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Seasonal changes in thermoregulatory responses to hypoxia in the Eastern chipmunk (*Tamias striatus*)

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SUMMARY

Mammalian heterotherms are known to be more tolerant of low oxygen levels than homeotherms. However, heterotherms demonstrate extreme seasonality in daily heterothermy and torpor expression. Because hypoxia depresses body temperature (T_b) and metabolism in mammals, it was of interest to see if seasonal comparisons of normothermic animals of a species capable of hibernation produce changes in their responses to hypoxia that would reflect a seasonal change in hypoxia tolerance. The species studied, the Eastern chipmunk (*Tamias striatus*, Linnaeus 1758), is known to enter into torpor exclusively in the winter. To test for seasonal differences in the metabolic and thermoregulatory responses to hypoxia (9.9 kPa), flow-through respirometry was used to compare oxygen consumption, minimum thermal conductance and T_b under fixed ambient temperature (T_a) conditions whereas a thermal gradient was used to assess selected T_a and T_b in response to hypoxia, in both summer- and winter-acclimated animals. No differences were observed between seasons in resting metabolism or thermal conductance in normoxic, normothermic animals. Providing the animals with a choice of T_a in hypoxia attenuated the hypoxic drop in T_b in both seasons, suggesting that the reported fall in T_b in hypoxia is not fully manifested in the behavioural pathways responsible for thermoregulation in chipmunks. Instead, T_b in hypoxia tends to be more variable and dependent on both T_a and season. Although T_b dropped in hypoxia in both seasons, the decrease was less in the winter with no corresponding decrease in metabolism, indicating that winter chipmunks are more tolerant to hypoxia than summer animals.

Key words: hypoxia tolerance, heterothermy, hibernator, thermal conductance, resting oxygen consumption, body temperature, behavioural thermoregulation.

INTRODUCTION

The relationship between heterotherms and hypoxia tolerance has long been of interest (Hiestand et al., 1950; Faleschini and Whitten, 1975; Barros et al., 2001; Drew et al., 2004). Hibernating species are known to survive periods of anoxia for longer than non-hibernating species (Lutton, 1982) and have a more moderate physiological response when confronted with a hypoxic stress (Faleschini and Whitten, 1975). A common hypoxic response that small mammals and other vertebrates exhibit, whether they hibernate or not, is a rapid and reversible decrease in body temperature (T_b) and metabolic rate (Wood, 1995; Wood and Gonzales, 1996; Tattersall et al., 2002; Tattersall and Milsom, 2003). The higher tolerance to hypoxia in hibernators does not necessarily dictate the magnitude of this thermoregulatory response to hypoxia. Some hypoxia-tolerant animals show a greater response to hypoxia when compared with non-hypoxia-tolerant species and achieve this through a greater drop in T_b and rate of oxygen consumption ($\dot{V}_{O_2}$) (Wood and Stabenau, 1998; Barros et al., 2001), hence utilising less oxygen than if they had remained normothermic (*sensu stricto* 'cenothermic') (IUPS Thermal Commission, 2003). Conversely, hypoxia tolerance can also manifest itself in a blunted physiological response to hypoxia, in which metabolism is not decreased but rather maintained, allowing the animal to continue to function relatively normally (Lutton, 1982; Frappell and Mortola, 1994; Scott et al., 2008). This is, presumably, because other homeostatic mechanisms [e.g. changes in blood oxygen affinity, chemosensory feedback and ventilatory responses (Kilgore et al., 2008)] offset any hypoxemia and obviate the need to conserve oxygen by reducing metabolism and T_b.

Mammalian heterotherms are well adapted to changing T_b because they do so under normoxic conditions while hibernating. The propensity to change T_b, which they do upon entry to, and arousal from, torpor is believed to predispose them to be more flexible to changes in T_b during exposure to hypoxia (Frerichs and Hallenbeck, 1998; Drew et al., 2007). As such, they have been found to show relatively large decreases in T_b and metabolism in hypoxia, which can provide large reductions in oxygen requirements (Barros et al., 2001; Tattersall and Milsom, 2003; Drew et al., 2004). However, hibernators are also more capable than non-hibernators of sustaining high rates of metabolism during exposure to hypoxia while active (Lutton, 1982). Thus, predicting how naturally heterothermic animals will thermoregulate in hypoxia is complicated by their increased tolerance to low oxygen, as well as by their propensity to drop T_b naturally. Furthermore, hibernators typically only enter torpor during winter; however, most hypoxic studies on hibernators (Faleschini and Whitten, 1975; Barros et al., 2001; Tattersall and Milsom, 2003) have been conducted on animals when they are normothermic (i.e. in the summer). These studies, therefore, did not account for seasonal variation in physiological characteristics that could affect thermoregulation and thus the hypoxic metabolic and thermoregulatory responses. Additionally, the majority of mammalian hypoxic studies have focused on either fat-storing hibernators, such as ground squirrels, or non-heterothermic laboratory rats. Food-storing hibernators, such as chipmunks, provide a critical physiological mid-point without thermoregulatory and metabolic complications associated with fat-storage. Although a previous study has shown that chipmunks can have either an equal

or lessened tolerance to hypoxia than their fat-storing counterparts (Faleschini and Whitten, 1975), their hypoxic response has yet to be fully characterised.

In addition, the behavioural response to hypoxia in chipmunks is currently unknown. Given that hypoxia suppresses thermogenesis (Barros et al., 2001), providing access to external sources of heat during exposure to hypoxia can help provide an indication as to what temperature an animal would defend when given the capacity to do so. Studies on other mammal species have reported a decrease in selected ambient temperatures (T_a) in hypoxia (Gordon and Fogelson, 1991; Wood, 1995), while others have reported that these species did not change selected temperatures at all (Gordon and Fogelson, 1991) or chose higher temperatures (Dupré et al., 1986; Gordon and Fogelson, 1991; Dupré and Owen, 1992; Gordon, 1997), although the later could partially be attributed to the animals having had insufficient time to acclimate to the gradient before hypoxia commenced (Gordon, 1997). Nevertheless, the decrease in T_b was much greater in studies in which the animals were kept at lower T_a than in studies where they were given a choice of T_a (Gordon, 1997; Wood and Stabenau, 1998), suggesting that mammals will utilise behaviour to assist in defending T_b in hypoxia.

Both field and laboratory studies have shown that chipmunks rarely enter torpor outside of the winter months (Wang and Hudson, 1971; Humphries et al., 2003). Due to the seasonal expression of torpor and the accompanying natural reduction of metabolic rate and T_b, it is of interest to see if the thermoregulatory response to hypoxia changes with season and what insight this will provide into the normal regulation of metabolism and T_b. We hypothesised that natural seasonal changes in thermoregulatory characteristics, such as metabolic rate, thermal conductance and propensity to exhibit torpor in the Eastern chipmunk (*Tamias striatus*), would manifest in seasonally altered hypoxic metabolic and thermoregulatory responses. Should a seasonal difference occur, however, it could result from one of two ways: an increased, more dramatic hypoxia-induced drop in T_b and metabolic rate (if thermoregulatory mechanisms are the dominant seasonal influence) or a decreased, more conserved, drop in T_b and metabolism (if hypoxia tolerance is the dominant seasonal influence). Our objectives were therefore to test for seasonal differences in physiological and behavioural thermoregulatory responses to hypoxia by measuring changes in metabolic rate, thermal conductance and preferred T_a and T_b.

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 in the winter. Animals were collected from Tea Lake Campsite and the Swan Lake Forest Reserve in Algonquin Provincial Park, Ontario, Canada (45 deg.35′N, 78 deg.30′W). During the summer phase of the experiments (June–August 2007), nine chipmunks (five males, four females) were captured and 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 it 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 h–21:00 h, 16 h:8 h light:dark), changing throughout the study period to match daily natural light–dark rhythms.

For the winter phase of the experiments (October 2007–March 2008), nine animals (four males, five females) were housed in an environmental control room at Brock University, Ontario, Canada. The room was kept at temperatures mimicking the known seasonal changes in burrow temperatures (ranging from 4°C–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 until 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.

Surgical implantation of T_b telemeters and collection of T_b

All animals were implanted (peritoneal cavity) with temperature-sensitive telemeters (single-stage radio transmitters, Sirtrack^{TM}, Havelock North, New Zealand) weighing no more than 3 g (<3% body mass), providing accurate measurements of core T_b. 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 ≤±0.1°C). Animals were provided with an analgesic (acetaminophen, at 80 mg ml^{-1} in drinking water or in jelly at 300 mg kg^{-1}) both pre- and post-surgery. All equipment, including the telemeters, had been sterilised overnight using a gluteraldahyde cold-sterilisation solution (Germex, Vétoquinol, Lavaltrie, QC, Canada). Anaesthesia was induced and maintained using 5% and 1.5–2.5% Isofluorane, respectively. The sterilised telemeter was implanted into the peritoneal cavity, and the muscle and skin were sutured using dissolvable poliglecaprone sutures (Ethicon-Brand Monocryl, Novartis, Basel, Switzerland). The animals were placed in a fresh cage lined with paper towel and allowed to recover overnight under a heat lamp before being returned to the regular housing area. T_b data were collected *via* the use of a radio receiver (R1000 Receiver, Communications Specialists, Orange, CA, USA) connected to a Tach3 Intelligent Tachometer (Sable Systems, Las Vegas, NV, USA) and recorded to a computer (Expedata software, v. 1.0.18, Sable Systems). The tachometer provided the period interval between pulses (s), which was subsequently converted into temperature using the unique 5th-order polynomial determined for each telemeter. All procedures involving the use of these animals were approved by the Brock University Animal Care and Use Committee (AUPP # 06-09-01). Collection of the animals was authorised by the Ontario Ministry of Natural Resources.

Experimental protocol

The experimental protocol consisted of two series of experiments to determine the effects of both season and hypoxia on thermoregulation. Physiological responses were measured using flow-through respirometry under fixed T_a conditions (Series 1). Behavioural responses were assessed using a thermal gradient apparatus, which allowed for the determination of preferred T_a (Series 2). During the summer, each animal was utilised in an experiment once every 4–5 days. The experimental procedure they were subjected to was determined by assigning each experiment–animal combination a random number and then using the numbers to rank the order in which they would be run. Slight changes were made to the schedule during the winter phase to allow for the animals to hibernate naturally. Animals were used in experiments on days when they were observed to be normothermic spontaneously (i.e. during their inter-bout arousal period).

Series 1: respirometry measurements

Flow-through respirometry was used to obtain values for $\dot{V}_{O_2}$, volumetric rate of carbon dioxide produced by the animal ($\dot{V}_{CO_2}$), rate of evaporative water loss (EWL), T_b and wet thermal conductance (C_{wet}) during exposure to normoxia and hypoxia at fixed temperatures. Experiments were run at two T_a values (15°C and 22°C) and two oxygen levels (20.8 kPa, 20.95%, normoxic and 9.9 kPa, 10%, hypoxia) to record changes in thermoregulatory characteristics between individuals in summer and normothermic individuals in winter. These temperatures were used because the responses to hypoxia below the thermal neutral zone (28–32°C) (Wang and Hudson, 1971), where thermogenesis is of greater importance, were of primary interest. Temperatures of 15°C and 22°C were chosen because they fall sufficiently below the thermal neutral zone to provide a thermoregulatory challenge to the animals (Wang and Hudson, 1971). The change in metabolism between basal rates and the rates at these temperatures is not influenced significantly by season (Levesque, 2008). To ensure that the recordings approximated resting rates, the animals were fasted for 8 h before the experiment to ensure that they were post-absorptive, and the trials were conducted between 19:00 h and 04:00 h in order to coincide with the natural rest phase.

Each experiment consisted of a period of 4 h in which the animal was placed in a cylindrical chamber (700 ml, 8.5 cm diameter, Animal Chamber, Qubit Systems, Kingston, ON, Canada). During the hypoxic experiments, the animal was allowed to habituate to T_a for one hour at 20.8 kPa O_2 before the nitrogen–air mix was initiated. Hypoxia (9.9 kPa) was maintained for 2 h before returning the animal to normoxic conditions. The final hour was used to record the animal's recovery from hypoxia. The cylindrical chamber was placed inside an environmental chamber, consisting of a cooler (Rubbermaid®, Atlanta, GA, USA), in which the temperature 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}. This 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™ 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 and H_2O analysers at 180 ml min^{-1} (Sable PP2 Dual Pump System). The air was first routed through a relative humidity analyser (Model RH200, Sable Systems) to record the water vapour density (μg ml^{-1}), which was later used to calculate EWL. The respirometer gas was subsequently diverted through a tube containing Drierite™ prior to entering a CO_2 analyser (Model CD-3A; AEI Technologies, Naperville, IL, USA 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 O_2 analyser (FC-1B O_2 Analyser, Sable Systems). Channels were set up in Sable System's data acquisition software, Expedata (v. 1.0.18) in order to record (at one sample s^{-1}) the fractional concentrations of O_2 and CO_2, as well as water vapour density (WVD) in the air leaving the chamber, the incurrent flow rate, the respirometer temperature, the environment chamber temperature and the T_b telemeter pulse rate. Temperatures in the environmental chamber and in the respirometry chamber were monitored using a thermocouple meter (Model TC-2000; Sable Systems). 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, although not calibrated to provide tidal volume, provided breathing rate (f_R). To ensure accuracy in detecting breathing frequency, 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 AcqKnowledge (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 used throughout produced 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, consisting of dry CO_2-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 was programmed to control which air stream (the respirometer or the baseline) entered the gas analysers.

Series 1: data analysis

To prepare the raw data files for analysis, initially all fractional gas concentrations were corrected for analyser drift throughout the experimental period. Values for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, EWL, T_b, C_{wet} and respiratory exchange ratio (RER) were also calculated by inputting the recorded values for proportions of O_2 and CO_2 going into and leaving the respirometer, WVD, flow rate, chamber temperature and T_b 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 temperature. Values were only selected from a minimum of 20 min after the first hour and up until the third hour of the experiment. Experiments in which the animal did not rest for the entire 4 h period were re-attempted on a different date. Steady-state $\dot{V}_{O_2}$ data from the hypoxic experiments were only taken following one hour after the onset of hypoxia. The values for O_2, CO_2 and WVD leaving the chamber were converted into $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$ and EWL using equations from Withers (Withers 2001). To account for potential effects of slight differences in body mass on the metabolic variables measured, all mass-dependent values were corrected to body $mass^{0.75}$ (Blaxter, 1989). $\dot{V}_{O_2}$ was then used to calculate C_{wet} using equation 3 from McNab (McNab, 1980). f_R was determined using the peak detection function in AcqKnowledge.

Series 2: behavioural thermoregulation assessment

A rectangular thermal gradient chamber (1.2 m×0.20 m) was designed to provide the animals with a thermal choice environment. The bottom of the box contained a copper sheet, coated with white Contact® paper to provide the visual contrast required for the video detection software in order to distinguish the animal. The copper sheet was cooled on one end and heated on the other end using two water baths, one hot (50°C) and one cold (0°C), connected to copper tubing exchangers. The copper tubing was placed under the ends of the copper sheet in loops that were decreasingly in contact with the copper sheet as they approached the centre of the gradient. This created a linear gradient in floor temperatures spanning from 13°C to 47°C. A similar gradient in air temperatures was also maintained by using fans connected to heat exchangers connected to the same water baths. The gradient chamber was constructed to be airtight, allowing oxygen levels to be lowered during the hypoxic trials. The

box was sealed using a Plexiglas lid that allowed a full view of the gradient from above.

The position of the chipmunk within the thermal gradient chamber was recorded with an ordinary webcam connected to visual detection software (ICFish v. 2, Brock University Electronics Shop, St Catharines, Ontario, Canada), which tracked the position of the animal using contrast against the white background. The gradient was visually isolated from the rest of the room and lit to provide a constant contrast for optimal position detection. Animal position in the gradient chamber was recorded once a second and was later transformed into selected T_a. Temperatures in the gradient were measured at 5 cm intervals and an interpolation function was used to fill in the connecting values. A lookup table in Microsoft Excel was used to convert animal position as detected by the visualisation software to selected temperature. T_b was recorded simultaneously as described in Series 1.

Individuals used in the present study were first habituated to the apparatus prior to experimentation. Tests were also undertaken to ensure that the animal's placement in the gradient apparatus was the result of selected T_a and not a bias for a particular area of the box. The animals were placed in the apparatus with the temperature throughout the box held at 28°C for at least 6 h. At no time during these trials did the animals select a position in the apparatus that coincided with any of the positions chosen when the temperature gradient was activated (T_7=2.97, P=0.02, N=8). During the hypoxic experiments, the animals were given 1.5 h to habituate to the gradient before hypoxia was initiated. Nitrogen gas was then pumped into the gradient using an oxygen controller (Pro-Ox, Biosherix, Redfield, NY, USA) to create a hypoxic atmosphere (9.9 kPa, stabilised 2 h into experiment), which lasted until 5.5 h into the experiment, after which oxygen levels were returned to normal. The animal was left in the gradient and allowed to re-adjust to normoxia until the sixth hour. To adjust to observed seasonal changes in daily activity patterns, the experiments were run between 11:30 h–17:30 h during the summer phase and 10:00 h–16:00 h in the winter.

Series 2: data analysis

Data from each thermal gradient experiment were entered into a custom-designed spreadsheet that converted the detected position into selected T_a. The data from each trial were averaged into 10 min periods for time course visualisation. Given that steady-state values were also of interest, a 3 h period, between hours 1–4 of exposure to hypoxia was used in the calculation of the mean values reported. All selected T_a and T_b reported from this time period are presented as the means ± s.d. of the median value from each individual. Median values of individual responses were used to ensure that extreme variables, such as what would occur should the animal spend time exploring either end of the gradient, did not unduly influence the reported values. To obtain a clear picture of the temperatures selected by each animal over the course of the steady-state period, the selected T_a was also binned into 1°C increments, and the mean percentage of the total experiment time in which the animals spent at each temperature was then calculated. Finally, to assess the overall variability of both selected T_a and of T_b throughout this period, the central 95% range of the data (referred to as ΔT_a or ΔT_b) was computed as the difference between the 5th and 95th percentiles.

Statistical analysis

All statistics were performed using SigmaStat 3.0.1 or Systat 12 (Systat Software, Systat Systems, Inc., Point Richmond, CA, USA) and resultant P values were compared with an α value of 0.05. Summer and winter steady-state values for $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, C_{wet}, RER, f_R, T_b and selected T_a (the latter two values obtained in both Series 1 and 2) were compared using two-way repeated-measures analysis of variance (RM ANOVA), with season and experiment type (T_a and oxygen level combined within one factor) 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 equal variance, transformations (log, square-root, reciprocal) were conducted.

RESULTS

Series 1: effects of season and hypoxia on thermoregulatory variables at fixed T_a

$\dot{V}_{O_2}$, T_b and f_R showed immediate responses to the reduced oxygen levels (Fig. 1). While there was no overall seasonal effect in $\dot{V}_{O_2}$ ($F_{1,16}$=0.119, P=0.734), during the summer phase $\dot{V}_{O_2}$ decreased in hypoxia (Table 1; Fig. 2) at 15°C ($F_{3,47}$=136.19, P<0.001). In the winter, although there was a slight initial decrease in oxygen consumption in hypoxia (Fig. 1), steady-state values were not significantly different between normoxia and hypoxia. Seasonal effects in hypoxic $\dot{V}_{O_2}$ were only observed at 15°C ($F_{1,16}$=7.33, P<0.001), where winter animals achieved higher $\dot{V}_{O_2}$. Changes in

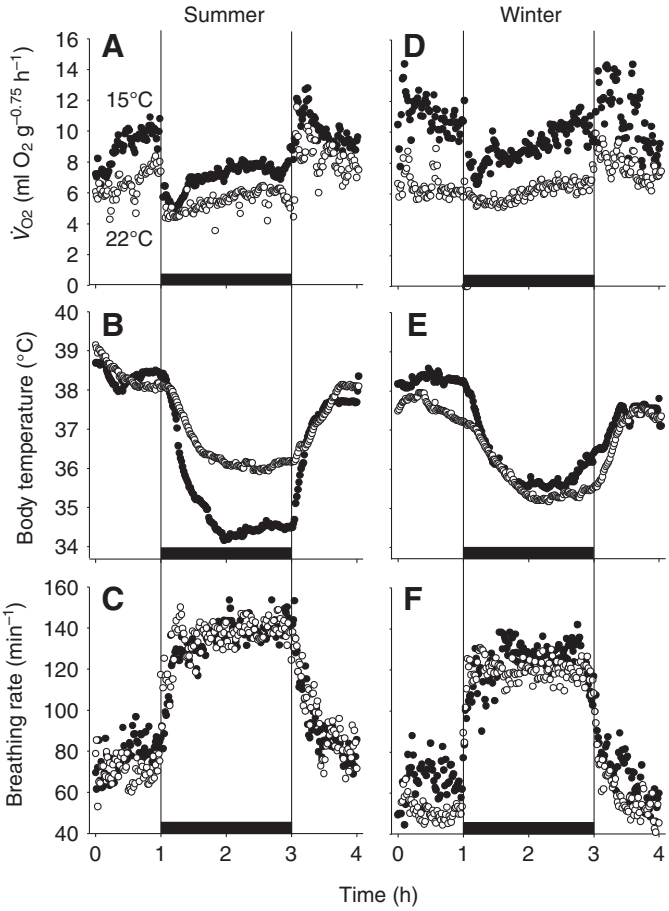

Fig. 1. Time course for rate of oxygen consumption ($\dot{V}_{O_2}$), body temperature (T_b) and breathing rate (f_R) during the transition from normoxia (20.8 kPa) to hypoxia (solid black bar, 1 h, 9.9 kPa O_2) and subsequent normoxic recovery in Eastern chipmunks. Black circles represent mean values, collated from all animals, from experiments at an ambient temperature of 15°C, open circles are those from 22°C. A, B and C are traces for $\dot{V}_{O_2}$, T_b and f_R, respectively, from the summer. Similarly, D, E and F are traces from the winter phase.

Table 1. A summary of thermoregulatory variables from Eastern chipmunks in the summer (S) and winter (W) phases during the experiments in Series 1

O_2 T_a (°C)		Normoxia 15	Hypoxia 15	Normoxia 22	Hypoxia 22
$\dot{V}_{O_2}$ (ml O_2 $g^{-0.75}$ h^{-1})	S	8.63±0.73 (9)	7.26±0.62 (9)*	5.30±0.53 (9)	5.07±0.56 (9)
	W	7.86±0.69 (8)	8.25±0.96 (9)	4.99±0.24 (9)	5.41±0.73 (9)
$\dot{V}_{CO_2}$ (ml CO_2 $g^{-0.75}$ h^{-1})	S	5.70±0.57 (9)	4.92±0.40 (9)*	3.78±0.29 (9)	3.83±0.34 (9)
	W	5.49±0.53 (8)	5.31±0.64 (8)	3.56±0.27 (9)	3.44±0.48 (9)
RER	S	0.68±0.03 (9)	0.68±0.07 (9)	0.72±0.04 (9)	0.76±0.08 (9)
	W	0.70±0.02 (8)†	0.64±0.02 (9)*,†	0.71±0.04 (9)†	0.64±0.05 (9)*,†
T_b (°C)	S	38.1±0.7 (7)	34.3±1.3 (7)*,†	37.9±1.1 (7)	36.0±0.41 (7)*
	W	37.2±0.8 (5)	35.2±0.5 (3)*,†	37.5±0.5 (5)	35.1±0.7 (7)*
C_{wet} (ml O_2 $g^{-0.75}$ h^{-1})	S	0.38±0.05 (7)	0.37±0.03 (7)	0.33±0.03 (7)*	0.37±0.03 (7)*
	W	0.35±0.05 (5)	0.38±0.04 (3)	0.32±0.03 (5)*	0.38±0.03 (5)*
EWL (mg $g^{-0.75}$ h^{-1})	S	3.90±0.93 (3)†	3.82±0.50 (5)†	3.96±0.93 (4)†	3.44±0.69 (5)†
	W	3.11±0.15 (3)†	3.07±0.61 (3)†	2.89±0.28 (6)†	3.10±0.63 (9)†
f_R (min^{-1})	S	86±8 (9)†	138±21 (9)†	63±15 (9)†	127±24 (9)†
	W	57±13 (8)†	122±8 (9)†	37±11 (9)†	117±22 (9)†

All data presented are means ± s.d. (*N*). *Indicates significant effects of oxygen, †indicates a significant difference between seasons. RER, respiratory exchange ratio; C_{wet}, wet thermal conductance; EWL, rate of evaporative water loss; f_R, breathing rate; T_b, body temperature; T_a, ambient temperature; $\dot{V}_{O_2}$, rate of oxygen consumption; $\dot{V}_{CO_2}$,volumetric rate of carbon dioxide produced by the animal.

$\dot{V}_{CO_2}$ during hypoxia mirrored those of the values for $\dot{V}_{O_2}$; the only significant seasonal differences were found in hypoxia at 15°C ($F_{3,47}$=3.03, P=0.038), where winter animals sustained higher values than summer animals. There was an overall seasonal difference in RER ($F_{1,16}$=7.7, P=0.013), which was lower in the winter. There was also an effect of experiment type on RER ($F_{1,16}$=4.99, P=0.005) but the only relevant significant differences observed were in the winter where hypoxia induced a significant decrease in RER. RER values were also lower in the winter compared with the summer at both hypoxic T_as ($F_{1,16}$=7.38, P<0.001) (Table 1).

Overall, f_R was significantly higher in the summer than in the winter ($F_{1,16}$=12.85, P=0.002) and increased during hypoxia at both T_as in both seasons ($F_{1,16}$=164.78, P<0.001) (Fig. 1; Table 1), although it did not differ between T_a in hypoxia. A similar RM ANOVA performed on the ratio of individual normoxic:hypoxic f_R indicated a significant effect of season ($F_{1,16}$=21.18, P<0.001) and of temperature ($F_{1,16}$=15.98, P=0.002) on the hypoxia-induced increase in f_R despite the lack of difference in the absolute hypoxic f_R between experiments. However, neither hypoxia nor T_a affected EWL ($F_{3,16}$=1.08, P=0.385), which remained relatively constant between T_a and oxygen levels although it was significantly higher in the summer than in the winter ($F_{1,15}$=5.51, P=0.027).

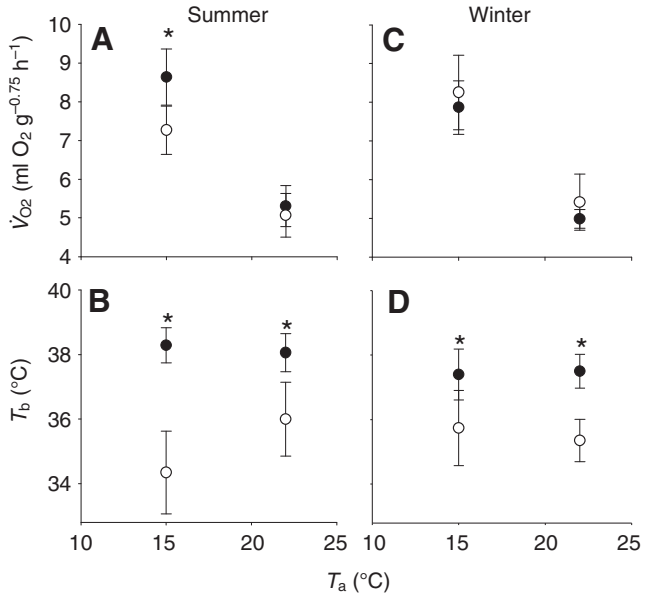

Fig. 2. Steady-state measurements of the rate of oxygen consumption ($\dot{V}_{O_2}$) (A,C) and body temperature (T_b) (B,D) for Eastern chipmunks in normoxia (20.8 kPa, closed circles) and hypoxia (9.9 kPa O_2, open circles) during summer (A,B) and winter (C,D). All data represented are means ± s.d. *Indicates significant differences between normoxic and hypoxic values. T_a, ambient temperature.

T_b dropped significantly in response to hypoxia ($F_{3,28}$=41.67, P<0.001) (Figs 1 and 2; Table 1). Although there were no overall significant seasonal differences in T_b ($F_{1,13}$=0.008, P=0.93), the response to the different T_a differed between seasons ($F_{3,28}$=5.19, P=0.006). The hypoxic decline at 22°C was similar in both seasons, with T_b dropping to 35–36°C. A difference was observed at 15°C, where in the summer, T_b at 15°C dropped significantly lower than at 22°C, reaching as low as 32°C in some individuals. In the winter, although T_b still dropped in comparison with normoxic levels, T_a did not significantly influence hypoxic T_b (Fig. 2). Accompanying these changes in T_b and $\dot{V}_{O_2}$, hypoxia induced an increase in C_{wet}, although the increase was only significant at 22°C ($F_{3,27}$=4.89, P=0.008). C_{wet} did not however differ significantly between seasons ($F_{1,12}$=0.01, P=0.918).

Series 2: effects of season and hypoxia on behavioural thermoregulation

During the experimental trials, a large amount of variation in selected T_a was observed both within and between individuals. Although all individuals remained at a single temperature for significant periods of time, some were more active than others. Some individuals even exhibited a shuttling behaviour, i.e. moving frequently between the hot and the cold ends of the thermal gradient. T_b often fluctuated, even while the animal remained at the same T_a. Time courses for T_b during the thermal gradient experiment are provided in Fig. 3. There was little discernable pattern in selected T_a within the normoxic experiments. There was however a consistent change in T_b in response to hypoxia ($F_{1,10}$=3.33, P<0.001) but not between seasons ($F_{1,13}$=32.92, P=0.09) (Fig. 4). The pattern of this change in T_b was similar to that observed in the respirometry experiments (Series 1). T_b decreased upon initiation of hypoxia and reached a plateau within one hour. However, the pattern differed slightly in

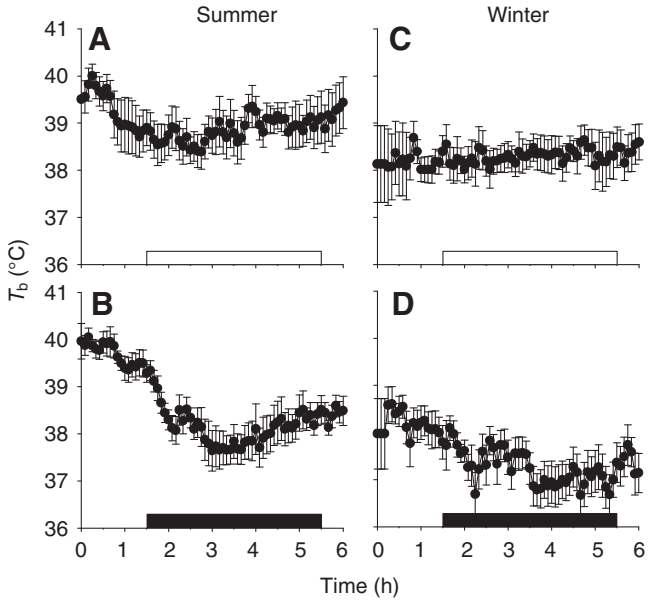

Fig. 3. Time courses of body temperature (T_b) in Eastern chipmunks given a choice of ambient temperatures during the normoxic (A,C) and hypoxic (B,D) thermal gradient trials in the summer (A,B) and in the winter (C,D). Shown are means (±s.e.m.) of the values from each individual taken every ten minutes. Standard error is used instead of standard deviation for visual clarity. Black bar denotes the period of hypoxic (9.9 kPa) exposure in B and D; white bar denotes the corresponding time periods for the normoxic, control experiments.

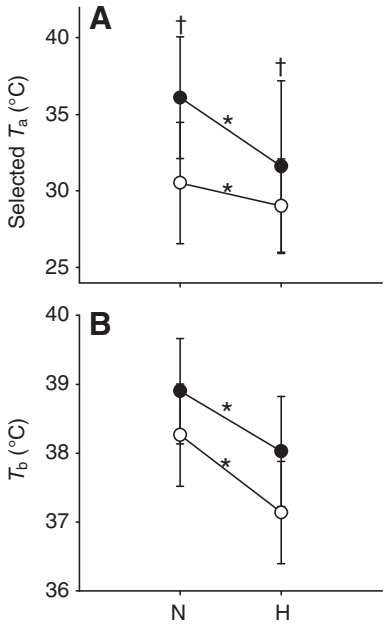

Fig. 4. Mean (±s.d.) ambient temperature (T_a) (A) and body temperature (T_b) (B) selected by Eastern chipmunks in the thermal gradient chamber under normoxic (N, 20.8 kPa) and hypoxic (H, 9.9 kPa) conditions in both seasons. Closed circles are values from the summer phase and open circles are from the winter. *Indicates significant effects of oxygen; †indicates a significant difference between seasons.

that in the thermal gradient apparatus, at least in the summer, T_b started to return to normoxic values nearing the end of the 4 h hypoxic exposure period (Fig. 3).

Selected T_a differed significantly between both season ($F_{1,16}$=5.84, P=0.028) and oxygen level ($F_{1,15}$=4.76, P=0.045), although there was no season–oxygen level interaction effect ($F_{1,15}$=1.66, P=0.22). Selected T_as were significantly higher in the summer than in the winter (Fig. 4; Table 2) and were also higher overall in normoxia than in hypoxia. It should be noted, however, that the decrease in selected T_a in the winter was slight and therefore the significant difference found between oxygen levels could have been primarily driven by the high summer values. The full spread of the temperatures commonly selected by the animals could be gleaned by observing the histograms of the percentage of time spent by the animals at each T_a (Fig. 5). Generally, the winter data were more centralised, with the majority of the selected temperatures being close to the mean values of 29–31°C. The effects of hypoxia are more visible in the summer where there was a notable increase in the selection of lower temperatures.

In terms of variation in selected T_a, there were no significant trends in the 95% distribution for selected T_a (ΔT_a), both season and oxygen level had no significant effect (Table 3). Variability in T_b (ΔT_b) in the thermal gradient, however, was significantly influenced by oxygen levels ($F_{1,13}$=9.82, P=0.008) but not by season ($F_{1,13}$=2.16, P=0.16). Hypoxia caused an increase in T_b variability in both seasons (Table 3).

DISCUSSION

As hypothesised, normothermic Eastern chipmunks exhibited distinct seasonal differences in thermoregulatory responses to hypoxia. Animals from the summer phase showed a hypoxic response similar to that observed in other mammals; decreases in metabolic rate and T_b in hypoxia became larger as T_a and oxygen levels decreased (Hiestand et al., 1950; Wood, 1995; Barros et al., 2001; Tattersall et al., 2002; Scott et al., 2008). The relationship between T_a, metabolic rate and T_b is consistent with a reduction in thermogenesis in hypoxia, accompanied by a selection of lower temperatures, leading to a temperature-dependent decrease in T_b. Interestingly, winter-acclimated animals exhibited attenuated changes in T_b to hypoxia and showed no corresponding decline in $\dot{V}_{O_2}$. These results are consistent with the notion that the hibernation state, which involves major adjustments in metabolic and thermoregulatory capacities, bestows hypoxia tolerance to animals (Drew et al., 2004), which, in our present study, manifests as changes to the hypoxic thermoregulatory response in normothermic animals.

Hypoxic thermoregulatory response in Eastern chipmunks

In the present study, changes in steady-state $\dot{V}_{O_2}$ in hypoxia generally did not occur, except for summer-acclimated animals at 15°C; however, T_b decreased significantly in hypoxia at both temperatures and in both seasons. The drop in T_b could, therefore, not be solely attributed to a drop in $\dot{V}_{O_2}$. A possible explanation for

Table 2. Mean ± s.d. (N) selected T_a and T_b of Eastern chipmunks in normoxia (20.8 kPa) and hypoxia (9.9 kPa) during the experiments in Series 2

	Treatment	Selected T_a (°C)	T_b (°C)
Summer	Normoxia	36.1±4.0*,† (9)	38.9±0.8* (7)
	Hypoxia	31.6±5.6* (9)	37.8±0.7* (7)
Winter	Normoxia	30.5±4.0*,† (9)	38.3±0.7* (7)
	Hypoxia	29.4±3.1* (9)	37.1±0.7* (7)

*Indicates significant effects of oxygen, †indicates a significant difference between seasons. T_a, ambient temperature; T_b, body temperature.

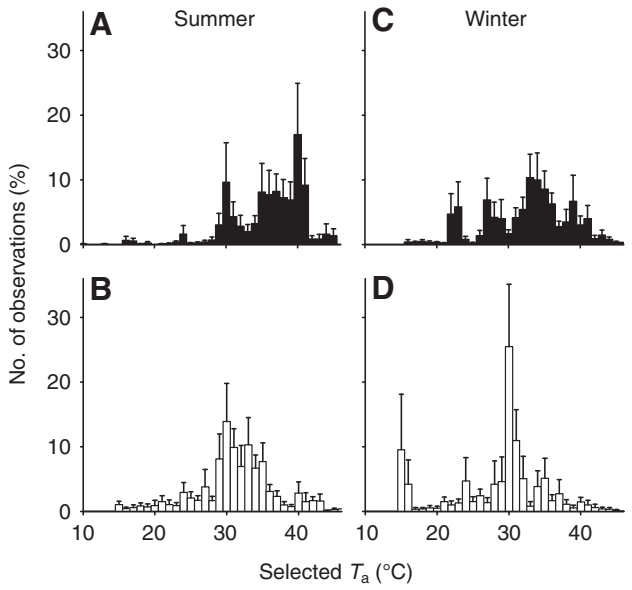

Fig. 5. Histograms of selected ambient temperature (T_a) in normoxia (20.8 kPa, A,C) and hypoxia (9.9 kPa, B,D) from both the summer- (A,B) and winter- (C,D) acclimated animals. All data presented are means (±s.e.m. for visual clarity). Each graph represents a mean of the percentage of the time, within hours 2.5 and 5.5 of the experiment, in which the animal spent at each selected T_a. Selected T_a was binned at 1°C intervals.

the decrease in T_b while $\dot{V}_{O_2}$ remained elevated is that elevated rates of heat loss were sustained throughout hypoxia (Tattersall and Milsom, 2003). The increased f_R observed during hypoxia may contribute to this heat loss, increasing pulmonary convective heat loss as well as evaporative cooling, which in turn would lead to the lower T_b observed (Gordon, 1997). The initial, non-sustained decrease in metabolic rate (Fig. 1) at both temperatures could have been enough to initiate the fall in T_b; however, $\dot{V}_{O_2}$ had recovered by the time steady-state values were recorded, 30 minutes or so after the initiation of hypoxia while T_b remained at a new, lower value. Similarly, while $\dot{V}_{O_2}$ remained unchanged at 22°C, C_{wet} increased in hypoxia, indicating greater potential for heat loss.

The drop in T_b was dependent on T_a in the summer. This response, especially the absence of a significant decrease in $\dot{V}_{O_2}$ at 22°C, indicates a more blunted hypoxic response compared with that of golden-mantled ground squirrels (Barros et al., 2001). This is most probably due to the more mild level of hypoxia (9.9 kPa) used in the present study compared with previous studies (Gordon and Fogelson, 1991), where lower inspired oxygen induced a greater decrease in T_b. Because greater survival in extreme hypoxic conditions has been linked to the ability to reduce T_b (Wood and Stabenau, 1998), a lower T_b would benefit the animal but only if the hypoxia was severe enough to require such a change. As hibernators and semi-fossorial animals, both attributes that coincide with greater tolerance to hypoxia, it is not surprising that 9.9 kPa did not invoke large changes in the T_b of chipmunks.

Table 3. Mean ± s.d. variability (N) of selected T_a and T_b (95th minus 5th percentile) of Eastern chipmunks in normoxia (20.8 kPa) and hypoxia (9.9 kPa) during the experiments in Series 2

	Oxygen level	ΔT_a (°C)	ΔT_b (°C)
Summer	Normoxia	3.3±2.3 (9)	1.6±0.6 (7)
	Hypoxia	4.0±1.8 (9)	1.9±0.7*(7)
Winter	Normoxia	3.9±3.0 (9)	1.9±0.7 (7)
	Hypoxia	5.7±4.6 (9)	2.7±1.0* (7)

*Indicates significant effects of oxygen. Season had no effect on either parameter. T_a, ambient temperature; T_b, body temperature.

The decrease in T_b in hypoxia has often been attributed to a lowering of the T_b set-point and a leftward shift in the lower critical limit of the thermal neutral zone (Branco et al., 2006; Tattersall and Milsom, 2003; Wood, 1991). At times, however, this hypothesis is only partially supported by the data because it can be difficult to distinguish changes in set-point with other factors, such as non-specific increases in heat loss or decreases in thermosensitivity, which could also cause T_b to decrease (Refinetti, 2003; Romanovsky, 2004). In situations such as fever, behavioural thermoregulation acts in the same direction as the metabolic responses to help increase T_b (Gordon, 1993). Similarly, when the hypothalamus is artificially cooled, a process that evokes heat generation mechanisms (Hensel, 1974), animals select higher temperatures (Satinoff, 1964; Adair, 1971) to keep warm. However, in other instances, such as circadian changes in T_b, the behavioural response acts in the opposite direction to that of T_b (Briese, 1985; Gordon, 1994), becoming uncoupled from the response of other thermoeffectors (Briese, 1985). Previous studies on the hypoxic response in mammals have demonstrated such an uncoupling between behavioural and physiological thermoeffectors (Dupré et al., 1986; Gordon and Fogelson, 1991; Gordon et al., 1991; Dupré and Owen, 1992; Gordon, 1997), although it was not the case in the present study. In both seasons, the selected T_a was lower in hypoxia, in parallel with the decrease in T_b. Interestingly the fluctuations in T_b were greater in hypoxia than normoxia (Table 3) and might be attributed to a hypoxia-induced decrease in thermosensation and thermoregulatory precision (Cadena and Tattersall, 2009).

Although hypoxia induced a decrease in T_b during the thermal gradient trials, the seasonal differences in T_b, both in normoxia and in hypoxia, observed in the fixed temperature respirometry experiments were not readily apparent in the gradient trials. Even though T_b in the thermal gradient from the winter animals was consistently lower than in the summer animals, the difference was not significant. The seasonal difference in T_b in response to hypoxia that was apparent in the metabolic experiments did not translate into differences in hypoxic T_b in the thermal choice experiments. Furthermore, although the difference between normoxic- and hypoxic-selected T_a is greater in the summer than in the winter, it stems from a higher normoxic value, not from a profound decrease in hypoxia. The net result is that when allowed to exhibit behavioural control over T_b, chipmunks select lower T_a, which serves to lower their T_b in hypoxia (Fig. 6), although to a lesser degree than observed under fixed, low T_a values. When given the ability to mitigate the effects of hypoxia behaviourally, chipmunks maintained T_b values closer to normoxic levels, indicating that they are capable of attenuating the hypoxia-induced drop in T_b (Fig. 6). Therefore, it would appear that the response to hypoxia, at least in terms of T_b regulation, is flexible and under the control of the animal rather than being an uncoordinated hypothermic response. It is likely to be the result of complex interactions between a suppression of thermogenesis, an increase in breathing rate and peripheral heat loss, as well as a decrease in thermosensitivity and thermoregulatory precision. Furthermore, hypoxia has recently been shown to reduce thermoregulatory precision in an ectotherm (Cadena and Tattersall, 2009). The increase in shuttling and variability of selected T_a in hypoxia in chipmunks implies a similar change in thermoregulatory precision in an endotherm, providing a more universal understanding

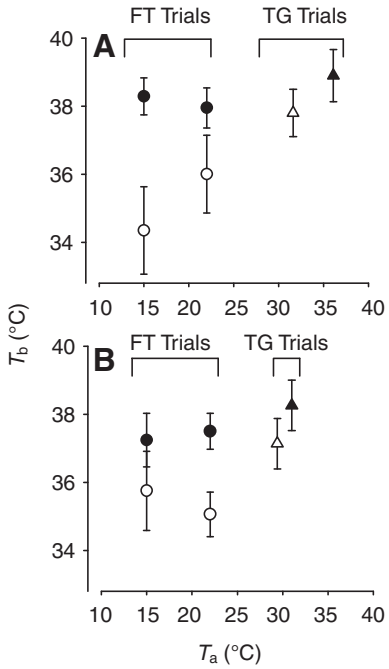

Fig. 6. Mean body temperature (T_b) (±s.d.) from Eastern chipmunks during the summer (A) and the winter phases (B) during normoxia (20.8 kPa, closed symbols) and hypoxia (9.9 kPa, open symbols) across both series of experiments. Circles denote data collected during the Series 2 respirometry experiments (fixed temperature trials, FT); triangles represent data from the Series 2 thermal gradient experiments (thermal gradient trials, TG).

of thermoregulatory control, and suggests that the drop in T_b observed in hypoxia may be partially due to alterations in thermosensation and not exclusively due to a regulated decrease in T_b.

Seasonal changes in hypoxia tolerance

The chipmunks in the present study demonstrated a seasonality characteristic of mammalian heterotherms. Torpor expression was restricted to the months of December through to April, and normothermic T_b values that occurred during the periodic arousals were consistently lower during this period (Levesque, 2008). This seasonality in T_b regulation manifested as a relatively large increase in EWL in the summer, primarily due to an increase in f_R but only as slight changes in $\dot{V}_{O_2}$ and no change in thermal conductance. As hibernators, it is perhaps not surprising that chipmunks undergo very few metabolic changes in response to cold acclimation. Instead of increasing basal metabolic rate or insulation, as is common in cold-adapted animals (Hart, 1971; Chappell, 1980), chipmunks respond to cold temperatures through torpor.

The data presented in the current study indicate that there were indeed seasonal differences in the responses of normothermic Eastern chipmunks to hypoxia. Winter-acclimated animals showed attenuated responses to hypoxia, with respect to T_b and heat production. This blunted response to hypoxia is believed to be related to the phenotypic traits that allows for the extended periods of torpor that occur in the winter but not in the summer. However, many physiological traits (body mass, basal metabolic rate, minimal thermal conductance, the placement of the thermal neutral zone) scarcely differ seasonally (Levesque, 2008). In other hibernating species, biochemical traits, however, such as blood glucose, mitochondrial and whole tissue properties have been found to have seasonal differences (Woodward and Condrin, 1945; Pehowich and Wang, 1984; Carey et al., 2003). Although the low blood glucose levels observed in hibernators are most likely to be the result of torpor expression itself, and not a factor in promoting it (Hannon and Vaughan, 1961), changes in temperature sensitivity at the mitochondrial level appear to be essential for torpor to occur (Pehowich and Wang, 1984). Interestingly, the RER decreased to values below 0.7 in hypoxia in the winter. This transient decrease below the lowest levels normally expected (Blaxter, 1989) could mean either CO_2 retention (Frappell et al., 1992) or increased gluconeogenesis (Schutz and Ravussin, 1980) was occurring in hypoxia in the winter. If the winter phenotype bestows metabolic changes that allow for providing glycolytic fuels for the brain (Carey et al., 2003), it is possible that an altered carbohydrate metabolism also accompanies the hypoxia-tolerant phenotype. Despite a wide ranging interest in the hypoxia tolerance in hibernators as a potential biomedical model for understanding natural mechanisms of ischemia-reperfusion injury (Drew et al., 2004; Drew et al., 2007; Nathaniel, 2008), no study has empirically tested how seasonal differences would influence hypoxia tolerance, *per se*.

Nevertheless, there are a few possible explanations as to why winter animals showed a blunted response to hypoxia. The first is that, similar to hypoxia-tolerant geese (Scott et al., 2008) and hamsters (Walker et al., 1985), winter chipmunks were slightly less responsive to hypoxia because of a lower sensitivity to low levels of oxygen. In the wild, hibernating animals may be pre-conditioned to hypoxia due to the propensity for their burrows to exhibit lower than normal oxygen levels (Kuhnen, 1986). Maclean (Maclean, 1981) found oxygen levels as low as 18% in chipmunk burrows, although he reported lower levels for other species of burrowing mammals; European rabbits had concentrations as low as 13%. Nevertheless, as animals in the present study were housed under normoxic conditions throughout, the attenuated hypoxic response observed in winter animals must be innate; the more likely explanation is that it relates to torpor expression. Hibernating species are more hypoxia-tolerant than non-hibernators (Hiestand et al., 1950; Drew et al., 2004); therefore, it follows that within a species, individuals from the hibernation season would be more hypoxia tolerant than individuals from the non-hibernation season. In fact, Ma and colleagues (Ma et al., 2005) found the blood of normothermic winter ground squirrels was slightly hypoxic when compared with rats or torpid ground squirrels, suggesting that they exhibit and tolerate lower levels of oxygen in the winter. However, although Ma and colleagues (Ma et al., 2005) reported low blood oxygen partial pressure (P_{O_2}) values, the oxygen affinity of ground squirrel blood is higher in the winter than in the summer (Maginniss and Milsom, 1994), suggesting that oxygen content and delivery are sustained throughout the year, and that winterised, normothermic animals are not, in fact, hypoxemic. Furthermore, processes associated with entry into, maintenance and arousal from torpor could precondition the animal to hypoxia. If this is the case, exposure to low oxygen would be met with little or no change in metabolism, because the animals would not have perceived the hypoxia as a challenge to their normal physiological responses. In addition, the relatively larger increase in f_R in hypoxia in the winter may serve to promote more effective blood–gas regulation, thereby mitigating the influence of hypoxia on thermoregulation and metabolism.

However, because the seasonal differences in the thermoregulatory response to hypoxia were only apparent at 15°C and not at 22°C, another explanation may account for the more blunted response to hypoxia in the winter animals. Our winter

experiments were undertaken during inter-bout arousals when their physiological imperative would have been to maintain an elevated T_b (Willis, 1982; Heldmaier et al., 2004). Hibernators do this in order to cope with a number of physiological exigencies, ranging from the disposal of accumulated wastes, repaying sleep debt, repairing damaged neurons, augmenting protein synthesis to dealing with osmoregulation (Willis, 1982; Daan et al., 1991; Popov et al., 1992; Thomas and Geiser, 1997; Martin et al., 2004). Regardless of the cause, during their inter-bout arousals, animals would have been primed for maintaining normothermia at this time rather than exhibiting pronounced decreases in thermoregulatory states. Similarly, the selection of T_a by chipmunks in hypoxia was also blunted in the winter. The animals in the summer phase had a greater decrease in preferred T_a than those in the winter. However, it is interesting to note that, despite the greater decrease in selected T_a (~1°C) in the summer compared with the winter (~5°C), selected T_a and T_b in hypoxia did not differ between the two seasons. There was also some overlap between the selected temperatures in normoxia and hypoxia in the winter. Therefore, the decrease in selected T_a was likely driven by the elevated summer values. In both seasons, animals in hypoxia appeared to shuttle, moving from the cold side to the hot side of the gradient; however, this response was more evident in the summer, suggesting fundamental differences in thermal detection between the seasons as well. Regardless, it appears that the behavioural thermoregulatory response acted in parallel to metabolic heat production to facilitate a decrease in T_b in hypoxia.

Concluding remarks

Although seasonal differences in T_b regulation in response to acute hypoxia were observed in the present study, it remains unclear whether these responses can be attributed to an increase in tolerance to hypoxia or a decrease in sensitivity to low oxygen levels during the hibernation season. Part of the problem may be the use of the term 'hypoxia tolerance'. Some studies, including the present one, have used this term to indicate decreased responsiveness to hypoxia (Hiestand et al., 1950). Others have used it to refer to the ability to maintain normal functions in low ambient oxygen concentrations, either *via* normal, sustained rates of metabolic activity in moderate hypoxia or by increased survival times in anoxia (Faleschini and Whitten, 1975; Lutton, 1982; Wood and Stabenau, 1998). Although at first these two possibilities would seem interchangeable, difficulties arise given the fact that greater decreases in T_b and metabolism in hypoxia, i.e. a greater response to acute hypoxia, can be beneficial in terms of long term survival (Faleschini and Whitten, 1975). Golden-mantled ground squirrels (a hibernating species) are known to survive anoxia longer than rats but their acute metabolic and thermoregulatory response to hypoxia is much greater than that of rats (Barros et al., 2001). However, if forced to exhibit activity in hypoxia, this sciurid was also capable of maintaining higher aerobic metabolic rates than rats (Lutton, 1982). Thus, it is unclear whether their hypoxia tolerance is bestowed by their greater decline in T_b and metabolism or by an inherent difference in the activation of cellular and metabolic defence mechanisms that mitigate the influence of low oxygen. It would appear, therefore, that the term 'hypoxia tolerance' can refer to either a decrease in sensitivity to hypoxia or a greater ability to compensate for low levels of ambient oxygen. In these cases, the attenuated response to hypoxia can be considered a sign of greater hypoxia tolerance. Future examination of these kinds of questions requires an integrated approach, incorporating natural changes (e.g. seasonal) in an animal's physiology as well as behavioural responses, in order to determine what factors contribute the most to increased survival in low oxygen concentrations.

LIST OF ABBREVIATIONS

C_{wet}	wet thermal conductance ($ml\,O_2\,g^{-0.75}\,h^{-1}\,°C^{-1}$)
EWL	rate of evaporative water loss ($mg\,g^{-0.75}\,h^{-1}$)
f_R	breathing rate (min^{-1})
RER	respiratory exchange ratio (ratio of $\dot{V}_{CO_2}$:$\dot{V}_{O_2}$)
T_a	ambient temperature (°C)
T_b	body temperature (°C)
$\dot{V}_{CO_2}$	volumetric rate of carbon dioxide produced by the animal ($ml\,CO_2\,g^{-0.75}\,h^{-1}$)
$\dot{V}_{O_2}$	metabolic rate assessed as the volumetric rate of oxygen consumed by the animal ($ml\,O_2\,g^{-0.75}\,h^{-1}$)
WVD	water vapour density (absolute humidity) of the air leaving the chamber ($mg\,ml^{-1}$)

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