Resource-mediated thermoregulation and energetics of a northern population of red squirrels (*Tamiasciurus hudsonicus*) in winter

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Abstract

Winter is an energetically challenging season for endotherms in temperate and northern climates because seasonally low food availability is paired with high costs for thermoregulation. In response to this apparent energetic mis-match, animals can use both physiological and behavioural adaptations to decrease energy requirements. I investigated the effects of resource levels and air temperatures (T_a) on body temperature patterns (T_b; chapter 1), as well as activity patterns and field metabolic rate (FMR; chapter 2) of a northern population of red squirrels (*Tamiasciurus hudsonicus*) in winter. Squirrels provided with supplemental food maintained consistently elevated T_b compared to control animals. T_b was positively related to T_a during the day, but not at night. FMR was measured over three years with varying levels of natural resources, and in two of these FMR was paired with activity patterns (time spent out of their well-insulated nest). During the two years of low resource abundance T_a had a positive effect on FMR, whereas in the one year of high resources T_a had a negative effect on FMR. This difference may be explained by differences in activity patterns. Time spent active was equal across both resource levels (one year of each low and high food), and all T_a. However, animals under low resources timed activity such to be exposed to the warmest part of the day, thereby decreased thermoregulatory requirements, particularly at cold T_a. Collectively, these results show that squirrels adjust energy requirements as a response to resource abundance, and that the effect of T_a on energy expenditure is resource-mediated.

Résumé

L’hiver pose un défi énergétique pour les endothermes dans les climats tempérés et nordiques. La nourriture est souvent moins disponible et les couts de thermorégulation sont plus élevés. Les animaux peuvent utiliser des adaptations physiologiques et du comportement pour diminuer leur demande énergétique. J’ai exploré l’effet de niveau de nourriture et de la tempérture de l’air (T_a) sur les profils de température corporelle (T_b; 1^{er} chapitre), ainsi que les profils d’activité et les taux métaboliques sur le terrain (FMR; 2^e chapitre) d’une population nordique d’écureuil roux (*Tamiascirus hudsonicus*) en hivers. Les écureuils qui avaient accès a la nourriture supplémentaire maintenaient une T_b élevée comparés aux animaux témoin. T_a avait un effet positif sur T_b durant le jour mais pas la nuit. J’ai mesuré FMR durant trois années avec différent niveaux de nourriture naturelle, et pendant deux de ces années (une de nourriture elevée et une de nourriture basse) j’ai aussi mesuré des profils d’activité. Pendant les deux années de faible taux de nourriture T_a avait un effet positif sur FMR, tandis que durant l’année de taux élevé de nourriture T_a avait un effect négatif sur FMR. Les différences de profils d’activité expliquent la différence de la relation entre T_a et FMR. La proportion du temps passé a être actif ne différait pas entre les différents taux de nourriture et de T_a. Par contre, les écureuils exposés à des taux de nourriture plus bas étaient actif durant la partie la plus chaude de la journée pour diminuer leur demande de thermorégulation, particulièrement par journées froides. Ces résultats démontrent que les écureuils ajustent leur demande énergétique en réponse au niveau de ressources, et que l’effet de T_a sur la dépense d’énergie est dépendante des taux de ressources.

Contribution of authors

This thesis is presented as two data chapters, each intended for publication. These are preceded by a general introduction and proceeded by a general conclusion. For each data chapter the candidate was responsible for developing the research questions, experimental design, field work, data management and analysis, interpretation and writing.

Chapter 1 is co-authored by Franziska Kohl, Stan Boutin, Brian Barnes and Murray Humphries. Franziska Kohl provided input into study design and logistics, particularly preparation for surgeries. She also assisted with fieldwork and performed surgeries. Stan Boutin provided assistance with field work, provided input into the manuscript at the early stages and commented on a later version of the manuscript. Brian Barnes provided input into study design, logistical support for surgeries and comments on the manuscript. Murray Humphries provided assistance with study design, logistics, and provided comments on the manuscript.

Chapter 2 is co-authored by Stan Boutin and Murray Humphries. Stan Boutin provided the opportunity to do this field research at Kluane, and is responsible for the development of the research in red squirrel ecology which provided the foundation for more physiological questions in this system. Stan also assisted with study design and field work. As my supervisor, Murray Humphries came up with the original research questions and provided assistance with their development. He also assisted with study design, interpretation of the results and provided comments on the manuscript.

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General Introduction & Literature Review

Metabolism is one of the most important factors in determining an animal's ecology (Brown 2004). It represents the energy throughput of an animal thus provides a link between energy requirements and food resources. Therefore, understanding the collective of factors that influence metabolism is critical to gaining insight into an animal's ecology, or its relationship with its environment (Schmidt-Nielson 1983). The field metabolic rate (FMR) of free-ranging endotherms (animals with endogenous heat production) is a collective term that incorporates a variety of techniques to estimate energy expenditure. The key difference between FMR and RMR (resting metabolic rate measured in the laboratory at thermo-neutrality) is that FMR represents the energy expended by a free-living animal in its natural environment. FMR is influenced by both intrinsic and extrinsic effects on energy expenditure (Goldstein 1988; Bryant 1997; Speakman 1997; Zerba et al. 1999; Gillooly et al. 2001; McNab 2002; Brown 2004; Suarez et al. 2004). In other words, FMR is a complex integration of a number of variables including, but not limited to body mass, body temperature, RMR, taxonomy, reproduction, activity, wind, solar radiation, conduction and diet.

Low ambient temperatures (T_a) associated with winter in temperate and northern climates impose a high energetic cost of thermoregulation for endotherms (Brown 2004; Anderson and Jetz 2005). Actively thermoregulating animals produce costly endogenous heat to maintain body temperatures (T_b) that center around 37°C in mammals and 40°C in birds (Hart 1963). Metabolic rate is influenced by the thermal gradient between T_b and prevailing T_a (Hart 1963; McNab 2002), therefore energy expenditure increases at lower T_a. In the laboratory this relationship is described by the Scholander-Irving model (Scholander et al. 1950), which shows a range of temperatures over which animals maintain a constant level of metabolism (thermal neutral zone), below which metabolic rate must increase to maintain thermal homeostasis.

The high costs of thermoregulation in winter are associated with a period of low primary production. To cope with this paradox between seasonally high energy demands and seasonally low energy availability endotherms have evolved strategies to decrease winter energy expenditure. Extreme adaptations include migration to warmer and more productive climates and hibernation (McNab 2002). However, animals that remain active throughout winter can make use of a number of behavioural and physiological mechanisms to decrease energetic costs. Behavioural adaptations are effective at decreasing the thermal gradient between T_b and T_a, and reducing the rate of heat exchange to the environment. These include posture changes (Stelzner and Hausfater 1986; Byman et al. 1988; de Lamo et al. 1998), seasonal changes in thermal conductance (Hart 1963), activity patterns (Pauls 1978; Long et al. 2005), and the occupation of thermal refuges (McNab 1980; Pauls 1981). The most effective refuges are sufficiently buffered to allow animals to decouple temperature experienced from prevailing ambient temperatures (McNab 1980; Pauls 1981; Long et al. 2005). Some animals are able to exploit these microhabitats, such as the subnivean environment, throughout the entire winter (Casey 1981; Courtin et al. 1991) however many animals must leave their refuge to forage or to express ecologically relevant behaviours (Long et al. 2005). Metabolic adjustments include seasonal drops in RMR (Hart 1963) and shallow, daily drops of T_b in heterothermic animals (Chaplin et al. 1984; Cooper and Swanson 1994). These drops in T_b both decrease the thermal gradient between T_b and T_a and reduce metabolism by Q_{10} processes (Geiser 1988; Snyder and Nestler 1990; McNab 2002). T_b patterns are influenced by patterns in activity (McNab 2002) however these T_b fluctuations have significant effects on energy expenditure above and beyond the energetic consequences of activity (McNab 2002).

Animals employ these strategies as a response to the coupled effects of seasonally low food and seasonally high energy demands for thermoregulation. Therefore, variation in energy availability and in the cost of thermoregulation as determined by T_a should affect the use and intensity of the aforementioned strategies. Evidence that food can affect energy expenditure comes from caloric restriction and some supplementation experiments which indicate that animals decrease energy expenditure by reducing activity (Enoksson 1989; but see Godfrey and Bryant 2000), T_b (Chaplin et al. 1984) and RMR (Nagashima et al. 2003) in response to decreased energy availability. Since food availability varies spatially and/or temporally we would expect variation in the intensity of strategies in time and in space. Air temperature also has a strong effect on metabolic rate (Speakman 1997; McNab 2002), and these can also vary daily and within a single day, therefore the cost of thermoregulation is also expected to vary on these time scales. Collectively, this evidence supports predictions that there should be variation in energy expenditure as a response to T_a, and that this response should vary with resource availability.

Broad-scale inter-specific studies show that energy expenditure tends to be highest at low air temperatures (Speakman 2000; Anderson and Jetz 2005), which is attributed to thermoregulatory costs. However, within species seasonal comparisons show that FMR does not tend to be highest during the coldest season, but rather during reproductive season (Oftedal 1984; but see Bozinovic et al. 2004), or that FMR is constant across seasons due to energy re-allocation (Weathers and Nagy 1980). This provides further evidence that thermoregulatory costs may not be very significant for free-ranging animals. It is interesting that despite the evidence to support the importance of behavioural and physiological strategies to decrease energy expenditure during cold, few studies incorporate such measures when trying to explain broad-scale patterns in FMR.

North American red squirrels (*Tamiasciurus hudsonicus*) are small arboreal rodents that are found throughout North America. They are territorial conifer seed specialists (Steele 1998) and in winter rely on cones, and to a lesser extent mushrooms, that they hoard in autumn (Steele 1998; Smith and Reichman 1984). Despite being larder hoarders they experience large variation in winter food supply due to extreme seasonal variation in cone abundance (Fletcher et al. submitted). Squirrels are winter-active, do not hibernate (Pauls 1979), and breed in early spring. During inactive periods of the day squirrels occupy arboreal grass or moss nests located on their territory (Klugh 1927; Rothwell 1979; Woods and

Humphries personal observations). These high quality refuges appear to decouple thermoregulatory demands from prevailing T_a (Pauls 1979). Surprisingly little is known about T_b regulation and activity patterns of red squirrels, particularly in natural systems and during winter. One study of T_b patterns of captive red squirrels (n=2; Pauls 1979) showed that squirrels are heterothermic and express daily T_b variation of 2-3 ^{o}C in winter. The author suggested reduced nightly T_b was a strategy for energy conservation. Anecdotal observations suggest that squirrel activity decreases at colder temperatures (Pruitt and Lucier 1958; Pauls 1978) and ceases at very cold temperatures (-35^{o}C, Dice 1921; -32^{o}C, Pruitt and Lucier 1958) or severe weather (Klugh 1927). Humphries et al. (2005) also showed a weak positive relationship between the proportion of squirrels observed active and T_a. Collectively this evidence suggests that squirrels use heterothermy and vary activity as an energetic response to winter T_a.

Red squirrels are an interesting model to study resource effects on energy expenditure. They experience extreme annual variation in food abundance as a result of periodic spruce masting (large production of cones, *Picea glauca*; LaMontagne and Boutin 2007), which can easily be quantified (LaMontagne et al. 2005). This variability in natural food abundance allows us to examine the role of natural food availability in influencing energy expenditure. Furthermore, the territorial nature of squirrels facilitates food supplementation experiments (Boutin and Larsen 1993; McAdam and Boutin 2003; Kerr et al. 2007). The importance of food to the ecology of the system has been well documented; cone production in the fall has direct consequences on reproductive effort the following spring (Larsen et al. 1997; Kerr et al. 2007; McAdam et al. 2007). Despite this distinct link between food and subsequent reproduction, there has been little focus on understanding how food levels affect squirrel ecology and energetics during the winter, even though winter is a critical period of high mortality that links fall cone harvest to spring reproduction. Furthermore, the evidence that red squirrels maintain low energy expenditure during winter (Humphries et al. 2005) supports predictions that they employ behavioural and/or physiological energy saving strategies during winter.

Research Objectives

In this thesis, I investigate how red squirrels modify body temperature and activity patterns in response to food availability. I study these relationships during winter, when the likelihood of low resource availability and high thermoregulatory requirements puts a premium on energy conservation. In the first chapter, I use a food supplementation experiment to investigate the influence of resource levels on body temperature patterns of red squirrels. In the second chapter, I investigate how natural levels of resources influence the overall energy expenditure of free-ranging animals and explore the role of activity patterns in mediating the relationship between FMR and T_a in winter.

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Chapter 1: Resource-dependent cold climate heterothermy in free-ranging red squirrels (*Tamiasciurus hudsonicus*)

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Abstract

Although not as dramatic as daily or prolonged torpor expression, shallow reductions in body temperature (T_b) expressed by homeothermic endotherms may be significant contributors to their energy conservation during daily or seasonal periods of energy shortage. I documented T_b variation of a cold climate population of free-ranging red squirrels (*Tamiasciurus hudsonicus*) during winter using surgically implanted data loggers that recorded T_b every 15 minutes for approximately one month. Among the 24 individuals monitored, minimum, average and maximum recorded T_b was 34.1, 38.1, and 42.3 ^{o}C, respectively. T_b was higher during activity than when in the nest and during the day than at night, with an average daily range (max T_b – min T_b expressed by one individual within the same 24 hour period) of 3.5 ^{o}C (range 2.2-6.5 ^{o}C). Daytime T_b was positively related to ambient temperature (T_a), which ranged from -38 to -3 ^{o}C during the study period. Half of the monitored individuals that were provided with ad libitum food prior to and during T_b measurements were characterized by slightly but significantly warmer T_b than controls across the range of encountered T_a (38.3 vs. 37.9 °C). T_b differences between food supplemented and control individuals were smallest on the warmest days and coldest nights. Although T_b does not vary greatly within and among individual red squirrels, its responsiveness to both ambient temperature and food availability demonstrates that shallow reductions in T_b are facultatively employed by red squirrels during cold periods of food scarcity.

Introduction

Variation in body temperature (T_b) has a fundamental influence on the metabolic rates of all organisms and is the primary reason endotherms have higher metabolic rates than ectotherms. The maintenance of a constant, elevated T_b allows endotherms to remain active in low ambient temperatures (T_a) but under these conditions endothermy has very high energy costs. One of the most effective ways for endotherms to conserve energy is to reduce the set-point of body temperature regulation (McNab 2002). T_b depression reduces the T_b-T_a gradient that must be countered with heat production and further reduces metabolic rate through Q_{10} or activation energy processes (Geiser 1988; McNab 2002). Maintenance of a high T_b is particularly costly for small endotherms characterized by high basal rates of mass-specific metabolism (Brown 2004), high surface-area to volume ratios, and a limited capacity to offset negative energy budgets with stored body fat. These species have many adaptations to counter the energetic costs of maintaining homeothermy in cold climates, including occupation of thermally-buffered microhabitats (Casey 1981; Mayer et al. 1982) and food hoarding (Smith and Reichman 1984; Vander Wall 1990), in addition to reversible T_b reductions (Mayer et al. 1982; see review by Geiser 2004).

Endotherm species known to express some regular form of T_b reduction are frequently assigned to one of three categories – daily heterothermy consisting of shallow decreases in T_b lasting less than 24 hours, daily torpor consisting of slightly deeper reductions in T_b also occurring within a 24 hour period, or prolonged torpor, or hibernation, consisting of deep reductions in T_b ranging in duration from a few days to more than a month. Although not as dramatic as prolonged or daily torpor, shallow, daily cycles in T_b have been documented in a wide array of endotherm species and can account for substantial energy savings (Heldmaier and Ruf 1992; Geiser and Brigham 2000). Because a 10^{o}C change in body temperature usually generates a two- to three-fold change in metabolic rate (i.e., Q_{10} is usually between 2 and 3; McNab 2002), reductions as small as 2^{o}C are expected to reduce metabolic rate by 13-19%. Furthermore, some authors have found that the energy savings of shallow T_b reductions are greater than would be expected through Q_{10} processes alone (Heldmaier and Ruf 1992). Unlike deeper forms of T_b reductions, there is some evidence that shallow hypothermia is associated with little or no impairment of locomotor and sensory capacity (Wooden and Walsberg 2004; but see Cossins and Bowler 1987, Bennett 1990). In general, daily T_b cycles reach their daily minima during sleep in the inactive phase and their daily maxima during bursts of activity.

The amplitude of daily T_b cycles is notably variable among species and has been hypothesized to be greater in small species than large species (Aschoff 1982) and greater in species exposed to widely varying T_a than those experiencing more constant environments (Lovegrove and Heldmaier 1994). However, a comparison of 11 small mammal species maintained under identical captive conditions revealed no effects of body size or natural climate variability on the amplitude of daily T_b cycles, but only that diurnal species tended to be characterized by larger amplitude cycles than nocturnal species (Refinetti 1999a). There is a surprising paucity of research on daily heterothermy expressed by free-ranging animals, which is critical because T_b patterns often differ substantially between captivity and the wild (Grossman and West 1977; Geiser and Ferguson 2001).

Little is known about the extent, causes, and consequences of intra-specific variation in the amplitude of daily T_b cycles occurring in free-living animals. Field studies performed mainly on birds have shown more hypothermia is expressed during winter than other times of the year (Chaplin 1974; Reinertsen and Haftorn 1983; Brigham et al. 2000) and by lean individuals than fat individuals (French 1982; Audet and Thomas 1997). Laboratory studies of birds and mammals have shown T_b is reduced in response to caloric restriction (Chaplin et al. 1984; Lane et al. 1996; Sakurada et al. 2000; Nagashima et al. 2003) and hypoxia (Barrow et al. 2001). Some species have also been shown to employ less hypothermia during breeding than non-breeding seasons (Csada and Brigham 1994; Geiser and Masters 1994; Barnes 1996; but see Willis et al. 2006). In general, these results suggest T_b is adaptively reduced when cold T_a increases energy demands, when low resource availability limits energy supply, and when the absence of breeding or other activities reduces the lost-opportunity cost of T_b reductions.

North American red squirrels (*Tamiasciurus hudsonicus*) are an excellent model system for field studies of daily T_b cycles. Because red squirrels are small (they are among the smallest mammals active above-ground during winter in the boreal forest; Pruitt and Lucier 1958), exposed to extensive T_a variation (the boreal forest is the world's coldest and most seasonal forest; Henry 2002), and diurnal (Klugh 1927; Gurnell 1987), they fulfill all three criteria previously hypothesized to promote large amplitude daily T_b cycles (Aschoff 1982, Lovegrove and Heldmaier 1994, Refinetti 1999a). A previous outdoor enclosure study of two female red squirrels implanted with temperature-sensitive radio transmitters confirmed the occurrence of pronounced daily T_b cycles with a maximum winter (T_a<0°C) amplitude of 5.2°C (Pauls 1979). Red squirrels are highly amenable to documenting T_b patterns in the field using surgically-implanted data loggers because their diurnal visibility, occupation of a single territory throughout the year, and willingness to enter live-traps makes it relatively easy to recapture implanted individuals (McAdam et al. 2007). Furthermore, a recent study of the field metabolic rate of a cold climate population of red squirrels, measured with the doubly labeled water technique, revealed remarkably low rates of energy expenditure during winter despite exposure to very low T_a (Humphries et al. 2005). This result encourages examination of the role of daily T_b cycles, or possibly longer and deeper T_b reductions than previously documented in red squirrels (Pauls 1979) and other tree squirrels (Pereira et al. 2002), as a contributor to winter energy conservation in free-ranging red squirrels.

In the present study, I examine the occurrence and extent of heterothermy in a northern population of free-ranging red squirrels during winter, and establish the role of heterothermy in their energetic responses to cold climates and reduced energy availability. I surgically implanted 28 squirrels with temperature data loggers in January, released squirrels back to the wild, then successfully recovered

24 of these data loggers 5-6 weeks later. I provided half of the implanted squirrels with ad libitum food prior to surgical implantation of data loggers and throughout the T_b recording period. The objectives of the study were to determine 1) the average T_b of free-ranging, cold climate, red squirrels in winter and the magnitude of their daily T_b fluctuations and 2) the influence of air temperature and food supplementation on average T_b and the magnitude of daily T_b fluctuations.

Methods

Study Site and Species

Research was conducted on a free-ranging population of red squirrels (*Tamiasciurus hudsonicus*) in the Kluane region, south-western Yukon, Canada (61°N, 138°W). Red squirrels occupying this study site have been the focus of long-term research on resource-mediated ecology, evolution and, more recently, energetics. Details about the study site, red squirrel ecology, and general methodology are described elsewhere (McAdam et al. 2007). Briefly, red squirrels occupy and defend year-round territories that contain at least one primary midden or food cache. During winter and spring, red squirrels feed primarily on white spruce cones (*Picea glauca*) and mushrooms that they larder hoard within the midden or scatter hoard throughout their territory in summer or autumn. When inactive, squirrels occupy tree nests constructed with grass and/or moss. Although some red squirrel populations have been noted to occupy underground nests during cold winter periods (Pruitt and Lucier 1958; Yahner 1980), we rarely observe prolonged underground nest occupation at our study site.

The study was conducted on two ~3.2 hectare study grids, approximately 1 km apart. Squirrels on one of these grids (n=11) were used as controls and those on the other grid (n=17) were food supplemented with peanut butter, provided ad libitum on their primary midden. Twenty-four days prior to the start of body temperature measurements, one kilogram of peanut butter contained in 10 L buckets was hung in a tree near the centre of the midden approximately 2 meters above-ground. Peanut butter levels in buckets were checked twice during the period (Jan 26 and Feb 9), and replenished when necessary to ensure individuals had access to an ad libitum source until the completion of body temperature measurements. Squirrels consumed differing amounts of peanut butter, which may reflect the amount of natural food available to them or food preference. Middens surrounding the study squirrels were also supplemented with peanut butter to reduce the possibility that other squirrels would pilfer from the study animals. Squirrel middens were located based on recent activity (e.g., cone bract piles, tracks, direct visual or auditory detection of squirrels) at locations known to be active in previous years (Boutin et al. unpublished data), and by walking transects to find additional middens. Midden ownership was determined by visual observation of squirrels during midden visits and by trapping (McAdam et al. 2007). All animal use procedures were approved by McGill University Faculty of Agricultural and Environmental Sciences Animal Care Committee (AUP #5274; AUP #4728).

Body temperature

Temperature sensitive data loggers (DS1922L iButtonTM, Dallas Semiconductor, Texas) were implanted into the peritoneal cavities of 28 (control: 5 males, 6 females; food supplemented: 8 males, 9 females) free-ranging adult red squirrels (mass 205-315g, mean 250g) January 16-21, 2007. Loggers were waterproofed with Elvax (Minimitter, Corp, OR), and once coated weighed 4-5g, less than 2.5% of the mass of the smallest squirrel. Squirrels were captured on middens using Tomahawk live traps baited with peanut butter and transported to a temporary facility 1 km from the study sites. Animals were anesthetized using isofluorane, weighed to the nearest gram, and tagged with permanent numbered ear tags. Coloured wire and pipecleaners were attached to the eartags to facilitate identification in the field following release (McAdam et al. 2007) and each squirrel was injected with 1 mg/kg ketoprophin analgesic and 30 mg/kg trimethoprim/sulfadiazine antibiotics. A logger was inserted intraperitoneally via a 1.5 cm incision in the linea alba posterior to the umbilical scar. Following surgery, animals were provided with apple, carrot and peanut butter, housed overnight and returned to site of capture the following day. Animals were not housed longer because they appeared to have recovered well enough to be released, and to minimize the chance of territory loss to other squirrels.

For all study individuals, logger temperature representing core T_b was measured every 15 minutes from January 30-February 27, 2007 and T_b measurements did not begin until at least 8 days after surgery. Of the 28 animals implanted, 24 were successfully recaptured at the end of the study period for data logger recovery (control: 4 males, 5 females; food supplemented: 7 males, 8 females). Animals were euthanized using an over-dose of isofluorane for tissue collection as part of another study and data loggers were removed. Logger resolution is 0.0625°C and accuracy is ± 0.5 °C, and they were calibrated after retrieval to 0.1 °C using a glass thermometer and a water bath at 32, 38 and 43 °C.

Activity

A subset of squirrels (n=14) were fitted with externally mounted temperature data loggers (iButton DS1922L) to evaluate the effects of nest attendance on body temperature patterns. Detection of nest occupation vs. activity outside the nest with externally mounted temperature data loggers was based on the premise that recorded temperatures would be influenced by a combination of air and body temperatures, as well as the posture and movement of the squirrel wearing the collar (Osgodd and Weigl 1972). Data loggers were re-encased in an epoxy to facilitate collar mounting (iBCollar, Alpha Mach Inc., Quebec) which I then attached around the necks of squirrels (McAdam et al. 2007). Loggers were set to record temperature every minute, and at this setting had enough memory to record for up to 5 days.

A method for extracting nest attendance patterns from these collar records was developed and validated within the same study population during the winter of 2006. Twenty-six squirrels were collared with both data loggers and radio transmitters (McAdam et al. 2007) to facilitate locating and visually observing the behaviour of squirrels with actively recording data loggers. Squirrels were visited 1 to 18 times (mean=8.5) and behaviour (whether the squirrel was in the nest or active outside of the nest) was recorded every minute for a minimum of 5 minutes. Of the focal visits (n=204), half (n=109) were randomly selected to develop criteria used to classify a collar temperature reading as either in the nest or active. When squirrels were in a nest, the data logger recorded high, stable temperatures, whereas when squirrels were active temperature readings were lower and more variable. Based on comparisons of collar temperature records and visually-observed behaviour, animals were classified as active when 1) collar temperature was 6 ^{o}C colder than the mode of all collar temperature records for that animal or 2) the coefficient of variation of five records centered on the record of interest was > 0.07. Using these criteria I was able to correctly classify 79% of the observations. The criteria were then tested against the second half of the data set (n=95) and could correctly classify nest attendance 77% of the time compared to visual observations. Although collar temperatures indicative of animals outside the nest are labeled as active, I cannot infer the actual behaviour of the animal when outside the nest. However, during winter, when air temperatures are low and solar radiation minimal, squirrels are rarely observed resting or basking outside the nest and instead spend the vast majority of outside-the-nest-time traveling or feeding (Woods and Humphries, unpublished observations).

Air temperature and day length

Air temperature (T_a) was measured hourly on the food supplemented grid using a shaded ibutton (DS1922L) suspended at a height of 1.5 meters. Sunrise and sunset were used to define day and night periods. These were determined using the National Research Council Canada's calculator (www.hia-iha.nrc.cnrc.gc.ca) with the study site's latitude and longitude as inputs.

Statistical analysis

I used a nested mixed model (PROC MIXED) to evaluate whether T_b varied according to sex, mass, food treatment, air temperature, period of day (daytime, nighttime) and interactions among the latter three variables. Both sex and mass were dropped sequentially because they were not found to be significant. I used an autoregressive covariance structure (AR[1]) in the model to account for temporal autocorrelation in the body temperature measurements. PROC CORR was used to evaluate the correlation of T_b and the proportion of the day spent active. Statistical analyses were performed using SAS 9.1 (SAS Institute INC., Cary, NC, USA). All values are shown as mean $\pm$ standard deviation unless otherwise stated.

Results

Activity patterns of red squirrels ascertained with information from collar data loggers indicated individuals spent nearly all (mean 95.4%, inter-individual range 84.5-99.2%) nighttime periods in the nest, as well as an average of 60.5% (inter-individual range 42.4-83.2 %) of daylight hours in the nest. Most activity outside the nest occurred during mid-day and peaked at 13:00 (Fig. 1).

Average core T_b of all monitored red squirrels was 38.1 ± 1.0 °C, with a maximum and minimum recorded value of 34.1 and 42.3 °C, respectively. Within individuals, the average T_b range observed across the entire study period was 4.7 ± 0.6 °C (range 4.0-6.9 °C). The daily range in individual T_b (max T_b – min T_b expressed by one individual within the same 24 hour period) averaged 3.5 ± 0.5 ^{o}C (range 2.2-6.5 ^{o}C). There was no significant difference in the mean daily T_b range of food supplemented and control squirrels (t-test, $df_{1,22}$, p=0.6426).

Overall, T_b was higher during activity than when animals were in the nest (ANOVA with squirrel ID as a random factor; $df_{(1,12e4)}$, p<0.0001). Across all paired observations of T_b and collar-inferred activity, modal T_b when active was 40.4 ^{o}C, which was 2.5 ^{o}C warmer than modal T_b when inside the nest (37.9 ^{o}C; Fig. 2a). Across all individuals with implanted and collar data loggers, there was a significant correlation between the proportion of daylight hours spent outside the nest and average daytime T_b (Pearson r=0.7212, p<0.0001, n=57). Because activity was concentrated during the day and was associated with elevated T_b, day vs. night differences in T_b mirrored active vs. nest T_b differences. Across all individuals, modal night time T_b was 37.7 ^{o}C whereas daytime T_b was bimodal with peaks at 38.0 ^{o}C and 39.7 ^{o}C (Fig. 2b). The upper mode (39.7 ^{o}C) is only slightly lower than the active T_b mode, whereas the lower mode of 38.0 ^{o}C likely reflects the T_b of squirrels occupying their nest during the day.

A mixed model combining time of day (day vs. night), treatment (food supplemented vs. control) and T_a as multiple explanatory variables, with individual included as a random factor was used to examine determinants of T_b values. The original model also included mass, which was not significant (p=0.8826) and subsequently dropped. The final model showed that, across all observations, the T_b of food supplemented squirrels (38.3±0.9°C) was consistently warmer than that of control squirrels (37.9±1.0°C; df $_{1,\ 22}$; p=0.0026; Fig. 3a), and that daytime T_b (39.0±1.0°C) was significantly warmer than night time T_b (38.6±1.2°C; df $_{1,\ 67e3}$; p<0.0001). Food supplemented squirrels had significantly warmer T_b than control squirrels both during the day (39.0 ± 1.0 vs 38.6 ± 1.2) and at night (37.9 ± 0.6 vs 37.5 ± 0.7; Fig. 3b,c), and this was supported by a non significant interaction between food treatment and time of day (df $_{1,67e3}$; p=0.4818). T_b was positively related to T_a during the day, but not during the night, as supported by a significant interaction between T_a and time of day (df $_{1,67e3}$; p<0.0001; Fig. 4). Finally, a significant three-way interaction between treatment, T_a, and time of day reflected that T_b differences between food supplemented and control squirrels were largest on cold days and warm nights (df $_{1,67e3}$; p=0.0002; Fig. 4).

Discussion

My results show that the body temperature of a free-ranging, cold climate red squirrel population is labile, and that the extent of this lability varies slightly but significantly within and among individuals according to environmental circumstances. Average T_b measured across all individuals throughout the study was 38.2 ^{o}C, which is within the range of body temperatures previously measured in captive red squirrels (Pauls 1979), other tree squirrels (Golightly and Ohmart 1978; Pereira et al. 2002), and rodents in general (Refinetti 1996; 1998). The lowest and highest T_b recorded on any individual generated a population T_b range of 8.2 °C, which is over 2 °C greater than that reported before in red squirrels (n=2; Pauls 1979) and other tree squirrels (Golightly and Ohmart 1978; Pereira et al. 2002). Within individuals, one outlier was characterized by a maximum daily T_b range of 6.5 °C, while the remainder of study individuals had maximum daily ranges between 3.6-5.2 °C. This range is similar to daily T_b ranges of other tree squirrel (Golightly and Ohmart 1978; Pereira et al. 2002), other rodents (Refinetti 1998) and hypothermic birds (Maddocks and Geiser 1997), and is less than the range commonly used to categorize daily torpor (>10 °C; Geiser and Ruf 1995) therefore, red squirrels in this population do not use daily torpor or hibernation.

Red squirrel T_b shows a distinct daily pattern, with daytime temperatures on average 1.1 °C higher than nighttime temperatures. This pattern of increased T_b during the species' active phase of the day is frequently observed in endotherms, and is attributed to increased activity (Heldmaier et al. 1989; Weinert and Waterhouse 1998) and/or circadian rhythms independent of activity (Refinetti and Menaker 1992; Refinetti 1999b). Indeed, red squirrel T_b was consistently increased during activity bouts outside the nest (Figure 2; Pauls 1979).

There is little evidence of an effect of T_a on T_b among animals that have small daily ranges in body temperature (Golightly and Ohmart 1978; but see references in Refinetti and Menaker 1992), yet my results clearly show that T_b and T_a are positively related during the day. This may be, in part, an indirect relationship driven by increased T_b during activity and increased activity during warm T_a (Pruitt and Lucier 1958; Pauls 1979; Humphries et al. 2005). Consistent with this, T_a does not affect T_b as strongly at night when red squirrels are inactive.

Food supplemented red squirrels maintained consistently higher T_b than controls during day and night (Fig. 3b,c) and across a 35 °C T_a gradient ranging from -38 to -3 °C (Fig. 4). The persistence of these T_b differences across all environmental conditions and active-inactive periods suggests that food supplemented squirrels increase their set-point T_b relative to control animals. The three-way interaction between food, time of day, and T_a results from food supplemented and controls having more similar T_b during cold nights and warm days. This may result from all individuals experiencing similar challenges of maintaining thermal homeostasis during the coldest nights and opportunities for extensive activity on the warmest days, with individual variation in thermal strategies becoming more clearly revealed during intermediate environmental conditions. The association between abundant resources, high T_b, and presumably elevated metabolism, is consistent with laboratory studies showing tree squirrels reduce T_b in response to caloric restriction (Pauls 1979; Pereira et al 2002) and laboratory and field studies showing reduced expression of daily or prolonged torpor in response to abundant resources (French 1982; Munro et al. 2005; Landry-Cuerrier et al. 2008). I interpret this T_b-resource response in red squirrels and other species as suggesting that T_b reduction is an energy conservation strategy, and under conditions of surplus energy availability individuals maintain higher T_b at the expense of increased energy requirements. More research is required to identify the benefits of maintaining a high T_b that outweigh the sizeable energetic costs (Humphries et al. 2003).

Most laboratory studies manipulate food levels by reducing diets from ad libitum to maintenance or sub-maintenance levels, whereas my food manipulations in the field elevate individuals from natural to ad libitum levels. The ability of these field manipulations to generate significant T_b responses suggests that animals are generally calorically restricted under natural circumstances, but the extent of this caloric restriction will differ over time and among individuals. The present study was conducted in a winter of intermediate food supply, with spruce cone production in the previous autumn (the staple winter food source of this red squirrel population) ranking 4th among the 18 years we have monitored food production at this site (LaMontagne and Boutin 2007). I expect T_b differences between control and food supplemented squirrels would be greater in years of lower natural food availability and less in years of higher natural food availability. T_b variation of the magnitude described in this study is not widely incorporated in field studies of their metabolism and energetics. However, even small reductions in T_b may lead to substantial energy savings (Wooden and Walsberg 2002) because metabolism increases exponentially with

T_b (McNab 2002). Assuming a Q_{10} of 2.5 (McNab 2002), the metabolic rate of a red squirrel constantly maintaining T_b = 42.3 ^{o}C (highest T_b measured in this study) at thermal neutral temperatures would be more than double the metabolic rate of a squirrel maintaining a constant T_b = 34.1 ^{o}C (lowest T_b measured in this study) under the same conditions. Even the 1.1 ^{o}C average difference between daytime and nighttime T_b and the 0.4 ^{o}C average difference between food supplemented and control T_b would lead to a 10% and 3% reduction in metabolism, respectively.

Effects of food availability and T_a on T_b have been previously documented in several studies, but few studies have examined these effects simultaneously, and fewer still in free-ranging animals experiencing extreme environmental conditions. My research confirms that in a free-ranging northern population, red squirrels express heterothermy during winter and that their use of heterothermy varies according to resource and climate conditions. Winter is an energetically challenging season for endotherms that are year-round residents of temperate and polar environments because elevated energy demands, associated with thermoregulation in cold temperatures, coincide with reduced energy supply, associated with seasonal declines in resource availability. This seasonal energetic challenge is particularly severe for small endotherms like red squirrels that have high rates of mass-specific metabolism, high surface-area to volume ratios, and limited capacity to offset negative energy budgets with stored body fat. Yet, despite being the smallest mammal active above snow in boreal environments, red squirrels have remarkably low energy expenditure during winter (Humphries et al. 2005). Shallow, facultative reductions in T_b appear to be an important element, expressed in combination with extensive occupation of well-insulated nests (Klugh 1927; Humphries and Woods personal observations) and heavy reliance on an energy-rich larder hoard, that facilitate winter energy conservation in this species.

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Figures

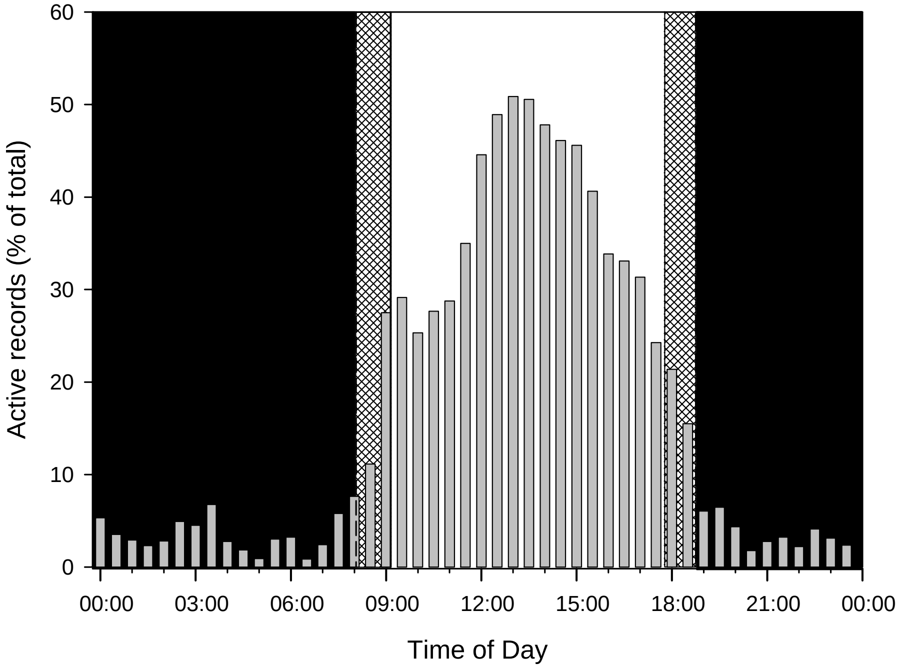

Figure 1. Proportion of time spent out of the nest by red squirrels as inferred from data logger collar records. Dark areas indicate night, white area indicates day, and hatched areas indicate the range in timing of sunrise and sunset.

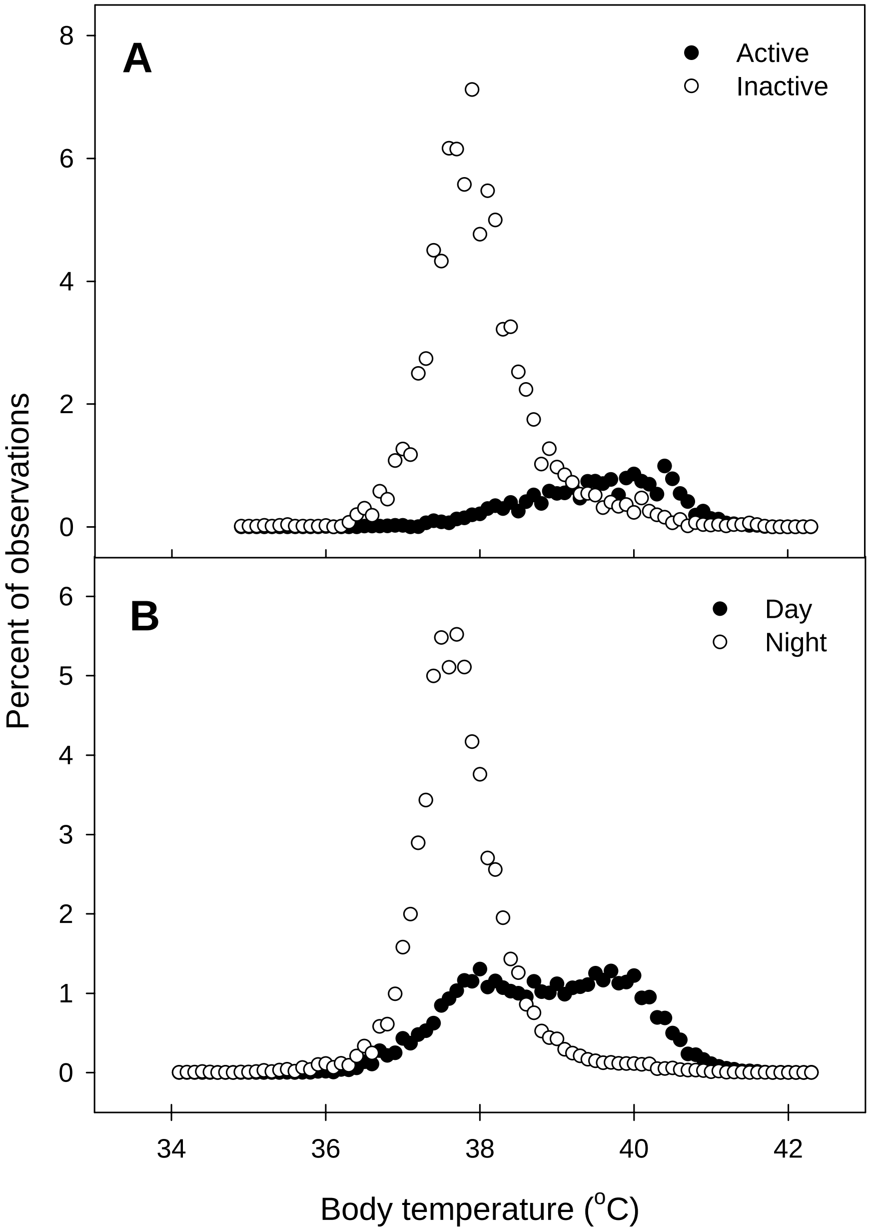

Figure 2. A – Frequency distribution of body temperature measurements of active (closed circles) and inactive (open circles) squirrels as determined for the subset of squirrels for which nest attendance was measured using collars. B - Frequency distribution of T_b measurements of all red squirrels during day and night as defined by sunrise and sunset.

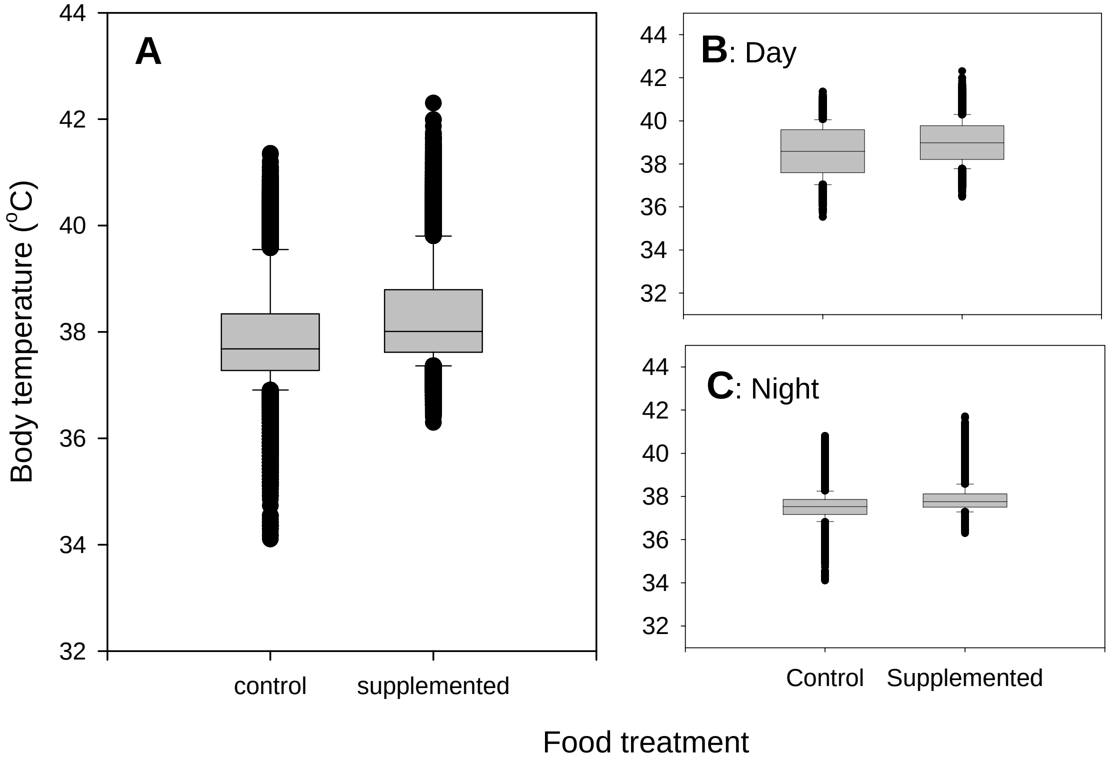

Figure 3. A – Boxplots (line: median, box: 25th/75th percentiles, bars: 10th/90th percentiles, circles: outliers) showing body temperature differences across treatments during the 29-day study period for control (n=9) and food supplemented (n=16) red squirrels. B – Same as A, including only the measurements that were recorded between sunrise and sunset (i.e., day). C – Same as A, except including only measurements that were recorded between sunset and sunrise (i.e., night). A mixed model including individual as a random effect revealed a significant difference in overall mean T_b across treatments (A; df $_{1,\,22}$; p=0.0026), and a non-significant interaction between food treatment and time of day (df $_{1,67e3}$; p=0.4818) suggests that these differences hold both during the day (B) and at night (C).

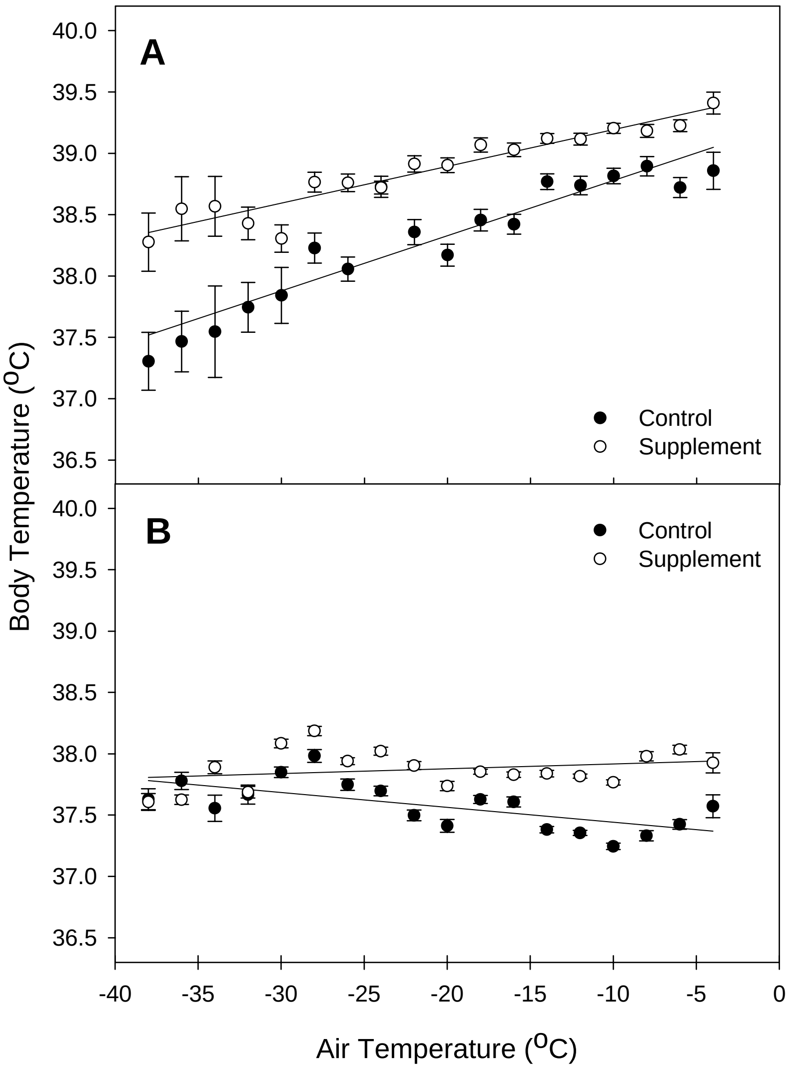

Figure 4. Relationship between body temperature and air temperature for control (closed circles) and food supplemented (open circles) red squirrels, for both A) daytime and B) night time. For presentation purposes, the mean and standard error of all T_b measurements recorded within 2 °C T_a increments are presented for each treatment, as well as the slope for the treatment-specific T_b-T_a relationship. Statistical analysis described and reported in the text treated T_a as a continuous variable and included individual as a random factor.

Connecting Statement

In this thesis I investigate the effects of resource levels on the thermoregulation and energetics of red squirrels in winter. In the first chapter I showed that squirrel body temperature is labile and that it increased in response to resource supplementation. This confirmed that squirrels make use of physiological adaptations to decrease winter energy expenditure. In the second chapter I will examine the role of behaviour in decreasing winter energy expenditure. Specifically, I will investigate the role of resources in mediating the effect of air temperature on the field metabolic rate of squirrels through activity patterns.

Chapter 2: The cost of living in the cold: resource mediated effects of air temperature on field metabolic rate of North American red squirrels (*Tamiasciurus hudsonicus*)

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Abstract

Energy expenditure of endotherms is expected to increase at lower air temperature (T_a) due to the cost of thermoregulation, however field studies suggest that the relationship can be in either direction. We used the double labeled water technique to document the effect of T_a on FMR in free-ranging red squirrels across three years of different natural resource levels. Mean FMR was similar among the three years, however in years of low and intermediate food abundance T_a had a positive effect on FMR (this relationship was significant in one year only), whereas in the year of high food abundance the effect was significantly negative. We used datalogging thermocrons to infer activity patterns during the high and low food year. The proportion of a 24-hr day that squirrels spent active (daily activity) did not vary with T_a or across years of varying resource levels. However, when activity was examined on an hourly basis, the proportion of individuals active increased significantly with T_a under low resources, but did not vary with T_a under high resources. This is because animals in the low food year timed their activity to coincide with the warmest part of the day, whereas animals in the high food year were active earlier in the day when T_a was colder. The observed reversal in the relationship between FMR and T_a across varying levels of resources, and the influence of activity on this relationship, are partially but not fully congruent with endotherms optimizing energetic status by modifying time spent inactive in thermal refuges and active in the cold.

Introduction

The metabolic rate of a thermoregulating endotherm is expected to increase with declining air temperature (T_a) because maintaining a constant body temperature (T_b) requires that heat production matches heat loss (McNab 2002). In addition to heat loss due to the T_b-T_a gradient, thermal exchange with the environment is influenced by convection, conduction and radiation (Oke 1978; McNab 2002). To counter heat loss animals make use of solar radiation and energetically demanding heat production via shivering and non-shivering thermogenesis (Hart 1963). Activity derived thermogenesis may also replace energy required for thermoregulation however the degree to which this occurs is unresolved because heat substitution is paired with heat loss due to an increased rate of convection (Hart 1963; Pauls 1981; McNab 2002). The heat increment of feeding also contributes to heat production (Bech and Præsteng 2004; White and Seymour 2005).

Thermal influences on the energy expenditure of free-ranging endotherms are complicated by their tendency to adjust behavioural activity and occupation of thermal refuges according to prevailing air temperatures. Behavioural activity alters energy expenditure through the costs of muscular movement, and activity patterns alter thermal exposure. Animals make use of a variety of refuges, such as nests (e.g. *Tamiasciurus hudsonicus*, Rothwell 1979; *Glaucomys sabrinus*, Cotton and Parker 2000), the subnivean space (e.g. *Lemmus trimucronatus*, Casey 1981; *Microtus pennsylvanicus*, Courtin et al. 1991), caves (e.g. *Rhinolophus ferrumequinum*, Park et al. 2006) and burrows (e.g. *Geomys bursarius*, Benedix 1994) to decrease the rate of heat exchange with the environment, and basking sites to take advantage of solar radiation (DeJong 1976, McNab 2002). Effective refuges are able to decrease thermoregulatory costs by decoupling the environment experienced by an animal from prevailing air temperatures. However, animals that must leave their thermal refuge to feed are exposed to low temperatures and therefore high thermoregulatory costs.

Studies of field metabolic rate, measured with the doubly labeled water technique, have documented both negative and positive effects of air temperature on field metabolic rate (Anderson and Jetz 2005; Humphries et al. 2005; Sheriff et al. submitted). A bioenergetic model of an endotherm seeking to maximize daily energy gain (or minimize daily energy loss) in the cold predicts that the strength and direction of the relationship between FMR and T_a should vary according to the thermal properties of refuges and resource availability (Humphries and Umbanhowar 2007). In most situations, FMR is expected to increase as T_a decreases due to elevated thermoregulatory costs inside and outside of thermal refuges (McNab 1980; Casey 1981). However, when thermal refuges are of high quality (i.e., thermoregulatory requirements of animals occupying refuges are minimal even on the coldest days) and resources not abundant, animals should spend a large proportion of cold days inactive in refuges because the costs of foraging with low efficiency outweigh the costs incurred while in the refuge. Animals are expected to take advantage of decreased thermoregulatory costs on warm days and rapidly increase activity as T_a increases (Pauls 1978; Humphries et al. 2005; Humphries and Umbanhowar 2007). As a result of more activity and more cold exposure as T_a increases, the relationship between T_a and FMR is expected to reverse into a positive relationship.

Red squirrels are one of the smallest boreal mammals active above the snow in winter (Pruitt and Lucier 1958), and thus have the potential to face very high thermoregulatory requirements during cold winter conditions. However, red squirrels spend a large proportion of cold winter days in well-insulated grass nests (Rothwell 1979). Pauls (1981) found that the metabolic rate of winter-acclimatized red squirrels occupying nests at sub-zero temperatures was similar to metabolic rates measured within their thermal neutral zone. Furthermore, squirrels can also have access to abundant resources in winter because they establish a large hoard of energy-rich conifer cones in autumn, then consume these throughout the winter (Smith and Reichman 1984). However, for red squirrel populations occupying conifer forests characterized by annually variable cone production (LaMontagne and Boutin 2007), there will be extensive year-to-year variation in the size of cone hoards and therefore winter resource availability (Humphries and Boutin 2000).

In the present study, we test the hypothesis that the direction and strength of the relationship between T_a and FMR in endotherms varies according to resource availability. This hypothesis is tested by extending the winter FMR study conducted by Humphries et al. (2005) on a high latitude, free-ranging population of red squirrels to an additional low food year plus a high food year. We also continuously monitor the nest occupancy of squirrels in both of these additional years to document the role of behavioural activity in determining how temperature variation affects endotherm energetics. In particular, we test the prediction that the relationship between FMR and T_a will switch from positive in low resource conditions to negative when resources are abundant, because activity increases and/or becomes less temperature-dependent in resource rich circumstances.

Methods

Field work was carried out on a free-ranging population of red squirrels in South-Western Yukon Territory, Canada (61°N, 138°W) in collaboration with the Kluane Red Squirrel Project. Details about the study site and the ecology of this population are described elsewhere (McAdam et al. 2007). Work was carried out during the winter, prior to reproduction, at a time when squirrels were dependant upon food cached the previous autumn. The primary winter food source of red squirrels in this population is white spruce cones (*Picea glauca*), with less preferred mushrooms and spruce buds.

We measured field metabolic rates (or daily energy expenditure) of red squirrels using the doubly-labeled water technique (Speakman 1997; Humphries et al. 2005) in three winters (2002, n=40; 2006, n=18, 2007 n=19). Data for 2002 were first published by Humphries et al. (2005). Females were studied in 2002 and 2006, and both females (n=16) and males (n=3) in 2007. Squirrels were injected with 0.5ml of labeled water which contained both hydrogen (^{2}H) and oxygen (^{18}O) isotopes (Speakman 1997). An initial blood sample was taken by nail clip after a one hour equilibration period do determine initial concentrations of body isotopes, and the final sample taken at a 24-hour interval, 2-9 days (mean 3.8) following initial sample in 2002, 3-5 days (mean 3.5) in 2006, and 3-4 days (mean 3.25) in 2007. Analysis of isotope concentrations was conducted according to methods described by Ergon and colleagues (2004). We estimated CO_2 production using the plateau method from Speakman (1997) and converted CO_2 production to FMR (kJ day -1) assuming RQ = 0.8. Since mass generally has a significant effect on FMR and we are interested in the other factors that influence FMR, figures are presented as the residuals of the relationship between FMR and mass (resFMR), calculated for each year independently.

Levels of cone availability were determined in each of the study years. Average levels of cones per tree were determined in late July/early August in each of the study years, prior to caching by red squirrels. The number of cones visible in the top 3 m of one side of a sample of trees ($n \geq 83$ trees) was ln (x+1) transformed to yield a cone index (McAdam et al. 2007).

In 2002 and 2006 air temperature (T_a) was measured hourly at a height of 1.5m at a weather station located within 1 km of the study site. In 2007 air temperature was measured on site using a shaded iButton (DS1922L iButtonTM, Dallas Semiconductor, Texas) suspended 1.5 meters in a tree. In analyses when only data from daylight hours are included, sunrise and sunset were calculated daily using the National Research Council Canada's calculator (www.hia-iha.nrc.cnrc.gc.ca) with the study site's latitude and longitude as inputs.

Activity was assessed on a subset of squirrels in both 2006 (n=25) and 2007 (control group n=6) using externally mounted temperature sensitive data loggers (iBCollar, Alpha Mach Inc., Quebec) to evaluate the effect of food level on the timing of activity. These loggers recorded temperature every minute for a period of 3 days. Because collars are influenced by air temperature, as well as body temperature, records were cold and more variable when squirrels were out of a nest and warm and stable when squirrels were inactive in their nest. We developed criteria using collar temperature and variability in the records to determine when squirrels were active or in their nest. See Woods et al. (Chapter 1) for more details about how activity was determined.

We measured activity during the FMR period on a subset of squirrels in 2006 (n=14) and 2007 (n=6) to test the effect of activity levels on resFMR. Activity was measured as the proportion of observations when squirrels were out of the nest during the FMR period.

To evaluate the effect of T_a on activity in different food treatments we evaluated the proportion of daylight hours spent active at each degree of T_a observed (instantaneous T_a) for the natural high (2006; n=25) and natural low (2007; n=6) treatments. Because squirrels are diurnal and the bulk of activity occurs during the daytime (see Chapter 1), only records taken between sunrise and sunset were evaluated. Individual activity records (measured every minute) were matched with the nearest T_a record (measured hourly and rounded to 1^{o}C). The number of activity records at each temperature thus varied depending on the occurrence of a given degree of temperature during the study period. Only air temperatures with a minimum of 50 records was obtained were included in analysis. Using this criterion, the average number of activity records at a given degree of T_a was 757 (range 60 to 3489).

To determine if squirrel daily activity varied according to mean daily T_a, we compared the proportion of the 24hr day spent active to the mean daily T_a in each of the food treatments [natural high n=25 (2006); natural low n=6 (2007)]. Each individual was monitored for 3 days, therefore in all models that used this data set squirrel ID was included as being nested within food treatment.

Squirrels under different food conditions may select warmer air temperatures by modifying daily activity patterns such that their peak levels of activity (measured by the highest proportion of observations that were out of their nest) coincide with peak daily T_a. To evaluate this, we calculated the proportion of time that squirrels were active during each half-hour time period over a 24-hour period.

All procedures were approved by McGill University Faculty of Agricultural and Environmental Sciences Animal Care Committee (AUP #4728).

All analyses were performed using Statistica 6.0 (StatSoft, Inc., Tulsa, OK, USA). Results are presented ± standard error, unless otherwise indicated.

Results

Average FMR was 211.3±6.4, 221.0±11.6, and 247.3±9.6 kJ day^{-1} in 2002 (cone index = 1.22), 2006 (cone index = 2.55) and 2007 (cone index = 0.25), respectively. FMR was significantly lower in 2002 than in 2007 (one-factor ANOVA, $F_{2,74}$=4.52, p=0.014; Bonferroni post-hoc test, p=0.011), but other differences were not significant. Overall mean body mass of squirrels was 248±3.6, 232±5.2, and 239±5.1 g in 2002, 2006 and 2007, respectively. Mass was significantly lower in 2006 than in 2002 (one-factor ANOVA $F_{2,74}$, p=0.033; Bonferroni post-hoc test, p=0.038), but other differences were not significant. FMR increased significantly with body mass in all three years (2002: FMR=7.014+0.823mass, $F_{1,38}$=10.51, p=0.002, R^2=0.217; 2006: FMR=-57.670+1.201mass, $F_{1,16}$=6.70, p=0.020, R^2=0.295; 2007: FMR=10.868+0.991mass, $F_{1,17}$=6.61, p=0.020, R^2=0.230). With mass included as a covariate, FMR continued to vary significantly among years ($F_{2,73}$=9.38, p<0.001), and there was no interaction between year and mass ($F_{2,71}$=0.31, p=0.737). Therefore for models that include FMR, mass is always included as a predictor variable, however for presentation purposes, figures are presented as resFMR, calculated from the equations given above.

The relationship between FMR and T_a differed among years, as indicated by a significant year by T_a interaction ($F_{2,68}$=9.66, p<0.001) in a model including mass, T_a and year as explanatory variables, as well as T_a by year and mass by year interactions. There was no significant interaction between year and mass ($F_{2,68}$=1.48, p=0.233) therefore it was removed from the model. In the resulting model the year by T_a interaction remained significant ($F_{2,70}$=8.44, p<0.001) as was mass ($F_{1,70}$=28.65, p<0.001). The relationship between T_a and resFMR (calculated as the residuals from the FMR:mass relationships given above) was strongly positive in the intermediate cone year (2002: resFMR = 37.562 + 2.467T_a,

R^2=0.138, n=40), weakly positive in the low cone year across a narrower range of T_a variation (2007: resFMR = 30.431 + 2.184T_a, R^2=0.044, n=19), and strongly negative in the high cone year (2006: resFMR = -52.607 – 3.963T_a, R^2=0.472, n=18; Figure 1).

A multiple regression analysis revealed that among squirrels on which we measured activity levels during their FMR sampling interval, the proportion of time spent active had a significant ($F_{1,18}$=7.96, p=0.011), positive effect on FMR and mass was marginally non-significant ($F_{1,18}$=3.49, p=0.078; FMR=7.157+3.795*Act+0.642*Mass, R^2_{adj}=0.366; figure 2). Excluding mass from the model in a simple regression did not change the interpretation of the activity results ($F_{1,19}$=8.91, p=0.008; FMR=150.352+4.211*Act, R^2=0.319).

Average activity (proportion of 24 hr day spent active) was 15.6±0.7% and 17.7±2.1% in 2006 (high cones) and 2007 (low cones), respectively. Activity did not vary with body mass (regression of average activity per individual against mass, $F_{1,38}$=0.199, p=0.658). A mixed model including year, T_a, and individual squirrel nested within year revealed that the proportion of a 24hr day spent active was equal among years ($F_{1,60}$=2.06, p=0.157). Activity did not vary significantly with 24 hr average T_a ($F_{1,60}$=1.77, p=0.904; Figure 3) and there was no significant year by T_a interaction ($F_{1,60}$=2.60, p=0.111). In other words, squirrels were active for similar proportions of a 24 hr period on days that were, on average, warm or cold. When the interaction was removed from the model there was still no overall effect of either year ($F_{1,61}$=1.46, p=0.232) or T_a ($F_{1,61}$=0.01, p=0.904). However, thermal influences on activity emerge when T_a is analyzed as an hourly rather than daily variable by relating the proportion of monitored individuals that were active during a given hour to recorded T_a during that hour. In this case, the relationship between activity and T_a differed with year, as indicated by a significant year by T_a interaction ($F_{1,58}$=37.12, p<0.001). Activity was independent of T_a among squirrels in 2006 when animals had access to high food levels (simple linear regression, β=0.142, $F_{1,39}$=0.89, p=0.351; model R^2=0.022) but increased significantly with T_a in 2007 when animals had access to low food (simple linear regression, β=2.57, $F_{1,19}$=62.68, p<0.001; model R^2=0.767; Figure

4). The stronger correspondence between hourly T_a and activity among squirrels with low natural food than high natural food occurs because the former's activity closely tracked daily T_a trends (low activity in morning and evening when T_a is low, high activity in late afternoon when T_a is high) whereas the latter' activity was concentrated earlier in the day when prevailing T_a remained low (Figure 5).

Discussion

As predicted, the direction and the strength of the relationship between T_a and red squirrel FMR varied across three years of divergent resource supply. FMR was positively related to T_a in two years of low and intermediate food supply, but increased strongly with declining T_a in a year of high food supply. Thus, the pattern of increasing FMR with increasing T_a reported by Humphries et al. (2005) studying the same red squirrel population in a low food year does not generalize to years of high food abundance. However, the tendency for red squirrels to be characterized by a surprisingly low FMR during winter, in relation to their resting metabolic rate (RMR; Humphries et al. 2005) and the FMR of other similar-sized mammals (McNab 2002), does generalize across multiple years of widely divergent resource supply.

A reversal in the relationship between T_a and FMR between low and high resource conditions was anticipated by a bioenergetic model described by Humphries and Umbanhowar (2007). This model tracks the daily energetic status of an endotherm optimizing its time allocation to activity and refuge occupation across a range of T_a, refuge quality and resource availability. In most circumstances the model predicts a negative relationship between FMR and T_a. It is only in situations of low or intermediate resource availability and high refuge quality that the relationship between FMR and T_a should switch to a positive slope. These criteria are consistent with the low and intermediate resources in 2002 and 2007, and high quality nests of red squirrels (Pauls 1981). Under these circumstances foraging efficiency is low therefore animals must spend more time foraging to meet energetic demands. At low T_a net thermoregulatory costs associated with foraging are greater than costs incurred while in their refuge therefore animals remain inactive. Animals take advantage of warm days to forage and the time that they spend active quickly increases with increasing T_a. Because of this rapid increase in activity animals are subject to higher thermoregulatory costs on warm days, causing a positive slope between FMR and T_a. Humphries and Umbanhowar's model predicts activity to vary with resources and T_a on a daily scale. In this way our results differ from the expectations of the model because we did not find differences in daily activity across food treatments or T_a, but only at a smaller time scale.

Activity had a strong effect on FMR, and the degree to which activity tracked T_a variation differed between low and high resource conditions. It is surprising that the proportion of the day that squirrels spent active did not differ significantly with daily average T_a or between years of different food availability. On both warm and cold days throughout high and low food years, squirrels were active for a rather consistent portion of the day (15.6% and 17.7% for high and low food, respectively). However when we summarized activity on an hour-to-hour basis (by calculating the proportion of observations that were active outside their nest during a given hour) and regressed this proportion against hourly T_a much clearer, but still annually variable effects of T_a on activity emerged. Under low food conditions, the proportion of squirrels active increased from 0 at T_a < -30 ^{o}C to 0.6 or more at T_a > -12 ^{o}C, whereas under high food conditions, the proportional activity was independent of T_a. The enhanced thermal selectivity of squirrels exposed to low food is achieved by timing daily activity to coincide with the warmest part of the day. In this way our results support the expectations of Humphries and Umbanhowar's (2007) model for squirrels under low resources to be more selective toward activity at warmer T_a, however we found the selectivity to be at an hourly rather than daily time scale. Animals with access to more food expose themselves to colder T_a's by being active earlier in the day. Further research is required to identify why squirrels opt for earlier activity and higher thermoregulatory costs under high resource conditions, but we speculate that territory defense and exploration may be involved, based on the year-round territorial nature of red squirrels, combined with previous observations of frequent cone pilferage and occasional territory takeovers.

The previously established relationships between T_b, activity and food (Chapter 1) may contribute to, but are unlikely to be the sole determinants of the food and temperature influences on FMR reported here. The independent effects of T_a on T_b and T_a on activity are difficult to separate because activity has a strong influence on T_b (Chapter 1; McNab 2002). As a result of the universal effects of T_b on metabolic rate (Gillooly et al. 2001), the consistently higher T_b in squirrels with access to abundant food (Chapter 1) should have led to higher average FMR in high cone than low cone years. Although there were differences in FMR across years (low food 2007 > high food 2006 > intermediate food 2002), they are not in the direction predicted by T_b responses. This does not discount energetic consequences of T_b, but suggests that other determinants of energy expenditure, such as resting metabolism or activity patterns, are more important contributors to overall annual patterns in FMR.

There is considerable interest among physiologists and ecologists regarding the effects of temperature on endotherm metabolism (Humphries et al. 2002; Johnston and Schmitz 2003; Brown 2004; Both et al. 2006). Although temperature has direct and largely predictable physiological influences on resting metabolism (McNab 2002), when metabolism is measured in a manner that incorporates behaviour, then these direct physiological effects can be counteracted or amplified by behavioural responses to temperature. The metabolism of active and inactive animals differs as a result of the energetic costs of activity (Hart 1963; Weathers et al. 1984; McNab 2002), plus differences in the temperature exposure and heat exchange of animals inside and outside of thermal refuges (McNab 1980; Pauls 1981; Long et al. 2005). Given this and that resources alter the extent and nature of activity (Hart 1963; Stephen and Krebs 1986, Godfrey and Bryant 2000), our results showing that behavioural and energetic responses to temperature variation depend on resource availability should come as no surprise. Nevertheless, behaviour and resources are rarely considered in broadscale studies of thermal influences on metabolic ecology (Anderson and Jetz 2005; Speakman

2000), which is unfortunate, because they are likely to be widespread determinants of how temperature affects the realized metabolism of free-ranging animals.

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Figures

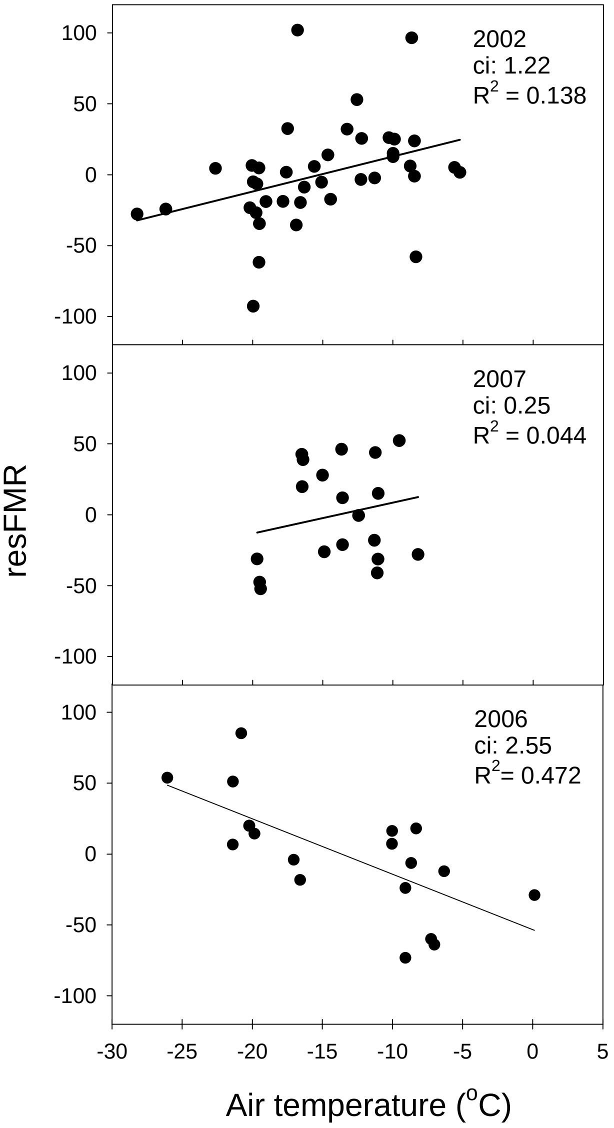

Figure 1. Residual FMR was positively related to air temperature in years of low cone abundance and negatively related to air temperature during years of high cone abundance. The first panel (2002) is re-drawn from Humphries et al. (2005). For presentation purposes, resFMR was calculated independently each year using the residuals from the mass-FMR relationship.

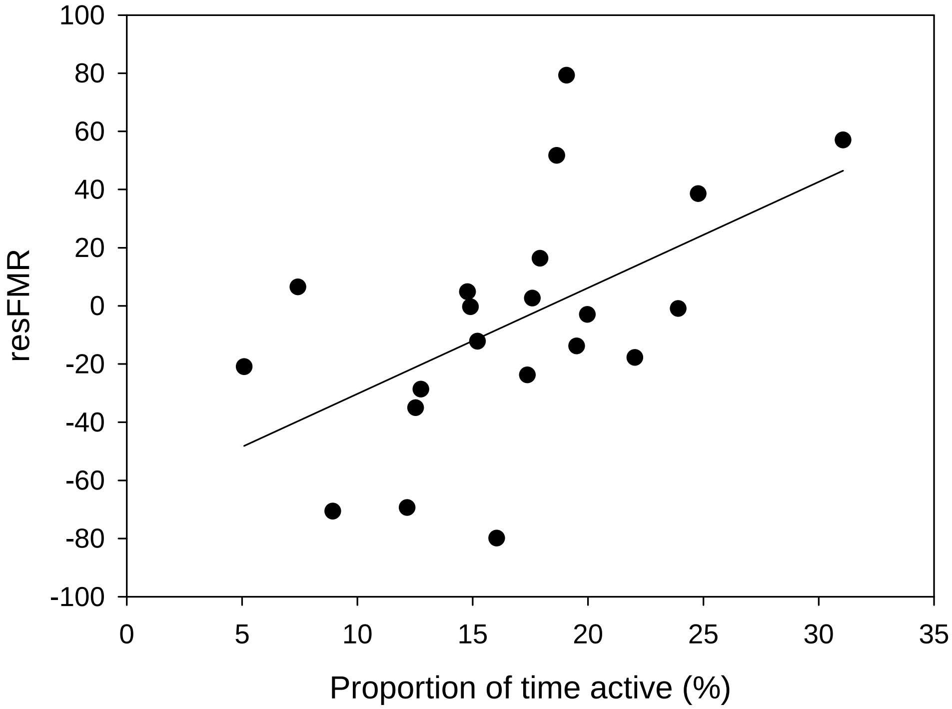

Figure 2. Residual FMR was positively related to the proportion of time that squirrels spent active during the FMR period. Data are presented as mass-corrected resFMR, however in analysis both mass and activity were included in a multiple regression.

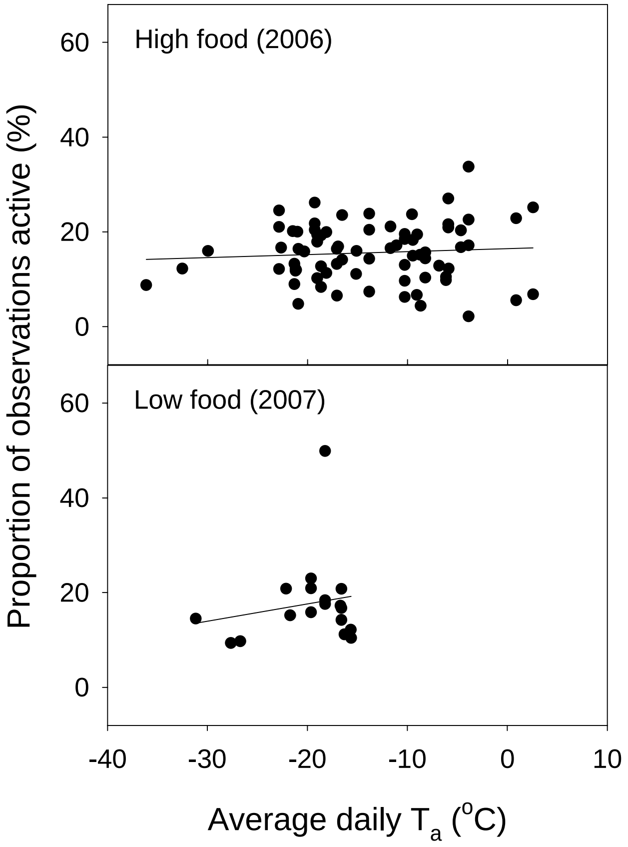

Figure 3. Proportion of a 24hr day that squirrels were active in a year of high natural food (top) and a year of low natural food (bottom). Daily activity budgets did not vary significantly with T_a during either year. There are 3 data points (each a 24hr average) presented per squirrel, however in analyses squirrel was included as a random factor.

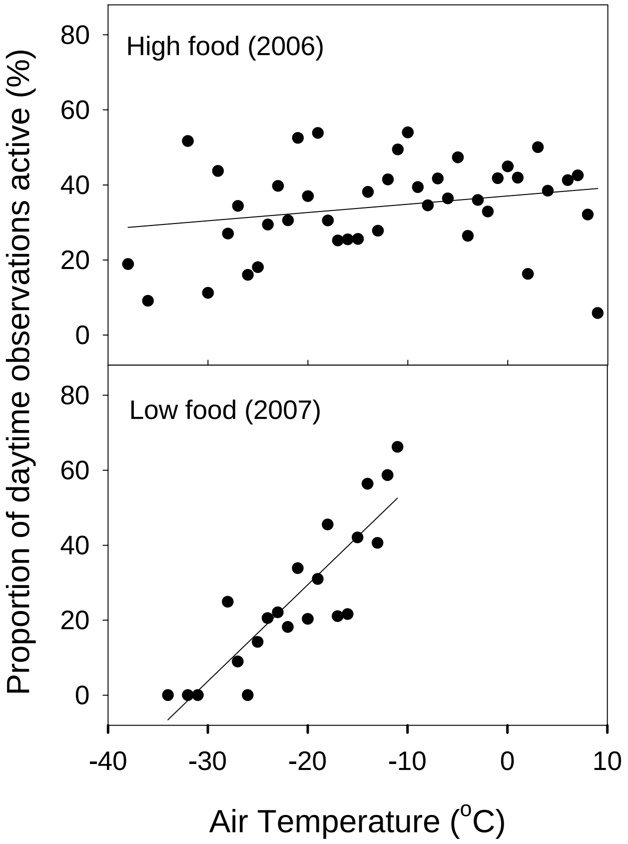

Figure 4. When the proportion of activity is expressed against hourly T_a, there is no relationship between T_a and the proportion of time spent active during a year of high food resources (top panel). However, there was a significant positive effect of T_a on the proportion of active records during a year of low food resources (bottom panel).

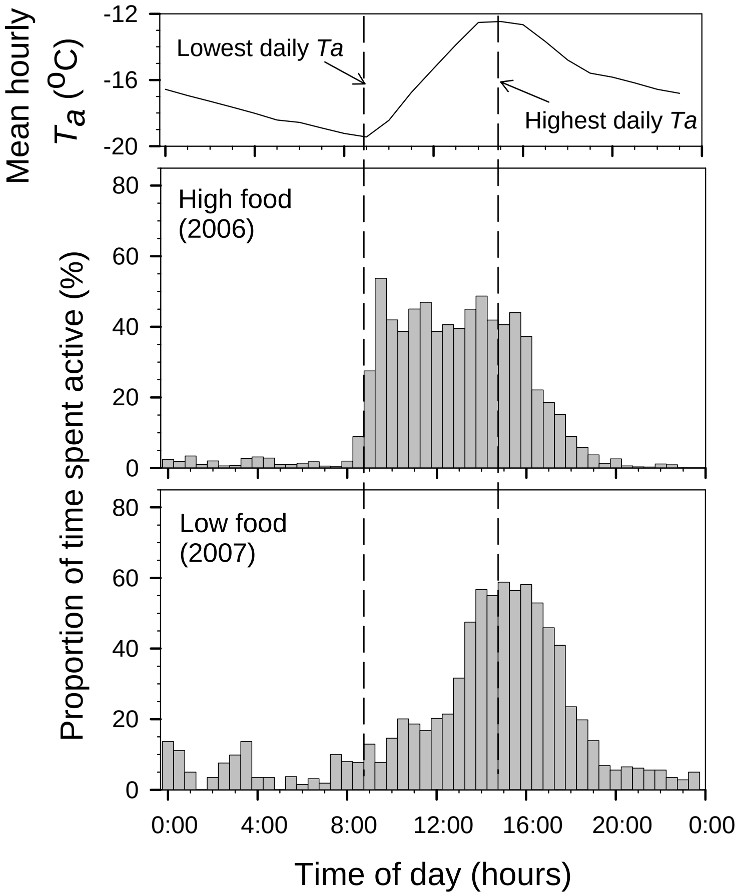

Figure 5. Proportion of time spent active at different times of the day as inferred from collar records, and mean hourly T_a during the same period in 2006. Squirrels with naturally high food were active early in the day therefore were exposed to cold morning temperatures. Squirrels with low food tended to be most active at the warmest part of the day. The dashed lines indicate maximum and minimum mean hourly temperatures.

General Conclusion

This thesis clearly demonstrates that resource abundance has a significant effect on the energy expenditure of red squirrels in winter, and that it these resource levels mediate the effects of T_a on FMR. It documents both physiological and behavioural strategies used by red squirrels to cope with low food resources and cold temperatures.

Body temperature of red squirrels increased when provided supplemental resources. The T_b differences among food treatments, particularly the persistent nighttime differences, suggest that T_b depression is an adaptive strategy for energy conservation in red squirrels in winter. Given that food supplemented squirrels maintain elevated T_b suggests that there is an advantage to doing so. Further research is required to determine the advantages of maintaining elevated T_b, but I speculate that even slight elevations in T_b will decrease reaction time due to enhanced enzyme activation. Pauls (1979) also showed that red squirrels increase T_b by approximately 2 ^{o}C during the hour prior to emergence from nest. Even the small differences in T_b of 0.4^{o}C as observed between control and food supplemented squirrels in my study, and differences observed by Pauls (1979) should alter reaction times due to Q_{10} effects on the rate of metabolism and enzyme activity (Gillooly et al. 2001). Variation in T_b of this magnitude has been shown to affect human reaction time (Kleitman et al. 1937) however more research is needed to determine whether the differences observed in this study are ecologically significant for red squirrels.

Annual variation in natural resources mediates the relationship between T_a and FMR in free-living animals. Although T_b patterns may contribute to this relationship, annual variation appears to be driven by resource-mediated patterns in activity. These results are consistent with the energetic model proposed by Humphries and Umbanhowar (2007) that predicts a positive association of FMR with T_a in cases of high quality refuge and low food resources. These results also challenge literature that predicts a single, negative effect of T_a on energy expenditure due to thermoregulation and provides further evidence that activity patterns and the use of thermally buffered microhabitats play an important role in winter energy expenditure.

The overall negative relationship between inter-specific FMR and T_a found by Anderson and Jetz (2005) is not congruent with either the low energy expenditure expressed by red squirrels in winter, nor the positive relationship of T_a on FMR of snowshoe hares (Sheriff et al. submitted) and red squirrels under low food conditions. This highlights a weakness of broad-scale studies on FMR in providing causal insight into determinants of FMR at the population level. Anderson and Jetz were very successful at explaining variation in FMR using a simple model that included mass (which I also showed to be significant in determining FMR in Chapter 2) and a negative effect of temperature. However, they did not find energy availability (as measured by net primary production) to have a significant effect on FMR (but see Mueller and Diamond 2001). Here, I have shown that at a population level food is an important mediator of the effect of T_a on FMR. This study also highlights the importance of including resource levels in studies of FMR at the population level. These are often overlooked, possibly because of the difficulties in measuring resources in natural systems.

A better understanding of the ecological determinants of FMR at a population level requires the inclusion of resource levels and an understanding of the physiological and behavioural adaptations to resource abundance. This would require simultaneous data on T_b, T_a, activity and FMR collected at different food treatments. Food supplementation experiments are ideal because they control confounding variables such as habitat, animal density, inter-population variability and inter-annual variation in climate. The response of T_b to food supplementation suggests that, at least in this squirrel system, animals are under food stress during winter and will to respond to supplementation. One could also take advantage of natural variation in space and time, but these could be confounded by heterogeneity in habitat and resource-dependant animal density. Another alternative is to examine the influence of small scale special patterns on energy expenditure (Landry-Cuerrier et al. 2008).

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