

# Associations between over-winter survival and resting metabolic rate in juvenile North American red squirrels

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## Summary

1. Resting metabolic rate (RMR) varies considerably among and within species. Two central questions in physiological ecology are whether values of RMR are repeatable and whether an association exists between RMR and fitness.

2. First, we investigated the repeatability of RMR in food hoarding, juvenile, North American red squirrels (*Tamiasciurus hudsonicus* Erxleben). Second, we explored links between RMR and survival. A low RMR may enhance survival if it reduces winter expenditure costs and/or allows more energy to be allocated towards autumn food hoarding. Alternately, a high RMR may enhance survival if it enables juveniles to hoard more food by increasing the throughput of energy available for investment in hoarding activities.

3. Resting metabolic rate adjusted for body mass, was repeatable in both males and females ( $r = 0.77$ ) over a short-term (mean 24.3 days) but only among females ( $r = 0.72$ ) over a long-term interval (mean 192 days).

4. Heavier juveniles and those with a lower RMR relative to their body mass were more likely to survive over-winter. Multiple selection models found significant selection for a decreased RMR ( $\beta' = -0.56 \pm 0.16$ ) and increased mass ( $\beta' = 0.69 \pm 0.17$ ). Survivors also tended to have more food stored within their hoard.

5. A low RMR relative to body mass and large body mass may have allowed individuals to minimize the expenditure costs related to a larger body mass, while maximizing thermal inertia.

**Key-words:** resting metabolic rate, individual, survival, red squirrel, food, juvenile

## Introduction

In endotherms, minimal metabolism is most often measured as the basal or resting metabolic rate (BMR, RMR) which sets the pace of life and continues to be of paramount importance for a number of evolutionary and ecological theories (Ricklefs, Konarzewski & Daan 1996; Koteja 2000; Lovegrove 2000; Brown *et al.* 2004; Hulbert & Else 2004; Speakman, Król & Johnson 2004a; Speakman 2008). Both BMR and RMR are standardized measures that represent the minimal rate of metabolism necessary to maintain basic physiological processes in a thermoneutral animal. RMR differs from BMR in that it allows violation of certain standard conditions, usually the maintenance of a post-absorptive state, while still

requiring the animal to rest in thermoneutrality (Speakman, Król & Johnson 2004a). Currently, the two terms are interpreted similarly for comparative purposes and thus for simplicity we use the abbreviation RMR to be reflective of minimal metabolism. Values of RMR vary widely among and within species (Kleiber 1961). After accounting for the large proportion of variation in RMR explained by body mass and higher-level taxonomic affiliation, comparative studies have linked residual variation to numerous abiotic and biotic factors including climate, altitude, and environmental productivity (Daan, Masman & Gronewold 1990; Lovegrove 2000; Mueller & Diamond 2001; McNab 2002; Cruz-Neto & Bozinovic 2004). At the intra-specific level, variation in RMR remains substantial (Jackson, Trayhurn & Speakman 2001; Johnson, Thompson & Speakman 2001; Boratynski & Koteja 2009) and has been receiving increasing attention in an attempt to

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identify a functional basis for its existence (Speakman, Król & Johnson 2004a).

Resting metabolic rate comprises a large component of a free-living endotherm's energy budget, averaging 30–40% of total daily energy expenditure (Drent & Daan 1980; Nagy, Girard & Brown 1999; Speakman 2000). A lower RMR could provide a fitness advantage for an individual by reducing foraging requirements, which may simultaneously prolong the use of a limited food supply, reduce exposure to predators, and increase the time available for investment in other fitness-enhancing activities. On the other hand, a high RMR may facilitate a greater maximum sustainable metabolic rate (susMR) or burst energy expenditure (maximum metabolic rate; MMR), thus permitting greater resource acquisition and processing rates. This latter hypothesis is comparable to the 'aerobic capacity model' for the evolution of endothermy (Bennett & Ruben 1979; Taigen 1983; Bozinovic 1992; Hayes & Garland 1995) or to the related 'sustained maximal limit model' (Drent & Daan 1980), both of which offer explanations for inter-specific differences in RMR.

Inter-specifically, susMR appears to be linked to RMR (Taigen 1983; Peterson, Nagy & Diamond 1990; Bozinovic 1992; Hammond & Diamond 1997; Rezende *et al.* 2002; White & Seymour 2004). However, intra-specifically, correlations are weaker or are not consistent (Hayes & Garland 1995; Książek, Konarzewski & Lapo 2004; Johnson *et al.* 2007). Attempts to link a high RMR to greater reproductive success have also been unsuccessful (Derting & McClure 1989; Earle & Lavigne 1990; Hayes, Garland & Dohm 1992; Stephenson & Racey 1993; Johnson, Thompson & Speakman 2001; Johnson *et al.* 2007) but few studies have considered how individual differences in RMR may affect survival and expenditure during periods of limited resource availability and adverse environmental conditions (Jackson, Trayhurn & Speakman 2001; Boratynski & Koteja 2009; Welckler *et al.* 2009).

Repeatability is a measure of the consistency of individual differences in a trait and as such can be considered an estimate of the upper limit to the trait's heritability (Falconer & Mackey 1996; but see Dohm 2002 for additional considerations). As a result, quantifying a trait's repeatability provides a useful tool for determining the maximum potential of a trait to evolve under natural selection (Bennett & Harvey 1987; Lynch & Walsh 1998; Bech, Langseth & Gabrielsen 1999; Nespolo & Franco 2007). Currently, only four field studies have quantified the repeatability of RMR, three of which report significance (Bech, Langseth & Gabrielsen 1999; Szafarska, Zub & Konarzewski 2007; Boratynski & Koteja 2009) and one that does not (Bozinovic 2007). The estimation of repeatability must therefore precede discussions of the evolution of physiological traits (Hayes & Jenkins 1997; Boratynski & Koteja 2009).

Our objectives were two fold. First, we estimated the short and long-term repeatability of body mass and RMR in juvenile free-ranging North American red squirrels (*Tamiasciurus hudsonicus*) (Fig. 1) and second, we evaluated whether the RMR of juveniles experiencing naturally limited food



**Fig. 1.** Juvenile North American red squirrel (*Tamiasciurus hudsonicus* Erxleben). Photograph taken in late 2008 from one of the project study grids by Julia Shonfield, a masters student on the project.

supply influenced over-winter survival. Red squirrels are territorial, food-hoarding rodents that specialize on conifer seeds (Steele 1998). At our field site in Kluane, Yukon, Canada, squirrels rely on seeds extracted from the cones of white spruce trees (*Picea glauca*) as a dominant food source (LaMontagne & Boutin 2007). The Kluane Red Squirrel Project offers an exceptional opportunity to study the fitness implications of metabolic variation in free-living animals as food availability can be monitored at multiple scales and local recruitment is related to fitness in this population (Boutin & Larsen 1993; Berteaux & Boutin 2000; Humphries & Boutin 2000; Réale *et al.* 2003; McAdam, Boutin & Sykes 2007; Larivée 2009).

In autumn, red squirrels larder-hoard cones within a central area of their territory called a midden (Steele 1998; Boutin *et al.* 2006). Squirrels must clip cones off trees and hoard them before seeds are released for wind dispersal. Hoarded cones are the primary food source used throughout winter and into the following breeding season (Boutin *et al.* 2006). The autumn hoarding period is characterized by high rates of daily energy expenditure (Q.E. Fletcher & M.M. Humphries unpublished data) which may constrain hoarding efficiency as individuals approach proposed physiological limits (Hammond & Diamond 1997). In winter, squirrels adopt an energy conservative lifestyle which prolongs the use of stored food (Humphries *et al.* 2005). Low survivorship during a red squirrel's first year provides strong opportunity for selection on energetic traits (McAdam, Boutin & Sykes 2007). Possessing a low RMR may enhance over-winter survival by reducing energy demands during a period of limited energy supply. However, these effects of RMR on over-winter survival may be amplified or attenuated by influences of RMR on autumn hoard accumulation. From an allocation perspective (Deerenberg *et al.* 1998; Nilsson 2002; Vezina, Speakman & Williams 2006), possessing a low RMR could increase the energy available for non-resting activities. In this case, a low RMR should be associated with a larger hoard size. Alternately, from a performance perspective (Drent & Daan 1980; Peter-

son, Nagy & Diamond 1990; Hammond & Diamond 1997), possessing a high RMR could enhance sustained metabolic endurance. A high RMR might enable higher rates of expenditure and thus may be associated with a larger hoard size, thus offsetting the predicted positive effects of a low RMR on survival from the allocation model. Here, we test these predictions with data on RMR, DEE, hoard accumulation, and over-winter survival. The independent and collective effects of body mass on metabolism are also examined, because of its potential influence on metabolism, behaviour, and survival.

## Materials and methods

Juvenile red squirrels were studied in conjunction with the Kluane Red Squirrel Project located in southwestern Yukon, Canada (61°N, 138°W) from 12 August 2007–30 March 2008. We used three 40-ha study grids to target juveniles that had settled a territory in autumn 2007 (10 August–1 October). Trapping and animal handling procedures are those described in McAdam, Boutin & Sykes (2007). Settlement was confirmed by observation of repeated territorial vocalizations and trapping of the same individual at the same midden location. The Biological Sciences Animal Policy and Welfare Committee at the University of Alberta approved all protocols for the capture and handling of red squirrels (363803).

### RESTING METABOLIC RATE

We measured RMR instead of BMR to avoid periods of hyperactivity or lowering of body temperature which may occur when small mammals are starved to obtain a post-absorptive state during a metabolic trial (Speakman, Król & Johnson 2004a). Resting metabolic rate was measured using a positive pressure, flow-through system contained in a mobile laboratory located close (<5 km) to the study grids. Juveniles were live trapped and immediately taken to the laboratory where they were provided with *ad libitum* peanut butter and an apple slice for moisture. Prior to a trial, individuals were weighed using a  $\pm 0.1$  g balance (Mettler Toledo PG12001 SDR, 2915 Argentia Road, Unit 6, Mississauga, ON, Canada L5N 866) and sexed. Squirrels were then placed in two litre metabolic chambers and positioned in environmental incubators set to a constant temperature (27 °C) within the thermoneutral zone of red squirrels (Paul 1981). Each metabolic chamber rested on a motion activity detector that provided an index of the animal's activity throughout the trial. Fresh air that had been scrubbed of CO<sub>2</sub> and water via Ascarite® and Drierite® respectively, was pumped through each chamber at a constant rate of 700 mL min<sup>-1</sup>. Ex-current air from each chamber was sub-sampled at 200 mL min<sup>-1</sup>, scrubbed of CO<sub>2</sub> and moisture, and sent to an oxygen analyzer (Sable Systems, Oxzilla-II, Henderson, NV, USA). A computerized data acquisition system (Sable Systems, RM8 Intelligent Multiplexer) was used to baseline the oxygen analyzer with fresh scrubbed air at the beginning and end of the trial and at 15 min intervals throughout the trial. Oxygen concentrations from the chambers were measured at pre-determined intervals and intermittently compared to the baseline. Digital signals from the oxygen analyzer were stored and analyzed using Expedata 1.0.18 data management software by Sable systems. Trial runs were corrected for equipment drift and subsequently RMR was calculated using the lowest level of oxygen consumption recorded for a minimum of 5 min during a 2–3 h run. Because red squirrels are diurnal, metabolic trials were initiated 2 h following sunset so that individuals were more likely to rest. Squirrels were held in captivity for a maximum of 6 h and were returned to their territories prior to sunrise.

### DAILY ENERGY EXPENDITURE

The daily energy expenditure (DEE) of a subset of individuals was determined using the doubly-labelled water method (Butler *et al.* 2004). This method has been widely validated in small mammals including small rodents (e.g. Speakman & Król 2005) and provides an estimate across individuals with a mean accuracy of about 4% (validations reviewed in Speakman 1997). This involved capturing, weighing, and intraperitoneally injecting 0.5 mL of doubly labelled water [10% APE enriched <sup>18</sup>O water (Enritech, Rehovot, Israel) and 99% APE enriched <sup>2</sup>H water (MSD Isotopes, Pointe-Claire, QC, Canada) mixed in a ratio of 20:1]. Following injection, juveniles were left in the trap for 60 min to allow equilibration of isotopes (Król & Speakman 1999) and then bled via a clipped toenail to obtain an initial blood sample for isotope analysis. Blood samples were obtained from unlabelled individuals to estimate the background isotope enrichments of <sup>2</sup>H and <sup>18</sup>O (Speakman & Racey 1987; –method C). Juveniles were recaptured, weighed, and bled again 48–72 h following the initial blood sample. Measurement intervals spanning multiples of 24 h minimize the large day to day variation in DEE (Speakman *et al.* 1994; Berteaux *et al.* 1996). Recaptures were timed to minimize deviations from 24 h intervals (Speakman & Racey 1988). For analysis, we used the average mass of squirrels obtained with a Pesola balance (Pesola AG, Switzerland) at the initial and final blood samples. Capillaries that contained the blood samples were then vacuum distilled (Nagy 1983), and water from the resulting distillate was used to produce CO<sub>2</sub> and H<sub>2</sub> (methods in Speakman *et al.* 1990 for CO<sub>2</sub> and Król, Murphy & Speakman 2007 for H<sub>2</sub>). The isotope ratios <sup>18</sup>O: <sup>16</sup>O and <sup>2</sup>H: <sup>1</sup>H were analysed using gas source isotope ratio mass spectrometry (Isoprime for hydrogen and Isochrom µG for oxygen, both machines by Micromass, Manchester, UK). Isotope enrichment were converted to values of daily energy expenditure using a single pool model as recommended for this size of animal by Speakman (1993). There are several alternative approaches for the treatment of evaporative water loss in the calculation (Visser & Schekkerman 1999). We chose the assumption of a fixed evaporation of 25% of the water flux (equation 7-17: Speakman 1997) which has been established to minimize error in a range of conditions (Visser & Schekkerman 1999; Van Trigt *et al.* 2002). We converted CO<sub>2</sub> production to DEE (kJ day<sup>-1</sup>) assuming RQ = 0.85. Because total body water and fat content are inversely correlated (Robbins 1993), use of the doubly labelled water technique provides a measure of total fat content of an animal. The amount of energy stores possessed by an animal are of interest as larger fat stores may increase the odds of over-winter survival.

### ESTIMATING NUMBER OF CONES IN MIDDENS

The majority of cones remaining on trees were open by September 29th while the first major snow fall occurred on October 1st. A noticeable decline in red squirrel hoarding activity was noted during this time. The number of cones hoarded in middens was estimated by quadrat sampling using a 70 × 70 cm plot. Most juveniles had one midden in their territory, any additional middens were sampled and an average was taken from the two final values. The sampling area on a midden was selected based on visual cues of repeated and recent use by the midden owner. Such cues included fresh diggings, new cones on the surface or within holes, fresh shed bracts from recently consumed cones, and well-used trails. The approximate mid-point of the sampling area was marked by the cross-section of two pieces of 1.8 m long rope placed across the longest width and length of the midden. This pattern formed four sampling quadrats. Within each quadrat we sub-sampled four locations, generating 16 samples plots per territory.

In each quadrat, the first plot was placed closest to the cross-section at 15.5 cm from each rope; the second plot was placed 30.5 cm away from the first plot, and 15.5 cm from the upper rope. The last two plots were similarly spaced beneath the upper two plots. It was noticed that red squirrels tended to hoard cones in clumps beneath woody debris or beneath the surface in holes. Less often, cones were buried singly beneath the surface. In each plot, we enumerated cones that were visible on the surface and buried within  $\sim 13$  cm of the surface. Cones that were hoarded in holes were removed as much as possible without damaging structural integrity. We were confidently able to remove the majority of cones hoarded in holes ranging in depth from  $\sim 7$  cm and  $\sim 35$  cm and replace them after they were counted. The average number of cones per square meter in a midden was calculated by summing the number of cones for each plot and dividing by the area sampled to arrive at a single value (hereafter referred to as hoarded cone estimate – HCE).

#### OVER-WINTER SURVIVAL

Over-winter survival was assessed by a grid-wide population census performed in March 2008. Our ability to confidently estimate survival and fitness based on local recruitment within this study system is possible due to complete enumeration of the study population coupled with the limited dispersal distances of red squirrels (Larsen & Boutin 1994; McAdam, Boutin & Sykes 2007). Population censuses were again performed in May 2008 and August 2008 and all juveniles that were not trapped in March were also not trapped in May or August. Twenty-five juveniles survived the winter and maintained ownership of their original territory. Four additional survivors changed territories and so were excluded from analyses, as we were interested in RMR in relation to the number of cones originally hoarded. The survival of offspring to the spring has been used previously as a measure of fitness in this system (McAdam & Boutin 2003).

#### STATISTICAL ANALYSIS

Repeatability of body mass and RMR was evaluated across three different intervals: during the autumn hoarding season (12 August–1 October 2007), across seasons (August/September, 2007–March, 2008), and during spring (March, 2008) (Table 2). The autumn and spring repeatability periods are referred to as short-term repeatability and the across season period as long-term repeatability. Two successive measurements of RMR were obtained on 26 individuals during the autumn hoarding period. Four individuals were excluded from analyses either due to restlessness ( $n = 2$ ) or to equipment malfunction ( $n = 2$ ). In spring, repeated measurements were successfully attained for 12 yearlings. Because of the possible confounding effects of pregnancy on the repeatability of RMR, pregnant females were not measured in March. Of the 53 individuals measured for RMR in autumn, 29 were re-captured in March. For repeatability measurements across seasons, successive measurements were obtained on 27 individuals.

Since body mass typically accounts for a portion of the observed variation in metabolism, it is necessary to incorporate measures of body mass when calculating the repeatability of metabolic rates. The residuals of the simple regression of mass on RMR in each season and across seasons were used to calculate the repeatability of mass-residual RMR (hereafter known as residual RMR). Repeatabilities for mass and residual RMR were calculated using Pearson's product-moment correlation coefficient ( $r$ ), which assesses the consistency of a trait relative to mean of all measures (van Berkum *et al.* 1989; Hayes

& Chappell 1990; Garland & Carter 1994; Speakman *et al.* 1994; Chappell, Bachman & Odell 1995). Due to the allometric relationship between RMR and mass, metabolic data were log-transformed prior to analyses (McNab 2002). A paired  $t$ -test was used to verify whether juveniles gained mass between autumn and spring and a repeated measures ANCOVA with mass as a covariate, was used to test for changes in RMR between seasons while controlling for the effects of body mass. All variables were tested for normality using Shapiro-Wilk tests. Juvenile body mass measurements were those taken from a digital scale prior to a metabolic trial.

Univariate and multiple logistic regressions were used to test the effect of RMR and DEE on over-winter survival. There were fewer individuals with measures of both DEE and RMR than for measures of RMR alone, and thus a separate model was performed using solely RMR so as to maximize power of detecting an effect of RMR on survival. Additional explanatory variables included in the full models were: mass, sex, study grid, and corresponding interaction terms. Juvenile age was not included in the full models as it was strongly correlated with body mass ( $r_{45} = 0.63$ ,  $P < 0.01$ ) and because values were missing for two individuals. Backwards stepwise model selection was used to select the final, most parsimonious model and a deviance Chi-square test provided an index of the goodness-of-fit between nested models (Zar 1999).

Due to adverse winter weather conditions shortly after the end of the hoarding season, it was not possible to obtain an estimate of hoarded cones for every midden for which we also had metabolic measurements; resulting in a reduced sample size for models with hoarded cone index as a predictor (18 estimates for survivors, 17 for non-survivors). Performance of a Shapiro-Wilk test indicated that hoarded cone index data significantly differed from a normal distribution ( $W = 0.6103$ ,  $P < 0.001$ ), but was normalized by a log transformation.

Multivariate selection analyses estimate selection gradients as measures of the strength of selection acting on a trait while holding the effects of other traits constant (Lande & Arnold 1983). Standardized linear selection gradients were estimated for two separate models because of the reduced sample size in the model that included HCE. Relative fitness was calculated and used as the dependent variable in a multiple linear regression and traits were standardized prior to analysis (Lande & Arnold 1983). The binomial nature of survival data does not bias the estimated selection gradients from this multiple regression (Mitchell-Olds & Shaw 1987), but parametric inference of significance depends on normally distributed residuals. As a result, we calculated standard errors for selection gradients by jackknifing and confidence intervals were estimated to determine the significance of the selection gradients. All analyses were performed in R (R Development Core Team 2006).

## Results

#### REPEATABILITY OF BODY MASS AND RESTING METABOLIC RATE

Juveniles that survived over-winter gained on average 30.6 g between autumn and spring (Table 1) Body mass was repeatable during the short-term in autumn ( $r_{29} = 0.73$ ,  $P < 0.001$ ) and spring ( $r_{27} = 0.94$ ,  $P < 0.001$ ), as well as during the long-term across seasons ( $r_{25} = 0.73$ ,  $P < 0.001$ ) (Table 2). There was a significant positive relationship between RMR and body mass in autumn ( $r_{53} = 0.73$ ,

**Table 1.** Mean and standard errors for juvenile red squirrel body mass, RMR and DEE measured in autumn 2007 and in spring 2008. Values of RMR are converted from their original units of mL O<sub>2</sub> h<sup>-1</sup> to kJ day<sup>-1</sup> to facilitate comparisons with values of DEE

Season and sex	Body mass (g)	RMR (kJ day <sup>-1</sup> )	DEE (kJ day <sup>-1</sup> )
Fall (both sexes)	203.10 ± 14.20	205.52 ± 27.65	339.11 ± 48.61
Female	201.92 ± 26.62	200.02 ± 29.24	319.60 ± 51.23
Male	216.00 ± 19.10	209.62 ± 24.51	340.62 ± 44.97
Spring (both sexes)	242.90 ± 18.70	181.27 ± 19.64	–
Female	238.20 ± 19.10	182.32 ± 19.42	–
Male	246.30 ± 18.30	180.50 ± 20.36	–

$P < 0.001$ ) and a weaker but significant relationship in spring ( $r_{27} = 0.39$ ,  $P = 0.034$ ) (Table 2). Repeatability of residual RMR was significant during autumn ( $r_{20} = 0.83$ ,  $P < 0.001$ , Fig. 2a) and spring ( $r_8 = 0.88$ ,  $P < 0.001$ , Fig. 2b), but not over the long-term across seasons ( $r_{25} = 0.098$ ,  $P = 0.63$ ) (Table 2). However, when the sexes were separated, residual RMR was repeatable over the long-term for females ( $r_{10} = 0.72$ ,  $P = 0.008$ , Fig. 2c) but not for males ( $r_{13} = -0.02$ ,  $P = 0.99$ ) (Table 2). Values of whole-animal RMR were significantly higher in autumn (Table 1,  $t_{80} = -2.66$ ,  $P = 0.01$ ). This was also the case once differences in body mass were considered ( $F_{2,79} = 25.62$ ,  $P < 0.001$ ).

#### OVER-WINTER SURVIVAL

The final selected multiple logistic regression model identified juvenile RMR ( $P = 0.004$ ) and body mass ( $P < 0.001$ ) as significant predictors of over-winter survival (Fig. 3, Table 3). The model yielded an AUC score (area under ROC curve) of 0.84, indicating good ability of the model to correctly classify survivorship (Hosmer & Lemeshow 2000). As per the methods described in Vittinghoff *et al.* (2004), we calculated odds ratios to reflect the change in odds across the 25th and 75th percentile for a given variable. This method requires that the difference between the two percentiles is obtained and then multiplied by the regression coefficient generated by the logistic model. The resultant value is then exponentiated to arrive at the desired odds ratio for the variable

of interest. Based on the odds ratio for RMR (Table 3), a juvenile occupying the 25th percentile (378.61 mL O<sub>2</sub> h<sup>-1</sup>) was 7.13 times more likely to survive over-winter than a juvenile occupying the 75th percentile (444.10 mL O<sub>2</sub> h<sup>-1</sup>). The odds ratio for body mass indicated that a juvenile occupying the 75th percentile (227 g) was 11.35 times more likely to survive over-winter than a juvenile occupying the 25th percentile (200 g). Individuals that survived over-winter did not have significantly higher or lower whole-animal RMR in the previous autumn (non-survivors;  $413 \pm 56.06$  mL O<sub>2</sub> h<sup>-1</sup>, survivors;  $417.60 \pm 40.19$  mL O<sub>2</sub> h<sup>-1</sup>,  $t_{47} = -0.028$ ,  $P = 0.77$ ). Thus, heavier squirrels and those with a lower RMR relative to their body mass were more likely to survive over-winter (Fig. 3a). Results of multivariate selection models found strong significant selection for a decreased RMR relative to the squirrel's body mass and increased mass, but no significant selection for age (Table 4).

Incorporating autumn DEE into a multiple logistic regression model for over-winter survival with RMR, body mass, sex, and study grid as additional predictors, did not significantly improve the fit of the model (Deviance Chi-squared test,  $P = 0.76$ ). In a model including solely autumn DEE and body mass, DEE was not significantly related to survival ( $P = 0.52$ ). Model fit did not improve by incorporating mean ambient temperature (Environment Canada: Haines Junction, Yukon, 608 N, 1368 W; approx. 30 km from study areas), for initial and final blood samples ( $P = 0.69$ ). Total percent body water in juveniles ranged from 65.08 to 77.17 and did not differ significantly between survivors and non-survivors ( $t = 0.79$ ,  $P = 0.43$ ,  $n = 23$ ), thus survivors did not appear to have more fat stores compared to non-survivors.

It should be recognized that because juvenile red squirrels were growing throughout this study, they were not in energy balance. Thus it is necessary to explore the potential implications of metabolism on growth and growth on over-winter survival, as a negative correlation between RMR and growth rate may underlie the association between RMR and over-winter survival. Due to a negative correlation between initial body mass measurements and growth rate (calculated as grams of mass gained over the number of days between two consecutive measures of mass) over the short-term in autumn ( $r_{22} = -0.59$ ,  $P = 0.003$ ) and a positive correlation for the same variables over the long-term (from autumn to spring)

**Table 2.** Repeatability estimates for body mass and residual RMR over short and long-term time periods. Repeatability is estimated as Pearson's product moment correlation coefficient ( $r$ )

Trait	Term	Sex	Period (days)	$n$	$r$	$t$	$P >  t $	95% CI
Body mass (g)	Short-term autumn	Both	25.01 ± 9.2	31	0.73	5.719	< 0.001	0.49–0.86
	Short-term spring	Both	23.30 ± 3.0	11	0.94	8.389	< 0.001	0.78–0.98
	Long-term	Both	192.20 ± 9.5	27	0.73	5.373	< 0.001	0.48–0.87
Residual RMR (mL O <sub>2</sub> h <sup>-1</sup> )	Short-term autumn	Both	25.01 ± 9.2	22	0.77	5.380	< 0.001	0.51–0.90
	Short-term spring	Both	23.30 ± 3.0	11	0.77	4.127	0.004	0.34–0.94
	Long-term	Both	192.20 ± 9.5	27	0.09	0.494	0.626	–
	Long-term	Male	195.34 ± 9.8	15	-0.00	-0.010	0.916	–
	Long-term	Female	192.10 ± 9.6	12	0.72	3.273	0.008	0.24–0.91

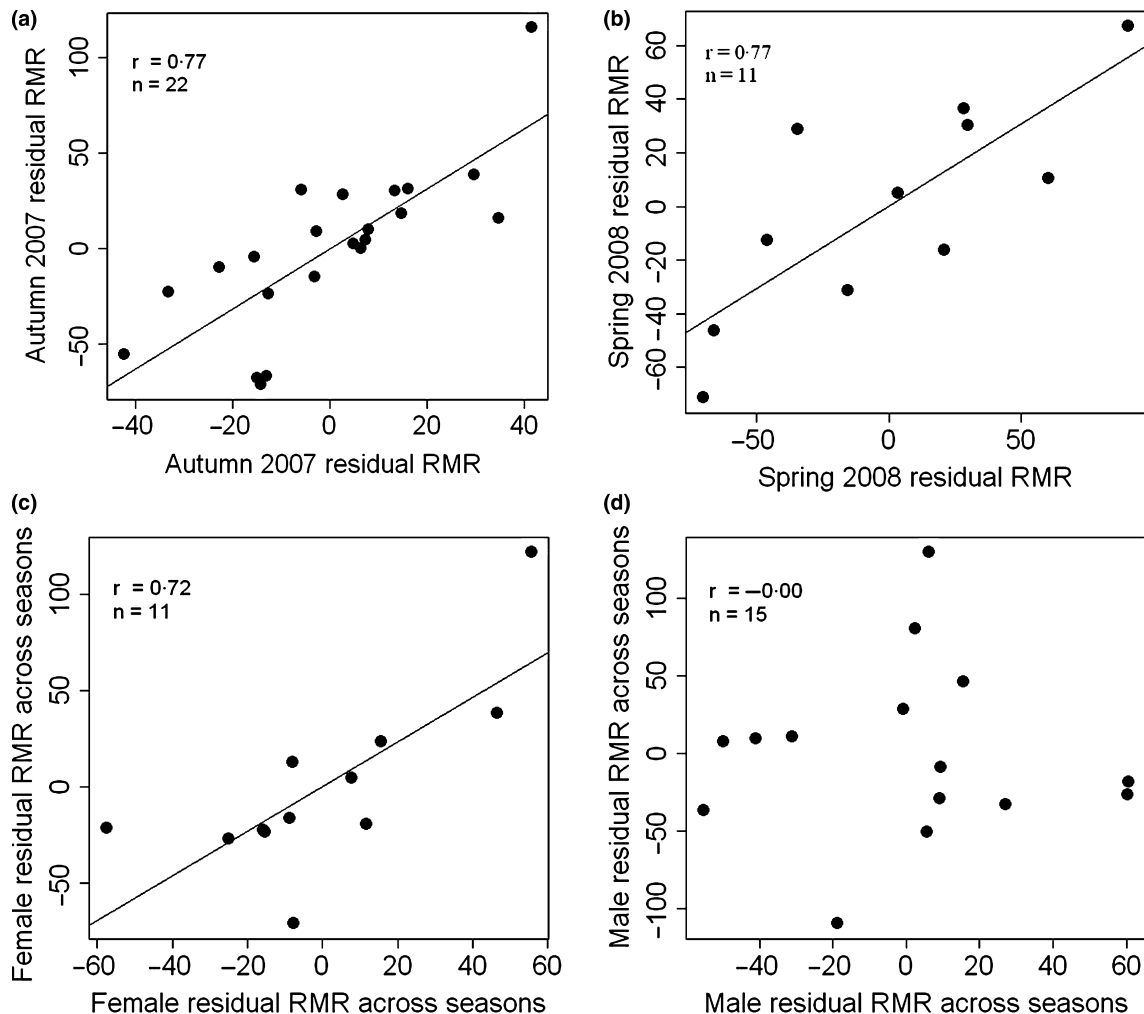


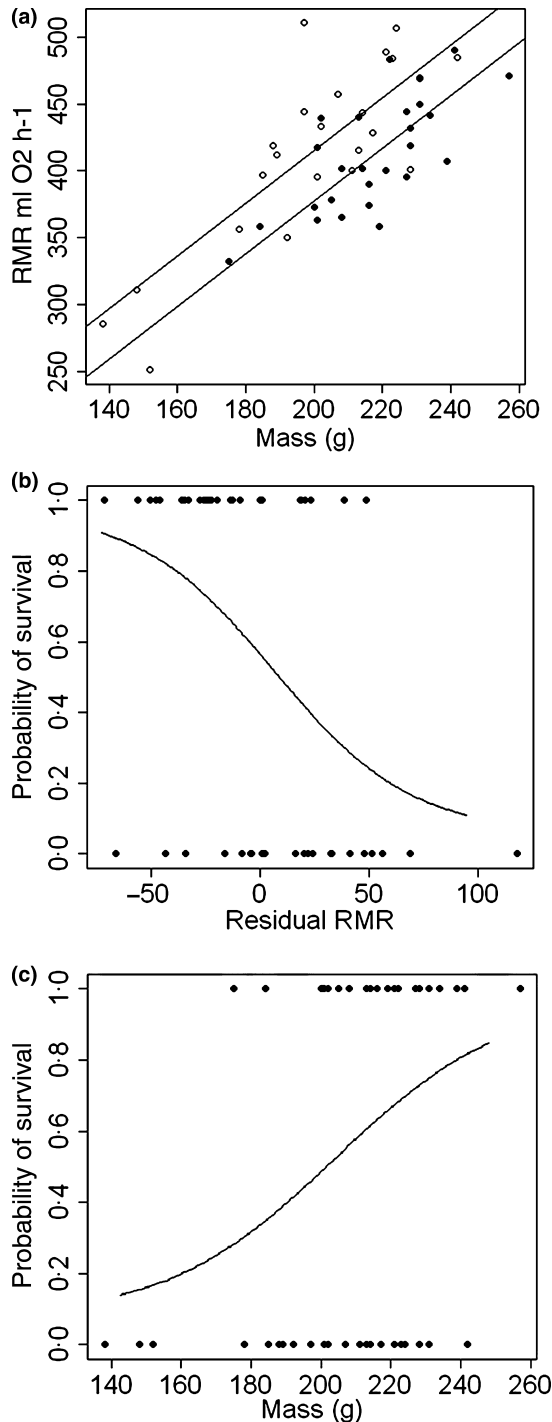
Fig. 2. Repeatability of residual RMR estimated as Pearson's product moment correlation calculated for (a) autumn 2007, (b) spring 2008 and, (c) from August/September 2007–March 2008 (long-term) for females only.

( $r_{27} = 0.82$ ,  $P < 0.001$ ), residual RMR was used to investigate the relationship between RMR and growth rate. No significant relationship was found between residual RMR and growth rate over the short-term (mean growth rate:  $0.94 \pm 0.72$  g day $^{-1}$ ,  $r_{22} = -0.089$ ,  $P = 0.69$ ) or over the long-term (mean growth rate:  $2.46 \pm 0.23$  g day $^{-1}$ ,  $r_{27} = 0.18$ ,  $P = 0.36$ ). Thus, the effect of metabolism on over-winter survival was not significantly influenced by juvenile growth rate.

In a multiple logistic regression including HCE and with RMR, body mass, sex and grid as additional predictors, HCE was identified as the sole predictor in the final model ( $P = 0.008$ , Table 2). An odds ratio of 2.48 for the log of HCE indicated that a juvenile belonging to the 75th percentile was 7.79 times more likely to survive over-winter than an individual in the 25th percentile. Because the results of previous multivariate analyses had consistently identified RMR (with the influence of body mass held constant) to be a significant predictor of over-winter survival, it was suspected that multicollinearity may have been a factor in interpreting models including mass, RMR and HCE. Residual RMR was negatively correlated with log HCE ( $r_{33} = -0.45$ ,  $P = 0.013$ , CI:

$-0.74$  to  $-0.20$ , Fig. 4). Because the relationship between log HCE and body mass bordered significance ( $r_{33} = 0.12$ ,  $P = 0.09$ ), we tested the former relationship again, using mass-adjusted HCE values and found that significance was maintained ( $r_{33} = -0.50$ ,  $P = 0.002$ , CI:  $-0.72$  to  $-0.21$ ). No significant relationship was found between juvenile age and HCE ( $r_{31} = 0.18$ ,  $P = 0.29$ ). In a multiple selection model, including standardized HCE, RMR, mass, and age, there was no significant selection for a larger hoard size. The magnitude of the selection gradients for RMR and mass were very similar to those estimated from the model without HCE (Table 4), but the selection gradient for RMR was no longer significant ( $P = 0.06$ ). This was likely due to the reduction in statistical power, because of missing data for HCE (sample size was reduced from 47 individuals to 33 in the model that included HCE).

The nature and extent of correlations between metabolic traits, body size and the hoarded cone estimate provided insight into their collective influence on over-winter survival. Despite the relationship between residual RMR and HCE, there was no significant relationship between HCE and DEE ( $r_{22} = 0.33$ ,  $P = 0.12$ ) or between HCE and mass-adjusted



**Fig. 3.** (a) ANCOVA scatter-plot for the relationship between RMR and body mass for individuals that survived over-winter (filled diamonds) and those that did not (unfilled diamonds) ( $r = 0.72$ ,  $P < 0.01$ ). Symbols along axes refer to mean values for survivors (filled) and non-survivors (unfilled). For any given mass, individuals that survived had on average lower RMR than those that did not survive. Figures (b) and (c) represent the relationship between probability of survival of juvenile red squirrels in relation to autumn residual RMR and body mass respectively.

DEE ( $r_{22} = 0.18$ ,  $P = 0.39$ ). There was also no significant relationship between HCE and whole-animal RMR ( $r_{33} = -0.18$ ,  $P = 0.92$ ). RMR and DEE were not signifi-

cantly correlated ( $r_{30} = 0.24$ ,  $P = 0.15$ ), as was also the case when variation due to mass was considered ( $r_{30} = 0.013$ ,  $P = 0.93$ ). Thus a relatively high RMR does not appear to enable relatively higher rates of DEE.

Daily energy expenditure, represented as a multiple of RMR (factorial metabolic scope: DEE/RMR), averaged  $1.7 \pm 0.29$ . There was a significant negative relationship between factorial metabolic scope and RMR ( $r_{30} = -0.60$ ,  $P < 0.001$ , CI:  $-0.78$  to  $-0.34$ ) as well as with residual RMR ( $r_{30} = -0.46$ ,  $P = 0.004$ , CI:  $-0.68$  to  $-0.16$ ). Thus a low RMR/residual RMR consisted of a lower proportion of total DEE/residual DEE.

## Discussion

The majority of studies on the repeatability of metabolic traits have focused on adult organisms in laboratory settings (Nespolo & Franco 2007). Currently, three (Bech, Langseth & Gabrielsen 1999; Szafranska, Zub & Konarzewski 2007; Boratynski & Koteja 2009) of the four (Bozinovic 2007) published field studies report significant repeatability for residual RMR while residual field MMR was significantly repeatable in both cases where it was measured (Chappell, Bachman & Odell 1995; Hayes & O'Connor 1999). In our red squirrel population, residual RMR and body mass were repeatable traits over the short- and long-term. We are aware of only one other study on the repeatability of residual RMR across ontogenetic stages. Lu, Wen-Qin & Wang (2007) found that in *Lasiopodomys brandtii*, residual RMR was not repeatable between the juvenile and adult stages, while studies on the repeatability of residual MMR in *Gallus gallus* (Chappell & Bachman 1995) and *Spermophilus beldingi* (Chappell, Zuk & Johnsen 1996) report significant repeatability in adults over relatively long periods, but not between juvenile and adult stages. In red squirrels, the significant repeatability of residual RMR in non-reproductive females but not in reproductively active males suggests that the initiation of reproduction may be associated with a reordering of individual metabolic rankings. If this is the case, juvenile measurements of males cannot be used to predict the residual RMR of reproductive adults. A better understanding of the effect of ontogeny on metabolic phenotypes is required to determine what processes are responsible for the lack of repeatability across these ontogenetic stages.

Juvenile red squirrels were more likely to survive over-winter if they had, on average, larger food stores and if they possessed a higher than average body mass and lower RMR relative to their body mass. We hypothesized that if possessing a high RMR enhances sustained energy expenditure, juveniles with a high RMR in autumn might be enabled to invest more energy in hoarding activity, thus supporting the hypothesis that a high RMR enables greater performance. In contrast, we found that individuals with the lowest residual RMR accumulated the largest hoards. Furthermore, there was no relationship between DEE and RMR (whole animal or residual) in autumn, but a tendency for individuals with the highest RMR to have the lowest differential between

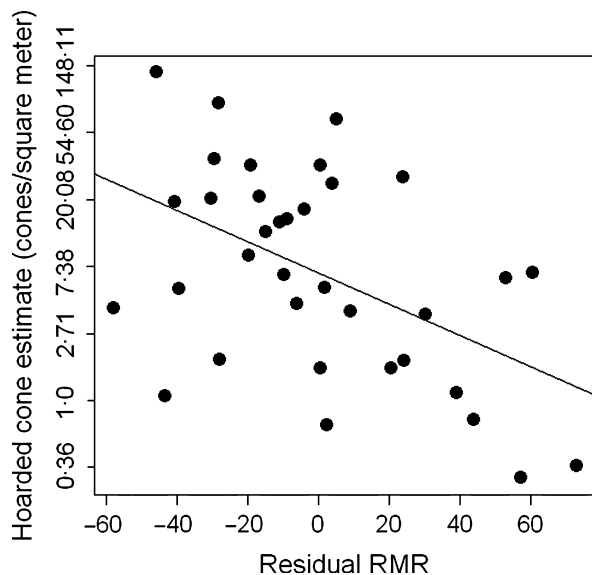
**Table 3.** Finalized\* multiple logistic regression models, with over-winter survival as the dependent variable ( $n = 49$ ). The second model contains hoarded cone estimate (HCE) as a predictor ( $n = 35$ )

Variable	Coeff.	SE	$z$	$P >  z $	Odds ratio	95% CI for odds ratio
RMR	-0.03	0.01	-2.82	0.004	0.96	0.94-0.98
Body mass	0.09	0.03	3.35	<0.001	1.10	1.05-1.17
Constant	-6.89	3.46	-1.99	0.046	-	-
Log hoarded cone index	0.911	0.34	2.64	0.008	2.48	1.32-6.24
Constant	-13.59	6.25	-2.18	0.029	-	-

\*Initial models also included as predictor variables: study grid, sex, and corresponding interaction terms. These variables were removed during stepwise backwards elimination model selection with  $P > 0.05$ .

**Table 4.** Standardized selection gradients ( $\beta'$ ) and standard errors (SE) for traits that significantly affect survival and/or are correlated with traits that affect survival. Boldface gradients are selectively different from zero based on confidence intervals (95% CI) estimated by jackknifing. Gradients calculated from two separate models are given, (model A, model B) and are based on differences in sample size once hoarded cone estimate (HCE) is added to the model

Traits (A, $n = 47$ )	$\beta' \pm SE$	95% CI	Traits (B, $n = 33$ )	$\beta' \pm SE$	95% CI
RMR	<b>-0.56 <math>\pm</math> 0.16</b>	-0.88 to -0.25	RMR	-0.54 $\pm$ 0.27	-1.09 to 0.02
Mass	<b>0.69 <math>\pm</math> 0.17</b>	0.35 to 1.03	Mass	<b>0.75 <math>\pm</math> 0.26</b>	0.22 to 1.28
Age	0.14 $\pm$ 0.15	-0.17 to 0.44	Age	-0.01 $\pm$ 0.16	-0.34 to 0.31
-	-	-	HCE	0.22 $\pm$ 0.22	-0.22 to 0.70

**Fig. 4.** Relationship between residual RMR and back-transformed values for the log of the average number of cones hoarded on an individual's midden (cone estimate).

DEE and RMR. A correlation between residual RMR and HCE, lends support for the allocation hypothesis as opposed to the performance hypothesis (Steyermark 2002), in that juveniles allocating less energy to maintenance requirements may be able to allocate more to cone hoarding. RMR accounted for 60% of average DEE, which is nearly double the reported average of 30–40% (Drent & Daan 1980; Speakman 2000). As a result, juvenile red squirrels are operating at a much lower metabolic scope (DEE/RMR = 1.7) than adult red squirrels in autumn (3.8; Q.E. Fletcher and M.M.

Humphries unpublished data) and most other mammals (Hammond & Diamond 1997; Speakman 2000). Juveniles being characterized by a lower metabolic scope than adults have also been shown in *Spermophilus beldingi* (Chappell & Bachman 1995) and *Lepus americanus* (M. J. Sheriff unpublished data). The demands of growth may limit juveniles to lower metabolic scopes than adults and thus may render them more susceptible to physiological limitation during periods requiring high levels of sustained activity (Czarnoleski *et al.* 2008). Interestingly, juvenile red squirrels in this population typically hoard fewer cones than adults (Q.E. Fletcher and M.M. Humphries unpublished data) and these differences do not appear to be related to differences in territory quality (LaMontagne 2007). Exploration of the interactions among food availability, RMR, DEE, and hoarded food may advance our understanding of the relationship between residual RMR and HCE within this population.

Further energetic explanations for why juvenile red squirrels with low RMR, relative to their body mass, had higher survival than those with high RMR, relative to their body mass, must accommodate the fact that survivors and non-survivors did not differ in whole-animal RMR. Although survivors and non-survivors would have thus had similar total energy requirements in a thermoneutral environment with constant access to resources, survivors tended to be larger than other individuals with similar metabolic rates and have lower metabolic rates than other individuals with a similar body mass. These size and metabolic differences may offer relative advantages in energy acquisition (e.g. larger gut surface area per unit resource requirement) and conservation (e.g. lower conductance and greater thermal inertia per unit resource requirement) in thermally challenging and resource limited environments (McNab 2002). Moreover, the rela-

tively low residual RMR of survivors appeared not to be attributed to individuals having greater fat stores compared to non-survivors. This suggests a metabolic basis to the physiological discrepancy that exists between groups rather than simply a difference in the proportion of generally metabolically inactive tissues. Little variability in fat stores among individuals may be reflective of the arboreal nature of red squirrels such that fat stores may impair agility (Vander Wall 1990; Witter & Cuthill 1993). Alone, the effect of mass on survival may have stemmed from heavier individuals also being older, thus allowing them to potentially settle a territory earlier than later born squirrels. This action could have provided them with more time to hoard cones or to improve cone hoarding techniques. However, we found neither a significant relationship between body mass and HCE, nor between juvenile age and HCE and accordingly, there was no significant selection for age (Table 4).

Presently, only two studies have investigated the association between RMR and survival in endotherms. Jackson, Trayhurn & Speakman (2001) found that in *Microtus agrestis*, individuals with high residual RMR in autumn were more likely to be recaptured the following spring. To minimize the potentially confounding effects of emigration, Boratynski & Koteja (2009) manufactured an island of *Myodes glareolus* and assessed the influence of BMR and MMR (whole-animal and residual) on survival over a 2 years period during winter and breeding seasons. Although no consistent associations between metabolic traits and survival were found within any one season, the presence of stabilizing selection on male MMR (potential influence of mass considered by use of multivariate model) was detected over breeding seasons. In a study investigating natural selection on the thermogenic capacity of high-altitude *Peromyscus maniculatus*, Hayes & O'Connor (1999) found evidence of directional selection for MMR (potential influence of mass considered by use of multivariate model) in one of two consecutive years. The authors proposed that cold, wet conditions may have favoured individuals with potentially higher thermogenic capacities. At our field site in Kluane, prevailing environmental conditions are routinely 35 °C below the lower critical temperature of red squirrels (Woods 2009) and few individuals in the population are able to hoard enough cones in autumn to support metabolic requirements much higher than RMR throughout winter (Q.E. Fletcher and M.M. Humphries unpublished data). Winter survival is possible only by spending most of the time in well-insulated nests and minimizing the duration and thermoregulatory costs of foraging bouts (Woods 2009). Under these conditions, being larger and simultaneously having a lower metabolic rate may minimize total winter energy requirements as defined by required levels of activity and thermoregulation in addition to RMR. The finding of strong negative directional selection (Kingsolver *et al.* 2001) on RMR ( $\beta' = -0.56$ ) and strong positive selection on body mass ( $\beta' = 0.69$ ) highlights the importance of considering the combined affect of these two physiological traits on over-winter survival. Future exploration of this hypothesis

will require comparing measures of DEE of individuals during the winter season.

While a lack of selection studies on metabolic rates in endotherms makes direct comparisons of our results difficult, our findings are in agreement with recent studies that have used ectotherms as a model for investigating contemporary selection on standard metabolic rate (SMR), a trait similarly defined as RMR but recognizing the inconsistency of body temperature characterizing these animals. Bochdansky *et al.* (2005) provided indirect evidence of selection favouring lower SMR in juvenile fish (*Ulvaria subbifurcata*); while recently, Artacho & Nespolo (2009) showed a combination of negative and stabilizing selection on SMR, such that *Helix aspersa* with average to reduced SMR boasted higher survivorship over a defined period in a semi-natural environment. Both studies considered mass as a potentially confounding variable.

The persistence of a substantial intra- and inter-specific variation in resting and active rates of metabolism suggests that a single best strategy does not apply. Though we did not find benefits of a high residual RMR in juvenile red squirrels in the winter of 2007–2008, it is possible that this phenotype is favoured in years when resources are abundant and consequently survivorship is high (McAdam & Boutin 2003). In this study system, the white spruce cone crop resource fluctuates in abundance over three orders of magnitude (LaMontagne & Boutin 2007). High cone production occurs every three to four years, with limited cone production in intervening years (LaMontagne & Boutin 2007). Cone production was low in 2007 and thus supported an environment in which differences in individual energy expenditure might conceivably influence over-winter survival (LaMontagne 2007). By contrast, in high food years, the disadvantages of possessing a high residual RMR may be moderated, as resource levels are sufficient to permit the persistence of individuals with higher metabolic rates. Alternatively, a high residual RMR may be consistently detrimental for over-wintering juveniles but beneficial either prior to autumn settlement or once they recruit into the population as adult breeders. Theoretically, such a situation could arise from a change in the direction of the relationship between residual RMR and absolute metabolic scope during ontogeny, due perhaps to the alleviation of growth related constraints on expenditure. Even so, studies attempting to link a high residual RMR to greater reproductive success have been unproductive (Johnson *et al.* 2007), thus it is not clear how this trait may be favourably selected either in juveniles or adults. Possibly, benefits may be realized through a positive relationship with thermogenic capacity (Haim & Izhaki 1993; Jackson, Trayhurn & Speakman 2001) or with the reduced production of harmful free-radicals (Speakman *et al.* 2004b). A lack of repeatability from juvenile to adult stages suggests that permanent alterations of metabolic phenotypes may occur during ontogeny and warrants investigation of trait selection in both juvenile and adult stages.

## Acknowledgements

We thank the 2006–2008 members of squirrel camp for their assistance with field data collection, especially Laura Turmel and Noemie Laplante, and Ainsley Sykes for coordinating research efforts. Thanks to Paula Redman and Peter Thomson for technician assistance with isotope analysis. Research support was provided by the Natural Sciences and Engineering Council of Canada (NSERC), Northern Scientific Training Program Grants (S. Boutin and M.M. Humphries), and National Science Foundation (A.G. McAdam). An NSERC Postgraduate Graduate Scholarship provided personal support to M.L. Larivée. This is paper number 51 of the Klauane Red Squirrel Project.

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Received 8 May 2009; accepted 3 December 2009  
Handling Editor: Marek Konarzewski