

# Seasonal stage differences overwhelm environmental and individual factors as determinants of energy expenditure in free-ranging red squirrels

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## Summary

1. Despite the central importance of the rate of energy expenditure in the lives of animals, the major drivers of within-species variation in energy expenditure remain uncertain, largely because most intraspecific studies focus on one or only a few potential determinants of expenditure.

2. Here, we examine the determinants of daily energy expenditure (DEE) in free-ranging female North American red squirrels (*Tamiasciurus hudsonicus* Erxleben) occupying a highly seasonal environment. By relating variation in 260 measurements of DEE from 176 individuals to key sources of seasonal (reproductive and foraging stages), environmental (resources and air temperature) and individual (body mass and individual identity) variation, our comprehensive analysis examines the relative importance of DEE predictors that have been more commonly examined in isolation.

3. Red squirrels demonstrated extensive variation in DEE with 5th (177 kJ per day) and 95th (660 kJ per day) percentile DEE levels that would correspond to mammals on an interspecific scale ranging in mass from 148 to 1120 g.

4. Seasonal stage differences accounted for most variation in DEE, with high expenditure during lactation and autumn hoarding, and very low expenditure during winter. Contrary to interspecific studies, energy expenditure increased with increasing ambient temperature and it was weakly related to body mass in all seasons except for winter. High resource availability was associated with reduced energy expenditure in winter, but elevated expenditure during lactation and hoarding.

5. Collectively, these results highlight substantial intraspecific variation in energy expenditure, most of which can be explained by a combination of seasonal stages and environmental conditions, and fundamental differences in the importance and direction of determinants of energy expenditure when examined at the intra- versus the interspecific level.

**Key-words:** body mass, doubly labelled water, energetics, food hoarding, lactation, resource availability, Sciuridae, thermoregulation, Yukon

## Introduction

An individual's rate of energy expenditure is a trait of central importance. Energy expenditure is important physiologically because it reflects the sum of metabolic processes operating within an organism (McNab 2002), how these processes are affected by environmental challenges to

homeostasis (Scholander *et al.* 1950; Bartholomew 1964; Sapolsky, Romero & Munck 2000), and the potential somatic damage they impose (Beckman & Ames 1998; Finkel & Holbrook 2000; Dowling & Simmons 2009; Monaghan, Metcalfe & Torres 2009). Energy expenditure is important ecologically because it reflects the resources and activity (Masman *et al.* 1989; Lima & Dill 1990; Arnould, Boyd & Speakman 1996; Welcker *et al.* 2009; Zub *et al.* 2009) required for individuals to maintain energy balance,

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in addition to representing a loss term in trophic energy flows (Yodzis & Innes 1992; Hairston & Hairston 1993; McCann 2011). Energy expenditure is important evolutionarily because it mediates the allocation of acquired resources to life-history traits, and thus, potentially plays a central role in determining reproductive success and survival (Gadgil & Bossert 1970; van Noordwijk & de Jong 1986; Roff 1992; Stearns 1992; Zera & Harshman 2001; Speakman 2008).

Despite the well-recognized importance of energy expenditure in physiology, ecology and evolution, we know little about the general circumstances that cause free-ranging animals to have low or high rates of energy expenditure. In fact, we know quite a lot about how energy expenditure varies throughout the year (Kenagy, Sharbaugh & Nagy 1989a; Weathers & Sullivan 1993; Williams, Anderson & Richardson 1997; Speakman 2000; Bozinovic *et al.* 2004; Gilbert *et al.* 2009) and the energy costs of specific activities and environmental challenges, such as resting (Speakman, Król & Johnson 2004), locomotion (Schmidt-Nielsen 1972; Taylor & Heglund 1982), digestion (Secor 2009), thermoregulation (Scholander *et al.* 1950) and reproduction (Naya *et al.* 2008). But we know comparatively little about how potential contributors interact to define variation in the energy expenditure expressed in nature by different individuals at different points in time. This knowledge gap exists largely because studies examining within-species variation in energy expenditure have generally focused on one or only a few potential determinants of energy expenditure, typically confined to one of three major axes of emphasis.

One research axis has focused on energetically demanding activities, with the expectation that energy expenditure reflects what an animal was doing when the measurement was taken. The most consistent claim of intraspecific examinations of mammalian energy expenditure is that lactation is the most energetically demanding activity (Millar 1975, 1978; Oftedal 1984; Gittleman & Thompson 1988; Kenagy, Stevenson & Masman 1989b; Kenagy *et al.* 1990; Hammond & Diamond 1992, 1994; Rogowitz 1996; Speakman & McQueenie 1996; Rogowitz 1998; Johnson, Thomson & Speakman 2001; reviewed in Naya *et al.* 2008). Other studies have quantified the energy expenditure of animals under a diversity of energetically demanding conditions (reviewed in Karasov 1992) including flight (Flint & Nagy 1984; Birt-Friesen *et al.* 1989), migration (Wikelski *et al.* 2003; Buehler & Piersma 2008), food hoarding (Humphries *et al.* 2002), harem maintenance (Kunz, Robson & Nagy 1998), hunting (Gorman *et al.* 1998), cooperative breeding (Scantlebury *et al.* 2002), mounting an immune response (Martin, Scheuerlein & Wikelski 2003) and being parasitized (Delahay, Speakman & Moss 1995; Scantlebury *et al.* 2007; Careau, Thomas & Humphries 2010).

A second axis is ecological in nature, with the expectation that energy expenditure reflects the environmental conditions when the measurement was taken. Air temperature has direct effects on the energy expenditure of endotherms (McNab 2002). Winter can be more energetically demanding for non-hibernating endotherms because of the thermoregulatory requirements associated with air temperatures below the

thermal neutral zone (Scholander *et al.* 1950; Mullen 1971; Holleman, White & Feist 1982; Kenagy, Stevenson & Masman 1989b; Nagy & Gruchacz 1994; reviewed in McNab 2002). However, other research demonstrates that the energetic responses of animals to seasonal variation in ambient temperatures are difficult to predict because microhabitat selection and behavioural strategies may facilitate surprisingly low levels of energy expenditure during winter (Chappell 1980; Bozinovic *et al.* 2004; Humphries *et al.* 2005; Sheriff *et al.* 2009; Zub *et al.* 2009). Other lines of research have focussed on how energy expenditure is affected by environmental resource availability. Even though environmental resource availability is difficult to quantify well for most species, field studies examining the relationship between energy expenditure and proxies of resource availability have found both positive (Bozinovic *et al.* 2009) and negative relationships (Thomas *et al.* 2001).

A third research axis has focused on individual differences in energy expenditure and the adaptive consequences of this variation, with the expectation that energy expenditure reflects characteristics of the individual on which the measurement was taken. Body mass is consistently identified as an important determinant of energy expenditure (Speakman, Król & Johnson 2004; Zub *et al.* 2009), and a seasonal reduction in body mass is often interpreted as an adaptive, energy conservation strategy (Croll, Gaston & Noble 1991; Speakman 2000; Li & Wang 2005; Lovegrove 2005). Other studies go beyond measurement of body mass to examine how energy expenditure varies according to body composition (Bozinovic, Novoa & Veloso 1990; Konarzewski & Diamond 1995; Selman *et al.* 2001; Książek, Konarzewski & Łapo 2004). Repeatable individual differences in energy expenditure are also of interest because of the potential that energetic traits are targets of selection on their own (Koteja 2004; Rønning, Moe & Bech 2005; Nespolo & Franco 2007; Duarte *et al.* 2010; Larivée *et al.* 2010), or part of a suite of correlated behavioural, physiological and life-history traits (Ricklefs & Wikelski 2002; Careau *et al.* 2008).

We feel that a multi-axis approach examining intraspecific variation in energy expenditure is necessary because by continuing to examine the axes in isolation, it is impossible to determine the relative importance of factors influencing energy expenditure by free-ranging animals. Moreover, how all these potential contributors to energy expenditure combine to define general variation in energy expenditure is likely to be more important physiologically, ecologically and evolutionarily than isolated energetic costs. Here, we relate variation in 260 measurements of daily energy expenditure (DEE) from 176 individual North American red squirrels (hereafter, red squirrels, *Tamiasciurus hudsonicus*; Fig. 1) to key forms of seasonal (reproductive and foraging stages), environmental (resources and air temperature) and individual (body mass and individual identity) variation. The large number of individuals, years and energetic measures included in the dataset, as well as the breadth of predictor variables quantified year-round on this free-ranging, non-hibernating population, renders this the most complete analysis of intraspecific variation



**Fig. 1.** Lactating female North American red squirrel (*Tamiasciurus hudsonicus*) moving a new-born offspring between nests. Photo credit: Ryan W. Taylor.

in endotherm energy expenditure in the wild. Previous intraspecific studies clearly recognize that variables from all three axes can interact with shape patterns of energy expenditure [e.g. seasonal schedules of reproduction coincide with particular environmental conditions (Moffatt, Devries & Nelson 1993)], and studies focused within the identified axes often incorporate variables from other axes as covariates [e.g. body mass as a covariate in ecological studies (Jodice *et al.* 2002); identity as a random factor in studies examining reproductive energetics (Welcker *et al.* 2010)]. Nevertheless, to our knowledge, variables from all three axes (activity-specific, ecological and interindividual) have never been simultaneously evaluated as determinants of intraspecific variation in energy expenditure.

## Materials and methods

We studied a free-ranging population of red squirrels between 2002 and 2009, in the Kluane region of south-western Yukon, Canada (61°N, 138°W). The average temperature in the coldest and warmest months ranges between -22 and 12.8 °C (Environment Canada: 1967–2006; Burwash, Yukon; 61°N, 139°W; c. 70 km from study area). This population has been the subject of long-term research, and details of research methodology, the study site and this population's ecology and evolutionary biology are published elsewhere (e.g. McAdam *et al.* 2007). Briefly, female red squirrels were examined on two c. 40 ha study areas. All individuals on these study areas were marked with unique alphanumeric ear-tags. Red squirrels are non-hibernating mammals (Pauls 1981). They occupy year-round food-based territories and often keep the same territory for their entire life (McAdam *et al.* 2007). Red squirrels preferentially hoard white spruce (*Picea glauca*) cones underground within their territory after cones mature in late summer/early autumn (Fletcher *et al.* 2010). Hoarded spruce cones are an important food source used to meet the energetic demands of winter and reproduction the following spring (Boutin *et al.* 2006). All animals were captured and handled in compliance with the guidelines of the Canadian Council on Animal Care.

We determined the DEE of female red squirrels engaged in three different seasonal/reproductive stages (hereafter referred to as seasonal stages): (i) winter ( $n = 85$  over 4 years), (ii) non-breeding during summer ( $n = 17$  over 2 years; henceforth non-breeding), (iii)

lactation ( $n = 130$  over 7 years) and (iv) hoarding ( $n = 28$  over 3 years). All red squirrels experience winter and engage in hoarding activity in the autumn. However, during spring and summer, a proportion of females are in non-breeding condition (i.e. no dependent offspring) when others are lactating. Winter DEE measures were collected between 9 January and 23 February, which was prior to the date of first mating on study areas in each year (gestation = 35 days; see Appendix S1 in Supporting information for how parturition dates were determined). Sixty-two of these winter DEE estimates were originally presented in Humphries *et al.* (2005). Lactation samples were collected from red squirrels attempting their first litter of the season when females were confirmed to be lactating and were between 35 and 40 days post-partum. This time window reflects a period when females likely experience peak energetic demands of lactation because pups have grown to their largest body size prior to first emergence from the nest when they start supplementing their diet with solid food (42 days; S. Boutin, A. G. McAdam & M. M. Humphries unpublished data). Non-breeding samples were collected on non-pregnant, non-lactating females during summer overlapping in time with samples collected during lactation. Non-breeding females were either 1-year-old individuals that did not attempt to breed in that year ( $n = 12$ ) or females that were > 1 year of age that lost their first litter and did not attempt to re breed ( $n = 5$ ). Hoarding samples were obtained during the peak of hoarding activity. The number of cones hoarded per day at this study site increases from low levels in late August to a peak in early- to mid-September (Fletcher *et al.* 2010) when hoarding measures of energy expenditure were made (7- to 15-September).

We used the doubly labelled water (DLW; Lifson, Gordon & McClintock 1955; Nagy 1983; Speakman 1997; Butler *et al.* 2004) technique to quantify the DEE of free-ranging red squirrels over a period of at least 2 days. Individuals were captured in live-traps and injected intraperitoneally with 0.50 mL of DLW. The DLW consisted of 10% enriched  $^{18}\text{O}$  water (Enritech, Rehovot, Israel) and 99% enriched  $^2\text{H}$  water (MSD Isotopes, Pointe-Claire, QC, Canada) mixed in a ratio of 20 : 1 (determined using serial dilution; Speakman 1997). Individuals were left in traps for 60 min to allow the isotopes to equilibrate with the body water pool (Król & Speakman 1999) and then were bled by slightly cutting into the quick of a toenail to obtain replicate initial blood samples into glass capillary tubes. Between 48 and 120 h after the initial blood sample, red squirrels were recaptured, weighed and bled to obtain replicate final blood samples. The final blood sample was primarily taken from a different toenail as the initial blood sample; however, initial and final blood samples were collected from the same toenail in some instances if only a small amount of the quick was exposed during the initial blood sample. Ninety-two percent of final blood samples were obtained within 2 h of a 24-h interval from the initial blood sample, which controlled for circadian rhythms of activity (Speakman & Racey 1988). The combination of the DLW injection and initial blood sampling appears to reduce the proportion of time red squirrels are active by 20% immediately after the initial blood sample is collected, but this effect fades progressively over time, and there is no difference between DLW and control squirrels after 7 h (D. W. Archibald, M. M. Humphries, A. G. McAdam & S. Boutin, unpublished data). The effect of the DLW technique on activity has been noted in other studies (Schultner *et al.* 2010; but see Speakman, Racey & Burnett 1991). However, by collecting samples over multiple days, we minimized the effect of this initial transient decline in activity (Speakman *et al.* 1994; Berteaux *et al.* 1996). For details regarding the laboratory processing of blood samples and the calculation of DEE in kJ per day, see Appendix S2 in Supporting information. Briefly,  $\text{CO}_2$  production was calculated based on the

differential washout of the hydrogen ( $^2\text{H}$ ) and oxygen ( $^{18}\text{O}$ ) isotopes, and this value was converted to an estimate of DEE in kJ per day ( $\log_{10}$  transformed). The body mass of individual red squirrels used in the analyses was the average mass of the individual just prior to the DLW injection and at the final blood sample ( $\log_{10}$  transformed). The ambient temperature ( $T_a$ ) experienced by individuals during the DLW interval was taken as the average of all hourly temperature readings between the initial and final blood samples collected from a weather station on one of our study areas. When these data were not available, we used data from an Environment Canada weather station c. 35 km from our study site (Haines Junction, Yukon).

We also calculated the daily energy requirements (DER;  $\log_{10}$  transformed) of lactating females that included the energy that was exported to their offspring. The DEE estimate yielded by the DLW technique does not include the metabolizable food energy intake that is exported in milk; therefore, the DER of lactating females is underestimated by the DLW technique (Król & Speakman 2003). We calculated the energy exported in milk for each individual based on the litter size and juvenile growth rate supported by that lactating female and a calibration equation for the DEE of juvenile red squirrels (Appendix S1 in Supporting information). The DER of lactating females was the sum of their DEE and the energy exported in milk.

The timing of parturition, litter size and the number of litters attempted by females depends on white spruce cone availability in the previous year (Boutin *et al.* 2006; McAdam *et al.* 2007). Resource availability was therefore quantified using annual counts of white spruce cone production, conducted during late summer, prior to when red squirrels hoard cones (McAdam *et al.* 2007; Fletcher *et al.* 2010). We counted all of the cones visible on one side of the top 3 m of approximately 80 trees per study area,  $\ln(x + 1)$  transformed the counts for each tree and calculated an index of cone availability as the average of the  $\ln(x + 1)$  transformed values. The  $\ln$  transformation was necessary because white spruce cone crops range from mast years with very high levels of population-wide cone production, interspersed with non-mast years when little or no cones are produced (LaMontagne & Boutin 2007, 2009). This index of cone availability is related to the amount of cones squirrels hoard (Fletcher *et al.* 2010), over-winter survival (LaMontagne 2007) and parturition dates the following spring (McAdam & Boutin 2003; Boutin *et al.* 2006). Therefore, cone availability for a given late-summer period was used as a correlate of energy expenditure measured during the upcoming hoarding season, winter and spring/summer (lactating and non-breeding) periods. That is, hoarding DEE was related to cone availability quantified in the same calendar year, whereas all non-hoarding samples (i.e. winter, non-breeding and lactation) were related to cone availability in the previous year. Henceforth, we refer to this variable as 'resources'.

We adopt two analytical approaches, the first of which uses an information-theoretic model comparison approach (Burnham & Anderson 2002) to evaluate the coarse-scale predictors of DEE and DER, integrating the three axes identified in the Introduction. In this analysis, we were unable to examine full models including all two-way, and multi-way, interactions because we lacked a large sample size of replicate measures across all seasons and resource levels. Because of this and the possibility that our global analysis would obscure important within stage predictors of expenditure variation, we conduct an alternative analysis based on seasonal stage-specific linear models. In these two analytical approaches, we consider two response variables (DEE and DER), because combining lactating and non-lactating individuals in the same analysis necessitates a

distinction between comparisons based on energy expenditure (DEE) and energy requirements (DER, reflecting DEE plus energy exported in milk). In the DER analysis, the DER of non-breeding and hoarding individuals was set equal to their DEE because all energy requirements are quantified by the DLW technique.

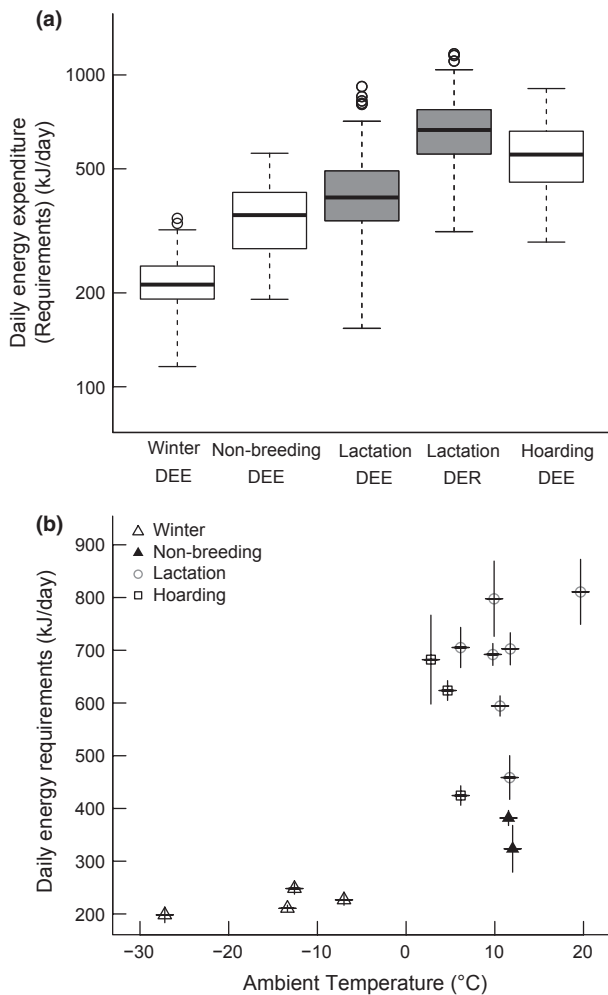
For the information-theoretic model comparison approach, our candidate model set included all possible combinations of the ordinary least squares regression models involving at least one of the predictor variables (seasonal stage,  $T_a$ , resources, and mass; 15 models) with no interactions (R version 2.9.1; R Development Core Team 2009). For each model, we also fit an identical mixed-effects model (R library: nlme; Pinheiro *et al.* 2009) with a random effect of each red squirrel's identity (henceforth, ID) for a total of 30 candidate models. We evaluated relative support for the fixed- and mixed-effects candidate models using conventional, rather than conditional (Vaida & Blanchard 2005), Akaike Information Criterion with small sample bias adjustment ( $\text{AIC}_c$  – calculated using maximum likelihood; Burnham & Anderson 2002). The model with the lowest  $\text{AIC}_c$  value was the most parsimonious model. We inferred support for the best model by examining the  $\text{AIC}_c$  differences ( $\Delta\text{AIC}_c$ ), and the  $\text{AIC}_c$  weights ( $w_i$ ). The most parsimonious model had a  $\Delta\text{AIC}_c$  value of zero, but models with  $\Delta\text{AIC}_c$  values  $\leq 2$  were considered to have substantial support (Burnham & Anderson 2002). If a model's  $\text{AIC}_c$  value was larger than another model's by up to 2 units, and it had only one more parameter (all other terms in the two models were identical), then the larger model was not considered to be supported or competitive (Burnham & Anderson 2002). In these instances, the model with one additional parameter falsely appears to have support because of the presence of the other terms in the model (Burnham & Anderson 2002, p. 131).  $\text{AIC}_c$  weights ( $w_i$ ) sum to one across all models and are the probability that each model provides the best fit among the candidate models.

For the seasonal stage-specific linear modelling approach, we used five separate linear models to examine the effects of mass, resources and  $T_a$  on DEE during the four seasonal stages (winter, non-breeding, lactation and hoarding) and on DER during lactation. These linear models included all repeated measures on individuals because the  $\text{AIC}_c$  analyses suggested repeatable individual differences did not influence energy expenditure (see Results).

## Results

Median levels of DER and DEE, grouping all seasonal stages, were 497.7 and 349.2 kJ per day, respectively. Levels of both DER and DEE were highly variable (Fig. 2a,b). The 75th percentile of both DER and DEE was at least double the 25th percentile (DER = 244.1–675.5 kJ per day; DEE = 231.6–462.5 kJ per day), and coefficients of variation for DER and DEE were 49.2% and 43.7%, respectively.

Repeatable individual differences did not influence levels of DER and DEE. Models that included the ID random effect were always ranked lower than the models with identical fixed-effects structure that excluded the ID random effect (results not shown). The  $\text{AIC}_c$  values of these model pairs differed by  $< 2.15$  (models including ID random effect always had higher  $\text{AIC}_c$  values), suggesting that ID falsely appeared to have support because of the presence of the other terms in



**Fig. 2.** Season stage and ambient temperature ( $T_a$ ) were the two best predictors of daily energy expenditure (DEE) and daily energy requirements (DER). (a) Box (1st and 3rd quartiles with median line) and whisker (95% CI) plot comparing levels of DEE and DER (DEE plus energy intake exported to offspring) among the seasonal stages of winter, non-breeding during summer, lactation and hoarding in female red squirrels. Grey lactation DEE and DER boxes were calculated for the same individuals; DEE and DER values are equal in all other seasonal stages. Raw values of DEE and DER are plotted on a  $\log_{10}$  scaled axis. (b) Yearly average DER values ( $\pm$ SE) plotted against the average  $T_a$  values ( $\pm$ SE) within these years.

the model. Moreover, likelihood ratio tests comparing paired models with and without ID were never significant ( $P > 0.71$ ;  $\chi^2$  distribution, d.f. = 1). Thus, we excluded the models including ID from the DER and DEE analyses (Table 1).

On the basis of comparison of models with only one predictor, the single best predictor of both DER and DEE was seasonal stage, followed by  $T_a$ , mass and resources (Table 1). The model with only seasonal stage explained 82% and 56% of the variation in DER and DEE, respectively, and the  $AIC_c$  for these models was  $\geq 69$  units smaller than all other models with only one predictor (Table 1). Considering median levels of DER within each seasonal stage, lactation was the most energetically demanding stage by 111 kJ per day over hoard-

ing, by 311 kJ per day over non-breeding and by 453 kJ per day over winter (Fig. 2a). However, in terms of median levels of DEE within each stage, hoarding was the most energetically demanding period by 151 kJ per day over lactation, by 200 kJ per day over non-breeding and by 343 kJ per day over winter (Fig. 2a). The median DER of lactating females was 261 kJ per day greater than the median DEE of lactating females (Fig. 2a). The model with only  $T_a$  as a predictor variable explained 63% and 41% of the variation in DER and DEE, respectively, with an overall positive relationship between  $T_a$  and year-round variation in energy expenditure (Fig. 2b).

Mass and resources explained less than a quarter of the variation in DER and DEE as univariate predictors (mass:  $R_{DER}^2 = 0.21$ ,  $R_{DEE}^2 = 0.10$ ; resources:  $R_{DER}^2 = 0.01$ ,  $R_{DEE}^2 = 0.01$ ). Mass was a significant predictor of DER in a simple linear regression pooling all seasonal stages ( $F_{1,258} = 68.3$ ,  $P < 0.0001$ , Fig. 3 – solid line). However, it is apparent from Fig. 3 that the positive effect of mass pooling all seasonal stages partially resulted because squirrels had smaller masses during less energetically demanding seasonal stages. When simple linear regressions are performed within each seasonal stage, mass is only a significant predictor of DER within winter ( $F_{1,83} = 5.0$ ,  $P = 0.03$ , Fig. 3 – dashed line; non-breeding:  $P = 0.56$ ; lactation:  $P = 0.35$ ; hoarding:  $P = 0.08$ ).

Examining models that included all predictors, there were three models with substantial support predicting both DER and DEE (Table 1a,b; bolded models). The best models for DER and DEE explained 83% and 57% of the variation in energy expenditure, respectively. Seasonal stage was included in all six models,  $T_a$  and mass were included in five of six models and resources was included in three of six models.

Seasonal stage-specific linear models indicated that winter DEE was the only measure of energy expenditure for which mass, resources and  $T_a$  were all significant predictors (Table 2). Mass was only positively related to DEE during winter (Fig. 4, column 1; Table 2). Resources had a positive effect on lactation DEE and DER, and a marginally positive effect on hoarding DEE (Fig. 4, column 2; Table 2). Conversely, resources had a negative effect on winter DEE (Fig. 4, column 2; Table 2).  $T_a$  had a positive effect on DEE during winter and lactation that was reversed to being negative during hoarding (Fig. 4, column 3; Table 2). The proportion of variation in DEE explained by  $T_a$  was much greater during winter ( $sr^2 = 0.32$ ) and hoarding ( $sr^2 = 0.29$ ) as compared to the proportion of variation explained by DEE during lactation ( $sr^2 = 0.05$ ). DER during lactation was positively related to resources, but not significantly related to body mass or  $T_a$  (Fig. 4, column 4; Table 2).

## Discussion

Most previous studies of intraspecific variation in energy expenditure have focused on the energetic costs or correlates of particular activities (e.g. lactation: Naya *et al.* 2008), environmental conditions (e.g. Zub *et al.* 2009), or individual

**Table 1.** AIC<sub>c</sub> analysis of the predictors of (a) daily energy requirements (DER; kJ per day) and (b) daily energy expenditure (DEE; kJ per day) for female red squirrels estimated using the doubly labelled water (DLW) technique; DER equals DEE plus energy intake exported to offspring

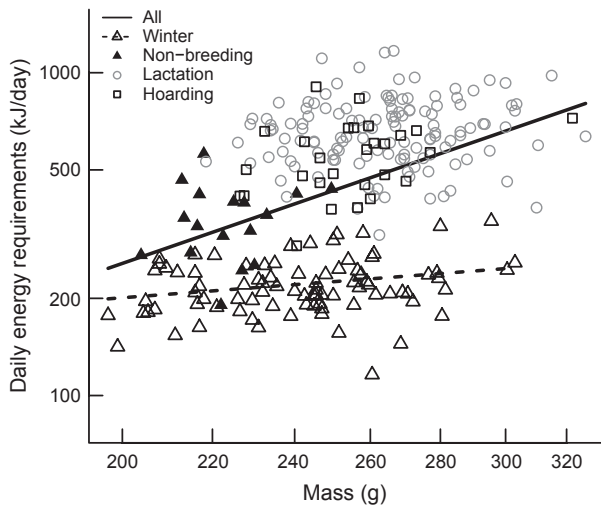
|   | <i>K</i> | logLik       | AIC <sub>c</sub> | ΔAIC <sub>c</sub> | <i>w</i> <sub>i</sub> |
|---|----------|--------------|------------------|-------------------|-----------------------|
| (a)   |          |              |                  |                   |                       |
| <b>Seasonal stage + <i>T</i><sub>a</sub> + resources + mass</b> | <b>8</b> | <b>234.5</b> | <b>-452.5</b>    | <b>0</b>          | <b>0.4</b>            |
| <b>Seasonal stage + mass + resources</b>                        | <b>7</b> | <b>232.9</b> | <b>-451.3</b>    | <b>1.2</b>        | <b>0.22</b>           |
| <b>Seasonal stage + <i>T</i><sub>a</sub> + mass</b>             | <b>7</b> | <b>232.6</b> | <b>-450.7</b>    | <b>1.7</b>        | <b>0.17</b>           |
| Seasonal stage + mass   | 6        | 231.3        | -450.4           | 2.1               | 0.14                  |
| Seasonal stage + <i>T</i> <sub>a</sub> + resources              | 7        | 230.6        | -446.7           | 5.8               | 0.02                  |
| Seasonal stage + resources                                      | 6        | 229.5        | -446.7           | 5.8               | 0.02                  |
| Seasonal stage  | 5        | 228          | -445.8           | 6.7               | 0.01                  |
| Seasonal stage + <i>T</i> <sub>a</sub>                          | 6        | 228.8        | -445.2           | 7.2               | 0.01                  |
| <i>T</i> <sub>a</sub> + mass                                    | 4        | 161.3        | -314.4           | 138.1             | 0                     |
| <i>T</i> <sub>a</sub> + mass + resources                        | 5        | 162          | -313.8           | 138.7             | 0                     |
| <i>T</i> <sub>a</sub>   | 3        | 133.8        | -261.6           | 190.9             | 0                     |
| <i>T</i> <sub>a</sub> + resources                               | 4        | 133.9        | -259.7           | 192.7             | 0                     |
| Mass + resources  | 4        | 36.5         | -64.9            | 387.6             | 0                     |
| Mass  | 3        | 33.6         | -61.1            | 391.4             | 0                     |
| Resources   | 3        | 4.6          | -3.1             | 449.4             | 0                     |
| (b)   |          |              |                  |                   |                       |
| <b>Seasonal stage + <i>T</i><sub>a</sub> + mass</b>             | <b>7</b> | <b>177.7</b> | <b>-341</b>      | <b>0</b>          | <b>0.34</b>           |
| <b>Seasonal stage + <i>T</i><sub>a</sub> + resources + mass</b> | <b>8</b> | <b>178.2</b> | <b>-339.9</b>    | <b>1.1</b>        | <b>0.2</b>            |
| <b>Seasonal stage + <i>T</i><sub>a</sub></b>                    | <b>6</b> | <b>175.9</b> | <b>-339.5</b>    | <b>1.5</b>        | <b>0.16</b>           |
| Seasonal stage + <i>T</i> <sub>a</sub> + resources              | 7        | 176.4        | -338.3           | 2.7               | 0.09                  |
| Seasonal stage + mass   | 6        | 175.3        | -338.2           | 2.8               | 0.09                  |
| Seasonal stage  | 5        | 173.9        | -337.7           | 3.3               | 0.06                  |
| Seasonal stage + mass + resources                               | 7        | 175.6        | -336.7           | 4.3               | 0.04                  |
| Seasonal stage + resources                                      | 6        | 174.2        | -336.1           | 4.9               | 0.03                  |
| <i>T</i> <sub>a</sub> + mass                                    | 4        | 142.5        | -276.8           | 64.2              | 0                     |
| <i>T</i> <sub>a</sub> + mass + resources                        | 5        | 143          | -275.8           | 65.2              | 0                     |
| <i>T</i> <sub>a</sub>   | 3        | 137.1        | -268             | 73                | 0                     |
| <i>T</i> <sub>a</sub> + resources                               | 4        | 137.3        | -266.5           | 74.5              | 0                     |
| Mass + resources  | 4        | 83.7         | -159.2           | 181.8             | 0                     |
| Mass  | 3        | 81.4         | -156.7           | 184.3             | 0                     |
| Resources   | 3        | 69.5         | -132.9           | 208.1             | 0                     |

The seasonal stages include winter, non-breeding during summer, lactation and hoarding. Mass and ambient temperature (*T*<sub>a</sub>) values were obtained during in conjunction with the DLW procedure. Resources were an index of the availability of white spruce (*Picea glauca*) cones, which contain seeds that are the primary food source of red squirrels. The models were ranked in ascending order according to ΔAIC<sub>c</sub> values. Bolded columns represent models with ΔAIC<sub>c</sub> values ≤ 2 that were considered to have substantial support. AIC<sub>c</sub> weights (*w*<sub>i</sub>) represent the probability that each model provides the best fit among the candidate models. *K* represents the number of parameters estimated, and logLik is the log likelihood for each of the DEE and DER models.

traits (e.g. personality: Careau *et al.* 2008). In the present study, we assess the relative importance of predictors from these three axes by quantifying year-round variation in the DEE of a free-ranging mammal. Our results demonstrate that: (i) there is extensive variation in DEE at an intraspecific level, (ii) variables from all three axes contribute to explaining a large proportion of this variation and (iii) an animal's seasonal stage accounts for most variation in DEE, followed by the effect of *T*<sub>a</sub>, mass and food availability. To place the extensive variation in DEE, we observed into the context of interspecific studies of DEE, the 5th and 95th percentiles of squirrel DEE (177 and 660 kJ per day, respectively) would correspond to mammals with masses of 148 and 1120 g,

respectively, on an interspecific scale (Speakman 2000, eqn. 4; range in DEE = 116–919 kJ per day, range of predicted masses = 77–1861 g).

Overall, red squirrels that occupy predictable, seasonal environments engage in activities that are energetically demanding when ambient temperatures are warm, and they invest more in energetically demanding activities when resources are readily available. Interestingly, our results are in accord with recent research on birds that also suggests that favourable environmental conditions, in the form of increased water and resource availability, are associated with increased resting metabolic rate (RMR; Smit & McKechnie 2009). Although our results seem unsurprising,



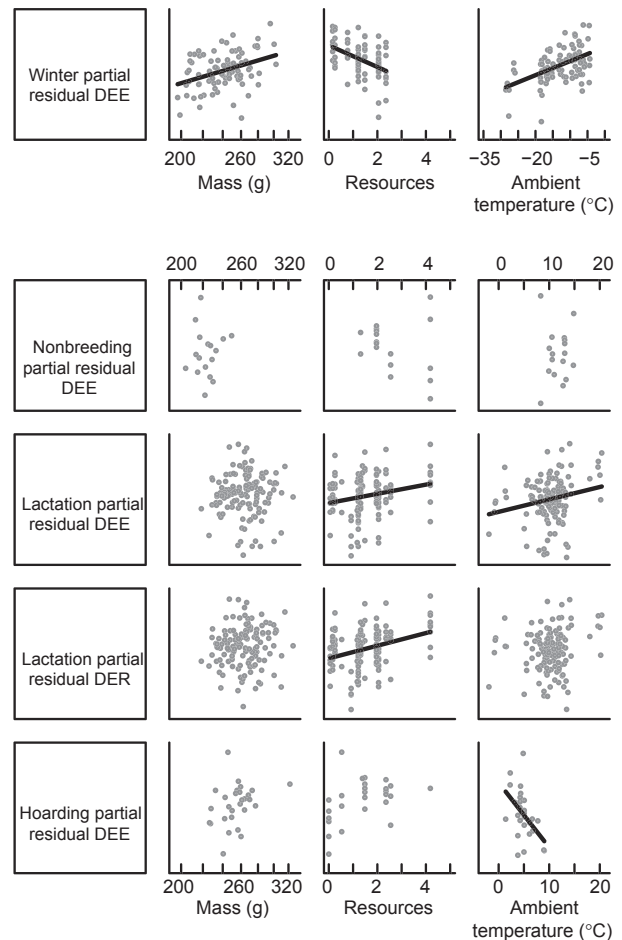
**Fig. 3.** The effect of mass on daily energy requirements (DER). Raw values of DER and mass are plotted on  $\log_{10}$  scaled axes. Lines of best fit (coefficients from analysis on raw values) are plotted pooling all seasonal stages (All – solid line) and for the winter seasonal stage (dashed line), which was the only seasonal stage in which mass was a significant predictor of DER.

**Table 2.** Linear models examining the predictors (mass,  $T_a$  and resources) of daily energy expenditure (DEE) and daily energy requirements (DER) within the seasonal stages of winter, non-breeding, lactation and hoarding

|  | Coefficient | SE    | F    | d.f.   | P        | $sr^2$ |
|--|-------------|-------|------|--------|----------|--------|
| <b>Winter <math>\log_{10}</math> (DEE)</b>       |             |       |      |        |          |        |
| $\log_{10}$ (mass)                               | 0.66        | 0.19  | 12.5 | 1, 81  | 0.0007   | 0.11   |
| $T_a$  | 0.01        | 0.001 | 22.3 | 1, 81  | < 0.0001 | 0.19   |
| Resources  | -0.05       | 0.01  | 16.8 | 1, 81  | 0.0001   | 0.14   |
| <b>Non-breeding <math>\log_{10}</math> (DEE)</b> |             |       |      |        |          |        |
| $\log_{10}$ (mass)                               | 0.33        | 1.64  | 0.04 | 1, 13  | 0.84     | 0.003  |
| $T_a$  | 0.004       | 0.02  | 0.1  | 1, 13  | 0.81     | 0.004  |
| Resources  | -0.03       | 0.03  | 0.6  | 1, 13  | 0.45     | 0.04   |
| <b>Lactation <math>\log_{10}</math> (DEE)</b>    |             |       |      |        |          |        |
| $\log_{10}$ (mass)                               | 0.09        | 0.38  | 0.1  | 1, 126 | 0.81     | 0.0004 |
| $T_a$  | 0.01        | 0.004 | 4.2  | 1, 126 | 0.04     | 0.03   |
| Resources  | 0.03        | 0.02  | 5.5  | 1, 126 | 0.02     | 0.04   |
| <b>Lactation <math>\log_{10}</math> (DER)</b>    |             |       |      |        |          |        |
| $\log_{10}$ (mass)                               | 0.28        | 0.27  | 1.1  | 1, 126 | 0.30     | 0.01   |
| $T_a$  | 0.004       | 0.003 | 1.7  | 1, 126 | 0.19     | 0.01   |
| Resources  | 0.03        | 0.01  | 11.2 | 1, 126 | 0.001    | 0.08   |
| <b>Hoarding <math>\log_{10}</math> (DEE)</b>     |             |       |      |        |          |        |
| $\log_{10}$ (mass)                               | 1.05        | 0.64  | 2.7  | 1, 24  | 0.11     | 0.08   |
| $T_a$  | -0.03       | 0.01  | 5.1  | 1, 24  | 0.03     | 0.15   |
| Resources  | 0.04        | 0.02  | 3.7  | 1, 24  | 0.07     | 0.11   |

Semipartial correlation coefficients ( $sr^2$ ) represent the percent of variation explained by the predictor of interest accounting for the effect of the other predictors.

they generate substantial within population variation in energy expenditure, driven by complex environment–activity–energetic relationships that completely contradict inter-



**Fig. 4.** The effect of mass, resources and ambient temperature ( $T_a$ ) on partial residual daily energy expenditure (DEE) and daily energy requirements (DER). The effect of these predictors is plotted within the seasonal stages of winter, non-breeding during summer, lactation and hoarding. Lines of best fit (coefficients in Table 2) are plotted only if the predictor was significant in the analysis within that seasonal stage (Table 2). Raw values of DEE and daily energy requirements (DER) are plotted on a  $\log_{10}$  scaled axis.

specific analyses of the relative importance and direction of predictors of energy expenditure.

Our results suggest that intraspecific variation in DEE is primarily determined by what an animal was doing when the measurement was taken. Research is required to determine the implications of this finding for interspecific studies that do not incorporate information regarding the seasonal stages animals are engaged in (Nagy, Girard & Brown 1999; Speakman 2000; Anderson & Jetz 2005; Nagy 2005). In intraspecific studies of mammals, lactation is generally considered the most energetically demanding period of the year (Naya *et al.* 2008; but see Lane *et al.* 2010). Our results are surprising because DER during lactation was only 20% higher than DEE during hoarding. Moreover, we quantified DER during lactation when females were likely facing their peak energetic demands; therefore, at less energetically demanding times over the lactation period (*c.* 70 days are required to wean a litter; S. Boutin, A. G. McAdam & M. M. Humphries

unpublished data), DEE during hoarding would have presumably exceeded DER during lactation. Moreover, our finding that DEE during hoarding exceeded DEE during lactation is interesting because it suggests that hoarding species that are capital breeders (Jönsson 1997) expend more energy accumulating capital during the hoarding period than they do when translating that capital into milk for their dependent offspring.

Our finding that squirrels engage in energetically demanding activities when ambient temperatures are warm suggests that intraspecific variation in DEE is strongly influenced by the environmental conditions when the measurement was taken; however, the positive relationship between energy expenditure and  $T_a$  is opposite to interspecific studies (Lovegrove 2003; Anderson & Jetz 2005). The positive relationship between  $T_a$  and energy expenditure presumably results because red squirrels have a suite of adaptations, including enhanced insulation, warm nests and a concentrated hoarded food source, which allow them to minimize energy expenditure at cold temperatures (Pruitt & Lucier 1958; Pauls 1978, 1981; Humphries *et al.* 2005). Previous studies of seasonal variation in energy expenditure have also demonstrated lower levels of RMR during winter when  $T_a$ s are colder (McDevitt & Andrews 1995; Szafrńska, Zub & Konarzewski 2007); however, our quantification of DEE and DER suggests that the amount of energy a free-ranging animal actually requires to remain in energy balance decreases to a much greater extent in response to cold  $T_a$ s as compared to what would be inferred from measuring RMR in captive animals. An increasing number of empirical studies that quantify DEE at below freezing temperatures also find surprisingly low levels of expenditure in the cold (Sheriff *et al.* 2009; Zub *et al.* 2009). Thus, it appears that behavioural flexibility, microhabitat use and morphological adaptations strongly counteract thermoregulatory challenges in non-hibernating mammals. Future empirical research focussing on the effect of  $T_a$  on energy expenditure, incorporating behaviour and refuge quality, is required to fully resolve the mechanisms driving this empirical pattern (Humphries & Umbanhowar 2007).

Our results suggest that intraspecific variation in energy expenditure is influenced to a lesser extent by individual traits like body mass. A weaker relationship between mass and expenditure at the intraspecific level, compared to interspecific level (Nagy, Girard & Brown 1999; Speakman 2000; Anderson & Jetz 2005; Nagy 2005; Speakman & Król 2010), is expected, given the limited amount of intraspecific mass variation, but the lack of correlation between mass and red squirrel expenditure remains surprising given the predictive power of mass in other mammals (e.g. Zub *et al.* 2009). Body mass was only positively related to DEE during winter when energy expenditure was extremely low ( $1.47 \times \text{RMR}$ ; Humphries *et al.* 2005; Humphries & Umbanhowar 2007). During periods of increased energy expenditure, other sources of variation influencing energy expenditure presumably increase which reduces the influence of mass. Our results are also intriguing because the lack of a relationship between body mass and DEE and DER during most of the year is in

direct contrast to the strong positive relationship between body mass and RMR in all species studied to date (McNab 2002) including red squirrels at our study site (M. M. Humphries unpublished data). The combination of these results means that female red squirrels with large masses elevate their energy expenditure (DEE and DER) above their RMR to a smaller extent (i.e. have smaller metabolic scopes) than red squirrels with small masses. One recent hypothesis suggests that large endotherms are less able to dissipate heat than small endotherms, which may constrain the energy expenditure of large endotherms (heat dissipation limit hypothesis; Speakman & Król 2010). However, this interpretation is difficult to reconcile with the positive influence of  $T_a$  on energy expenditure during lactation.

In our study, we also found no evidence that individual differences influenced variation in DEE or DER even after controlling for other drivers of energy expenditure (e.g. seasonal stage, temperature, mass, food availability). In other words, these results suggest that energy expenditure (DEE or DER) is not a repeatable trait in our population. Berteaux *et al.* (1996) found significant, but low levels of repeatability of DEE in meadow voles (*Microtus pennsylvanicus* Ord.) living in natural enclosures. In general, studies examining RMR in captive and free-ranging animals find this measure of energy expenditure to be repeatable (Nespolo & Franco 2007; Szafrńska, Zub & Konarzewski 2007; Larivée *et al.* 2010; for examples where RMR is not repeatable, see Bozinovic 2007; Russell & Chappell 2007). Repeatability can be generally considered an estimate of the upper limit to the trait's heritability (Falconer & Mackay 1996; but see Dohm 2002). Even though recent research is detecting genetic correlations between RMR and a diversity of traits (Sadowska *et al.* 2005, 2009; Wone *et al.* 2009; Careau *et al.* 2011), the lack of repeatability in the DEE and DER of red squirrels casts doubt on the possibility that these traits are heritable and thus may respond to selection (Hayes 2010), or be genetically correlated with other traits (e.g. animal personality; Careau *et al.* 2008).

The contrast between our intraspecific findings, and the conclusions of interspecific analyses, are characteristic of the classic problem of scale in ecology whereby the direction and strength of relationships depend on the scale of comparison (Levin 1992). At the interspecific level of analysis, mass is the primary predictor of energy expenditure and  $T_a$  has a negative effect on energy expenditure (Anderson & Jetz 2005; Speakman & Król 2010), whereas in our intraspecific analysis, mass was unrelated to energy expenditure during most seasonal stages, energy expenditure increased rather than decreased with  $T_a$  and seasonal stage emerged as the major predictor of energy expenditure. Many important large-scale patterns have been successfully explained using interspecific energetic analyses (Brown *et al.* 2004; Clarke & Gaston 2006), but our findings clearly caution against using interspecific patterns to understand intraspecific variation in energy expenditure (Konarzewski, Książek & Łapo 2005). Moreover, our findings highlight that there is substantial intraspecific variation in energy expenditure that is often concealed by species averages used in interspecific studies.



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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Determination of female parturition dates and milk energy output to juveniles.

**Appendix S2.** Laboratory processing of doubly labelled water blood samples.

**Appendix S3.** Literature cited only in the appendices.

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