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The fitness consequences of variation in resting metabolic rate in juvenile North American red squirrels (*Tamiasciurus hudsonicus*)

by

Meghan Larivée

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Stan Boutin, Biological Sciences
Erin Bayne, Biological Sciences
Robert Hudson, Renewable Resources

ABSTRACT

Resting metabolic rate (RMR) is the minimum energy expenditure necessary for survival. RMR varies widely both among and within species and a central question in evolutionary physiology concerns the functional basis for this variation. Juvenile North American red squirrels were used to investigate fitness consequences of variation in RMR by considering how expenditure relates to differences in food availability and to overwinter survival. Additionally, this thesis examines whether red squirrels exhibit phenotypic plasticity in RMR in response to varying levels of food availability. Results indicate that heavier juveniles with relatively low RMRs were more likely to survive overwinter. Moreover, these juveniles were capable of allocating more energy towards mechanical work and possessed larger food stores. Food supplemented yearlings exhibited higher RMRs than unsupplemented controls at the onset of the breeding season, while no difference in RMR was detected following termination of supplementation.

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CHAPTER I: GENERAL INTRODUCTION AND THESIS OVERVIEW

THE MYSTERY OF VARIATION IN METABOLIC TRAITS

An animal's physiology influences its food requirements, its activity budget and its capacity for sustained energy expenditure (Pough 1980). Consequently, understanding the selective forces responsible for the evolution of physiological traits is of interest to ecologists. Observed differences in metabolic performance among species are often considered adaptive, yet rarely has the selective significance of physiological traits been examined (Speakman et al. 2004). Basal metabolic rate (BMR) is the minimal rate of metabolism required to keep a non-growing, post-absorptive animal alive under thermoneutral conditions (Kleiber 1961). The concept of BMR originated in recognition of the need for a standardized measurement that would facilitate inter-specific comparisons of energy expenditure and provide an estimate of the basic cost of living (Harris and Benedict 1919, Kleiber 1961, McNab 1992, Speakman et al. 2004). However, early analyses highlighted great variability in BMR, even among species of comparable body mass and among individuals of the same species (e.g. Brody 1945, Kleiber 1961). This observation has since generated considerable debate (e.g. Ricklefs et al. 1996, Lovegrove 2000, Tieleman et al. 2003) concerning the functional basis of variation in BMR.

BMR has been measured in over 600 mammals and 300 species of birds (McNab 2002, White and Seymour 2004); making it the most widely measured metabolic parameter for endothermic vertebrates (McNab 1992). After accounting for variation due to body mass (allometric scaling) and higher-level taxonomic affiliation, there remains a 6-fold range in BMR (Blaxter 1989). Correlative studies have linked residual variation to numerous abiotic and biotic factors such as temperature, latitude, altitude, population growth rate, density, food availability and life-history traits (McNab 1988, Hayes 1989, Hayes et al. 1992, Lovegrove 2000, 2003, Brown et al. 2004, White and Seymour 2004, Broggi et al. 2007). Mueller and Diamond (2001) proposed that a potential unifying thread derived from these correlates might be the net primary productivity of the environment where a species is found. A common garden experiment using five species of *Peromyscus* mice collected from differing environmental locations supports this hypothesis and suggests that inter-specific variation in BMR may indeed reflect genetic adaptation to environmental heterogeneity (Mueller and Diamond 2001). A similar study on stonechats (*Saxicola torquata*) found marked differences in resting metabolic rates (RMR; similar to BMR though animals are not post-absorptive when measured) when birds from different populations were kept under controlled laboratory conditions (Wikelski et al. 2003). A BMR that is adapted to local environmental characteristics likely reflects past selection on individual traits (Schluter et al. 1991). Because natural selection acts on differences among individuals, inter-specific studies can only speculate on proximate factors responsible for phenotypic differences in a trait (*sensu* Bennett 1987, Bozinovic et al. 2007). However, despite an interest in the evolutionary significance of variation in BMR, studies on the selection of proximate factors underlying variation among individuals have only recently received attention.

Variation in BMR at the individual level is considerable, even when measurement error and variation in body mass is considered (Speakman et al. 2004). Because the fitness costs of wasteful expenditure are likely severe, the persistence of variation in intra-specific BMR is somewhat surprising. Life history trade-offs are founded on the basis that energy allocated to a particular activity such as reproduction, competes with other components of the energy budget such as growth, mechanical work, storage, and maintenance (Gadgil and Bossert 1970). Such trade-offs are thought to be driven by physiological constraints on an organism's ability to acquire resources and export energy for work and reproduction (Weiner et al. 1992). An individual with a high BMR would need to forage for longer periods, potentially increasing risks related to predation and exposure, and may have less surplus energy available to allocate to other activities (Speakman et al. 2004). Hypotheses put forth to explain variation in BMR have thus focused on the fitness advantages that a high BMR may accrue.

The dominant hypotheses for the evolution of endothermy propose that high rates of BMR reflect the high maintenance costs of the metabolic machinery required for supporting either greater maximum metabolic rates (MMR, aerobic capacity hypothesis, Bennett and Ruben 1979) and/or sustained metabolic rates (susMR, sustained-limit hypothesis, Drent and Daan 1980). Accordingly, a high BMR could allow for greater resource acquisition, growth rates, and ultimately increase reproductive performance and/or survival probability. Drent and Daan (1980) proposed that possession of a larger alimentary tract and other supportive organs (e.g. liver, heart, and kidneys) may allow individuals to maximize their investment in reproduction or self preservation. However, organs involved in the assimilation and management of energy have relatively high mass-specific metabolic rates that contribute disproportionately to BMR (Drent and Daan 1980, Daan et al. 1990). Thus increasing susMR would require a more proficient alimentary system, resulting in a relationship between susMR and BMR (Chappell et al. 1999, Selman et al. 2001, Piersma 2002, Moe et al. 2004, 2005). A trade-off between a conservative life-style which minimizes costs related to high expenditure and one that is relatively wasteful but enables high aerobic performance is therefore produced. Inter-specifically, a high BMR has been linked with a high maximal and sustained metabolic rate (Peterson et al. 1990, Weiner 1992, Hammond and Diamond 1997). However, such correlations are not ubiquitous for all clades or species (Hayes and Garland 1995). Meta-analyses considering phylogenetically independent contrasts report strong relationships within mammals but not within birds or marsupials (e.g. Koteja et al. 1991, Ricklefs et al. 1996). Results from intra-specific studies are similarly equivocal; however individual variation in energy budget management may play a critical role in determining our ability to detect a relationship between BMR and susMR (Hayes 1989, Chappell and Bachman 1995, Meerlo et al. 1997, Nilsson 2002, Speakman et al. 2003, Vezina et al. 2006). For example, reallocation of energy away from maintenance processes may allow greater investment in reproduction or survival (Nilsson 2002).

The identification of seasonal or ontogenetic bottlenecks, when BMR and related physiological traits (e.g. body mass, organ size) become particularly important to future fitness, is central for intra-specific studies. Correlations between life-history parameters and BMR are more likely to be detected when physiological limits are approached. Physiological constraints on sustained energy expenditure can be grouped into two general, non-mutually exclusive categories. Limits on the rate at which resources can be acquired (foraging rate) and processed (digestion and absorption) are referred to as central limitations (Speakman and Krol 2005). Whereas limits on the rate at which energy is expended (mechanical muscle work, tissue growth, thermoregulation) are referred to as peripheral limitations (Speakman and Krol 2005, Speakman 2008). While there is no consensus as to which type of limitation is primarily responsible for limits on expenditure (Speakman and Krol 2005), possession of a higher BMR may reflect an extension of either of these limits, allowing for greater productivity overall.

Within mammals, one of the most energetically demanding periods occurs during lactation (Thompson and Nicoll 1986, Thompson 1992). There is evidence for an association between BMR and susMR in that the higher energy demands of lactating mice are positively correlated with a higher BMR (Hammond and Diamond 1992, Hammond et al. 1994, Konarzewski and Diamond 1995, Rogowitz and McClure 1995, Rogowitz 1998, Speakman 2008). However, lactating individuals with high BMRs do not necessarily have larger organs (Speakman and Johnson 2000, Krol et al. 2003, Johnson et al. 2007, but see Daan et al. 1990), nor is their reproductive success superior to individuals with lower BMRs (Derting and McClure 1989, Earle and Lavigne 1990, Hayes et al. 1992, Stephenson and Racey 1993, Johnson et al. 2001). Unlike results found at the inter-specific level (Drent and Daan 1980, Peterson et al. 1990, Weiner 1992, Hammond and Diamond 1997) intra-specific relationships between life-history traits and BMR generally disappear once shared variation due to body mass is taken into account. Aside from physiological constraints during reproduction, other bottlenecks that may improve the probability of detecting natural selection on metabolic rates could occur during juvenile stages or during periods of seasonal food limitation or cold ambient temperatures. However, these periods have seldom been examined, with the exception of a possible link between metabolic rate and non-shivering themogenesis in small mammals (Speakman 1996, Jackson et al. 2001).

The primary goal of this thesis was to investigate the selective importance of individual variation in body mass and resting metabolic rate during a period of strong selection pressure in juvenile North American red squirrels (*Tamiasciurus hudsonicus*). This objective was accomplished by first determining the repeatability of RMR in order to evaluate the propensity of natural selection to act on individual differences. Most vertebrate species boast a relatively high coefficient of variation in BMR, but depending on what proportion of this variation is attributed to differences between individuals rather than within individuals is important to consider. For example, populations that exhibit a low degree of consistent inter-individual variation may be poor candidates for studies of contemporary selection. Second, I considered how individual differences in overwinter food supply interact with particular energy requirements of individuals to potentially result in differential overwinter survival. An additional goal was to determine whether red squirrel yearlings exhibit phenotypic flexibility of RMR in response to variation in food availability. The identification of potential instigators of phenotypic flexibility in metabolic traits could permit a better understanding of the causes and consequences of intra-specific variation in metabolic traits. This objective was accomplished experimentally, by food supplementing a subset of individuals throughout winter and then comparing RMR measurements to those of unsupplemented individuals at the onset of the breeding season.

STUDY SYSTEM

Data for this thesis was obtained from a population of free-ranging North American red squirrels in conjunction with a long-term field study entitled the Kluane Red Squirrel Project, located near Kluane Lake in southwest Yukon, Canada (61° N, 138°

W) (KRSP; http://www.redsquirrel.msu.edu/). Founded in 1987, the KRSP is an interdisciplinary effort that combines the fields of behavioural ecology, evolutionary ecology, and physiological ecology to address questions often requiring close monitoring of individuals from birth until death (e.g. McAdam et al. 2007). Climate in the Kluane area is cold and continental, with snow remaining on the ground from early October until May of the following year (Boutin et al. 1995). The KRSP provides an ideal system for the investigation of contemporary selection on BMR (McAdam et al. 2007). Red squirrels are diurnal, tree-dwelling sciurids that are active in all seasons and rely on the seeds of conifer cones as their primary food source. Individuals occupy non-overlapping, year-round territories. In autumn, conifer cones are cached in a central hoard (midden), which provides energy for overwinter survival and for reproduction in the following spring. Females generally give birth between March and June and wean juveniles around 70 days of age (McAdam et al. 2007). Newly independent juveniles must secure a territory in order to survive overwinter (Smith 1968, Larsen and Boutin 1994). Energy allocated to maintenance and growth likely competes with and/or constrains energy allocated to other activities that require mechanical work, such as hoarding cones, territory defense, and foraging. Juveniles are inexperienced in the majority of these activities and generally take longer to complete the same tasks performed by adults. High energy demands combined with a short growing season prior to winter likely contribute to the severe early mortality that characterizes juveniles from this population (McAdam et al. 2007). The sole conifer species in the Kluane area, the white spruce (*Picea glauca*), undergoes episodic and fulfilled synchronous production of large cone crops (masts) (LaMontagne and Boutin 2007). At smaller scales, temporal and special heterogeneity in food availability may lead to the persistence of a slow-fast continuum of BMR. The Biosciences Animal Policy and Welfare Committee at the University of Alberta approved all protocols for the capture and handling of red squirrels.

THESIS CHAPTERS

The research component of this thesis is comprised of two chapters: Chapter II is entitled "The influence of resting metabolic rate and body mass on overwinter survival in juvenile red squirrels" and is related to my primary objective as previously stated. The results in this chapter provides evidence that body mass and resting metabolic rate play a role in determining the overwinter survival of juvenile red squirrels and that individual differences in the amount of food hoarded in central caches may be linked to intrinsic constraints related to differences in basal energy expenditure. Chapter III is entitled "Effects of food supplementation on the resting metabolic rates of yearling red squirrels at the onset of the breeding season**".** This chapter provides evidence that red squirrels display phenotypic plasticity in RMR in response to variation in food availability such that greater expenditure is associated with more food. Overall, this thesis provides preliminary evidence that despite the plastic nature of RMR, among-individual differences are consistent over considerable time periods and variation in RMR is important to overwinter survival.

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CHAPTER II: THE INFLUENCE OF RESTING METABOLIC RATE ON OVERWINTER SURVIVAL IN JUVENILE RED SQUIRRELS

INTRODUCTION

Basal metabolic rate (BMR) sets the pace of life and continues to be of paramount importance for a number of evolutionary and ecological theories (Ricklefs et al. 1996, Koteja 2000, Lovegrove 2000, Brown et al. 2004, Hulbert and Else 2004, Speakman et al. 2004a). Although BMR is a standardized trait that represents the minimal rate of energy expenditure necessary to maintain basic physiological processes in a thermoneutral animal, values vary widely among and within species (Kleiber 1961). After accounting for the large proportion of variation in BMR explained by body mass and higher-level taxonomic affiliation, comparative studies have linked residual variation to numerous abiotic and biotic factors including climate and environmental productivity; suggesting that variation in RMR is adaptive (Cruz Neto et al. 2004, McNab 2002, Mueller and Diamond 2001, Lovegrove 2000, Daan et al. 1990). At the intra-specific level, variation in BMR remains substantial, though comparatively fewer studies have investigated its biological significance (Speakman 2004a).

For selection on a trait to occur, the trait must influence individual performance, must display sufficient variation among individuals, and must be heritable. Repeatability is a measure of the consistency of individual differences in a trait and is a useful tool for determining the potential of a trait to evolve under natural selection (Bennett and Harvey 1987, Lynch and Walsh 1998, Bech et al. 1999). A trait's repeatability can be considered an estimate of upper-limit heritability (Falconer and Mackay 1996; but see Dohm 2002 for additional considerations). As such, the repeatability of a trait should first be demonstrated prior to the investigation of its selective significance (Hayes and Jenkins 1997, Bozinovic 2007). While laboratory studies generally indicate consistent among-individual differences in metabolic traits over time (for review see Nespolo and Franco 2007), the reduction of environmental variation may inflate repeatability estimates (Szafranska et al. 2007, Nespolo et al. 2003). Moreover, the highly plastic nature of BMR may decrease a value's relative performance consistency over longer periods and limit its potential to respond to selection (Chappell and Bachman 1995, Tieleman et al. 2003, McKechnie et al. 2006). Finding low repeatability in field conditions would signify a limited capacity of BMR to respond to natural selection (Berteaux et al. 1996). Currently, four field studies exist on the repeatability of BMR, three of which report significant repeatability (Bech et al. 1999, Szafranska et al. 2007, Boratynski and Koteja 2008) and one that does not (Bozinovic 2007). The estimation of repeatability must therefore precede discussions concerning natural selection of metabolic traits (Boratynski and Koteja 2008).

BMR comprises a large component of an individual's daily energy budget, averaging 30-40% of total daily energy expenditure (Drent and Daan 1980, Nagy et al. 1999, Speakman 2000). A lower BMR could provide a fitness advantage 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 other fitness-enhancing activities. On the other hand, a high BMR may facilitate a greater throughput of maximum sustainable metabolic rate (susMR) or burst energy expenditure (maximum metabolic rate; MMR), thus permitting greater rates of resource acquisition and processing. The existence of such a relationship might increase reproductive output from the possibility that individuals with a high RMR could invest more energy into fitness enhancing activities. This hypothesis is a derivation of the "aerobic capacity model" for the evolution of endothermy (Bennett and Ruben 1979, Taigen 1983, Bozinovic 1992, Hayes and Garland 1995) and is similar to the "sustained maximal limit model" (Drent and Daan 1980), both of which offer explanations for inter-specific differences in RMR. At least inter-specifically, susMR appears to be linked to BMR (Taigen 1983, Peterson et al. 1990, Bozinovic 1992, Hammond and Diamond 1997, Rezende et al. 2002, White and Seymour 2004). However, intra-specifically, correlations are weaker or are not consistent (Hayes and Garland 1995, Ksiazek et al. 2004, Johnston et al. 2007). Attempts to link a high BMR to greater reproductive success have been unsuccessful (Derting and McClure 1989, Earle and Lavigne 1990, Hayes et al. 1992, Stephenson and Racey 1993, Johnson et al. 2001). Critically, few studies have considered how individual differences in BMR affect survival and susMR during periods of limited resource availability and adverse environmental conditions (Jackson et al. 2001, Boratynski and Koteja 2008). Presumably, the ability to conserve energy during such times would provide a potential survival advantage.

My objectives were two fold: First, I investigated the short and long-term repeatability of body mass and resting metabolic rate (RMR; an estimator of BMR, see Materials and Methods) in juvenile free-ranging North American red squirrels (*Tamiasciurus hudsonicus*). Because repeatability estimates reflect both environmental and genetic variation, it is possible that the attainment of significant repeatability estimates is driven mainly by consistent differences in extrinsic conditions over-time.

Considering the close link between energy expenditure and food availability at the interspecific level (Bozinovic et al. 2007, 2009), I explored whether differences in red squirrel food supply influenced repeatability estimates of RMR by investigating repeatability in animals experiencing naturally limited and experimentally supplemented food supply. I predicted that if differences in individual food supply were a primary driver maintaining repeatability, then eliminating differences by food-supplementation (equalizing resources) would either greatly reduce repeatability estimates and the coefficient of variation, or result in a non-significant repeatability estimate. My second objective was to evaluate if the RMR of juveniles experiencing naturally limited food supply influenced overwinter survival.

North American red squirrels are territorial, food-hoarding rodents that specialize on conifer seeds (Steele 1998). Red squirrels in Kluane, Yukon, Canada rely on seeds extracted from the cones of white spruce trees (*Picea glauca*) as a dominant food source (LaMontagne and Boutin 2007). In autumn, cones are larder-hoarded within a central area of a squirrel's 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. Cached cones are 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 (Fletcher and Humphries, unpublished data) which likely constrain hoarding rates as individuals approach proposed physiological limits (Hammond and 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 et al. 2007).

Juvenile experience higher overwinter mortality likely because they are smaller, less experienced, hold poorer-quality territories, and have the added energetic demands of growth (LaMontagne 2007, McAdam et al. 2007). I predict that possessing a low RMR may enhance over winter survival by reducing energy demands during a period of limited energy supply. Alternatively high RMR may be linked to greater thermogenic capacity (Jackson et al. 2001) and thus individuals with high RMR may be more capable of surviving periods of extreme cold exposure. However, these effects of RMR on over-winter survival could be amplified or contradicted by influences of RMR on autumn hoard accumulation. From an allocation perspective, possessing a low RMR could increase the energy that can be allocated to non-resting activities (Deerenberg et al. 1998, Nilsson 2002, Vezina et al. 2006). In this case, a low RMR should be associated with larger hoard size, and the survival benefits of a low RMR should be amplified. From a performance perspective, possessing a high RMR could enhance sustained metabolic endurance (Drent and Daan 1980, Hammond and Diamond 1997). In this case, a high RMR should be associated with large hoard size, and the survival benefits of a low RMR may be subdued. Here, I test these hypotheses with data on RMR, DEE, and hoard accumulation, and over-winter survival. I also examine the independent and interactive effects of body mass, because of its potential influence on metabolism, behaviour, and survival.

MATERIAL 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 August 12, 2007 - March 30, 2008. I used five 40 ha study grids, two of which are food supplemented annually from mid-October until May as part of long-term food-supplementation project. Red squirrels were marked and monitored for survival and reproduction using standardized methodology (McAdam et al. 2007). Trapping efforts were targeted towards juveniles that had settled a territory in autumn 2007 (August 10th – October 1st). Settlement was confirmed by observation of repeated territorial vocalizations and trapping of the same individual at the same midden location.

Food supplementation

The territorial behaviour of red squirrels provides the opportunity to food supplement individuals. Following the hoarding season, a total of 32 juveniles from food-supplemented grids were provided with additional food by suspending 5-litre buckets containing 1 kg of natural peanut butter above each squirrel's midden (completed in conjunction with the KRSP's long-term food-supplementation study). This unnatural food source was chosen because of its high caloric value and because it is not hoarded by squirrels. Buckets were refilled in December, February, April, and May to ensure squirrels had access to *ad libitum* peanut butter throughout winter and early spring.

Flow-through Respirometry

Resting metabolic rate was measured using a positive pressure, flow-through respirometry system contained in a mobile laboratory located close (< 5 km) to the study grids. RMR differs from BMR only in that animals are not post-absorptive prior to measurements. Denying access to food can result in periods of hyper-activity in small mammals which is not conducive for measuring resting state expenditure (Speakman

2004a). Juveniles were live trapped and immediately taken to the mobile 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 ± 0.1 g balance (Mettler PG12001 SDR) and sexed. Because red squirrels are diurnal, metabolic trials were initiated two hours following sunset so that individuals were more likely to rest. Juveniles were returned to their territories prior to sunrise.

Juveniles were placed in two liter 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_2 and water via Ascarite© and Drierite9© respectably, was pumped through each chamber at a constant rate of 700 ml/min. Ex-current air from each chamber was sub-sampled at 200 ml/min, scrubbed of CO_2 and moisture, and sent to an oxygen analyzer (Sable Systems, Oxzilla-II, Henderson, Nevada, U.S.A.). 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 hr run. In autumn

2007, juveniles were measured for RMR prior to food supplementation and again during supplementation in spring 2008.

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 and Krol 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 labeled water (10% APE enriched ^{18}O water [Enritech, Rehovot, Israel] and 99% APE enriched ^{2}H water [MSD Isotopes, Pointe-Claire, Quebec, Canada] mixed in a ratio of 20:1). Following injection, juveniles were left in the trap for 60 min to allow equilibration of isotopes (Krol and 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 ^{2}H and ^{18}O (Speakman and Racey 1987 – method C). Juveniles were recaptured, weighed, and bled again 48-72 hrs following the initial blood sample. Measurement intervals spanning multiples of 24h minimize the large day to day variation in DEE (Speakman et al. 1994; Berteaux et al. 1996). Recaptures were timed to minimize deviations from 24h intervals (Speakman and Racey, 1988). For analysis, the average mass of squirrels was 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_2 and H_2 (methods in Speakman *et al*. 1990 for CO_2 and Krol *et al*. 2007 for H_2). The isotope ratios ^{18}O: ^{16}O and ^{2}H: ^{1}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 and Schekkermann 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_2 production to DEE (kJ/day) assuming RQ = 0.85.

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. I estimated the number of cones hoarded in middens by quadrat sampling using a 70 x 70 cm plot. Most juveniles had one midden in their territory. If more than one midden was present both were sampled and an average 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. Additionally, due to repeated behavioural observations of midden owners throughout the study, I was familiar with preferred hoarding areas. 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 I 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, I enumerated cones that were visible on the surface and buried within ~ 13 cm of the surface. Cones that were hoarded in holes were removed as much as possible without damaging structural integrity. I was confidently able to remove the majority of cones hoarded in holes ranging in depth from ~ 7 cm and ~ 35 cm. It is possible that cones hoarded beyond arms' length were missed. Following enumeration, cones were replaced. The average number of cones per square meter on 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)).

STATISTICAL ANALYSIS

Repeatability of Body Mass and RMR

Repeatability of body mass and RMR was evaluated across three different intervals: during the autumn hoarding season (August 12th - October 1st, 2007), across seasons (August/September, 2007 – March, 2008), and during spring (March, 2008). The autumn and spring repeatability periods are referred to as short-term repeatability and the across season period as long-term repeatability. In addition, long-term repeatability was measured before and after food-supplementing a subset of individuals. The average number of days between measurements was approximately 24 for short-term repeatability, while the average for long-term repeatability was approximetly192 (Table 2-1). 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 32 food-supplemented and 53 control individuals measured for RMR in autumn, 25 and 29 respectively were alive in March. For repeatability measurements across season, successive measurements were obtained on 16 food supplemented and 27 control individuals.

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. 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). Repeatability of mass and residual RMR was 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, Speakman et al.1994, Hayes and Chappell 1990, Garland 1994, Chappell et al. 1995). Pearson's correlation coefficient is more appropriate than the intraclass correlation coefficient (ICC; Sokal and Rholf 1981, Lessells and Boag 1987) for calculating the repeatability of traits in growing organisms, as the ICC is sensitive to changes in the means of repeated measures (Hayes and Jenkins 1997). 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.

Overwinter survival

Overwinter survival was assessed by a grid-wide population census performed in March 2008 (McAdam et al. 2007). Complete enumeration of the study population coupled with the limited dispersal distances of red squirrels provides a robust and reliable measure of survival (Larsen and Boutin 1994, McAdam et al. 2007). Population censuses were again performed in May and August of the same calendar year 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 I was interested in RMR in relation to the number of cones originally hoarded. Univariate and multiple logistic regression was used to test the effect of RMR and DEE on overwinter survival. For presentation purposes, figures are presented as the relationship between the variable of interest and residual RMR. 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. Backwards stepwise model selection was used to select the final, most parsimonious model. 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 the estimated number of comes hoarded as a predictor (18 estimates for survivors, 17 for non-survivors). Performance of a Shapiro-Wilk test indicated that HCE data significantly differed from a normal distribution ($W = 0.6103$, $p < 0.001$), but was normalized by a log transformation. All analyses were performed in R (R Development Core Team 2006).

RESULTS

Repeatability of body mass

Juveniles that survived overwinter gained on average 30.6 g between autumn (203 ± 14.2 g) and spring (242.9g ± 18.737 g). 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 2-1).

Repeatability of resting metabolic rate

There was a significant positive relationship between RMR and body mass in autumn (control; $r_{53} = 0.73$, $p < 0.001$, food-supplemented; $r_{30} = 0.59$, $p < 0.001$) and a weaker but significant relationship in spring (control; $r_{27} = 0.39$, $p = 0.034$, food-supplemented; $r_{21} = 0.48$, $p = 0.019$) (Table 2-1). Repeatability of residual RMR was significant during autumn ($r_{20} = 0.83$, $p < 0.001$, Fig. 2-1a) and spring ($r_8 = 0.88$, $p < 0.001$, Fig. 2-1b), but not over the long-term across seasons ($r_{25} = 0.098$, $p = 0.63$) (Table 2-1). However, when males and females were considered separately, residual RMR was repeatable over the long-term for females ($r_{10} = 0.72$, $p = 0.008$, Fig. 2-1c) but not for males ($r_{13} = -0.02$, $p = 0.99$) (Table 2-1). Similarly, in the food-supplemented group, residual RMR was not repeatable across seasons for both sexes combined, but female residual RMR was repeatable ($r_{10} = 0.62$, $p = 0.031$, Table 2-1).

Values of whole-animal RMR were significantly higher in autumn (control; 426.96 ± 52.89 ml O_2 h^{-1}, food-supplemented; 465.12 ± 40.20 ml O_2 h^{-1}) than in spring (control; 368.42 ± 39.43 ml O_2 h^{-1}, food-supplemented; 436.42 ± 52.77 ml O_2 h^{-1}) (control; $t_{80} = -2.66$, $p = 0.01$, food-supplemented; $t_{22} = -2.70$, $p = 0.01$). This was also the case once differences in body mass were taken into account (control; $F_{2,78} = 25.62$, $p < 0.001$, food-supplemented $F_{2,52} = 714.16$, $p < 0.001$). Moreover, food supplemented squirrels had significantly higher RMR and residual RMR in spring compared to unsupplemented squirrels ($t_{50} = -5.24$, $p < 0.001$, $F_{2, 49} = 33.496$, $p < 0.001$).

Overwinter survival

Resting metabolic rate

The final selected multiple logistic regression model identified juvenile RMR ($p = 0.004$) and body mass ($p < 0.001$) as significant predictors of overwinter survival (Fig. 2-2, Table 2-2). The model yielded an AUC (area under ROC curve) of 0.84, signifying good predictive ability for cases chosen a random within the bounds of the dataset (Hosmer and Lemeshow 2000). Based on the odds ratio adjusted for body mass (Table 2-2), a juvenile occupying the 25th percentile of RMR was 5.5 times more likely to survive overwinter than a juvenile occupying the 75th percentile. The odds ratio for body mass adjusted for RMR indicates that a juvenile occupying the 75th percentile for adjusted body mass was 13.5 times more likely to survive overwinter than a juvenile occupying the 25th percentile. Individuals that survived overwinter did not have significantly higher or lower whole-animal RMRs in the previous autumn (non-survivors; 413 ± 56.06 g, survivors; 417.60 ± 40.19 g, $t_{47} = -.028$, $p = 0.77$). Thus, larger, older squirrels were more likely to survive overwinter and for any given mass, possessing a lower RMR increased the probability of survival (Fig. 2-2a).

Daily energy expenditure

Daily energy expenditure was successfully measured in 37 individuals from two control grids. Two of the collected values were identified as influential outliers (Cook's distance plots) and were thus excluded from analyses. Incorporating DEE into a multiple logistic regression model for overwinter survival with RMR, body mass, juvenile sex, and study grid as additional predictors, did not significantly improve the fit of the model (Deviance Chi-squared test, $p = 0.76$). Despite a smaller sample size, RMR ($p = 0.008$) and body mass ($p = 0.004$) were again retained as predictors in the final selected model.

Cones hoarded

I obtained HCEs for 39 individuals in autumn 2007. In a multiple logistic regression with RMR, body mass, sex and grid as additional predictors, HCE ($p = 0.008$) was identified as the sole variable in the final model (Table 2-2). An odds ratio of 2.48

for HCE indicated that a juvenile belonging to the 75th percentile was 10 times more likely to survive overwinter than an individual in the 25th percentile. Because the results of previous analyses had consistently identified RMR to be a significant predictor of overwinter survival, once differences in mass were accounted for, I suspected that multicollinearity may have been a factor in interpreting models including both mass-corrected RMR and HCE. Indeed, further exploration revealed that once variation due to body mass was considered, RMR was negatively correlated with HCE ($r_{33} = -0.45$, $p = 0.013$, CI: -0.74 - -0.20, Fig. 2-4). No significant relationship was found between HCE and autumn body mass ($r_{33} = 0.12$, $p = 0.09$).

Relationship between energy expenditure and cones hoarded

There was no significant relationship between HCE and DEE ($r_{22} = 0.33$, $p = 0.12$) or between HCE and residual DEE ($r_{22} = 0.18$, $p = 0.39$). There was also no significant relationship between HCE and RMR ($r_{33} = -0.18$, $p = 0.92$). As previously noted, a significant inverse relationship was found between residual RMR and HCE. RMR and DEE were not significantly correlated ($r_{30} = 0.24$, $p = 0.15$), as was also the case when variation due to mass was considered ($r_{30} = 0.013$, $p = 0.93$).

DEE, represented as a multiple of RMR (factorial metabolic scope: DEE/RMR), averaged 1.7 ± 0.29. There was a significant negative relationship between factorial metabolic scope and RMR ($r_{32} = -0.60$, $p < 0.001$, CI: - 0.78 - 0.34) as well as with residual RMR ($r_{32} = -0.46$, $p = 0.004$, CI: - 0.68 - 0.16). Thus a low RMR/residual RMR consisted of a lower proportion of total DEE, possibly allowing for more energy to be invested into non-maintenance activities. Subtracting RMR from DEE provided a measure of the energy available for work or thermoregulation (absolute metabolic scope). For unsupplemented juveniles in autumn, absolute metabolic scope averaged 129.44 ± 46.65 kJ/day and was inversely related to residual RMR ($r_{33} = -0.43$, $p = 0.013$, CI: -0.67 - -.09, Fig. 2-3). Mass-corrected absolute metabolic scope was also inversely related to residual RMR ($r_{33} = -0.54$, $p < 0.001$, CI: -0.74 - -0.26). Thus, juveniles with lower RMR for their body mass had more energy available to potentially allocate to mechanical work. Despite this, a significant relationship was not found between energy available for work and HCE ($r_{26} = 0.15$ $p = 0.43$, Fig. 2-5).

DISCUSSION

Repeatability of body mass and RMR in juvenile red squirrels

Residual RMR and body mass are repeatable traits in juvenile red squirrels. Residual RMR remained repeatable in juveniles that had been food supplemented throughout winter. This suggests that differences in food availability minimally influence individual metabolic rankings and provides additional confidence that differences in metabolic values may have a genetic basis.

The majority of studies on the repeatability of metabolic traits have focused on adult organisms (Nespolo and Franco 2007). However, in many species, juveniles experience important selection events that strongly influence future population dynamics (McAdam et al. 2007). I am aware of only one other study on the repeatability of residual RMR across ontogenetic stages. Lu et al. (2007) found that in Brandt's voles (*Microtus brandti*) residual RMR was not repeatable between juvenile and adult stages. Similarly, studies on the repeatability of MMR in red jungle fowl (*Gallus gallus*) and Belding's ground squirrels (*Spermophilus beldingi*) report significant repeatability in adults over long periods but not between juvenile and adult stages (Chappell and Bachman 1995, Chappell et al. 1996, 1999). In this study, the finding of significant repeatability 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 in red squirrels. Determining when RMR is repeatable and over what time period will allow for an improved definition of the trait itself. 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.

The primary goal of this study was to assess the influence of autumn RMR on overwinter survival, thus long-term repeatability estimates are of direct interest. Although residual RMR in males was not repeatable across seasons, it is possible that it remained repeatable prior to male reproductive maturation. If so, selection during this period could have acted on predictable rankings of RMR values. However, consecutive measurements of RMR were not collected throughout winter and thus it is not certain at what point male RMR rankings could no longer be predicted from autumn measurements. Despite this, the significant influence of residual RMR on overwinter survival, regardless of sex, suggests that selection events occurred prior to the reduction of repeatability estimates.

RMR and overwinter survival

Juvenile red squirrels were more likely to survive overwinter if they possessed in autumn a higher than average body mass and a comparatively low RMR for their body mass. This accounts for why whole-animal RMR did not differ between survivors and non-survivors, although survivors were heavier than non-survivors and there was a significant positive relationship between body mass and whole-animal RMR. I am aware of only two studies that have investigated the association between RMR and survival. Jackson et al. (2001) found that short-tailed field voles (*Microtus agrestis*) were more likely to survive overwinter if they possessed higher residual RMRs. However, interpretation of this result is compromised as emigration of animals from the study area was not considered. Recently, Boratynski and Koteja (2009) manufactured an island population of bank voles to assess the importance of BMR and MMR for survival over two years while nearly eliminating the confounding influence of emigration. Although no consistent associations were found within seasons, the presence of stabilizing selection on male MMR was detected over breeding seasons, suggesting that both extremes of the MMR continuum incurred fitness costs.

Potential energetic explanations for why juvenile red squirrels with low RMRs for their body mass had higher survival than those with high RMRs for their mass must consider 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 thermal neutral 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 combined 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. Kluane red squirrels rarely hoard enough cones to support metabolic requirements much higher than RMR throughout winter (Fletcher and Humphries unpublished data), despite prevailing environmental conditions that are routinely 35°C below their lower critical body temperature (Humphries et al. 2005). Survival is possible only by spending most of the time in well-insulated nest and minimizing the duration and thermoregulatory costs of foraging bouts (Woods and Humphries unpublished data). Under these conditions, being larger than other individuals with a similar metabolic rate and having lower metabolic rates than individuals with a similar body mass may minimize total winter energy requirements as defined by required levels of activity and thermoregulation in addition to RMR.

It was predicted that if possessing a high RMR enhances sustained energy expenditure, juveniles with a high RMRs in autumn may be enabled to invest more energy in hoarding activity; thus supporting the hypothesis that a high RMR enables greater performance capacity. However, neither whole-animal RMR and DEE or residual RMR and DEE were positively correlated in autumn. Moreover, the finding of a negative relationship between residual RMR and HCE provides support for the allocation hypothesis as opposed to the performance hypothesis, in that juveniles with lower expenditure tended to have more hoarded cones in their middens. However, although juveniles with lower residual RMRs also had more energy available to invest in mechanical work, HCE was not significantly related to energy available for work. While it is possible that small sample size may be partly responsible, it is suggested by figure (2-5) that perhaps the variable nature of measures of DEE (Berteaux et al. 2006) coupled with differences in individual energy budgets may also have been important. The number of cones hoarded in a midden likely depends on an individual's physiological capacity for work and how it invests available energy. Although two juveniles may have similar absolute metabolic scopes, their energy budgets may differ through disproportionate investment in hoarding activity or, if both invest equally, in returns for investment (i.e. some individuals may invest similarly in hoarding but obtain fewer cones based on differences in the spatial distribution of cones on a territory). In figure 2-5, four individuals appear to have relatively high absolute metabolic scope though relatively few cones hoarded. Indeed, exclusion of these points results in a significant positive relationship ($r_{22} = 0.56$, $p = 0.004$). Survivors overwinter had more cones hoarded in their middens and more energy available for work, suggesting that possessing a greater capacity for mechanical work may allow an advantage in hoarding more cones overall, despite differences in individual efficiency or accessibility. Thus, the survival benefits of a low RMR are potentially amplified through thermoregulatory advantages and through larger hoards, though at this time it is not possible to determine whether the relationship between residual RMR and HCE is causal or correlative.

Considering the potential fitness advantages associated with a low residual RMR, juveniles with high RMRs are presumably either incapable of reallocating energy from maintenance metabolism towards work or are unable do so without incurring fitness costs which nullify the benefits of reallocation. Growing mammals have high maintenance costs, even when variation due to thermoregulatory costs and body mass are removed (Wieser 1994). For red squirrel juveniles, increasing an already high RMR to accommodate the energy required to increase investment in hoarding activity may not be physiologically feasible or optimal if digestive organs are operating near or at maximum assimilation capacity to optimize growth (Hammond and Diamond 1997). A remaining option would be to reallocate energy away from maintenance metabolism towards work, but this would require diverting energy away from growth processes. Subsequently, juveniles may be constrained in their ability to alter energy expenditure to optimize both growth and work demands, compared to adults. Indeed, RMR accounted for 60% of autumn DEE, which is a notable proportion compared to an average range of 30-40% of total energy demand (Drent and Daan 1980, Speakman 2000).

Hammond and Diamond (1997) suggested that there is an upper sustained metabolic scope that animals can sustain over extended periods. Based on a report of 50 vertebrate species with the highest metabolic scope, the average scope was 3 (Hammond and Diamond 1997). Previously collected measurements of DEE in hoarding adults in this population were 3.8 times RMR (Fletcher and Humphries unpublished data), signifying that individuals are working close to proposed physiological maximums. Surprisingly, juvenile scope in autumn is almost half that of adults (1.7). Similarly, Chappell and Bachman (1995) found that juvenile Belding's ground squirrels had significantly smaller metabolic scopes than adults. This suggests either that juveniles are characterized by a lower average DEE or by a combination of a low DEE and high RMR. A small sample of RMR measurements from juvenile ($n = 10$) and adult ($n = 11$) Kluane red squirrels, collected following the 2006 hoarding season (mid-October), lends support to the latter hypothesis in that juveniles had significantly higher residual RMRs compared to adults (ANCOVA, $F_{2,18} = 6.28$, $p = 0.02$), though values of post-hoarding RMRs were lower compared to 2007 hoarding measurements. While small sample sizes prevent confident conclusions as of yet, the continuation of this work will result in a better understanding as to how the expenditure profiles of juveniles may differ from adults.

The persistence of a high-low continuum of metabolic rates in endotherm populations suggests that a single best strategy does not apply. Though no benefits of a high RMR were identified, it is possible that these phenotypes are retained in years when resources are abundant and consequently survivorship is high (McAdam and Boutin 2003). In this study system, the white spruce cone crop resource fluctuates in abundance over three orders of magnitude. High cone production occurs every three to four years, with limited cone production in intervening years (McAdam and Boutin 2003). Cone production was low in 2007 and thus supported an environment in which differences in individual energy expenditure might conceivably influence overwinter survival (Lamontagne 2007). By contrast, in high food years, the disadvantages of possessing a high residual RMR would be moderated, as resource levels would be sufficient to permit the persistence of individuals with higher metabolic rates. It is also important to consider that a high RMR in an adult squirrel may not pose the same costs to fitness as in a juvenile. Theoretically, such a situation could arise from a change in the direction of the relationship between 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, thus it is not clear how a high RMR may be favourably selected either in juveniles or adults (Johnston et al. 2007). Although, there is some evidence of a link between a high RMR and the reduced production of harmful free-radicals (Brand 2000, Echtay et al. 2002, Speakman et al. 2004b).

Concluding remarks

Studies of individual variation in metabolic rates are necessary for determining the extent to which traits are under selection. Exploring how environmental factors and physiological mechanisms affect the consistency of individual differences will permit a better understanding of whether inter-specific variation in metabolic traits is primarily the result of genetic adaptation or of plastic responses to environmental heterogeneity (McKennie et al. 2006). The use of repeatability estimates provides relatively rapid way to determine a trait's likelihood to respond to selection. However, without emphasis on the fitness consequences of individual variation it is difficult to discuss the biological significance of the trait in question. I have demonstrated that red squirrels express consistent inter-individual differences in residual RMR that are not especially affected by physiological acclimatization to seasonal changes or changes in food supply. These findings suggest a strong potential for heritability of juvenile RMR. This is one of first studies to find an association between RMR and survival, moreover during a period previously demonstrated as important to future population dynamics of the study species (McAdam et al. 2007).

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Table 2-1. Repeatability estimates for body mass and residual RMR over short and long-term time periods, including repeatability of residual RMR before and after food-supplementation (Food). Repeatability is estimated as Pearson's product moment correlation coefficient (r).

Variable	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_2 h^{-1})								
	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
	Long-term (Food)	Female	193.92±10.5	12	0.62	2.4991	0.032	0.07 – 0.88

Table 2-2. Finalized* multiple logistic regression models, with overwinter 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 (HCE)	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 using a deviance Chi-square test of fit.

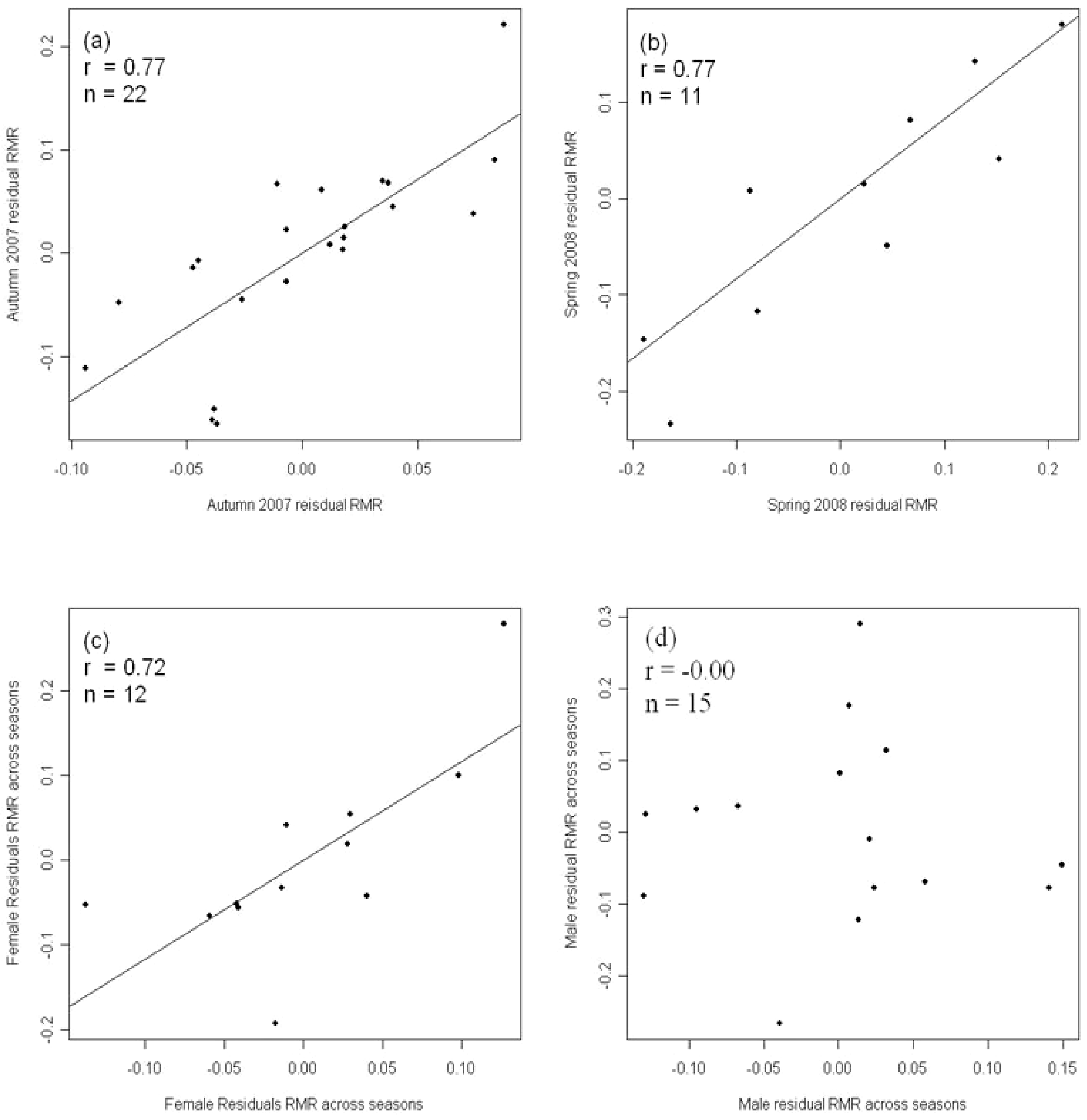
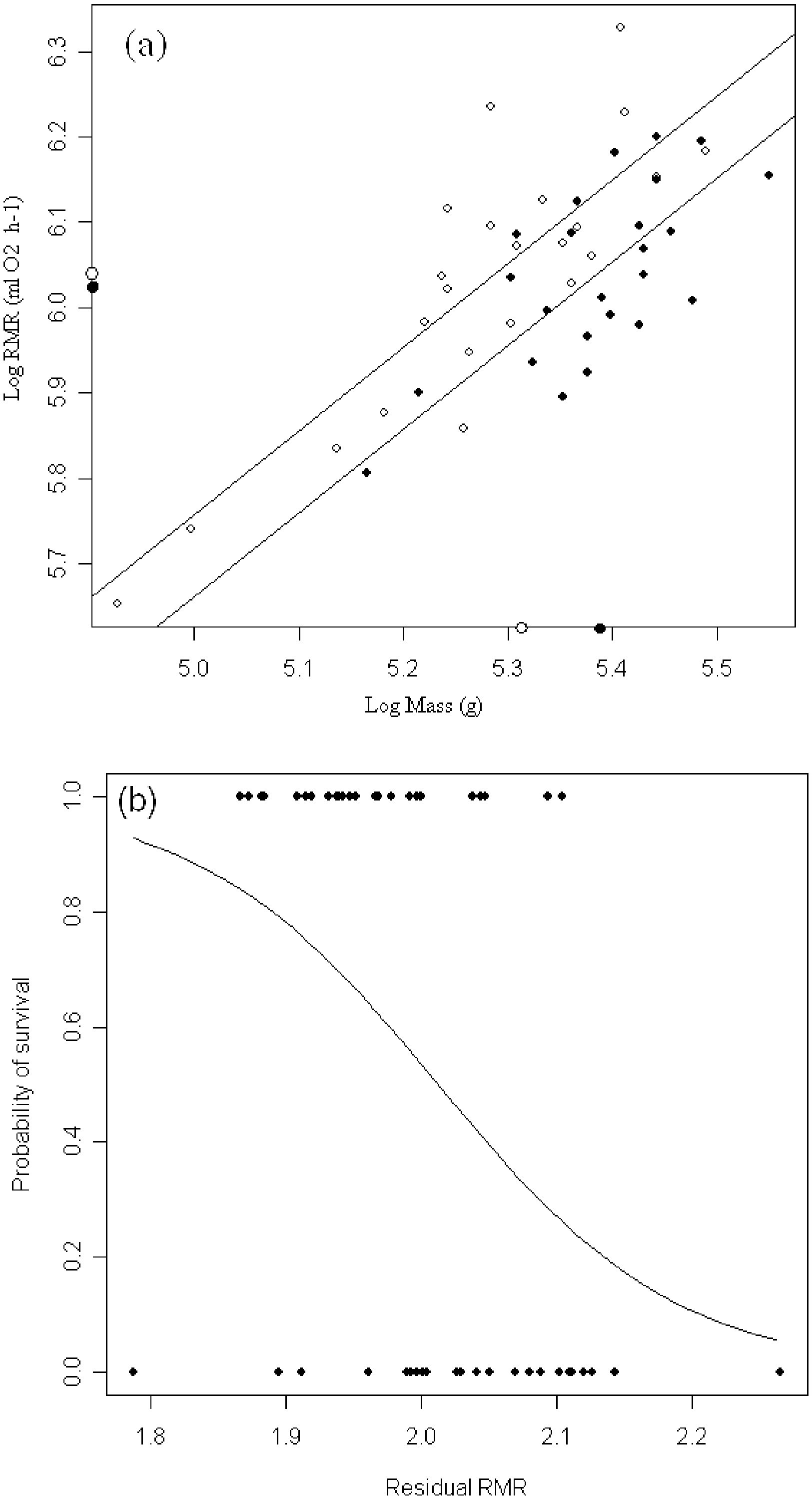


Figure 2-1. Repeatability of residual RMR estimated as Pearson's product moment correlation calculated for (a) autumn 2007, (b) spring 2008 and, (c) from August/September 2007 – March 2008 (long-term) for females only.



(a)
Log RMR (ml O2 h-1)
6.3
6.2
6.1
6.0
5.9
5.8
5.7
5.0
5.1
5.2
5.3
5.4
5.5
Log Mass (g)
(b)
Probability of survival
1.0
0.8
0.6
0.4
0.2
0.0
1.8
1.9
2.0
2.1
2.2
Residual RMR

.

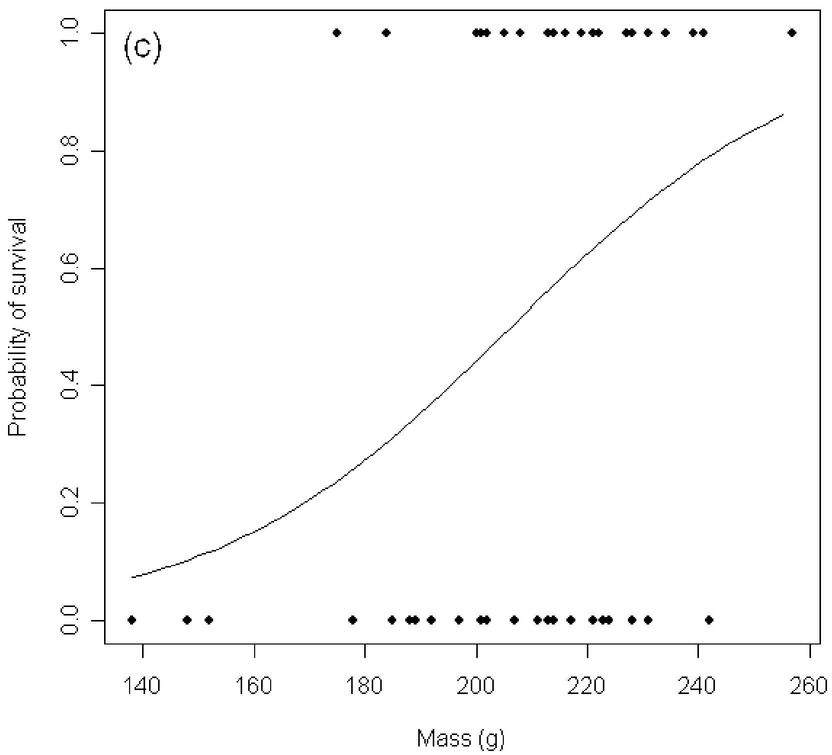


Figure 2-2. (a) ANCOVA scatter-plot for the relationship between the log of RMR and the log of body mass for individuals that survived overwinter (filled diamonds) and those that did not (unfilled diamonds). Symbols along axes refer to mean values for survivors (filled) and non-survivors (unfilled). For any given mass, individuals that survived had on average lower RMRs 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 (calculated from the regression of RMR on body mass and transformed by adding two to each residual in order to fit a logistic curve) and body mass respectively.

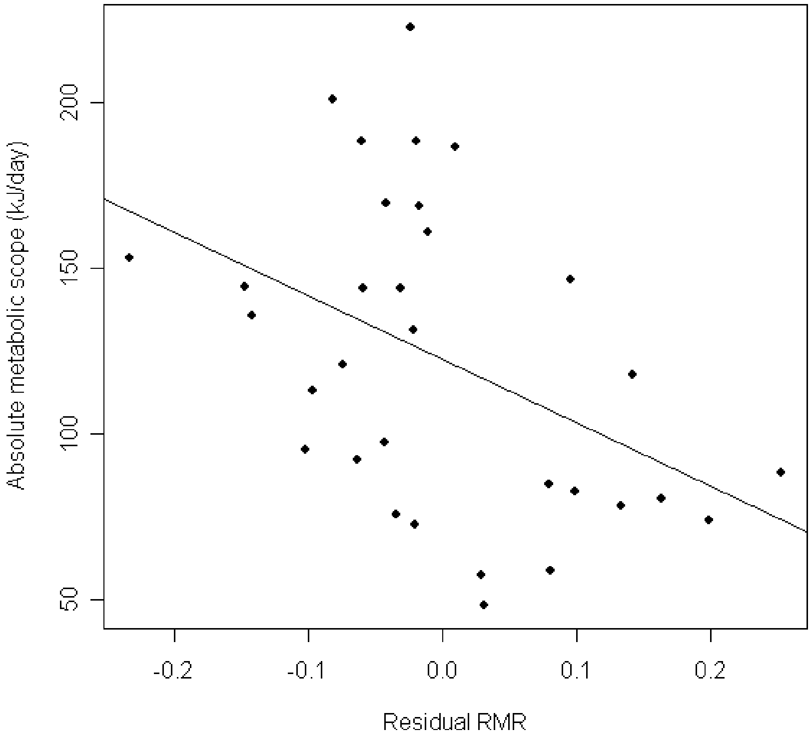


Figure 2-3. Relationship between absolute metabolic scope (kJ/day) and RMR (kJ/day) for juvenile red squirrels in autumn.

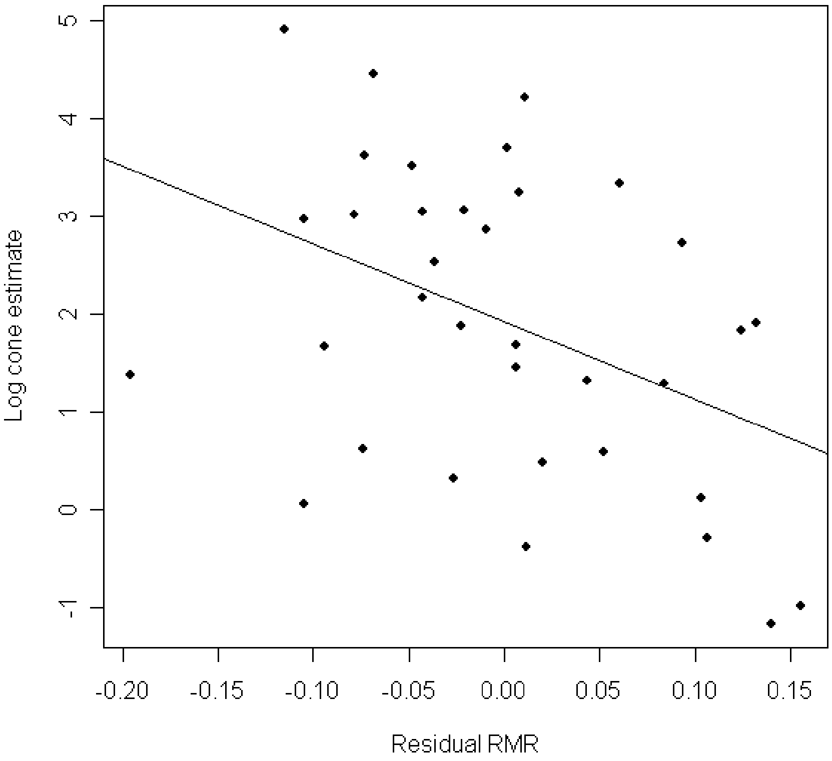


Figure 2-4. Relationship between residual RMR and log of the average number of cones hoarded on an individual's midden (cone estimate).

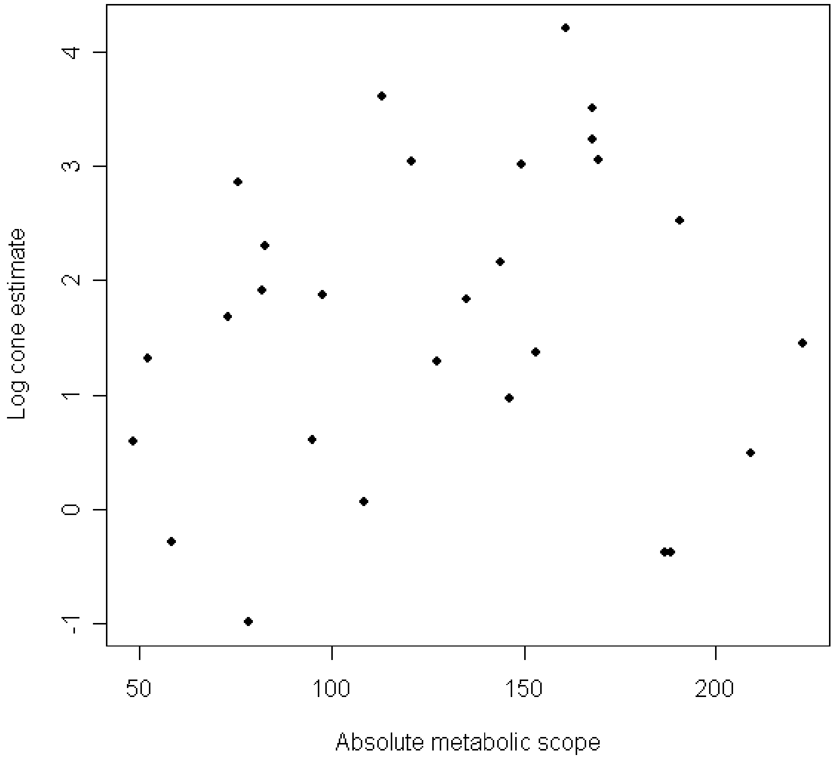


Figure 2-5. Relationship between absolute metabolic scope and log of the average number of cones hoarded on an individual's midden (cone estimate).

CHAPTER III: EFFECTS OF FOOD SUPPLEMENTATION ON RESTING METABOLIC RATE AND BODY MASS OF YEARLING RED SQUIRRELS AT THE ONSET OF THE BREEDING SEASON

INTRODUCTION

For ecological and physiological ecologists interested in the maintenance of metabolic variation among species, consideration of the phenotypic flexibility of metabolic traits is required, as traits are not fixed through time or within individuals (Speakman et al. 2004). Phenotypic flexibility refers to reversible changes in a trait due to changes in intrinsic or extrinsic conditions (Piersma and Drent 2003). As a physiological trait, resting metabolic rate (RMR) exhibits high phenotypic flexibility while also displaying consistent inter-individual variation (repeatability) (Chapter 1 of this thesis, Nespolo et al. 2008). Phenotypic flexibility in RMR has been documented in birds (Piersma et al. 1996, Broggi et al. 2004, McKennie et al.2006, 2007, McKennie 2008, Tieleman et al. 2003) and mammals (Lovegrove 2005, Naya et al. 2008) and is an important component of seasonal acclimatization and short-term acclimation to changes in ambient temperatures, resource levels and physiological status (McKennie 2008). One potential instigator of phenotypic change in RMR is food availability (Lalonde 1991, Moe et al. 2005). Small vertebrates are limited in the extent that their body reserves can supply energy demands for extended, energetically expensive activities (Kenagy et al. 1989, Oftedal 2000, Speakman and Krol 2005). By increasing the size and proficiency of relevant digestive organs, it is possible for animals to increase the rate of food ingestion without compromising processing and assimilation rates (Speakman 2008). However, such organ remodeling often necessitates an increase in RMR, as larger organs are energetically more expensive to maintain (McBride and Kelly 1990, Alexander 1999, Wang et al. 2001, Naya et al. 2008).

The availability and quality of food resources within an environment as well as an animal's current physiological state influences the magnitude and direction of energy expenditure and how animals balance investment in survival, growth, and reproduction (Veloso and Bozinovic 2000, Mueller and Diamond 2001, Speakman 2008, Tieleman et al. 2008). An increase in food ingestion or in the mobilization of body reserves is necessary during energetically demanding life-history events such as reproduction (Kenagy et al. 1989). Animals facing large energy deficits can experience physiological consequences that often compromise fitness (Lima and Dill 1990, Daan et al. 1996, Naya et al. 2008). Reallocation of energy away from maintenance metabolism towards other activities may reduce consumption needs, but can compromise somatic repair processes, and increase damage related to oxidative stress (Wiersma et al. 2004, Wiersma and Verhulst 2005, Alonso-Alvarez et al. 2006). Consequently, a life-history trade-off exists between the degree of energetic investment in an activity and the ability to of an organism to compensate for high energetic overheads associated with increases in expenditure. High food-availability could facilitate greater investment in fitness related activities, while compensating for associated higher maintenance costs. In support of this, species of *Peromyscus* mice living in environments with high net primary productivity are characterized by both higher RMRs and daily energy expenditures (DEE) compared to those in poorer environments (Muellar and Diamond 2001). Speakman et al. (2003) found that reproductive free-living field voles (*Microtus agrestis*) in high quality habitats had higher RMRs and DEE than voles from poorer quality habitats during the reproductive season, whereas the opposite was true during the off-season in winter.

Despite a hypothesized link between energy expenditure and food availability; few studies have investigated this relationship. Laboratory food restriction experiments generally report reductions in RMR, suggesting that animals down-regulate expenditure to reduce energy costs when conditions are not ideal (Moe et al. 2005, Wiersma et al. 2005, Kristan and Hammond 2006). Field studies that address the effects of food availability on metabolic rate report equivocal findings. A study on free-ranging, black legged kittywakes (*Rissa tridactyla*) found that supplemented birds raising dependent young increase body mass and reproductive output, but decrease daily energy expenditure (DEE) (Jodice et al. 2002). In contrast, the same authors later found that kittiwakes increase DEE in response to naturally high food availability during the chick rearing period (Jodice et al. 2006). I am unaware of any study on the response of RMR to food variability in free-living mammals. Food-supplementation experiments on free-living organisms could provide a useful approach to explore how individuals respond metabolically to high-food conditions (Boutin 1990).

I compared resting metabolic rate and body mass in food supplemented and natural populations of yearling North American red squirrels at the beginning of the reproductive season. Being smaller and less experienced than adults, yearlings are likely to benefit from an increase in food-availability (Clutton-Brock and Albon 1985, Duquette and Millar 1995). An increase in RMR in females may enable weight gain and improve the probability of entering estrous (Wauters and Dhondt 1989, Becker et al. 1998). In males, the pursuit of estrus females during spring mating chases requires sustained physical effort which may be facilitated by increasing RMR and body reserves (Smith 1968, Lair 1985). I predicted that RMR and body mass would be higher in pre-estrous yearling females and yearling males exposed to *ad libitum* food. Alternately, RMR may be lower in supplemented individuals if yearlings in low food conditions are forced to maintain higher expenditures (Speakman et al. 2003, Thomas et al. 2001). A reduction in RMR in supplemented yearlings may thus reflect the alleviation of forced workloads, allowing leftover energy to be allocated towards reproduction. With these hypotheses I investigate whether red squirrels respond to high food availability by increasing maintenance expenditure or whether low food conditions force higher expenditures, such that when conditions are favourable individuals reduce expenditure (alleviating effect). Finally, if RMR responds plastically to high food levels, I expect no apparent differences in RMR or body mass following the termination of supplementation.

METERIALS AND METHODS

Red squirrels were studied near Kluane National Park, in southwestern Yukon, Canada (61°N, 138°W) from March 2008 to July 2008 as part of the Kluane Red Squirrel Project. All squirrels were marked and monitored for survival and reproduction using standardized methodology (McAdam et al. 2007). Pre-estrous females and scrotal male yearlings were targeted for metabolic measurements on two control grids (~ 30 – 40 ha) and on one food supplemented grid (~ 40 ha). Pre-estrus females were chosen as to avoid variability in RMR potentially caused by differences in pregnancy stages. The reproductive activity of females was monitored from March - August 2008 according to a standardized protocol (McAdam 2007). In conjunction with a separate on-going study, all females on one control grid were fitted with VHF radio collars and located each morning by telemetry to confirm whether or not they were in estrus. A female was considered to be in estrus if she was actively pursued by one or more courting males. Females from the second control grid and food supplemented grid were monitored for estrus and early pregnancy by frequent behavioural observations (mating chases for estrus females are loud, noticeable events and are easily detected) and by regular trapping to confirm pregnancy status through change in body mass and by palpation for the presence of embryos (McAdam et al. 2007).

Two months following the termination of food supplementation (mid-June - July), yearlings were once again measured for RMR. Females that had produced a litter were captured approximately 5-10 days after having weaned offspring in order to ensure offspring viability during the mothers' absence. Females that did not become pregnant during the breeding season were considered as "non-breeders". As only a proportion of Kluane yearlings breed in their first year (Descamps et al. 2006), I additionally measured non-yearling males and females to increase power for detecting a difference in RMR between treatment grids and between breeding females and non-breeding females/males following the termination of food supplementation.

Food supplementation

Since red squirrels defend year-round food based territories, it is possible to target particular individuals for food-supplementation. On the food supplemented grid, food addition was initiated in 2004. Since then, territory owners have annually been provided with *ad libitum* natural peanut butter by a bucket hung between two trees located on a territory. Supplemental food is provided each year from October until approximately mid-May. No additional peanut butter is added after this period in an effort to deter attention from bears and other non-target animals living within the study area. Control grids consisted of two un-manipulated 30-40 ha plots. Natural food production on these grids was quantified by obtaining an index of the total number of cones (cone index) produced by select trees (LaMontagne et al. 2005).

Flow-through respirometry

Procedures to obtain measures of RMR in red squirrels are the same as those described in chapter II of this thesis.

STATISTICAL ANALYSIS

The 2007 cone index revealed that cone production on control grids was relatively low compared to visual cone indices calculated for medium cone crops and masting events (large scale production of high numbers of cones) in past years. Thus food levels on unsupplemented grids provided a good contrast to food availability on supplemented grids. All data were explored *a priori* for departure from normality using the Shapiro-Wilk test for normality (Zar 1999). Diagnostic plots (regression of residuals, cook's distance) were used to identify potential outliers in the dataset. Although, resting metabolic rate varies allometrically with body mass (Huxley 1932), log transforming data did not change statistical outcomes. Results are thus presented as untransformed values. Pearson's product moment correlation coefficient was used to test the significance of the relationship between RMR and body mass. Analysis of variance (ANOVA) and student's t-tests were used to examine differences in whole-animal RMR and body mass between control and supplemented grids, sexes (spring), and breeding versus non-breeding females/males (summer). Analyses of covariance (ANCOVA) with mass as a covariate were used to test for differences in RMR once differences in mass have been corrected for (RMR_{mc}). ANCOVAs were performed to test for differences between control and supplemented grids, sexes (spring), and between breeding females and non-breeding females/males (summer). Due to the reduced number of yearlings trapped in late summer, the same calculations were performed for a combination of yearlings and non-yearling squirrels to increase sample size. Significant interaction terms were tested for in each analysis. Post hoc tests were performed when necessary using Tukey Honestly Significant Differences (HSD) test. All statistical analyses were performed in R (R Development Core Team 2006).

RESULTS

Spring

RMR - In March 2008, RMR and body mass measurements were obtained from 23 and 30 yearlings from food supplemented grids respectively, and from 29 and 32 yearlings from unsupplemented grids. Body mass was positively correlated with RMR ($r_{50} = 0.50$, $p < 0.001$). Data from the two control grids were pooled as there were no significant differences in RMR or in RMR_{mc} ($t_{27} = 1.49$, $p = 0.15$, $F_{2, 26} = 2.85$, $p = 0.11$). Supplemented yearlings exhibited significantly higher RMR ($F_{3,48} = 29.29$, $p < 0.001$, Fig. 3-1, Table 3-1) and RMR_{mc} ($F_{3, 48} = 33.11$, $p < 0.001$, Fig. 3-1, Table 3-1) than controls. There was no significant difference in RMR ($F_{3, 48} = 1.58$, $p = 0.23$) or RMR_{mc} ($F_{3, 48} = 1.69$, $p = 0.20$) between sexes.

Body Mass - Body mass did not differ between control grids (spring: $t_{27} = 1.44$, $p = 0.16$), thus values were pooled. A two-factor ANOVA yielded a significant main effect of food availability ($F_{3,58} = 9.96$, $p = 0.002$) and sex ($F_{3,58} = 11.25$, $p = 0.001$). However, the corresponding interaction term approached significance at alpha = 0.05 ($F_{3,58} = 3.55$, $p = 0.064$) and was thus explored further. Closer examination of the data (Fig. 3-2), suggested that the effect of food availability depended on sex, but overall, supplemented squirrels (252.74 ± 17.87 g) were larger than controls (239.43 ± 18.60 g), and males (252.51 ± 18.50 g) tended to be larger than females (238.78 ± 17.88 g). A single factor ANOVA revealed significant differences in mean body mass for the four treatment-sex combinations ($F_{3,58} = 5.23$, $p = 0.001$) and a post-hoc test confirmed that average body mass was significantly greater for supplemented males (263.92 ± 14.21 g) compared to supplemented females (241.56 ± 16.89 g, adjusted $p = 0.0023$), control females (236.01 ± 18.21 g, adjusted $p < 0.001$), and control males (242.45 ± 15.12 g, adjusted $p = 0.003$). Remaining categories did not differ significantly from one another (Fig. 3-2). Low sample size for each treatment-sex combination may have resulted in reduced ability to detect a statistically significant interaction term.

Summer

RMR - RMR measurements were obtained on 20 supplemented yearlings and 17 unsupplemented yearlings. Body mass was positively correlated with RMR ($r_{35} = 0.37$, $p < 0.023$). Two months following the removal of supplemental food, RMR ($t_{14} = 1.89$, $p = 0.081$) and RMR_{mc} ($F_{2,13} = 3.56$, $p = 0.09$) did not differ significantly between control grids (Table 3-2). No difference in RMR_{mc} was detected between food supplemented and control grids, although the main effect of breeding type (breeding female, non-breeding female, male) was significant ($F_{4,32} = 4.41$, $p = 0.02$, Table 3-2). A post hoc test revealed that RMR_{mc} differed significantly between breeding females and non-breeding females (adjusted $p = 0.043$), while no difference was detected between breeding females and males (adjusted $p = 0.09$) or between males and non-breeding females (adjusted $p = 0.8$). Use of an extended data set which included yearlings as well as non-yearlings detected a stronger effect of breeding type on RMR_{mc}: Similarly, RMR ($t_{41} = -0.27$, $p = 0.78$) and RMR_{mc} ($F_{2,40} = 0.093$, $p = 0.76$) did not differ significantly between control grids and no difference in RMR_{mc} was found between supplemented and control grids ($F_{4,80} = 0.11$, $p = 0.74$). However, breeding status significantly influenced RMR_{mc} ($F_{4,80} = 7.268$, $p < 0.001$) and a post hoc test revealed that breeding females had significantly greater RMR_{mc} than non-breeding females (adjusted $p = 0.05$) and males (adjusted $p = 0.001$).

Body mass – There was no significant difference in body mass between the control grids ($t_{14} = -1.41$, $p = 0.18$). Mass did not differ significantly between supplemented and control yearlings ($F_{4,32} = 2.33$, $p = 0.96$), however the main effect of breeding status was significant ($F_{2,32} = 0.026$). A post hoc test revealed that males (251.23 ± 21.13 g, adjusted $p = 0.021$) were significantly larger than non-breeding females (232.4 ± 18.74 g).

DISCUSSION

It is well documented that increased energy demands correspond with increases in RMR, partly due to higher maintenance costs associated with the enlargement of metabolically expensive organs (Drent and Daan 1980, Speakman and McQueenie 1996, Hammond and Kristan 2000, Koteja 2000, Derting and Hornung 2003, Naya et al. 2008).

Food levels in the environment dictate the ability of an organism to attain, assimilate and export nutrients for investment in fitness related activities (Martin 1987, Rogowitz 1996, Koskela et al. 1998, Therrien et al. 2008). In this study, yearling red squirrels exposed to *ad libitum* food experienced an increase in both whole-animal RMR and RMR_{mc}. Consideration of the ecology of red squirrels and the temporal availability of food within this system supports such a response to high food availability as opposed to an alleviation response, where individuals reduce costs associated with mechanical work and thereby reduce RMR. First, red squirrels rely on cones stored in the previous autumn to meet energy requirements necessary for reproduction (McAdam et al. 2007). Thus, available food reserves in spring are predictable and limited such that increases in foraging activity would provide little compensation for greater effort. Secondly, high annual variability in the size of autumn cone crops coupled with red squirrels being relatively long lived (Lamontagne and Boutin 2007, McAdam et al. 2007), suggests that expenditure should be maximized when adequate food reserves allow and that in low food conditions, individuals should mediate expenditure relative to current and future reproduction events (Daan et al. 1990, 1996, Deerenberg et al. 1997, Tieleman et al. 2008). In agreement with results of this study, Speakman et al. (2003), found that voles living in “good” quality habitats had higher RMRs compared to voles in “poor” habitats. However, because habitat quality was defined according to empirical differences in reproduction demographics, it is not possible to attribute these results directly to differences in food availability. Indeed, Speakman and colleagues recognized the need to identify extrinsic factors responsible for plastic changes in RMR; this study concludes that food availability is among them.

Overall, whole animal RMR was 16% greater in supplemented yearlings than in un-manipulated controls. Differences in RMR could have reflected an increase in the size of relevant organs involved in processing efficiency and/or could have resulted from supplemented yearlings being structurally larger than controls (i.e. greater muscle mass, fat deposits, larger bone structure). However, structural tissues typically contribute minimally to maintenance costs and thus do not provide a strong basis for the observed differences in metabolic rate (Rolfe and Brown 1997). Supplemented yearlings increased expenditure per gram of body mass by 12%, suggesting a modulation of metabolic intensity at a cellular level. Ronning et al. (2008), recently provided evidence that changes in maintenance metabolic rate during the breeding season in female black-legged kittiwakes were due not only to changes in organ size and body composition, but also in the metabolic intensity of particular organs at the cellular level. Specifically, thyroid hormone plasma T3 is a known determinant of individual variation in metabolic rates in both mammals and birds (Chastel et al. 2003). Thus, potential mechanisms underlying the observed increase and decrease in RMR in this study include both larger organ size and higher levels of molecular activity.

It is important to note that during this study, supplemented grids were also characterized by higher densities of squirrels owing to higher annual juvenile recruitment during food supplementation (food supplemented mean density: 2.93 squirrels/ha, control mean density: 1.65 squirrels/ha). In this population an increase in density is coupled with a decrease in territory size (LaMontagne 2007). High density conditions could have increased the frequency of territorial behaviours and antagonistic interactions, resulting in higher energy expenditure (Barnett and Pankhurst 1996, Macdonald et al. 2004, Blanchet et al. 2006). However, this phenomenon would have likely remained present following the termination of food supplementation as the number of squirrels did not decline. If an increase in territorial interactions was responsible for higher RMRs found in supplemented individuals during spring, the intensity of this factor would have been similar or magnified in late summer by a large number of dispersing juveniles (Berteaux and Boutin 2000). Thus, the positive metabolic responses of supplemented yearlings in spring were likely due to an increase in food-availability rather than a difference in density between the two treatment groups.

Following the termination of supplemental food addition, squirrels no longer exhibited higher RMRs in comparison to controls, suggesting a down-regulation of RMR in response to a decrease in food availability. Females at this time had just weaned offspring and were no longer lactating, while the majority of males were no longer scrotal. It is possible that the lack of difference in RMR between treatment groups arose because of a considerable decrease in energy demands in concurrence with the end of the breeding season. However, results indicate that breeders maintained high RMRs compared to non-breeders, implying that high maintenance energy demands persisted at the time of measurement. Shortly after lactation, female mammals invest a large amount of energy in anabolic processes involved in rapid bone regeneration and in re-building body reserves lost during lactation (Millar et al. 2005, Speakman et al. 2008). A positive relationship exists between the rate of bone regeneration and RMR, suggesting that individuals would have benefited from a higher RMR if adequate food had been available to support the increase (Khomullo 1962). Moreover, superior food quality has been shown to increase BMR even in the non-reproductive season in degus (*Octodon degus*)

(Veloso and Bozinovic 1993). Therefore, red squirrels likely exhibit a flexible response to food-supplementation, characterized by an increase in RMR when food availability is high and a down-shift when availability decreases.

I hypothesized that the mean body mass of supplemented yearlings would be greater than in controls. In spring, yearlings exhibit continued growth demands that may compete concurrently with reproduction (Boutin, unpublished data). In females, a small body mass constrains the probability of entering estrus (Becker et al. 1998), whereas in males, a small body mass could limit endurance capacity during mating chases and hinder recovery of body condition afterward. In general, food supplemented squirrels tended to be heavier than controls and males tended to be heavier than females. However, the body masses of control males, supplemented females and control females were not statistically different from each other, while supplemental males were significantly heavier than all other groups. A potential reason for this result must consider that energy demands for males were considerable at the time of measurement (Lane et al. 2009). Yearling males and older males expend more energy than mature males during the breeding season (Lane et al. submitted). Breeding males expand their home ranges by almost 10-fold as individuals search for females in or approaching estrus (Lane et al. 2009). Although male body mass does not covary with reproductive success in this population, increases in body mass could facilitate long-distance searching for estrus females by reducing the frequency at which an individual would have to stop to refuel, while additionally minimizing negative impacts on body condition over the course of the breeding season (Lane et al. 2009). Additionally, if the larger body mass of supplemented yearling males signifies reduced investment in growth, more energy may be available for investment in reproduction. In this study, supplemented males were 8 % heavier than supplemented females, while control males were only 3 % heavier than female counterparts; suggesting that males on un-manipulated grids were restricted from growth by lower food availability. Indeed, red squirrels display slight but consistent sexual dimorphism (males are 5-10% heavier than females) (Boutin & Larsen 1993). One possible explanation for a lack of significant weight gain in supplemental females may be the presence of an abundant food supply. An increase in body reserves may not be necessary if food availability is predicted to be sufficient for the duration of the reproductive period. Moreover, carrying potentially heavy internal reserves may negatively affect agility and maneuverability (Sullivan 1990).

There is little information about how RMR and other metabolic traits respond to changes in environmental temperatures, resource levels, seasonality and other potential inducers of change. Knowledge of how the magnitude and rapidity of a response is modified with respect to differences in physiological state or within different populations could increase our understanding of life-history trade-offs associated with constraints on energy allocation (McKechnie 2008). Factors such as the predictability of food, the degree of seasonality of an environment, whether an animal is a capital or income breeder, or whether an animal is a specialist or generalist will likely influence the shape of a species' or populations' reaction norm for metabolic traits. This study demonstrates that yearling red squirrels exhibit phenotypic flexibility in RMR in response to variation in food conditions and supports the hypothesis that high food availability is associated with greater levels of resting metabolism.

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Table 3-1. Mean body mass and resting metabolic rate with associated sample sizes for supplemented and unsupplemented yearlings measured in March 2008.

Group	n	Mass (g)	n	RMR (O2 $ml^{-1}hr^{-1}$)
Supplemented (total)	30	252.7±19.3	23	436.42±52.77
Female	15	241.6±16.9	12	417.85±41.53
Male	15	263.9±13.9	11	456.65±58.01
Unsupplemented (total)	32	239.9±17.6	29	368.42±39.93
Female	15	236.0±18.9	12	370.57±39.48
Male	17	242.4±18.3	17	366.91±41.38

Table 3-2. Mean body mass and resting metabolic rate with associated sample sizes for supplemented and unsupplemented yearlings and for primiparous females and non-breeding females (N-b) / males in summer 2008 post food supplementation.

Group	n	Mass (g)	n	RMR (O2 $ml^{-1}hr^{-1}$)
Supplemented	20	245.96±54.78	20	393.64±26.04
Unsupplemented	17	255.59±25.80	17	402.81±54.81
Breeding females	23	259.1±22.09	23	409.00±32.23
N-b females/males	17	246.89±25.50	17	370.37±41.17

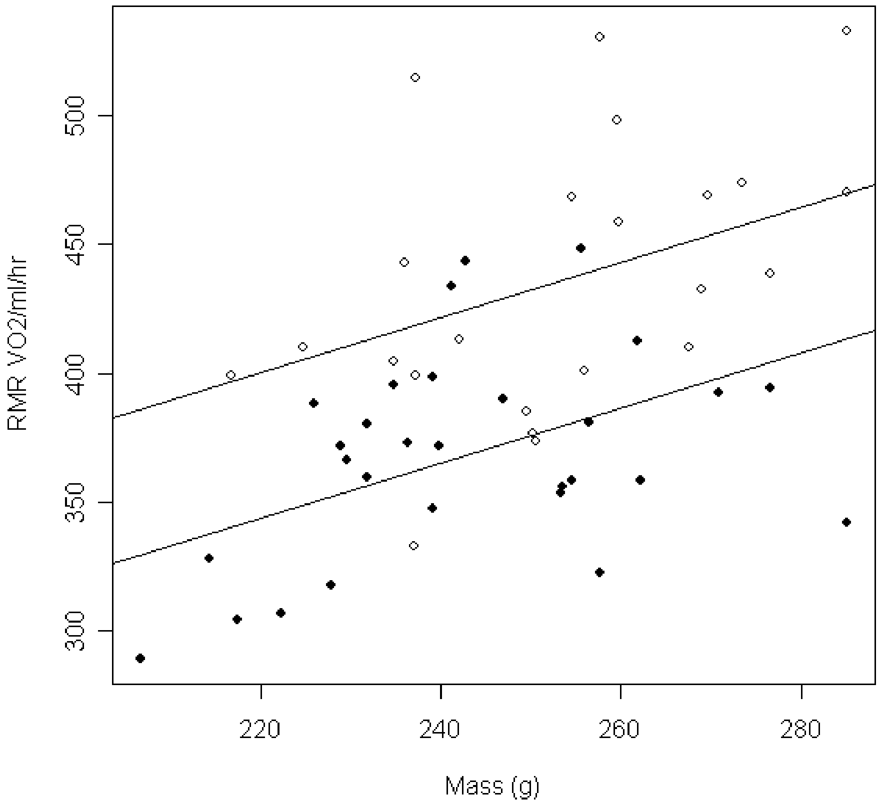

Figure 3-1. Relationship between body mass and resting metabolic rate (RMR) for food-supplemented yearlings (unfilled diamonds) and unsupplemented yearlings (filled diamonds). For any given mass, supplemented individuals tended to have higher RMRs.

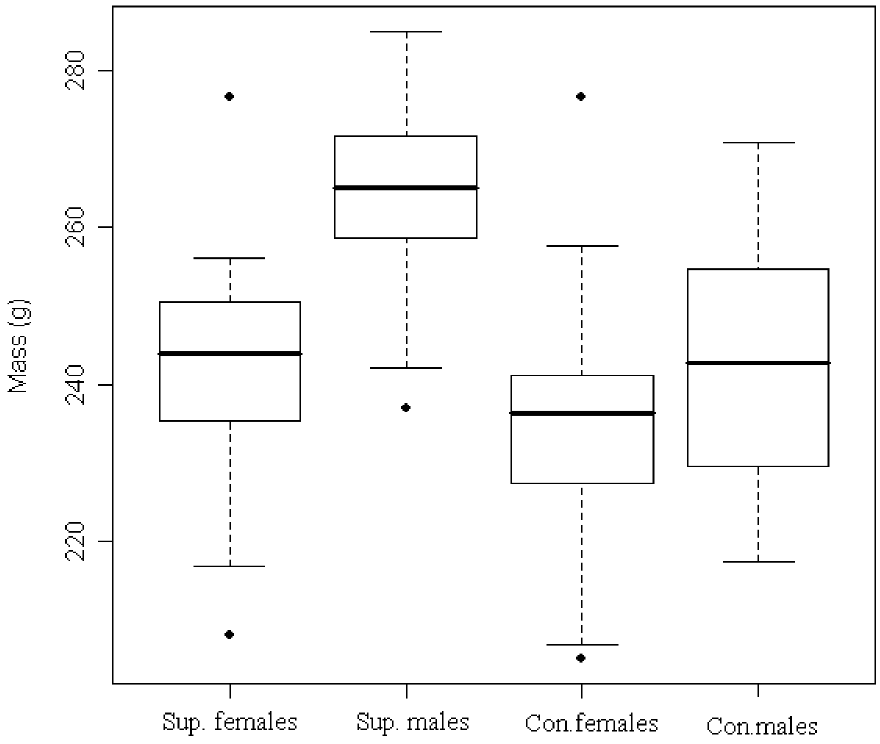

Figure 3-3. Box plot comparing body mass of supplemented males and females (Sup.) and control males and females (Con.). Body mass in supplemented males was significantly greater than in all other groups. Overall, body mass was larger in supplemented individuals and males.

GENERAL DISCUSSION AND CONCLUSIONS

Resting metabolic rate (RMR) is the most widely measured physiological trait in endothermic vertebrates (McNab 2002, Cruz-Neto and Bozinovic 2004). RMR varies greatly at the inter-specific and intra-specific level, even when variation due to body mass and higher-level taxanomic affiliation is considered (Daan et al. 1990). Correlative studies have linked variation in RMR to a variety of biotic and abiotic factors which have provided insight into potential evolutionary processes responsible for the maintenance of variation (e.g. Mueller and Diamond 2001). However, the selective value of a trait can only be assessed at the individual level. As such, many questions concerning how organisms respond physiologically to environmental variation and the functional significance of individual variation in metabolic traits remain unanswered (Bozinovic 2007).

The primary objective of this study was to investigate the fitness consequences of individual variation in RMR and body mass in juvenile North American red squirrels. This was accomplished by first documenting the repeatability of RMR so as to assess its likelihood of responding to natural selection over pre-defined short and long-term time intervals. I then examined whether individual differences in rates of energy expenditure influenced overwinter survival by considering how RMR interacted with inter-individual differences in overwintering food supply. A secondary objective was to examine how variation in food availability influenced RMR at the onset of the subsequent breeding season. Understanding how environmental variables affect energy expenditure provides an underlying basis for the study of life-history theory. Overall, this study investigates the ecological and evolutionary importance of variation in RMR and provides insight into some underlying factors which influence intra-specific variation.

SUMMARY OF RESULTS

***Chapter II*:** Both body mass and RMR were found to be repeatable traits in juvenile red squirrels, however long-term repeatability for RMR was dependent on an individual's sex; RMR remained repeatable in pre-estrus females, but not in scrotal males. Indeed, the majority of physiological traits are not perfectly repeatable over longer time scales as they are affected by age and by environmental heterogeneity (Hayes and Jenkins 1997). It is possible that the lack of repeatability in males reflects a reordering of individual metabolic rankings associated with sexual maturation, suggesting that metabolic ranking may differ from juvenile to adult stages and that juvenile RMR cannot be used to predict adult values (Chappell and Bachman 1995, Chappell et al. 1996, 1999).

Of the juvenile red squirrels measured in autumn, 54% survived until spring. The probability of a juvenile surviving overwinter was significantly related to a squirrel's body mass and RMR, once differences in body mass were considered. However, despite being larger than non-survivors, juveniles that survived had similar whole-animal energetic requirements as those that did not survive. In winter, red squirrels face thermoregulatory trade-offs attributed to living in a severely cold environment with limited food availability. Although, a larger body size conveys a smaller surface-to-mass ratio and thus reduces heat loss (James 1970, McNab 1970), it is also associated with greater energy requirements both while resting and when active. Selection favouring a larger body size may be counterbalanced by costs associated with higher energy requirements. Surviving juveniles minimized total winter energy requirements as defined by required levels of expenditure and thermoregulation by optimizing body mass and expenditure requirements. However, as I was not able to detect a benefit of possessing a higher RMR, it remains uncertain as to how variation in RMR is maintained within the Kluane population. Although it is possible that a high RMR may allow a greater allocation of energy towards reproduction or other fitness enhancing events, studies to date have failed to find a relationship between metabolic rate and correlates of reproductive success (e.g. Johnston et al. 2007).

Juveniles with higher residual RMRs did not have significantly higher daily energy expenditure and tended to have fewer cones stored in their middens at the end of the hoarding season. Moreover, they had less energy available for investment in work related activities, such as clipping and hoarding cones. This suggests a complex interplay of several factors that when combined, may contribute to high juvenile mortality. Studies on the evolutionary importance of individual variation in physiological traits must consider that the advantages and disadvantages of possessing one extreme of a physiological trait may be heightened by a multitude of other variables that influence the current selective importance of that trait. For example, food availability, nest insulation properties, and ambient temperature are likely to influence how important an individual’s RMR is to overwinter survival. Red squirrel juveniles require longer food processing times (Larivée, unpublished data), have smaller body masses, higher residual RMR, and generally own smaller territories (LaMontagne 2007). During periods of low food abundance, it is possible that even small differences in energy savings could determine whether an individual survives until spring. The possibility of constraints on the flexibility of metabolic expenditure due to growth demands may also play a role in selection acting against high rates of expenditure. For example, although RMR is characteristically flexible, juveniles may be constrained in their ability to either increase DEE or to reallocate energy away from maintenance processes without compromising growth.

Chapter III: I detected an increase in both RMR and mass-corrected RMR at the onset of the 2008 breeding season in yearlings exposed to supplemental food throughout winter. This result supports the hypothesis that high food availability is associated with an increase in maintenance energy expenditure. Kluane red squirrels rely primarily on the seeds of white spruce cones which are hoarded in autumn when cone crops mature (McAdam et al. 2007). Because red squirrels are territorial and food is limited, increases in foraging effort are likely to be counterproductive. Moreover, increasing expenditure without sufficient compensatory resources may entail long-term fitness costs (e.g. Alonso-Alvarez et al. 2006). Supplemented yearlings also tended to have larger body masses than controls; however supplemented males were on average 8% heavier than control males, while the body masses of control males, supplemented females and control females did not differ significantly from one another. A greater body mass in males may facilitate searches for estrus females by improving physiological endurance and offsetting expenditure normally directed towards growth during the breeding season.

FUTURE DIRECTIONS

The popularity of intra-specific studies is increasing, mainly because this approach sheds light on the factors responsible for divergence in physiological traits and because reliable, repeatable measures of such traits can be acquired. However, a holistic approach should be taken in order to address questions concerning the evolution of metabolic traits.

Large-scale differentiation observed at the inter-specific level represents the end point of evolutionary processes. Although, contemporary studies at the individual level can be used to test hypotheses formed from higher level observations, traits may be under different selection processes then they were in the past. Nevertheless, valuable insight can be gathered by considering contemporary selection processes, including alterations to existing hypotheses and the formation of new ones that further the field of physiological ecology.

The Kluane red squirrel project provides an ideal system with which to investigate inter-individual variation in physiological traits. Because it is possible to quantify spatial and temporal variation of the red squirrel's primary food source, the ability to tease apart factors affecting the maintenance of variation in metabolic traits is greatly improved. Research questions considered by the KRSP will allow studies of free-living organisms to be compared to the more commonly performed laboratory studies. While laboratory studies are informative, there is a need for more field based studies which consider natural variation in extrinsic factors. The course of my research has led me to identify certain avenues of research that I believe deserve attention.

- How does adult metabolic rate differ from that of juveniles? Does energy expended on growth constrain juvenile metabolic scope and the ability to reallocate energy away from maintenance metabolism? Under what circumstances will individuals compromise growth to increase short-term survival and what physiological costs are involved in doing so?
- What processes are responsible for linking RMR to food stores in red squirrels? How does spatial variation in the distribution of food stores affect individual work

effort versus gain during hoarding, and does physical activity during hoarding reflect measures of daily energy expenditure?

- What are the selective disadvantages and advantages of possessing a higher RMR versus a lower RMR? Although this thesis provided evidence that variation in RMR influences survival during periods of low food availability, more research is required to identify alternative strategies by which selection operates to preserve metabolic variation within a population. Long-term studies, such as the KRSP, will contribute disproportionately to questions of this nature. Systems which experience a high degree of environmental variability within the lifetime of the target species should be targeted as they may be more likely to exhibit a higher degree of inter-individual variation rather than within individual variation.
- What mechanism is responsible for the lack of repeatability between juvenile and sexually mature adult stages?
- Is resting metabolic rate heritable in red squirrels? To what extent do maternal effects and developmental plasticity account for individual variation in RMR?
- Do small mammals consistently respond to high levels of food by increasing metabolic rates during periods requiring high energy expenditure (e.g. reproduction, dispersal, food hoarding)? Do they do the same in periods of low activity (e.g. during winter)?

Studies of inter-individual variation in metabolic traits offer the potential to link physiological ecology to other burgeoning fields such as behavioural ecology, evolutionary ecology and even conservation ecology. Often, researchers use energetic rationale to explain given behaviours or different phenomena occurring among populations. However, metabolic traits are rarely measured. Explanations for the evolutionary importance of intra-specific variation in metabolic traits will most likely be generated by consideration of the population's natural history and the formation of hypotheses based on life-history tradeoffs individuals may face.

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