26	0.27
CV	16.1	16.1	6.7	11.4	11.0	42.9	50.0	40.0	45.3	50.1
Females										
N	23	23	23	23	23	21	21	23	23	23
Range	3.8-7.8	4.4-9.1	605-785	153-223	18-25	0.02-0.53	0.03-0.46	0.18-1.53	0.12-1.05	0.10-2.02
Mean	6.3	7.3	664.7	190	22	0.14	0.15	0.58	0.49	0.56
SD	1.0	1.2	36.2	18	2	0.12	0.11	0.34	0.27	0.44
CV	16.4	16.4	5.4	9.6	8.6	75.0	72.5	59.2	56.1	78.5

prior to drying: the five dissected fat depots as described above, the kidneys and heads that were kept for other studies, tail and paws as their availability varied with each carcass provided by trappers and pelt due to its commercial value. However, with the exception of the five fat depots, fat extraction included all intra- and inter-muscular, as well subcutaneous fat layers typically left with the carcass during professional skinning.

The dried carcass was submersed in liquid nitrogen and mechanically ground into a homogenate using a custom-made steel pestle and a 4-l steel cylinder. Since wolverines are relatively large, grinding resulted in heterogeneous material ranging from fine powder to coarse bone fragments. To minimize the effect of heterogeneity on fat extraction, the ground material for each animal was separated into three grain sizes (fine, medium and coarse) using 8-mm and 4-mm mesh sieves prior to fat extraction. As we decided to extract fat from aliquots of approximately 100 g for each carcass, each of the three grain sizes was weighed, and a proportional amount (typically 20-40g) was placed into a pre-weighed 250-ml Erlenmeyer flask. With the development group, extraction was done in duplicate (i.e. two flasks per grain size and carcass) to measure error in estimated fat content based on 20-40 g samples. Fat extractions were performed by adding enough petroleum ether to each flask to cover the entire aliquot, as per Robitaille & Cobb (2003). The flasks were kept at room temperature and agitated periodically over 24-hour periods after which petroleum ether was decanted through filter paper (coarse porosity and fast flow rate; Fisher Scientific Company, Ottawa, Ontario, Canada). New ether was used and extraction continued until the solution was clear, indicating that no more fat was extracted. This extraction method removed all lipids, phospholipids and triacylglycerols in structural adipose tissue, as well as reclaimable storage triacylglycerols. The dry, fatless samples were then weighed and the proportion of fat (sample fatness, i.e. g of fat/100 g homogenate) extracted from each flask was calculated from weight difference before and after extraction. Since we did not use a grinding mill and Soxhlet apparatus for extraction, we were still concerned that the material heterogeneity due to the particular grinding method used, as well as the *ad hoc* procedure for fat extraction, would result in less than total extraction. We performed a paired t-test on the sample fatness between pairs of replicates in the development group ($N = 29$) to determine if 100-g aliquots were sufficient to give a consistent and accurate reading of fat mass. This analysis revealed that there was no significant difference in fatness between the replicated aliquots ($t = 0.811$, $P = 0.42$), indicating that a total of approximately 100 g of wolverine material was sufficient to yield accurate fat content. Furthermore, as we anticipated that fat load would vary with grain size, we performed an analysis of variance (ANOVA) to determine if each of the three different grain sizes varied in fatness. The ANOVA revealed that the different grain sizes yielded significantly different fatness values ($P < 0.001$). This indicated that the ground material was heterogeneous, and that the sieving and separate

fat extraction for each grain size was justified. Thus, with the test group, 100 g of wolverine material was sieved into three grain sizes and fat was extracted as described above. We acknowledge that standardized chemical methods include milling organic material to a fine powder, and that the Soxhlet apparatus makes maximal use of solvent. However, given the amount of solvent used in our 'open' system, and given uniform results from replication, we are confident that our estimates of fatness are realistic and precise.

Pond et al. (1994) defined and measured fatness as "the mass of all (14) dissectible adipose tissue (except the cardiac depots) expressed as a percentage of the estimated total body mass". In this study, fatness was defined as the mass of fat/100 g of total body mass, which was calculated by combining the mass of five dissected depots and fat mass estimated from the total fat extraction procedure. This combination of two data sources, one fresh (depots) and one dry (fat extraction) forced us to estimate non-dissectible fat for the entire body using known pre-drying water content and body mass. Thus, we calculated fatness using the following sequential equations:

$$\text{SampleFatness}\,(\text{g fat/g sample}) = (\text{InitialMass} - \text{FinalMass})/\text{InitialMass}*100 \quad (1),$$

where SampleFatness refers to the proportion of fat in the dry fat extraction aliquot. Initial and final masses were the masses of the aliquot before and after extraction, respectively;

$$\text{BodyFatMass}\,(\text{g}) = \text{SampleFatness}*\text{BodyDryMass} \quad (2),$$

where BodyFatMass is the amount of fat in the entire carcass milled for extraction; SampleFatness is the amount of fat in 100 g of homogenate;

$$\text{Fatness}\,(\text{g fat/100 g body mass}) = (\text{BodyFatMass} + \text{OilMass} + \text{FatDepotMass})/\text{Total body mass}*100 \quad (3),$$

where fatness is defined as the percent fat in the entire animal; oil mass refers to the fluid found in drying pans after drying to constant mass. Total body mass was obtained by correcting skinned carcass mass*1.165 to account for skin mass (Pond et al. 1994). Although other researchers have measured fatness using other denominators (e.g. skinned carcass mass or ingesta-free carcass mass), we preferred to use body mass (i.e. including pelt mass) because of the availability of such a correction factor for pelt (which was not weighed by the trapper and thus not available for our study), and the fatness figures reflected better those of live animals.

Index development and test

To estimate the performance of each depot at predicting known percent fat, fatness (from equation 3) was regressed on the fresh mass (g) of each of the five fat depots. Calculations were done using Microsoft Excel and Statistica Version 8.0.

In developing predictive equations, genders were treated separately due to strong sexual dimorphism in body size. Possible effect of age was also considered, but was assumed to be negligible as juvenile wolverines have nearly reached adult size at the time of harvest (J-F. Robitaille & T.S. Jung, unpubl. data). Model functions were obtained from separate least squares linear regressions of selected depots against fatness, from which explained variance (R^2 values of each regression line) was used to assess accuracy of each depot in representing fatness. In the validation phase, predicted fatness was calculated using the predictive equations and its relationship with known fatness as well as its frequency distribution were compared with that of known fat levels (fatness) using Pearson correlation and paired t-test.

Results

The mean fresh body mass of male wolverines was 11.3 kg (N = 32) whereas that of females was 7.3 kg (N = 23), indicating that females were on average 64% the size of males (see Table 1). As expected, omentum, mesentery and perirenal fat depots were relatively heavy (0.10-2.02% of body mass) in both males and females, whereas popliteal and sternal depots were 3-7 times smaller (0.02-0.53%; see Table 1).

Fatness varied extensively in both male and female wolverines (males: 1.5-15.1% and females: 1.0-16.6%) in 2005/06 and average fatness (males: 7.8 ± 3.5%, N = 32 and females: 6.5 ± 3.8%, N = 23) did not differ significantly between genders, although in 2006/07, females were on average one third (33%) leaner than males (Table 2). Furthermore, coefficients of variation were relatively high despite relatively good sample sizes with each gender/year, suggesting a natural variability of individual fatness in wolverine. Genders were combined to examine effects of body size, age, seasonality and harvest year. There was no relationship between fatness and

Table 2. Fatness (in g fat/100 g of body mass) estimated from whole body fat extraction in free-ranging wolverines collected during winter in the Yukon over two harvest years (2005/06 and 2006/07).

		Fatness (in g fat/100 g body mass)		
Harvest season/sex	N	Range	Mean ± SD	CV
2005/06				
Males	18	1.5-15.1	7.5 ± 3.7	48.8
Females	14	1.0-16.6	7.2 ± 4.6	63.7
2006/07				
Males	14	3.8-15.1	8.2 ± 3.4	41.8
Females	9	3.4-8.7	5.4 ± 1.7	30.8

body mass, indicating that all size cohorts were as likely to include lean or obese animals (Fig. 1). There was also no significant relationship between fatness and age (Fig. 2). This suggests that all age cohorts, including young-of-the-year (i.e. age 0 in our study) and mature adults, were as likely to show signs of food deprivation (emaciation) or excessive energy intake (obesity). There was also no significant seasonal trend in fatness (Fig. 3). Finally, average fatness was only slightly higher in 2005/06 (7.39 ± 4.02%, N = 32) than in 2006/07 (7.06 ± 3.12%, N = 23).

Fatness of females was better predicted by fat depots than fatness of males. With both males and females, the popliteal fat depot was not useful in predicting fatness ($R^2 = 0.30$-0.45; Fig. 4). In males, the fat depot that provided the best estimate for fatness was the sternal depot ($R^2 = 0.73$), followed by the perirenal depot ($R^2 = 0.60$). By comparison, omentum and mesentery fresh mass performed poorly ($R^2 = 0.44$ and 0.39, respectively) as a few males possessed quite large depots for their fatness (see Fig. 4). In females, the omentum fat depot performed particularly well in predicting fatness ($R^2 = 0.94$; see Fig. 4), although sternal and perirenal de-

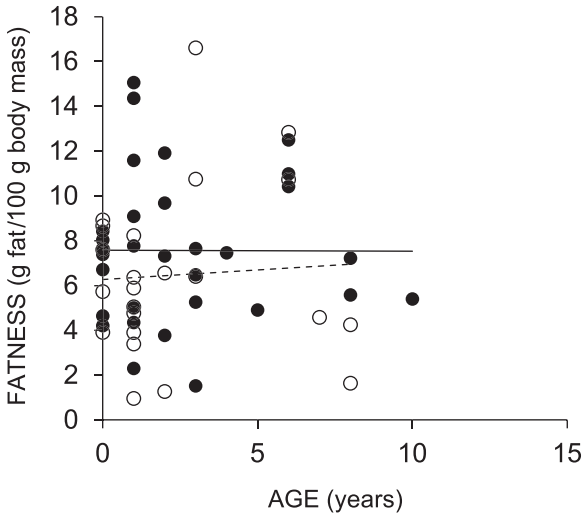

Figure 2. Relationship between age (in years) and fatness in male (● and —) and females (○ and - - -) from the Yukon, Canada. The non-significant relationships ($R^2 = 0.00$ for each gender) indicate no effect of age, in either gender, on the ability to store more or less fat.

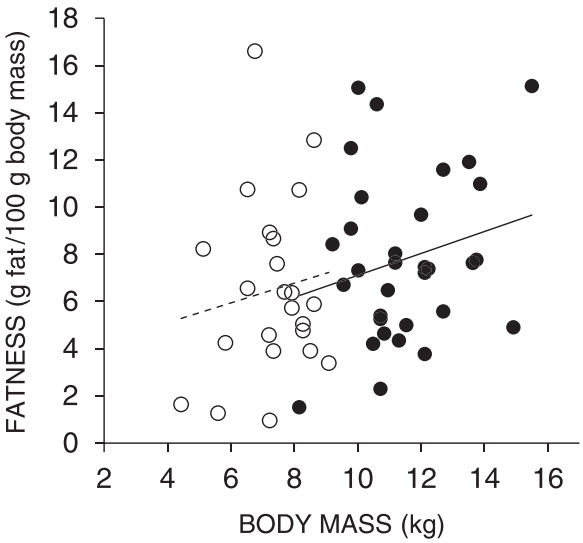

Figure 1. Relationship between fatness and body mass in male (● and —) and female (○ and - - -) wolverines from the Yukon, Canada. The non-significant relationships ($R^2 = 0.05$ and 0.02 for males and females, respectively) indicate no effect of body mass, in either gender, on the ability to store more or less fat.

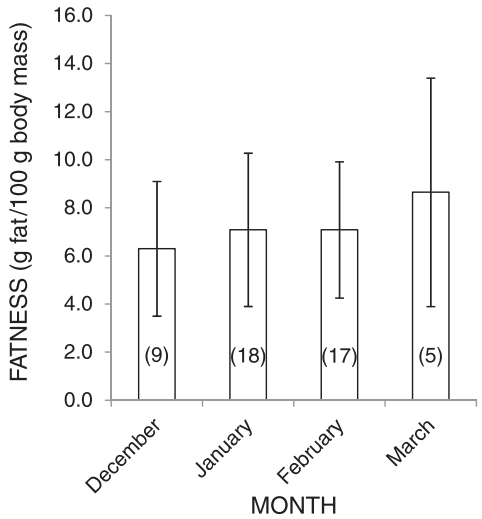

Figure 3. Seasonal trend in fatness (in g fat/100 g body mass) of male and female wolverines combined from harvests 2005/06 and 2006/07 (N = 49).

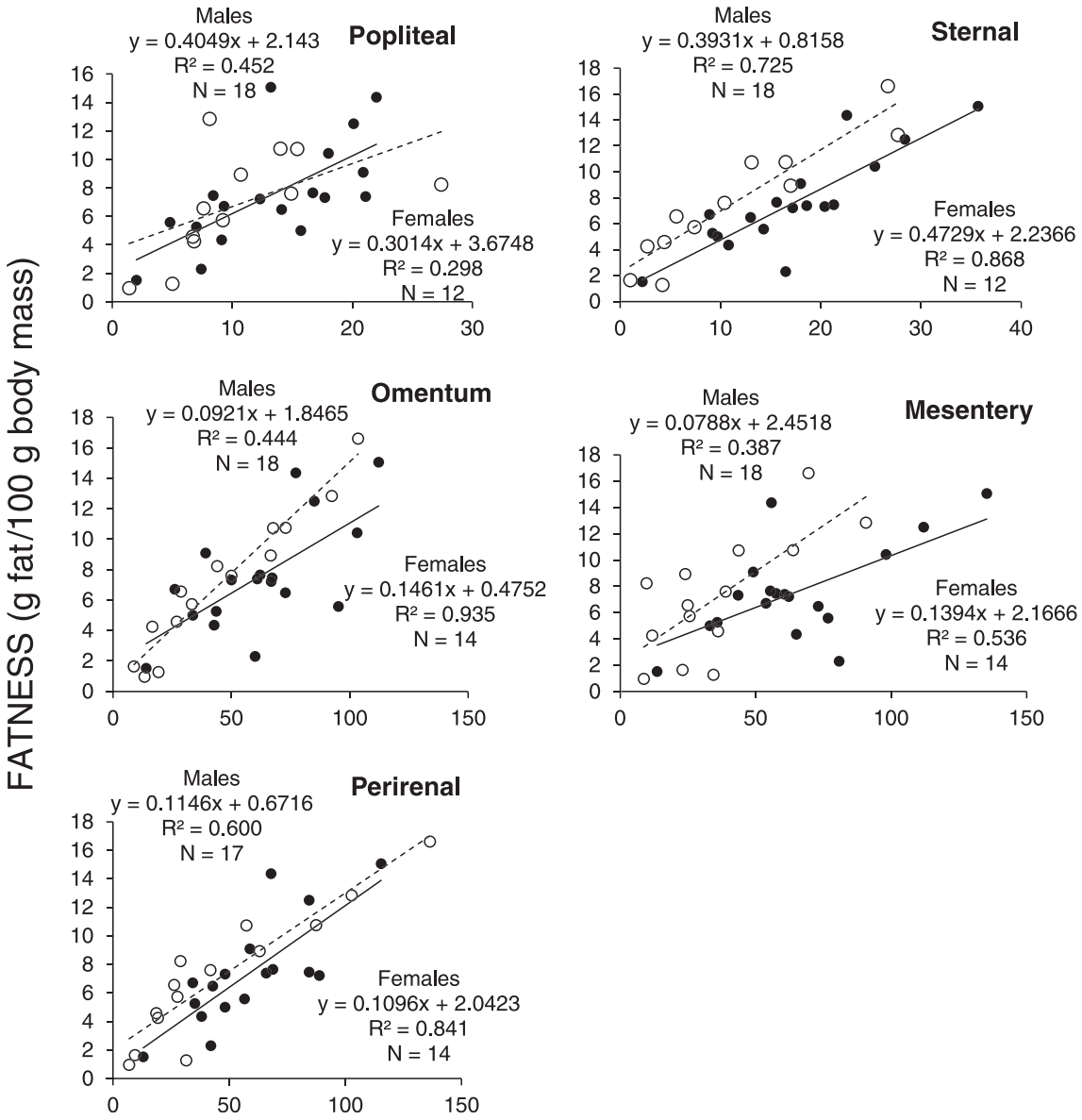

Figure 4. Predictive relationships between fresh mass (in g) of five fat depots (popliteal, sternal, omentum, mesentery and perirenal) and fatness (in g fat/100 g body mass) in male (● and —) and female (○ and ---) wolverine.

pots were also accurate in predicting fatness ($R^2 =$ 0.87 and 0.84, respectively). In comparison, mesentery fat depot ($R^2 = 0.54$) was not as accurate.

In order to validate the performance of the sternal fat depot as a fatness index, the test group of wolverines (N = 23 including 14 males and nine females), for which fatness was known from total body fat extraction, was used to compare fatness predicted by the regressions developed with the first group with known fatness (Fig. 5). This test revealed that average predicted fatness did not differ significantly from actual fatness (7.75 and 7.06%, respectively; $t = 1.78$, $P = 0.08$). However, individual error on the predicted fatness estimates was high, ranging between -33 (underestimate) and 44% (overestimate). A similar calculation was performed using the omentum, an index validated for a number of mustelids (see above), and for female wolverines in our study. As expected, this fat depot did not perform better than the sternal depot, as actual fatness (7.06%) differed significantly from predicted fatness (7.95%; $t = 2.47$, $P < 0.05$). Again, individual error on predicted fatness was variable, ranging between -27 and 76%.

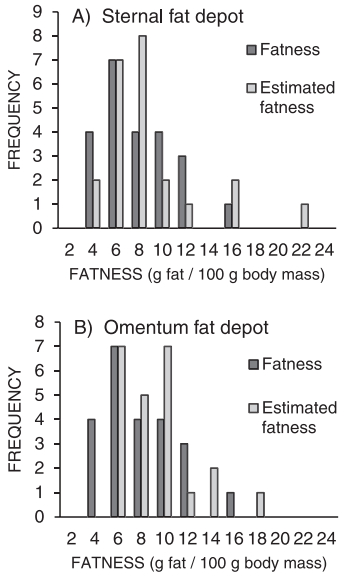

Figure 5. Distribution of actual fatness and fatness estimated from the regression between fatness and A) sternal fat depot fresh mass and B) omentum fat depot.

Discussion

An important issue in the development of a fat index is the choice of a fatness estimate, which should be expressed as grams of fat/100 g of the animal's total body mass (Pond et al. 1994). Thus, we corrected body mass of skinned carcasses using a constant pelt mass (as a proportion of body size), allowing for a small error related to allometric variation in skin-to-body size (i.e. surface-to-volume) ratio. Fur trappers, as trained professionals, were efficient at skinning carcasses without removing subcutaneous fat. Also, as heads were needed for other studies, head fat content was assumed to be proportional to the amount of body fat as seen in fishers (Robitaille & Jensen 2005). We are thus confident that our fatness data are accurate.

In many carnivores, the relative masses of major depots are variable, thus it was suggested (Pond et al. 1995) that indices of fatness that depend upon the dimensions of only one or a few depots (e.g. Holand 1992, Matlack & Evans 1992) are not accurate over more than a narrow range of fatness, unless corrected for changes in fat distribution with body size. In order to control for sexual body size dimorphism, sex differences in fat distribution (Robitaille & Jensen 2005) and in life histories (Schulte-Hostedde et al. 2001), we analyzed males and females separately.

Using fresh mass rather than dry mass will simplify the collection of data in large-scale studies. However, we advise caution in the selection of carcasses. The condition of each carcass should preferably be rated on an ordinal scale (e.g. 1: fresh and complete specimen; 2: specimen has some missing, but correctable body parts such as paws; 3: specimen with evidence of drying, scavenging, decay or freezer burns) to help with the selection of quality sample animals. Also, only tightly wrapped carcasses should be used.

Our study revealed a number of patterns that have a direct impact on the predictability of body fat levels. First, over the two years of study combined, we failed to detect any significant difference in fatness between genders, either in the range or average of fatness, and this was observed in other carnivores (Cobb 2000, Pond et al. 1995, Robitaille & Jensen 2005). However, our data also suggest the occurrence of annual contrasts in fatness between genders.

It also appears that, despite being as fat as female wolverines, males stored fat relatively less in fat depots than through the deposition of fat at other locations, including the thick and widespread subcutaneous lumps and layer, especially in the inguinal region (J-F. Robitaille, pers. obs.; Pond et al. 1994). For instance, the relatively slow enlargement of male omentum and mesentery depots is illustrated by the relatively gentle slopes of regressions for each of these two depots. Pond & Ramsay (1992) clearly showed that in carnivores ranging in body size by several orders of magnitude, the superficial adipose tissue grows isometrically with body size, while intra-abdominal depots become relatively smaller with increasing body mass. It would thus appear that this pattern occurs intra-specifically in wolverines with their clear sexual size dimorphism. Conversely, females' thinner subcutaneous fat did not appear to limit their energy reserves as they had, on average (over two consecutive years), the same amount of fat than males. With sufficient data, fat dynamics of males and females could be further examined with respect to their respective food supply, as well as other life history traits (e.g. metabolism and habitat use).

Using our entire sample (N = 34 males and 23 females), we detected no significant relationship between body mass (including fat mass) and fatness. Our data clearly indicate that fatness occurred in small as well as larger wolverines for both genders. The slight, although insignificant, positive relationship observed may have resulted from including fat mass in body size. It would appear that the fatness of

wolverines is not conditioned (i.e. restricted or relaxed) by body size.

We also found no evidence that fatness was related to age (range: 0-8 years). Thus, it appears that no age cohort could be limited in their nutritional condition. We also found no evidence of seasonal trend in fatness, a result consistent with previous studies of body condition in the American marten (Cobb 2000). In small and medium-sized mustelids sustaining on patchy resources, food deprivation probably occurs on a short-term basis, which would explain the wide range of fatness registered in any month of the harvest season (December-March in the Yukon). Studies of non-hibernating mustelids have documented the speed at which food deprivation depletes lipid reserves (Buskirk & Harlow 1989, Harlow 1994), and how fast they recover from it (Mustonen et al. 2006, Nieminen et al. 2006), suggesting that fatness can vary in the short term. The lack of a seasonal trend in fatness further suggests that there was no seasonal shortage in food during that time period, possibly since wolverines are capable of exploiting a wide range of prey, including large ungulate carcasses. Conversely, ungulate carcasses are a patchy resource, and this could explain the wide range of fatness recorded at any one time.

Finally, we did not detect significant differences in fatness between harvest years. However, because of our particular selection of animals for our study, annual changes in body condition status in wolverine populations should be monitored using entire harvest cohorts over several years in varying food availability conditions.

The large body mass and high fat content of wolverines (compared to other mustelids) was reflected in fat depots that were as numerous, large and easy to delineate as previously noted by Pond et al. (1994) and by Robitaille & Jensen (2005) in fisher. While Pond et al. (1994) used all fat depots (including subcutaneous layers), our goal was to seek the performance of individual, readily accessible depots in order to streamline the procedure for population-level monitoring of body condition. Thus, we selected the same five fat depots as studied in fisher (Robitaille & Jensen 2005). While the excision of most superficial fat depots (popliteal and sternal) was quick and easy, that of the deeper depots (omentum, mesentery and perirenal) required more time and skill. Contrary to our hypothesis, these three heavier fat depots performed poorly in wolverine compared to other depots and to other medium-sized mustelids (Robitaille & Cobb 2003, Robitaille & Jensen 2005). As noted by Pond et al. (1994), the mesentery and perirenal fat depots contained non-fat tissues (e.g. structural protein, vascular tissue and lymph nodes), which are associated with functions other than energy storage, and thus the mass of the depot is less tied to the overall fatness of the animal. Also, the delineation of these depots was somewhat less clear. The superficial popliteal depot was small and often not discernable or easily excised.

Our analyses indicate that the depots that most accurately predicted body fat content included the smaller, single sternal depot, which in females was second only to the mesentery depot. The sternal depot was also the quickest to excise due to its superficial location and relatively clear delineation. Also, it appears that this depot was not constrained by sex-dependent fat distribution (see above), and is the only one that performed well as a fatness index for both males and females. If the stomach was intended to be removed for other studies, the omentum depot could also be used to estimate fat levels in females.

Except for the sternal depot, fat depot mass was a better predictor of fatness in female wolverines than in males. There may be two possible explanations for this. First, the limited development of internal fat depots in males compared to their subcutaneous fat may limit the predictive power of any depot. Conversely, a few individual males exhibited very large sternal depots. For example, one of them possessed a large (50 g) sternal depot for its fatness (15%), which resulted in a 37% overestimate in its predicted fatness (20%; see Fig. 5). The presence of a few outliers in body size and/or fatness has also been observed in the American marten and the fisher (J-F. Robitaille, unpubl. data). We suggest that outliers could be omitted from monitoring of nutritional status because they tend to overestimate fatness in the population. We propose to exclude outlying individuals by a quick examination of fat depot sizes relative to the body size. However, as shown during our test phase, outliers (e.g. extremely lean or obese animals) still occurred, and the problem remains. Pond et al. (1994) suggested that fatness indices would be more accurate in a narrower range of depot masses. Alternatively, monitoring programs could focus on females only.

Conclusion

Fat depots performed variably in predicting fatness levels. The sternal fat depot appeared to be the most

powerful index in predicting fatness; separate regression functions should be used for male and female wolverines. The predictive power of fat depots in wolverines is, like in other mustelids, limited by the periodic occurrence of extra large or obese individuals, often in males. This could be due to the ability of male wolverines to store subcutaneous body fat with a limited gain in internal fat depot (except the sternal depot). If males are being monitored, case-by-case exclusion of outlying individuals, based on multiple morphometrics, should be considered.

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