Eurasian Red Squirrels Show Little Seasonal Variation in Metabolism in Food-Enriched Habitat

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ABSTRACT

Energy expenditure and ambient temperature (T_a) are intrinsically linked through changes in an animal's metabolic rate. While the nature of this relationship is stable, the breadth of change in thermoregulatory cost varies with body size and physiological acclimatization to season. To explore seasonal metabolic changes of small mammals, we studied a population of Eurasian red squirrels (*Sciurus vulgaris*) in a seminatural environment with a year-round supply of natural and supplemented food. In each season we measured the metabolic rate of wild-caught red squirrels, using open-flow respirometry, and hypothesized that individuals would make adjustments to contend with seasonal weather conditions. In comparison to summer animals, we predicted that winter squirrels would show (1) an increase in metabolic rate within the thermoneutral zone, (2) a decrease in the lower critical temperature of the thermoneutral zone, (3) a shallower slope of resting metabolic rate with decreasing T_a, and (4) lower thermal conductance. Surprisingly, we observed only minor changes in resting metabolic rate, and energetic modeling suggested that the scope of change was unlikely to be of ecological consequence. Hair area density was higher in winter than in summer, corresponding to a slightly elevated thermal conductance in summer, while body mass was reasonably constant year-round. We conclude that the scope of physiological seasonal adaptation is minimal when food is abundant and that squirrels instead rely on adjustments in activity to reduce exposure to low T_a. We suggest that this may explain the squirrel's success in a wide range of habitats, including urban areas, which require a rapid and flexible response to environmental changes and may indicate the capacity of other small mammal species to cope with environmental disturbance.

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Introduction

The energetic costs of small endotherms are directly linked to ambient temperature (T_a). These animals must contend with changes in T_a, and therefore metabolic rate (MR), on timescales ranging from seconds (e.g., leaving a nest) to years (seasonal and annual climatic trends). This translates into involuntary adjustments in the amount of endogenous heat production required for physiological thermoregulation and the maintenance of an elevated body temperature (T_b) independent of T_a. This expense is lowest within a narrow T_a range (the thermoneutral zone [TNZ]) that varies among species, within species inhabiting different climatic zones, and seasonally within individuals (Lovegrove 2005). Resting MR (RMR) increases linearly with decreasing T_a below the TNZ because the cost of thermoregulation increases as the difference between T_b and T_a becomes larger. Similarly, RMR increases rapidly above the TNZ as animals actively dump heat to avoid hyperthermia. This is a dynamic relationship, and metabolism varies in response to physiological status; for example, digestion, lactation, locomotion, and stress all influence MR, and their effects are compounded by T_a (Kleiber 1961; Hammond and Diamond 1992; Speakman 1999; Withers et al. 2016). Winter is empirically more expensive than summer, particularly for smaller animals with proportionally high rates of heat loss, requiring many species to undergo seasonal acclimatization to manage energy budgets.

Small, nonmigratory mammals have an array of strategies available for decreasing energetic demands and surviving the winter/leaner season. Torpor is the most powerful tool for reducing MR but is restricted to heterothermic species (Ruf and Geiser 2015). Other strategies include reducing body mass and/or size, nest sharing and huddling, using insulated nesting sites, basking, peripheral vasoconstriction, increasing fur density, decreasing normothermic T_b and increasing thermolability, increasing thermogenic capacity, shortening activity periods, and food storage (Pauls 1981; Heldmaier 1989; McNab 2002; Humphries et al. 2005; Lovegrove 2005; Zhao and Wang 2005; Warnecke et al. 2010; Withers et al. 2012, 2016; Marchand 2014).

Body mass can show large seasonal variation in some small-mammal species, while others are able to maintain a stable body mass year-round and instead rely on changes in body composition and thermogenic capacity to cope with varying environmental conditions. For example, cold-acclimated desert ham-

sters (*Phodopus roborovskii*) maintained their body mass at the same level as warm-acclimated animals by increasing basal MR (MR within the TNZ [BMR]), nonshivering thermogenesis capacity, gut mass, and food intake while decreasing T_b and fat mass (Chi and Wang 2011). Similarly, plateau pikas (*Ochotona curzoniae*) increased brown adipose tissue (but not total fat mass), mitochondrial protein, cytochrome *c* oxidase, and UCP1 expression in winter (Wang et al. 2006). Metabolic capacity (i.e., maximum MR in relation to cold exposure) can also increase in winter compared to that in summer, with or without concomitant changes in BMR (Heldmaier et al. 1986; Feist and White 1989; Holloway and Geiser 2001).

The Eurasian red squirrel *Sciurus vulgaris* (hereafter "squirrel") is a strictly homeothermic, small-bodied mammal species with an extremely wide distribution that encompasses a range of climatic zones from Nearctic to temperate to Mediterranean. While it usually inhabits closed coniferous forest with abundant tree seeds, it is also common in urban areas (Bosch and Lurz 2012). Like other arboreal squirrels, it has never been observed using torpor (Brigham and Geiser 2012; Dausmann et al. 2013), and it compensates for the colder winter months by reducing activity (Tonkin 1983; Humphries et al. 2005), spending more time in insulated dreys, and consuming food scatter-hoarded in autumn. Previous studies in its natural habitat found that its body mass peaks in winter and is lowest in summer, when little natural food is available in the landscape (reviewed in Bosch and Lurz 2012), similar to other tree squirrel species (e.g., Short and Duke 1971; Dubock 1979; Koprowski 2005). Thus, it shows a body mass cycle that does not correspond to physiological strategies used to endure seasonal T_a patterns: it does not store energy by fattening before winter, nor does it reduce overall body mass to save energy. Whether this species expresses seasonal changes in its metabolic physiology is unknown, yet this information is crucial for understanding its survival strategies in response to climatic variation. Therefore, we studied individuals inhabiting a large, forested cemetery where the amount of food energy available is relatively high and stable year-round (Reher et al. 2016) but large interseasonal differences in T_a occur. We hypothesized that squirrels would make adjustments in their metabolism to contend with changing weather conditions over the course of the year. Specifically, we predicted that the cost of maintaining homeothermy at colder T_a would be lower in cold-acclimated (winter) than in warm-acclimated (summer) animals. In comparison to summer animals, we expected winter squirrels to show (1) an increase in RMR within the TNZ, (2) a decrease in the lower critical temperature of the TNZ (T_{lc}), (3) a shallower slope of RMR with decreasing T_a, and (4) lower thermal conductance.

Methods

Study Site and Animals

This study was conducted in four seasons in 2014: winter (January), spring (April), summer (July), and autumn (October) in Ohlsdorf Cemetery, Hamburg, Germany (53°37.393′N, 10°3.516′E). Ohlsdorf is a 391-ha cemetery densely forested in parts with Douglas fir *Pseudotsuga menziesii*, Norway spruce *Picea abies*, European larch *Larix decidua*, and beech *Fagus sylvatica* and contains 17 km of roads and more than 280,000 burial sites. It also contains hundreds of bird feeders that are supplemented year-round by visitors. Squirrels were captured with cage traps (20 cm × 20 cm × 50 cm; Tomahawk Live Trap, Hazelhurst, WI) placed in bushes or trees and baited with hazelnuts, walnuts, sunflower seeds, peanut butter, and apple. Traps were opened in the early morning and checked every 2 h. Trapped individuals were transferred to a handling cone (adapted from Koprowski 2002), weighed, sexed, and implanted with subcutaneous transponders for individual identification (ID-100B; Trovan, Hessle, UK), and nonreproductive adults of both sexes were transported to the University of Hamburg ($n = 7$ in each season). Squirrels were kept in individual outdoor aviaries (4.4–7.2 m² floor area and 2.3–2.7 m in height) to maintain exposure to natural photoperiod and T_a. Cages contained a partial roof and branches to climb and had a leaf/woodchip substrate, and each squirrel had access to a single insulated, wooden nest box. Squirrels were fed daily with whole and shelled nuts, seeds, and apple and were provided with water.

Respirometry

Open-flow respirometry measurements were conducted inside a darkened climate chamber (Type WK; Weiss Umwelttechnik, Reiskirchen-Lindenstruth, Germany) set to T_a's of 0°, 10°, 20°, 25°, 30° (all seasons), and 33°C (winter excluded). During winter, subzero outside T_a allowed additional measurements at approximately −7° and −2°C, which were made by placing respirometry chambers in the outdoor aviaries. Each squirrel was allowed to become habituated to captive conditions for at least 48 h before the first measurement commenced. All measurements were made during the rest phase (Bosch and Lurz 2012), beginning at 17:25 ± 37 min, and ran for a duration of 5.4 ± 0.5 h ($T_a = 0$°–30°C) or 3.4 ± 0.1 h ($T_a = 33$°C); the O_2 consumption measurement duration required for small mammals to reach low and stable BMR is at least 4.3 h (Cooper and Withers 2009). Individual squirrels were transferred from their nest boxes via pillowslip to a 10.8-L plastic respirometry chamber. All individuals settled almost immediately and were monitored for well-being during each measurement under infrared light with a modified webcam (c170, Logitech Europe, Lausanne, Switzerland). Room air dried with silica gel was pulled through the animal chamber at a flow rate (FR) of ~100 L h⁻¹ (standard temperature and pressure, dry [STPD]). Incurrent and excurrent ports were located at opposite ends of the rectangular chamber, offset diagonally and vertically, and were covered with plastic mesh to facilitate air mixing. Excurrent air was dried with silica gel and then analyzed for O_2 content with an OxBox field oxygen analyzer (T. Ruf and T. Paumann, University of Veterinary Medicine, Vienna; see Fietz et al. 2010) that incorporated an electrochemical fuel-cell O_2 sensor (7OX-V CiTicel, City Technology, Portsmouth, UK). The OxBox was programmed to alternate between 55 min of sample air and 5 min of reference air and to record a data point every 10 s. The OxBox gas analyzer was

calibrated before each season with room air and N_2 mixed with a gas-mixing pump (type 2KM300/a, H. Wösthoff Messtechnik, Bochum, Germany). The OxBox flow meter was calibrated during manufacture against a certified mass flow meter (Type 358-11, 0–2 L min^{-1}, Analyt-MTC, Müllheim, Germany) corrected to STPD. T_a was measured in the excurrent air immediately after the respirometry chamber with a temperature-sensitive data logger (DS1922L-F5 Thermochron iButton, Maxim Integrated Products, San Jose, CA).

O_2 traces (in mV) were corrected for drift, using every reference air measurement within a measurement run, with Clampfit, v10.3.1.4 (Molecular Devices, Sunnyvale, CA). The rate of O_2 consumption ($\dot{V}_{O_2}$; mL O_2 g^{-1} h^{-1}) during 13-min periods where values were minimal and steady (variation < 10%) was calculated after Heldmaier and Steinlechner (1981) with the equation $\dot{V}_{O_2} = 10 \times FR(L\,h^{-1}) \times \Delta vol\%\,O_2$/body mass (g), where FR is converted to STPD and $\Delta vol\%\,O_2$ is the difference in $[O_2]$ between animal and room air, assuming a respiratory exchange ratio of 0.85. Each value was corrected for the presence of exhaled CO_2 in the airstream by adding $\dot{V}_{O_2} \times 0.0818$, which is a constant provided by the manufacturer of the O_2 measurement unit (Gasmonitor O2-25; Bieler and Lang, Achern, Germany). Squirrels were weighed immediately before and after measurements, and a linear rate of mass loss was assumed for $\dot{V}_{O_2}$ calculations.

Measurements of BMR require postabsorptive, nonreproductive, inactive, adult individuals during the rest phase at T_a within the TNZ. Because some squirrels stored food inside their nest boxes, we are unable to say for certain that all individuals were postabsorptive; therefore, we do not present BMR measurements and instead describe RMR (i.e., MR of normothermic animals that do not fulfil the requirements of BMR), both within and below the TNZ. The lowest RMR for each individual within the TNZ (see below) was termed "RMR$_{TNZ}$" and the corresponding T_a value "T_a at RMR$_{TNZ}$." Calculations of the linear relationship between RMR and T_a for each season were made by using only values below the T_{lc} of the TNZ. The RMR$_{TNZ}$ for each season was compared to the BMR predicted by a rodent-specific allometric curve presented by Withers et al. (2016). Wet thermal conductance (C_{wet}) was calculated as $\dot{V}_{O_2}/(T_b - T_a)$ with mean daily T_b data published by Dausmann et al. (2013) averaged for the time period corresponding to our MR measurements for each season. Standard C_{wet} (i.e., at T_a at RMR$_{TNZ}$) values were compared to those predicted by Aschoff (1981).

Hair Area Density and Ambient Temperature

At the conclusion of each seasonal measurement period ($\sim$3 wk), squirrels were released at their point of capture. In winter and summer, a small patch of fur (0.5 ± 0.2 cm²; $n = 11$, $N = 12$) was shaved from the right flank of each individual before release and weighed to the nearest 0.1 mg for a hair area density analysis. The T_a in Ohlsdorf Cemetery and the outdoor aviaries at the University of Hamburg was measured every 30 min with iButtons placed 1 m above the ground in the shade.

Statistical Analysis

The TNZ was determined with linear mixed-effects models for each season, with the dependent variable "RMR," factors "Set T_a" and "Season," and random factor "Individual," identifying the range of high T_a within which RMR did not differ significantly among T_a steps (i.e., $P > 0.05$; Tukey's post hoc comparisons). Similarly, the difference in C_{wet} among T_a steps was determined, with C_{wet} as the dependent variable. This method determines the deviance of each individual from the common regression, adjusting for repeated measures and thereby taking individuals into account. The intercept of the fitted line below the TNZ and the line within the TNZ with a slope of 0 (i.e., average RMR within the TNZ) was taken as the T_{lc}. Body mass at the start of each measurement was included as a factor in each seasonal model, but because it reduced the model fit, based on the Akaike information criterion, it was subsequently excluded from analyses (results not shown). We used the packages nlme (mixed models; Pinheiro et al. 2017) and lsmeans (Tukey's post hoc comparisons; Lenth 2016) in R (R Core Team 2017).

Further analyses were conducted with statistiXL v1.9 and SPSS v21. Univariate general linear models with Tukey's post hoc tests were used to compare body mass, minimum and maximum outside T_a, RMR$_{TNZ}$, and T_a at RMR$_{TNZ}$ among seasons. The relationships between RMR and T_a and between C_{wet} and T_a below the TNZ for each individual in each season were examined with ordinary least squares regression, and slopes and intercepts of RMR-T_a and C_{wet}-T_a curves were compared among seasons with ANCOVA. A t-test was used to compare hair area density between summer and winter. Sexes were not compared because of restricted sample sizes. We present means $\pm$ SD; n indicates the number of individuals and N the number of measurements.

Results

Ambient Temperature

Average daily maximum and minimum outside T_a during the study period and the preceding 30 d (allowing for seasonal acclimatization) differed among all seasons (maximum: $F_{3, 24} = 35.28$, $P < 0.001$; minimum: $F_{3, 24} = 28.26$, $P < 0.001$; fig. 1), except for average minimum T_a in winter and spring ($P = 0.105$; fig. 1).

Body Mass and Hair Area Density

Average body mass at the time of capture was 363.2 ± 22.0 g and did not differ among seasons ($F_{3, 25} = 0.42$, $P = 0.739$, $n = 26$). Hair area density was higher in winter (34.2 ± 16.8 mg cm^{-2}, $n = 6$) than in summer (14.8 ± 7.1 mg cm^{-2}, $n = 6$) animals ($t_{6.7} = 2.60$, $P = 0.035$).

Metabolic Physiology

The T_{lc} of the TNZ was 24.5°, 31.7°, 29.8°, and 28.0°C in winter, spring, summer, and autumn, respectively. The T_a at RMR$_{TNZ}$ differed among seasons ($F_{3, 25} = 6.82$, $P = 0.002$): the winter value ($28.1° \pm 2.4°$C) was lower than the autumn
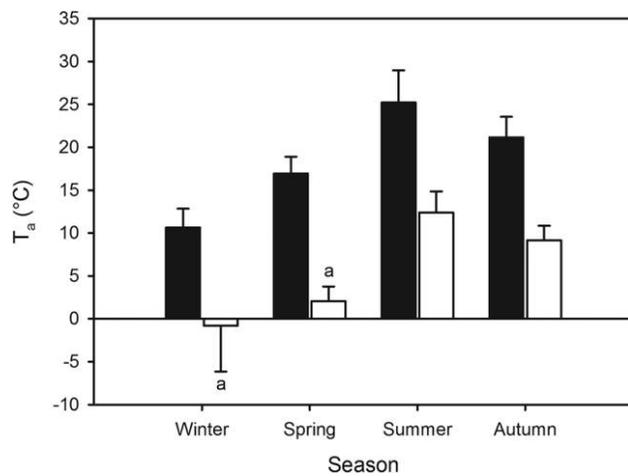
Figure 1. Average daily maximum (filled bars) and minimum (open bars) outside ambient temperature $\pm$ SD (T_a) measured during each seasonal respirometry session, and for the 30 preceding days, in a seminatural habitat in Hamburg, Germany, in 2014. Columns with the same letter did not differ significantly from one another.

(31.8° $\pm$ 1.7°C; $P = 0.019$) and summer (33.6° $\pm$ 1.4°C; $P = 0.002$) values, but that for spring did not differ from that for any other season (30.8° $\pm$ 2.5°C; $P \geq 0.125$), and the summer and autumn values did not differ ($P = 0.503$; fig. 2). Changes in RMR$_{TNZ}$ reflected the changes in T_a at RMR$_{TNZ}$. There was an overall seasonal effect on RMR$_{TNZ}$ ($F_{3,25} = 6.86, P = 0.002$); the winter RMR$_{TNZ}$ (0.97 $\pm$ 0.06 mL O$_2$ g^{-1} h^{-1}) was higher than the spring (0.75 $\pm$ 0.12 mL O$_2$ g^{-1} h^{-1}; $P = 0.003$) and summer (0.76 $\pm$ 0.02 mL O$_2$ g^{-1} h^{-1}; $P = 0.010$) values, but the summer and spring RMR$_{TNZ}$ did not differ from each other ($P = 0.997$), and the autumn RMR$_{TNZ}$ (0.87 $\pm$ 0.13 mL O$_2$ g^{-1} h^{-1}) did not differ from that of any other season ($P \geq 0.141$; fig. 2). The RMR$_{TNZ}$ values for each season were higher than predicted (110%–142% of predicted).

RMR was affected by T_a ($F_{7,119} = 143.27, P < 0.001$) but not by season ($F_{3,119} = 0.94, P = 0.425$; all seasonal comparisons: $P \geq 0.386$). RMR increased linearly as T_a decreased below the TNZ (table 1; fig. 3). Including the two subzero T_a's into the winter curve did not change the significance of the model and improved the fit ($R^2 = 0.82$ vs. 0.73), so we present these data hereafter. The model comparing slopes was nonsignificant ($F_{3,89} = 2.70, P = 0.051$), with a common slope of -0.042. No common intercept was found ($F_{3,92} = 4.18, P = 0.008$), and the intercept for summer was higher than both the winter ($P = 0.018$; summer intercept was 117% of winter intercept) and spring ($P = 0.010$; 124%) intercepts.

C_{wet} was affected by T_a ($F_{7,119} = 239.90, P < 0.001$) but not by season ($F_{3,119} = 2.47, P = 0.065$), although C$_{wet}$ in summer was slightly higher than that in spring ($P = 0.046$; all other seasonal comparisons: $P \geq 0.366$). Below the TNZ, C_{wet} increased slightly with T_a in each season (table 1; fig. 4). The model comparing slopes was nonsignificant ($F_{3,89} = 2.04, P = 0.114$), with a common slope of 0.018. No common intercept was found ($F_{3,92} = 2.97, P = 0.036$), and the intercept for summer was higher than that for spring ($P = 0.035$; 132%). Standard

C_{wet} values were as follows: 2.01, 2.17, 3.61, and 2.77 J g^{-1} h^{-1} °C^{-1} in winter, spring, summer, and autumn, respectively, which were between 37.4% and 67.2% of the predicted values.

Discussion

Squirrels showed only a minimal physiological response to season via small changes in metabolism and conductance. Nevertheless, they were able to maintain a high body mass and positive energy balance year-round despite living in a climate with large seasonal changes in T_a. We suggest that the small scale of the responses likely reflects the higher-than-average food energy availability of their seminatural habitat (compared to the energy available during an average crop year in native habitat; Bosch and Lurz 2012; Reher et al. 2016) due to diverse food trees, bird and squirrel food supplemented by cemetery visitors, and the modification of activity patterns. Our results highlight the importance of considering behavior and habitat when interpreting the physiological adaptations that a small mammal species uses to cope with seasonality, particularly when a heterothermic response is unavailable.

Our study animals showed stable body masses year-round. In fact, the mass of nonreproductive adults trapped in
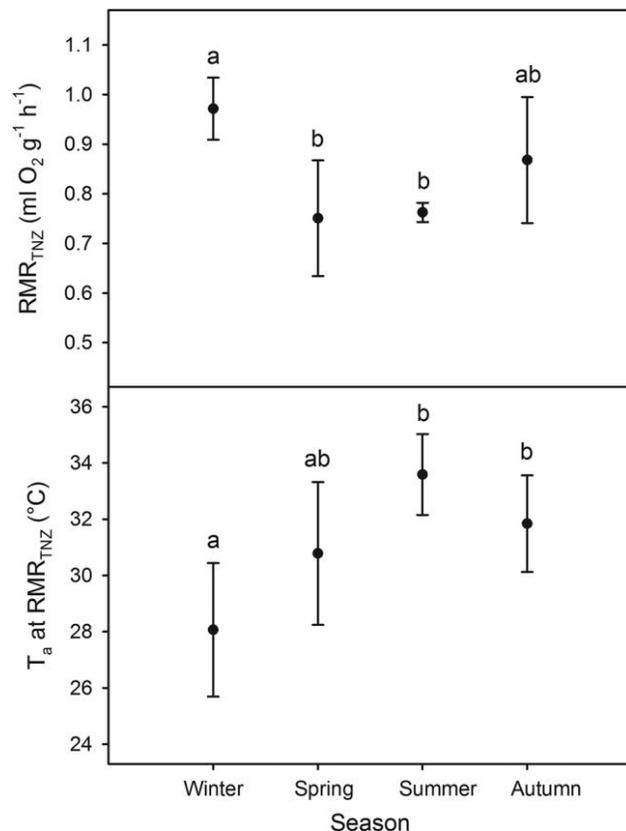

Figure 2. Lowest average resting metabolic rate $\pm$ SD (RMR$_{TNZ}$; *top*) for red squirrels from seminatural habitat in Hamburg, Germany, and corresponding average ambient temperature $\pm$ SD (T_a at RMR$_{TNZ}$; *bottom*), in each season of 2014. Differing letters above points indicate significant differences among seasons.

Table 1: Linear regression equation details and statistical results describing the response of RMR and C_{wet} to T_a below the TNZ for each season in 2014 for red squirrels in seminatural habitat in Hamburg, Germany

Season	n (N)	Slope	Intercept	R^2	F (df)	P
RMR:						
Winter	6 (24)	−.040	2.043[B]	.82	96.80 (1, 22)	<.001
Spring	7 (26)	−.034	1.926[B]	.71	59.67 (1, 24)	<.001
Summer	5 (19)	−.051	2.389[A]	.80	66.17 (1, 17)	<.001
Autumn	7 (25)	−.047	2.217[AB]	.90	198.16 (1, 23)	<.001
C_{wet}:						
Winter	6 (24)	.014	1.077[AB]	.52	23.84 (1, 22)	<.001
Spring	7 (26)	.026	.918[B]	.59	33.82 (1, 24)	<.001
Summer	5 (19)	.016	1.213[A]	.45	13.77 (1, 17)	.002
Autumn	7 (25)	.019	1.093[AB]	.61	35.52 (1, 23)	<.001

Note. Superscript letters indicate differences in intercept among seasons. C_{wet} = wet thermal conductance; RMR = resting metabolic rate; T_a = ambient temperature; TNZ = thermoneutral zone.

Ohlsdorf Cemetery did not differ among seasons or between sexes over a period of 4 yr (361.2 ± 26.6 g; $F_{4, 136}$ = 1.28, P = 0.282 [general linear model], n = 50, N = 137; J. M. Turner, S. Reher, L. Warnecke, and K. H. Dausmann, unpublished data). This contradicts most previous studies, which report a peak in late winter and a minimum in late summer, with slight differences among habitat types (Tonkin 1983; Wauters and Dhondt 1989; but see Lurz and Lloyd 2000), similar to other species of Holarctic tree squirrel (e.g., Dubock 1979; Knee 1983; Koprowski 2005). Red squirrels evolved to live in a seasonal environment characterized by annual changes in T_a that affect food availability and drive the body mass cycles seen in natural habitat (Wauters and Dhondt 1989; Bosch and Lurz 2012). Therefore, the high and stable mass of animals in our seminatural study site likely indicates that they can take advantage of the abundant and predictable food resources, which were as

high as 36 kg wk^{-1}, even during the normally leaner seasons (Lurz and Lloyd 2000; Reher et al. 2016). Indeed, in the year before this work the mass of food resources in the cemetery changed seasonally, but food energy availability (including both the natural seed crop and supplemented food in bird feeders) did not. It also appeared that squirrels modified their movement patterns to maximize energy intake and minimize energy expenditure and that home ranges were, on average, smaller than those reported for squirrels in native habitat (Reher et al. 2016). Combined with activity pattern alteration to avoid cold stress (Tonkin 1983; Rong et al. 2009), the abundant food would have ensured a high and stable body mass and resulted in the low seasonal variation in RMR that we observed. It would be of interest in future studies to examine body composition in squirrels to see whether fat and muscle content changes seasonally, which may help clarify the patterns we observed.

Squirrels molt every year in spring and autumn (Raspopov and Isakov 1980). It is possible that the higher RMR intercept in summer was related to reduced insulation (e.g., if squirrels lost their denser coat between the spring and summer measurements, this could result in the higher RMR intercept below the TNZ in summer animals that we observed), given that their body mass remained constant (Innes and Lavigne 1979; Heldmaier 1989; Steudel et al. 1994) and hair area density changed. Although insulation year-round was high compared to the predicted value for placental mammals, we found that conductance was slightly higher in summer than in spring, indicating a change in insulation. While this may allow the squirrels in our study to dump excess heat in summer, it could also indicate an ability to take advantage of solar radiation and rapidly uptake heat during colder times of the year (Cooper and Withers 2012). Degus (*Octodon degus*) show no change in BMR or body mass among seasons, but their activity patterns, which are similar to those of squirrels, are related to sun exposure and allow them to live under near-thermoneutral conditions in winter (Bozinovic et al. 2004). Also, our observed magnitude of change was small (RMR of summer animals at 0°C was only 121% that of spring animals), which led us to question whether these differences are meaningful within an ecological framework. For an approxi-
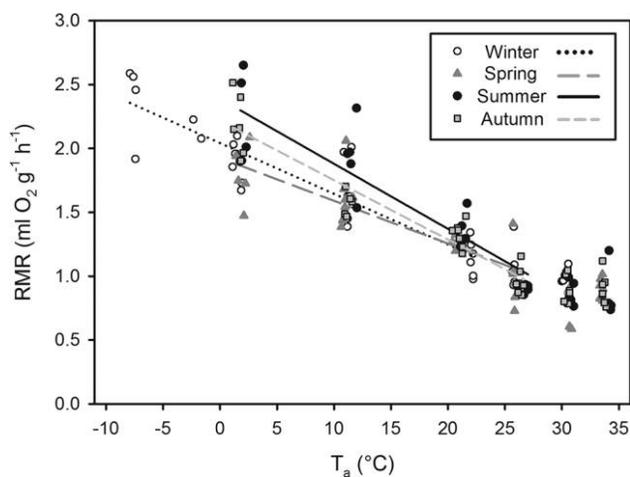

Figure 3. Metabolic rate for red squirrels from seminatural habitat in Hamburg, Germany, at each experimental ambient temperature (T_a) for each season in 2014. The relationships between resting metabolic rate (RMR) and T_a below the thermoneutral zone in winter (open circles, dotted line), spring (triangles, dashed line), summer (filled circles, solid line), and autumn (squares, dashed line) are shown by significant linear regressions (see table 1 and text for details).
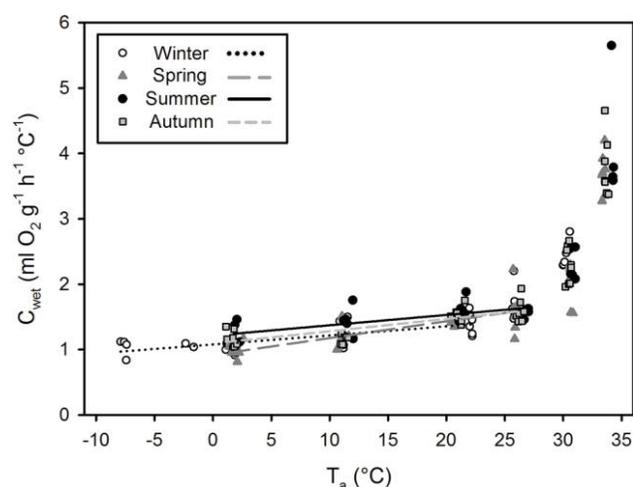
Figure 4. Wet thermal conductance (C_{wet}) for red squirrels from seminatural habitat in Hamburg, Germany, at each experimental ambient temperature (T_a) for each season (winter: open circles, dotted line; spring: triangles, dashed line; summer: filled circles, solid line; and autumn: squares, dashed line) in 2014. Lines represent significant linear regressions for data below the thermoneutral zone (see table 1 and text for details).

mate estimation, at $T_a = 0°C$ the daily energy expenditure (DEE) of spring squirrels equates to 315.1 kJ, versus 385.1 kJ for summer squirrels (the two most divergent seasons for our animals), assuming that all animals were at rest at this T_a for 24 h (1 L O_2 = 20.083 kJ; Schmidt-Nielsen 1997). If we include 8.0 h for foraging and traveling activity in spring and 9.9 h in summer (Tonkin 1983), at active MRs of 3.7 and 4.0 mL O_2 g^{-1} h^{-1} (trotting speed of 0.81 m s^{-1}, after Flaherty et al. 2010; active MR calculated with eq. [4] in Wunder and Morrison 1974), DEE increases to 425.4 and 513.1 kJ, respectively. Therefore, the difference in DEE between the seasons is only 87.7 kJ. This equates to the energy contained in 16–31 beech seeds (Grodziński and Sawicka-Kapusta 1970), which would require the squirrels to spend an extra 13 min per day processing food in summer, compared to spring (Wauters et al. 1992). If the squirrels ate sunflower seeds (readily available in bird feeders) instead of beech seeds, processing time would be even faster (Makowska and Kramer 2007). In our study site of approximately 33 ha, there is an average of 27,203 and 11,332 kJ of food energy available on any given day in spring and summer, respectively (Reher et al. 2016). Therefore, because there are few diurnal predators in the site to make increased traveling time to food sources disadvantageous, we conclude that the small difference in metabolism among seasons could be easily accommodated by the large amount of food available in the landscape, having little impact on the squirrels' ecology.

In the only published work we are aware of examining red squirrel energetics, Bryce and colleagues (2001) found that free-ranging animals inhabiting a mostly coniferous forest in Scotland had a higher DEE (measured using doubly labeled water) in spring (389.8 kJ d^{-1}) than in autumn (313.5 kJ d^{-1}). For an illustrative comparison, we extrapolated our data to calculate DEE by using the average daily T_a for each season,

the corresponding average RMR curves (table 1), and activity patterns determined as described above and found the same relationship with similar values (360.4 kJ d^{-1} in spring vs. 319.1 kJ d^{-1} in autumn). This pattern is perhaps indicative of a congruent seasonal response in metabolism and activity of squirrels from seminatural and natural habitat. In a review of North American Holarctic tree squirrel energetics for three species (*Sciurus carolinensis*, 301–619 g; *Sciurus aberti*, 624 g; and *Tamiasciurus hudsonicus*, 165–344 g), Reynolds (1985) showed that mass-specific BMR ranged from 1.03 to 1.5 mL O_2 g^{-1} h^{-1} for winter animals and from 0.72 to 1.7 mL O_2 g^{-1} h^{-1} for summer animals, similar to our measured RMR_{TNZ} values (winter: 0.97 mL O_2 g^{-1} h^{-1}, summer: 0.76 mL O_2 g^{-1} h^{-1}). Our estimations of the T_{lc} are on the upper end of the values listed in Reynolds (1985; 15°–25°C in winter vs. 20°–25°C in summer) and have a larger seasonal change. The slope of the RMR curves below the TNZ presented in figure 3 by Reynolds (1985) was also qualitatively higher: −0.054 to −0.085 versus −0.040 (our study) in winter and −0.51 to −0.085 versus −0.051 (our study) in summer. Taken together with the lower-than-predicted C_{wet}, we suggest that the Eurasian red squirrel is better adapted physiologically to withstand colder T_a, given that the slope of its RMR in summer was shallower than that of the lowest of the North American species' range in winter, indicating lower thermoregulatory costs.

Besides modification of activity, postural adjustments are likely to contribute significantly to the squirrels' heat exchange dynamics and costs of endogenous heat production. At low T_a, animals reduced their body surface area exposed to the air by sitting on their feet, adopting a hunched posture with their nose between their legs and laying their tails over their backs; at high T_a, animals lay down with their limbs outstretched and held their tails away from their bodies in an apparent attempt to dissipate heat (this latter behavior would have specifically increased dry thermal conductance; Withers and Cooper 2009). The relationship between C_{wet} and MR is evidence that in red squirrels these variables are inherently linked via behavioral thermoregulation. While changes in physical features that reduce conductance, such as increased hair density or subcutaneous fat deposits, take weeks or months to develop, behavioral thermoregulation can provide an instant response to prevailing conditions and therefore likely plays a major role in maintaining a positive energy balance. Indeed, squirrels reduce the duration of activity periods with decreasing T_a (Pulliainen 1973; Tonkin 1983; Wauters et al. 1992; Rong et al. 2009; Dausmann et al. 2013), utilizing insulated nests that can maintain temperatures up to 30°C above T_a when a squirrel is inside (Pulliainen 1973; Raspopov and Isakov 1980; although this heat is rapidly lost from a nest when the squirrel departs), and this behavioral flexibility is likely vitally important for minimizing energy expenditure and consequently survival (Reynolds 1985). When squirrels were in their nests in winter, they were presumably resting at a nest temperature close to the T_a at RMR_{TNZ}; Pauls (1981) also found that when *T. hudsonicus* were in nests, the T_{lc} was effectively decreased by a further 20°C and that MR remained only slightly above basal levels down to a T_a of −20°C. The additional cost of

thermoregulation at thermoneutrality of winter squirrels was similar to that of other species (e.g., *S. carolinensis*; Innes and Lavigne 1979) and likely mitigated by the high food-energy availability at the site (Reher et al. 2016).

Red squirrels are homeothermic, but small daily and seasonal changes in T_b occur that may convey some further energy savings (Dausmann et al. 2013). For example, Pereira et al. (2002) found that the circadian T_b oscillations of captive *S. carolinensis* dropped to lower levels at night in winter than during summer, indicating a possible energy-saving mechanism via a decrease in thermoregulatory costs. This finding is similar to, but slightly larger in scope than, those for North American red squirrels (*T. hudsonicus*; Brigham and Geiser 2012) and Eurasian red squirrels (Dausmann et al. 2013). To save energy, numbats decreased their T_b (and BMR) in summer in response to limited food availability (Cooper and Withers 2012).

Generally, tree squirrels cache food for winter, thereby making it the season with the highest food availability, despite decreased T_a. In turn, the decrease in RMR_{TNZ} in summer is a response not only to higher T_a but also to seasonal food paucity; a smaller body mass in summer in squirrels in native habitat would also decrease energy requirements. However, squirrels in urban or seminatural environments, such as those in our study, can rely on supplementary food year-round. Therefore, they did not rely on seasonal changes in metabolism or body mass. Nevertheless, the T_a at RMR_{TNZ} was higher in summer than in winter, to reduce the costs of exposure to high T_a. The risk of an energy deficit caused by seasonal changes in T_a was offset by only minor changes in metabolic physiology, indicating the importance of behavioral thermoregulation for this species. Squirrels in our site and tree squirrels in general appear well equipped to deal with extremes in low T_a common in their native habitats and would rarely experience T_a high enough to cause hyperthermia. In the face of a warming climate, with a predicted increase in heat wave events (Meehl and Tebaldi 2004), investigations into the ability of squirrels to contend with exposure to high T_a—which is pertinent, given their high insulation—will likely aid predictions of their future habitat requirements and distribution. In light of dramatic population decreases in parts of Europe caused by invasive species and disease, these data would be highly useful for informing strategies aimed at species conservation.

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