RESEARCH ARTICLE

Hibernation energetics of free-ranging little brown bats

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SUMMARY

Hibernation physiology and energy expenditure have been relatively well studied in large captive hibernators, especially rodents, but data from smaller, free-ranging hibernators are sparse. We examined variation in the hibernation patterns of free-ranging little brown bats (*Myotis lucifugus*) using temperature-sensitive radio-transmitters. First, we aimed to test the hypothesis that age, sex and body condition affect expression of torpor and energy expenditure during hibernation. Second, we examined skin temperature to assess whether qualitative differences in the thermal properties of the hibernacula of bats, compared with the burrows of hibernating rodents, might lead to different patterns of torpor and arousal for bats. We also evaluated the impact of carrying transmitters on body condition to help determine the potential impact of telemetry studies. We observed large variation in the duration of torpor bouts within and between individuals but detected no effect of age, sex or body condition on torpor expression or estimates of energy expenditure. We observed the use of shallow torpor in the midst of periodic arousals, which may represent a unique adaptation of bats for conservation of energy during the most costly phase of hibernation. There was no difference in the body condition of hibernating bats outfitted with transmitters compared with that of control bats captured from the same hibernaculum at the same time. This study provides new information on the energetics of hibernation in an under-represented taxon and baseline data important for understanding how white-nose syndrome, a new disease devastating populations of hibernating bats in North America, may alter the expression of hibernation in affected bats.

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INTRODUCTION

Winter is a period of negative energy balance for many mammals. The cost of defending body temperature (T_b) increases as ambient temperature (T_a) decreases, particularly for small-bodied mammals (Aschoff, 1981). Additional energy intake is required to meet these high thermoregulatory costs, but seasonal reductions in food availability often coincide with low T_a. Hibernators survive this energetic bottleneck by entering torpor and drastically reducing their metabolism so that stored food or fat reserves can match energetic demand (Lyman, 1982). The energy savings provided by deep torpor are accompanied by physiological costs including build-up of metabolic wastes, dehydration stress (Thomas and Geiser, 1997) and diminished immunocompetence (Burton and Reichman, 1999). Longer, deeper bouts of torpor will minimize energy expenditure but may also exacerbate these physiological costs. Consequently, torpor bouts are interrupted by periodic arousals, brief returns to euthermic T_b that appear to be important for rectifying the physiological imbalances accrued during torpor (Geiser, 2004). Although hibernators are euthermic for a small fraction of their time during hibernation, arousals account for the vast majority of winter energy requirements (Heldmaier et al., 1993; Wang, 1978). It has been suggested that a trade-off between energetic benefits and physiological costs of torpor should cause individuals to optimize, rather than maximize, their expression of torpor during hibernation, and avoid the use of deep torpor when possible (Humphries et al., 2003). The torpor optimization hypothesis predicts that individuals

should: (1) increase the use of torpor if post-hibernation energy reserves are important for reproduction; and (2) use excess energy reserves to mitigate the costs of torpor by reducing its depth and duration (Humphries et al., 2003).

Bats are second only to rodents as the most diverse order of mammals and, like rodents, include many heterothermic and hibernating species. However, in contrast to rodents (e.g. Landry-Cuerrier et al., 2008; Michener, 1992; Munro et al., 2005), only a handful of studies of thermoregulation during hibernation exist for free-ranging bats and many of these are for tropical or sub-tropical species (Cory Toussaint et al., 2010; Liu and Karasov, 2010; Stawski et al., 2009; Turbill, 2006; Turbill and Geiser, 2008). Very few studies have reported long-term body temperature profiles of temperate-zone bats in winter (Hope and Jones, 2012; Park et al., 2000). Temperate-zone bats may spend energy reserves differently from rodents during hibernation. Smaller hibernators face a greater energetic challenge because fat storage capacity is directly proportional to body mass, but mass-specific energy requirements are highest for small-bodied species (Capellini et al., 2010). Therefore, small hibernators must expend a greater proportion of their fat reserves per unit time compared with larger species (Morrison, 1960). The limitations of small body size present a greater challenge for bats than for similarly sized granivorous rodents because bats' insectivorous diet prevents them from supplementing fat reserves with cached food. Many bat species hibernate in caves, which are substantially different from the burrow hibernacula of

rodents. Burrows are small, 'dug to fit' and often insulated by their occupants, allowing greater potential for rodents to use metabolic heat to regulate microclimate during periodic arousals. Large air volumes in cave hibernacula may limit the potential for even large clusters of bats to modulate microclimate. Suitable cave hibernacula are also patchily distributed across the landscape, which may require bats to travel tens or hundreds of kilometres to reach them (e.g. Fenton, 1970). This rarity can result in the formation of large aggregations, often of multiple species, that may place bats at greater risk of disturbance from predators or disease.

The little brown bat, *Myotis lucifugus* (LeConte 1831), is a wide-ranging, cave-hibernating species for which metabolic rates have been quantified under a variety of captive conditions. Consequently, measurements of T_b and T_a could potentially be used to make inferences about energy consumption in free-ranging individuals (i.e. Boyles and Brack, 2009). Little brown bats are also a useful species for testing the torpor optimization hypothesis because females use fat reserves remaining after hibernation to support pregnancy in spring, whereas males invest in reproduction during the autumn and early winter mating season (Buchanan, 1987; Gustafson and Shemesh, 1976; Thomas et al., 1979; Wimsatt and Kallen, 1957). This leads to the prediction that females should be 'thrifty' with their fat reserves relative to males and young-of-the-year to increase their likelihood of successful reproduction (Jonasson and Willis, 2011). To achieve this, adult females must either (1) arouse less frequently, exhibit shorter arousals and/or be less active during arousals, or (2) decrease their T_b and torpid metabolic rate more than other bats.

Most studies of torpor and hibernation in free-ranging mammals report on parameters associated with T_b or skin temperature (T_{sk}) such as torpor bout duration or minimum torpid T_{sk} (Hope and Jones, 2012; Michener, 1992; Munro et al., 2005; Park et al., 2000) but the commodity of interest for these studies is energy (Brigham et al., 2011; Willis, 2007). Estimates of hibernation energy requirements are important for answering fundamental and applied questions about hibernation, such as identifying crucial winter habitats for bats, and predicting impacts of disturbance, disease, year-to-year variation in weather patterns or climate change (Boyles and Brack, 2009; Boyles and Willis, 2010; Frick et al., 2010; Humphries et al., 2002; Landry-Cuerrier et al., 2008). The recent emergence of white-nose syndrome (WNS), which has devastated populations of hibernating bats in Eastern North America (Frick et al., 2010), underscores the need to collect this kind of energetic data for bats. White-nose syndrome is caused by infection with the cold-tolerant, invasive fungus *Geomyces destructans*, which invades the skin of the face and wing membranes of bats (Blehert et al., 2009; Gargas et al., 2009; Lorch et al., 2011; Warnecke et al., 2012). Bats with WNS are emaciated and have been observed flying out of hibernacula mid-winter, potentially in search of food, and it is now clear that infected bats arouse from torpor too frequently, prematurely depleting their winter fat reserves (Boyles and Willis, 2010; Warnecke et al., 2012). Thus, one hypothesis is that bats with WNS spend too much time out of torpor during hibernation and prematurely deplete their fat reserves (Boyles and Willis, 2010). Energetic models are needed to address this and other conservation issues facing hibernating bats, but these have limited applicability unless they can be validated against measurements from free-ranging animals.

We used temperature radio-telemetry and energetic calculations, first, to evaluate one prediction of the torpor optimization hypothesis: that adult female little brown bats exhibit a more energetically conservative pattern of hibernation than males or young-of-the-year

(Jonasson and Willis, 2011). Second, we tested the hypothesis that little brown bats would exhibit differences in their patterns of hibernation compared with those previously reported for rodents, because of the constraints of a small body size and large air volume inside bat hibernacula. Specifically, we predicted that bats would exhibit relatively infrequent, brief arousals compared with those of rodents. Finally, we used measurements of body condition to evaluate the impact of carrying transmitters for hibernating bats.

MATERIALS AND METHODS

All methods were approved by the University of Winnipeg Animal Care Committee and conducted under Manitoba Conservation Wildlife Scientific Permit WB06122.

This study was conducted in Dale's cave, central Manitoba, Canada (53°12′N, 99°19′W). This limestone cave is accessed by a 3 m deep sinkhole and consists of a bell-shaped chamber (approximately 7 m long, 5 m wide with a 4 m ceiling) that slopes away from the entrance. Approximately 80–200 bats, depending on the year, over-winter in Dale's cave from mid-September until mid-May (J. Dubois and C.K.R.W., unpublished data). We monitored cave T_a every 2 h with temperature data loggers (N=3; DS1922L iButtons, Maxim Integrated Products, Dallas, TX, USA) wrapped in foam to attenuate ultrasonic noise (Willis et al., 2009) and placed in domed areas of the ceiling known to be used by bats.

Torpid bats were captured by hand on 29 November 2009. Bats were sexed and weighed to the nearest 0.01 g (Durascale-100, MyWeigh, Vancouver, BC, Canada), and their forearm length was measured to the nearest 0.05 mm. We defined body condition index (BCI) as mass divided by forearm length (Pearce et al., 2008; Reynolds et al., 2009). We classified individuals as adult or young-of-the-year based on ossification of metacarpal–phalangeal joints and evidence of prior reproduction (Davis and Hitchcock, 1965; Racey, 1974). At the time age was determined we were blind to the body condition of individuals. All bats were outfitted with a numbered, lipped aluminium forearm band (2.9 mm, Porzana Ltd, Icklesham, East Sussex, UK) prior to their release at the site of capture. To reduce energetic costs to bats outfitted with transmitters and minimize disturbance to other bats in the hibernaculum, we limited the duration of our cave entrance and total handling time to less than 2 h.

Radio-telemetry

We used T_{sk} as a proxy for T_b because surgical implantation of transmitters would have been too disruptive in the midst of hibernation and, for small-bodied bats, T_{sk} provides a reasonable approximation of T_b (Willis and Brigham, 2003). T_{sk} was recorded by attaching temperature-sensitive radio-transmitters (BD-2NT; Holohil Systems Ltd, Carp, ON, Canada; mass 0.64–0.80 g) to 22 bats (N=5 adult females, N=11 adult males, N=3 juvenile males, N=3 juvenile females) on 29 November 2009; lighter transmitters were attached to smaller bats so that all bats carried transmitters that weighed a similar percentage of their body mass. Transmitters were affixed to the interscapular region with a non-toxic, latex-based adhesive (Osto-bond; Montreal Ostomy, Vaudreuil, QC, Canada). A data-logging receiver (Lotek SRX400; Lotek Engineering Inc., Newmarket, ON, Canada) and an array of four 30 cm omnidirectional whip antennas were used to record T_{sk} every 10 min, although sampling intervals occasionally varied if an individual's signal was temporarily lost. To test for an impact of carrying the transmitters on the bats, and to obtain data on the loss of body mass to test our energetic models, we re-entered Dale's cave on 23 January 2010 and re-captured 12 bats already outfitted with transmitters (N=2 adult
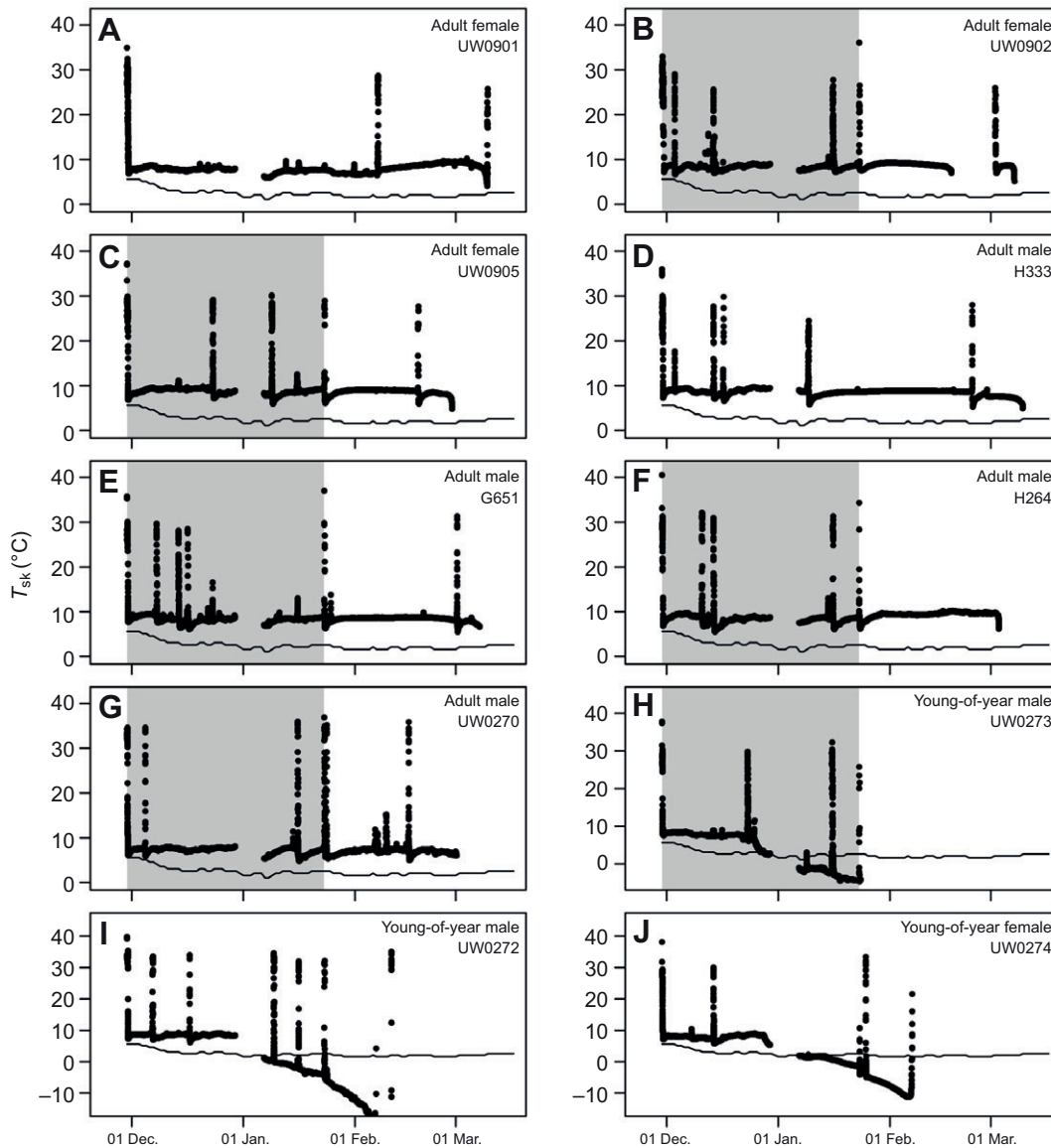
Fig. 1. Representative traces of skin temperature (T_{sk}) obtained for hibernating little brown bats, *Myotis lucifugus*. Panel labels show demographic information and forearm band number. Filled circles denote the bat's T_{sk}, the thin solid line denotes ambient temperature (T_a) and the shaded regions on some plots represents the time between capture and recapture for individuals that were used to validate energetic models. Note the prolonged torpor bouts in D and E. Towards the end of the recording period, transmitter signals drifted as batteries began to fail, especially at low T_{sk} (H–J).

female, $N=9$ adult male, $N=1$ juvenile male) as well as 7 new individuals ($N=1$ adult female, $N=6$ adult male) that had not previously been captured.

We divided each individual's time course into four phases: steady-state torpor bouts, warming, euthermic periods between torpor bouts, and cooling. The start and end of each phase was always readily distinguishable because of the large difference between torpid and euthermic T_{sk} in the cold, stable microclimate of the cave (Fig. 1). Steady-state torpor bouts were defined as periods of reduced (i.e. below a threshold of 25°C) and stable T_{sk} between the obvious and abrupt warming and cooling phases associated with each periodic arousal. The warming phase was defined by an abrupt increase in torpid T_{sk} that resulted in an elevation of T_{sk} by more than 5°C over 30 min until T_{sk} stabilized at an elevated steady-state greater than 25°C. We used 25°C as the T_{sk} boundary between torpor and euthermia, rather than 28 or 30°C, which have often been used for studies of bats in warmer or more variable microclimates (e.g. Stawski et al., 2009; Turbill and Geiser, 2008), because consistently cold T_a in the cave appeared to increase ambient cooling of external transmitters, leading to slightly reduced euthermic T_{sk} values (Willis and Brigham, 2003). The euthermic period was defined as the time

between the end of this rapid warming period and the initiation of cooling. The onset of cooling was defined as an initial abrupt decline in T_{sk} resulting in a decrease of more than 5°C within 30 min, while the end of the cooling phase was defined as the time at which the slope of the cooling curve fell to less than –1. The cut-off point for the end of cooling preceded strict steady-state torpor as it often took several hours for the bat to cool the last ~3–5°C. However, the metabolic rate of bats cooling at a T_{sk} only ~3–5°C above steady-state torpor would not be substantially different from torpid metabolic rate (TMR). Including this time period in the cooling phase would have inflated our estimates of the cost of cooling and reduced our sample size because we lost signals for some individuals when their transmitters were in the final stages of cooling.

Energetic models

We used our T_{sk} data to estimate energy expenditure for each individual using two published models and our own model incorporating more detailed calculations of arousal and cooling costs. To limit the influence of disturbance associated with entering the cave on our results, we excluded measurements recorded during the 24 h immediately after bats were captured. Because of occasional gaps in

Table 1. Models used to estimate hibernation energy expenditure in *Myotis lucifugus* based on T_{sk} profiles and T_a

	Thomas et al., 1990	Humphries et al., 2006	Composite
Steady-state torpor (ml O_2 g^{-1} h^{-1})	0.02	$0.03 \times Q_{10}^{(T_a - T_{tor,min})/10}$; if $T_a > T_{tor,min}$ $0.03 + (T_{tor,min} - T_a)C_t$; if $T_a \leq T_{tor,min}$	$0.0051 \times T_a + 0.0198$; if $T_a > 2°C$ $-0.0553 \times T_a + 0.1407$; if $T_a < 2°C$
Euthermic phase (ml O_2 g^{-1} h^{-1})	8.29	$BMR + (T_{lc} - T_a) \times C_{eu}$	$0.0193(T_b - T_a)^2 + 0.268$
Warming (ml O_2 g^{-1} h^{-1})	5.86	$(T_{b,eu} - T_{b,tor}) \times S$	$(T_{b,eu} - T_{b,tor}) \times S$
Cooling (ml O_2 g^{-1} h^{-1})	3.95	None specified, therefore treated as torpor (see above)	$(T_{b,tor} - T_{b,eu}) \times S \times 0.672$

T_{sk}, skin temperature; T_a, ambient temperature; $T_{tor,min}$, lower set point temperature, 2°C (Hock, 1951); T_{lc}, lower critical temperature; T_b, body temperature; $T_{b,eu}$, euthermic body temperature; $T_{b,tor}$, torpid body temperature; S, specific heat capacity of tissue, 0.131 ml O_2 g^{-1} C^{-1} (Thomas et al., 1990); C_{eu}, conductance at euthermic T_b, 0.2683 ml O_2 g^{-1} h^{-1} (Stones and Wiebers, 1967); C_t, conductance below $T_{tor,min}$, 0.055 ml O_2 g^{-1} h^{-1} (Hock, 1951); BMR, basal metabolic rate, 2.6 ml O_2 g^{-1} h^{-1} (Stones and Wiebers, 1967).

the data resulting from these cave entrances and an 8 day period when receiver batteries failed and we could not access the site on snowmobile, we divided the hibernation period into three sampling windows: 1 December to 28 December 2009 (28 days); 08 January to 21 January 2010 (14 days); and 25 January to 20 February 2010 (27 days). To make sure we quantified energetic costs for different individuals as precisely as possible, we excluded an individual's entire time course from the analysis for a given sampling window if there were any gaps between consecutive T_{sk} measurements >30 min long during an arousal (i.e. no signal detected by the receiver). This was important because inaccurate estimates of arousal duration, the most energetically expensive phase of hibernation, could have led to large errors in our energetic estimates.

We evaluated three different models of energy expenditure calculated based on assumptions about the costs of each phase of hibernation combined with measurements of T_{sk} (Table 1). (1) A highly simplistic representation of energy expenditure, based on a single value of metabolic rate for each of the four thermoregulatory phases (Thomas et al., 1990). This model did not account for any variation in T_a or T_{sk}. (2) A model following Humphries et al. (Humphries et al., 2006) that accounted for the effect of T_a on the cost of torpor, warming and euthermia, but did not estimate the rate of energy expenditure during cooling. (3) A new composite model integrating values from a range of literature sources that seemed intuitively most likely to account for variation in T_{sk} within each phase of hibernation. For this model, to calculate metabolic rate during torpor we used measurements of mean metabolic rate at different T_a (Hock, 1951) to create a linear regression equation for TMR at T_a between 2 and 10°C (when TMR was positively correlated with T_a) and a second linear regression for TMR at T_a between 0.5 and 2°C (when TMR was negatively correlated with T_a); linear regressions were used because Hock (Hock, 1951) reported too few measurements of metabolic rate at low T_a to plot an exponential curve. To calculate metabolic rate during an arousal, we extrapolated from Studier's curve quantifying the relationship between metabolic rate and the $T_b - T_a$ differential (Studier, 1981). Studier's measurements were obtained at a T_a of 16°C, higher than the ~3°C in Dale's cave. However, these rates were obtained across a range of T_b values and a clear relationship was observed (Studier, 1981), so in the absence of other data, we predicted that extrapolating out to larger $T_b - T_a$ differentials should provide a more precise estimate of energy costs during arousals than if we did not account for variation in T_b. Metabolic rate during warming was calculated as per Humphries et al. (Humphries et al., 2006), and metabolic rate during cooling was considered to be 67.2% of the cost of warming following Wang (Wang, 1978). We assumed T_b was 3°C greater than T_{sk}, which is consistent with the $T_b - T_{sk}$ differential for bats at low T_a (Willis and Brigham, 2003), and used these estimates of T_b, T_a and body mass at the time of first capture as inputs for the models.

Models yielded measurements in ml of O_2, which we converted to mg of fat based on the assumption that 1 ml O_2=20.1 J and 1 mg of fat=39.3 J (Schmidt-Nielsen, 1997).

Statistics
Values are reported as means ± s.d. and sample sizes as n=number of measurements and N=number of individuals. We used mixed effects models in R (R Development Core Team, 2009) to investigate fixed effects of intrinsic (i.e. age, sex, BCI) and extrinsic (i.e. T_a) factors on characteristics of torpor bouts and arousals (i.e. torpor bout duration, torpid T_{sk}, arousal duration, arousal T_{sk}), while controlling for the random effect of individual. For each response variable we tested the full model, a model with the extrinsic factor (T_a), models of intrinsic factors (sex, age, sex×age, BCI) and models composed of a single predictive factor. We did not examine all possible combinations of explanatory variables (i.e. all interactions) because there was no reason to predict that factors other than age and sex would interact and we aimed to avoid inclusion of meaningless models, which can inflate type I error rates (Johnson and Omland, 2004). We ranked all predictive models using the Akaike information criterion (AIC) corrected for small sample sizes (AIC_C). Models with $\Delta AIC_C < 2$ were considered equally parsimonious (Burnham and Anderson, 2003) and AIC weights (w) were used to determine the relative support for each model. ΔAIC_C and w were calculated using the aictab function of the AICcmodavg package in R (Mazerolle, 2010).

We tested the influence of our predictor variables on the duration of torpor bouts, bounded on either side by an arousal, with generalized linear mixed models (GLMMs) and poisson error distributions using the lmer function of the lme4 package (Bates and Maechler, 2009). We also used general linear models (GLMs) to test for the influence of predictor variables on torpid T_{sk}, a measure of torpor depth, during each individual's first torpor bout. T_{sk} during subsequent torpor bouts was not examined, to exclude the possible effects of transmitter drift as battery power declined. We tested for the effects of predictor variables on the duration of arousals and T_{sk} during arousals with GLMMs using the lme function of the lme4 package (Bates and Maechler, 2009). Arousal duration was transformed using a square-root function to achieve normality.

To test the strength of the three energetic models from the literature, we compared the rates of energy expenditure they predicted between 29 November 2009 and 23 January 2010 against the decline in body mass observed for re-captured bats over this same interval using least squares regressions with the lm function in R. We assumed that mass loss during this period reflected fat loss based on data from a related species, *Myotis grisescens*, which exhibits no annual variation in lean mass (Krulin and Sealander, 1972). This period included an 8 day gap due to equipment failure (see above) and we assumed individuals
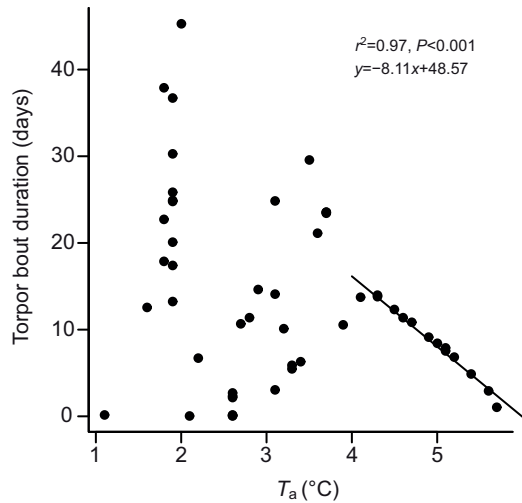
Fig. 2. Negative relationship observed between T_a and torpor bout duration in *M. lucifugus*. Data from *N*=20 individuals. Note the dramatic change in the scatter of the relationship at 4°C; when T_a>4°C there is a negative correlation between torpor bout duration and T_a but there is no correlation when T_a<4°C.
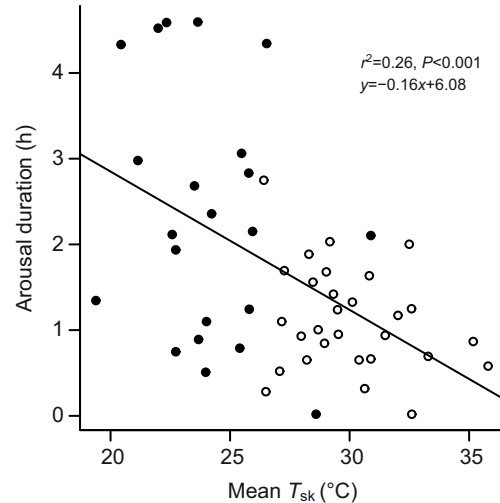

Fig. 3. Negative relationship between arousal duration and mean T_{sk} during arousals in hibernating *M. lucifugus*. Open circles represent arousals classified as plateau, filled circles represent arousals classified as heterothermic.

were torpid during this period for energetic calculations. If this gap had included an arousal for a given individual it would have appeared as a clear outlier as each arousal accounts for approximately 5% of the over-winter energy budget (Thomas et al., 1990). The effects of our predictor variables on estimated rates of energy expenditure were examined in two ways. For bats from which we had data during all three sampling windows, we examined mean energy expenditure over the entire study period. However, for some individuals we were not able to record data from all bats in each of the sampling windows because of periodic signal loss and/or transmitter failure towards the end of the study. Therefore, dividing the sampling period and repeating the analysis for each interval separately allowed us to increase our sample size within each interval and improve the chances of detecting significant effects of our predictor variables.

RESULTS
Torpor and arousal patterns

We collected 1511 bat-days of data from *N*=5 adult female, *N*=11 adult male, *N*=3 juvenile female and *N*=3 juvenile male bats (72.0±21.3 days bat^{-1}; Fig. 1; supplementary material Table S1). Bats spent 99.6% of recording days in bouts of deep, steady-state torpor, interspersed with brief and rapid returns to euthermia. Torpor bouts lasted for 13.1±10.8 days (*n*=54, *N*=20) and ranged from 6 min to over 48 days. The best-fit model for torpor bout duration included T_a as the only fixed effect, with the longest torpor bouts occurring at colder T_a (GLMM, *P*=0.01, *n*=54, *N*=20). However, the scatter in this relationship increased substantially below T_a=4°C and both the longest and the shortest torpor bouts were exhibited at T_a between 1 and 2°C (Fig. 2). At T_a above 4°C there was a significant negative relationship between torpor bout duration and T_a (regression, r^2=0.97, *P*<0.001) but there was no significant relationship at T_a below 4°C. The best-fit model for the maximum torpor bout duration exhibited by individual bats included only the number of recording days as a predictor. Longer torpor bouts were observed in bats with the most recording days (GLM, *P*=0.012, r^2=0.31, *N*=20), which reflects the fact that torpor bout duration got longer as the season progressed and T_a declined. The best-fit model for

mean T_{sk} during each individual's first torpor bout included T_a as the only predictor and torpid T_{sk} was positively correlated with T_a (GLM, *P*<0.001, r^2=0.65, *N*=14). The mean T_{sk}–T_a differential (ΔT) during each individual's first torpor bout was 4.3±0.5°C (*N*=14).

We recorded 66 spontaneous arousals (2–5 per bat). Arousals lasted between 5 min and 4.6 h, and mean T_{sk} was 27.5±3.9°C, ranging from 11 to 36°C (*n*=51, *N*=19). The best-fit model for number of arousals per individual was non-significant (GLM, *P*=0.73, r^2=0.01, *N*=19), meaning that none of our models were strong predictors of the total number of arousals per bat. There was a significant negative relationship between the square root of arousal duration and mean T_{sk} during arousals (GLM, r^2=0.23, *P*=0.002; Fig. 3), but all other models were non-significant. Long arousals (>1 h) never occurred at high (34–37°C) T_{sk}, but shorter than predicted arousals did occur in conjunction with low T_{sk}. The majority of arousals (60%, *n*=29, *N*=18) were plateau shaped with a stable euthermic T_{sk} that was defended until re-entry into torpor (mean T_{sk}=29.7±2.0°C, s.d. T_{sk}=1.4±0.8°C). We termed these 'plateau arousals' (Fig. 4A). However, 40% (*n*=22, *N*=18) of arousals were characterized by clear reductions in T_{sk}, often to levels below those normally considered euthermic, yet clearly distinct from the deep torpor characteristic of hibernation (mean T_{sk}=23.8±2.6°C, s.d. T_{sk}=3.3±1.4°C; Fig. 4B). Although these were clearly arousals from deep, steady-state torpor, T_{sk} during these periods often dropped below our threshold of 25°C for up to 3.5 h. We termed these patterns 'heterothermic arousals' because bats appeared to be expressing shallow torpor in the midst of a periodic arousal. Heterothermic arousals were observed in almost all individuals, and bats that exhibited heterothermic arousals often subsequently expressed plateau arousals (7 of 11), indicating that this pattern was not an artifact of transmitters becoming detached or transmitter batteries failing. We tested for the influence of predictor variables on arousal type (plateau *vs* heterothermic) with GLMMs and bimodal error distributions using the lmer function of the lme4 package (Bates and Maechler, 2009). None of the models examined were good predictors of the likelihood of a plateau *vs* heterothermic arousal (GLMM, *P*>0.05, *n*=51, *N*=20).

In addition to heterothermic arousals, some bats warmed rapidly to near-euthermic temperatures (~20°C) but immediately re-entered
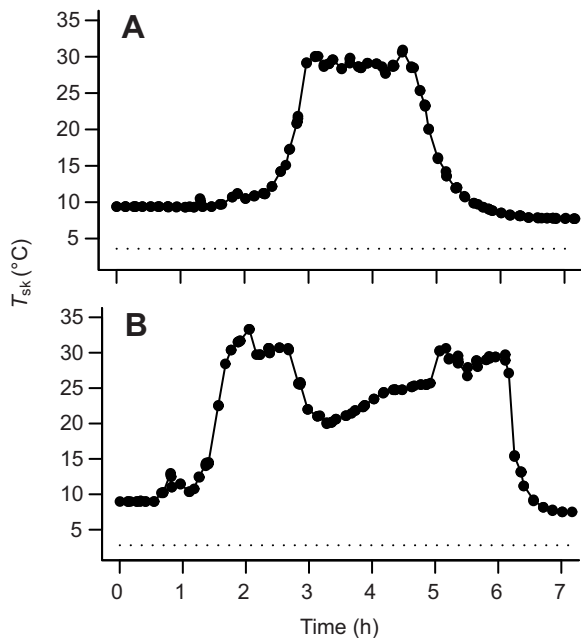
Fig. 4. An example of (A) a plateau arousal and (B) a heterothermic arousal from one adult female *M. lucifugus* (forearm band: UW0906). The solid line and filled circles denote the bat's T_{sk}, and the dotted line denotes T_a.

the cooling phase ($n=5$, $N=4$). We termed these 'partial arousals' and did not include them in analyses of torpor–arousal patterns because bats did not defend elevated T_{sk}. However, we did incorporate partial arousals into energetic analyses.

Estimated energy expenditure

All three models of energy expenditure from the literature [the models of Humphries et al. and Thomas et al. (Humphries et al., 2006; Thomas et al., 1990) and our composite model] provided estimates of fat depletion that were significantly correlated with observed rates of decline in body mass of bats between 29 November 2009 and 23 January 2010, although all models overestimated the actual rate of fat depletion (Fig. 5). The model that best predicted the decline in body mass was that of Thomas et al. (Thomas et al., 1990) (regression, $P<0.001$, $r^2=0.87$), followed by that of Humphries et al. (Humphries et al., 2006) (regression, $P=0.004$, $r^2=0.72$). Our composite model, incorporating more of the variation in T_a and T_{sk} than the other models, explained the least amount of variation (regression, $P=0.024$, $r^2=0.54$). Therefore, we used the energetic model of Thomas et al. (Thomas et al., 1990) for all subsequent calculations and analyses.

Over the entire study period bats consumed an estimated 7.6 ± 1.8 mg fat day^{-1}, ranging from 5.5 to 11.2 mg fat day^{-1} ($N=9$ total; $N=3$ adult females, $N=6$ adult males). There was no significant difference in the calculated rate of fat consumption between adult males (5.9–8.1 mg fat day^{-1}, $N=6$) and adult females (5.5–11.2 mg fat day^{-1}, $N=3$; two-sample t-test, $t=1.2$, $P=0.25$). Arousals accounted for $64.6\pm6.1\%$ of energetic costs over the study period ($N=9$). The mean rate of estimated energy expenditure in a given sampling window decreased as T_a declined (GLMM, $P=0.022$; Table 2) but no other predictor variables were significant.

Effect of radio-transmitter attachment

The mass of transmitters as a percentage of body mass was $7.5\pm0.7\%$. The BCI of bats outfitted with transmitters that were recaptured after 55 days (0.24 ± 0.03, $N=11$) was virtually identical
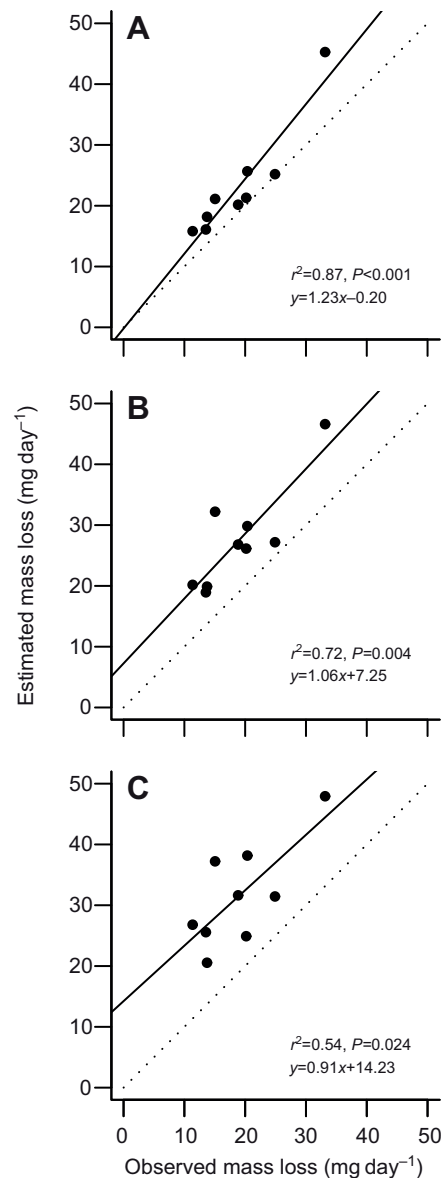

Fig. 5. Test of three models of energy expenditure in hibernating *M. lucifugus*. The solid line denotes regression and the dotted line denotes a 1:1 relationship. (A) Data from Thomas et al. (Thomas et al., 1990); (B) data from Humphries et al. (Humphries et al., 2005); (C) our composite model. Note that all models over-estimate the actual rate of mass loss.

to that of newly captured individuals that had not been carrying radio-transmitters over the same period (0.24 ± 0.02, $N=7$; two-sample t-test, $t=0.011$, $P=0.99$).

DISCUSSION

This study provides some of the first data on torpor–arousal cycles of free-ranging little brown bats during hibernation and is one of the first to estimate energy expenditure for a hibernator based on radio-telemetry measurements of T_{sk}.

Torpor and arousal patterns of free-ranging bats

Our measurements of T_{sk} did not support predictions of the torpor optimization hypothesis. We observed no significant influence of age, sex or BCI on the duration or depth (torpid T_{sk}) of torpor bouts.

Table 2. Estimated rate of fat depletion during each of our three sampling windows

Sampling window	Recording days	Rate of energy use (mg fat day^{-1})	Energy consumption (mg fat)	T_a (°C)	N
31 November to 29 December 2009	28	8.2±3.6 (2.7–15.6)	230.0±101.2 (75.3–437.2)	3.5±1.0 (5.6–2.1)	13
08 January to 22 January 2010	14	5.7±4.3 (1.2–14.5)	158.6±119.2 (32.8–405.5)	2.1±0.4 (2.6–1.1)	13
25 January to 22 February 2010	27	4.9±3.3 (2.3–10.0)	132.7±88.8 (61.7–269.0)	1.9±0.3 (2.6–1.6)	12

Data calculated using the skin temperature (T_{sk}) and ambient temperature (T_a) we measured for free-ranging *M. lucifugus* and Thomas et al.'s model of energy expenditure (Thomas et al., 1990).
Values are means ± s.d. with ranges in parentheses.

Similarly, the duration of bats' arousals and mean T_{sk} during arousals were not influenced by age, sex or BCI. Most likely, the absence of a sex difference in patterns of T_{sk} reflects low statistical power due to a limited sample size. Despite the large number of bat-days of data collected, standard deviations of torpor bout and arousal durations were large, often as great as the mean, and the number of individuals we monitored was low compared with our previously reported dataset for BCI, which found support for this hypothesis (Jonasson and Willis, 2011). The large, between-individual variation we observed suggests that sample sizes larger than typical for studies in ecophysiology may be needed to fully characterize the hibernation energetics of bats. An alternative method that could be used to increase sample size for this type of study would be to monitor large clusters of bats with thermal imaging video cameras (e.g. Hristov et al., 2008).

Large variation in the duration of torpor bouts appears to be characteristic of little brown bats and our data support Twente and colleagues' (Twente et al., 1985) hypothesis that this variation and abbreviated torpor bouts (<1 day) are not artifacts of captivity. Torpor bout duration increased as T_a declined, which is consistent with the negative correlation reported for numerous hibernating species (e.g. Buck and Barnes, 2000; Dunbar and Tomasi, 2006; Geiser and Kenagy, 1988). However, the scatter in this relationship increased dramatically below 4°C. The reversal of the relationship between torpor duration and T_a near the set-point for T_b during hibernation has been noted in several species (e.g. Buck and Barnes, 2000; Geiser and Kenagy, 1988; Thomas and Geiser, 1997). In our study, the large variation in torpor bout duration below 4°C could reflect the fact that as T_a first declined below this lower critical temperature, bats immediately aroused in an attempt to find a warmer microclimate. Given the small size of Dale's cave this was probably not possible so they re-entered torpor and exhibited long torpor bouts at this same T_a. This pattern could also be due to increased evaporative water loss (EWL) in colder, drier air, which may increase the pressure for bats to move to a more humid roost or cluster with other individuals to reduce EWL (Thomas and Cloutier, 1992).

Unlike torpor bouts, the duration of arousals and mean T_{sk} during arousals were not significantly related to T_a. The within-individual flexibility in the duration of arousals and in T_{sk} during arousals, independent of T_a, suggests that hibernation energy expenditure may be less dependent on hibernaculum T_a than has been assumed for previous models (i.e. Boyles and Brack, 2009; Humphries et al., 2002). To our knowledge, this study is the first to report what we term heterothermic arousals by any hibernator. We observed multiple occurrences of these arousals from almost all individuals and many individuals that exhibited this pattern subsequently expressed plateau-style arousals. This makes it extremely unlikely that the heterothermic arousals we observed reflected an artifact of transmitter detachment or failing batteries. In addition to heterothermic arousals, we also observed several partial arousals during which bats warmed to near-euthermic temperatures but returned to torpor without defending elevated T_{sk}. This may reflect a response to disturbance from conspecifics or possibly a response to predators entering the cave (e.g. red squirrels *Tamiasciurus hudsonicus*).

Modelling energy expenditure

All three of the energetic models we evaluated were significantly correlated with the actual energy expenditure of free-living bats based on measurements of the decline in body mass between captures. However, all models slightly overestimated energy expenditure. This is surprising as none of the models accounted for energetic costs other than thermoregulation (e.g. flight, mating) and we expected all models to underestimate actual energy expenditure. This suggests that these energetic calculations failed to incorporate some behavioural (e.g. clustering, microclimate selection) or physiological (e.g. heterothermic arousal) factors that reduce the energetic demands of free-living bats. Values of metabolic rate for all models were based on measurements obtained using open-flow respirometry. It is possible that respirometry studies overestimate metabolic rates of hibernating bats in part because of the stress of metabolic measurements but also because ambient conditions in metabolic chambers are likely to differ from conditions in caves with little airflow and high humidity (Davis, 1970). Air being pumped through respirometry chambers may increase thermal conductance and, therefore, the need for metabolic heat production. Available respirometry measurements also fail to incorporate potential energy savings from clustering, or movement to warmer regions of the hibernacula during arousal (Boyles and Willis, 2010; Hardin and Marion, 1970). The simplest energetic model (Thomas et al., 1990) best matched the decline in body mass observed in free-ranging bats. The lower fit of Humphries et al.'s model (Humphries et al., 2006) may be attributed to omission of the costs of cooling period. The composite model we devised was poorest at estimating energy expenditure, probably because we extrapolated Studier's (Studier, 1981) equation for metabolic rate during euthermic periods to greater T_b–T_a differentials. Furthermore, the resting metabolic rates of the pregnant, lactating and post-lactating bats Studier measured (Studier, 1981) were undoubtedly greater than those of hibernating bats. We recommend that future studies obtain more detailed estimates of energy expenditure for little brown bats, and other species, during each phase of thermoregulation associated with hibernation. In the meantime, studies using T_{sk} to estimate energy expenditure should employ Thomas et al.'s (Thomas et al., 1990) model or the model used by Humphries et al. (Humphries et al., 2006), if costs during cooling can be incorporated.

The rate of estimated fat consumption decreased as hibernation progressed and as T_a declined within the cave (Table 2), which is consistent with our previous finding that bats declined in body mass more rapidly early in the season when cave T_a was higher (Jonasson and Willis, 2011). The trend for reduced energy expenditure later during hibernation results from longer torpor bouts at low T_a, and

hence less frequent arousals. However, the model we used to calculate energy expenditure for this analysis (Thomas et al., 1990) did not account for the relationship between torpid and euthermic metabolic rates and T_a. The actual energetic savings resulting from less frequent arousals and reduced TMR at low T_a may be smaller than predicted by our calculations because, even though TMR falls with declining T_a, euthermic metabolic rate, and the cost of arousals, will increase. Again, this suggests that existing models of hibernation energy expenditure need to be further refined based on new measurements.

Torpor–arousal cycles and, therefore, rates of energy expenditure varied widely between individuals, even though all animals were exposed to the same range of environmental conditions (Table 2). This between-individual variation means that bats' likelihood of surviving hibernation cannot be entirely predicted by the size of immergence fat reserves, T_a and winter duration. Individual variation in hibernation energy expenditure could reflect heritable differences among individuals and, therefore, be subject to natural selection or it may represent phenotypic plasticity, in which case individual hibernating bats may have a wider range of available responses to disturbance and environmental change than previously thought (e.g. Boyles and Brack, 2009). In particular, given recently established links between WNS and hibernation energetics (Warnecke et al., 2012), better understanding the heritability and/or phenotypic flexibility of hibernation patterns has potential importance for understanding responses of bat populations to this emerging wildlife disease. More work is needed to better understand factors causing between- and within-individual variation in torpor–arousal cycles of this species and other hibernators.

Hibernation patterns of bats *vs* rodents

The little brown bats in our study exhibited an energetically conservative pattern of hibernation compared with other hibernators. The percentage of time bats from our study spent in torpor (99.6%) is considerably greater than that for other fat-storing hibernators such as western jumping mice [*Zapus princeps*, 95% (Cranford, 1978)], Columbian ground squirrels [*Spermophilus columbianus*, 95.4% (Young, 1990)], white-tailed prairie dogs [*Cynomys leucurus*, 85.8% (Bakko and Nohorniak, 1986)] and yellow-bellied marmots [*Marmota flaviventris*, 88.6% (Armitage et al., 2003)]. Moreover, bats appeared to express periods of shallow torpor in the midst of periodic arousals, which could increase energy savings during hibernation. Heterothermic arousals could be a unique adaptation of bats linked to conditions in their hibernacula and/or their morphology. In contrast to most mammalian hibernators, which use small, enclosed, insulated burrows, little brown bats spend the winter in large, open-air hibernacula. They are also unable to curl into the characteristic ball-like posture of most hibernators because they must hang upside down by their feet. Therefore, they may face increased heat loss to the environment and incur proportionally greater costs of arousal. Heterothermic arousals may allow them to save energy during arousals by defending a T_b lower than euthermic T_b while still repaying (albeit perhaps at a reduced rate) the physiological debt accrued during the preceding deep, multi-day torpor bout. Heterothermic arousals, brief plateau arousals and very long torpor bouts appear to be adaptations of this species for extreme energy savings during winter.

Effect of transmitter attachment on hibernating bats

Aldridge and Brigham used models of flight performance to recommend that radio-transmitters should not exceed 5% of body mass (Aldridge and Brigham, 1988), a standard that has become widely known as the '5% rule' in telemetry studies. Even though, at approximately 7.5% of bat body mass, transmitters in this study exceeded the 5% rule, we detected no energetic cost of carrying transmitters for hibernating bats. Bats subjected to capture, handling and transmitter attachment in November, followed by 2 months of carrying transmitters, were in virtually identical body condition to bats captured for the first time in late January. This supports the suggestion of Rojas and colleagues that, in certain circumstances, exceeding the 5% rule can substantially improve data quality without having a significant impact on the animals, as long as the ecology of the study species is taken into account (Rojas et al., 2010). The cost of carrying transmitters while roosting should be negligible for bats because little muscular effort is required for grasping the roosting substrate at rest (Quinn and Baumel, 1993). During hibernation, bats may also tolerate heavier transmitters than during the active season because they typically only fly for short distances to switch roosting locations or find water, tasks that require less manoeuvrability than foraging during the summer. Caution should still be used when conducting temperature telemetry during hibernation because transmitters could remain attached for several months until the end of hibernation, when bats emerge from hibernation and depend more heavily on long-distance flying. However, our findings suggest that the process of conducting temperature telemetry studies has little impact on hibernating bats if disturbance is kept to a minimum.

CONCLUSIONS

We found that little brown bats exhibited an energetically conservative pattern of hibernation compared with many rodents and appeared to reduce the cost of hibernation by spending a greater proportion of their time in deep torpor and expressing heterothermic arousals (i.e. shallow torpor bouts in the midst of arousals). We found that a simple model of hibernation energetics best predicted actual decline in body mass but more detailed estimates of energy expenditure in hibernating bats are needed. Despite the results of our previous study (Jonasson and Willis, 2011), we found no effect of age, sex or energy availability (i.e. fat stores) on hibernation patterns or energy expenditure. Thermal cameras may be needed to gain large enough sample sizes to adequately test for optimization of torpor expression in free-living hibernating bats. However, given that we found no evidence that transmitters exceeding 5% of body mass had a negative impact on bats, it may be possible and more cost effective to obtain adequate data employing temperature transmitters or temperature dataloggers.

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