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Do red squirrels (*Tamiasciurus hudsonicus*) use daily torpor during winter?¹

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Abstract: Given their relatively small body size, high thermoregulatory costs, and low metabolic rate, we tested the hypothesis that red squirrels (*Tamiasciurus hudsonicus*) would employ bouts of daily torpor to save energy during winter. We collected data on body temperature (T_b) using surgically implanted data loggers for squirrels in the Cypress Hills region of Saskatchewan, where extended periods of cold snowy weather make foraging difficult and should lead to high levels of energy expenditure. Based on over 8000 measurements from 4 animals over 3 winters, we found no evidence for torpor use. However, T_b was lowest in January and highest in September and May, and mean monthly T_b was correlated with mean monthly ambient temperature (T_a). Given that taxonomically related species can and do use torpor, it remains to be determined what makes heterothermy in this species costly to the extent that its use is precluded.

Keywords: energetics, heterothermy, red squirrels, temperature dataloggers, torpor.

Résumé: Étant donné leur relative petite taille corporelle, leurs coûts élevés de thermorégulation et leur faible taux métabolique, nous avons évalué l'hypothèse que les écureuils roux (*Tamiasciurus hudsonicus*) utiliseraient des périodes journalières de torpeur pour économiser de l'énergie en hiver. Nous avons récolté des données de température corporelle en utilisant des enregistreurs de données implantés par chirurgie chez des écureuils dans la région de Cypress Hills en Saskatchewan où des épisodes prolongés de temps froid et neigeux font que la quête alimentaire devient difficile et devrait ainsi entraîner des dépenses énergétiques élevées. Sur la base de plus de 8000 mesures sur quatre individus durant trois hivers, nous n'avons trouvé aucune évidence d'utilisation de la torpeur. Cependant, la température corporelle était la plus basse en janvier et la plus élevée en septembre et en mai. La température corporelle moyenne mensuelle était corrélée à la température moyenne mensuelle de l'air ambiant. Étant donné que d'autres espèces proches sur le plan taxonomique sont capables de torpeur et l'utilisent, il reste à déterminer pourquoi l'hétérothermie serait si coûteuse chez cette espèce qu'elle n'est jamais utilisée.

Mots-clés: écureuils roux, énergétique, enregistreurs de température, hétérothermie, torpeur.

Nomenclature: Wilson & Reeder, 2005; Moss, 1994.

Introduction

Anderson and Jetz's (2005) review of studies employing the doubly labeled water method to estimate metabolic rates suggests that ambient temperature (T_a) is among the strongest and most consistent environmental predictors of field metabolic rate (FMR) in birds and mammals. They found that the highest levels of energy expenditure coincided with the coldest T_a , a relationship that is not surprising, but until Humphries *et al.* (2005), there were no published FMR data for animals exposed to T_a s below -3 °C. Humphries *et al.* found that red squirrels (*Tamiasciurus hudsonicus*) expended the least energy on the coldest days during winter because they spent more time in warm nests. Humphries *et al.* argued that this was an adaptation to low resource availability and low energy requirements during winter, when animals are not growing or reproducing.

At 200–250 g, red squirrels (henceforth squirrels) are the smallest boreal mammal that exhibits activity above the snow in winter, meaning they should face high thermoregulatory energy costs due to their relatively high surface area to volume ratios (Pruitt & Lucier, 1958). Red squirrels are specialized to eat conifer seeds, relying on a larger hoard of cones collected from trees in late summer/autumn that they eat during winter and spring (Steele, 1998). Cones are stored underground within middens, which are the focal points of each individual's territory. Territories are maintained year-round by both sexes and consist of one to several middens and the surrounding cone-bearing trees. Based on this natural history, Humphries *et al.* (2005) hypothesized that red squirrels have such exceedingly low winter energy costs for 3 reasons: 1) access to a secure food source stored in middens; 2) mostly remaining inactive in well-insulated nests, which are preferentially constructed with southern exposures to absorb heat from winter sunlight; and 3) the tendency to be active out of the nest only on warmer days. That much of the winter is spent inactive in nests and outside activity is timed to coincide with the

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warmest periods of the warmest days has been reported elsewhere (Pruitt & Lucier, 1958; Pauls, 1978a,b). Based on levels of winter activity, Pauls (1978b) concluded that captive red squirrels exposed to natural conditions do not hibernate, but the sampling period of 12–24 h meant that it was unclear whether squirrels could employ bouts of daily torpor. This would represent a fourth reason for the exceedingly low energy use by these animals in winter. Heterothermic responses are common in a wide variety of mammals (Geiser *et al.*, 2000), including close relatives of red squirrels amongst the rodents such as ground squirrels, chipmunks, and marmots. Thus, we predicted that red squirrels would employ daily torpor during winter, especially at a location where extended periods of cold snowy weather would make access to middens difficult and lead to relatively high levels of energy expenditure.

Methods

Our study took place in the West Block of Cypress Hills Provincial Park, Saskatchewan, Canada (49° 34' N, 109° 53' W) at the University of Regina Research Station, which is adjacent to Fort Walsh National Historic Site (for details see Willis & Brigham, 2004). In brief, the Cypress Hills are a raised upland that is typically wetter and colder than the surrounding mixed-grass prairies. We chose the study area due to the extreme winter weather conditions it imposes on squirrels relative to many other parts of their range (Steele, 1998). The nearest Environment Canada weather station that records long-term (since 1981) weather data is in the Centre Block of Cypress Hills InterProvincial Park, about 35 km east of our study area and approximately 200 m lower in elevation. Data from that weather station (http://climate.weatheroffice.gc.ca/climate_normals/index_e.html; accessed on 5 October 2011) show that the mean minimum daily T_a is below -10 °C during December–February, and extreme low T_a s of < -40 °C have been recorded in all 3 months. On average there are 2–10 d per month from October to March when T_a is < -20 °C. Extreme daily snowfalls of > 20 cm have been recorded for all months from September to May, and mean monthly snowfalls exceed 20 cm for all months from October to May. Compared to the location of the weather station, the higher elevation of our specific study site means that winter temperatures squirrels were exposed to were likely slightly colder, and precipitation levels were likely slightly higher on average. T_a data from the weather station were used for all of our analyses.

We live-trapped squirrels in Havahart squirrel traps (Woodstream Corp., Lititz, Pennsylvania, USA) during sessions that lasted from 3 to 18 d in late August or early September and again in mid-May, beginning in the fall of 2004 and concluding in spring 2008. We did not trap in fall 2006 because access to the park was denied due to an extreme threat of forest fire. All trapping occurred at the same stations in the same 1-ha block of mature white spruce (*Picea glauca*) forest. During trapping sessions we caught 7–25 different individuals, which were temporarily marked (for the remainder of that trapping session) by clipping approximately 1 cm of fur from the end of the tail.

To collect data on squirrel body temperatures (T_b) during winter, we used temperature data loggers (DS1921 Thermochron iButtons, Maxim Integrated Products Inc., Sunnyvale, California, USA; mass of ~ 3.1 g after being coated in paraffin). The data loggers represented between 1 and 2% of squirrel body mass. Temperature loggers recorded T_b to the nearest 0.5 ± 1 °C (loggers were calibrated by the manufacturer) and were implanted intraperitoneally under oxygen/isoflurane anesthesia (for details see Körtner & Geiser, 2009). Squirrels recovered from the anesthesia within 15 min and were released at the site of capture usually within 90 min. Loggers were implanted during the August–September trapping sessions in 2004 (7), 2005 (11), and 2007 (14). In different years, data loggers were programmed to record T_b at 1-, 2-, or 3-h intervals beginning at approximately midnight each day until 2048 records had been stored, which filled the memory capacity.

All unmarked squirrels captured after fall 2004 were anesthetized and checked for implanted data loggers. Loggers were recovered in the same manner as they were implanted, and we assumed that there had been minimal drift. We conservatively defined torpor entry when T_b fell below and then rose above a threshold of 30 °C, following Körtner & Geiser (2000; 2009), but see also Boyles, Smit, and McKechnie (2011a) and Brigham *et al.* (2011).

All protocols were approved by the University of Regina President's Committee on Animal Care in accordance with guidelines of the American Society of Mammalogists (Gannon *et al.*, 2007) and those of the Canadian Council for Animal Care.

Results

We recovered temperature loggers from 4 individuals. All 4 of the squirrels with loggers (1 male and 3 females) were recaptured during spring trapping sessions, and all were reproductively active. Two of the females yielded data for the winter of 2004–05 (recordings every 3 h from 30 August until 13 May), the male yielded data for the winter of 2005 (hourly recordings from 27 September until 21 December), and the remaining female was monitored for the winter of 2007–08 (records every 2 h from 1 October to 19 May). The low rate of data logger recovery was presumably due to squirrels migrating from the small 1-ha trapping grid and/or mortality. We contend that excessive mortality due to our protocol was unlikely given that we regularly recaptured squirrels with clipped tail fur (2–6 d) after they had been anesthetized to check for loggers and post-surgery health during the course of each fall trapping session. For example, during the spring and fall trapping sessions in 2007, we caught 25 and 15 different individual squirrels a total of 86 and 78 times, respectively, implying that many if not all squirrels were recaptured during trapping sessions and that, at least in the short term, our protocol had relatively little if any effect on animals.

We found no evidence of torpor use by any of the 4 individuals from which we recovered data loggers, which yielded a total of 8190 temperature/time readings during a total of 30 months between October and May of 3 different winters. As shown for a female measured in 2004–05 (Figure 1), T_b fluctuated daily by ~ 1.5 to 2.5 °C.

The T_b showed a curvilinear response over time, with the lowest T_b measured in January and the highest in September and May.

The minimum T_b recorded for any animal was 34.5 °C, a temperature that was recorded on just 3 occasions for a single female (Figure 2). Due to the different measurement intervals and time periods involved, direct statistical comparison is not valid; however, the mean T_b of the 4 individuals varied by only 1.7 °C (37.2–38.9), although the mean T_b s for the male were about 1.5 °C higher than those of the females. In total, nearly 84% (6866 of 8190) of all temperature recordings were between 36.5 and 39.5 °C. There is no apparent difference between individuals in the distribution of T_b measurements (Figure 2). The mean monthly T_b of individual squirrels was a linear function of the mean

monthly T_a ($P < 0.001$), with the exception of the female measured in 2007-08, likely because it was exposed to a narrower T_a range than the other individuals (Figure 3).

Discussion

In contrast to the prediction of our hypothesis, we found no evidence that red squirrels employed anything resembling a torpor bout during 3 different winters in the Cypress Hills. This conclusion is valid even if the loggers drifted by 1–2 °C. Although our sample size of individuals is small, the fact that our data come from long uninterrupted periods of time, for both genders and for 3 different winters, gives us confidence that our conclusion is robust. We acknowledge that physiological traits are variable given varying conditions and locations. For example, a study on

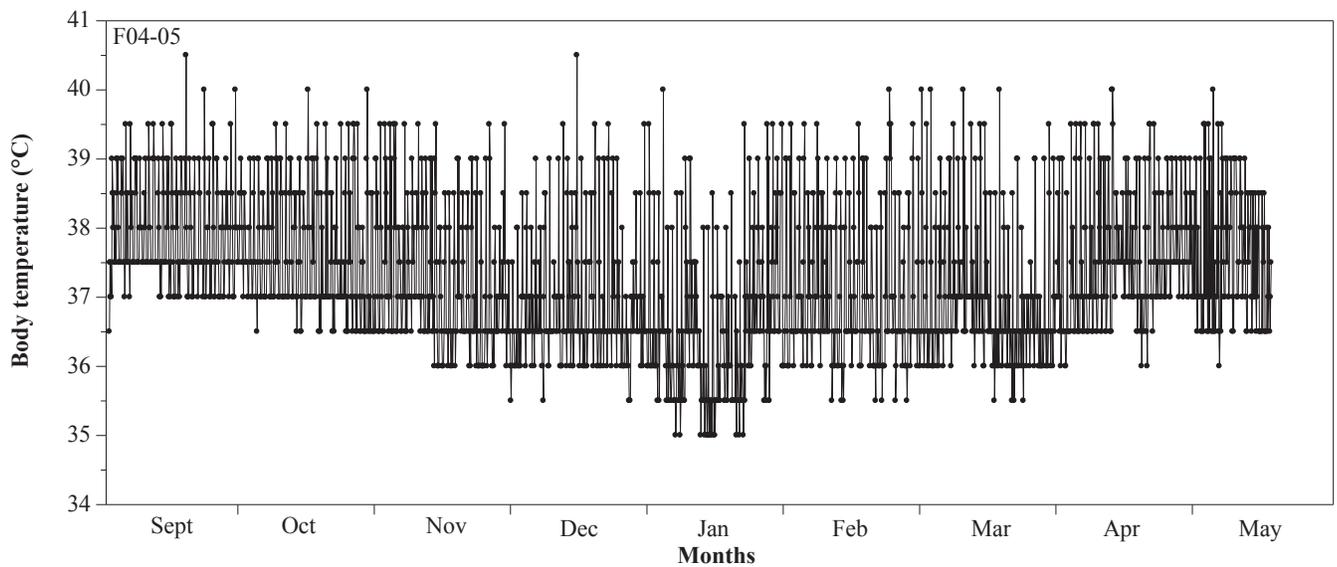


FIGURE 1. T_b measurements as a function of date during the winter of 2004-05 for a female in the Cypress Hills, Saskatchewan.

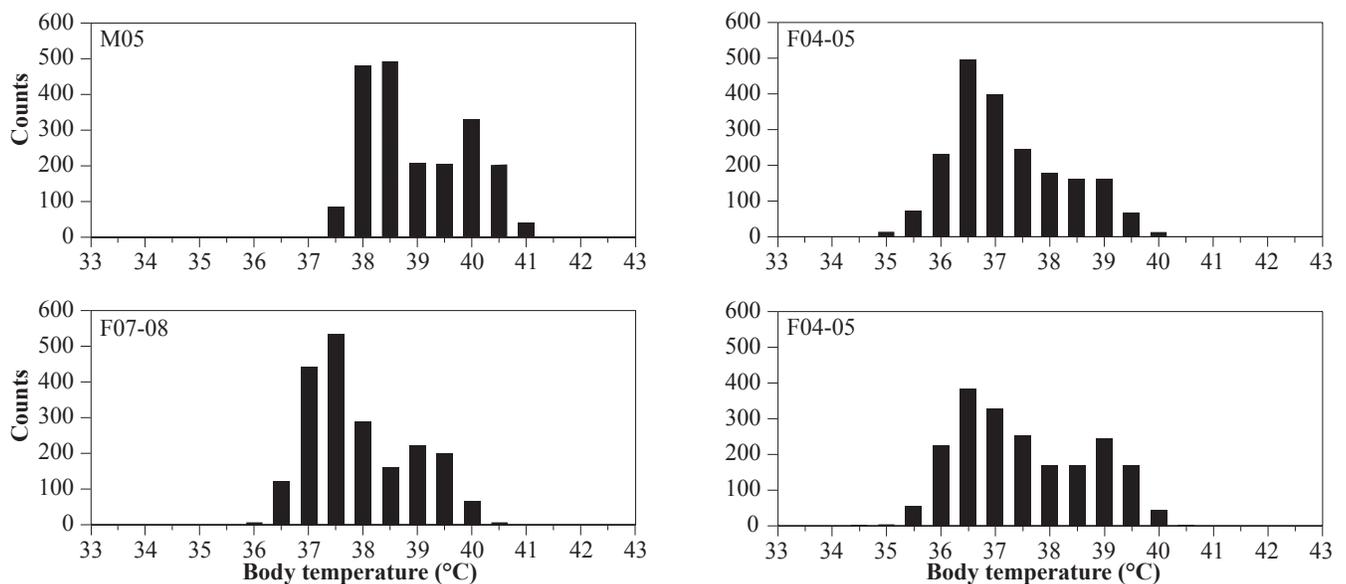


FIGURE 2. Frequency distributions of T_b measurements collected during winter in the Cypress Hills, Saskatchewan for each of 4 red squirrels. Data are equally spread amongst 4 individuals (either 2047 or 2048 measurements for each of 1 male and 3 females) recorded during 3 winters. Panels are labelled with squirrel gender and year of data collection.

African lesser bush babies (*Galago moholi*) suggested that the species was entirely homeothermic (Mzilikazi, Masters & Lovegrove, 2006); however, subsequent work on the same species showed that they can use torpor, although they do so infrequently (Nowack, Mzilikazi & Dausmann, 2010). Thus, our data can only be used to conclude that red squirrels did not use torpor under the natural conditions to which they were exposed; it does not mean that they cannot, but it strongly suggests that torpor is not regularly used anywhere. Given that close relatives of red squirrels (marmots, ground squirrels, and chipmunks) all employ torpor and even hibernate, what remains to be determined is whether or not red squirrels have lost the physiological ability to use torpor or whether they retain the ability but the costs of using it are so high that they do not employ it.

One possibility is that the savings that might accrue to these animals due to their naturally low FMR are simply not enough to warrant using torpor (Figure 4). Our calculations based on the mean monthly minimum for the coldest month, January, suggest a savings of only 18% of RMR would result per h of torpor use, not including the cost of arousal. The ~ 1.5 °C decline of average T_b at low T_a observed for the 2 females measured in 2004-05 would result in a reduction of energy expenditure by about 3%.

The fact that squirrels store food that they access over winter is not a sufficient reason to expect that torpor would not be used. Winter thermoregulation patterns by eastern chipmunks (*Tamias striatus*) provide an interesting context for our data on squirrels. Chipmunks are facultative mammalian hibernators that subsist primarily on stored food and less on the internal fat stores (they do fatten to some extent) that most hibernators rely on during the winter hibernation period (Humphries, Kramer & Thomas, 2003; Landry-Cuerrier *et al.*, 2008). Further, unlike most mammals that hibernate or use torpor, they do not require food deprivation to initiate bouts of torpor. Chipmunks with larger food stores employ torpor less often and reduce T_b to a lesser degree (Humphries, Kramer & Thomas, 2003; Landry-Cuerrier *et al.*, 2008). Thus, food storage does not obviate the need for a benefit to using torpor. For red squirrels, cone crop failure in many years (Smith, 1968) and in many parts of their range means that in some years, food stores may be limited. During the course of our study there was no spruce cone crop failure, but qualitative observations suggest there was some annual variation in cone production.

We employed a highly conservative metric to define torpor, namely a threshold of $T_b < 30$ °C, following Körtner and Geiser (2000; 2009). Levesque and Tattersall (2010) employed a less conservative threshold of 35.5 °C to define heterothermic episodes in eastern chipmunks. Levesque and Tattersall used the calculations of Willis (2007) and respirometry data from 3 chipmunks that entered torpor to derive the threshold value. Assuming the same metabolic patterns and thus a similar temperature threshold for closely related squirrels, we would conclude that 2 different individuals (on 14 and 7 occasions, respectively), had T_b fall below this threshold. However, on only 1 occasion were 2 or more consecutive T_b readings for either individual below the 35.5 °C threshold. Given this and the fact that the measurement error of iButtons is ± 1 °C and it is possible that some drift occurred, we argue that even using a

highly liberal threshold value, we had only 21 measurements from more than 8000 suggesting any type of heterothermic response. Further, only 3 of these 21 measurements were > 0.5 °C below the threshold, making it unreasonable to conclude definitively that heterothermy was used. Boyles, Smit, and McKechnie (2011b) proposed a new metric termed the H-Index for identifying heterothermic responses in endotherms based on a combined assessment of the depth and duration of any decline in T_b . The metric does not employ a threshold T_b value below which torpor is defined to have occurred (see Brigham *et al.*, 2011). However, using Boyles, Smit, and McKechnie's approach would not lead to the conclusion that red squirrels clearly exhibit heterothermy.

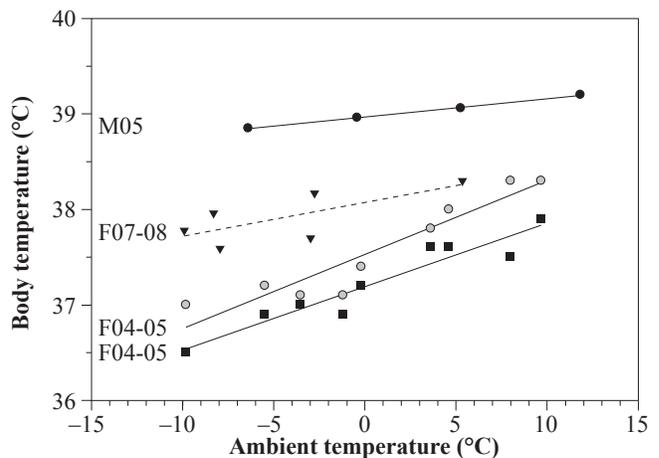


FIGURE 3. Mean monthly T_b as a function of mean monthly T_a of the 4 squirrels measured. All least squares regressions were significant ($P < 0.001$) with the exception of the female in 2007-08 ($P = 0.11$). Equations were Male M05 (black circles): $y = 38.97 + 0.019x$, $r^2 = 0.99$; Female F07-08 (black triangles): $y = 38.05 + 0.031x$, $r^2 = 0.51$; Female F04-05 (grey circles): $y = 37.53 + 0.077x$, $r^2 = 0.88$; Female F04-05 (black squares): $y = 37.19 + 0.066x$, $r^2 = 0.91$.

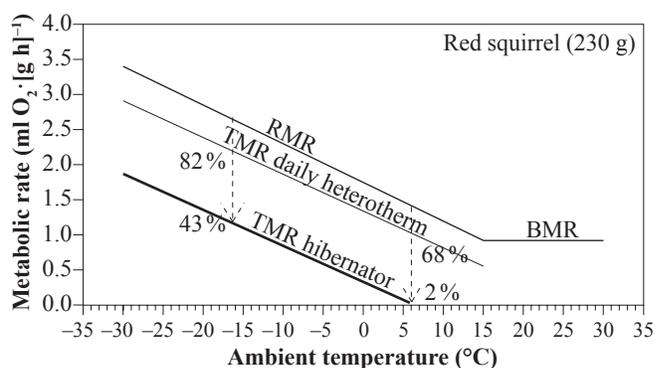


FIGURE 4. Predicted metabolic rate for a 230-g red squirrel over the range of T_a they experience in the Cypress Hills. Basal (BMR) and resting (RMR) metabolic rates were taken from Pauls (1978) and so were the slopes for torpid metabolic rate (TMR) for daily heterotherms and hibernators because thermal conductance in thermoregulating individuals is similar during torpor and normothermia (Geiser, 2004). Minimum T_b for daily heterotherms and hibernators were calculated from Geiser and Ruf (1995). The dotted lines indicate thermo-conforming hibernators. Vertical arrows indicate the percent reduction of TMR at $T_a = -16.5$ °C, the mean minimum T_a in January, and at $T_a = 6$ °C where torpid hibernators begin to thermoregulate on average, in relation to the RMR of daily heterotherms and hibernators.

Red squirrels are highly territorial year round (Steele, 1998), meaning that it is unlikely that significant long-distance migration from the study area took place. However, the fact that we always trapped at the same stations on the same small grid means that small changes in territory area might have reduced our probability of recapturing them. Another potential explanation for the low level of recovery of individuals with loggers is that our protocol contributed to high mortality rates. Although this is possible, Mzilikazi and Lovegrove (2005) found no evidence of a long-term effect of implanting the same loggers in rock elephant shrews (*Elephantulus myurus*) using a similar protocol. We suggest that the large numbers of raptors that eat squirrels and the fact that American marten (*Martes americana*), which are well known to depredate red squirrels and were successfully reintroduced to the area near the time our study commenced, likely contributed to mortality and thus low recovery. Halvorson and Engeman (1983) found that red squirrels rarely survived to be more than 5-y-old in the wild. However, future studies could employ PIT tagging to ensure that the surgical protocol is not responsible for increasing mortality rates.

In conclusion, we found no evidence that red squirrels employed torpor and would thus agree that the 3 reasons proposed by Humphries *et al.* (2005) for low energy use by these animals do not need to be expanded to include heterothermy.

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