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Energy availability influences microclimate selection of hibernating bats

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Summary

Many species hibernate to conserve energy during periods of low food and water availability. It has long been assumed that the optimal hibernation strategy involves long, deep bouts of torpor that minimize energy expenditure. However, hibernation has ecological (e.g. decreased predator avoidance) and physiological (e.g. sleep deprivation) costs that must be balanced with energy savings; therefore, individuals possessing sufficient energy reserves may reduce their use of deep torpor. We tested the hypothesis that energy (fat) availability influences temperature selection of two fat-storing bat species during hibernation. We predicted that individuals with small energy reserves would select colder temperatures for hibernation in order to minimize energy expenditure, while individuals with larger energy reserves would choose warmer temperatures to minimize the costs of hibernation. Results from our field experiment indicate that little brown myotis (*Myotis lucifugus*) hibernating in warm microclimates were significantly heavier than individuals hibernating in cooler microclimates. To determine if energy availability was mediating this relationship, we limited fatty acid availability with mercaptoacetate (MA) and quantified its effect on torpid metabolic rate (*TMR*) and thermal preference of big brown bats (*Eptesicus fuscus*). Administration of MA caused a 43% drop in *TMR* at 10°C and caused bats to choose significantly colder temperatures for hibernation. Our results suggest that fat-storing bats minimize torpor expression using both physiological and behavioral mechanisms.

Key words: *Eptesicus fuscus*, fat storing, hibernation, metabolic depression, *Myotis lucifugus*, respirometry, thermal preference.

Introduction

Limited food availability during winter threatens survival for most temperate-zone mammals; therefore, many species have evolved mechanisms to increase winter survival. Some small mammals maintain a constant, elevated body temperature (T_b) through increased energy consumption (usually by food hoarding). Conversely, some marsupials, rodents and bats exhibit a significant decrease in T_b, thereby regulating energetic expenditures through hibernation (Geiser, 2004). Consequently, hibernating mammals survive winter on a fraction of the energy required to remain euthermic.

During hibernation, torpid metabolic rate (*TMR*) is largely dependent on temperature (Geiser, 1988; Geiser, 2004). Torpid metabolic rate drops exponentially to a minimum temperature (T_{min}) and then increases rapidly as ambient temperature (T_a) falls below T_{min}. The T_a at which *TMR* is minimal varies by species and is often considered the optimal temperature for energy conservation during hibernation. In addition, the length of torpor bouts increases as T_a decreases, thereby minimizing the frequency of energetically expensive arousal bouts (Brack and Twente, 1985; Dunbar and Tomasi, 2006). Individuals may maximize winter survival and optimize their energetic condition for spring emergence by choosing a hibernaculum with T_a near T_{min} (Richter et al., 1993; Tuttle and Kennedy, 2002). Migration and gestation in spring often start shortly after hibernation (Kunz et al., 1998; Michener, 1985), so emerging from hibernation with a larger energy reserve may confer a reproductive advantage over individuals with small energy reserves (Hackländer and Arnold, 1999; King et al., 1991).

However, factors other than energetic constraints may also influence hibernation decisions. For instance, it has been proposed that hibernation imposes ecological costs such as decreased detection of predators (Humphries et al., 2003a) and increased likelihood of freezing (Clawson et al., 1980). Hibernation may also have physiological costs such as reduced motor function (Choi et al., 1998), decreased immune response (Luis and Hudson, 2006; Prendergast et al., 2002), sleep deprivation (Daan et al., 1991; Trachsel et al., 1991) and reduced protein synthesis (Frerichs et al., 1998; Van Breukelen and Martin, 2002). An optimization approach predicts that hibernating mammals should minimize these negative aspects of hibernation while maintaining sufficient energy reserves to survive winter (referred to herein as the 'hibernation optimization hypothesis') (Humphries et al., 2003a). As such, individuals with high energy availability should exhibit less frequent, shorter and shallower (i.e. maintenance of a higher T_b) torpor bouts compared with individuals with smaller energy

reserves. As predicted by the hibernation optimization hypothesis, food-caching eastern chipmunks, *Tamias striatus*, express shorter and shallower hibernation bouts when food hoards are supplemented (French, 2000; Humphries et al., 2003b). This suggests that the physiological and ecological costs of hibernation are substantial and may be avoided when energetically feasible.

Recent evidence suggests that fat-storing hibernators also minimize the expression of hibernation when stored energy (body fat) is abundant. By spending less time in torpor and maintaining a higher T_b during torpor, individuals with sufficient energy stores may minimize the costs of hibernation (Wojciechowski et al., 2007). Fat-storing species generally cannot increase energy intake as can food-caching species (e.g. Humphries et al., 2003b); however, they can behaviorally regulate the length and depth of torpor by selecting favorable microclimates (Brack and Twente, 1985; Kokurewicz, 2004). Hibernating at warm temperatures leads to increased energy expenditure (Dunbar and Tomasi, 2006; Geiser, 2004) but a high T_b also minimizes the expression of torpor and should therefore lessen negative aspects of hibernation (Humphries et al., 2003a). Individuals with large fat stores should be more energetically capable of hibernating at warmer temperatures than individuals with small fat stores and may therefore choose warmer temperatures to lessen the ecological and physiological costs of hibernation (Munro et al., 2005).

We tested the hypothesis that energy availability affects microclimate selection of fat-storing hibernators using two species of vespertilionid bats, the little brown myotis, *Myotis lucifugus* (LeConte 1831), and the big brown bat, *Eptesicus fuscus* (Beauvois 1796). In a correlative field study, we predicted that hibernating individuals would select hibernation temperatures according to their body mass: energetically constrained individuals (i.e. individuals with less body fat) should minimize energy expenditure by hibernating at cooler temperatures, while less energetically constrained individuals should minimize the costs of hibernation by selecting warmer temperatures. In a laboratory study, we sought to determine if experimental manipulation of energy availability [*via* the drug mercaptoacetate (MA)] influenced microclimate selection during hibernation. We predicted that experimental limitation of energy availability would cause bats to choose cooler microclimates than control individuals.

Materials and methods

Experimental animals

Little brown myotis hibernate in caves and mines and generally form small, loose clusters except at temperatures near freezing. They hibernate across a wide range of temperatures and congregate in large numbers in some hibernacula (Brack, 2007). Therefore, little brown myotis were used to examine microclimate selection in a natural setting. Big brown bats hibernate in buildings, mines, caves and trees and are known for their hardiness, ease of care in captivity (Wilson, 1988) and ability to hibernate and become active across a wide range of temperatures (Boyles et al., 2006). They are an ideal model for testing microclimate selection in the laboratory through experimental energy manipulations.

Field experiment

We studied a population of hibernating little brown myotis in an abandoned limestone mine in western Ohio, USA, during the winter of 2006–2007. The mine contains approximately 71 km of passages and is surveyed biennially for endangered Indiana myotis, *Myotis sodalis*. During these surveys, rock temperature is mapped throughout the mine, and little brown myotis typically hibernate across approximately a 12°C (0–12°C) thermal gradient (Brack, 2007). The winter of 2006–2007 was an El Niño year, and thus weather patterns were atypical. The regional temperature was approximately 4°C above the long-term monthly mean in December and January and 7°C below the mean in February (National Oceanic and Atmospheric Administration, Dayton, OH Weather Station). Because of the delayed onset of cold temperatures, the rock temperatures across which little brown myotis were hibernating was only 3°C and bats were not as abundant as in previous years (Brack, 2007).

We collected data on three dates during the hibernation season: 2 December 2006, 14 January 2007 and 12 February 2007. For each bat or cluster, we measured rock temperature within 3 cm of the cluster and air temperature within 7 cm of the cluster using a thermocouple thermometer (Model 52 II; Fluke Corporation, Everett, WA, USA) accurate to 0.1°C. The thermometer was factory-calibrated and the calibration was verified in an ice bath in the field before measurements were taken. We weighed bats using a digital balance accurate to 0.1 g (Scout II; Ohaus, Pine Brook, NJ, USA) and measured forearm length to the nearest mm to adjust mass for body size. Mass alone (or mass corrected for forearm length) is not necessarily indicative of fat mass. However, body mass and fat mass are highly correlated in little brown myotis (Kunz et al., 1998). Following data collection, we released bats on a surface near where they were collected.

We split the collection area into two halves (front and back), designated by an obvious physical break in the mine (Brack, 2007). We predicted that bats hibernating in the warmer, back half of the mine would be larger than bats hibernating in the cooler, front half of the mine; therefore, we tested for differences in body mass of individuals in the two areas using one-tailed *t*-tests (Zar, 1999).

Laboratory experiments

We collected 20 adult big brown bats (10 male, 10 female) from December 2006 to February 2007 in the attic of an unused school building in western Indiana and from an abandoned limestone mine in western Ohio. We transferred bats to holding facilities at Indiana State University where they were kept communally in soft mesh cages (43×43×38 cm) at 8.5±1°C. Bats were not fed before trials to ensure they were post-absorptive but were fed mealworm, *Tenebrio molitor*, larvae prior to release, and water was available *ad libitum*.

Thermal preference

Stored body fat is the main source of energy for bats during hibernation (Dark, 2005). Therefore, we assessed the thermal preference of hibernating big brown bats before and after manipulating their energetic state with mercaptoacetate (Sigma, St Louis, MO, USA). MA inhibits fatty acid availability by reducing the mitochondrial oxidation of fatty acids (Bauche et

al., 1983; Dark and Miller, 1998). MA increases food intake in rats on high-fat diets (Scharrer and Langhans, 1986) and affects torpor expression in placental golden-mantled ground squirrels, *Spermophilus lateralis* (Dark and Miller, 1998), and marsupial eastern pygmy possums, *Cercartetus nanus* (Westman and Geiser, 2004). In eastern pygmy possums, T_b and *TMR* of individuals injected with MA did not differ significantly from food-deprived individuals (Westman and Geiser, 2004). Dosages of MA used herein are sufficient to cause a substantial increase in food intake in rats (Scharrer and Langhans, 1986), indicating an inhibition of fatty acid oxidation. However, they are low enough that ground squirrels were not aroused from hibernation (Dark and Miller, 1998), which is important because our experiment requires bats to enter torpor.

To test the thermal preference of hibernating bats, we established a thermal gradient on a 127×61×0.3 cm sheet of aluminum placed in a dark, walk-in environmental chamber set at 6.0±1°C. The apparatus was laid flat on a shelf and covered with soft mesh so bats could move along the gradient. We placed a strip of terrarium heat tape (Flexwatt, Calorique Ltd, West Wareham, MA, USA) running at full power on one end of the aluminum sheet and a second strip of terrarium heat tape running at half power near the midpoint of the aluminum. We covered the underside of the aluminum sheet, including the heat tape, with insulation. This created a thermal gradient running from approximately 6.0°C on one end of the aluminum sheet to 12.5°C on the opposite end. We covered the apparatus with a large plastic container to keep bats on the gradient. We hung black cloth inside the container such that it lightly touched the gradient (Fig. 1). This homogenized the structure within the apparatus, allowing hibernation location to be selected based on temperature; without the cloth, bats hibernated exclusively touching one edge of the lid.

During each trial, we placed a single bat on the center of the apparatus at a point perpendicular to the thermal gradient. We left bats on the apparatus for 6–18 h to select a temperature and enter a bout of hibernation. After removing each bat, we measured the temperature perpendicular to the direction of thermal change 5 cm from the point selected by the hibernating bat (to avoid changes in temperature caused by the bat). We measured surface temperature of the gradient using a thermocouple thermometer covered by a 10×10 cm piece of insulation. The insulation minimized the effect of air temperature on the temperature reading and insured that only the surface temperature of the gradient was measured. In a few instances, bats were not torpid when removed from the gradient, so they were immediately placed back on the center of the thermal gradient for another trial. If the individual did not enter torpor during the second attempt, they were excluded from analyses (N=2). After removing bats from the gradient, we immediately administered a subcutaneous injection of either MA (500 μmol kg^{-1} body mass in a reverse osmosis water vehicle, N=9) or a blank control (N=9) using the same total volume of reverse osmosis water (Dark and Miller, 1998). Treatment for each individual was determined by a random number generator. After injection, we held bats at room temperature (22±1°C) for 15–20 min before placing them onto the thermal gradient. We left bats on the gradient for 6–18 h, removed them and recorded the temperature where they hibernated as described above. Trials were conducted in January and February 2007.

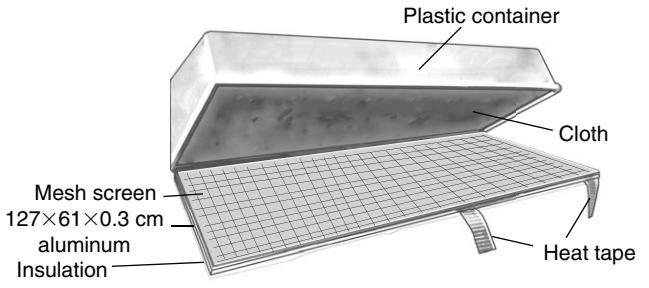

Fig. 1. Thermal gradient apparatus used to test the thermal preference of hibernating big brown bats, *Eptesicus fuscus*.

Treatment with MA should mimic energy limitation. According to the hibernation optimization hypothesis, energy-limited bats should choose colder temperatures for hibernation to maximize energy savings, while energetically capable individuals should choose warmer temperatures to minimize the negative aspects of hibernation. We predicted that bats treated with MA would exhibit a significantly larger decrease in temperature of hibernation than bats given the control. Therefore, we used one-tailed *t*-tests to determine if the difference in temperature selected before and after the injection was larger in the MA group than in the control group (Zar, 1999).

Respirometry

Mercaptoacetate lowers *TMR* and T_b in some eastern pygmy possums, a hibernating marsupial (Westman and Geiser, 2004). In placental mammals, T_b is not lowered (Dark and Miller, 1998) or is lowered only slightly (Stamper and Dark, 1997), and the effect of MA on *TMR* is unknown. A drop in *TMR* caused by MA should lessen the strength of the effect in the thermal preference experiment because a smaller change in hibernating temperature would be needed to lower *TMR* to the necessary level.

To determine whether handling stress and injection of MA influenced *TMR*, we measured oxygen consumption of torpid adult female big brown bats not used in the thermal preference experiment, using open-airflow respirometry before and after injection of MA (400 μmol kg^{-1}). We placed individual bats in a metabolic chamber consisting of a sealed 0.24-liter glass jar lined with nylon netting so bats could hang freely. The metabolic chambers were placed in a temperature-controlled chamber maintained at 10°C. Temperature was not measured in the metabolic chambers, so the bat's body heat could have slightly raised T_a. Ambient air was dried and pushed through the metabolic chamber at 100 ml min^{-1}. Incurrent air flow was measured with a factory-calibrated mass flow controller (Sable Systems, Las Vegas, NV, USA). Excurrent air was scrubbed of carbon dioxide and water with soda lime and anhydrous calcium sulfate, respectively, before passing into an oxygen analyzer (FC-10A; Sable Systems). We calibrated the oxygen analyzer to ambient air prior to respirometry trials. We kept the bats in the metabolic chamber for approximately 4 h before each trial to allow oxygen consumption to stabilize before measurements

were recorded. We ran trials for 2 h and began all trials during the inactive part of the bats' daily cycle. Fractional oxygen concentrations were recorded at 1 min intervals, and we identified the minimum value (from 10 min means) for each individual before and after administration of MA. We calculated oxygen consumption corrected for standard temperature and pressure ($\dot{V}_{O_2}$) using the methods of Withers (Withers, 2001). Data were analyzed for whole-animal (ml O_2 h^{-1}) and mass-specific values (ml O_2 h^{-1} g^{-1}). We used 0.179 ml O_2 h^{-1}=1 mW for conversion from $\dot{V}_{O_2}$ to *TMR* (Willis et al., 2005).

Respirometry measurements were limited because of the availability of bats and time constraints on equipment. All trials were performed at 10°C, near the temperature in the center of the thermal gradient, because our goal was only to determine if handling effects or injection of MA caused a drop in *TMR*, not to quantify a change in *TMR* across a range of temperatures. Further, we did not use a control group in respirometry experiments to differentiate between handling effects and treatment effects, because this is inconsequential to the results of our study. We used respirometry results only to strengthen our conclusion in the thermal preference experiment.

We calculated statistics in Minitab version 14 (State College, PA, USA). Results are reported as means (± s.d.). All field and laboratory methods were approved by the Indiana State University Animal Care and Use Committee under protocol JOW/JB 9-18-2006.

Results

Field experiment

Rock temperature where bats were sampled was warmer in the back section of the mine than in the front section (overall ANOVA, $F_{1,1558}$=1298.66; P<0.005) during December (9.00±0.54°C in the front of the mine *vs* 9.93±0.59°C in the back of the mine), January (9.2±0.73 *vs* 10.2±0.59°C) and February (6.28±0.48 *vs* 8.28±0.88°C). Air temperatures were warmer in the back section of the mine than in the front section ($F_{1,1558}$=326.02; P<0.005) during December (9.17±0.59 *vs* 10.37±5.9°C), January (9.99±0.71 *vs* 10.47±1.27°C) and February (7.29±0.73 *vs* 9.54±0.84°C). We weighed and measured 590, 529 and 443 little brown myotis on the three trips, respectively. When evaluated by gender, individuals in the warmer back section of the mine were significantly heavier than individuals in the front section in all comparisons (one-tailed *t*-tests; P<0.05) (Fig. 2). All comparisons remained significant after adjusting body mass for forearm length (P<0.05). The effect sizes were small during all sampling periods (Hedges' $\hat{g}$ 0.25–0.47 for all comparisons) but were detectable and were always in the predicted direction (i.e. larger bats were at warmer temperatures). We would not expect all six comparisons to be significant in the same direction if this significance is purely artifactual. These differences, if assumed to be solely related to differences in fat mass, represent ⩾10% of fat mass acquired prior to the onset of hibernation in little brown myotis (Kunz et al., 1998).

Laboratory experiments

Thermal preference

Neither body mass (t=0.54, d.f.=16, P=0.60) nor preferred temperature (t=1.69, d.f.=16, P=0.11) differed significantly between the control and treatment groups prior to injection of MA. In addition, injection of the blank control did not change the temperature chosen for hibernation from before (9.3±1.6°C) to after (9.1±1.6°C) injection (–0.21±0.26°C; paired *t*-test, d.f.=8, t=0.81, P=0.44). However, MA caused a significant decrease in the chosen hibernation temperature from before injection (10.5±1.5°C) to after injection (8.8±2.0°C; –1.68±0.71°C; paired *t*-test, d.f.=8, t=2.46, P=0.045). This included two individuals that chose the coldest spot on the gradient both before and after injection of MA. If these individuals are removed, the change in temperature after injection of MA is larger (–2.14±0.83°C; paired *t*-test, N=7, t=2.57, P=0.043). Further, the relative change in temperature was significantly larger in the MA group than in the control group (one-tailed *t*-test, t=1.94, d.f.=10, P=0.04).

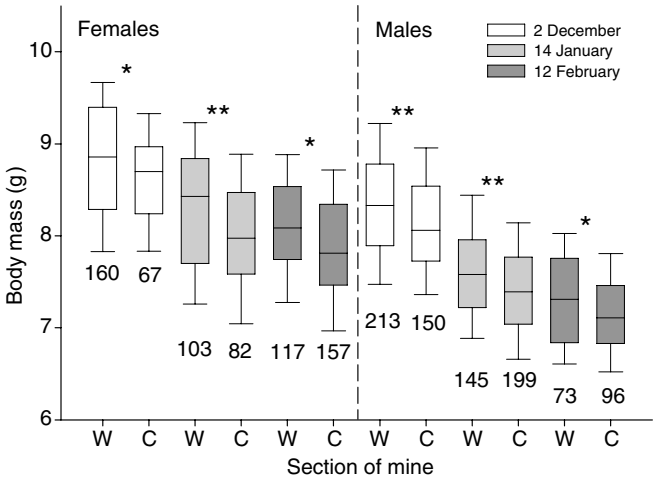

Fig. 2. Body mass of little brown myotis, *Myotis lucifugus*, in an abandoned limestone mine in western Ohio on three dates during winter 2006–2007. Each pair of box-plots represents the mass of bats weighed in two sections of the mine, a cold section near the front of the mine (C) and a warmer section deeper in the mine (W). Sample sizes are under the box-plot. In all samples, bats from the warmer section were larger than those from the colder section. Significance level of one-tailed *t*-tests indicated by: *P<0.05; **P<0.01.

Respirometry

Injection of MA caused a drop in whole-animal $\dot{V}_{O_2}$ at 10°C from 0.39±0.29 ml O_2 h^{-1} to 0.22±0.15 ml O_2 h^{-1} and a drop in mass-specific $\dot{V}_{O_2}$ from 0.022±0.015 ml O_2 h^{-1} g^{-1} to 0.019±0.008 ml O_2 h^{-1} g^{-1}. This equates to a marginally significant drop in whole-animal *TMR* from 2.18±1.63 mW to 1.25±0.88 mW (paired *t*-test, d.f.=8, t=2.22, P=0.057) and a drop in mass-specific *TMR* from 0.124±0.084 mW g^{-1} to 0.072±0.045 mW g^{-1} (paired *t*-test, d.f.=8, t=2.15, P=0.069). This change represents a 43% decrease in whole-animal *TMR* and a 42% decrease in mass-specific *TMR* after injection of MA. Although the drop is non-significant, the effect size of MA is very large on both whole-animal *TMR* (Hedges' $\hat{g}$=2.04) and mass-specific *TMR* (Hedges' $\hat{g}$=1.87).

Discussion

This is the first study to explicitly test the hibernation optimization hypothesis proposed by Humphries et al.

(Humphries et al., 2003a; Humphries et al., 2003b) using fat-storing hibernators. Our results suggest that energy availability affects microclimate selection during hibernation in these two fat-storing bat species. As predicted by the hibernation optimization hypothesis, body mass was related to microclimate selection in little brown myotis. Field data indicate that heavier, and presumably fatter (*sensu* Kunz et al., 1998), individuals selected warmer temperatures for hibernation than did smaller individuals. Natural variation in hibernation microclimate matched the predicted pattern in both sexes on all three sampling occasions, despite the unusually warm winter and narrow thermal gradient in the mine. We expect the difference in microclimate selection to be more pronounced during a normal winter when a larger thermal gradient is present. Furthermore, this pattern was apparent despite evidence that individuals in clusters are heavier than solitary individuals (Fenton, 1970) and nearly all clusters in our experiment were in the front, colder section of the mine.

Likewise, experimental reduction of fat availability with MA caused individuals to choose colder temperatures for hibernation. This pattern emerged despite a nearly significant drop in *TMR* due to handling and the injection of MA. The nearly significant *P*-value and large effect size give us cause to believe this relationship is biologically important. It suggests that physiological mechanisms did not decrease *TMR* sufficiently and bats used microclimate selection to slow *TMR* even further. Our results suggest that energetic state influences the thermal preference of hibernation sites in fat-storing hibernators such as big brown bats.

Unlike food-caching species, fat-storing hibernators generally cannot adjust energy intake during hibernation; therefore, survival costs are potentially large if an animal expends energy too quickly or if an unusually harsh winter extends the hibernation season (Dark, 2005). Regardless, our results, and those of Wojciechowski et al. (Wojciechowski et al., 2007), indicate that fat-storing bats minimize their expression of torpor when energy reserves are sufficient. Our results suggest that fat-storing bats regulate the expression of torpor through microclimate selection, while Wojciechowski et al. (Wojciechowski et al., 2007) suggest torpor is regulated by changes in depth and length of torpor bouts. These differences may be explained by interspecific variation, but it is likely that both microclimate selection and changes in depth and length of torpor bouts are important mechanisms of hibernation regulation.

Our results add to the growing literature suggesting that the optimal hibernation strategy may not be characterized by long, deep bouts of torpor to minimize energy expenditure (Humphries et al., 2003b). Rather, the optimal hibernation strategy for fat-storing bats (and likely other fat-storing species) may be to hibernate at a relatively warm T_a (which will lead to a high T_b and short torpor bouts), thereby minimizing the ecological and physiological costs of hibernation. The optimal expression of torpor is likely to be different for each individual. This may explain intraspecific variation in temperature selection by hibernating bats, and thus the locations and temperatures at which individuals are found hibernating in caves and mines. To our knowledge, this is the first mechanistic hypothesis explaining intraspecific variation in microhabitat selection by fat-storing species during hibernation. It may also provide clues for understanding interspecific variation in microclimate selection (McNab, 1974), although this is speculative without further experiments. Other factors, such as the decreased probability of predation in the back of a hibernacula (Kokurewicz, 2004), may help explain why not all individuals hibernate at the temperature that minimizes energy expenditure. However, the ability to take advantage of any such benefits is ultimately constrained by energetic considerations.

This study has important conservation and management implications for bats during the hibernation season. It has been suggested that ideal hibernacula are cold and thermally stable (*sensu* Tuttle and Kennedy, 2002). Hibernacula meeting these criteria may minimize energy expenditure but they also limit the opportunity for individuals with sufficient energetic reserves to choose warmer microclimates and minimize negative aspects of hibernation. In addition, the energetic savings from hibernating at cold temperatures may be outweighed by unknown long-term fitness costs (e.g. lowered survival or reproduction) of hibernating at those temperatures.

In summary, our results suggest that energy availability affects microclimate selection of hibernating bat species. Minimizing energy expenditure may be the ultimate goal of hibernation, but hibernation is not a cost-free strategy and may be avoided when possible. Presumably, there must be a benefit to hibernating at warmer temperatures to offset the added energetic costs; otherwise, all individuals would hibernate at the temperature that minimizes energy expenditure. Further research is needed to determine what these benefits are and the ecological (e.g. risk of freezing or predation) and physiological (e.g. buildup of metabolic waste) costs that cause hibernators to avoid cold temperatures when energetically capable.

List of abbreviations

MA	mercaptoacetate
T_a	ambient temperature
T_b	body temperature
TMR	torpid metabolic rate
T_{min}	temperature at minimum torpid metabolic rate
$\dot{V}_{O_2}$	rate of oxygen consumption

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