Trade-offs between activity and thermoregulation in a small carnivore, the least weasel *Mustela nivalis*

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We studied factors influencing daily energy expenditures (DEE) of male least weasels (*Mustela nivalis*) using the doubly labelled water technique. The relationship between ambient temperature and DEE formed a triangular pattern, characterized by invariance of the maximum DEE and an inverse relationship between minimum DEE and temperature. A simple energetic model relating the DEE of male weasels to activity time (AT) and ambient temperature predicted that, across seasons, less than 10 per cent of measurements approach the upper bound of observed DEE. Male weasels were able to maintain a relatively constant maximum energy output across varying temperatures by adjusting their AT to changes in temperature. They achieved maximum energy expenditures in winter due to high thermoregulatory costs, and in spring and summer due to high levels of physical activity. This pattern exemplifies a ‘metabolic niche’ of a small mammal having extremely high energy expenditures primarily driven by ambient temperature.

Keywords: daily energy expenditure; ambient temperature; activity time; metabolic niche; weasel; doubly labelled water

1. INTRODUCTION

Energy expenditure measured under natural conditions provides essential information about the relationship between an organism and its environment (Speakman 2000; Brown *et al.* 2004; Costa & Sinervo 2004). It has important consequences for many aspects of animal life, including effects on their survival and reproduction (Jonsson *et al.* 1996; Hayes & O'Connor 1999; Tinbergen & Verhulst 2000; Jackson *et al.* 2001; Costa 2008). Over the past few decades, studies on energetics of free-ranging animals have been revolutionised by measurements of daily energy expenditure (DEE) through the use of the doubly labelled water (DLW) technique (Lifson & McClintock 1966; Butler *et al.* 2004). The application of DLW has dramatically increased the available data on factors affecting DEE under a wide range of environmental conditions (Nagy *et al.* 1999; Speakman 2000; Anderson & Jetz 2005; Miles *et al.* 2007). In interspecific studies, body mass is the most important predictor of variation in DEE, followed by ambient temperature, phylogeny, diet and habitat (Nagy *et al.* 1999; Speakman 2000; Anderson & Jetz 2005).

Several authors have suggested that DEE may be affected not only by environmental (extrinsic) factors, but also by physiological (intrinsic) factors (Speakman 1997a; Speakman *et al.* 2003; Costa 2008). The notion that intrinsic factors may be an important constraint is related to the simple fact that when food is unlimited the capacity to harvest it and expend energy can not increase indefinitely (Weiner 1992; Bacigalupe & Bozinovic

2002). Across mammals and birds, a possible interplay between extrinsic and intrinsic factors has been suggested by Anderson & Jetz (2005). They demonstrated the invariance of an upper bound of DEE with latitude in birds and mammals, and its inverse relationship with temperature in mammals. Anderson and Jetz suggested that the lower boundary of a triangular pattern of DEE variation is driven by environmental factors, whereas the upper one may represent physiological ones. A limitation of their analysis, however, is that it has been carried out on the species-specific average values of DEE. This may not be appropriate for identifying the boundaries of DEE variation, as energy expenditures vary substantially at the species level (e.g. Speakman *et al.* 1994; Humphries *et al.* 2005; Costa 2008). This variation is particularly important, since most of the time animals expend energy at sub-maximal rates, albeit exceeding the minimum possible level of DEE which is equal to the resting metabolic rate (RMR) at a given environmental temperature (Hammond & Diamond 1997). To our knowledge, the significance and consistency of upper and lower boundaries on DEE variation across the wide range of environmental conditions has not been identified intraspecifically. This is most probably owing to the fact that typical DEE datasets are too limited with respect to sample size and the range of prime environmental factors (chiefly temperature).

Here we analyse 52 DEE and 106 concomitant locomotory activity measurements in male least weasels (*Mustela nivalis* Linnaeus 1766), which were free-ranging under ambient temperatures from -22 to 23°C . Least weasels, the smallest member of the order Carnivora, are characterized by extremely high metabolic rates and

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high levels of activity (Casey & Casey 1979; Szafrńska *et al.* 2007). The goal of our study was to identify possible trade-offs between environmental conditions, locomotor activity and thermoregulation, as represented by the upper and lower boundaries of DEE variation. We identified the boundaries by means of quantile regression (Koenker 2005) and explored the effects of ambient temperature on DEE and potential behavioural compensation mechanisms at very low environmental temperatures. We also aimed to break down the total variation of DEE with respect to the enormous variation in body mass (typical for male weasels), their locomotor activity, ambient temperature, season, and habitat type.

2. MATERIAL AND METHODS

(a) Study area

The study was carried out in the central part of the Białowieża Forest (23.86° E, 52.70° N), NE Poland. The study area, in the Białowieża Glade and adjacent forest, encompassed different types of habitats. The open areas included two habitats, meadows and river valleys, which have distinct water regimes due to persistence, in the latter, of floodwater in spring and autumn. A relatively high water table in river valleys affects resting site selection by weasels in different seasons, which in turn might influence their energy expenditure (Zub *et al.* 2008).

Over the last 30 years, mean temperatures have averaged 19.2°C in summer (July) and –3.2°C in winter (January) in Białowieża Forest. Our study period covered three winters ('winter') encompassing from the beginning of November until the middle of March, which were characterized by mean daily temperatures ranging from –23.3 to 15.8°C (averaging 0.5°C in 2004/2005, –2.2°C in 2005/2006, and –0.3°C in 2007/2008).

Ambient temperature was recorded by a meteorological station located in the central part of the study area, and expressed as a daily mean value from four measurements, taken every 6 hours.

(b) Animal handling

From summer 2004 until winter 2007/2008 we made 72 measurements of DEE from 47 male weasels. We removed repeated measurements made in the same season for the same individuals from the analyses ($n=10$). We also excluded cases where final samples had an unusually high k_o/k_d ratio (proportion of fractional turnover for oxygen and hydrogen respectively), as these high ratios suggest a problem with sealing of the blood samples ($n=10$). Therefore in the final analysis we included 52 samples (43 individuals), including nine measurements performed for the same individuals, but in different seasons. Weasels were captured as described by Jędrzejewski *et al.* (1995) under collecting permits from the nature conservancy authorities (permits no. DOPweg-4201-04-6/03/jr, DOPog-4201-04-43/05/aj, LKE 2003/04 and LKE 2004/06).

Captured animals were immediately transported to the laboratory, anaesthetized, marked by ear punching, and sexed. To allow for the restoration of body condition, captured weasels were provided with water *ad libitum* and 1–2 laboratory mice per day, depending on body mass. The next day, animals were re-weighed and this body mass was used in calculations. On the second day we measured RMRs (for details see Szafrńska *et al.* 2007),

and on the third day weasels were anaesthetized with a ketamine–xylazine mixture and fitted with a neck-collar radio transmitter. Animals were kept in the laboratory 1 or 2 more days to habituate to the collars. On the morning of the fifth or sixth day the weasels were anaesthetized with Halothane, weighed (± 0.1 g) and injected with 0.5 ml of DLW 66 atom per cent enrichment (APE) oxygen and 33 APE deuterium. To precisely determine the quantity of injected isotope, the syringe was weighed immediately before and after the injection (± 0.0001 g). The weasel was returned to the cage for 1 hour to allow the isotopes to equilibrate in body water (Król & Speakman 1999). After the equilibrium period, a small (approx. 100–150 μ l) blood sample was obtained to estimate initial isotope enrichments. The sample was taken from the tail vein into 50 μ l capillary tubes, by sectioning the tip of the tail. Upon completion of all procedures the animals were released at the location of capture.

We used radio-telemetry to check the position and activity of released animals every 15 min. Bearings were taken from a distance of 50–100 m, to avoid disturbance by the observer, and animals were classed as active when they changed position between two consecutive locations. We finished radio-tracking around 1 hour after sunset, because weasels are not active during the night (Jędrzejewski *et al.* 2000), and continued observation after sunrise. Occasionally we checked the position of animals during the night to provide an accurate estimate of activity time (AT). After 2 days (approx. 48 hours) we attempted to recapture the animals by setting several traps at the night resting site. Recapture intervals were timed to be close to 24 hours to avoid effects of different activity periods on DEE estimates (Speakman & Racey 1988). In the morning, weasels explore areas near their overnight shelter before they begin hunting, so usually they were captured immediately after leaving the nest. When we failed to recapture animals we continued radio-tracking and repeated the capture attempt the following morning.

After recapture, weasels were transported to the laboratory where we collected a final blood sample, usually by removing the scab left from the first bleeding. To estimate the background isotope enrichments of ^2H and ^{18}O a 100 μ l blood sample was obtained from unlabelled animals (Speakman & Racey 1987—method C). Upon completion of all procedures radio transmitters were removed and on the next day weasels were returned to the wild in the place of last capture.

(c) Isotope analysis

Blood samples were vacuum distilled (Nagy 1983), and the resulting distillate was used to produce CO_2 and H_2 (methods in Speakman *et al.* (1990) for CO_2 and Speakman & Król (2005) for H_2). The isotope ratios $^{18}\text{O}:^{16}\text{O}$ and $^2\text{H}:^1\text{H}$ were analysed using gas source isotope ratio mass spectrometry (Optima, Micromass IRMS and Isochrom μ G, Manchester, UK). Samples were run alongside three laboratory standards for each isotope (calibrated to International standards) to correct delta values to ppm. Isotope enrichments were converted to DEE using a single pool model as recommended for this size of animal (Speakman 1993, 1997b). There are several approaches for the treatment of evaporative water loss in the calculation (Visser & Schekkerman 1999). We assumed evaporation of 25 per cent of the water flux (Speakman 1997b, eqn (7.17)) which minimizes error in a range of conditions (Visser & Schekkerman 1999; Van Trigt *et al.* 2002).

(d) Statistical methods

The effect of intrinsic (body mass and AT), and extrinsic (habitat type and temperature) factors on DEE was determined using general linear models. Habitat type was included as a random categorical variable. DEE and body mass were left untransformed because they were normally distributed and the relationship between them was linear. We transformed ambient temperature using a cubic polynomial function and AT using natural logarithm as $\ln(AT+1)$.

To explore the possible effect of life history on DEE we distinguished two groups. The first group included adult individuals, which had survived a previous winter, and the second group consisted of juvenile animals, which had been born the year in which measurements were taken. We distinguished adult animals from juveniles based on trapping records from previous seasons (for details see Zub et al. (2008)), and some external features (size of testis, development of *baculum*, coloration of ears, wearing of teeth). All males from the first group were mature and could reproduce, whereas only some of the young males matured and reproduced in the same year that they were born.

To examine the combined effect of all factors on DEE, we constructed a graphical model, superimposing smoothed contour lines indicating equal residual DEE (corrected for effect of body mass and habitat type) on the relationship between ambient temperature and AT. Next, we added to the graph points collected for all radio-tracked male weasels. In this way we were able to identify average energy expenditures (after removing the effect of body mass and habitat type) for given combinations of ambient temperature and AT. We compared linear models fitted to these data using Akaike's Information Criterion (AIC_c) (Burham & Anderson 2002). To estimate maximum DEE and locomotor activity in relation to ambient temperature we used a quantile regression (Koenker 2005), fitting data with linear functions for different τ -values. The significance and standard errors for estimated coefficients were calculated by bootstrapping. For statistical procedures we used STATISTICA v. 7.0 and the R package.

3. RESULTS

We identified four significant factors which, in combination, explained 76.4 per cent ($p < 0.00001$) of the variation in DEE of male weasels (table 1). The most important factor affecting DEE was body mass, followed by AT and the interaction of habitat with ambient temperature. Without accounting for the effect of other factors, body mass explained half of the variation in DEE of male weasels ($R^2 = 0.50$, $p < 0.0001$). Male weasels inhabiting the river valley had a higher DEE (208.2 kJ d^{-1} , s.e. = 8.9, $n = 16$) than animals living in the meadows (192.6 kJ d^{-1} , s.e. = 6.2, $n = 36$). This difference was significant when energy expenditures were corrected for the effects of body mass and AT ($F_{1,51} = 13.39$, $p = 0.0006$). DEE, corrected for the effects of body mass, AT and habitat type, did not differ between winter and summer ($F_{1,50} = 0.80$, $p = 0.38$), despite significant differences in mean ambient temperatures between these two seasons (summer: 15.5°C , winter: -2.4°C , $F_{1,50} = 97.02$, $p < 0.0001$).

When examining variation of DEE corrected for the effects of body mass, AT and habitat type in relation to ambient temperature we found a triangular pattern, with

Table 1. Factors affecting DEE in weasel males. (Semipartial correlation coefficient (sr^2)—per cent of variation explained after accounting for the effect of other factors. Only significant factors are reported.)

factor	effect	<i>F</i>	d.f.	sr^2	<i>p</i> -value
body mass	fixed	68.82	1,47	0.35	<0.00001
activity time	fixed	41.62	1,47	0.21	<0.00001
habitat $\times$ temperature	random	16.06	2,47	0.19	0.00001

greater variation at high temperatures (figure 1a). Maximum residual DEE determined by linear quantile regression was equal across the whole range of temperatures and, for τ ranging from 0.85 to 0.95, not significantly different from zero ($p > 0.10$). Also the relationship at $\tau = 0.50$ was not different from zero (slope = -0.49 , $p = 0.18$). In contrast, at the lowest quantiles ($\tau < 0.15$) the slopes were significantly negative ($p < 0.05$, figure 1a).

An opposite, but again triangular pattern, was found for the relationship between ambient temperature and AT. The quantiles from 85th until 95th had significantly positive slopes (slopes varied from 0.13 to 0.18, $p < 0.001$), but the lower boundary for this relationship was not significantly different from zero (for $\tau < 0.15$, the slope varied between 0.03 and 0.07, $p > 0.05$). For the median τ ($= 0.50$) the slope was also positive and significantly different from zero (slope = 0.11, $p = 0.02$, figure 1b).

To examine possible effects of different life histories on DEE during the summer, which was characterized by the highest variation of energy expenditures, we distinguished two groups of weasels as previously described: adult males and juvenile males. We estimated upper and lower limits of energy expenditures using quantile regression and including group as a factor. The maximum DEE was not different between groups. For τ ranging from 0.85 to 0.95, the relationship between ambient temperature and DEE, corrected for the effects of body mass, AT and habitat, was significantly negative, but not affected by group (figure 2). For the lowest quantiles (τ ranging from 0.05 to 0.15) this relationship differed between groups, i.e. for adults, the relationship was significantly negative, but for juveniles the relationship was not significantly different from zero (figure 2). For adult males the mean DEE was also negatively correlated with ambient temperature ($r = -0.74$, $p = 0.001$), whereas for juvenile males we did not find any significant correlation ($r = -0.22$, $p = 0.36$). In adult males this relationship was not due to decreasing activity across reproductive season because we did not find a significant correlation between DEE and calendar date ($r = -0.32$, $p = 0.22$).

The combined effect of ambient temperature and AT on DEE (corrected for the effect of body mass) was best explained by the quadratic polynomial function ($R^2 = 0.47$, $p = 0.00005$, figure 3), which fitted data much better than a simple linear model which explained only 28 per cent ($p = 0.0002$) of DEE variation ($F_{3,46} = 5.67$, $p = 0.002$). To account for the different number of parameters, we also used AIC_c to compare these two models. The second model was more parsimonious and fitted data better ($AIC_c = 464.54$, AIC_c weight $\omega_i = 0.99$) than the first model ($AIC_c = 473.21$). Analysis of the graphical model revealed that maximum energy

Figure 1. Ambient temperature versus DEE, corrected for the effect of body mass, AT and habitat type (a), and (b) AT. Lines correspond to linear quantile regression for $\tau=0.9, 0.5$ and 0.1 (starting from the top).

Figure 2. Effect of ambient temperature on DEE in adult male (filled circles) and juvenile male weasels (open circles). Lines correspond to linear quantile regression for $\tau=0.1$ and 0.9 .

expenditures occurred mostly at intermediate temperatures (between 0 and 10°C), where the combined effects of relatively high thermoregulatory and activity costs presumably incurred high DEE. Low DEE occurred primarily at relatively high T_a (above 0°C) when daily activity was low (figure 3). This model suggests that the lowest DEEs could not be achieved at temperatures below -10°C, even if animals were completely inactive (figure 3).

4. DISCUSSION

When corrected for the effects of body mass, AT and habitat type, DEE of male weasels formed a triangular pattern, with an upper boundary that was invariant with ambient temperature, but with a lower boundary that increased with decreasing temperature (figure 1a). The constraint space imposed by temperature on weasels' DEE was similar, but not identical, to that reported by Anderson & Jetz (2005) for interspecific comparisons of mammals. Unlike our study on weasels, the upper boundary of interspecific variation in mammalian DEE was inversely related to temperature (Anderson & Jetz 2005, fig. 2). However, at both the inter- and intraspecific level the range of variation of DEE considerably decreased with temperature, which clearly indicates that the lowest ambient temperatures considerably reduce the capacity for variation in mammalian energy expenditures.

Figure 3. Relationship between ambient temperature, AT and predicted DEE, corrected for the effects of body mass and habitat type. The contour lines for DEE were fitted using a quadratic polynomial function ($DEE = -44.71 + 25.28 \times Act_time - 1.45 \times Act_time^2 - 0.67 \times Temp - 0.01 \times Temp^2 - 0.35 \times Act_time \times Temp$). The points represent values of mean daily AT from 81 radio-tracked male weasels. Individuals radio-tracked in different seasons are presented as separate data points. Circles indicate data collected for male weasels used in the DLW study, squares—other individuals. Solid symbols represent positive residuals.

Although the distribution of points in figures 1a and 2 shows that male weasels generally expend energy at a sub-maximal rate, the independence of the upper limit of DEE from temperature leads us to question the nature of the invariance of the maximum observed DEE. The significance of physiological limits to DEE in free-living endotherms is a matter of debate (Speakman 2000; Bacigalupe & Bozinovic 2002; Costa 2008). In general, demonstration of the existence of such limits requires experimental manipulation by forcing the animals to expend energy at the maximum rates, constrained only by centrally or peripherally driven physiological processes (Tinbergen & Verhulst 2000). One can expect that, under most environmental conditions, animals are unwilling to operate at the maximal rates even if food is available to excess, because it most probably entails physiological costs (Miles *et al.* 2007; Speakman 2008). Such costs might include, for example, an increased rate of reactive oxygen species production (Vanholt *et al.* 2008) or

immunosuppression (Svensson *et al.* 1998). These effects may ultimately lead to reduced survival (Wolf & Schmid-Hempel 1989; Daan *et al.* 1996). However, the effects of increased energy expenditure on free-radical production and lifespan are far from straightforward, with some experimental studies showing no effect (Selman *et al.* 2008) and yet others suggesting the association between lifespan and metabolism may be positive (Lin *et al.* 2002; Speakman *et al.* 2004; Schulz *et al.* 2007).

Nevertheless, it is probable that individual energy expenditures are sometimes close to physiological limitations. The invariance of the upper boundary of the temperature-DEE relationship (figure 1a) may indicate the presence of such a constraint on maximal energy expenditure. The nature of this limitation however remains obscure. On the other hand it is worth noting that only 10 out of 106 points (i.e. less than 10% of observations) depicted on the activity-temperature plane (figure 3) fall on or between the uppermost contour lines (isoclines) of DEE. This demonstrates that a robust analysis of the upper bound of DEE in unmanipulated mammalian populations is only achievable when a large dataset is available.

The invariance of the upper boundary of the DEE space remains a striking contrast to the strong, positive relationship between temperature and maximum values of locomotor activity (figure 1b). This discrepancy is probably caused by high winter costs of thermoregulation during activity, contributing to high DEE, rather than high RMR. Two sources of evidence support this claim. First, Chappell (1980) demonstrated that the use of underground nests by wintering small mammals considerably reduced costs of thermoregulation. During cold periods, male weasels in our study rested almost exclusively in the subnivean nests of rodents, additionally lined with rodent fur, which created a thick layer of insulating material (K. Zub, P. A. Szafrńska, M. Konarzewski, P. Redman & J. R. Speakman 2007, unpublished data). Second, RMR in male weasels is lower in winter than in summer (Szafrńska *et al.* 2007). Moreover, energy expenditures of sexually active males during the breeding season were higher at low temperatures, even after correction for AT. This may indicate that thermoregulation costs increase disproportionately in individuals forced to spend more time outside the nests.

High thermoregulatory costs paid during locomotion in winter are also the most probable reason for the inverse relationship between the lower boundary of DEE with temperature (figure 1a). This pattern indicates the importance of the combined costs of activity and thermoregulation. In cold seasons, weasels are active only when hunting, because they do not reproduce in winter (King 1983; Jędrzejewska 1987).

Despite anatomical adaptations for hunting in rodent burrows, weasels are often active above the ground, even in cold conditions. In many parts of their range they may be exposed to low winter temperatures, especially if snow cover is absent. At a mean body mass of approximately 100 g, they are among the smallest high-latitude mammals routinely active in winter out of protective cover (Casey & Casey 1979; Chappell 1980). Many animals respond to winter climates by reducing body mass, which decreases overall energy requirements and thereby mitigates increased energy demands at low ambient temperature

(Speakman 2000). However, male weasels maintain their body mass (Szafrńska *et al.* 2007) and instead respond to lower temperatures by reducing AT. Similar responses have been observed in red squirrels (*Tamiasciurus hudsonicus*) in North America (Humphries *et al.* 2005).

Our results suggest that a decrease of the lower DEE boundary with increasing temperature (figure 1) was mainly driven by high thermoregulatory costs in the cold, because the combination of low daily activity and relatively high T_a (above 0°C) also produced low DEE values (figure 3). According to our graphical model the lowest levels of energy expenditure, achievable at higher temperatures, cannot be maintained at temperatures below -10°C, even if the animals remained completely inactive (figure 3). Nevertheless, the observed range of variation of DEE and activity with respect to temperature is wide down to ca -5°C (figure 1), which explains why the mean DEE in male weasels does not differ significantly between summer and winter. This, by itself, raises the question of which factors affect DEE that falls between the upper and lower boundaries.

The large variation of DEE at higher temperatures might be at least partly related to different life histories of male weasels. During spring and summer adult males maximized their energy expenditures because they are forced to reproduce due to their short expected lifespan (King 1989). This group of individuals also demonstrated a strong correlation between energy expenditure and ambient temperature, whereas in the same range of temperatures, juvenile males presented different strategies, exhibiting either high or low DEE, probably according to their reproductive status. Because male weasels mature at the age of 4–5 months, at least some juveniles are able to reproduce in the summer of the year they are born (King 1989). Thus variation of DEE in this group of animals is not necessary related to ambient temperature, because non-reproducing individuals may operate at a lower DEE when environmental conditions are unfavourable.

To our knowledge, the only other study on DEE variation in small mammals similar to ours is that by Humphries *et al.* (2005) on red squirrels. However, Humphries *et al.* (2005) reported only one measurement of DEE during the warm period (a mean of three individuals measured), and were therefore not able to demonstrate whether maximum energy expenditure differed between seasons. Moreover, we could expect that this species would also express more variation in their DEE at a higher temperature. Looking at the association between activity of squirrels outside the nest and ambient temperature we observed the same pattern in male weasels, i.e. a triangular relationship with increasing variation towards higher temperatures (Humphries *et al.* 2005). Thus we suspect that both male weasels and squirrels adopt the same behavioural strategies, reducing their activity at low temperatures. The only difference is higher energetic effectiveness of behavioural compensation in squirrels; they can rely on a food store collected prior to the winter season, whereas weasels have to hunt regularly to satisfy their energy needs. Although weasels are able to increase killing rates and feed on previously stored food during sub-zero days, this behaviour is limited by the abundance of prey (Jędrzejewska & Jędrzejewski 1989). Surplus killing may further reduce energy

expenditures of weasels, but it forces animals to increase their activity when the temperature increases.

Variation of DEE in male weasels is reduced at low temperatures but increases along with increasing ambient temperatures (and AT). What is more surprising, however, is that across seasons maximum energy expenditure did not change, probably due to compensation of high winter thermoregulatory costs by increased summer locomotory activity. We postulate that the most profitable approach to reveal the effects of temperature on DEE is to focus on intraspecific studies that incorporate the possible influence of other environmental factors as well as behaviour. The results of our study indicate that, when analysing the effect of external factors (e.g. temperature) on DEE, one must also consider individual differences in life histories.

Anderson & Jetz (2005) formulated the concept of 'metabolic niche' defined as the range of animals' energy expenditures within the space set by the interplay between extrinsic (environmental) and intrinsic (physiological) factors. Unlike Anderson & Jetz (2005), who applied the concept interspecifically, we have demonstrated its usefulness at the intraspecific level, where, by definition, the notion of 'niche' should be most relevant. Small endotherms by virtue of their high surface to volume ratios are probably particularly prone to the effects of heat loss at cold winter temperatures. Unless they compensate for the effects of low temperatures by adjusting their activity patterns it seems probable that they would be unable to expend the required large amounts of energy because of physiological limits as suggested by Anderson & Jetz (2005). The implications for larger endotherms, where the surface to volume ratio is much lower and consequently the lower critical temperature is also much lower, are less certain. It would consequently be instructive to know how the energy demands of larger terrestrial mammals differ seasonally in response to ambient temperature changes. Unfortunately the high cost of the isotope labelling method used here means that studies of larger mammals are rare (reviewed in Westerterp & Speakman 2008) and none include comparisons between seasons.

The weasels were collected and subject to all procedures with permission from the nature conservancy authorities (permit nos DOPweg-4201-04-6/03/jr, DOPog-4201-04-43/05/aj), and the Local Research Ethics Committee in Białystok (permit nos LKE 2003/04, LKE 2004/06 and LKE 2005/08).

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