

Seasonal torpor and normothermic energy metabolism in the Eastern chipmunk (*Tamias striatus*)

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Abstract To assess the changes in thermoregulatory characteristics that accompany the seasonal expression of torpor we measured seasonal differences in body mass adjustments, body temperature (T_b) and metabolic rate (MR) in both summer- and winter-acclimated individuals from a species of food-storing hibernator, the Eastern chipmunk (*Tamias striatus*). Torpor occurred only in the winter and was associated with lower normothermic T_b , during inter-bout arousal periods than in the summer. Chipmunks increased body mass before the initiation of torpor in winter, and steadily lost mass as the hibernation season progressed. Torpor expression was correlated to initial mass gain, with the individuals who showed the largest mass increase in the fall showing the highest degree of torpor. Acclimation to winter-like conditions produced a decline in normothermic MR at all ambient temperatures examined. The findings indicate that torpor expression is accompanied by a decrease in T_b and MR during normothermy, indicating that a conservation of energy metabolism occurs, not only in torpor, but also during the inter-bout arousal periods.

Keywords Seasonality · Cold-acclimation · Metabolism · Hibernator · Torpor · Body temperature · Acclimatisation

Abbreviations

C_{Wet} Wet thermal conductance ($\text{W}^\circ\text{C}^{-1} \text{kg}^{-1}$)
 C_{Dry} Dry thermal conductance ($\text{W}^\circ\text{C}^{-1} \text{kg}^{-1}$)

E Evaporative cooling (W kg^{-1})
 MR Metabolic rate (W kg^{-1})
 BMR Basal metabolic rate (W kg^{-1})
 EWL Rate of evaporative water loss ($\text{mg kg}^{-1} \text{h}^{-1}$)
 f_R Breathing rate (min^{-1})
 T_a Ambient temperature (chamber temperature)
 T_b Core body temperature
 $\dot{V}O_2$ Volumetric rate of oxygen consumed by the animal ($\text{mLO}_2 \text{kg}^{-1} \text{h}^{-1}$)
 $\dot{V}CO_2$ volumetric rate of carbon dioxide produced by the animal ($\text{mLCO}_2 \text{kg}^{-1} \text{h}^{-1}$)
 RER Respiratory exchange ratio (ratio of $\dot{V}CO_2 : \dot{V}O_2$)
 WVD Water vapour density (absolute humidity) of the air leaving the chamber ($\text{mg H}_2\text{O mL}^{-1}$)

Introduction

In general, mammals adapt to the cold by increasing the capacity to produce heat, or decreasing the rate of heat loss to the environment (Hart 1971; Bartholomew 1972). However, the small size of many mammals, especially hibernators, prevents large seasonal adjustments in insulation (Gordon, 1993). This relative incapacity to increase insulation, coupled with the high costs of maintaining elevated rates of metabolism (MR) in the cold, along with seasonal fluctuations in food availability, are considered to be the primary factors which promote the widespread use of seasonal torpor expression in small mammals, which consists of periodic adjustments in MR and body temperature (T_b) over the hibernation period (Lyman et al. 1982; Heldmaier et al. 2004). Most thermoregulatory studies on hibernators, however, focus on MR and T_b control during

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torpor (Barnes and Buck 2000), or on characterising the costs of arousal and the subsequent normothermic periods (Wang 1978). While seasonality in MR has been demonstrated for some heterothermic species—the Djungarian hamster (Heldmaier et al. 1982) the marmot (Ortmann and Heldmaier 2000)—for the most part, thermoregulatory control, thermogenic capacity, and basal metabolic turnover rates (BMR) of hibernators during their normothermic periods in winter are relatively unknown. Knowledge of the rates of MR during the normothermic state can provide critical insights into the physiological mechanisms at play which support and prepare the animals for torpor, and is, therefore, an important state to consider.

Eastern chipmunks (*Tamias striatus*, Linnaeus, 1758) provide an interesting model for studying mammalian thermoregulation. They are one of the few species of hibernators that subsist primarily on stored food and not internal fat stores during the winter hibernation period (Humphries et al. 2003b; Munro et al. 2008), and unlike some mammals (e.g., hamsters), do not require food deprivation to initiate torpor. Although their hibernation patterns are relatively similar to those of fat storing hibernators, food storing hibernators exhibit shorter torpor bout length and longer inter-bout arousal times (Humphries et al. 2003b; Munro et al. 2005; Landry-Cuerrier et al. 2008). This is, in part, because they need to eat during arousals to assimilate the energy required to sustain them in hibernation until their next arousal (Willis 1982; Humphries et al. 2003b). Recent studies have also found that the length and depth of torpor bouts in chipmunks are dependent both on the amount of stored food and the fatty acid composition of the food hoard (Geiser and Kenagy 1987; French 2000; Humphries et al. 2003a; Munro et al. 2005; Landry-Cuerrier et al. 2008) generally, animals with larger food hoards expressed less torpor (French 2000; Humphries et al. 2003a; Humphries and Rodgers 2004; Landry-Cuerrier et al. 2008), highlighting that chipmunk hibernation is conditional on predictive energy availability. Torpor expression appears to be a purely seasonal occurrence; there have been no reported cases of torpor outside of winter (Wang and Hudson 1971; Pivorun 1976a).

The ability to store most of the energy needed for hibernation in the form of food, and not fat, makes Eastern chipmunks nearly unique among hibernators in that they do not increase in mass dramatically (<25% compared to 40–100% body mass change in related Sciuridae) prior to the start of winter (Panuska 1959; Geiser and Kenagy 1987; Trombulak 1989; Michener and Locklear 1990; Ortmann and Heldmaier 2000; Humphries et al. 2003b; Kauffman et al. 2004). In theory, this makes them well-suited for seasonal comparisons of metabolic traits because the confounds of changing body size and body fat composition on mass-dependent traits are minimised (Singer et al. 1995).

In addition, longer inter-bout arousal periods provide greater opportunities to characterize thermoregulation and energetics in normothermic animals, and assess the presence of diurnal fluctuations common to circadian regulation of homeostatic processes. Chipmunks, therefore, provide a good model from which to determine whether acclimation to winter-like conditions leads to metabolic and thermoregulatory adjustments outside of torpor expression (i.e., during normothermic periods). We hypothesized that seasonal torpor expression, manifested as frequent entries into and arousals from torpor, would influence the MR and T_b of normothermic animals throughout the hibernation season. Based on the premise that metabolic adjustments that promote torpor carry throughout the hibernation period, we predicted that animals undergo torpor would have reduced MR expenditure in the normothermic state. In order to assess this, we characterised the energetics and thermoregulation in both summer- and winter-acclimated Eastern chipmunks by recording body mass and daily T_b in both summer- and winter-acclimated animals and measuring MR over a range of ambient temperatures (T_a).

Materials and methods

Animals, husbandry, and seasonal acclimation

To assess potential seasonal differences in the variables tested, separate cohorts of nine individual Eastern chipmunks were collected in the summer and the winter. Animals were collected from Tea Lake Campsite and the Swan Lake Forest Reserve in Algonquin Provincial Park, Ontario (45°35'N, 78°30'W). During the summer (June–August 2007), nine chipmunks (five males, four females) were housed indoors at the Wildlife Research Station in Algonquin Park. The chipmunks were fed a diet of standard rat chow and sunflower seeds which were hidden throughout the cage as a form of enrichment. Temperature in the room fluctuated according to outside temperatures, but was not allowed to drop below 15°C or rise above 30°C. Lighting was provided via overhead fluorescent tubes, maintained on a light cycle coinciding with natural light periods (approx 05:30–21:00 h, 16 h light:8 h dark) changing throughout the study period to match daily natural light–dark rhythms.

For the winter phase (October 2007–March 2008), nine animals (four males, five females) were housed in an environmental control room at Brock University. The room was kept at temperatures mimicking the known seasonal changes in burrow temperatures (ranging from 4 to 20°C throughout the winter) of chipmunks from Southern Quebec (D. Munro personal communication; Landry-Cuerrier et al.

2008). Light cycles were maintained according to sunrise and sunset times in Algonquin Park to mimic natural light:dark cycles. Rat chow was provided up to mid-November, a period in which chipmunks experience a more mixed diet in the wild (Humphries et al. 2001), after which they were exclusively fed sunflower seeds until the end of the hibernation period in April when their feed was once again supplemented with rat chow.

Body mass was recorded at the start of each experiment, or once a week during cage cleaning. Certain individuals in the winter were weighed less frequently due to deep periods of torpor when they were left undisturbed. Data collection lasted from the end of June to the 3rd of September in the summer and from the first week of January to the first week of April in the winter. To ensure that the housing set-up was sufficient to induce torpor in the chipmunks, a preliminary study was undertaken in the fall of 2006. Ten animals (four females and six males) were housed under identical conditions to the winter-acclimated animals. Only body mass changes were collected from these individuals.

All animals were implanted with temperature-sensitive telemeters (single-stage radio transmitters, Sirtrack™, Havelock North, NZ) that were coated with biologically inert enamel and weighed no more than 3 g (<3% body mass). In the summer phase, animals were given a week to adjust to captivity before undergoing surgery for telemeter implantation. Due to early fall emergence dates for this species (Humphries et al. 2002), it was necessary to capture the animals for the winter phase a few months before the hibernation period. The limited battery life of the telemeters (4 months) prevented them from being implanted throughout the entire October–March captivity period, therefore surgery was undertaken in late-November to ensure that the telemeters would last throughout the experimental period (January–March). Before implantation, each telemeter was individually calibrated against a range of temperatures (5–42°C, NIST standardised) to determine the correct formula (a 5th order polynomial) to convert the pulse interval into temperature (precision is less than or equal to $\pm 0.1^\circ\text{C}$). The telemeters were implanted into the peritoneal cavity to provide accurate readings of core T_b throughout the study. Additional details on housing and the methods used for assessing T_b with telemeter implants are outlined in Levesque and Tattersall (2009).

Series I: spontaneous fluctuations in T_b

Telemeter implants allowed for the recording of daily T_b fluctuations throughout the entire period of study, except during periods when the recording equipment was utilised by the experiments in Series II. A radio receiver (R1000 Receiver, Communications Specialists Inc., Orange, CA, USA) was programmed to scan between channels, pre-

programmed to each telemeter's frequency at 60 s intervals, allowing for 1 min recordings from each individual. The period interval between pulses (s) was determined using the Tach3 Intelligent Tachometer (Sable Systems, Las Vegas, NV, USA) and recorded to computer (Expedata software, v1.0.18, Sable Systems). The period values were converted into T_b using a 5th order polynomial determined for each telemeter.

Data analysis: Series I

The data from the week following surgeries were excluded from all T_b analyses to ensure full recovery. To determine if there were diurnal patterns in T_b in the summer and the winter, T_b values recorded for each individual were binned into half-hour segments according to time of day. The seasonal comparison of T_b was divided into two parts. The first compared all T_b s from the summer to those from the winter, including torpid values. The second was a direct comparison of normothermic values, therefore the torpor bouts were excluded from the winter data. A spreadsheet was designed to separate the torpid T_b from the normothermic values. The cut-off T_b for designating torpor (<35.5°C) was chosen as described in Willis (2007); this value was the T_b at which resting metabolic rate was observed to drop below basal metabolic rate for three individuals that entered torpor during respirometry experiments at 8 and 15°C (data not shown).

A seasonal comparison was made between various T_b parameters. The first was between the absolute maximum and minimum T_b values recorded from each animal. To compare values that were more representative of overall trends, the daily maximum and minimum values from the half-hour segments (i.e., the time of day at which the individual had the highest and lowest average T_b , respectively) were used. Confidence (95%) limits of T_b for summer and winter normothermic periods, as well as winter torpor periods, were calculated for each animal. Torpor patterns were analysed by isolating each individual torpor bout and recording: the time at which the animals entered and aroused from torpor, the length of the torpor bout, and the length of the preceding normothermic (interbout) interval. Only bouts for which precise entry and exit times recorded were included in the averages. All animals occasionally showed brief periods (generally less than 1 h) where T_b declined to no less than 32°C. These bouts were considered short bouts and the length of these bouts were excluded from the calculation of average bout length.

Series II: respirometry experiments

Flow-through respirometry was used to obtain values for oxygen consumption ($\dot{V}\text{O}_2$), carbon dioxide production

($\dot{V}CO_2$), evaporative water loss (EWL) and thermal conductance during both seasons. Experiments were run over a range of T_a to record changes in thermoregulatory characteristics and minimum thermal conductance between seasons. Trials were conducted at T_{as} of 8, 15, 22 and 29°C; at least three of these T_{as} (8, 15 and 22°C) fall below the reported thermal neutral zone for this species (28–36°C; Neumann 1967; 28–32°C; Wang and Hudson 1971). Animals were fasted for 8 h before the experiment started to ensure a post-absorptive state. Experimental times were chosen to coincide with the time of day when the animals were most likely to be at rest. During the summer phase this was between 21:00 and 04:00 h but was more variable in the winter where the time of day at which each individual was most likely to rest was different. Earlier start times (1500–1700 h) were necessary for the few individuals that went torpid each night, and later times (2100–2200 h) for those that remained active later on in the day.

Each experiment consisted of a period of 4 h in which the animal was placed in a cylindrical respirometry chamber (700 mL, 8.5 cm diameter Animal Chamber, Qubit Systems, Kingston, ON, Canada). The chamber was placed inside a temperature-controlled environmental chamber consisting of a cooler (Rubbermaid®), in which the T_a was controlled using a water bath connected to an internally mounted heat exchanger/fan assembly. Dry CO_2 -free air was pumped into the chamber at a rate of 400 mL min^{-1} . The incurrent air was scrubbed of H_2O and CO_2 using a column containing a layer of Drierite™, a layer of soda-lime, and a final layer of Drierite™, necessary for absorbing H_2O vapour released by the soda lime. A subsample of the air from the respirometer was pulled through the O_2 , CO_2 , H_2O analysers at 180 mL min^{-1} using a Sable PP2 Dual Pump System, controlled via an analog mass flow controller. The air was first routed through a relative humidity analyser (Model RH200, Sable Systems), to record the H_2O vapour density ($\mu g mL^{-1}$). The respirometer air was subsequently diverted through a tube containing Drierite™ prior to entering a carbon dioxide analyser (Model CD-3A; AEI Technologies, Naperville, IL, or CA-2a; Sable Systems). From there, the air sample was passed through a tube containing soda lime followed by Drierite™ and finally through the oxygen analyser (FC-1B O_2 Analyser, Sable Systems). The following were recorded to a data acquisition system (Expedata, Sable Systems) at one sample per second: the fractional concentrations of O_2 and CO_2 , and WVD in the air leaving the chamber, the incurrent flow rate, respirometer T_a , environment chamber T_a , and T_b telemeter pulse rate. T_a in the environmental chamber and in the respirometry chamber were monitored using a thermocouple meter (Model TC-2000; Sable Systems). All thermocouples were pre-calibrated by the provider (Sable

Systems) to 0.2°C; calibrations were verified to within 0.1°C by submerging them in ice water and in boiling water. The positive side of a differential pressure transducer (Validyne model DP4510, Northridge, CA, USA) was also connected to the respirometer chamber, permitting the continuous recording of pressure within the chamber, which, though not calibrated to provide tidal volume, provided breathing rate (f_R). To ensure accurate detection of f_R , a higher sample rate, 100 samples s^{-1} , was necessary. Therefore, data were collected from the pressure transducer into a Biopac® MP150 and recorded in Acq-Knowledge (v. 3.8.1, BIOPAC Systems, Goleta, CA, USA).

The O_2 analyser was regularly calibrated using CO_2 -free, dried air (20.95% O_2). The CO_2 analyser was similarly calibrated using pure nitrogen as a zero value and a 1% CO_2 mixed gas (certified) as a span gas. The H_2O analyser was also calibrated regularly using pure nitrogen as the zero value, and air bubbled through water of a known temperature, and therefore known WVD, as the span gas. Empirically determined $CO_2:O_2$ values using the combustion of ethanol yielded values of 0.66–0.68, suggesting that the calibrations were accurate assessments of animal CO_2 production and O_2 consumption. To ensure that the incurrent O_2 , CO_2 and WVD remained constant throughout the experiment, a baseline measurement, consisting of dry carbon-dioxide free air, was set up to record for 3 min every 20 min. To do so, a gas flow distributor (Sable Systems, RM8 Intelligent Multiplexer) was placed just after the respirometer and programmed to control which air stream (the respirometer or the baseline) entered the gas analysers.

Data analysis: Series II

The respirometry files were analysed initially by correcting all fractional gas concentrations for analyser drift throughout the experimental period using the collected baseline values. Values for $\dot{V}O_2$, $\dot{V}CO_2$, EWL, T_b , C_{WET} and RER were calculated using equations adapted from page 456 of Withers (2001), by inputting the recorded values for O_2 and CO_2 , WVD, flow rate, T_a and telemeter pulse rate into a spreadsheet. To obtain steady-state values, a pre-recorded macro was used to locate multiple 3-min sections of data with the most stable trace in a resting state. The lowest of these values was used as the resting rate for that T_a . Experiments in which the animal did not rest for the entire 4 h period were re-attempted at a different date. Some animals would not rest at the lowest T_{as} (two at 8°C, one at 15°C) and values for these animals were omitted from the analysis. To account for potential effects of slight differences in body mass on the metabolic variables measured, all mass-dependent values were divided by body per

mass⁻¹ (Blaxter 1989). Initial analyses had been performed using mass^{-0.75}, however, using mass⁻¹ did not change any statistical outcomes and therefore, the latter (mass⁻¹ scaling) was retained for simplicity in data analysis and reporting. $\dot{V}O_2$ and EWL (in mL O₂ kg⁻¹ h⁻¹ and mg kg⁻¹ h⁻¹) were transformed into MR (W kg⁻¹) and E (W kg⁻¹) by multiplying them by energetic equivalence of oxygen, as determined by the RER (Blaxter 1989), and the latent heat of vaporization (McNab 2002), respectively. Wet thermal conductance (C_{WET}) was calculated using Equation 3 from McNab (1980). Steady state values of f_R (min⁻¹) obtained from the same periods of time where MR obtained were calculated by taking the inverse of the average time the animal required for ten breaths and multiplying this by 60-sec.

Due to the loss of function of some of the telemeters before the end of the study period, values for T_b and C_{WET} had smaller sample sizes than MR, $\dot{V}O_2$, $\dot{V}CO_2$, RER, E and f_R (Table 4). Measuring water loss at 8°C proved to be impossible because of excess condensation in the chamber, suggesting fully saturated air. Similarly, if the animal urinated during the experiment, which happened regularly at the lower T_a , accurate values for evaporation could not be recorded; these values were omitted from the calculation and analysis of E .

Statistical analysis

All statistics were performed using SigmaStat 3.0.1 or Systat 12 (Systat Software, Inc., Point Richmond, CA, USA) and resultant p values were compared to an α -value of 0.05 unless otherwise stated. One-way ANOVAs were used to analyse values for absolute maximum and minimum body mass as well as rates of mass change (% maximum mass day⁻¹) for summer and winter animals. For this analysis, the winter group included mass data from the Fall of 2006 and was separated into two groups animals who consistently entered torpor (winter torpid) and those who remained normothermic (winter normothermic). Summer values for T_b , as well as the time at which the maximum and minimum values were recorded were compared to overall winter T_b (including torpor), normothermic winter T_b and torpid winter T_b using multiple t tests or, if the data were non-normal, Mann–Whitney U tests. To compensate for the use of multiple t tests on data derived from similar values, the alpha value for these tests was Bonferroni-corrected to 0.01 for the summer/winter comparison and 0.01 for the comparison of normothermic values between seasons. Summer and winter values for MR, $\dot{V}CO_2$, T_b , C_{WET} , RER, E and f_R were compared using a two-way repeated measures ANOVA (RMANOVA), with season and T_a as the two factors tested. When significant differences were observed, the Holm–Sidak post

hoc method for multiple pair wise comparisons was used. If the data failed to meet the assumptions of normality or homoscedasticity, transformations (log, square-root or reciprocal) were used.

Results

With the exception of a single bout in the summer (where T_b dropped as low as 30°C for a period of 2 days) torpor was restricted to the winter months (December–April) and showed a high level of inter-individual variation in terms of degree and magnitude (Fig. 1; Tables 1, 2). While only eight of out of the eleven individuals from the Fall–Winter of 2006 expressed torpor, all but one (8/9) of the individuals from the Winter of 2007 expressed torpor (Tables 1, 2).

Seasonal variations in body mass

Animals in both seasons had variable masses at date of capture (83–111 g) and tended to gain approximately 10 g during the first few weeks in captivity. A slight increase in mass was observed in all individuals over the summer season, with some of the inter-individual variability

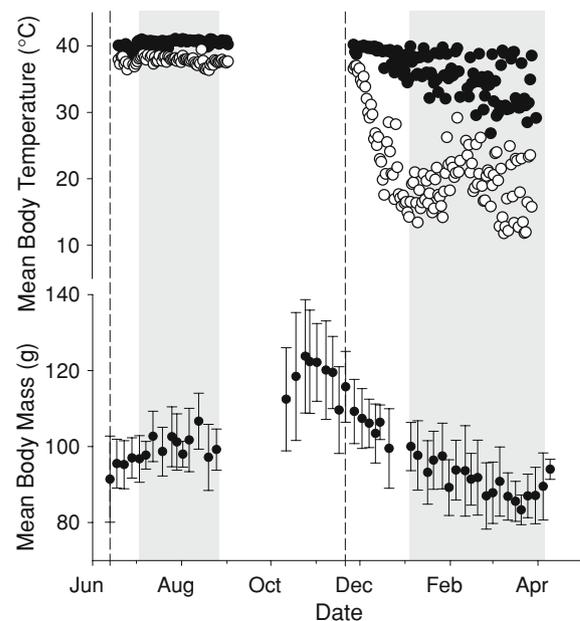


Fig. 1 Mean (\pm SD) body mass and mean maximum (filled circles) and minimum (open circles) body temperatures of summer- and winter-acclimated chipmunks over time. Standard deviation is only presented for the body mass for visual clarity. Summer animals were released around the 1st of September 2007 and the data as of October 2007 are from a different group of individuals. The shaded areas indicate the dates over which the animals were used in the respirometry studies. The telemeter implant dates are indicated by the dashed lines

Table 1 Dates of the initiation and termination of torpor expression in Eastern chipmunks in the winter

Individual	Date of onset of torpor	End of torpor	Proportion of time spent torpid
1	30 November 2007	after 2 February 2008 ^a	0.57
2	7 December 2007	31 March 2008	0.50
3	10 January 2008	22 January 2008	0.02
4	17 December 2007	30 March 2008	0.45
5	18 December 2007	After 3 March 2008 ^b	0.30
6	30 December 2007	After 3 March 2008 ^b	0.20
7	9 December 2007	16 April 2008	0.60
8	7 December 2007	20 April 2008	0.72

^a Final date of torpor unknown because the telemeter's battery died early, 2 February 2008; however, the animal was observed to be torpid after that date

^b The batteries in both animals' telemeters died around the 3 March 2008; subsequent bouts of torpor in both individuals were observed

Table 2 Characterization of torpor parameters in chipmunks from the winter phase

Individual	No. of short bouts	No. of long bouts	Average bout length (h)	Maximum bout length (h)	Minimum bout length (h)	Average interbout arousal time (h)	Minimum torpid body temperature (°C)	Average torpid body temperature (°C)
1	18	51	13.5	31.8	3.7	12.0	8.5	16.2
2	9	80	17.5	49.1	2.1	11.8	13.5	15.6
3	10	7	6.0	15.6	2.3	26.1	16.6	17.2
4	14	69	15.8	36.1	1.7	17.1	13.1	14.9
5	6	45	11.9	18.3	3.3	26.0	11.0	15.7
6	9	35	8.8	15.3	3.3	24.9	15.1	19.9
7	5	78	19.0	58.2	4.4	13.0	4.9	12.1
8	2	43	35.0	133.0	2.3	13.4	6.3	8.9
Mean ± SD	9 ± 5	51 ± 25	15.9 ± 8.8	44.7 ± 39.0	2.9 ± 0.9	18.0 ± 6.5	11.1 ± 4.2	15.1 ± 3.3

An animal was considered to be torpid whenever T_b dropped below 35.5°C for more than 30 min. All bouts of torpor lasting more than 30 min and less than 2 h in which T_b remained above 32°C were classified as short bouts. The remaining bouts, those lasting longer than 2 h, were classified as long bouts. Only long bouts for which the precise entry and exit times were known were included in the calculation of the average bout length

becoming dampened as the season progressed (Fig. 1). Body mass in the summer ranged between 90 and 110 g. The minimum value for mass of the summer animals was recorded at the date of capture (91.4 ± 11.3 g), and the maximum was recorded in mid-August (106.7 ± 7.4 g). Mass was much more variable in the winter, both at date of capture (83–122 g) and throughout the season (Fig. 1). A general trend, however, was still apparent with all animals initially increasing in body mass, and reaching a peak average of 123.7 ± 14.9 g near the end of October, before declining steadily until the end of March when most individuals ceased hibernation and commenced re-gaining mass. At the time of release, April 2008, mass reached levels similar to individuals caught in the summer (>90 g). The downward trend in mass in the winter began before the implant surgeries. An RMANOVA performed on the mass taken from each animal just before the metabolic experiments (Series II) indicated that, in spite of an initial mass increase in the fall, winter animals had lower body mass

than summer animals ($F_{1,17} = 9.0$, $p = 0.007$). Rate of mass loss differed in animals that regularly expressed torpor ($n = 15$), and was greater than the summer ($n = 9$) and winter animals ($n = 4$) that remained normothermic throughout the period of study (Fig. 2a; $F_{2,26} = 42.2$, $p < 0.001$). Similarly, animals that expressed torpor regularly had both higher maximum (Fig. 2b; $F_{2,26} = 12.6$, $p < 0.001$) and lower minimum mass values ($F_{2,26} = 9.3$, $p < 0.001$).

Series I: Torpor expression and spontaneous variation in T_b

Significant differences were observed between the summer and the winter values for absolute maximum and minimum T_b , as well as averaged daily maximum and minimum values ($p < 0.001$, t tests and Mann–Whitney tests). T_b was consistently lower in the winter even during periods of normothermy ($p < 0.01$, t tests and Mann–Whitney U tests;

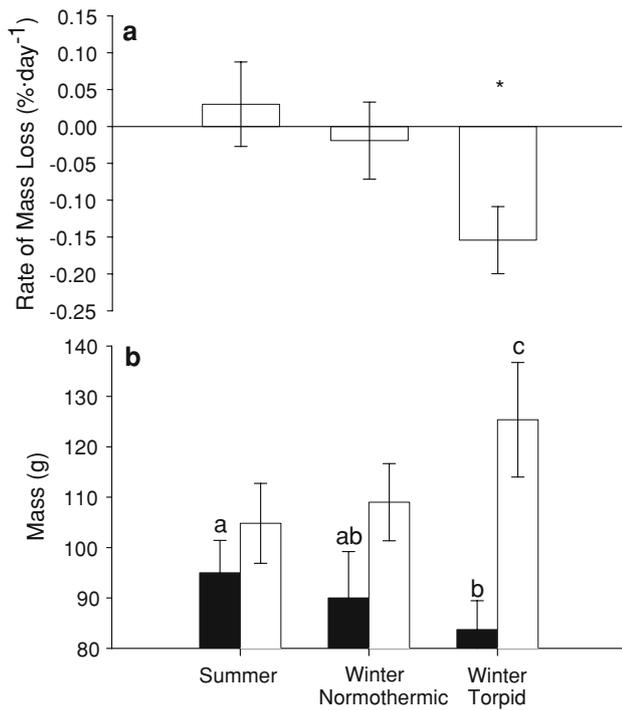


Fig. 2 **a** Mean (\pm SD) rate of mass change per day (calculated as the slope of mass change per day divided by maximum body mass and multiplied by 100; % maximum mass day⁻¹) from summer-acclimated Eastern chipmunks and winter-acclimated animals that did not exhibit torpor (winter normothermic) or went torpid (winter torpid) throughout the experimental period. *Indicates significant difference between groups. **b** Mean (\pm SD) of the maximum (white bars) and minimum (black bars) for body mass from summer, winter normothermic and winter torpid chipmunks. Different symbols represent a statistically significant difference for maximum or minimum values between groups

Fig. 1, Table 3). The time of day at which the maximum daily T_b was observed was also different; 06:37 h \pm 38 min in the summer compared to 14:48 h \pm 202 min in the winter ($T_{8,8} = 99$, $p < 0.001$). However, no differences were observed between the times at which the minimum T_b occurred; 00:27 h \pm 49 min in the summer 01:33 h \pm 189 min in the winter ($T_{8,8} = 61$, $p = 0.5$). All of the values recorded during the winter phase showed higher inter-individual variation than the summer values (Fig. 3). Clear diurnal patterns in T_b were apparent in animals from the summer phase (Fig. 3a); there was a visible distinction between nocturnal T_b (2000–0300 h), and diurnal T_b (0500–2000 h). In the summer, T_b reached a peak around 07:00 h coinciding with observed bouts of activity. T_b declined gradually throughout the afternoon, reaching a plateau just before nightfall, after which it declined to night-time values. In addition, the average daily maximum T_b differed from the average minimum ($t_8 = 20.88$, $p < 0.001$). Similar patterns were less apparent in the winter; although the daily maximum and minimum T_b were different for normothermic

values ($T_{8,8} = -36$, $p = 0.008$) no similar difference could be observed in the torpid values ($t_4 = 3.32$, $p = 0.029$, $\alpha = 0.01$).

Series II: respirometry

A lower MR was measured in winter-acclimated animals than in summer-acclimated ones ($F_{1,16} = 6.9$, $p = 0.017$) and MR increased at lower T_{as} ($F_{3,46} = 515.7$, $p < 0.001$; Table 4, Fig. 4) during both seasons. $\dot{V}CO_2$ levels were similarly affected by season ($F_{1,16} = 5.9$, $p = 0.026$) and T_a ($F_{3,45} = 357.0$, $p < 0.001$). While no differences were observed in RER between seasons ($F_{1,17} = 3.1$, $p = 0.09$), T_a had an effect ($F_{3,45} = 8.6$, $p < 0.001$); RER values for 29°C were higher than those for the other T_{as} and a T_a -season interaction was measured at 29°C ($F_{3,45} = 3.4$, $p = 0.027$); where the summer values were higher than the winter ones. Effects of season and T_a on f_R were measured ($F_{1,16} = 34.2$, $p < 0.001$; and $F_{3,43} = 101.1$, $p < 0.001$, respectively), although there was no T_a -season interaction ($F_{3,43} = 1.1$, $p = 0.34$). f_R was consistently lower in the winter than in the summer, and during both seasons increased as T_a decreased. Additionally, season affected E ($F_{1,11} = 9.1$, $p = 0.006$), which was consistently lower in the winter than in the summer, although no differences were measured in E among different T_a ($F_{2,11} = 0.63$, $p = 0.55$).

Seasonal differences were also apparent in T_b during the respirometry experiments; T_b was affected by season ($F_{1,13} = 12.8$, $p = 0.003$) and T_a ($F_{3,28} = 3.3$, $p = 0.037$), although no season- T_a interaction was observed ($F_{3,28} = 1.4$, $p = 0.25$). As with the daily T_b data, T_b during these experiments was lower in the winter than in the summer. The only within season difference was found between values from 8°C and those from 29°C, although the general trend was for T_b to increase with decreasing T_a (Fig. 4). C_{wet} did not change seasonally ($F_{1,13} = 2.4$, $p = 0.144$) and the only difference between T_a values was found at 29°C which was higher than all other recorded values for thermal conductance ($F_{3,27} = 20.5$, $p < 0.001$). C_{dry} equalled C_{wet} at all T_{as} , except for 29°C and is therefore not reported for the lower temperatures. At 29°C, C_{dry} was equal to the minimal levels found at the lower T_{as} (0.62–0.73 W°C⁻¹ kg⁻¹).

Correlations between torpor expression and energetics

Since torpor expression varied in the winter chipmunks, we were able to establish correlations between variables of interest. Average torpor bout length was strongly and inversely correlated with the average torpid T_b ($F_{1,6} = 30.8$, $p = 0.001$, $r^2 = 0.81$; Fig. 5a). This tight correlation allowed for the estimation of Q_{10} for torpor

Table 3 Mean \pm SD of the daily maximum and minimum T_b values from summer- and winter-acclimated Eastern chipmunks

	Summer		Winter (all values)		Winter (normothermic values only)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Average ^a	37.9 \pm 0.3	40.0 \pm 0.4	17.4 \pm 14.3	23.3 \pm 15.8	36.7 \pm 0.3	37.7 \pm 0.5
Time of day at average	00:00 h	06:30 h	01:00 h	14:00 h	21:30 h	12:00 h
95% confidence limits	Lower	Upper	Lower	Upper	Lower	Upper
	37.1 \pm 1.9	40.8 \pm 0.2	15.6 \pm 8.4	39.1 \pm 0.8	36.0 \pm 0.3	39.5 \pm 0.6

^a Average values refer to the time of day (when binned in half hour increments) that had the highest, or lowest, body temperature. The average 95% confidence limits of the body temperature derived from each individual are also presented. The second group of winter values (normothermic values only) were taken solely from the periods of normothermy between torpor bouts

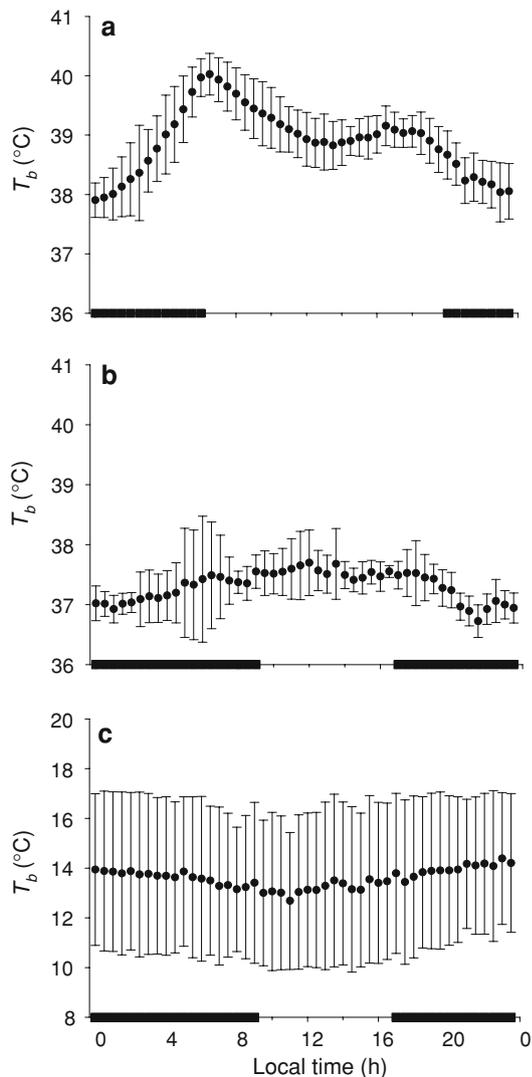


Fig. 3 Diurnal patterns in T_b in the two seasons of the study, the plots represent the means (\pm SD) of the T_b for each half hour of the day from the summer phase (a), normothermic only T_b values from the winter individuals (b), and torpid values only from the winter individuals (c)

duration (~ 4) by calculating the slope for \ln (bout length⁻¹) versus T^{-1} (K⁻¹) and deriving the activation energy. There was also a correlation between the rate of

mass loss and the proportion of time that the individuals spent in torpor (see next section for torpor assessment); a greater degree of torpor expression correlated to higher rates of mass change ($F_{1,6} = 8.1$, $p = 0.029$, $r^2 = 0.50$; Fig. 5b). BMR (MR from chipmunks at 29°C) was negatively correlated with the number of torpor bouts exhibited over the course of the entire winter ($F_{1,6} = 9.0$, $p = 0.024$, $r^2 = 0.53$; Fig. 5c). In association with changes in BMR, the normothermic T_b exhibited by chipmunks during their bouts of arousals was also negatively correlated with the degree of torpor expression ($F_{1,6} = 15.3$, $p = 0.008$, $r^2 = 0.67$; Fig. 5d).

Discussion

The chipmunks in this study showed yearly rhythms in both mass and T_b . Mass increased over the fall in preparation for hibernation, and decreased as hibernation progressed, and, in the winter, was elevated in animals that spent a greater proportion of time in torpor. Concurrent with the decline in mass, T_b fluctuated to a greater extent in the winter, with the majority of the animals expressing torpor throughout the season. Torpor expression also resulted in changes to the diurnal pattern of T_b regulation; daily peaks and nadirs in T_b were diminished in the winter animals, whether in torpor or during their periods of normothermia. As a result, even while normothermic, winter animals had lower T_b than the summer animals. This decrease in T_b was concurrent with a decline in MR with the seasonal difference becoming greater at lower T_a . In sum, the seasonal changes associated with hibernation in the chipmunk result in an energy sparing response during periods of normothermic arousal that appears to be fuelled by diminished diurnal changes in T_b , as well as a decrease in metabolic turnover (i.e. BMR).

Seasonality in body mass

Changes in body mass over the period of study were similar to those observed in other laboratory based studies

Table 4 A summary of thermoregulatory variables from the summer (S) and winter (W) phases

T_a (°C)	8	15	22	29
MR (W kg ⁻¹)				
S	19.41 ± 1.31(9)a	14.84 ± 1.19 (9)b	9.27 ± 0.98 (9)c	6.45 ± 0.50 (9)d
W	18.04 ± 1.53(8)a*	14.04 ± 1.26(8)b*	8.94 ± 0.38 (9)c*	6.22 ± 0.28 (9)d*
$\dot{V}O_2$ (mLO ₂ h ⁻¹ kg ⁻¹)				
S	3,561 ± 239 (9)a	2,745 ± 237 (9)b	1,687 ± 190 (9)c	1,131 ± 86 (9)d
W	3,303 ± 273(8)a*	2,568 ± 231(8)b*	1,627 ± 74 (9)c*	1,124 ± 52 (9)d*
$\dot{V}CO_2$ (mLCO ₂ h ⁻¹ kg ⁻¹)				
S	2,444 ± 190 (9)a	1,814 ± 191 (9)b	1,203 ± 107 (9)c	989 ± 62 (9)d
W	2,291 ± 216 (8)a*	1,792 ± 168 (8)b*	1,161 ± 69 (9)c*	826 ± 47 (9)d*
RER				
S	0.69 ± 0.03 (9)	0.68 ± 0.03 (9)	0.72 ± 0.04 (9)	0.84 ± 0.14 (9)
W	0.69 ± 0.02 (8)	0.70 ± 0.02 (8)	0.71 ± 0.04 (9)	0.74 ± 0.03 (9)*
T_b (°C)				
S	38.6 ± 0.6 (7)a	37.9 ± 0.5 (5)ab	37.9 ± 0.8 (7)ab	37.5 ± 0.5 (5)b
W	38.1 ± 0.7 (7)a*	37.2 ± 0.8 (5)ab*	38.0 ± 0.4 (7)ab*	37.3 ± 0.4 (7)d*
C_{WET} (W°C ⁻¹ kg ⁻¹)				
S	0.65 ± 0.05 (8)a	0.65 ± 0.07 (7)a	0.58 ± 0.07 (8)a	0.78 ± 0.07 (7)b
W	0.61 ± 0.04 (4)a	0.63 ± 0.09 (4)a	0.60 ± 0.03 (5)a	0.78 ± 0.06 (7)b
E (W kg ⁻¹)				
S	n/a	0.71 ± 0.13 (2)	0.86 ± 0.21 (4)	0.86 ± 0.12 (7)
W	n/a	0.69 ± 0.04 (3)*	0.64 ± 0.07 (7)*	0.59 ± 0.09 (7)*
f_R (min ⁻¹)				
S	120 ± 29 (9)a	104 ± 39 (9)b	63 ± 15 (9)c	45 ± 10 (9)d
W	74 ± 16 (8)a*	57 ± 13 (8)b*	37 ± 11 (9)c*	25 ± 7 (9)d*

All data presented are means ± SD (*n*)

Significant differences between ambient temperatures are indicated by different letters

* Significant difference between seasons

on Eastern chipmunks. Individuals from the summer were slightly heavier than the average wild-caught summer animal, which generally weighs less than 100 g (Pidduck and Falls 1973). This mass gain is consistent with a general increase in the body mass observed in some captive species (Kenagy 1981; Larcombe and Withers 2007 etc.), including Eastern chipmunks (Panuska 1959; Forbes 1966). More extreme mass gains were observed in the fall, during the beginning of winter, consistent with the pre-hibernation mass gain in wild chipmunks (Forbes 1966; Scott and Fisher 1972; Pivorun 1977). It is not surprising that the 15–20% mass gain before the onset of hibernation differs from the extreme, 40–100% mass gain observed in fat-storing hibernators (Panuska 1959; Trombulak 1989; Michener and Locklear 1990; Ortman and Heldmaier 2000; Kauffman et al. 2004), since chipmunks rely primarily on their food hoard to provide enough energy to sustain them for the winter (Humphries et al. 2003b). However, despite the fact that the animals were provided food ad libitum throughout the winter phase, a steady

decrease in the mass of the animals regularly exhibiting torpor was recorded as the season progressed. The onset of torpor expression resulted in a steady decrease in mass; furthermore, the rate of mass decrease was dependent on the amount of time spent torpid. Thus, despite the availability, and consumption, of food during the hibernation season (Humphries et al. 2001), chipmunks still utilize fat stores during torpor bouts or the subsequent inter-bout arousal periods, as witnessed by the lower RER in the winter (0.74 ± 0.03 compared to 0.84 ± 0.14 in the summer). Once the animals had ceased hibernating for the season, body mass returned to levels similar to those at capture, consistent with previous studies (Panuska 1959; Scott and Fisher 1972).

Seasonal differences in T_b and diurnal rhythms

T_b patterns were significantly more complex than changes in body mass. The first bouts of winter torpor were short, resembling the ‘test-drops’ that characterise the start of all

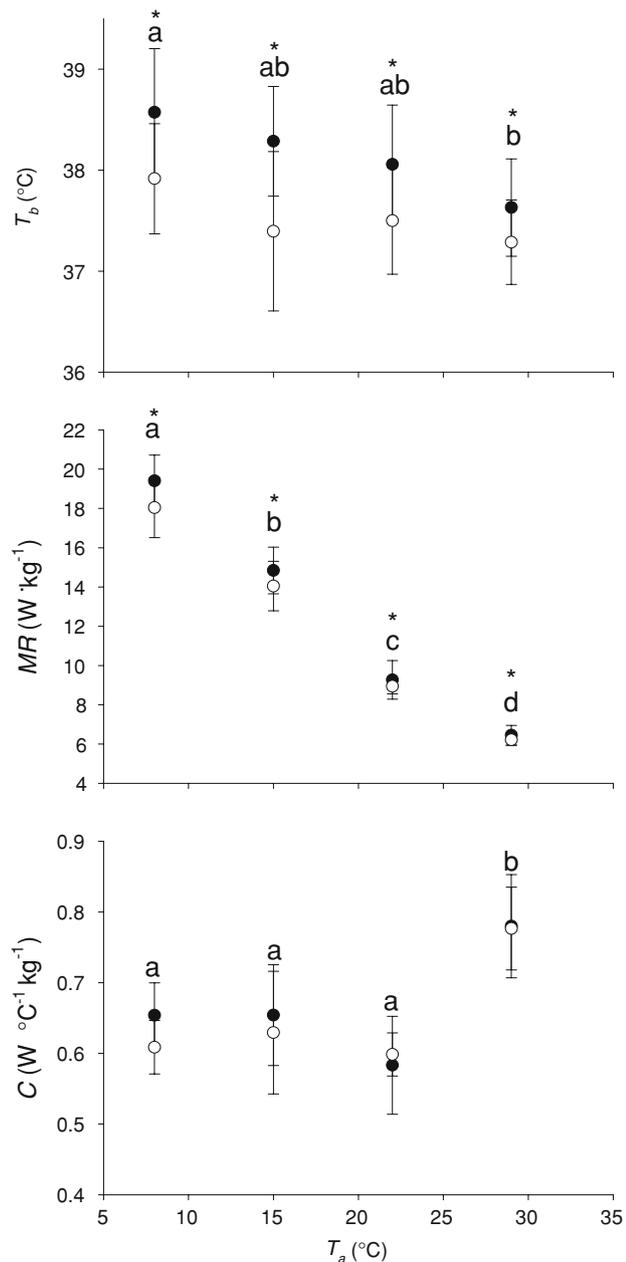


Fig. 4 Metabolic rate, T_b and thermal conductance in summer- and winter-acclimated chipmunks conducted during normothermic periods at 8, 15, 22, and 29°C. All values presented are means (\pm SD). Summer data are represented by *black circles*, while winter values are indicated in *white* ($n = 9$ for each season). *Different lettering* indicates significant effects of temperature. *Indicates seasonal differences

hibernation periods (Strumwasser 1958; Pivorun 1977; Lyman et al. 1982). However, three individuals never progressed past this stage into the deeper stages of hibernation. This variation in torpor expression is typical of laboratory-based studies on this species (French 2000; Humphries et al. 2001). Food supplementation throughout the winter may have had an adverse effect on torpor expression, since

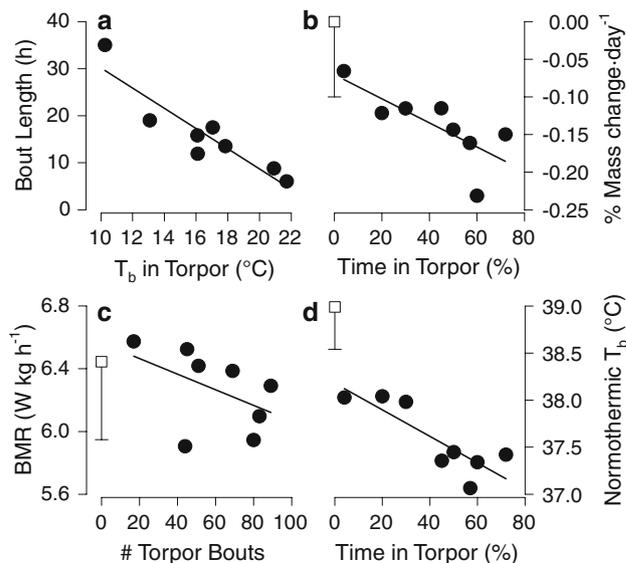


Fig. 5 Correlations among physiological variables measured in torpid chipmunks. **a** Demonstrates a negative correlation between average bout length and average T_b in torpor ($r^2 = 0.81$), **b** depicts the negative correlation between daily mass changes and the proportion of time spent in torpor ($r^2 = 0.50$), **c** shows the negative correlation between basal metabolic rate during periods of normothermic arousal and total torpor bouts exhibited throughout the winter ($r^2 = 0.53$), and **d** illustrates the influence that torpor expression has on normothermic T_b itself ($r^2 = 0.67$). *Filled circles* are derived from winter chipmunks exhibiting a variation in torpor expression. *Open squares* show the average (\pm SD) from summer, normothermic animals for comparison

increased hoard size negatively affects torpor expression (French, 2000; Humphries et al. 2003a; Landry-Cuerrier et al. 2008). Furthermore, the food provided (sunflower seed), is high in polyunsaturated fatty-acids, which, although important for hibernation, can also reduce torpor bout length at high dietary concentrations (Geiser et al. 1997; Munro et al. 2005). To avoid dietary confounds, future studies should include a more naturalized diet over the winter periods. Overall, it appears that although all animals had the ability to hibernate in the winter, most did not hibernate as deeply as has been observed in free-ranging individuals (Humphries et al. 2003a; Landry-Cuerrier et al. 2008), remaining at a torpid T_b greater than 14°C or only going torpid during the night. Similar patterns have been observed in the wild, and it is believed that, although hibernation benefits the animal in terms of energy savings, it is of greater benefit to the individual to reduce the time spent torpid when energy stores, in the form of stored food, are sufficient (French 1985; 2000; Humphries et al. 2003a, b; Landry-Cuerrier et al. 2008). Indeed, the tight correlation between average torpor bout duration and T_b during torpor in this study (Fig. 5a), yields a Q_{10} of ~ 4 , suggesting that the time invested in torpor, although partly explained by torpor T_b (French 1982; Thomas and Geiser 1997; Buck and Barnes

2000), is more likely driven by the metabolic rate experienced during torpor itself (Geiser and Kenagy 1988), shedding light on the inherent relationship between metabolism and torpor use patterns in hibernators. It should be noted, however, that while torpor in Eastern chipmunks, at least in the northern parts of their distribution range, is often reduced in the presence of large amounts of stored energy, it is rarely abandoned completely (Humphries and Rodgers 2004; Munro et al. 2005; Landry-Cuerrier et al. 2008). This indicates some benefit to hibernation outside of energy conservation. Indeed, it could also represent a bet-hedging strategy allowing them to carry unused food stores over to the next hibernation season (see Munro et al. 2008) or reduce the inherent costs of normothermic thermoregulation during periods of extreme cold (Landry-Cuerrier et al. 2008).

Torpor expression was not the only aspect of the chipmunk's T_b regulation to differ between seasons. Diurnal patterns in T_b were vastly different as well. A clear bimodal daily pattern was observed during the summer (Fig. 3a), consistent with the daily activity patterns of chipmunks (Elliot 1978; Richter 1978; Decoursey et al. 1998). Animals were most often at rest overnight, when the lowest T_b was observed. T_b generally increased shortly before sunrise, and the period of greatest activity was observed in the early morning. Diurnal patterns in normothermic T_b were much less evident, and nearly absent in some individuals in the winter. In general, peak normothermic T_b was observed during the light hours, which would be consistent with the higher levels of activity observed in the chipmunks during the day. Similarly, the lowest normothermic T_b values from all individuals were consistently recorded overnight. However, this pattern may have been influenced by the few individuals that barely hibernated or the two individuals that only ever went into torpor overnight and that were always active during the day. The four hibernators that had the longest torpor bouts showed very little in the way of diurnal normothermic T_b patterns; their patterns of entry to and arousal from torpor also did not follow any distinct pattern. This lack of daily T_b patterns in hibernating animals has also been observed in Anatolian ground squirrels (*Spermophilus xanthoprimum*) (Kart Gür et al. 2009).

Other studies monitoring T_b in chipmunks have failed to find seasonal differences in normothermic T_b s (Neumann, 1967; Wang and Hudson, 1971). In the present study, however, chipmunks had lower normothermic T_b in the winter. Although the difference was less than 1°C, the fact that normothermic winter T_b was consistently lower is significant. Interestingly, a similar drop in normothermic T_b , attributed to a reduction in activity or in energy expenditure, has been observed in both daily heterotherms (Christian and Geiser 2007) as well as hibernators (Kart Gür et al. 2009) in

periods immediately preceding torpor use. Defending a lower normothermic T_b could permit slight decreases in energy expenditure. In normothermic mammals, both hibernators and non-hibernators, daily oscillations in T_b are generally larger at lower T_a (Refinetti and Menaker 1992). This has been attributed to a larger drop in T_b when thermoregulation is partially shutdown during sleep (Gordon 1993), although corresponding changes in daily maximum T_b are rare (Refinetti and Menaker 1992). Given the lower normothermic T_b observed in winter-acclimated chipmunks, it suggests that animals in the present study spent considerable amounts of time resting or asleep during their arousal episodes. T_b was relatively low (<38°C; Fig. 5d) during the normothermic periods of individuals that hibernated more deeply, possibly because the animals were not active or awake during the entirety of these arousals. Indeed, it has been suggested that normothermic periods may be times when hibernators make up for the loss of sleep that occurs during torpor (Daan et al. 1991; Larkin et al. 2002). Although the animals must have been feeding, since normothermic periods are the only times in which the chipmunks can eat enough food to sustain themselves during their next hibernation bout (Humphries et al. 2001), based on absolute T_b values and visual inspections of the animals' cages, very little activity was observed during these times compared to similar times of day in the fall.

Seasonality in metabolic capability

This is the first study to observe and quantify the thermoregulatory characteristics of animals in their hibernation season during their brief periods of normothermia. All of the animals from the winter phase expressed torpor to some degree coinciding with a decrease in normothermic MR. The degree of torpor expression was negatively correlated to both normothermic BMR and T_b (Fig. 5c, d); trends previously observed at an inter-specific level (McNab 2002; Christian and Geiser 2007; Cooper and Geiser 2008). Interestingly, despite mass, MR and, T_b exhibiting seasonal differences, C_{wet} remained constant between seasons. The winter animals did not appear to gain any additional fur and often did not re-grow fur on the area of the stomach, which had been shaved for surgery. This is consistent with reports from the literature that indicate that chipmunks generally only moult once annually, around the middle of the summer (Yerger 1955). Field studies in which an individual's hair was clipped in the fall (a common form of identification) still maintained the same marking in the summer (Snyder 1982; Munro et al. 2008). This would indicate that changes in insulation are not part of the normal adaptations to the cold in this species. Similarly, the presence or absence of fur does not appear to affect the expression of hibernation. Kauffman et al. (2004) found that shaved

ground squirrels continued to hibernate as normal, although the costs of arousal and maintaining elevated metabolism during normothermic bouts increased. They concluded that although insulation during hibernation can permit greater energy savings, it is not necessary (Kauffman et al., 2001; Kauffman et al., 2004). Therefore, it is not surprising that the animals in this study showed no change in thermal conductance between seasons.

Another common adaptation to the cold that could have been employed by the animals, an increased in thermogenic capacity, was not supported by data in this study. It is possible that early in the winter, when the chipmunks reach their peak mass (Fig. 1), the animals would have shown a greater response to the cold. Mass gain is a common occurrence during cold acclimation in small mammals, accompanied by an increase in brown adipose tissue, which in turn increases the degree of non-shivering thermogenesis (Heroux et al. 1959). However, because neither shivering nor non-shivering thermogenesis were measured in this study, it is unknown whether or not the increase in mass seen at the start of the study was related to cold acclimation or to a seasonal mass increase in anticipation of hibernation. The subsequent decrease in mass, however, can be readily attributed to reducing fat stores during torpor bouts; the proportion of time spent torpid was correlated with rate of mass decrease (Fig. 5b). It is interesting to note that the chipmunks, at least at the lower $T_{a,s}$, did not switch metabolic fuel sources between seasons, as indicated by the comparable *RER* values below thermal neutrality. *RER* of 0.68–0.72 measured at those $T_{a,s}$ indicate the sole usage of fat as a substrate for MR (Blaxter 1989), consistent with work on other endotherms in the cold (Hart 1971; Walsberg et al. 1997).

General conclusions

Although previous studies have described either the seasonal changes in chipmunk T_b (see Neumann 1967; Wang and Hudson 1971; Scott and Fisher 1972; Pivorun 1976b; Kawamichi 1996; French 2000; Humphries et al. 2001; Munro et al. 2005), or the amplitude of daily T_b variation (Wang and Hudson 1971; Decoursey et al. 1998), ours is the first study to combine the analysis of both of these cycles with measurement of resting and basal rates of metabolism, and the potential influence of hibernation and winter acclimation on the normothermic arousal states. The chipmunks in our study demonstrated seasonality in both body mass and T_b regulation characteristic of mammalian heterotherms. While field-studies have found no correlation between mass at immergence and torpor expression (Humphries et al. 2003a; Landry-Cuerrier et al. 2008), animals in our study, in conditions which permitted the collection of body mass data unavailable from animals in

the wild, showed a strong relationship between fall mass gain and the degree of torpor expression; the individuals who put on the most mass spent more time in torpor. Torpor expression was restricted to the months of December through April, and normothermic T_b that occurred during the periodic arousals was consistently lower. This seasonality in T_b regulation manifested itself as a slight decrease in MR and evaporative water loss in the winter, but no change in thermal conductance. It appears that the physiological adaptations necessary for seasonal expression of torpor coincide with lower T_b and MR during the normothermic periods between torpor bouts. The fact that these levels are correlated with increased torpor expression strongly suggests that conferring greater energy savings to the animal during torpor and normothermia is an essential feature of winter survival in chipmunks and likely many other hibernating mammals.

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References

- Barnes BM, Buck CL (2000) Hibernation in the extreme, burrow and body temperatures, metabolism, and limits to torpor bout length in arctic ground squirrels. In: Heldmaier G, Klingenspor M (eds) Life in the cold: eleventh international hibernation symposium. Springer, New York, pp 65–72
- Bartholomew GA (1972) Body temperature and energy metabolism. In: Gordon MS, Bartholomew GA, Grinnell AD, Jorgensen CB, White FN (eds) Animal physiology: principles and adaptations. Macmillan, New York, pp 298–368
- Blaxter K (1989) Energy metabolism in animals and man. Cambridge University Press, Cambridge
- Buck CL, Barnes BM (2000) Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *Am J Physiol* 279:R255–R262
- Christian N, Geiser F (2007) To use or not to use torpor? Activity and body temperature as predictors. *Naturwissenschaften* 94:483–487
- Cooper CE, Geiser F (2008) The “minimal boundary curve for endothermy” as a predictor of heterothermy in mammals and birds: a review. *J Comp Physiol B* 178:1–8
- Daan S, Barnes BM, Strijkstra AM (1991) Warming up for sleep—ground-squirrels sleep during arousals from hibernation. *Neurosci Lett* 128:265–268

- Decoursey PJ, Pius S, Sandlin C, Wethey D, Schull J (1998) Relationship of circadian temperature and activity rhythms in two rodent species. *Physiol Behav* 65:457–463
- Elliot L (1978) Social behavior and foraging ecology of the eastern chipmunk (*Tamias striatus*) in the Adirondack mountains. *Smithson Contrib Zool* 265:1–107
- Forbes RB (1966) Fall accumulation of fat in chipmunks. *J Mammal* 47:715–716
- French AR (1982) Effects of temperature on the duration of arousal episodes during hibernation. *J Appl Physiol* 52:216–220
- French AR (1985) Allometries of the durations of torpid and euthermic intervals during mammalian hibernation—a test of the theory of metabolic control of the timing of changes in body-temperature. *J Comp Physiol (B)* 156:13–19
- French AR (2000) Interdependency of stored food and changes in body temperature during hibernation of the eastern chipmunk, *Tamias striatus*. *J Mammal* 81:979–985
- Geiser F, Kenagy GJ (1987) Polyunsaturated lipid diet lengthens torpor and reduces body temperature in a hibernator. *Am J Physiol* 252:R897–R901
- Geiser F, Kenagy G (1988) Torpor duration in relation to temperature and metabolism in hibernating ground squirrels. *Physiol Zool* 61:442–449
- Geiser F, Kenagy GJ, Wingfield JC (1997) Dietary cholesterol enhances torpor in a rodent hibernator. *J Comp Physiol B* 167:416–422
- Gordon CJ (1993) *Temperature Regulation in Laboratory Rodents*. Cambridge University Press, Cambridge
- Hart JS (1971) Rodents. In: Whittow G (ed) *Comparative physiology of thermoregulation vol II, mammals*. Academic Press, New York, pp 2–130
- Heldmaier G, Steinlechner S, Rafael J (1982) Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *J Comp Physiol B* 149:1–9
- Heldmaier G, Ortman S, Elvert R (2004) Natural hypometabolism during hibernation and daily torpor in mammals. *Respir Physiol and Neuro* 141:317–329
- Heroux O, Depocas F, Hart JS (1959) Comparison between seasonal and thermal acclimation in white rats. I. Metabolic and insulative changes. *Can J Biochem Physiol* 37:473–478
- Humphries MM, Rodgers B (2004) The energetic state-dependency of autumn immergence in eastern chipmunks. In: Barnes BM, Carey HV (eds) *Life in the cold: evolution, mechanisms, adaptation, and application twelfth international hibernation symposium*, Institute of Arctic Biology, University of Alaska Fairbanks, Alaska, USA, pp 101–112
- Humphries MM, Thomas DW, Kramer DL (2001) Torpor and digestion in food-storing hibernators. *Physiol Biochem Zool* 74:283–292
- Humphries MM, Thomas DW, Hall CL (2002) The energetics of autumn mast hoarding in eastern chipmunks. *Oecologia* 133:30–37
- Humphries MM, Kramer DL, Thomas DW (2003a) The role of energy availability in mammalian hibernation: an experimental test in free-ranging eastern chipmunks. *Physiol Biochem Zool* 76:180–186
- Humphries MM, Thomas DW, Kramer DL (2003b) The role of energy availability in mammalian hibernation: a cost-benefit approach. *Physiol Biochem Zool* 76:165–179
- Kart Gür M, Refinetti R, Gür H (2009) Daily rhythmicity and hibernation in the Anatolian ground squirrel under natural and laboratory conditions. *J Comp Physiol B* 179:155–164
- Kauffman AS, Cabrera A, Zucker I (2001) Torpor characteristics and energy requirements of furless Siberian hamsters. *Physiol Biochem Zool* 74:876–884
- Kauffman AS, Paul MJ, Zucker I (2004) Increased heat loss affects hibernation in golden-mantled ground squirrels. *Am J Physiol R* 287:R167–R173
- Kawamichi M (1996) Ecological factors affecting annual variation in commencement of hibernation in wild chipmunks (*Tamias sibiricus*). *J Mammal* 77:731–744
- Kenagy GJ (1981) Effects of day length, temperature, and endogenous control on annual rhythms of reproduction and hibernation in chipmunks (*Eutamias* spp). *J Comp Physiol* 141:369–378
- Landry-Cuerrier M, Munro D, Thomas DW, Humphries MM (2008) Climate and resource determinants of fundamental and realized metabolic niches of hibernating chipmunks. *Ecology* 89:3306–3316
- Larcombe AN, Withers PC (2007) Effects of long-term captivity on thermoregulation, metabolism and ventilation of the southern brown bandicoot (Marsupialia: Peramelidae). *J Comp Physiol B* 177:229–236
- Larkin JE, Franken P, Heller HC (2002) Loss of circadian organization of sleep and wakefulness during hibernation. *Am J Physiol* 282:R1086–R1095
- Levesque DL, Tattersall GJ (2009) Seasonal changes in thermoregulatory responses to hypoxia in the Eastern chipmunk (*Tamias striatus*). *J Exp Biol* 212:1801–1810
- Lyman CP, Willis JS, Malan A, Wang LCH (1982) *Hibernation and torpor in mammals and birds*. Academic Press, New York
- McNab BK (1980) On estimating thermal conductance in endotherms. *Physiol Zool* 53:145–156
- McNab BK (2002) *The physiological ecology of vertebrates: a view from energetics*. Cornell University Press, Ithaca
- Michener GR, Locklear L (1990) Over-winter weight loss by Richardson's ground squirrels in relation to sexual differences in mating effort. *J Mammal* 71:489–499
- Munro D, Thomas DW, Humphries MM (2005) Torpor patterns of hibernating eastern chipmunks *Tamias striatus* vary in response to the size and fatty acid composition of food hoards. *J Anim Ecol* 74:692–700
- Munro D, Thomas DW, Humphries MM (2008) Extreme suppression of aboveground activity by a food-storing hibernator, the eastern chipmunk (*Tamias striatus*). *Can J Zool* 86:364–370
- Neumann R (1967) Metabolism in the eastern chipmunk (*Tamias striatus*) and the southern flying squirrel (*Glaucomys volans*) during the winter and summer. In: Fisher K, Dawe A, Lyman CP, Schonbaum E, South FE (eds) *Mammalian hibernation III*. Elsevier, New York, pp 64–74
- Ortman S, Heldmaier G (2000) Regulation of body temperature and energy requirements of hibernating Alpine marmots (*Marmota marmota*). *Am J Physiol* 278:R698–R704
- Panuska JA (1959) Weight patterns and hibernation in *Tamias striatus*. *J Mammal* 40:554–566
- Pidduck ER, Falls JB (1973) Reproduction and emergence of juveniles in *Tamias striatus* (Rodentia: Sciuridae) at two localities in Ontario, Canada. *J Mammal* 54:693–707
- Pivrun EB (1976a) A biotelemetry study of thermoregulation patterns of *Tamias striatus* and *Eutamias minimus* during hibernation. *Comp Biochem Physiol* 53A:265–271
- Pivrun EB (1976b) A gradient calorimeter study of normothermic and hibernating eastern chipmunks, *Tamias striatus*. *Comp Biochem Physiol* 54A:259–261
- Pivrun EB (1977) Hibernation of a southern subspecies of *Tamias striatus*: thermoregulatory patterns. *Am Midl Nat* 98:495–499
- Refinetti R, Menaker M (1992) The circadian-rhythm of body temperature. *Physiol Behav* 51:613–637
- Richter CP (1978) Evidence for existence of a yearly clock in surgically and self-blinded chipmunks. *Proc Natl Acad Sci USA* 75:3517–3521

- Scott GW, Fisher KC (1972) Hibernation of eastern chipmunks (*Tamias striatus*) maintained under controlled conditions. *Can J Zool* 50:95–105
- Singer D, Schunck O, Bach F, Kuhn HJ (1995) Size effects on metabolic rate in cell, tissue, and body calorimetry. *Thermochim Acta* 251:227–240
- Snyder DP (1982) *Tamias striatus*. *Mamm Species* 168:1–8
- Strumwasser F (1958) Factors in the pattern, timing and predictability of hibernation in the squirrel, *Citellus beecheyi*. *Am J Physiol* 196:8–14
- Thomas DW, Geiser F (1997) Periodic arousals in hibernating mammals: is evaporative water loss involved? *Funct Ecol* 11:585–591
- Trombulak SC (1989) Running speed and body mass in Belding's ground squirrels. *J Mammal* 70:194–197
- Walsberg GE, Tracy RL, Hoffman TCM (1997) Do metabolic responses to solar radiation scale directly with intensity of irradiance? *J Exp Biol* 200:2115–2121
- Wang LCH (1978) Time patterns and metabolic rates of natural torpor in Richardson's ground squirrel. *Can J Zool* 57:149–155
- Wang LC-H, Hudson JW (1971) Temperature regulation in normothermic and hibernating eastern chipmunk, *Tamias striatus*. *Comp Biochem Physiol* 38A:59–90
- Willis JS (1982) The mystery of the periodic arousal. In: Lyman CP, Willis JS, Malan A, Wang LCH (eds) *Hibernation and torpor in mammals and birds*. Academic Press, New York, pp 92–103
- Willis CKR (2007) An energy-based body temperature threshold between torpor and normothermia for small mammals. *Physiol Biochem Zool* 80:643–651
- Withers PC (2001) Design, calibration and calculation for flow-through respirometry systems. *Aust J Zool* 49:445–461
- Yerger RW (1955) Life history notes on the Eastern chipmunk, *Tamias striatus lysteri* (Richardson), in Central New York. *Am Midl Nat* 53:312–323