

# Energetics of arousal episodes in hibernating arctic ground squirrels

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**Abstract** Arctic ground squirrels overwintering in northern Alaska experience average soil temperature of  $-10^{\circ}\text{C}$ . To examine energetic costs of arousing from hibernation under arctic compared to temperate conditions, captive ground squirrels were maintained in ambient temperatures ( $T_a$ ) of 2,  $-5$  and  $-12^{\circ}\text{C}$ . Rates of oxygen consumption and carbon dioxide production were used to estimate metabolic rate and fuel use during the three phases of arousal episodes: rewarming, euthermia, and recooling. Respiratory quotient comparisons suggest exclusive use of lipid during rewarming and mixed fuel use during euthermia. Animals rewarming from torpor at  $T_a -12^{\circ}\text{C}$  took longer, consumed more oxygen, and attained higher peak rates of oxygen consumption when compared to  $2^{\circ}\text{C}$ .  $T_a$  had no significant effect on cost or duration of the euthermic phase. Animals re-cooled faster at  $-12^{\circ}\text{C}$  than at  $2^{\circ}\text{C}$ , but total oxygen consumption was not different.  $T_a$  had no significant effect on the total cost of arousal episodes when all three phases are included. Arousal episodes account for 86% of estimated costs of a complete hibernation cycle including torpor when at  $2^{\circ}\text{C}$  and only 23% at  $-12^{\circ}\text{C}$ . Thus, due to the higher costs of steady-state metabolism during torpor, proportional metabolic costs of

arousal episodes at  $T_a$  characteristic of the Arctic are diminished compared to relative costs of arousals in more temperate conditions.

**Keywords** Oxygen consumption · Ground squirrel · *Spermophilus* · Metabolic rate · Arousal · Arctic

## Abbreviations

$T_a$	Ambient temperature
$T_b$	Body temperature
MR	Metabolic rate
BMR	Basal metabolic rate
RQ	Respiratory quotient

## Introduction

Hibernating ground squirrels alternate between extended bouts of torpor (1–3 weeks) characterized by profound hypometabolism and low body temperatures and short arousal episodes (1–2 days) (Twente and Twente 1965; Barnes and Ritter 1993). Arousal episodes include three phases: rewarming, euthermia, and recooling. During rewarming, metabolic heat production, originating from non-shivering thermogenesis in brown adipose tissue (BAT) and muscular shivering, increase to elevate core body temperature ( $T_b$ ) from minimum levels during torpor to euthermic, or normothermic levels. The 10–15 h interbout euthermic phase consists of limited activity, while animals usually remain inside their nests (Lyman 1948; Daan et al. 1991). The recooling phase begins when metabolic rate (MR) decreases below levels necessary for homeothermy, and  $T_b$  gradually declines (Heldmaier et al. 2004) to a new set point level that

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usually approximates ambient temperature in above freezing conditions. The universal presence of regular arousal episodes among small hibernating mammals suggests a fundamental limit to the length of time torpor can be sustained before a return to high  $T_b$  and metabolic rate is required (Willis 1982).

Energetics of hibernation in ground squirrels and other small mammals held at ambient temperatures ( $T_a$ ) representative of temperate or alpine environments where soils do not freeze have been well investigated. Thermal conforming animals in steady-state torpor maintain  $T_b$  0.5–2°C above  $T_a$  and have very minimal rates of metabolism, as low as 0.01 ml  $O_2$   $g^{-1}$   $h^{-1}$  or 1–2% of basal metabolic rate (BMR) (Geiser 1988, 2004; Buck and Barnes 2000). Metabolic rate during rewarming can reach levels that are several times BMR and during euthermia are typically maintained above basal rates due to the requirement for thermoregulation. Arousal episodes are reported to be the most energetically costly component of hibernation for ground squirrels overwintering in above freezing conditions and are estimated to represent the great majority of the energy expended over the entire heterothermic season. There are few reported measurements of metabolic rate during hibernation under freezing conditions, however. Two studies (Heller and Colliver 1974; Geiser and Kenagy 1988) included slightly subzero temperatures, and one study (Buck and Barnes 2000) measured metabolic rate, respiratory quotient, and  $T_b$  during steady-state torpor at  $T_a$ 's to  $-16^\circ\text{C}$ . None of these studies included the energetics of arousal episodes.

Arctic ground squirrels (*Spermophilus parryii*) overwintering in northern Alaska experience winter-long average soil temperatures at hibernacula depth (1 m) of  $-10^\circ\text{C}$  and minima of  $-23^\circ\text{C}$  (Buck and Barnes 1999), and they adopt minimum core  $T_b$  as low as  $-2.9^\circ\text{C}$  (Barnes 1989). These environmental conditions require that arctic ground squirrels are continuously thermogenic during most of the hibernation season. In the present study, we contrasted the metabolic costs of arousal episodes in arctic ground squirrels hibernating in above freezing conditions to costs during hibernation at  $T_a < 0^\circ\text{C}$  within the range of those experienced by animals in their natural burrows. We hypothesized that energetic costs and duration of the rewarming phase would increase with decreasing  $T_a$  and that animals would maintain interbout euthermia for shorter durations at lower  $T_a$  to minimize energetic costs. Finally, using data on the increased cost of maintaining steady-state torpor at  $T_a < 0^\circ\text{C}$  from Buck and Barnes (2000) in combination with field data on duration of torpor bouts (Buck et al. 2008; Barnes et al. unpublished), we calculated the proportional costs of arousal episodes within the entire heterothermic season.

## Materials and methods

### Animals

Arctic ground squirrels (*S. p. kenocottii*, 10 adult males, 2 adult females) were captured in August near Toolik Lake, Alaska ( $68^\circ38'\text{N}$ ,  $149^\circ38'\text{W}$ ) and transported by truck to the University of Alaska Fairbanks where they were housed individually in cages within environmental chambers. Animals were maintained on a 12L:12D photoperiod and in a  $T_a$  of  $2^\circ\text{C}$  and provided water and ad lib Masuri Rodent Chow supplemented with sunflower seeds, carrots, and apples.

At least 1 month prior to the start of hibernation, temperature-sensitive radiotransmitters (Minimitter Inc., Sunriver, OR) were implanted into the peritoneal cavity of each animal (body mass range 653–943 g). Before implant, transmitters were sealed in heat shrink tubing and triple coated in Elvax (Minimitter Inc.), creating a package weight of approximately 17 g, calibrated to the nearest  $0.1^\circ\text{C}$  at 0 and  $20^\circ\text{C}$  against a precision mercury glass thermometer, and gas sterilized (Long et al. 2007). Surgeries were performed under isoflurane anesthesia. Signals from the radiotransmitters were received using a model RA1010 receiver with a computer interface for data acquisition (Data Sciences International, Saint Paul, MN).  $T_b$  and an index of locomotor activity, indicated by movements of the animal in its cage relative to two orthogonally mounted bar antennae, were recorded each 5 min.

Animals were randomly assigned to experimental treatment groups of  $T_a$  2,  $-5$  or  $-12$  (each  $\pm 2^\circ\text{C}$ ) and maintained in hanging wire cages ( $28 \times 49 \times 20$  cm) with cotton batting for nest construction. Food and water were removed after hibernation began. Prior to metabolic trials, animals had been hibernating at each experimental temperature for at least 14 days. Metabolic rate and respiratory quotient (RQ) during arousal episodes were estimated by open flow respirometry (details below). Ground squirrels that had been torpid continuously for 6–11 days were placed individually into sealed lexan chambers ( $42 \times 22 \times 20$  cm) positioned over a radio receiver. Metabolic chambers contained 3–5 cm of wood chips and 85 g of cotton batting as nesting material. No food or water was available within the chamber. Transfer of the animal from cage to chamber initiated an arousal as discerned by sustained increases in oxygen consumption and  $T_b$ .

For measuring rates of oxygen consumption and carbon dioxide production, room air was pulled through the metabolic chamber with a vacuum pump at  $2 \text{ l min}^{-1}$ , or  $0.3$  followed by  $3 \text{ l min}^{-1}$ ; the low to high flow rate sequence was used for better resolution of rates of oxygen consumption during early rewarming. Flow rate was

determined by a two different mass flowmeters; model HFM-229H, 0–0.3 l/min and AFSC-10 K, 0–10 l/min, respectively (Teldyne Hastings-Raydist, Hampton, VA). Flow meters were calibrated by measuring weight loss of a cylinder of compressed, dry air as its contents passed through the flow meter (Tøien et al. 2001).

Excurrent air was drawn through calcium chloride to remove moisture prior to measurements of flow or gas concentrations. A subsample was passed through an oxygen analyzer (Applied Electrochemistry model S-3A, Thermo Instruments Division, Pittsburgh, PA) and then through an infrared carbon dioxide analyzer (Beckman model 864, Fullerton, CA). Each 2–10 h, CO<sub>2</sub> and O<sub>2</sub> analyzers were calibrated with ambient air and the CO<sub>2</sub> analyzer additionally with a span gas of 1% CO<sub>2</sub>.

The analyzers were connected to a 12-bit AD converter (Labmaster TM-40 PGL, Scientific Solutions Inc., Solion, OH). Averages of flow rate, CO<sub>2</sub>%, O<sub>2</sub>%, and room and metabolic chamber temperatures were logged onto a computer each minute. With 9,600 times oversampling the resolution was better than 0.0005% O<sub>2</sub> and 0.0001% CO<sub>2</sub>. The data acquisition software (modified version of LabGraph, Tøien 1992) linearized the output from the CO<sub>2</sub> analyzer and automatically corrected data from both gas analyzers for baseline and span drift by linear interpolation.

Metabolic rate, represented by mass specific rate of oxygen consumption (ml g<sup>-1</sup> h<sup>-1</sup>), was calculated by the data acquisition software using the following equations to compensate for respiratory volume change according to the principles of the Haldane transformation (Haldane 1912; Wagner et al. 1973).

$$\text{O}_2 \text{ consumption} = ((\text{Flow}_I \times \text{FI}_{\text{O}_2}) - (\text{Flow}_E \times \text{FE}_{\text{O}_2}))/\text{BM}$$

$$\text{Flow}_I = \text{Flow}_E \times (1 - \text{FE}_{\text{O}_2} - \text{FE}_{\text{CO}_2}) / (1 - \text{FI}_{\text{O}_2} - \text{FI}_{\text{CO}_2})$$

Flow<sub>I</sub> = air flow (STPD, l h<sup>-1</sup>) entering chamber, Flow<sub>E</sub> = air flow (STPD, l h<sup>-1</sup>) exiting chamber, FI<sub>O<sub>2</sub></sub> = fraction of O<sub>2</sub> entering chamber, FE<sub>O<sub>2</sub></sub> = fraction of O<sub>2</sub> exiting chamber, FI<sub>CO<sub>2</sub></sub> = fraction of CO<sub>2</sub> entering chamber, and FE<sub>CO<sub>2</sub></sub> = fraction of CO<sub>2</sub> exiting chamber, BM = body mass (kg). Temperature of the metabolic chamber was measured with a 30-ga copper-constantan thermocouple that was threaded through the lid of the chamber, extending 1–2 cm into the chamber and connected to a thermocouple amplifier (AD595, Analog devices Inc., Norwood, MA) and linearized in software (Tøien 1992). The resolution after digitizing was 0.05°C and further improved to 0.01°C by oversampling. It was calibrated in software against an NIST traceable reference thermometer in a temperature controlled water bath or stirred ice bath at 20 and 0°C. The integrity of the respirometry system was assessed every 30 days or less

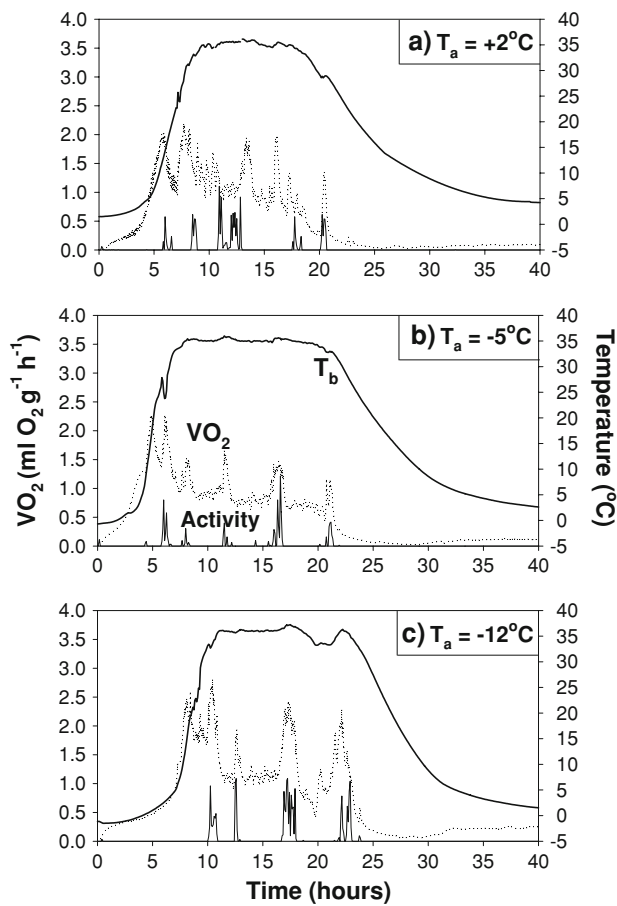
during data collection by burning known amounts of 100% ethanol in the respirometry chamber with a small lamp. The stoichiometric equation of ethanol combustion was used to verify mass loss of an ethanol lamp placed inside the metabolic chamber, allowed to burn until it extinguished, and reweighed after complete washout of chamber. The ethanol burn also provided a check of the calibration gas for the CO<sub>2</sub> analyzer because the RQ of burning ethanol is 0.6667. Oxygen consumption during ethanol burns deviated by 2.2% (0.025 ± 0.006 l/h), and RQ deviated by 1.8% (mean RQ of ethanol trials = 0.6783 ± 0.0116).

#### Data analysis

We delimited the beginning of the rewarming phase of arousal as when animals were moved into the metabolic chamber and the end as when *T<sub>b</sub>* increased to ≥30°C. The recooling phase began when *T<sub>b</sub>* decreased and remained ≤30°C and ended when *T<sub>b</sub>* fell to ≤5°C. The duration of the euthermic phase was thus defined as from *T<sub>b</sub>* ≥ 30°C to first *T<sub>b</sub>* ≤ 30°C (Barnes and Ritter 1993). Metabolic rate and *T<sub>b</sub>* during euthermia were averaged from the first *T<sub>b</sub>* > 35.0°C to first *T<sub>b</sub>* < 35.0°C, including and excluding periods of elevated MR associated with movement, as detected by activity units registered by the receiver. *T<sub>b</sub>* > 35°C was chosen for the average non-active MR rate instead of *T<sub>b</sub>* > 30°C to exclude depression in MR at the beginning of the recooling phase and decreases in MR associated with transient decreases in *T<sub>b</sub>*, as occurred in Fig. 1c.

The phases of each arousal episode (rewarming, euthermia, and recooling) were analyzed to determine average duration, average MR, and total oxygen consumed. Respiratory quotient was measured during rewarming and euthermia and was arcsine square root transformed prior to statistical analysis. Respiratory quotient values during recooling are not reported because these were considered to be unreliable as MR decreased rapidly while flow rates of air remained high. Peak (maximum) MR, *T<sub>b</sub>* at initiation of trial and duration from initiation of trial to peak MR during rewarming were determined. Total costs of arousal episodes were calculated by summing the oxygen consumption of each phase. The proportional cost of arousal episodes as part of a complete cycle of hibernation that includes the torpor bout was estimated. Costs of torpor were calculated by multiplying mean MR during torpor (Buck and Barnes 2000) by the average torpor bout durations from field animals (Buck et al. 2008; Barnes et al. unpublished) that correspond to the three *T<sub>a</sub>* treatments (2, –5 and –12°C) used in this study.

Data obtained from the three *T<sub>a</sub>* treatments were compared using the SAS general linear model for analysis of unbalanced designs. Pair-wise comparisons were obtained



**Fig. 1** Individual recordings of metabolic rate (MR), core body temperature ( $T_b$ ), and activity during an arousal episode in representative arctic ground squirrels hibernating at ambient temperatures ( $T_a$ ) **a** 2°C, **b** -5°C, and **c** -12°C

using least square means (Zar 1996). Results of statistical comparisons of metabolic parameters did not change when data were reanalyzed on a mass-independent basis using the general linear model with mass included as an independent variable (Hayes and Shonkwiler 1996). Each mean contains 1–2 females; interactions and covariation between sex and the parameters measured had no significant effect on the general linear model. Values are reported as mean  $\pm$  standard errors. For measurements of resting MR at each  $T_a$ , 30-min averages of rates of oxygen consumption were calculated, when MR was stable at minimal levels and not associated with activity. The corresponding 30-min average of  $T_b$  was also recorded during each minimum MR measurement.

## Results

Patterns of rewarming, euthermia, and recooling during arousal episodes in arctic ground squirrels were generally similar at the three  $T_a$ 's (Fig. 1).

### Rewarming phase

Body temperatures of torpid animals at the initiation of arousal differed among  $T_a$ 's, with  $T_b$  approximating ambient at  $T_a$  2°C and averaging  $-0.17^\circ\text{C}$  at  $T_a$  -5 and  $-1.44^\circ\text{C}$  at  $T_a$  -12°C (Table 1). During rewarming,  $T_a$  had a significant effect on both duration of the phase and the intensity of metabolism. Rewarming from the initiation of arousal to a  $T_b$  of 30°C took 45% longer (2.5 h increase) at  $T_a$  -12°C than at 2°C ( $P = 0.018$ ), while time to  $T_b$  30°C at -5°C was intermediate and not significantly different from the other  $T_a$  groups (Table 1). However, when the time to rewarm from a  $T_b$  of 5 to 30°C was compared, there was no difference among  $T_a$  groups ( $P = 0.12$ ; average for all groups  $2.29 \pm 0.13$  h).

Compared to animals hibernating at 2°C, time from the initiation of arousal to when MR reached peak levels was 53% (2.2 h increase) longer at -5°C ( $P = 0.034$ ) and 67% (2.8 h) longer for animals at  $T_a$  -12°C ( $P = 0.002$ ). Peak MR also averaged 28% higher at  $T_a$  -12°C than in 2°C ( $P = 0.002$ ) and 42% higher at -12°C than at -5°C ( $P = 0.002$ ). Animals rewarming at  $T_a$  -12°C consumed 42% more total oxygen to reach  $T_b$  30°C than animals rewarming at  $T_a$  2°C ( $P = 0.006$ ); total oxygen consumed at  $T_a$  -5°C for rewarming was intermediate and not significantly different from the other groups (Fig. 2a).

Respiratory quotient during the thermogenesis of rewarming did not differ among groups and averaged 0.71–0.72 (Table 1).

### Euthermic phase

There were no differences among groups in either duration of interbout euthermia, calculated as the amount of time with  $T_b > 30^\circ\text{C}$ , or in total oxygen consumed during that time (Table 1; Fig. 2a). Average MR, excluding intervals with activity, did differ. Animals consumed 47% more oxygen while resting at  $T_a$  -12°C as compared to at 2°C ( $P = 0.005$ ) (Table 1).  $T_b$  did not differ among groups when averaged either with or without intervals of activity. Respiratory quotient during interbout euthermia (0.77–0.79) was higher than during rewarming in all  $T_a$  groups ( $P = 0.002$ –0.03) (Table 1).

### Recooling phase

Ground squirrels reentered torpor more rapidly and sustained a higher MR while recooling at low compared to high  $T_a$ . It took approximately one-third as long at  $T_a$  -12°C ( $P = 0.005$ ) and one-half as long at  $T_a$  -5°C for animals to recool to a  $T_b$  of 5°C, compared to in animals at  $T_a$  2°C. Average MR during recooling at  $T_a$  -12°C was twofold higher than at 2°C ( $P = 0.014$ ), but when duration

**Table 1** Metabolic rate (MR) and body temperature ( $T_b$ ) during hibernation in arctic ground squirrels at ambient temperatures ( $T_a$ ) of 2, -5 and -12°C

$T_a$ (°C)	$T_b$ (°C)	Duration (h)	Average MR (ml g <sup>-1</sup> h <sup>-1</sup> )	Total O <sub>2</sub> (ml g <sup>-1</sup> )	Time to peak MR (h)	Peak MR (ml g <sup>-1</sup> h <sup>-1</sup> )	RQ
Initial rewarming phase							
2	2.37 ± 0.38a	5.65 ± 0.51a	0.96 ± 0.06	5.42 ± 0.28a	4.25 ± 0.30a	2.65 ± 0.22a	0.72 ± 0.01
-5	-0.17 ± 0.24b	7.04 ± 0.44a,b	0.91 ± 0.05	6.41 ± 0.38a,b	6.49 ± 0.41b	2.39 ± 0.15a	0.71 ± 0.01
-12	-1.44 ± 0.40b	8.20 ± 0.89b	0.94 ± 0.08	7.71 ± 0.34b	7.08 ± 0.65b	3.40 ± 0.18b	0.71 ± 0.01
Interbout euthermia							
2	35.38 ± 0.53	14.75 ± 2.23	0.94 ± 0.10a	13.87 ± 2.31	b	b	0.79 ± 0.02
-5	35.49 ± 0.30	17.49 ± 2.09	1.06 ± 0.07a	18.36 ± 2.47	b	b	0.78 ± 0.01
-12	35.60 ± 0.32	14.58 ± 2.74	1.38 ± 0.05b	20.12 ± 3.22	b	b	0.77 ± 0.02
Initial recooling phase							
2	30	20.15 ± 2.39a	0.06 ± 0.01a	1.21 ± 0.24	b	b	b
-5	30	10.65 ± 1.94b	0.09 ± 0.01a,b	0.96 ± 0.26	b	b	b
-12	30	7.68 ± 0.63b	0.12 ± 0.01b	0.92 ± 0.26	b	b	b
Steady-state torpor <sup>a</sup>							
2	2.2	230	0.014 ± 0.001a	3.22	b	b	0.72 ± 0.02a
-5	-0.5	389	0.053 ± 0.004b	20.67	b	b	0.77 ± 0.01b
-12	-1.5	470	0.134 ± 0.008c	62.98	b	b	0.82 ± 0.02c

Values are mean ± SE. For each table cell,  $n = 5-11$ , mean = 6.2. Values for average MR during euthermia do not include intervals when animals were moving

Significant differences among  $T_a$  groups are denoted with different letters

<sup>a</sup> Values for  $T_b$ , MR, and RQ during torpor are from Buck and Barnes (2000); duration of torpor bouts is from field data (Buck et al. 2008; Barnes et al. unpublished)

<sup>b</sup> Not applicable or not available

of the recooling phase is considered, total oxygen consumed during the recooling phase did not differ among groups (Fig. 2a).

#### Total and proportional costs of an arousal episode

The total amount of oxygen consumed over all three phases of the arousal episode was 20.50 ± 2.44 ml O<sub>2</sub> g<sup>-1</sup> for  $T_a$  2°C, 25.73 ± 2.43 ml O<sub>2</sub> g<sup>-1</sup> for  $T_a$  -5°C, and 28.75 ± 2.53 ml O<sub>2</sub> g<sup>-1</sup> for  $T_a$  -12°C and did not significantly differ among groups ( $P = 0.399$ ). These averages do not include increases in metabolism associated with activity and the energy required for warming between  $T_b$  30 and 35°C, as phases of the arousal episode were defined as  $\leq T_b$  30°C for rewarming and  $T_b > 35°C$  for euthermia.

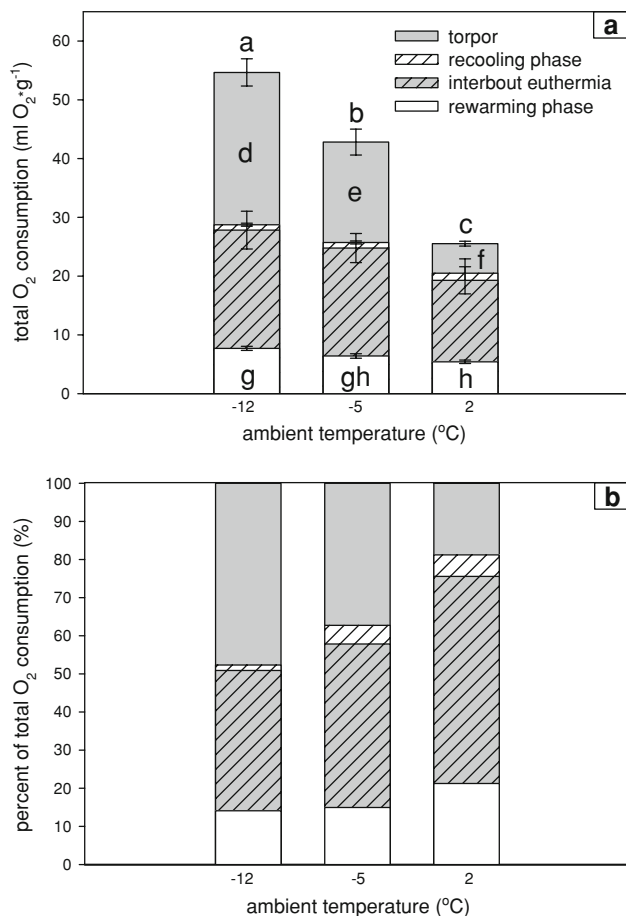
To calculate the total cost of a cycle of hibernation composed of an arousal episode followed by a torpor bout, we combined the average costs of an arousal episode at each  $T_a$  with the average metabolic costs of steady-state torpor in arctic ground squirrels at the same  $T_a$ 's from Buck and Barnes (2000) multiplied by the average duration of torpor bouts at the same  $T_a$ s as measured in field animals (from Buck et al. 2008; Barnes et al. unpublished) (Table 1). These results predict the total average cost of a

hibernation cycle in equivalencies of oxygen consumed as 23.7 ml O<sub>2</sub> g<sup>-1</sup> at 2°C, 46.4 ml O<sub>2</sub> g<sup>-1</sup> at -5°C, and 91.7 ml O<sub>2</sub> g<sup>-1</sup> at -12°C. Animals would consume 95% more oxygen during one hibernation cycle at  $T_a$  -5°C and 191% more at  $T_a$  -12°C, compared to a hibernation cycle at  $T_a$  2°C (Fig. 2a). In this model the cost of the arousal episode in proportion to the total hibernation cycle decreases at subzero  $T_a$ 's due to the relative increase in oxygen consumed that is necessary for thermoregulation during torpor. At  $T_a$  2°C rewarming represents 23%, euthermia 58%, recooling 5% (total for the arousal episode 86%), and torpor 14% of the hibernation cycle, compared to at  $T_a$  -5°C rewarming 14%, euthermia 39%, recooling 2% (total 55%), and torpor 45%, and at  $T_a$  -12°C, rewarming 9%, euthermia 17%, recooling 1% (total 27%), and torpor 73% (Fig. 2b).

#### Discussion

This study investigates the energetic costs of arousal episodes in arctic ground squirrels hibernating under ambient conditions representative of the Arctic. The population of arctic ground squirrels from which animals for this study were drawn regularly experience soil temperatures during

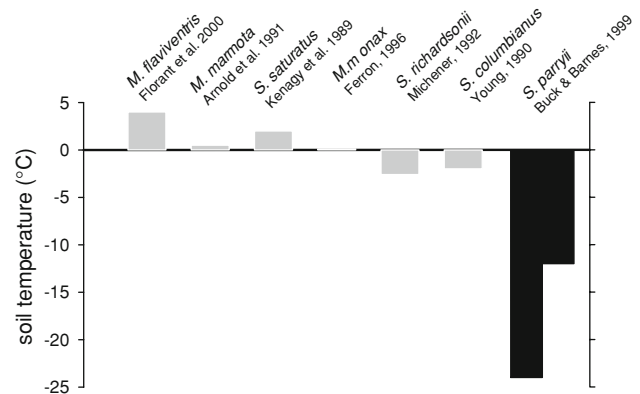




**Fig. 2** Effect of ambient temperature ( $T_a$ ) on each phase of hibernation expressed as totals associated with a single hibernation cycle, one arousal episode and one torpor bout (a) and as proportion of the total oxygen consumption for the hibernation season (b). Unlike letters on the bars represent statistically significant differences

winter that average between  $-5$  and  $-13^\circ C$  over the 7 month period of October–April, with minima to  $-23^\circ C$  (Buck and Barnes 1999); these conditions are extreme compared to those reported for hibernacula of other hibernators. Minimum soil temperatures reported for burrows of other ground squirrels and marmots remain mostly above freezing even in alpine and northern plains environments (Fig. 3). In these non-arctic environments, thick snow cover can insulate hibernacula that may be deeper than those of arctic ground squirrels, which are limited to depths of 70–100 cm by permafrost and typically have only 12–35 cm of snow cover (Buck and Barnes 1999). Winter air temperatures in the Arctic are also very low, averaging  $-25^\circ C$  October–April, near the site where animals were collected.

Extreme thermal conditions of the Arctic present a metabolic challenge not only for arousing animals rewarming to euthermia, but also for animals in torpor that maintain significant thermal gradients between



**Fig. 3** Minimum overwinter hibernacula temperatures (gray bars) reported for the Sciurids *Marmota flaviventris* (Florant et al., 2000), *M. marmota* (Arnold et al., 1991), *S. saturatus* (Kenagy et al., 1989), *M. monax* (Ferron, 1996), *S. richardsonii* (Michener, 1992), *S. columbianus* (Young, 1990) and minimum and mean overwinter hibernacula temperatures (black bars) for *S. parryii* (Buck and Barnes 1999) near Toolik Lake, Alaska

temperatures of the body and that of the surrounding soil over torpor bouts of 2–3 weeks in length (Barnes and Ritter 1993). Buck and Barnes (2000) demonstrated that MR during steady-state torpor increases proportionately as  $T_a$  is decreased below  $0^\circ C$ ; MR during torpor at  $T_a -5$  and  $-12^\circ C$  are 2.8- and 8.6-fold higher, respectively, than minimal values measured during steady-state torpor at  $T_a 2^\circ C$  (Table 1).

#### Arousing under arctic conditions

Even though animals hibernating at subzero  $T_a$  in the present study began the rewarming phase of an arousal episode with elevated MR compared to animals at  $2^\circ C$ , it took them longer to arouse. This was partly due to the greater rise in temperature required to reach  $T_b 30^\circ C$  for animals beginning the rewarming process from a lower  $T_b$ , but it was also due to a protracted early rewarming as  $T_b$  warmed to about  $5^\circ C$  as compared to animals in a  $T_a$  of  $2^\circ C$ . This was especially evident in animals at  $T_a -12^\circ C$  (Fig. 1c). This could be due to a low capacity for heat production at low  $T_b$  or a lesser degree of responsiveness of animals with subzero  $T_b$  to the stimulus to arouse. Once animals were actively rewarming, peak rates of thermogenesis and overall costs of rewarming were highest in animals at  $T_a -12^\circ C$ , as they worked to produce the largest gradient between  $T_b$  and  $T_a$  among groups, although rates of rewarming from  $T_b 5$  to  $30^\circ C$  did not differ among groups. Rate of energy expenditure and overall costs of arousal have also been demonstrated to increase with decreasing  $T_a$  in the eastern pygmy-possum, *Cercartetus nanus*, hibernating over a  $T_a$  range of  $0$ – $30^\circ C$  (Song et al. 2000).

## Euthermy

Average  $T_b$  during the euthermic phase of arousals was about 35.5°C and did not differ among  $T_a$  groups. This  $T_b$  is representative of the diurnal minimum reached each night in free-living arctic ground squirrels resting in their burrows during their active season (Long et al. 2005). Duration of the euthermic phase averaged 15.6 h and was not shorter at low  $T_a$ 's, which is contrary to our prediction that ground squirrels minimize costs of arousal episodes in colder conditions by shortening their time at high  $T_b$ . Duration of the euthermic phase in hibernating golden-mantled ground squirrels, *S. lateralis*, whose rates of heat loss and energetic costs of arousal episodes were increased by shaving dorsal fur, were also not different from non-shaved controls (Kauffmann et al. 2004). These results argue that there is an inviolate duration of euthermy that is required for arousal episodes. The functional significance of arousal episodes in hibernators remains controversial, but most hypotheses cite a requirement of high  $T_b$  to enable a physiological or neurobiological mechanism to proceed that is slowed or inhibited in torpor by low  $T_b$  (Galster and Morrison 1975; Trachsel et al. 1991; Barnes et al. 1993; Van Breukelen and Martin 2001; Prendergast et al. 2002). A requirement for a minimum duration of euthermy during arousal episodes is consistent with the need from arousals for a specific product that results from a temperature-dependent rate and time dependent process. Average resting metabolic rate during euthermia in aroused arctic ground squirrels was between 2 and 2.7 times that of BMR, which is estimated at 0.40–0.61 O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> (Scholander et al. 1950; Withers et al. 1979) for this species. This elevation in metabolism in part reflects requirements for thermoregulation to maintain constant  $T_b$  at  $T_a$  that were below the lower critical temperature of 6–10°C for arctic ground squirrels (Erikson 1956).

Total oxygen consumption during the euthermic phase including periods of activity did not differ statistically among  $T_a$  groups, although there was a trend of energetic costs increasing with decreasing  $T_a$ . When resting MR in the absence of detected movement was compared, average rates increased with decreasing  $T_a$ , as was expected as animals defended larger thermal gradients. Arctic ground squirrels mostly sleep during the euthermic phase of arousals with movement related to grooming and nest repair during short periods of wake (Daan et al. 1991).

## Recooling phase

During reentry into torpor, animals cooled faster at low compared to high  $T_a$ , a result that also occurred in shaved ground squirrels that were presumed to have had greater rates of heat loss (Kauffmann et al. 2004). These are not

unexpected results unless entry into torpor is considered to be a regulated process that follows a similar time-course regardless of  $T_a$ , as may be an assumption of the “sliding  $T_b$  set point” hypothesis. This hypothesis (Florant and Heller 1977; Ortman and Heldmaier 2000) suggests that hibernators control rates of change in  $T_b$  during cooling as they defend setpoint values that decrease regularly to a new minimum level that is defended during torpor. Rate of setpoint change during entry into torpor, however, may be affected by  $T_a$  and be reduced more quickly at lower temperatures to allow for a suppression of thermogenesis and faster cooling. Alternatively, the rate of decrease in the setpoint may be faster than the rate of  $T_b$  change in all groups and thus not influence cooling rates in this range of  $T_a$ .

Animals recooling in  $T_a$  -5 and -12°C anticipated the need for thermogenesis during torpor by increasing MR when  $T_b$  was approximately 5°C, well before animals neared the freezing point. Initiation of thermogenesis was evident for animals recooling only at subzero  $T_a$  values (Fig. 1b, c), while no thermogenesis was apparent at  $T_a$  2°C (Fig. 1a). Consequently, even though the duration of the recooling phase was extended at  $T_a$  2°C, the total oxygen consumed was not different than at -5 and -12°C.

## Respiratory quotient

The RQ when averaged over hours is an indicator of the type of fuel that is being oxidized through metabolism. The RQ of 0.71 measured during rewarming in all groups is consistent with a nearly exclusive use of fat as substrate for thermogenesis as animals aroused from torpor to  $T_b$  30°C. Rewarming during arousal is initiated through non-shivering thermogenesis in BAT which primarily uses free-fatty acids as fuel (Wilson et al. 1987; Isler et al. 1987). Shivering thermogenesis is present at a  $T_b$  of 5°C and higher during arousal in arctic ground squirrels (Tøien et al. 2001), and although shivering can be fueled either by carbohydrates or fatty acids in mammals (Haman 2006), from RQ values in the present study shivering also appears to almost exclusively use fatty acids as substrate. There was no transient rise in RQ seen during rewarming in this or in a previous study in this species (Tøien et al. 2001) to suggest that arousing arctic ground squirrels release stored CO<sub>2</sub>, as reported during arousals in the golden-mantled ground squirrel (Snapp and Heller 1981) and after daily torpor in deer mice (Nestler 1990). Due to the rapid decline in metabolic rate and an inability to switch to lower flow rates, we were unable to record gas exchanges with the high accuracy needed for reliable RQ measurements during the recooling phase. As a ratio, RQ is more sensitive to the accuracy of the measurements than O<sub>2</sub> consumption alone, and RQs at low rates of oxygen consumption will

contribute equally to the average, compared to oxygen consumption where low values will contribute less to the average. Thus we were not able to determine whether a transient decrease in RQ occurred to indicate CO<sub>2</sub> storage during reentry into torpor. Transient changes in RQ during arousal and recooling are thought to reflect a respiratory acidosis and its reversal that may be involved in the suppression of thermogenesis and metabolism during entry into torpor and arousal (Malan 1993; Snapp and Heller 1981).

Measures of RQ during the euthermic phase rose significantly to values of 0.77–0.79 that were sustained over 15 h, indicating the recruitment of protein and/or carbohydrates as metabolic fuels. Ground squirrels fast during the heterothermic season of hibernation and do not replace metabolized substrates through dietary intake; this may be an issue for maintaining glucose homeostasis during hibernation. Since rates of gluconeogenesis in the liver are greatly slowed or inhibited at temperatures representative of torpor (Staples and Hochachka 1998), one function of arousal episodes may be to replenish carbohydrates stores (Galster and Morrison 1970). Galster and Morrison (1975) demonstrated that arctic ground squirrels increase blood glucose and liver and muscle glycogen levels during arousal episodes. Gluconeogenesis through increased rates of protein catabolism occurs, as well as from glycerol released from fat metabolism, which may explain the increase in RQ measured in the present study. RQ also rises significantly during steady-state torpor in arctic ground squirrels hibernating at subzero  $T_a$ 's. This is thought to reflect the increased utilization of glucose to support the continuously elevated levels of metabolism necessary to maintain  $T_b$  above  $-2.9^\circ\text{C}$  (Buck and Barnes 2000).

#### Total and proportional costs of arousal episodes

Arousal episodes while hibernating under arctic, subfreezing conditions were not demonstrated to be energetically more costly compared to those occurring under more moderate conditions over the temperature ranges compared in this study. This conclusion follows from measuring the time and energy required by animals undergoing induced arousals under captive conditions and the assumption these do not differ from patterns associated with spontaneous arousals under natural conditions. This emphasizes that the major costs of arousal episodes in hibernating mammals result from the duration of the euthermic phase. To consider the costs of arousal episodes in the context of a complete hibernation cycle that includes the torpor bout, values of MR during torpor and lengths of torpor bouts were taken from other studies of arctic ground squirrels that compared animals across the same  $T_a$  range as the present study (Buck and Barnes 2000; Buck et al. 2008; Barnes et al.

unpublished). In the field at subzero  $T_a$ 's, the length of the torpor bout in free-living arctic ground squirrels increases (9.5 days at  $T_a 2^\circ\text{C}$ , 16 days at  $T_a -5^\circ\text{C}$ , and 19.5 days at  $T_a -12^\circ\text{C}$ ), as does the calculated cost per day of torpor due to increasing costs of thermoregulation as measured in captive animals (Table 1). Even though costs of individual arousal episodes do not differ with decreasing  $T_a$ 's, their relative cost within a single hibernation cycle and their overall cost as a proportion of the entire heterothermic season diminish with decreasing  $T_a$  due to the higher costs of steady-state metabolism during torpor. At  $T_a -12^\circ\text{C}$ , arousal episodes are estimated to represent only 27% of total costs. This contrasts with previous estimates under non-arctic conditions that emphasize the relative costs of arousals. At  $T_a 5^\circ\text{C}$ , Wang (1978) attributed 83% of the energetic cost of the hibernation season to arousals in Richardson's ground squirrels. Strijkstra (1999) calculates arousal episodes to comprise 86% of the energetic cost of the hibernation season in European ground squirrels at  $T_a 3-20^\circ\text{C}$ . If the hibernation season is considered as the total time animals remain sequestered underground in their hibernacula, for male arctic ground squirrels the pre-emergent euthermic interval, a 2–3 week period in spring when animals undergo reproductive maturation, while remaining in their burrows at high  $T_b$ , is also a significant energetic cost that is estimated to represent up to 50% of entire hibernation season (Barnes 1996).

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