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Thrifty Females, Frisky Males: Winter Energetics of Hibernating Bats from a Cold Climate

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ABSTRACT

Mammalian hibernation consists of energy-saving torpor bouts (periods of controlled reduction in body temperature [T_b]) interspersed with brief arousals to normothermic T_b . Frequency and duration of torpor bouts and arousals can affect winter survival and are thought to be influenced by an optimization balancing the energetic benefits of prolonged torpor against the physiological and ecological costs (e.g., accumulation of metabolic wastes). Female little brown bats (*Myotis lucifugus*) spend their fat reserves more slowly than males during winter, presumably so they can emerge from hibernation in good condition to initiate pregnancy. We used temperature telemetry over three winters to test a prediction of the optimization hypothesis that female *M. lucifugus* would use longer torpor bouts and/or shorter arousals than males. Females did conserve energy relative to males by adjusting the magnitude and duration of arousals but not the duration of torpor bouts. Although torpor bout duration did not vary by sex for adults, it did vary by age. Adults initially used longer torpor bouts than young-of-the-year, but this difference declined as cave temperature warmed in spring. Males and females in better condition spent more energy during hibernation, again via increased arousal duration rather than decreased torpor bout duration. Longer arousals by males could increase reproductive fitness if males mate with torpid females throughout winter. Our results highlight demographic differences in winter behavior for small hibernators facing extreme energy limitation in cold climates and illustrate the influence that reproductive costs have on hibernation energetics.

Keywords: hibernation energetics, torpor, optimization, chiroptera, thermoregulation.

Introduction

Seasonal variation in climate and environmental conditions strongly influence the energy budgets of animals (McNab 2002). In temperate areas, winter is a time of low ambient temperature (T_a) and reduced food availability, which makes it more difficult for endotherms to balance their energy budget. Temperate endotherms have therefore evolved behavioral and physiological adaptations to contend with unfavorable winters. Hibernating mammals, for example, spend the majority of their time in torpor, a state of controlled reduction in body temperature (T_b) and metabolic rate (MR; Geiser 2004, 2013). Reduction in MR confers energy savings and allows hibernators to survive the winter on a small store of food or fat (Lyman 1982; Wilkinson and South 2002).

Despite energetic benefits, long-term torpor has costs, including sleep deprivation, dehydration, and increased blood CO_2 and metabolic wastes (Daan et al. 1991; Thomas and Geiser 1997; Elvert and Heldmaier 2000; Ben-Hamo et al. 2013). Additionally, the immune system of hibernators is depressed, which could make them more susceptible to pathogens (Bouma et al. 2010; Moore et al. 2013). Presumably to counteract these physiological costs, all hibernators interrupt their torpor bouts with periodic arousals to normothermic T_b (Geiser 2004; but see Lovegrove et al. 2014). Although arousals are brief, due to the high metabolic cost of thermoregulation during normothermia at low T_a , they can account for up to 90% of overwinter energy expenditure (Wang 1978; Thomas et al. 1990; Geiser 2004).

In the past decade, the paradigm that hibernators should maximize energy savings by maximizing torpor expression has been challenged by the torpor optimization hypothesis (Humphries et al. 2003a, 2003b; Boyles et al. 2007; Wojciechowski et al. 2007). Torpor optimization predicts that hibernators with excess energy reserves should sacrifice the energetic benefits of long torpor bouts to avoid the physiological costs of torpor (Humphries et al. 2003b). Data from eastern chipmunks (*Tamias striatus*) and several bat species support the optimization hypothesis. Individuals with excess energy (i.e., greater access to food or fat) tend to express torpor less and, if available, select warmer microclimates (Munro et al. 2005; Boyles et al. 2007; Wojciechowski et al. 2007; Landry-Cuerrier et al. 2008).

In addition to effects of energy reserves, optimization also predicts that the timing of reproductive investment for males

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and females should influence energy expenditure and torpor expression during winter (Humphries et al. 2003b; Jonasson and Willis 2011). Female little brown bats (*Myotis lucifugus*) delay fertilization until after hibernation (Wimsatt 1944, 1945) and must emerge to initiate pregnancy in early spring, when the weather is still cold and food may be unavailable (Norquay and Willis 2014). Therefore, females should face selection pressure to emerge from hibernation with fat reserves left over. *Myotis lucifugus* in central Canada were, indeed, more “thrifty” with their fat reserves and declined in body condition more slowly during hibernation than males (Jonasson and Willis 2011). Males, not facing the imminent burden of reproductive investment in spring, presumably used more of their winter energy reserves to mitigate the physiological costs of prolonged torpor and, potentially, to mate with torpid females during hibernation (Thomas et al. 1979). However, despite detecting a difference in the rate of fat use between males and females, with a small sample of individual bats Jonasson and Willis (2012) were not able to detect sex differences in torpor expression on the basis of skin temperature (T_{sk}).

In addition to sex differences, torpor optimization also predicts differences in hibernation behavior between adults and young-of-the-year (YOY). YOY have more difficulty than adults accumulating fat stores during the fall swarming period (McGuire et al. 2009). The size of fat stores appears crucial for survival, as bats born earlier in spring, with more time to accumulate fat, are more likely to survive their first winter (Frick et al. 2010). If YOY have smaller energy stores than adults, they should rely more heavily on torpor expression during hibernation than adults. Complicating this prediction, though, is the fact that some YOY females, but apparently not males, reach sexual maturity before their first winter (Carter 1970; Gustafson and Shemesh 1976). This suggests that YOY females may face similar reproductive energetic constraints as adults despite a smaller fat reserve. If so, optimization predicts that YOY females should increase their expression of torpor even more than adult females. YOY males may behave more like adult males or, if their reserves are small, hibernate conservatively like adult females.

Our objective was to provide additional tests of Humphries et al.’s (2003b) torpor optimization hypothesis and Jonasson and Willis’s (2011) related thrifty-female hypothesis, which predict that arousal and/or torpor bout duration should vary with sex, age, and body condition index (BCI; i.e., mass/forearm length). We combined T_{sk} data published by Jonasson and Willis (2012) with 2 yr of additional data from the same study area. We used temperature telemetry to record the T_{sk} of free-ranging bats combined with a model of MR during different thermoregulatory phases to quantify the energetic costs of torpor and arousals. We predicted that (1) bats with higher BCI would exhibit a high-energy hibernation pattern, performing longer arousals and shorter torpor bouts; (2) females would exhibit a low-energy hibernation pattern, with shorter arousals and longer torpor, because of strong selection pressure on them to emerge from hibernation with fat remaining to support pregnancy; and (3) YOY would exhibit an extremely low-energy hibernation pattern, with longer torpor bouts and shorter arousals than adults, to conserve their smaller

fat reserves and, possibly in the case of some reproductive YOY females, support the potential for pregnancy in spring.

Methods

All procedures were approved by the University of Winnipeg Animal Care Committee and conducted under Manitoba Conservation Wildlife scientific permit WB0612. This study was conducted in two limestone caves (Firecamp Cave and Dale’s Cave) north of the town of Grand Rapids in central Manitoba, Canada (53°30’N; 99°24’W). We combined data collected in 2009–2010 by Jonasson and Willis (2012) with new data from the same study site collected during the winters of 2011–2012 and 2012–2013. From 2009 to 2013, the mean annual T_a in Grand Rapids ranged from -1.8° to 1.7°C , with a maximum T_a of 33.3°C and a minimum T_a of -40.7°C . Firecamp Cave is accessed by a sinkhole entrance approximately 50 cm in diameter and consists of a bell-shaped chamber with a floor about 7 m from the surface. Between 30 and 60 bats overwinter in Firecamp Cave from mid-September until late May (J. Dubois and C.K.R. Willis, unpublished data). Bats in this cave hibernate in one tight cluster within a crack 1 m long by 20 cm deep, on the ceiling of the main chamber. Dale’s Cave is accessed by a 3-m-deep sinkhole and consists of a bell-shaped chamber (approximately 7 m in length and 5 m in breadth, with a 4-m ceiling) that slopes away from the entrance. Approximately 80–200 bats overwinter in Dale’s Cave from mid-September until late May (J. Dubois and C.K.R. Willis, unpublished data).

Temperature data loggers (iButtons; Maxim Integrated Products, Dallas, TX) dipped in paraffin wax to attenuate ultrasonic noise (Willis et al. 2009) were used to record T_a ($\pm 0.5^\circ\text{C}$) every 2 h in Dale’s and Firecamp Caves. In 2013, we also placed data loggers in Dale’s Cave (HOBO Micro Station Data Logger H21-002; $\pm 0.2^\circ\text{C}$; Onset Computer, Cape Cod, MA). All data loggers were hung from the ceiling of the caves in areas known to be used by bats.

Bats were captured by hand on November 29, 2009 (Jonasson and Willis 2012); February 20, 2012; and January 24, 2013. They were then sexed and weighed to the nearest 0.01 g (Durascale-100; MyWeigh Canada, Vancouver, British Columbia), and their forearm length was measured to the nearest 0.05 mm. YOY were identified on the basis of unfused third-digit metacarpal-phalangeal joints and tapered rather than “knobby” joints, following Davis and Hitchcock (1965). In this northern study area, YOY can be readily identified during swarming and even into hibernation (Jonasson and Willis 2011, 2012; Czenze et al. 2013).

For small bats, T_{sk} provides a good approximation of T_b during torpor (Audet and Thomas 1996; Barclay et al. 1996; Willis and Brigham 2003). After clipping a small ($<1\text{ cm}^2$) patch of fur between the shoulders, T_{sk} was recorded by attaching temperature-sensitive radio transmitters (BD-2NT; 0.80–0.90 g; Holohil Systems, Carp, Ontario) using a nontoxic, latex-based adhesive (Osto-Bond; Montreal Ostomy, Vaudreuil, Quebec). The manufacturer modified the transmitters by reducing signal strength to maximize battery life. Bats from all

three winters weighed 9.73 ± 1.2 g on average, and transmitters represented more than 5% of body mass. This is greater than the guideline suggested by Aldridge and Brigham (1988) but similar to the size that Jonasson and Willis (2012) demonstrated had no negative impact on body condition in hibernating *Myotis lucifugus*. We calibrated transmitters before use in a water bath at 5°C increments from 0.3° to 45.3°C against a thermometer traceable to the National Institute of Standards and Technology.

In 2009–2010, we deployed a data-logging receiver (Lotek SRX400; Lotek Engineering, Newmarket, Ontario) outside Dale's Cave, and an array of four 30-cm omnidirectional whip antennas was used inside the cave to record T_{sk} every 10 min. In 2012 in Firecamp Cave and in 2013 in Dale's Cave, we used a similar data-logging receiver (Lotek SRX600; Lotek Engineering) powered by two solar panels and connected to one 30-cm omnidirectional whip antenna in 2012 and, to improve signal detection, three four-element yagi antennas in 2013. Receivers were programmed to record T_{sk} data every 10 min.

Large differences between cave T_a and normothermic T_{sk} led to obvious arousals in the T_{sk} trace, so we followed Jonasson and Willis (2012) to define the phases of torpor and arousal. Torpor bouts were defined as periods of reduced stable T_{sk} between the obvious warming and cooling phases associated with each arousal (fig. 1A). Skin temperature measured using Holohil transmitters occasionally appears to increase slightly and gradually after initial entry into a torpor bout and appears to decline gradually as the transmitter battery loses voltage at cold T_a (e.g.,

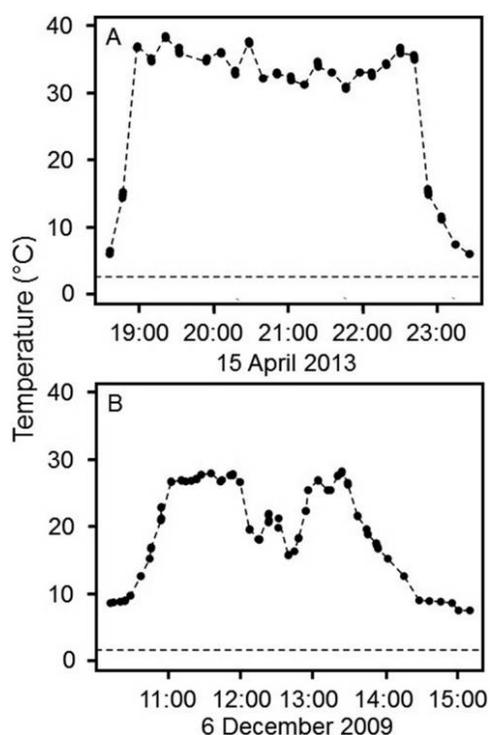


Figure 1. Representative skin temperature trace of (A) plateau arousal by a *Myotis lucifugus* on April 15, 2013, and (B) heterothermic arousal on December 6, 2009, by a hibernating adult male *M. lucifugus* in Dale's Cave in central Manitoba. Dashed lines represent cave temperature.

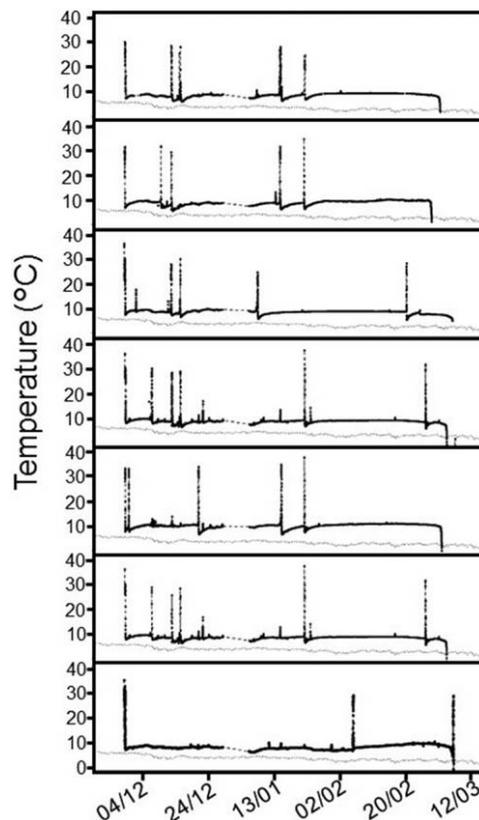


Figure 2. Representative skin temperature traces for seven little brown bats (*Myotis lucifugus*) in Dale's Cave in central Manitoba. Dotted lines represent cave temperature. Dashed lines represent periods when we lost the transmitter signal due to receiver drain.

Jonasson and Willis 2012; Czenze et al. 2013; figs. 2, 3). However, we could readily identify these artifacts in our T_{sk} traces, and it did not affect our analysis. Onset of the warming phase was classified as an abrupt increase in torpid T_{sk} that eventually stabilized at a normothermic steady state. The caves' cold T_a appeared to increase ambient cooling of external transmitters, leading to reduced T_{sk} values during the normothermic phase (Willis and Brigham 2003). However, the normothermic period could still be readily identified as the time between the end of the warming phase and the initiation of cooling, with cooling defined as an abrupt decline in T_{sk} preceding steady-state torpor. We excluded data from the first 24 h after releasing the bats to avoid the influence of disturbance on our results. Some bats also exhibited what Jonasson and Willis (2012) referred to as heterothermic arousals (fig. 1B). During heterothermic arousals, individuals appeared to allow T_b to fall to an intermediate level after the initial rewarming phase. Bats defended this intermediate T_b for up to 2.3 h and then rewarmed to a higher T_b typical of periodic arousals. Following Jonasson and Willis (2012), we defined heterothermic arousals as those during which the T_{sk} of an individual fell below 25°C for at least 20 min (i.e., two T_{sk} recordings). The warming, homeothermic, and cooling phases of plateau and heterothermic arousals were determined identically.

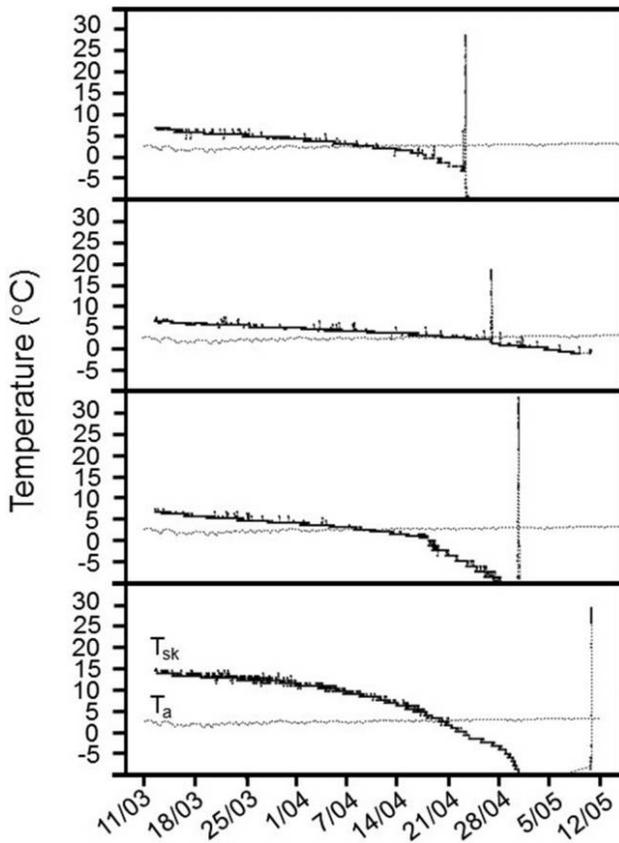


Figure 3. Representative skin temperature traces of winter torpor bouts for four little brown bats (*Myotis lucifugus*) in Dale's Cave in central Manitoba. Dotted lines represent cave temperature. The steady decline in skin temperature is due to a gradual decline in battery voltage at the end of the transmitter's battery life.

All analyses were conducted in R (ver. 2.10.1; R Development Core Team 2009), and values are reported as the mean \pm SD, with N as the number of animals and n as the number of events/arousals. We assessed significance at the $P < 0.05$ level. To assess potential differences in torpor and arousal duration while controlling for pseudoreplication and the fact that data were recorded over three winters in two different caves, we used linear mixed-effects models (packages nlme, lme4, and MuMIn in R; ver. 3.0.1; R Development Core Team 2009) with a conservative random-effects structure (i.e., individual nested within cave nested within year). We conducted model selection by comparing nested models using maximum likelihood tests until only significant variables remained. We examined whether sex, age, BCI, date, and mean cave T_a (during torpor bout or arousal) affected torpor bout duration, arousal duration, and estimated over-winter energy expenditure. We used cave T_a in our torpor bout duration models because date and mean cave T_a are positively correlated at our study site, and cave T_a predicted torpor bout duration better than date (Czenze and Willis 2015).

To convert the duration of arousals and torpor bouts into energetic units, we used a simple model first presented by Thomas et al. (1990), which Jonasson and Willis (2012) dem-

onstrated predicted declines in body condition (i.e., expenditure of fat) over time for hibernating *M. lucifugus* much more accurately than two alternative models. As hibernating bats in good condition rely primarily on fat metabolism (Yacoe 1983), we assumed 1 mL of $O_2 = 20.1$ J as a conversion factor (Thomas et al. 1990). We assumed that the warming phase of arousals cost $2.71 \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$, the homeothermic phase cost $166.6 \text{ J g}^{-1} \text{ h}^{-1}$, and the cooling phase cost 67.2% of the cost of warming (Wang 1978; Thomas et al. 1990). To estimate energy expenditure over time, we calculated power (W ; kJ s^{-1}). We did not differentiate between heterothermic and plateau arousals in our estimates of energy expenditure because of the difficulty of accurately estimating energetic costs during shallow torpor. Thus, the model likely overestimated energetic costs for some individuals using heterothermic arousals. The model was also unable to account for differences in energy costs associated with behavioral activity of bats during arousals (e.g., resting vs. grooming, mating, or flying). However, these costs may be relatively small compared with the overall thermoregulatory costs of rewarming and maintaining normothermic T_b at cave T_a . Flight is energetically expensive, and if bats spent a large proportion of their arousals in active flight, activity costs could have strongly influenced overall energy expenditure. On the other hand, thermogenesis during flight can substitute for a portion of thermoregulatory costs, even in small-bodied endotherms, reducing the impact of activity on overall energy costs (Humphries and Careau 2011; Minnaar et al. 2014; Klüg-Baerwald et al. 2016). The model we used accounted for variation in rewarming costs and costs during cooling associated with variation in cave T_a but did not capture variation in resting MR due to the effect of cave T_a on MR during the plateau phase of arousals. Cave T_a was relatively stable throughout the study ($1.1^\circ\text{--}3.9^\circ\text{C}$), but even this small range would have caused differences in energy costs. Nevertheless, we used Thomas et al.'s (1990) model because it more reliably predicted declines in energy reserves for free-ranging bats from this study area in an early study (Jonasson and Willis 2012), and it still provides useful information about overall energy expenditure.

We used a generalized linear mixed-effects model with a binomial distribution, sex as a fixed effect, and individual as a random effect to test for a difference between males and females in the proportion of arousals that included a bout of heterothermy. We also used this model to compare the proportion of individual females versus males that exhibited at least one heterothermic arousal.

Results

We recorded 3,247 bat-days of T_{sk} data from 63 individuals during the three winter field seasons from 2009 to 2013 (11 adult females, 11 YOY females, 35 adult males, and 6 YOY males; table 1). Bats exhibited long torpor bouts averaging 16.2 ± 11.4 d (range, 0.02–60.25 d; fig. 3) interspersed with brief periodic arousals averaging 3.9 ± 1.5 h (range, 1.1–7.9 h). During this time, we recorded 200 torpor bouts and 139 arousals.

Forearm length ($P = 0.27$, $N = 63$ bats) and body condition ($P = 0.50$, $N = 63$) were not different between age

Table 1: Summary of hibernation patterns and morphometric data for hibernating little brown bats (*Myotis lucifugus*) in Dale's (D) and Firecamp (F) Caves in central Manitoba

Year	Cave	Capture date	Bat-days	No. individuals	Mean mass at capture (g)	Mean BCI at capture (g/mm)	No. torpor bouts	No. arousals	Mean TBD (d)	Mean AD (min)
2009	D	Nov 29	1,511	21	10.2 ± .92	.27 ± .02	54	64	13.1 ± 10.8	260 ± 94
2012	F	Feb 20	459	15	9.7 ± 1.6	.26 ± .04	42	29	15.3 ± 8.9	198 ± 66
2013	D	Jan 24	1,277	27	9.4 ± 1.1	.25 ± .02	104	46	20.1 ± 14.6	190 ± 90

Note. AD = arousal duration; BCI = body condition index; TBD = torpor bout duration.

classes at the time of capture, but females had higher BCI than males ($P < 0.01$, $N = 63$). Torpor bout duration was not affected by sex, date, or BCI. However, torpor bout duration was related to the interaction between age class and cave T_a ($P < 0.01$, $N = 62$, $n = 158$ bouts; table 2; fig. 4). The duration of torpor bouts used by adults appeared to decline as cave T_a increased during late winter/spring (from 2.2° to 3.4°C), while bouts used by YOY increased in duration as cave T_a increased. As winter progressed maximum torpor bout duration increased ($P = 0.02$, $N = 63$, $n = 63$), but on average maximum torpor bout duration recorded for adults (31.0 ± 15 d) was longer than that of YOY (20.2 ± 10 d; $P = 0.03$, $N = 63$, $n = 63$). The model, with parameter estimates ± SE, was described by the following equation: torpor bout duration (d) = age × (−38.26 ± 11.84) + cave temperature × (−6.60 ± 2.77 + (12.68 ± 4.45 × age)) + (37.26 ± 7.66), where age is a categorical variable with YOY = 1 and adults = 0.

Age class, cave T_a , or date did not affect duration of arousals. However, BCI was a significant predictor of arousal duration, with bats in better condition exhibiting longer arousals ($P = 0.04$, $N = 52$, $n = 95$). There was also a significant sex effect, with arousals by male bats lasting 22% longer on average than those of females ($P < 0.01$, $N = 52$, $n = 95$). The model, with parameter estimates ± SE, was described by the following equation: arousal duration (min) = BCI × (838.83 ± 402.75) + sex × (72.98 ± 23.59) − (30.33 ± 115.66), where sex is a categorical variable with males = 1 and females = 0. The energetic model we used estimated that mean arousal cost was 2.78 ± 1.71 kJ. Surprisingly, this model was not able to detect increased energy expenditure resulting from the longer arousals we observed for males than females ($P = 0.6$, $N = 52$, $n = 95$). There were no effects of age, class, sex, T_a or date on calculated energy expenditure during arousals. However, BCI

was a significant predictor of energy expenditure during arousals, with bats in better condition spending more energy ($P > 0.01$, $N = 52$, $n = 95$; fig. 5). The model, again with parameter estimates ± SE, was described by the following equation: arousals costs (W) = BCI × (1.7 ± 0.52) − (0.23 ± 0.14).

Thirty percent (33/110) of arousals were heterothermic, during which individuals defended T_{sk} below 25°C for at least 20 min. The proportion of heterothermic arousals expressed by females (68%) was dramatically higher than that expressed by males (39%; $P < 0.01$, $N = 53$, $n = 110$). Furthermore, the proportion of individuals using at least one heterothermic arousal during the winter was higher for females (56%) than males (32%; $P < 0.01$, $N = 53$).

Discussion

We found that body condition impacts winter energy budgets of *Myotis lucifugus*, with individuals in better condition performing longer arousals and spending more energy during their arousals. This is consistent with the optimization hypothesis and suggests that bats reduce expression of torpor if they have fat remaining to offset thermoregulatory costs. We also found support for our second hypothesis, that the “thrifty” energetic strategy of females observed by Jonasson and Willis (2011) is achieved by expressing torpor differently than males. Interestingly, females did not exhibit longer torpor bouts but, instead, used arousals of shorter duration than males. Females were also more likely to express heterothermy in the midst of their arousals. We found no support for our final prediction that YOY would express the most torpor. Instead, we found that torpor bout duration was affected by an interaction between cave T_a and age. YOY increased torpor bout duration as cave T_a increased toward spring, while adults decreased torpor bout duration with increasing T_a . Our

Table 2: Significant predictors (i.e., age class, cave ambient temperature [cave T_a], and the interaction between the two) of torpor bout duration (TBD) in hibernating little brown bats (*Myotis lucifugus*) from Dale's and Firecamp Caves in central Manitoba

Predictor variable	t	P	No. individuals	No. torpor bouts
Age	−3.23	.002	62	158
Cave T_a	−2.38	.019	62	158
Age × cave T_a	2.85	.005	62	158

Note. Adults exhibited longer TBD than young-of-the-year (YOY), and increasing cave T_a in spring was associated with decreased TBD in adults but increased TBD in YOY.

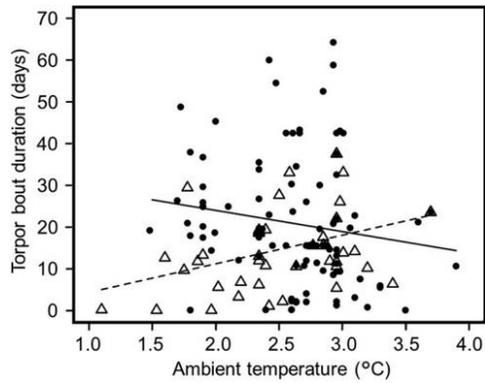


Figure 4. Effect of ambient temperature in the hibernaculum on torpor bout duration of hibernating little brown bats (*Myotis lucifugus*) in Dale's and Firecamp Caves in central Manitoba. A linear mixed model was used to analyze the data ($P = 0.02$, $t = 2.85$, $N = 62$ bats, $n = 158$ bouts), and ordinary least squares regression lines are shown here to illustrate the relationships. Filled circles and the solid regression line represent adults. Open triangles and the dashed regression line represent young-of-the-year.

data indicate that available energy reserves, sex, and age influence torpor expression and winter energy budgets of hibernating bats.

Our findings are consistent with the torpor optimization hypothesis. In yellow-bellied marmots (*Marmota flaviventris*), individuals with larger fat reserves spent more energy than smaller conspecifics (French 1985, 1990); in edible dormice (*Glis glis*), fatter individuals reduced torpor bout duration and increased arousal duration (Bieber et al. 2014). We found that bats in good body condition increased arousal duration, and, on the basis of Thomas et al.'s (1990) model, this translated into increased energy expenditure during the normothermic phases of arousals. These findings are consistent with optimization and suggest that longer, more energetically expensive arousals allow hibernators to compensate for the physiological costs of torpor more fully. As the winter progressed and fat reserves would have declined, individual bats also become more likely to exhibit shorter arousals, spend less energy during the normothermic period, and express longer maximum torpor bouts. This is also consistent with optimization because, as the end of hibernation approaches, the energetic cost of maintaining a long homeothermic period may begin to outweigh the benefit of mitigating the negative physiological consequences of torpor.

Our findings suggest that, for temperate bats, adjustments in the characteristics of arousals may be more important than adjustments in torpor bout duration for balancing the physiological costs of torpor against its energetic benefits. We predicted that females would exhibit longer torpor bouts than males, but we found no effect of sex on duration of torpor bouts. Several studies of hibernating bats have also reported the absence of a sex effect on torpor bout duration (Park et al. 2000; Dunbar and Tomasi 2006; Hope and Jones 2012; Halsall et al. 2012; Jonasson and Willis 2012), although most of these have had low statistical power. Despite a relatively large sample size, we cannot exclude the possibility that between-individual variability in

torpor bout duration masked a potential sex effect, but our results suggest that sex does not influence torpor bout duration in this species.

Although we did not detect longer torpor bouts in adult females, consistent with the thrifty-female hypothesis, they did exhibit shorter arousals and were dramatically more likely to express the heterothermic arousals observed by Jonasson and Willis (2012) than adult males. Sex effects on arousal duration have been observed in several studies of mammalian hibernators (Michener 1992; Munro et al. 2005), but not for bats (Park et al. 2000; Jonasson and Willis 2012). Our results suggest that the relatively slow decline in body condition of females compared with males observed by Jonasson and Willis (2011) reflected differences in arousal duration, not torpor bout duration. Males that do not face the same energetic constraints as females may use their energy stores during arousals to mitigate the negative physiological consequences of long torpor bouts more fully. Longer arousals could also be important for male reproductive success and may reflect mating attempts by males during winter, which have been observed in *M. lucifugus* from other parts of their range (Racey 1972; Thomas et al. 1979). These include multiple thermal/infrared imaging observations of attempts by some free-ranging bats, presumably males, to mate with torpid conspecifics (P. Cryan, personal communication). Interestingly, the "receivers" in these interactions often fail to arouse in response to observed mating attempts by conspecifics (P. Cryan, personal communication). If individual males in the best condition can spend more time in a normothermic state, they could engage in more mating attempts and potentially increase their reproductive success compared with individuals in poorer condition. Although the cost of behavioral activity during arousals may be relatively small compared with thermoregulatory costs and could help substitute for costs of thermoregulation (Humphries and Careau 2011; Klüg-Baerwald et al. 2016), we

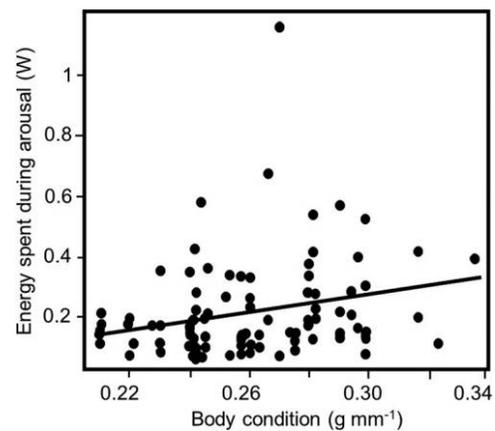


Figure 5. Relationship between body condition at capture and calculated energy expenditure during arousals for hibernating little brown bats (*Myotis lucifugus*) from Dale's and Firecamp Caves in central Manitoba. A linear mixed model was used to analyze the data ($P > 0.01$, $t = 3.28$, $N = 52$ bats, $n = 95$ arousals), and the ordinary least squares regression line is shown here to illustrate the relationship.

suggest that additional efforts be made to quantify the energetic implications of behavior the model cannot account for (e.g., grooming, mating, locomotion, drinking) during arousals to understand better this critical, cryptic, and potentially unique aspect of hibernation by bats.

Arousals by females were shorter than those of males, but when we calculated the energetic costs of arousal using Thomas et al.'s (1990) model, this difference did not translate into a significant effect of sex on energy expenditure. Although Thomas et al.'s (1990) model performed better than two alternatives at predicting the decline of body condition in free-ranging little brown bats during hibernation (Jonasson and Willis 2012), the model is potentially too simplistic to capture some of the variability in energy expenditure among bats from our study. For one, the model is based on respirometry measurements from captive animals in an artificial environment (small respirometry chambers). Thus, it could overestimate energy costs if animals were stressed during respirometry measurements or underestimate costs if respirometry fails to account for costs of behavioral activity of free-ranging animals during arousals. Moreover, while the model accounts for the influence of variation in T_a and T_b on warming and cooling costs, it assumes that MR during the plateau phase of arousal is constant (i.e., $166.6 \text{ J g}^{-1} \text{ h}^{-1}$; Thomas et al. 1990) regardless of the T_a in the hibernaculum or the T_b an individual is defending. This is clearly an oversimplification. First, although cave T_a was relatively stable, it still varied by 2.8°C over the course of the study, which could cause a difference in MR of as much as 10% for normothermic little brown bats (Jonasson and Willis 2012). Second, if all arousals were plateau shaped with stable T_{sk} , a single value for normothermic MR would reliably approximate MR, but, like Jonasson and Willis (2012), we found that 30% of arousals were not plateau shaped. Instead, some individuals (mostly females) defended a reduced T_b in the midst of their homeothermic phase. Thus, assumptions of Thomas et al.'s (1990) model likely led us to overestimate female energy expenditure. The model could also underestimate male energy expenditure if individuals engage in energetically costly behavior during arousal (e.g., flying to and copulating with females), although, as noted above, the costs of activity are likely relatively low compared with the thermoregulatory costs of arousal, and activity may compensate for thermoregulatory costs (Humphries and Careau 2011; Minnaar et al. 2014; Klüg-Baerwald et al. 2016).

Despite its limitations, the model was able to detect an effect of energy reserves (i.e., BCI) on energy expenditure. This difference in performance of the model for energy reserves versus sex likely reflects variability in the data and corresponding effect sizes for these two analyses. The effect size for BCI (Cohen's $d = 0.13$) was almost four times greater—and therefore more readily detectable at our sample size—than the effect size for sex ($d = 0.03$). This could partially explain why arousal duration differences translated into significant energetic differences when related to BCI but not to sex. As suggested by Jonasson and Willis (2012), more work is needed to quantify the energy expenditure of bats during different phases of torpor and arousal throughout hibernation, particularly during heterothermic arousals.

We found no support for our third prediction that YOY should have longer torpor bouts than adults because they tend to acquire smaller fat reserves during fall. In fact, we observed the opposite pattern, with YOY exhibiting shorter torpor bouts. The effect of age class on torpor bout duration during hibernation is not consistent across mammal species. In some species, age does not appear to affect torpor expression (Park et al. 2000; Munro et al. 2005), while juveniles of other species may exhibit longer (French 1990; Young 1990; Michener 1992) or shorter (Armitage et al. 2003; Landry-Cuerrier et al. 2008) bouts than adults. Reduced insulation and high thermal conductance in juvenile marmots led to higher O_2 consumption, resulting in shorter and more energetically expensive torpor bouts compared with those in adults (Armitage et al. 2003). If YOY little brown bats also have higher O_2 consumption, this could explain the shorter torpor bouts we observed. More data on O_2 consumption of YOY versus adult bats could help resolve this question.

When we considered YOY alone, we surprisingly found that individuals with the lowest BCI had the shortest torpor bouts. This is not consistent with optimization but has been observed for Natterer's bats (*Myotis nattereri*) in the United Kingdom (Hope and Jones 2012). Poor body condition is associated with shorter torpor bouts in some UK bat species, presumably to help these individuals exploit as many mild nights and potential feeding opportunities as possible to compensate for small fat reserves (Hope and Jones 2012). At our colder study site, this type of winterfeeding is impossible, and bats appear to synchronize arousals with huddle mates, possibly to take advantage of social re-warming (Czenze et al. 2013; Czenze and Willis 2015). YOY in the worst condition could be most likely to exploit warming huddle mates as a heat source during arousals, which could, in turn, drive their greater arousal frequency. In general, YOY could also be more sensitive to disturbance by colony mates (Turner et al. 2015) than adult bats that have learned or been selected to ignore disturbance by other bats. If YOY can benefit from social re-warming, YOY in the worst condition could be the most sensitive to disturbance, to ensure that their arousals coincide with those of other individuals.

Although variation in cave T_a throughout the study was small (1.1° to 3.9°C), especially compared with variation in T_a outside the cave (-39.8° to 26.8°C), rising cave T_a throughout winter likely affected MR, as cave T_a predicted torpor bout duration for both adults and YOY. However, the direction of this effect differed between age classes. Adults increased torpor bout duration at low cave T_a , while YOY reduced torpor bout duration. Similar to adult bats in our study, European ground squirrels (*Spermophilus citellus*) and golden-mantled ground squirrels (*Callospermophilus lateralis*) exhibited shorter torpor bouts at higher T_a , presumably because lower torpid MR at low T_a led to reduced sensitivity to and rate of accumulation of metabolic wastes (Geiser and Kenagy 1988; Nemeth et al. 2009). This could also explain the pattern we observed for adult bats. Increased torpor bout duration with increased cave T_a in YOY, however, was unexpected and could reflect the cold climate at our northern study site. In more southern populations, YOY *M. lucifugus* are indistinguishable from adults by their first winter. This is not

the case in central Manitoba, where at least some YOY bats can still be distinguished from adults during their first winter, likely due to a short growing season (Jonasson and Willis 2011, 2012; Czenze et al. 2013). There was no difference in body mass or forearm length between YOY and adults in our study, indicating that YOY had reached adult size by the time hibernation began. However, it is possible that adults and YOY differ in energy expenditure due to developmental processes for YOY that may be ongoing even during hibernation (e.g., dendritic remodeling, neuroprotection; Popov et al. 1992; Biggar and Storey 2014). Additionally, if hibernation performance is not fully developed by the first winter, YOY bats may be less able to tolerate colder cave T_a chosen by adults. For all hibernators, there is a set-point temperature (T_{set}) below which thermoregulatory costs and MR increase to defend a minimum T_b (Heller and Hammel 1972). For adult *M. lucifugus*, T_{set} is thought to occur around 0.5°C in laboratory-acclimated individuals (Hock 1951). There are few data on potential differences in T_{set} between age classes, but it is possible that inexperienced YOY bats have an elevated T_{set} compared with adults. At low T_a , YOY may therefore need to spend more energy defending T_b compared with adults and, in turn, face more rapid accumulation of metabolic wastes, more rapid water loss, and more frequent arousals. This suggests that if YOY have access to multiple microclimates during hibernation, they should choose warmer temperatures closer to their T_{set} . We recommend that studies using open-flow respirometry to quantify T_{set} and behavioral tests of thermal preferences be conducted to understand age differences more fully in this important aspect of hibernation behavior.

Our results are relevant to understanding potential impacts of white-nose syndrome (WNS), the recently emerged disease caused by the fungus *Pseudogymnoascus destructans*. *Myotis lucifugus* is among the most susceptible species (Frick et al. 2010, 2017) and, along with two other species, is now listed as endangered in Canada. Premature depletion of fat reserves due to increased arousal frequency is thought to be a primary cause of death from WNS (Reeder et al. 2012; Warnecke et al. 2012). Jonasson and Willis (2011) suggested that adult female bats may be buffered against WNS-related mortality because they accumulate more fat than males and spend it more slowly during winter, although increased use of fat could compromise reproductive success for WNS survivors. Our results are consistent with this hypothesis. Grieneisen et al. (2015) showed that colder hibernacula are more favorable for WNS-affected individuals. At our study site, the combination of hibernating in the coldest regions of caves, arousing for short periods of time, and/or using heterothermic arousals could allow adult females to spend less energy and lose less fat over the winter. However, our results suggest that YOY females in poor condition may be more likely to choose warmer microclimates and exhibit shorter torpor bouts, which would lead to higher overwinter energy expenditure.

This study shows that energy reserves, sex, and age influence patterns of torpor, arousal, and energy expenditure during hibernation. Our data support Jonasson and Willis's (2011) thrifty-female hypothesis that female body mass declines more slowly than that of males during hibernation. Specifically, we found that

females exhibit energetically conservative arousals compared with those of males, despite there being no difference in torpor bout duration. Our results also support a more general prediction of the optimization hypothesis, as bats in better condition spent more energy during hibernation. Again, this difference was a result of increased arousal duration, which suggests that altered arousals, rather than changes in torpor bout duration, are the more important driver of difference in the winter energy budgets of little brown bats. This pattern may allow males with a high BCI to increase fitness by providing them with increased mating opportunities. Although torpor bout duration did not vary by sex, it did vary by age class, which could reflect differences in torpid MR and T_{set} between adults and YOY.

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