

Hibernation Behaviour of Little Brown Bats

(Myotis lucifugus)

By

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ABSTRACT

For temperate endotherms (i.e., mammals and birds) energy costs are highest during winter but food availability is lowest and many mammals depend on hibernation as a result. Hibernation is made up of energy-saving torpor bouts [periods of controlled reduction in body temperature (T_b)], which are interrupted by periodic arousals to normothermic T_b . What triggers these arousals in free-ranging hibernators is not well understood. Some bats with access to flying insects during winter synchronize arousals with sunset, suggesting that feeding opportunities may influence arousal timing. I tested whether hibernating bats from a cold climate without access to food during winter also maintain a circadian rhythm for arousals. I used temperature telemetry to monitor skin temperature (T_{sk}) of free-ranging little brown bats (*Myotis lucifugus*) hibernating in central Manitoba, Canada. I found no evidence bats, during late winter, synchronized arousals with photoperiod. However, during early spring, as emergence date approached I found bats resumed a circadian pattern of arousals and aroused synchronously with sunset. Female bats give birth after emergence and must emerge earlier from hibernation in good body condition with fat reserves remaining. Difference in reproductive investment should lead to differences in torpor/arousal patterns and emergence timing. I found that males exhibited longer periodic arousals than females, that females emerged earlier than males, independent of environmental conditions, and that male emergence date was predicted by warming cave temperature. Longer arousals by males could reflect mating attempts by males and/or the strong selection pressure on females to be “thrifty” with their fat reserves and emerge in good condition to support pregnancy. These findings provide valuable new insights in hibernation behaviour, phenology, and energetics.

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CHAPTER 1: GENERAL INTRODUCTION

Both the intake and output components of an animal's energy budget equation can be strongly affected by environmental conditions (McNab 2002). This energy budget is, in part, affected by the net thermal exchange between the animal and the environment through radiation, convection, conduction, and evaporation (McNab 2002). Organisms must balance energy intake against expenditure in order to survive over a long period of time, and available energy must be partitioned between maintenance, somatic growth and reproduction.

Energy balance depends on abiotic factors such as climate, and biotic factors such as food availability. Endotherms living in temperate or seasonally variable environments face challenges in maintaining their energy balance and depending on the season these animals may have to tolerate vastly different environmental conditions. During summers individuals lose less heat to the environment and have greater access to food. In winter, the challenge of greater heat loss is compounded by the relative lack of food available compared to summer which leads to a significant challenge for energy balance.

Temperate animals have evolved several specializations to counteract the unfavourable winter months. One strategy is migration; animals leave their summer grounds and travel to wintering grounds where the climate and/or food availability is more favorable (e.g., Visser et al. 2006; Post and Forchhammer 2008). An alternative is seasonal dormancy or hibernation where hibernators accumulate a food cache or fat store during their active season and then reduce energy expenditure to a point where their energy reserves can balance winter energy costs (e.g., Lyman 1982, Geiser 2004). In

many species, these winter caches or fat stores are just large enough for the animal to survive and even small increases in energy expenditure during winter may have negative consequences for survival (Speakman et al. 1991; Boyles and Brack 2009).

Energetics of Torpor

Seasonal dormancy or hibernation has evolved in many clades of vertebrates as a strategy to conserve energy and avoid periods of severe environmental conditions and food scarcity/unpredictability (Wang 1989; Grigg et al. 2004). For many hibernators, these periods comprise, cumulatively, one-third to one-half of their total lifetime (Humphries et al. 2003a). In endotherms (i.e., birds and mammals), hibernation involves bouts of “torpor”, which is a physiological state characterized by drastically lowered metabolic rate (MR) and body temperature (T_b) to reduce energy requirements (Geiser 2004). Endotherm torpor is distinct from the dormancy exhibited by ectotherms (e.g., reptiles, amphibians, and some fish) in being a regulated process from which individuals can emerge to euthermy (i.e., high and constant T_b) via metabolic heat production. Ectotherms, unlike endotherms, cannot regulate torpid T_b and cannot interrupt torpor bouts with periods of euthermy during low ambient temperature (T_a) (McNab 2002; Geiser 2004).

During the winter season, hibernating mammals spend the majority of their time in bouts of prolonged torpor. Torpid MR (TMR) is typically less than 5-30% of basal MR (BMR) at the same T_a (Geiser 2004). The duration and depth of torpor is highly variable between species, sexes, and individuals. The little brown bat (*Myotis lucifugus*) is thought to exhibit among the longest known torpor bouts at 86 days (Menaker 1964) and arctic

ground squirrels (*Spermophilus parryii*) the coldest abdominal temperature at -3°C (Barnes 1989). Torpor is achieved by the downregulation of the hypothalamic set-point for T_b (internal thermostat), which reduces metabolic heat produced and therefore T_b (Heldmaier and Ruf 1992). The physiological mechanism of lowering MR to this level is thought to include temperature-independent metabolic inhibition along with high Q10 (MR change over 10°C) (Geiser 2004). While a contentious issue, there is evidence that respiratory acidosis causes reduced pH and this reduction leads to MR inhibition (Malan 1986; 1989). The respiratory exchange ratio (CO_2 eliminated / O_2 consumed) of ground squirrels (*Callospermophilus lateralis*) falls during hibernation and rises during arousal suggesting that a build-up of CO_2 during torpor may result in reduced pH and, thus, metabolic inhibition (Snapp and Heller 1981).

Costs of Torpor

Torpid MR can be reduced to less than 1% of normothermic levels resulting in significant energetic benefits (Geiser 2004). However, long-term expression of torpor also appears to result in costs to individuals. Heart rate during torpor is depressed and breathing occurs between bouts of apnea (Heldmaier et al. 2004). These physiological patterns result in an increase of CO_2 in the blood and, consequently, respiratory acidosis (Elvert and Heldmaier 2000). The inability to drink or urinate while torpid results in the buildup of metabolic wastes and dehydration stress (Thomas and Cloutier 1992; Thomas and Geiser 1997). The immune systems of hibernating animals appear to be depressed which can make individuals more susceptible to certain pathogens (Prendergast et al. 2002; Luis and Hudson 2006; Moore et al. 2011). Protein synthesis, gluconeogenesis and

cell division are all suppressed during torpor bouts (Frerichs et al. 1998; Staples and Hochachka 1998; van Breukelen and Martin 2002). Hibernators also appear to accumulate a sleep debt and may exhibit memory loss (Larkin and Heller 1999; Millesi et al. 2001; Thompson et al. 2013). To counteract these potentially harmful consequences of long-term torpor, hibernators briefly and periodically return to a euthermic state at regular intervals throughout hibernation. These periodic arousals are highly variable between and within species. The duration of arousals by little brown bats ranged from 0.65–2.17 hours (Jonasson and Willis 2011). These periodic arousals are thought to help counteract the physiological side-effects of long-term torpor (Thomas and Geiser 1997).

Although periodic arousals are brief they account for up to 90% of overwinter energy expenditure (Wang 1989; Thomas et al. 1990; Geiser 2004). This would seem counterintuitive since hibernators must conserve as much energy as possible and it highlights the likely physiological importance of arousals for survival. External stimuli may also elicit arousals and “emergency arousals” are seen in response to freezing, predation, consumption of food stores, and in some cases mating (Humphries et al. 2003a). There is a limit to how many arousals hibernators can perform so they must time them carefully and budget their energy meticulously. Therefore, the overall goal of my thesis was to improve understanding of hibernation energy budgets and factors influencing hibernation behaviour of healthy little brown bats hibernating in a cold climate. In Chapters 2 and 3 I tested the hypothesis that opportunities for nighttime feeding would affect little brown bats’ arousal timing. I predicted that a lack of winter opportunities for nighttime feeding would not lead to a circadian pattern of arousals. In the early spring when temperatures are warmer, I predicted more bats would synchronize

their arousals with sunset to take advantage of the emergence of flying insects.

Until recently, it was widely believed that hibernators should use the longest, deepest bouts of torpor possible, in order to conserve the most energy (sensu Körtner and Geiser 1998; Frank et al. 2000). However, over the past decade this view has been challenged by “the optimization hypothesis” which states that, as for other physiological and behavioural traits, expression of torpor should reflect a balance between costs and benefits (Humphries et al. 2003a; Jonasson and Willis 2011). The optimization hypothesis predicts that individuals with large energy stores should express less torpor than those with limited reserves and this prediction has been supported by several experimental studies. Humphries et al. (2003b) found that free-ranging eastern chipmunks (*Tamias striatus*) whose food caches were supplemented exhibited shallower bouts of torpor (i.e., defended higher skin temperature, T_{sk}) than controls. Fat-storing hibernators also show this pattern of optimization with fatter individuals selecting warmer microclimates and expressing torpor less compared to those who are energetically stressed (Boyles et al. 2007). Jonasson and Willis (2011) showed that male bats use their energy stores more rapidly than females during hibernation and that their body mass declined by as much as 130% that of females. Male little brown bats, unlike females, do not need conserve their fat to fuel post-emergence pregnancy resulting in faster mass decline (Jonasson and Willis 2011). However, when Jonasson and Willis (2012) examined T_{sk} traces from hibernating bats they did not find evidence to support the optimization hypothesis and variability in patterns of periodic arousal among individual hibernators is still not fully understood. Therefore, in Chapter 4 I revisited the hypothesis that little brown bats’ body condition affects torpor bout and arousal duration.

I predicted that, to save energy, individuals in poor condition would exhibit longer torpor bouts and/or shorter arousals than individuals in good condition. This is important because of the relevance of torpor expression during winter to other ecological factors such as the timing of emergence from hibernation and reproduction.

Over-winter differences in intersexual energy budgets occur in species where one sex's reproductive investment comes after spring emergence (Michener and Locklear 1990; Humphries et al. 2003a; Jonasson and Willis 2011). The sex that most depends on spring fat reserves for reproduction should rely more heavily on torpor during hibernation and spend less energy mitigating the costs of torpor (Humphries et al. 2003a; Jonasson and Willis 2011). Adult male Richardson's ground squirrels (*Urocitellus richardsonii*) emerge earlier than females to establish territories and compete intensely in the spring for mates. As a result they experience a slower rate of fat depletion than females during hibernation (Michener and Locklear 1990). In chapter 4, I tested the hypothesis that differences in reproductive timing would influence the energetic strategies of male and female bats differently. I predicted that male bats, not under the same energy constraints, should exhibit a more high-energy strategy (i.e., shorter torpor bouts and/or longer arousals) than females.

Hibernation in Bats

Most of our understanding of mammalian hibernation comes from studies of rodents that construct small, well-insulated burrows and hibernate singly or huddle in small groups (Karasov 1983; Geiser 2004). In contrast, many hibernating bat species spend the winter in caves and mines, which provide a very different kind of microclimate.

These sites may possess large air volumes, which means that metabolic heat does little to help bats regulate their immediate microclimate. Bats also differ from rodent hibernators in that they often huddle in aggregations of hundreds to thousands of individuals, which may help to reduce energetic costs (Henshaw 1970; Kunz 1980; 1982; McNab 1982; Kurta 1986), or reduce water loss (Hayes et al. 1992; Boyles and Brack 2009).

Energetically stressed individuals may benefit more from these huddles likely resulting in the considerable variation in huddle sizes ranging from small to very large (Humphrey and Kunz 1976; Kunz 1976; Raesly and Gates 1987; Whitaker and Gummer 1992).

In practice, we know very little about huddling in bats because they are difficult to observe during hibernation. Huddling is highly variable between and within species of bats with some individuals huddling in tight groups, others in loose aggregations, and others hibernating singly (Humphrey and Kunz 1976; Kunz 1976; Raesly and Gates 1987; Whitaker and Gummer 1992). In addition to benefits of passive re-warming, which allows an animal to reduce the energetic cost of arousal from torpor (Henshaw 1970; Kunz 1980; 1982; McNab 1982; Kurta 1986; Boyles et al. 2008), huddling may benefit euthermic bats that are out of torpor by reducing heat lost to the environment (Arnold 1990). Boyles et al. (2008) hypothesized that the main thermoregulatory benefit of clustering by hibernating bats is to minimize heat loss during rewarming from torpid to euthermic T_b levels and while euthermic. Huddling bats lost less mass than individuals and moreover, an individual-based energetic model of hibernation showed simulated little brown bats that huddled during winter had a survival rate of 0.96 compared to 0.73 for simulated bats that did not huddle (Boyles and Brack 2009).

Hibernation Phenology

Phenology refers to the timing of events, such as reproduction or hibernation, in the annual cycle and is often influenced by seasonal and interannual variations in climate and resource availability (Both et al. 2009). Climate change is shifting temporal abundance of resources and populations must adjust accordingly or face a lack of food, reduced active season, or population declines (Inouye et al. 2000; Root et al. 2003; Post and Forchhammer 2008). In some northern ecosystems, however, warmer winters and earlier springs may not impact certain hibernating species negatively and some species have benefitted. In yellow-bellied marmots (*Marmota flaviventris*) earlier emergence date has prolonged the active season and allowed animals to forage for longer periods (Ozgul et al. 2010). Prolonged active season due to climate change led to both higher juvenile over-winter survival and higher adult reproductive success (Ozgul et al. 2010).

To take advantage of a prolonged active season, hibernators could benefit from the ability to perceive environmental cues and adjust emergence timing accordingly. Hibernators may respond to a number of environmental cues including winter severity, snow cover, snow depth, food availability, and air temperature (Murie and Harris 1982; Inouye et al. 2000; Arlettaz et al. 2001; Blumstein 2009; Schorr et al. 2009; Sheriff et al. 2011; 2012). One long-term study suggested that yellow-bellied marmots, social hibernators, adjusted emergence timing in response to warming air temperatures (Inouye et al. 2000). The ability to rapidly respond to environmental variables is critical to the success of these animals and allows them to take advantage of warmer springs and avoid colder ones. Therefore, in Chapter 3 I tested the hypothesis that weather patterns affect emergence date of little brown bats. I predicted that bats would emerge on nights

characterized by environmental cues that predict availability of flying insects, especially temperature but also barometric pressure (Paige 1995; Turbill 2008), which may be more detectable inside the hibernaculum.

Animals hibernating in underground hibernacula may not experience conditions reflective of the surface and must also respond adaptively to non-environmental stimuli especially in habitats or hibernacula where external cues are difficult to detect (Williams Barnes and Buck 2012; Williams et al. 2012). For hibernators in these systems, phenological traits like parturition and emergence date may be less flexible and more strongly influenced by genetics (Reale et al. 2003; Kruuk and Hadfield 2007; Lane et al. 2011). The emergence date and body mass of Columbian ground squirrels (*Spermophilus columbianus*) are genetically heritable and body mass is positively correlated with number of young that survive their first winter (King et al. 1991; Lane et al. 2012). However, their northern, high elevation ecosystem is experiencing a delayed active season due to early spring snowstorms and the population has been declining over the past twenty years (Lane et al. 2012). This suggests that, contrary to marmots, not all northern hibernators are benefitting from climate change. However, if some individuals and their offspring are better able to adjust their phenology, they may have higher over-winter survival. Therefore, for Chapter 3 I also tested the hypothesis that body condition affects emergence timing of little brown bats. I predicted that females, because their larger fat reserve would buffer them against cold spring weather, would be the first to emerge from hibernation while males with large fat reserves would remain in hibernation as long as possible to avoid possible sources of extrinsic mortality during spring and summer, such as predation.

Study Species

The little brown bat is a small insectivorous bat that ranges throughout North America from Alaska to Mexico (Fenton and Barclay 1980). Depending on latitude, little brown bats hibernate for five to eight months each year in caves and mines which are cold (-4–13 °C), humid (>90% relative humidity), and have little airflow (Davis 1970; Fenton and Barclay 1980; Webb et al. 1996). Promiscuous mating occurs during fall swarms at the entrances of hibernacula and to a lesser extent during hibernation when males will mate with torpid females (Thomas et al. 1979; Hosken 1997). Females store sperm over the winter, delay fertilization until the spring and give birth to a single pup in late spring/early summer (Hosken 1997). Males are the first to arrive at the hibernacula followed closely by nulliparous females and then young of the year (YOY) some three weeks later (Thomas et al. 1979). Female and male YOY lose more mass over hibernation compared to their adult counterparts; this may be due to their inexperience in behaviours associated with hibernation (i.e., huddle partners, microclimate selection, etc.) (Kunz et al. 1998; Jonasson and Willis 2011).

Presumably because of differences in the timing of their reproductive investments, adult female and male bats use their energy stores differently (Jonasson and Willis 2011). Female bats are more conservative or “thrifty” in terms of energy expenditure compared to males likely so they can emerge in the spring with sufficient fat reserves to initiate pregnancy (Jonasson and Willis 2011). Spring fat reserves are especially critical for female little brown bats because, with the potential for only one pup per year, they do not have the option to manipulate litter size depending on the availability of resources.

Males' investment in reproduction, conversely, happens before hibernation and to a lesser extent during hibernation (Hosken 1997). Males in good body condition may have two, non-mutually exclusive strategies for increasing mating success. They may mate promiscuously with as many females as possible during swarming when resources are plentiful or, alternatively, what I term "megamales" may mate during the hibernation period, force copulations with torpid or semi-torpid females and benefit by displacing the sperm of rival males (Hosken 1997; Kunz et al. 1998). Sperm competition is common within many species of bats and the benefit of prolonged spermatogenesis is twofold, increased copulatory activities and increased probability of fertilization (Hosken 1997). Males that mate during winter must use fat stores to become euthermic and therefore these hibernation copulations are costly. Males with the largest fat reserves may arouse more frequently and/or for longer and this behaviour would allow for more copulations than those in poor condition. Therefore, in Chapter 4 I tested the hypothesis that differences in reproductive strategies between sexes would affect torpor expression and arousal duration. I predicted that males, not under the energy constraints of females, would use their fat stores more freely and spend less time in torpor to avoid physiological costs of prolonged heterothermy, and spend more time euthermic possibly to take advantage of winter copulations.

Juvenile bats of both sexes are thought to employ different energetic strategies than adults (Kunz et al. 1998), accumulate less fat before hibernation (Thomas et al. 1990), and experience higher over winter mortality than adults (Davis and Hitchcock 1965). While some YOY female bats may be capable of reproduction their first year, YOY male bats are not (Kunz et al. 1998). Since spermatogenesis by males occurs in summer, YOY

males born in the summer do not have enough time to complete this process (Kunz et al. 1998). Therefore, YOY males (and non-reproductive YOY females) may allocate fat reserves differently than any other sex/age class. While YOY females must use their reserves to survive hibernation and possibly reproduce, YOY males can use whatever reserves they accumulate exclusively for hibernation. Therefore, in Chapter 4 I also tested the hypothesis that sex/age class will affect torpor bout and arousal duration. I predicted that YOY males, with no reproductive constraints on spring energy reserves, would use their fat reserves more freely (i.e., shorter torpor bouts and/or longer arousals) than any other sex/age class.

Bats and White–Nose Syndrome

Populations of hibernating bats in northeastern North America are being devastated by an emerging disease called white-nose syndrome (WNS). A characteristic feature of WNS is a white fungus that infects the muzzle, ears, and wing membranes of affected bats (Gargas et al. 2009). The fungus was first discovered on bats in four caves during the winter of 2006–2007 in New York State and, since then, has spread rapidly to hibernacula throughout eastern North America (Frick et al. 2010). The fungus associated with WNS, *Pseudogymnoascus destructans* (*Pd*), is a recently described cold-tolerant species that invades the exposed skin of hibernating bats (Gargas et al. 2009; Blehert et al. 2009). Using energetic modeling Boyles and Willis (2010) provided the first evidence for the hypothesis that the fungus causes bats to spend more time out of torpor than normal, effectively depleting fat stores too early. Warnecke et al. (2012) subsequently confirmed this hypothesis experimentally and also confirmed that *Pd*, alone, is the cause

of mortality from WNS. Upon approaching depletion of critical fat reserves, some bats may emerge and attempt to forage or drink, or relocate to warmer microclimates within the hibernaculum, presumably to conserve energy (Boyles and Willis 2010; Warnecke et al. 2012). Bats may also vacate affected hibernacula prematurely to seek alternate roosts for the remainder of the winter and early spring. Combined with the bats that die from WNS inside their caves, in cold climates these behaviours contribute to extremely high mortality and, to date, 5.5–7.5 million bats are estimated to have died (<http://www.fws.gov/whitenosesyndrome/>).

The natural cycle of hibernation in little brown bats has allowed *Pd* to become highly successful for several reasons; the hibernating temperature of little brown bats (1–15°C) is within the range for optimal growth of the fungus (Cryan et al. 2010). In WNS-affected bats connective tissue, glandular structures, and muscle fibers of normal wing tissue are replaced as *Pd* invades and digests skin (Cryan et al. 2010). Bats are especially susceptible to dehydration during winter hibernation and this may interrupt the wing-dependant physiological processes such as water and gas exchange (Meteyer et al. 2009; Cryan et al. 2010). The wing membranes of bats are critical for not only gas exchange but also in maintaining water balance (Cryan et al. 2010; Willis et al. 2011). The large surface area of skin on the wings of bats make them prone to evaporative water loss (EWL), losses from skin can account for 99% of total water loss in healthy hibernating bats (Thomas and Cloutier 1992). Evaporative water loss is inversely related to the humidity of the air inside the hibernaculum, and the most EWL susceptible bat species select hibernacula with high humidity (Cryan et al. 2010). Little brown bats roost in the most humid parts of hibernacula, suggesting that they are susceptible to EWL and have

evolved compensatory behavioural strategies, such as roost selection or huddling (Cryan et al. 2010; Boyles and McKechnie 2010). These behavioural strategies are likely crucial for bats as they help to conserve energy and decrease water loss, thus increasing survival during hibernation (Cryan et al. 2010; Boyles and McKechnie 2010) but they may also facilitate fungal transmission and growth by elevating humidity, and increasing dispersal of *Pd* through contact with infected bats. Understanding the natural hibernation behaviour of healthy little brown bats is important for predicting how the disease might impact populations and, in particular, will allow for specific predictions as to which demographic may be most susceptible to WNS mortality. More generally, examining differences in the timing of torpor bouts and arousals during hibernation, as well as emergence phenology is important for understanding of the energetic strategies hibernators use to cope with winter.

**CHAPTER 2: STAYING COLD THROUGH DINNER: COLD-CLIMATE BATS
REWARM WITH CONSPECIFICS BUT NOT SUNSET DURING
HIBERNATION**

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Introduction

Many temperate animal species have evolved physiological and behavioural mechanisms to survive the challenging winter months. Hibernating mammals, for example, spend the majority of their time during winter in torpor, a state of controlled reduction in body temperature (T_b) and metabolic rate (Geiser 2004). Long-term torpor provides energetic benefits (Geiser 2004), and may provide additional benefits for some species by reducing exposure to predators and extending lifespan (Wilkinson and South 2002; Stawski and Geiser 2010). However, it also appears to result in physiological and ecological costs, which limit the duration of torpor bouts. For example, the inability to drink while torpid results in dehydration stress (Thomas and Cloutier 1992; Thomas and Geiser 1997) and immune responses of torpid hibernators may be suppressed increasing susceptibility to pathogens (Luis and Hudson 2006; Moore et al. 2011; Prendergast et al. 2002).

Presumably as a result of these costs of torpor, all hibernators exhibit periodic arousals throughout hibernation, during which they return to a normothermic T_b (e.g., Grahn et al. 1994; Jonasson and Willis 2012; Ruby et al. 2002).

Although periodic arousals are brief they account for up to 90% of winter energy expenditure due to the high metabolic cost of thermoregulation at low ambient temperatures (T_a) (Geiser 2004; Thomas et al. 1990; Wang 1978). Hibernators like temperate zone bats cannot store a food cache and must survive exclusively on stored fat which places a limit to how many arousals they can perform (Thomas et al. 1990). As a result, bats must balance the energetic, physiological and ecological costs and benefits of torpor and arousal. Presumably, the frequency of arousals must be timed carefully to offset the risk of exhausting fat stores prematurely (Humphries et al. 2003a; 2003b; Jonasson and Willis 2011; 2012; Racey and Swift 1981).

The mechanism that initiates periodic arousals is still unknown but one of several hypotheses is that circadian clocks may play a role (e.g., Malan 2010). The T_b of hibernating golden-mantled ground squirrels (*Callospermophilus lateralis*) fluctuates with circadian rhythms during deep torpor and individuals of this species are more likely to enter torpor at night and arouse during the day when exposed to a light/dark cycle (Grahn et al. 1994; Ruby et al. 2002). However, Ruby et al. (2002) found that the timing of entry into and arousal from torpor occurred at random times for animals housed under 24 h lights-on photoperiod. Free-ranging, cave-dwelling greater horseshoe bats (*Rhinolophus ferrumequinum*) and Natterer's bats (*Myotis nattereri*) from the United Kingdom (U.K.) receive little or no natural photoperiod cues in caves during winter. In spite of the lack of photoperiod, however, these species appear to maintain a circadian pattern to arousals (Hope and Jones 2012; Park et al. 2000). Hibernating bats of these species “warm up for dinner”, synchronizing arousals with sunset, likely to take advantage of flying insects during occasional mild winter nights (Hope and Jones 2012;

Park et al. 2000).

Greater horseshoe and Natterer's bats hibernate in relatively mild climates where temperatures during winter occasionally exceed 10°C. This is a threshold above which aerial insect abundance can increase dramatically (Jones et al. 1995), providing opportunities to forage. Even though foraging opportunities will be less common, bats from colder environments might also synchronize arousals with sunset. To my knowledge the maintenance costs of neural mechanisms underlying a circadian clock are unknown. However, it seems likely these costs would be small relative to the benefits of even unlikely potential foraging opportunities, especially in mid- to late-winter when fat reserves are dwindling. Thus, bats might still synchronize arousals with sunset, as selection against arousal at dusk might be relatively weak.

Another trigger of arousal, specific to communal hibernators like bats, could be cues from conspecifics. Such cues could be beneficial if groups of individuals can reduce energetic costs of arousal via social thermoregulation (Ruf and Arnold 2000). Alpine marmots (*Marmota marmota*), for example, hibernate in groups of related individuals and synchronize arousals with burrow mates to save energy. These benefits presumably increase over-winter survival, particularly for young-of-the-year (YOY), and may enhance inclusive fitness via kin selection (Ruf and Arnold 2000). On the other hand if arousals of different individuals are not well synchronized, disturbance by normothermic cluster mates could be detrimental to energy balance for some animals if it induces arousals more frequently than required to offset costs of torpor. For example, in bats, males may attempt to mate with torpid females during hibernation and these mating attempts may disturb females.

In contrast to most mammalian hibernators which spend the winter in well-insulated burrows, bats hibernate in caves and mines often in large, open-air chambers. These environments may reduce the potential for bats to use metabolic heat to influence their immediate microclimate and could explain the energetically conservative hibernation patterns of little brown bats (*Myotis lucifugus*), characterized by long torpor bouts, brief arousals and the expression of shallow heterothermy in the midst of arousals (Jonasson and Willis 2012). Thus, benefits of social thermoregulation for hibernating bats may be especially important. An individual-based population model, incorporating presumed thermoregulatory benefits of clustering for little brown bats predicted that 96% of clustering bats should survive a typical winter compared to 73% for solitary bats (Boyles and Brack 2009). This suggests that bats from the same cluster should exploit the energetic benefits of social thermoregulation and arouse synchronously with other individuals in the cluster.

I used temperature radio-telemetry to test two hypotheses about periodic arousals in hibernating bats. First, I tested whether arousal and photoperiod are synchronized in bats from a cold climate. I predicted that arousals would remain synchronized with sunset based on the hypothesis that maintaining neural mechanisms underlying a circadian clock would be inexpensive relative to potential benefits of obtaining even an unlikely meal during winter. Second, I tested whether individual bats would take advantage of social thermoregulation to reduce arousal costs. I predicted that individuals in the same cluster would arouse from torpor at the same time and that a majority of arousals would overlap in time. Alternatively, if bats tend to arouse from torpor on a similar timetable (e.g., on roughly the same days) but not at precisely the same times, this would suggest that

disturbance by conspecifics could be influencing torpor-arousal cycles. Such a pattern might be observed if, for example, bats gradually disturb each other as they rewarm, resulting in a cascade of arousal within a cluster.

Methods

This study was conducted in Firecamp cave, near the town of Grand Rapids, in central Manitoba Canada (53°29'25"N 99°20'50"W). This cave is accessed by a sinkhole entrance approximately 50 cm in diameter and consists of a bell-shaped chamber about 7 m deep. Between 30 and 60 bats over-winter in Firecamp cave from mid-September until mid-May. Bats in this cave all hibernate in one tight cluster within a crack 1 m long by 20 cm deep, on the ceiling of the main chamber.

All procedures were approved by the University of Winnipeg Animal Care Committee and by Manitoba Conservation Wildlife Scientific Permit WB0612. I entered the cave and handled bats during hibernation but to minimize disturbance I only entered once and limited the duration of my visit to less than 90 minutes. Twenty-four torpid bats were captured by hand on 20 Feb 2012, sexed, weighed to the nearest 0.01 g (Durascale-100, MyWeigh, ON, Canada) and their forearm length was measured to the nearest 0.05 mm. I identified YOY based on un-fused 3rd digit metacarpal-phalangeal joints, and tapered rather than “knobby” joints following Davis and Hitchcock (1965). In many parts of their range YOY little brown bats cannot be distinguished from adults by the time hibernation begins but in our northern study area we can readily identify at least some YOY late during swarming and even into hibernation (Jonasson and Willis 2011; 2012).

For small bats, skin temperature (T_{sk}) provides a good approximation of T_b during

torpor (Audet and Thomas 1996; Barclay et al. 1996; Willis and Brigham 2003). I recorded T_{sk} by attaching temperature-sensitive radio-transmitters (0.64-0.80 g, BD-2NT; Holohil Systems Ltd, Carp, Ontario) using a non-toxic, latex-based adhesive (Osto-Bond, Montreal Ostomy, Vaudreuil, Quebec, Canada) after clipping a small ($< 1 \text{ cm}^2$) patch of fur between the shoulders. Transmitters represented between 6.9 and 8.6% of body mass, greater than the 5% guideline suggested by Aldridge and Brigham (1988) but similar to the size which Jonasson and Willis (2012) demonstrated had no negative impact on body condition in hibernating little brown bats. I calibrated transmitters prior to use in a water bath at 5°C increments from just above 0°C to 45°C against a thermometer traceable to the National Institute of Standards and Technology. A datalogging receiver (Lotek SRX600; Lotek Engineering Inc., Newmarket, Ontario, Canada) powered by a solar panel was deployed outside the cave and connected to an omnidirectional whip antenna positioned in the cave, within 60 cm of the cluster of bats. The receiver recorded T_{sk} of each individual at six-minute intervals. Each bat was recorded for 15 seconds and a full cycle of recordings took 360 seconds. I recorded T_a inside the cave using iButtons (DS1922L iButtons, Maxim Integrated Products, Dallas, TX, USA) coated in paraffin wax to dampen ultrasonic noise (Willis et al. 2009) and positioned at the base of the crack within 60 cm of where the bats roosted while hibernating.

I defined the phases of arousal following Jonasson and Willis (2012). Due to the large difference between normothermic T_{sk} and T_a in the cave, arousals were always readily obvious in the T_{sk} trace. Torpor bouts were defined as periods of reduced stable T_{sk} between the obvious warming and cooling phases associated with each arousal. Skin temperature measured using Holohil transmitters occasionally appears to increase slightly

and gradually after initial entry into a torpor bout (e.g., Jonasson and Willis 2012). This appears to reflect an artifact of long periods at low temperature, which I could readily identify in our T_{sk} traces and did not affect my analysis.

Warming was defined by an abrupt increase in torpid T_{sk} that resulted in an elevation of T_{sk} until it stabilized at a normothermic steady-state. Cold T_a in the cave appeared to increase ambient cooling of external transmitters, leading to slightly reduced T_{sk} values during arousal (Willis and Brigham 2003). The normothermic period was defined as the time between the end of this rapid warming period and the initiation of cooling, which was in turn defined as an abrupt decline in T_{sk} preceding steady-state torpor. I excluded data from the first 24 hours after releasing the bats to avoid the influence of this disturbance on the results.

I recorded the date and time that arousals occurred from the T_{sk} trace and converted time of arousal from minutes before or after sunset into radians. I then used Rayleigh's test for circular distributions to determine if arousal times were significantly different from a random distribution. All analyses were conducted in R version 2.10.1 (R Development Core Team 2009) and values are reported as the mean \pm SD. I assessed significance at the $P < 0.05$ level.

Results

Although 9 transmitters failed, I recorded 459 bat-days of T_{sk} data from 15 individuals over 73 days following transmitter attachment and release. Average body mass at capture was 9.4 ± 1.3 g and body condition index (BCI) was 0.26 ± 0.04 g/mm. All bats exhibited long bouts of torpor averaging 15.3 ± 8.9 days with the longest bout

lasting 35 days, interspersed with brief periodic arousals averaging 3.3 ± 1.1 hours (Fig. 2.1). I recorded a total of 42 discrete torpor bouts and 29 arousals before the final transmitter battery failed on May 5, 2012.

I found no evidence of a relationship between arousal times and sunset. The circular frequency distribution of differences between arousal time and sunset time, expressed in radians, was no different from a random distribution ($Z=0.65$, $P=0.54$, mean vector=0.15, $N=15$ bats, $n=29$ arousals, Fig. 2.2a,b). This analysis was technically pseudoreplicated as I recorded multiple arousals from some, but not all individuals. However, I still found no relationship between sunset and arousal time when I limited the analysis to each individual's first arousal after capture ($Z=1.2$, $P=0.32$, mean vector=0.28) or calculated the average arousal time for each individual ($Z=0.41$, $P=0.67$, mean vector=0.16).

In keeping with my prediction that individuals in the same cluster should arouse from torpor at the same time, many (but not all) bats aroused on the same days although not all bats aroused at precisely the same times. For example, the 6 arousals I detected on Day 17 each lasted 2.9 ± 0.9 h and were spread out over a 14.9-hour period so that some bats had already re-entered torpor before other bats rewarmed. Of all the arousals that I observed occurring on the same day as at least one other arousal, 78% (14/18) were completely or partially overlapping with the arousal of at least one other bat (Fig. 2.3). In other words, bats were normothermic at the same times as other individuals in their cluster

Discussion

In contrast to previous studies of bats from warmer environments where winter

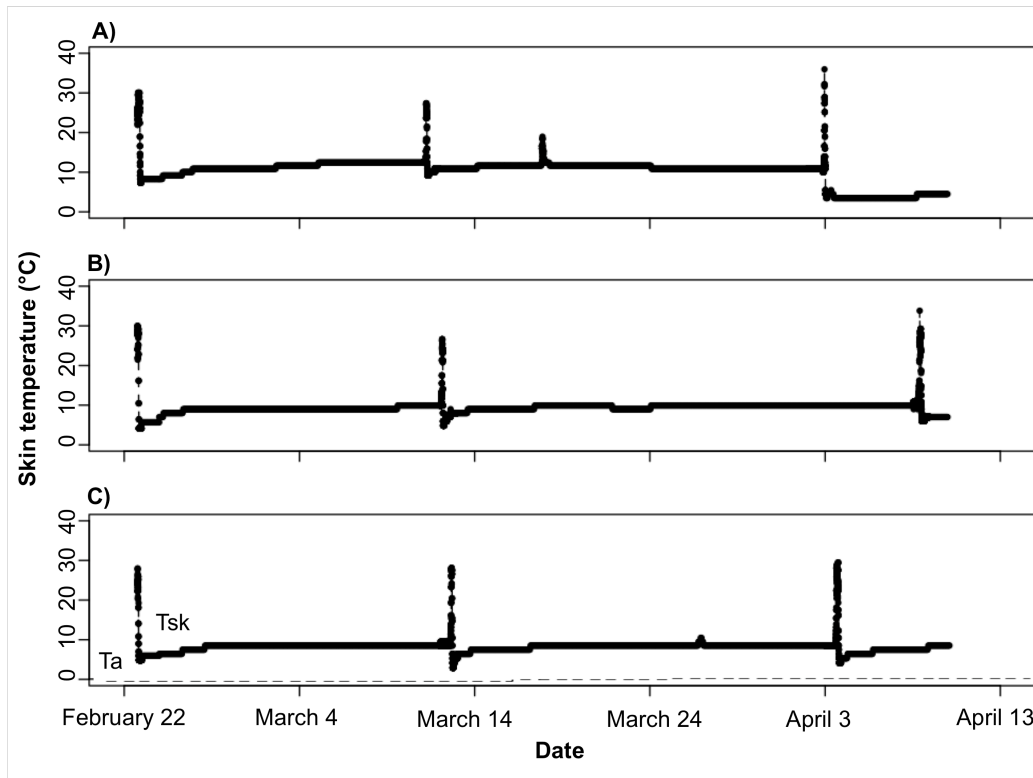


Figure 2.1. Skin temperature (upper trace) of three representative hibernating little brown bats A) female adult, B) male young of the year, C) female adult, dashed line shows the average cave temperature as measured by data loggers within 60 cm of the roosting bats.

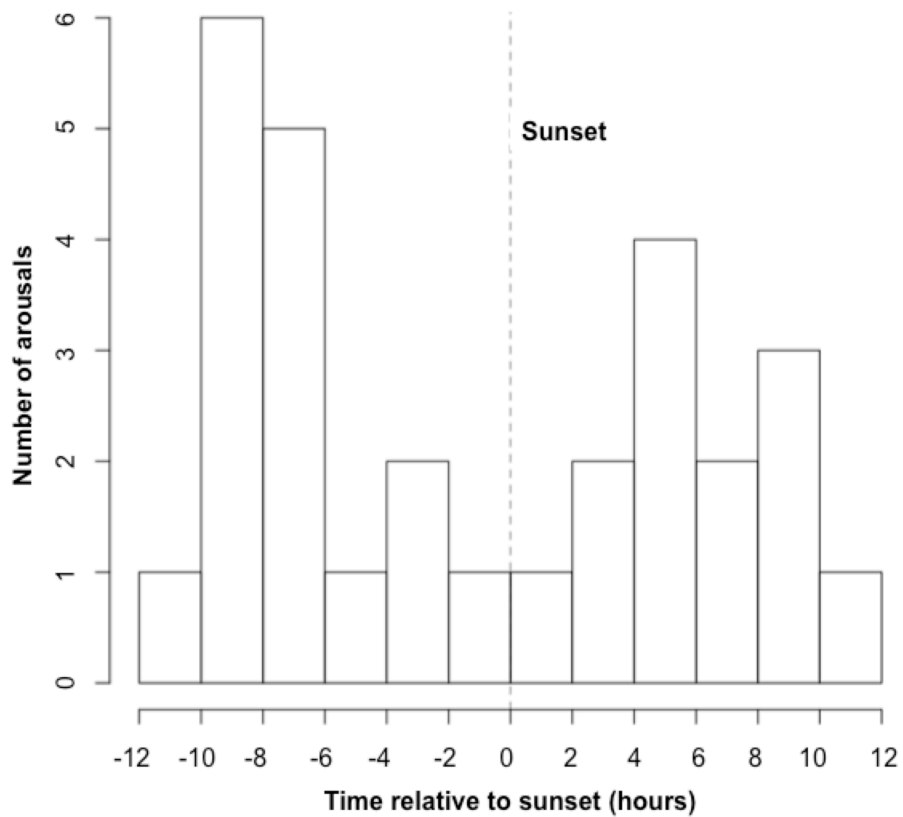


Figure 2.2a. Histogram showing frequency of arousals from torpor versus time relative to sunset for 15 little brown bats.

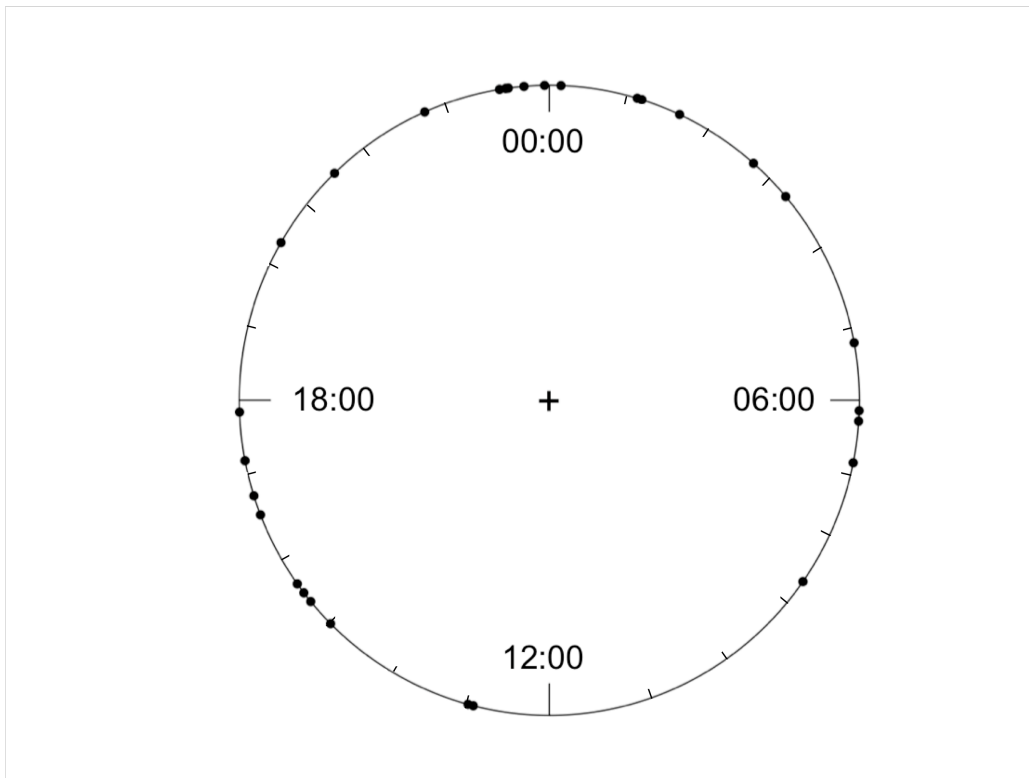


Figure 2.2b: Clock face representing the circular distribution of arousal times by individual little brown bats during hibernation. Average sunset time was $18:42 \text{ h} \pm 0.4$ ($Z=0.65$, $P=0.54$, mean vector= 0.15 , $N=15$ bats, $n=29$ arousals).

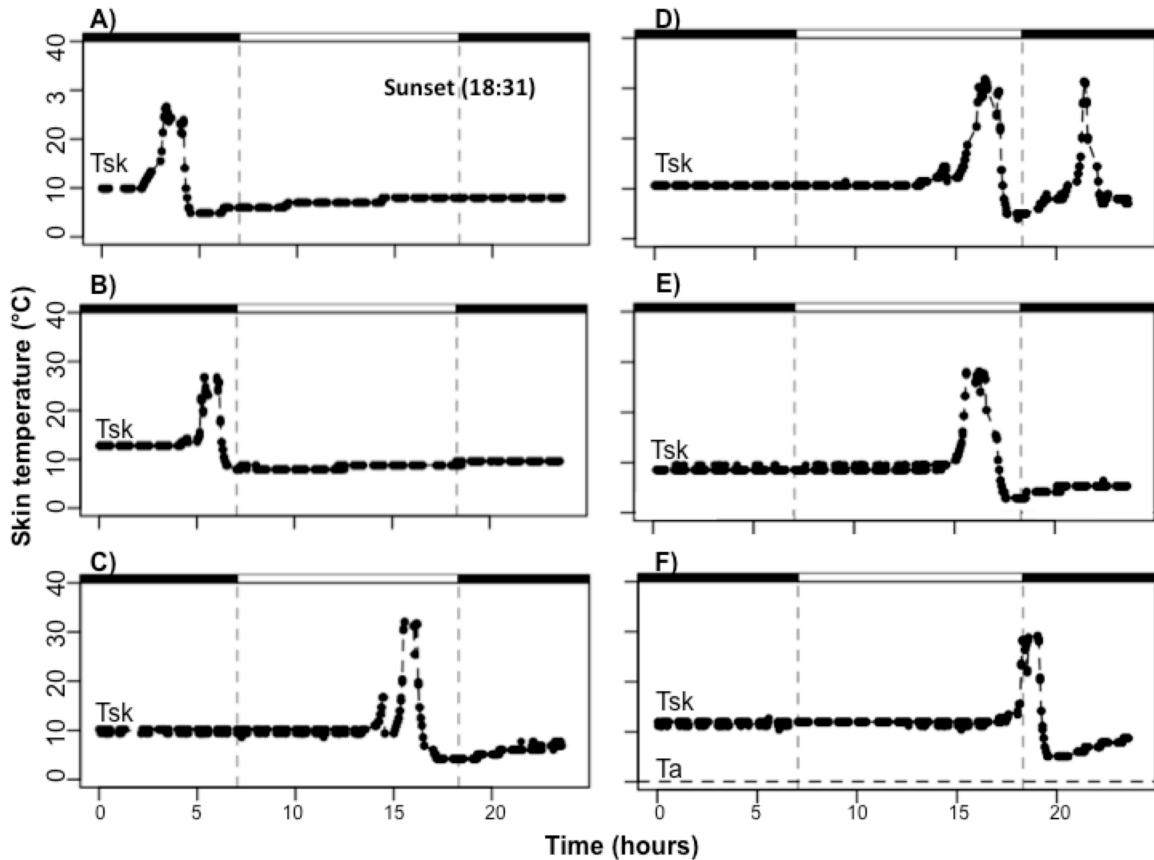


Figure 2.3: Skin temperature (upper trace) of six *M. lucifugus* showing the timing of arousals on Day 17 of the study. A) female young of the year, B) female young of the year, C) male young of the year, D) male Adult, E) female Adult, F) male Adult. The lower flat trace in F (dashed line) shows the average cave temperature as measured by a temperature data logger within 60 cm of the roosting bats. Dashed vertical lines indicate sunrise and sunset.

foraging is a possibility (Park et al. 2000; Hope and Jones 2012), I found no evidence of a circadian rhythm associated with arousal. Similar to a study using bat detectors in a hibernaculum (Thomas 1993), I found that bats did not synchronize arousals with sunset. The evidence showing that bats do arouse with sunset was obtained from the U.K. where T_a often exceeds 10°C during winter and flying insects are likely to be available, at least occasionally during hibernation (Park et al. 2000). Thus the maintenance of a circadian rhythm, and the ability to synchronize arousal with a prospective foraging opportunity could be highly beneficial, allowing bats to supplement their fat reserves and take advantage of warm nights. In central Manitoba, T_a never exceeds 10°C for five months of the year, and typically never exceeds freezing for at least 2-3 months (National Climate Data and Information Archive, 2012). Therefore, flying insects are consistently unavailable for a much longer period during winter. Despite this limitation on foraging opportunities, I predicted that bats in my study site would exhibit arousals linked to sunset because, presumably the cost of maintaining a circadian clock is relatively low and, especially as spring approaches, the potential for foraging opportunities does occasionally arise. For example, during late-winter (i.e., March) 2012, there were multiple nights during which temperatures were warm enough to support flying insects and foraging by bats (Czenze and Willis, unpublished data). However, my results suggest that, at least during mid- to late-winter in my study area, the costs of maintaining a daily rhythm appear to outweigh any possible benefits of winter foraging.

Although I found no daily pattern to arousals, I did find evidence that bats arouse synchronously with each other. I predicted that benefits of social thermoregulation would favour synchronous arousals, especially in this site as all bats in Firecamp cave hibernate

exclusively in one cluster within a crack in the ceiling. Thus, in contrast to many little brown bat hibernacula, individuals in this cave have the potential to warm their local environment and presumably could also gain significant energetic benefits by arousing synchronously (Boyles et al. 2008; Boyles and Brack 2009).

My results were consistent with the social thermoregulation hypothesis given that 78% of all the arousals I observed overlapped with the arousals of at least one other individual and often more than one. Therefore, although bats did not appear to maximize benefits of social thermoregulation by all arousing at the same times, they almost certainly gained some energetic benefit. Mating attempts by males, which occur during hibernation (Thomas et al. 1979), may also have influenced this synchrony. Interestingly, for the three sets of arousals I observed, each appeared to be initiated by a male bat, followed by multiple females. These arousals were not, however, completely synchronized. Given the tight energy budgets of hibernating bats, an interesting question for future studies is, why has selection not favoured mechanisms that allow all bats in a cluster to precisely synchronize arousals to minimize rewarming costs?

Multiple individuals accumulating physiological imbalance at about the same rate might explain approximately synchronous arousals by bats in the same microclimate within a hibernaculum. They might therefore arouse at about the same time to restore homeostasis. For example, assuming evaporative water loss (EWL) influences arousal timing, bats in the same cluster in my study may have experienced similar rates of EWL during torpor (Thomas and Cloutier 1992) and, therefore, may have almost simultaneously reached a point at which arousal was necessary to re-hydrate. All radiotagged bats aroused during handling on 20 February 2012 when I attached

transmitters, so it is also possible that this disturbance-induced arousal reset the mechanism underlying arousal (i.e., allowed all individuals to drink or excrete metabolic wastes) increasing the chance that their subsequent arousals would be synchronized. However, given large between-individual variation in most physiological processes, including those likely to influence arousals (e.g., metabolic rate, EWL) it seems unlikely that this is the exclusive explanation for the similar arousal times of multiple individuals in my study.

Another possible explanation for the timing of arousals I observed is that warm bats disturb torpid conspecifics. Even non-tactile disturbance by human visitors to hibernacula can stimulate arousals in bats (Thomas et al. 1990) and, it is possible that movement by other bats within a hibernaculum or within a cluster could have similar effects. This seems unlikely given strong selection pressure for bats to remain torpid for long enough during winter to conserve energy. Such a mechanism might be important if different individuals exhibit consistent individual differences in sensitivity to disturbance. For example, mated adult females may be especially resistant to disturbance because selection appears to favour mechanisms allowing females to save fat for spring reproduction (Jonasson and Willis 2011). At the other extreme, inexperienced juvenile bats may be more prone to disturbance during torpor and, to avoid exhausting fat reserves too quickly, may need to learn how to differentiate disturbances which warrant arousing (e.g., a potential predator) from those that do not (e.g., conspecifics).

If disturbance by bats can cause arousals, this could have consequences for bats hibernating in sites affected by white-nose syndrome (WNS). WNS is caused by cutaneous infection with the fungus *Pseudogymnoascus destructans* (*Pd*) (Blehert et al.

2009, Lorch et al. 2011) and infection causes bats to arouse from torpor more frequently during hibernation and prematurely exhaust energy reserves (Boyles and Willis 2010, Reeder et al. 2012, Warnecke et al. 2012). If active bats can induce arousals in other torpid individuals, the increased arousal frequency caused by infection with *Pd* could result in a positive feedback of arousals, accelerating the use of fat reserves even further. More work is needed to understand the physiological signals and environmental cues that trigger arousal in hibernators, especially in bats which are highly prone to disturbance and, in North America, now facing the threat of WNS.

This is the first study to examine the timing of arousal in free-ranging little brown bats, particularly in the extreme northern part of their range and provides, to my knowledge, the first evidence that bats in northern climates synchronize their arousals with huddle-mates rather than photoperiod. Future research on northern bats should aim to examine arousal patterns over the duration of the winter period of bats in caves with multiple clusters to determine if arousals remain asynchronous with photoperiod and to improve understanding of mechanisms underlying this critical aspect of hibernation.

CHAPTER 3: CUES FOR EMERGENCE FROM HIBERNATION IN LITTLE BROWN BATS

INTRODUCTION

Phenology is the study of the timing of life history events in plants and animals and how these events are influenced by seasonal and inter-annual variations in climate (Both et al. 2009). In plants, variation in ambient temperature (T_a) influences flowering dates with warmer spring T_a causing earlier flowering (Sparks et al. 2000). The seasonal peak of caterpillars on spring plants is associated with warming weather and Great Tits (*Parus major*) time their reproduction to exploit the seasonal abundance of food (Visser et al. 2006). Ensuring synchrony with temporally variable food, however, is especially difficult when individuals travel long distances between summer and winter. Some migratory birds, faced with shifting seasonal abundance of their insect prey with climate change, have failed to advance their migration phenology to take full advantage of the peak in prey (for a review see Visser and Both 2005). Although migration is less common in mammals, changing phenology of food plants has also affected some migratory herbivores. Due to climate change, caribou have experienced recent declines in both offspring production and survival because of a growing mismatch between reproductive timing and advancing plant phenology (Post and Forchhammer 2008). Caribou use photoperiod cues to time their migration to calving grounds, whereas the sympatric food plants they depend on respond to T_a (Post and Forchhammer 2008). For non-migratory animals, like hibernators, it is also critical to respond to specific environmental cues and

time phenological traits accordingly but much less is known about phenology in these species.

Most mammals remain in the same area over winter and many use hibernation (a physiological and behavioural adaptation to reduce energy expenditure) to deal with seasonally unfavorable conditions and unpredictable food availability (Lyman 1982; Geiser and Ruf 1995; Humphries et al. 2003a; 2003b). Some hibernators must endure a hibernation period of up to eight months and winter duration likely impacts over-winter survival and fitness in the following reproductive season (Lyman 1982; Twente et al. 1985; Wilkinson and South 2002; Lane et al. 2012; Norquay 2013). Over-winter survival and reproductive success are also impacted by the emergence date of the hibernator. During warmer springs, yellow-bellied marmots (*Marmota flaviventris*) emerge from hibernation earlier and experience greater reproductive success than in colder years (Ozgul et al. 2010). Spring weather varies inter-annually and cold years may delay emergence phenology adding additional costs to the hibernator's carefully budgeted over-winter energy reserve.

During the active season, and especially before immergence into hibernacula, hibernators accumulate a food cache or fat store to fuel their over-winter energy demands. They, then, reduce energetic demands by spending the majority of the hibernation period in multi-day bouts of reduced metabolic rate (MR) and body temperature (T_b) known as torpor (Geiser 2004). These torpor bouts are interspersed with brief periodic arousals to a normothermic T_b . Cumulatively these arousals, which typically last less than 24 h, account for up to 90% of the hibernator's over-winter energy budget (Wang 1989; Thomas et al. 1990; Geiser 2004). Hibernators face selection

pressure to carefully budget the frequency, duration, and timing of arousals to ensure they emerge from hibernation in adequate body condition to support a successful reproductive season.

Mechanisms underlying arousal timing during hibernation are still not fully understood. The biological clock within the suprachiasmatic nuclei (SCN) of the hypothalamus controls circadian rhythms and there is evidence that this clock is inhibited during multiday torpor bouts in hibernation (Tøien et al. 2011; Williams, Barnes and Buck 2012; Williams et al. 2012). However, persistence of a circadian rhythm to arousals has been observed during the hibernation period (Grahn et al. 1994; Heller and Ruby 2004; Zervanos et al. 2009; Park et al. 2010; Hope and Jones 2012). Variation in the rhythm of ecological and environmental factors may also influence the timing of periodic arousals from hibernation. In the United Kingdom (U.K.), cave-dwelling, greater horseshoe bats (*Rhinolophus ferrumequinum*) and Natterer's bats (*Myotis nattereri*) exhibit a circadian rhythm to arousals and warm up from torpor at sunset, likely because mild winter nights ($>10^{\circ}\text{C}$) with flying insects available occur relatively often (Park et al 2010; Hope and Jones 2012). In contrast, little brown bats (*Myotis lucifugus*), hibernating in a part of their range where ambient T_a on winter nights rarely exceeds 0°C , appear to abandon any circadian rhythmicity to their arousals (Chapter 2, Czenze et al. 2013). These bats are also more likely to arouse in synchrony with conspecifics, which could reflect individual benefits of passive or social rewarming (Chapter 2, Czenze et al. 2013). Thus, in cold northern environments where fat reserves cannot be augmented by winter-feeding, factors like social thermoregulation may be more important for hibernating bats.

Mammals living in highly seasonal temperate areas must be able to withstand cold temperatures in winter. Huddling may be beneficial by allowing individuals to reduce their exposed surface area and, therefore, heat loss, and computer models have shown huddling increases survival (Boyles et al. 2008). Huddling may also provide additional thermoregulatory benefits if individuals synchronize arousals from torpor with conspecifics and exploit social rewarming (Arnold 1993). Siberian hamsters (*Phodopus sungorus*) held in captivity with four litter mates synchronized their arousals more than 50% of the time and exhibited a smaller decline in mass over the winter compared to solitary individuals (Jefimow et al. 2011). Tactile social cues could be beneficial if groups of individuals can reduce energetic costs of arousal via social thermoregulation (Ruf and Arnold 2000). Alpine marmots (*Marmota marmota*), for example, hibernate in groups of related individuals and synchronize arousals with burrow mates to save energy. These benefits presumably increase over-winter survival, particularly for young-of-the-year (YOY), and may enhance inclusive fitness benefits via kin selection (Ruf and Arnold 2000). Little brown bats, with no opportunities for feeding during the middle of winter, synchronize their arousals with conspecifics, presumably to decrease energetic costs (Chapter 2, Czenze et al. 2013). However, whether this arousal synchrony is maintained into early spring, as emergence approaches and chances for occasional feeding increase, is unknown.

The hibernation period culminates with the emergence of an individual from its hibernaculum and emergence timing is an important aspect of hibernation phenology (Blumstein 2009). Emergence time of fat-storing hibernators is likely strongly influenced by fat reserves. When reserves are exhausted an individual must emerge to maintain

energy balance. Environmental cues may also influence emergence phenology as they may be good predictors of spring conditions. Many of these cues, and hibernator's responses to them, are affected by inter-annual variation in weather patterns and climate (Murie and Harris 1982; Inouye et al. 2000; Arlettaz et al 2001; Schorr et al 2009; Blumstein 2009; Sheriff et al. 2011). Along with fat reserves and environmental cues, there may be a genetic component influencing emergence date as well. Emergence date in Columbian ground squirrels (*Urocitellus columbianus*) is heritable and affects fitness (Lane et al. 2012). The timing of reproductive investment for males and females also strongly influences sex differences in emergence phenology. In some hibernators, males may emerge earlier than females and balance selective costs of enduring lingering winter weather against benefits of early spermatogenesis, access to mating territories, and improved ability to defend females from other males (Barnes et al. 1986; Buck and Barnes 2003; Hwang et al. 2007). The opposite pattern has been observed for fat-storing bats that mate before hibernation and take advantage of delayed fertilization (e.g., Jonasson and Willis 2011). In little brown bats, females are thriftier with their fat reserves and emerge from hibernation earlier than males presumably to exploit microclimates in maternity roosts (Jonasson and Willis 2011; Norquay 2013). Although more variable in T_a than hibernacula, maternity roosts will be much warmer than hibernacula on warm spring days which would expedite gestation. Females with larger fat stores have better reproductive success and pups born earlier in the year have higher overwinter survival so it is critical that females emerge in good body condition to initiate pregnancy (King et al. 1991; Hackländer and Arnold 1999; Frick, Reynolds and Kunz 2010). Males, whose reproductive investment takes place before and during early hibernation (Thomas et al.

1979), may remain in hibernacula for a longer period, and delay emergence until fat reserves have been exhausted (Jonasson and Willis 2011). By remaining in hibernacula, males may reduce their exposure to inclement weather and other sources of mortality such as predation (Barnes 1984; Strauss et al. 2007; Stawski and Geiser 2010; Healy et al. 2012). Although several studies have examined aspects of hibernation behaviour in this species there are none that attempt to examine the connection between arousal timing and emergence date.

I used temperature radio-telemetry, and passive integrated transponder tags (PIT-tags) to test hypotheses about links between torpor arousal cycles and emergence phenology in hibernating little brown bats. I first, revisited Czenze et al.'s (2013; Chapter 2) finding that little brown bats exhibit no circadian pattern to arousals during hibernation and tested the hypothesis that, as opposed to deep winter, opportunities for feeding in late winter/early spring do, in fact, influence arousal timing. I predicted that as warmer nights with flying insects become more common, bats would begin to exhibit a circadian pattern to arousal. Second, I tested whether hibernation duration would affect the propensity of bats to synchronize their arousals with conspecifics. I predicted that as energy reserves decline towards spring and the relative survival benefit of social rewarming increases, individuals would be more likely to synchronize their arousals with each other. Finally, I tested the hypothesis that sex, energy reserves, and environmental conditions influence emergence date as a result of differences in the timing of reproductive investment. This hypothesis predicts that females should emerge earlier than males and that energy reserves (assessed via body condition index (BCI)) should affect emergence date differently for males vs. females. Males in the best condition should remain in

hibernation the longest to minimize their exposure to sources of extrinsic mortality while females in the best condition should emerge earliest because they are best equipped to balance a trade-off between the cost of cold and unpredictable spring weather against the benefit of early gestation.

Methods

This study was conducted about 50km north of the town of Grand Rapids (53° 30' N; 99° 24' W), in central Manitoba, Canada. The region has extensive limestone karst topography and over 50 caves and sinkholes have been found in the area (S. Falkingham pers. comm.). Eight of these caves are known little brown bat hibernacula including my study site, Dale's cave. Dale's cave is a limestone cave accessed by a 3m deep sinkhole and consists of a bell-shaped chamber (approximately 7m in length, 5m in breadth with a 4m ceiling) that slopes away from the entrance. Approximately 80 to 200 bats overwinter in Dale's cave from mid-September until mid-May (J. Dubois and C.K.R. Willis, unpublished data).

The hibernation period in this region lasts approximately eight months from mid-September until mid-May (Norquay 2013). Temperature sensitive HOBO data loggers (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Cape Cod, Massachusetts, USA) were used to record cave T_a and humidity every two hours in the cave. I placed the datalogger in areas used by bats to ensure I recording biologically relevant microclimates.

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

WB0612. I entered the cave and handled bats during hibernation but to minimize disturbance I only entered once and limited the duration of my visit to less than 2 hours. Thirty torpid bats were captured by hand on 24 Jan 2013, sexed, weighed to the nearest 0.01g (Durascale-100, MyWeigh, Ontario, Canada) and their forearm length was measured to the nearest 0.05mm. I identified YOY based on un-fused 3rd digit metacarpal-phalangeal joints, and tapered rather than “knobby” joints following Davis and Hitchcock (1965). Although this distinction is difficult in more southern parts of their range, in my northern study area I can readily identify at least some YOY late during swarming and into hibernation (Jonasson and Willis 2011; 2012).

For small bats, skin temperature (T_{sk}) provides a good approximation of T_b during torpor (Audet and Thomas 1996; Barclay et al. 1996; Willis and Brigham 2003). To record T_{sk} I clipped a small ($<1\text{cm}^2$) patch of fur between the shoulders and attached a temperature-sensitive radio-transmitter (0.80-0.90g, BD-2NT; Holohil Systems Ltd, Carp, Ontario) using a non-toxic, latex-based adhesive (Osto-Bond, Montreal Ostomy, Vaudreuil, Quebec, Canada). Bats weighed, on average $9.44 \pm 1.08\text{g}$, and transmitters represented between 6.7 and 10.4% of body mass, greater than the 5% guideline suggested by Aldridge and Brigham (1988) but similar to the size which Jonasson and Willis (2012) demonstrated had no negative impact on body condition in hibernating little brown bats. I calibrated transmitters prior to use in a water bath at 5°C increments from 0.3°C to 45°C against a thermometer traceable to the National Institute of Standards and Technology. A datalogging receiver (Lotek SRX600; Lotek Engineering Inc Newmarket, Ontario, Canada) powered by two 64 W solar panels (US-64, Uni-solar, Auburn Hills, MI) connected to a solar charge controller (Sunsaver 10, Morningstar, Newtown, PA)

was deployed in a weatherproof box outside the cave and connected to 3 yagi antennas positioned in the cave. Receivers recorded T_{sk} of each individual at 10 min intervals. To assess individual emergence date passive transponders (PIT tags, Trovan Ltd. ID 100-01, <1g, Douglas, UK) were attached to each transmitter with epoxy. The mouth of the cave was outfitted with two 475 x 400 x 40mm panel antenna (ANT-612 Large Panel Antenna, Trovan Ltd., Douglas, UK). Panels were placed where their detection range exceeded the diameter of the cave mouth.

Large differences between cave T_a and normothermic T_{sk} made arousals obvious in the T_{sk} trace and I followed Jonasson and Willis (2012) and Czenze et al. (2013; Chapter 2) to define the phases of torpor and arousal. I defined torpor bouts as periods of reduced stable T_{sk} between the obvious warming and cooling phases associated with each arousal. Occasionally, Holohil transmitters exhibit a slight and gradual increase in temperature after initial entry into a torpor bout, which appears to be an artifact of these devices (e.g., Jonasson and Willis 2012; Czenze et al. 2013) as it does not occur for other temperature dataloggers attached to bats (e.g., Warnecke et al. 2012; Reeder et al. 2012). I could readily identify this artefact in my T_{sk} traces and it did not affect my analysis. The warming phase was classified as an abrupt increase in torpid T_{sk} that eventually stabilized at a normothermic steady state. The cave's cold T_a appeared to increase ambient cooling of external transmitters, leading to reduced T_{sk} values during arousal (Willis and Brigham 2003) but this effect was similar across individuals. The normothermic period of arousal was defined as the time between the end of the warming phase and the initiation of cooling; defined as an abrupt decline in T_{sk} preceding steady-state torpor. I excluded data from the first 24 hours after releasing the bats to avoid the influence of this disturbance

on my results.

All analyses were conducted in R version 2.10.1 (R Development Core Team 2009) and values are reported as the mean \pm SD with N equal to the number of animals and n equal to the number of events/arousals. I recorded the date and time that arousals occurred based on the T_{sk} trace and converted time of arousal from minutes before or after sunset into radians. I then used Rayleigh's test for circular distributions to determine if arousal times were significantly different from a random distribution. I tested the circular distribution of arousal times to determine if there was a temporal point when the distribution of arousal times became non-random. I tested the circular distribution of the first five recorded arousals and stored the subsequent P value. Next, I added sequentially later arousals to the distribution, increasing the amount of arousal times, and stored each P value. I continued adding later arousals until the distribution became non-random, I chose this day to split the study period into "winter" and "late winter/spring". To determine which bat-specific variables best predict emergence phenology I used linear mixed models with emergence date as the response variable and sex and BCI (body mass (g)/forearm length (mm)) at capture as predictors. I defined emergence date as days after 15 September 2012, the average recorded immergence date for little brown bats in this area (Norquay 2013). I compared nested models using maximum likelihood tests and ranked models based on their AIC scores (Pinheiro and Bates 2000). To determine which environmental cues best correlate with emergence time I used archived climatic data from the National Climate Data and Information Archive. I chose environmental factors which have been associated with bat activity in past studies: T_a (Anthony et al. 1981; Negraeff and Brigham 1995), barometric pressure (Paige 1995; Turbill 2008) and cave T_a (Perry

2012). To determine the influence of weather variables on the 17 nights during which I detected bats emerging between 27 April and 16 June 2013, I used a bootstrap resampling (with replacement) approach to generate a random sample of 17 “control” nights between those dates (for review see Crowley 1992). To test if weather variables were significantly different between these randomly selected nights and nights on which bats emerged I used a student’s t-test and stored the subsequent P-value. I then repeated this test 10000 times, each time using a new random sample of 17 “control” nights. This generated a distribution of 10000 P-values and I used the mean P-value from these 10000 iterations to assess significance. For all tests, I assessed significance at the $P < 0.05$ level.

Results

Average body mass of all bats at capture on 24 January 2013 was 9.4 ± 1.1 g and BCI was 0.25 ± 0.02 g/mm (Table 3.1, N=22 adult males, 7 female YOY, 1 adult female) Adult male bats had lower BCI ($t=2.8$, $P=0.02$, $df=10.2$) and were lighter ($t=2.9$, $P=0.02$, $df=9$) than the YOY (plus one adult) females at capture.

Although three transmitters failed, I recorded 1276.8 bat-days of T_{sk} data from 27 individuals over 110 days following transmitter attachment (mean= 48.2 ± 18.5 days per bat). Bats exhibited long bouts of torpor, the longest bout lasting 60.25 days, averaging 20.07 ± 14.55 days (Fig. 3.1). Torpor bouts were interspersed with periodic arousals averaging 190 ± 90 minutes (Fig. 3.2). I recorded a total of 104 discrete torpor bouts and 46 arousals before the final transmitter battery failed on 13 May 2013.

I split the study period into “winter” from 25 January-6 April and “late winter/spring” from 7 April-13 May. I chose 7 April to separate study periods because,

Table 3.1. Body mass, forearm length and body condition index of hibernating little brown bats captured in Dale's cave in Central Manitoba on 24 January 2013. Values represent the mean \pm SD. YOY refers to young of the year.

Demographic	Body Mass at Capture (g)	Forearm Length (mm)	Body Condition at Capture (g/mm)	No. of Individuals
Adult Male	9.1 \pm 0.7	38 \pm 0.8	0.24 \pm 0.02	22
Adult Female	9.7	37.9	0.26	1
YOY Female	10.3 \pm 1.2	39.2 \pm 0.9	0.26 \pm 0.03	7

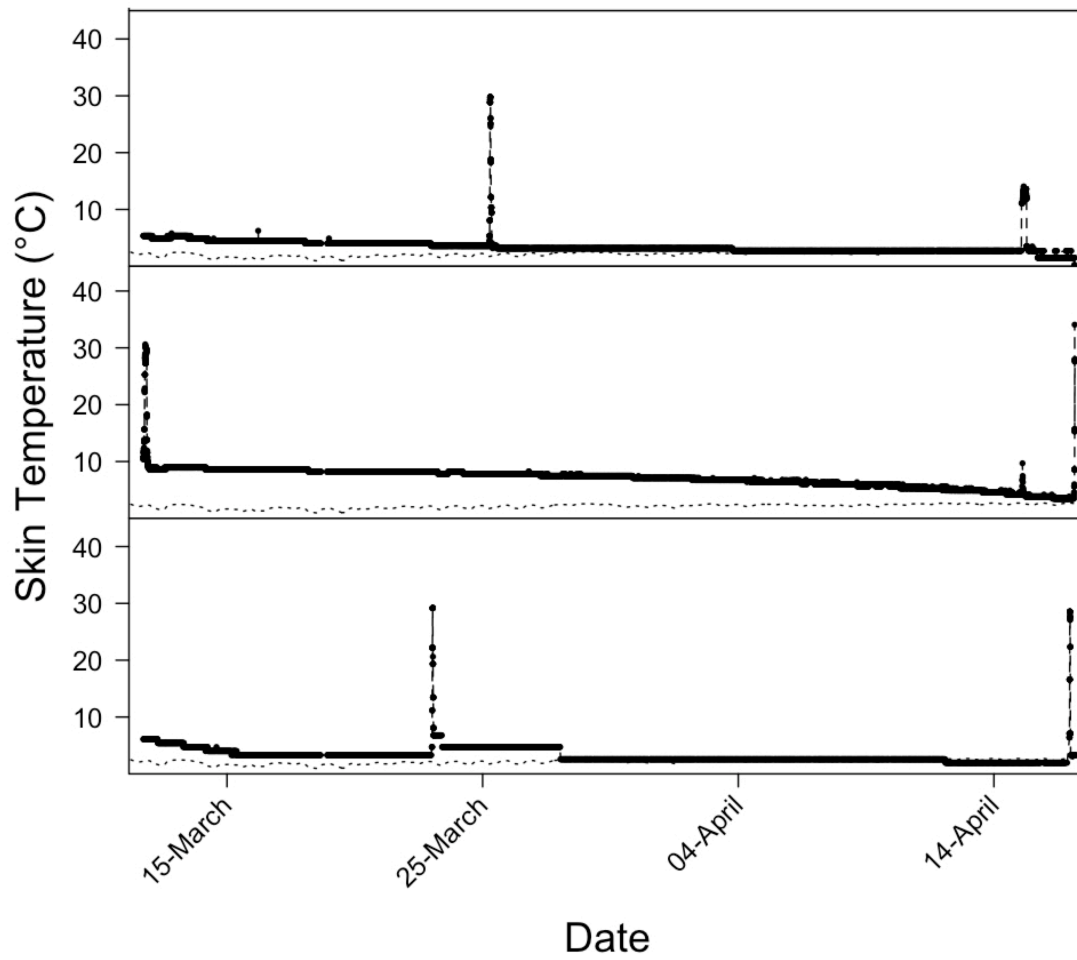


Figure 3.1. Representative skin temperature traces of three hibernating adult male little brown bats in Dale's cave, Central Manitoba. Dotted line represents cave T_a .

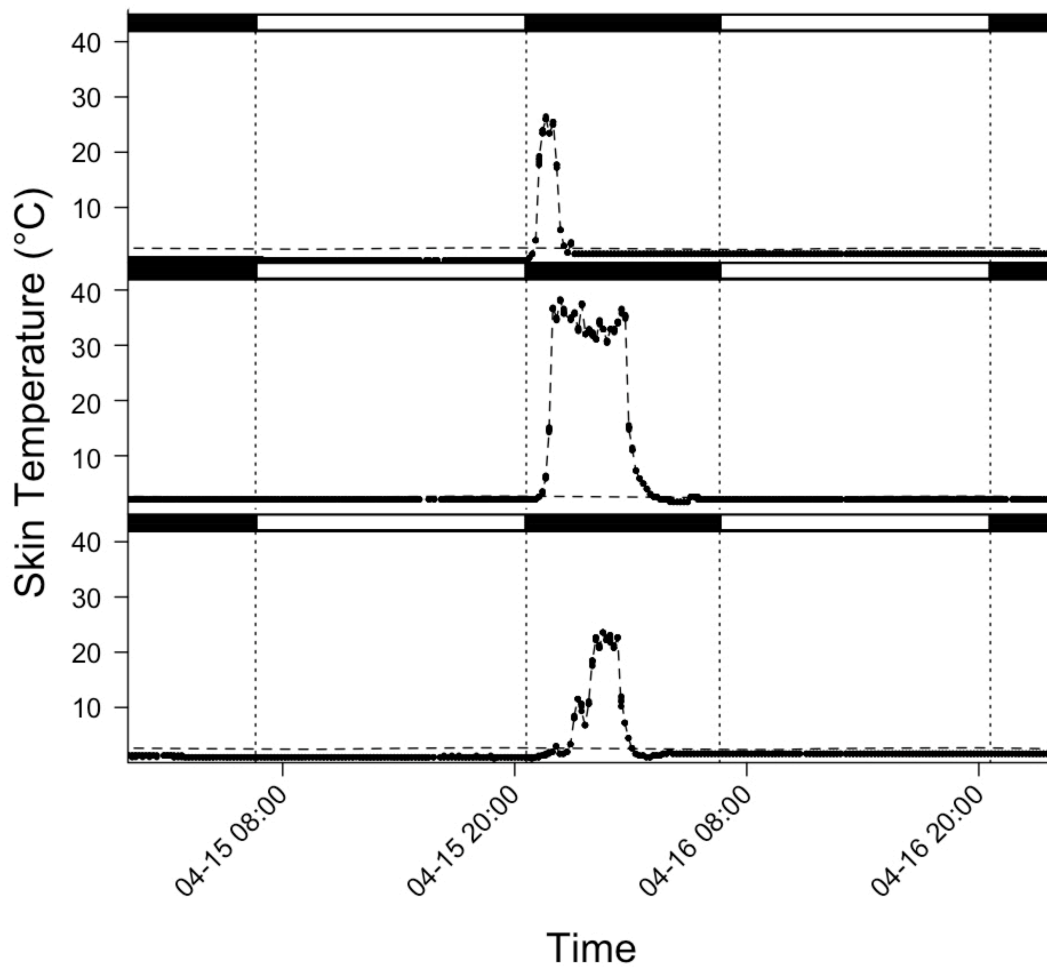


Figure 3.2. Representative skin temperature traces of three arousals of adult male little brown bats during 15 April 2013. Dotted vertical lines represent sunrise and sunset, dashed horizontal line represents cave T_a . Black horizontal bars represent dark phase and white horizontal bars represent light phase.

when analyzing the circular distribution of arousals, I found that prior to 7 April the circular distribution of arousal times was random and when I began including arousals after 7 April the distribution became significantly different from random (Fig. 3.3). During winter, prior to 7 April the circular frequency distribution of differences between arousal time and sunset time, expressed in radians, was no different from a random distribution (Fig. 3.4a, b, $Z=2.02$, $P=0.13$, mean vector=0.31, $N=17$ bats, $n=21$ arousals). Since I recorded multiple arousals from some, but not all individuals this analysis is technically pseudoreplicated. However, I still found no relationship between sunset and arousal time when I calculated the average arousal time for each individual ($Z=0.83$, $P=0.44$, mean vector=0.24) or limited the analysis to each individual's first arousal during this period ($Z=1.14$, $P=0.32$, mean vector=0.29). During this period 35% (6/17) of individuals aroused at least once with a conspecific and over 38% (8/21) of arousals were at least partially temporally overlapping with those of other individuals.

In late winter/spring, after 7 April (7 April–13 May) there was a relationship between arousal times and sunset. During this period bats were more likely to arouse within 0.6 ± 3.3 hours of sunset and the differences between arousal time and sunset time was different from a random distribution (Fig. 3.5a, b, $Z=14.8$, $P<0.01$, mean vector=0.77, $N=15$, $n=25$). As above, this analysis was technically pseudoreplicated but I still found the relationship when I limited the analysis to each individual's first arousal during this period ($Z=10.6$, $P<0.01$, mean vector=0.77) or calculated the average arousal time for each individual ($Z=12.8$, $P<0.01$, mean vector=0.84).

The degree of conspecific synchrony of arousals also increased during late winter/spring compared to the previous period. After 7 April, 80% (12/15) of individuals

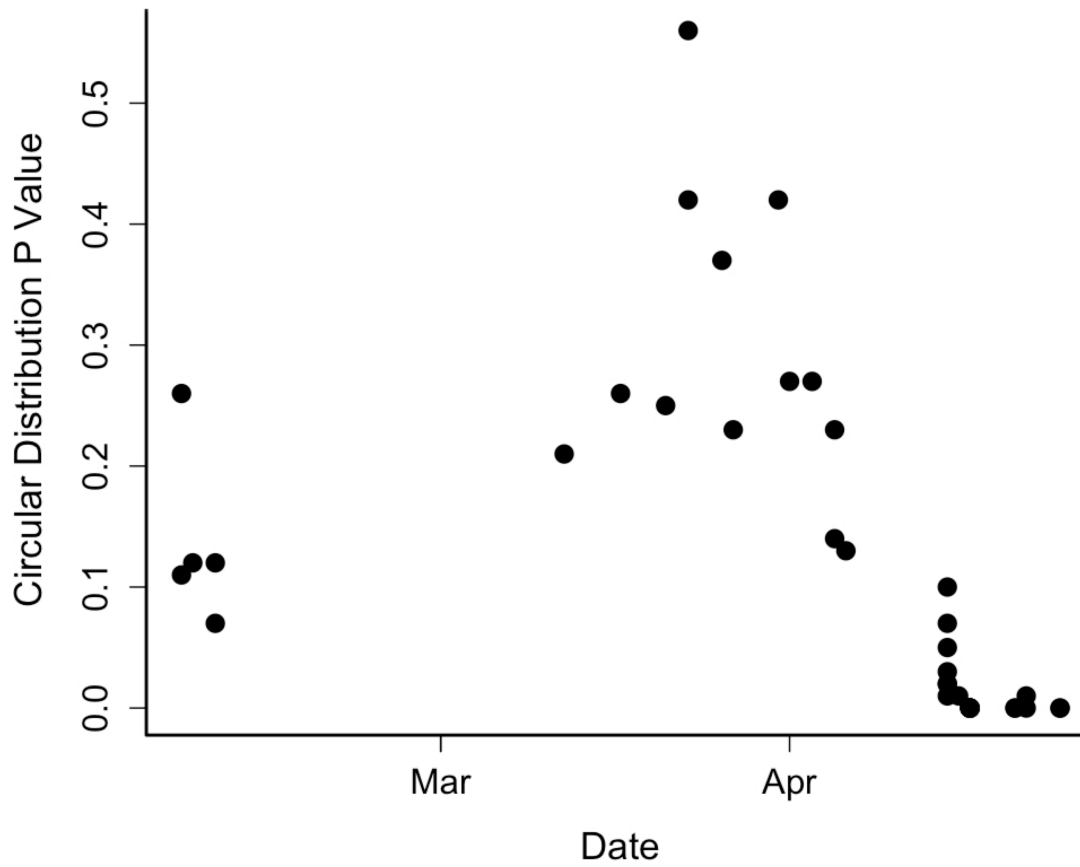
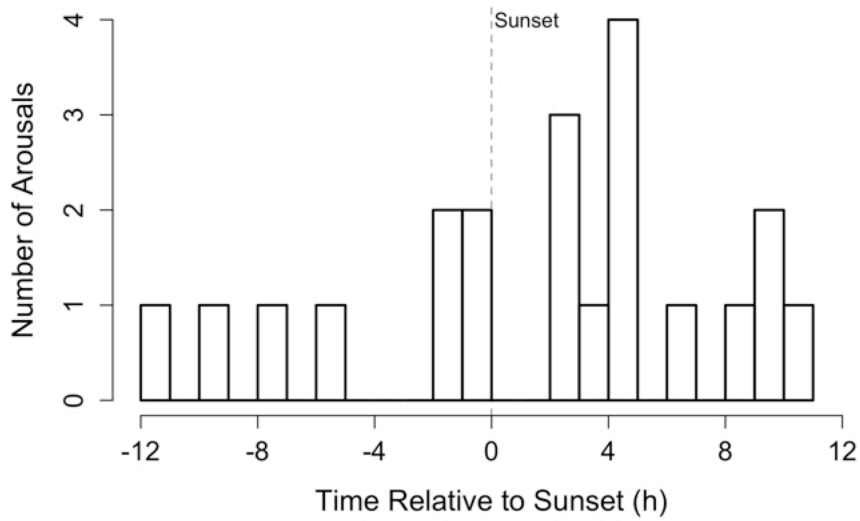


Figure 3.3. P values of the circular distribution of arousal time over the winter period of little brown bats in Dale's cave, Manitoba, Canada. See methods for an explanation of statistical comparisons.

a)



b)

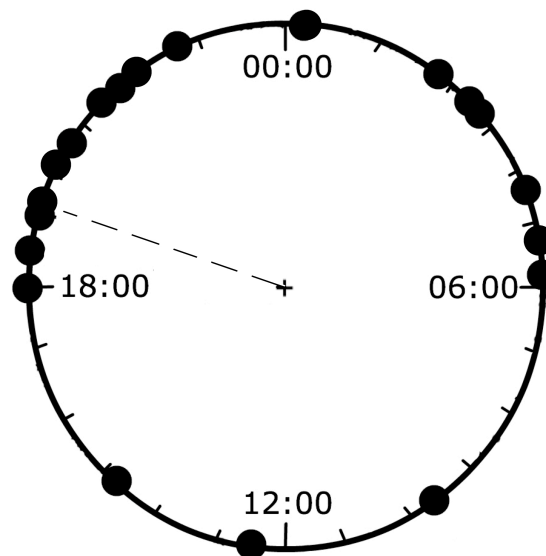
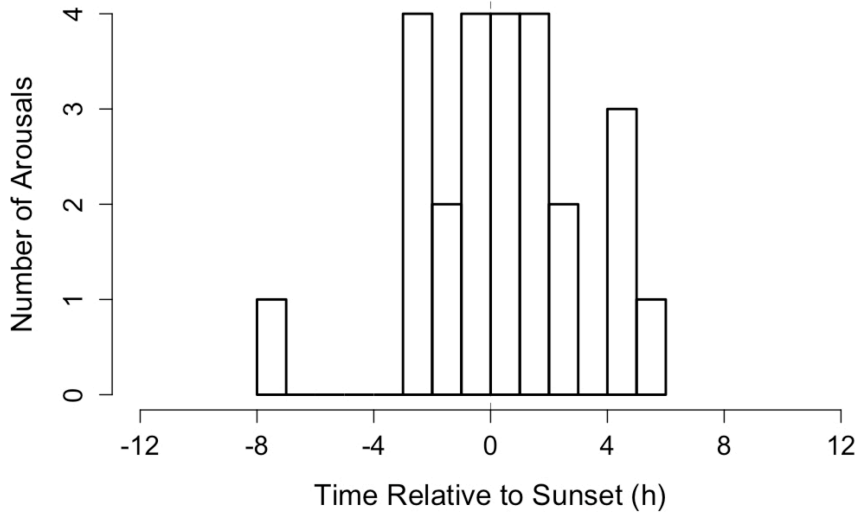


Figure 3.4. Frequency distribution of arousal time of individual little brown bats relative to sunset during hibernation (a); and (b) clock face representing circular distribution of arousals during hibernation before 7 April 2013. Dotted line represents mean sunset time (mean sunset=19:02, $Z=2.02$ $P=0.13$, mean vector=0.31, $N=17$, $n=21$).

a)



b)

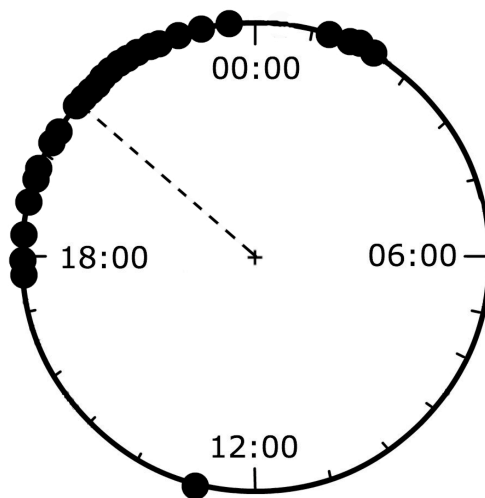


Figure 3.5. Histogram showing the timing of arousals relative to sunset for little brown bats during hibernation (a); and (b) clock face representing circular distribution of arousals during hibernation after 7 April 2013. Dotted line represents mean sunset time (mean sunset=20:47, $Z=14.8$, $P<0.01$, mean vector=0.77, $N=15$, $n=25$).

aroused at least once with a conspecific and 80% (20/25) of all arousals were temporally overlapping with those of another individual. This proportion was significantly greater than that prior to 7 April 2013 (Fig. 3.6, $Z=-2.9$, $P>0.05$). To further illustrate this point, I recorded arousals by five bats that rewarmed both singularly and socially. Four of these five aroused singularly before 7 April and socially after 7 April. I also observed three instances of what I termed “arousal cascades” during which one individual would initially arouse followed by one or more additional individuals.

Males emerged from hibernation 22.7 ± 6.9 days later than females (Fig. 3.7, $P<0.01$, $N=17$). The value of interaction term between sex and BCI approached significance, so I examined the effect of BCI on each sex separately. I found no significant effect for males ($P=0.41$, $N=12$ bats) but a significant effect of BCI on emergence date for females (Fig. 3.8, $P=0.026$, $N=5$ bats) with females in better condition emerging earlier. Cave T_a on the 17 nights during which I observed bats emerging was the only environmental variable that differed from a random sample of 17 nights within the 51-day emergence period ($P=0.03$, $N=17$, $n=17$) although the change in outside T_a over the hour prior to emergence and T_a at the time of emergence approached significance (Table 3.2). When I analyzed each sex separately, cave T_a predicted emergence timing of males ($P<0.01$) but not females.

Discussion

I found strong support for the hypotheses that declining energy stores in late hibernation influence arousal timing and that the timing of investment in reproduction influences hibernation phenology. As I found previously for little brown bats in a

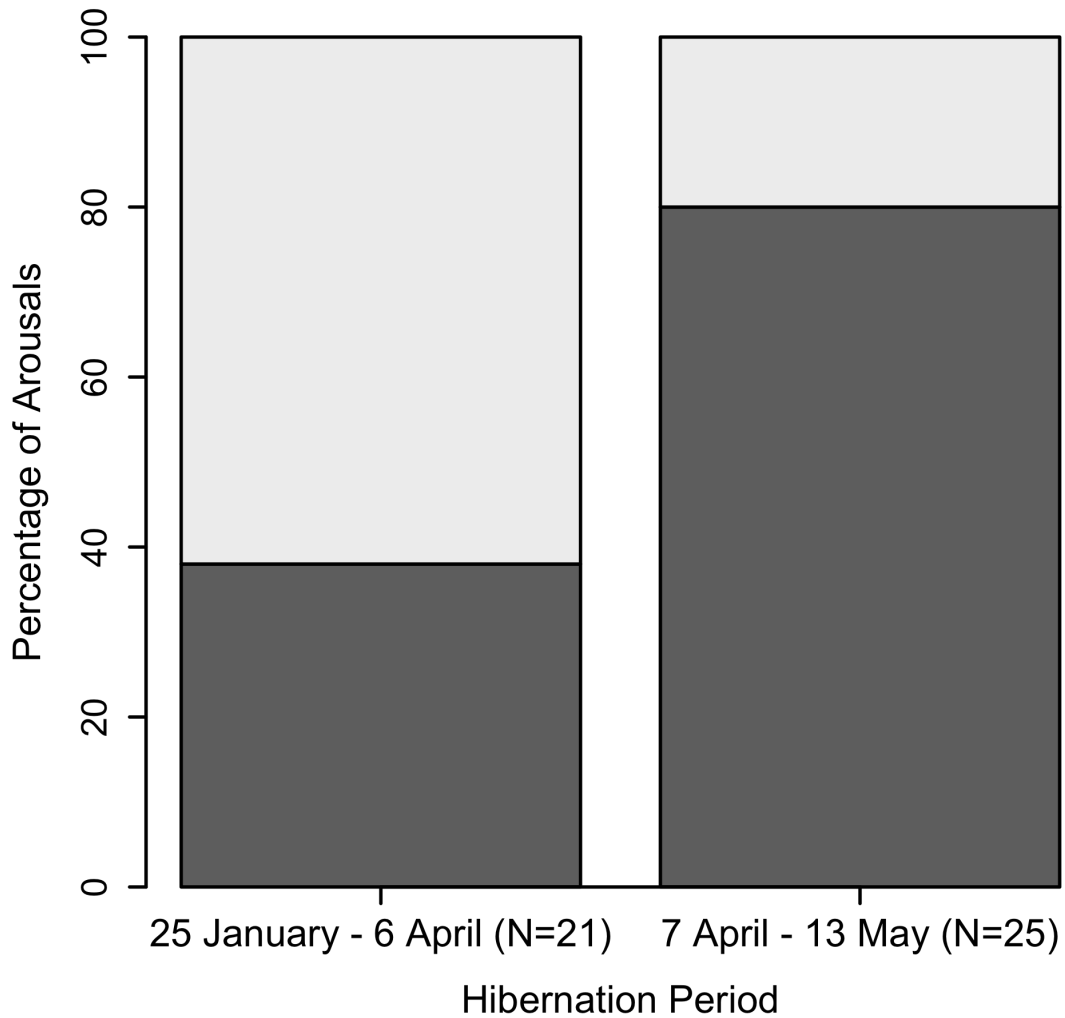


Figure 3.6. The proportion of arousals by hibernating little brown bats which overlapped with arousals of at least one other bat in the same cave (dark grey) vs. non-overlapping arousals before and after 7 April 2013. ($Z=-2.4$, $P=0.02$, $N=46$).

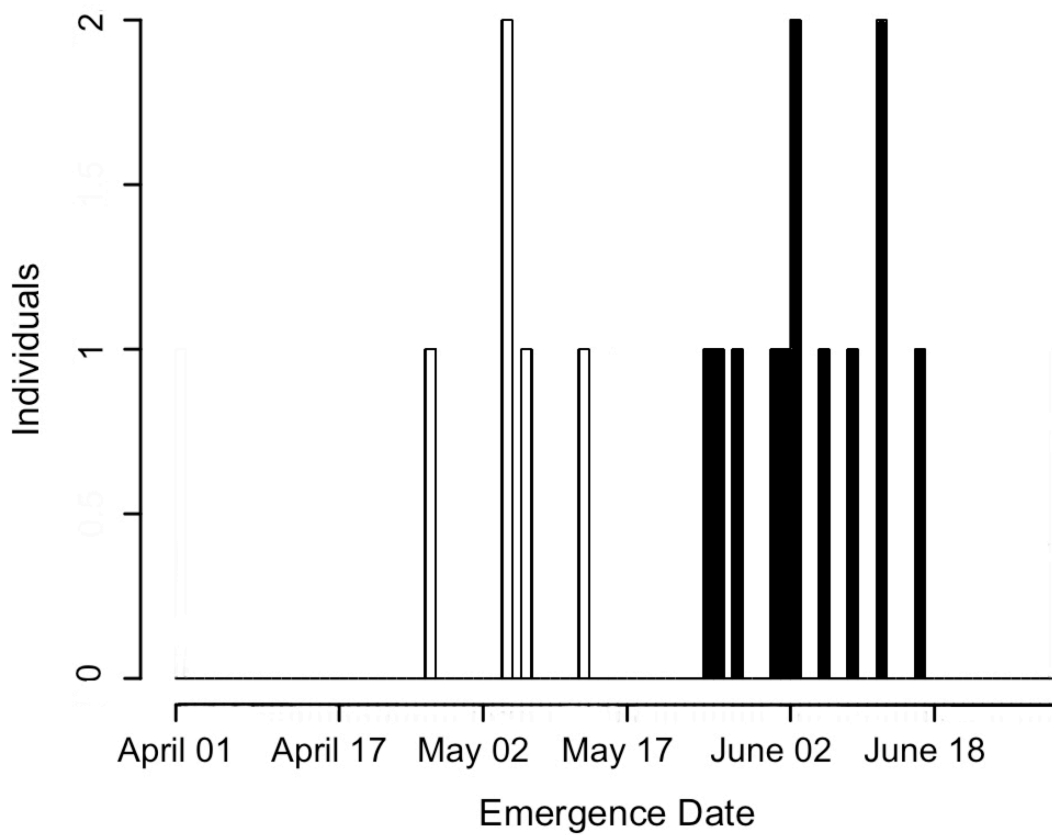


Figure 3.7. Histogram of recorded emergence dates of individual little brown bats. White bars represent females and black bars represent males (N=5 females, 12 males).

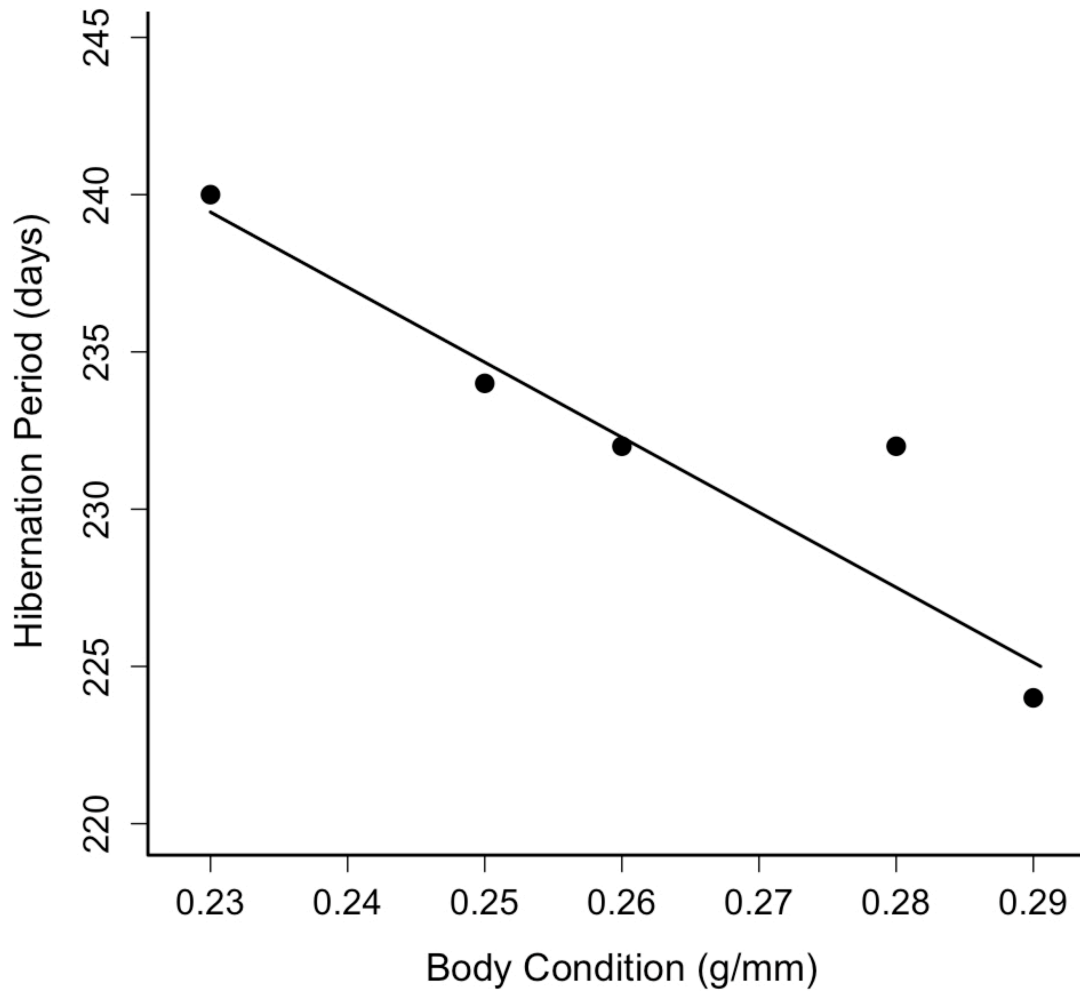


Figure 3.8. Emergence date of female little brown bats compared to body condition (body mass (g)/forearm length (mm)). Hibernation period represents the number of days since 15 September 2012 ($P=0.03$, $r^2=0.85$, $N=5$ bats).

Table 3.2. A comparison of climatic variables (measured value) recorded on the 17 nights during which I observed little brown bats emerge between 27 April and 16 June 2013 vs. corresponding values for 17 randomly selected nights during the same period. “Estimated value” T_a refers to ambient temperature outside the cave. Values represent the mean \pm SD. See methods for an explanation of statistical comparisons.

Climate Variable	Measured Value	Estimate Value	Mean P-Value	T-Value
Cave T_a ($^{\circ}\text{C}$)	3.7 ± 0.5	2.9 ± 0.5	0.03 ± 0.07	-6.1
T_a ($^{\circ}\text{C}$)	5.2 ± 4.9	-2.4 ± 7.0	0.07 ± 0.1	5.7
Hourly change in T_a before emergence ($^{\circ}\text{C}$)	-1.2 ± 0.9	-0.5 ± 1.1	0.07 ± 0.1	6.8
Ambient Pressure (kPa)	98.7 ± 0.7	98.9 ± 0.9	0.3 ± 0.3	28.8
Hourly change in pressure before emergence (kPa)	-0.02 ± 0.05	-0.01 ± 0.05	0.44 ± 0.2	14.1
Hourly change in cave T_a before emergence ($^{\circ}\text{C}$)	-0.001 ± 0.02	-0.005 ± 0.03	0.46 ± 0.3	44.9

different cave, a year before this study was undertaken (Czenze et al. 2013; Chapter 2), during the coldest months of the year (January–March) hibernating little brown bats exhibited no circadian pattern of arousals. During this study between 24 January and 6 April, bats aroused randomly with respect to sunset suggesting that they are either no longer sensitive to, or have abandoned maintenance of, their circadian rhythm. During this time the mean daily T_a outside the cave ranged from -31.4 to -1.7°C (National Climate Data and Information Archive, 2013) eliminating any opportunity for bats to forage. The complete lack of food availability for bats in my study area during this period makes nightly feeding impossible, and the costs of maintaining a circadian rhythm in the absence of an energetic benefit could explain the random timing of arousals with respect to sunset (Czenze et al. 2013, Chapter 2). Interestingly, however, after 7 April bats resumed a circadian pattern to arousals and synchronized their arousals with sunset. Some mammalian hibernators appear to maintain a circadian rhythm of arousals (Zervanos et al. 2009; Grahn et al. 1994; Heller and Ruby 2004; Hope and Jones 2012, Park et al. 2010) but, to my knowledge, this is the first evidence of a hibernator which abandons a circadian arousal pattern and then later resumes that pattern prior to emergence in the absence of photoperiod cues.

The resumption of the circadian rhythm was closely correlated with increasing air temperature, and during this period mean T_a outside the cave ranged from -12.2 to 13.2°C (National Climate Data and Information Archive, 2013). Although there were many nights below the 10°C threshold at which flying insects may become more available (Jones et al. 1995), on 21% (7/33) of nights between 7 April and 13 May, the outside T_a at sunset was greater 10°C . This suggests that, during early spring there is at least some

opportunity for bats to take advantage of flying insects and individuals may gain an energetic benefit from resuming a circadian rhythm to arousal on the chance they might arouse on one of these warm evenings.

The mechanism underlying suppression and subsequent resumption of a circadian rhythm could be found in the SCN of the hypothalamus. Removal of the SCN disrupts circadian rhythms of T_b in hibernating golden-mantled ground squirrels (*Callospermophilus lateralis*) (Ruby et al. 2002). The expression of circadian rhythms in bats may be similar to that of hibernating European hamsters (*Cricetus cricetus*) for which the expression of SCN clock genes is halted during the low T_b associated with deep torpor (Revel et al. 2007). Translation, transcription and protein synthesis involved in this process could all be depressed during long torpor bouts (Storey and Storey 2004). Alternatively, it may be that, although oscillators in the SCN are still functioning rhythmically during hibernation, the output pathways are inhibited (Williams et al. 2012). In arctic ground squirrels (*Urocitellus parryii*) this inhibition continues briefly in the presence of photoperiod cues even after emergence from hibernation (Williams et al. 2012). This suggests bats may arouse and emerge from hibernacula before flying insects are readily available to obtain photoperiod cues and begin the process of resuming their circadian rhythm in time for the active season.

In addition to scheduling arousals around sunset, I also found that bats synchronized arousals with each other, especially after 7 April. In the latter part of the hibernation period the proportion of arousals that were synchronized nearly tripled. Part of this effect could reflect the fact that bats were synchronizing arousals with sunset and, therefore, their arousals coincided. However, the fact that they aroused at sunset does not

necessarily require them to arouse on the same days and it seems unlikely that the arousal synchrony I observed is completely explained by resumption of a circadian pattern to rewarming. During long northern winters, as bats become more energetically stressed, the energetic savings provided by huddling and synchronizing arousals may become especially important. Larger huddles would increase the opportunity for social thermoregulation during early spring. This hypothesis predicts that huddle sizes should increase as the winter period progresses and I recommend studies aimed at testing this hypothesis in the lab or field.

In addition to a resumption of a circadian pattern to arousals and increased arousal synchrony among individuals in late winter, I found that female bats emerged earlier from hibernation than males, likely to take advantage of more variable but potentially warmer microclimates in maternity roosts as well as occasional warm spring nights for foraging. Both of these factors could expedite gestation and allow mothers to give birth sooner. Giving birth to pups as soon as possible is critical and pups born earlier have a better chance of survival (Frick, Reynolds and Kunz 2010). This strategy may be risky, however, as weather during early spring is unpredictable and cold temperatures/spring snowstorms may still occur. Despite a small sample size, I found a very strong relationship between BCI of females and emergence, with females in the best condition being first to emerge. This is consistent with observations from a previous study at a different cave further south in Manitoba (Norquay 2013) and suggests that females with low BCI may not have the necessary fat reserves to deal with unpredictable harsh spring weather. On the other hand, females with the largest fat reserves may be able to balance risks of early emergence against benefits of a longer active season.

I found no link between BCI and emergence date for males. I predicted that males in the best condition should emerge later to minimize their risk of encountering predators or harsh spring weather. Given that their reproductive investment does not occur until late summer/fall, selective pressure for early emergence on males should be weaker than that for females. Although the relationship was not significant, I did find that the male with lowest BCI was the first to emerge and the male with highest BCI was last to emerge from hibernation. Thus high inter-individual variation among males, combined with a small sample size, may have masked a potentially weak effect of body condition on emergence date. I recommend future studies place an emphasis on tagging male bats to determine the potential effect of BCI on their emergence patterns.

In addition to the effect of BCI on female emergence date, I also found that bats were more likely to emerge from their hibernaculum on days associated with warm cave T_a and that this relationship was driven by a preference for males to emerge on nights with relatively warm cave T_a . By returning to a circadian rhythm of arousals in April bats may be able to assess T_a at about sunset. For hibernators in northern latitudes the ability to adapt to changing climates may depend on the use of a combination of external and endogenous cues. This is certainly the case with male bats in my study, and increasing cave T_a influenced these individuals' emergence time. The effect of T_a as well as the hourly change in T_a which both approached significance, suggests that environmental cues may play a more important role in emergence timing for male bats compared to females.

I found no significant effect of barometric pressure on emergence date, given that, in contrast to T_a , it is likely to be identical inside and outside the cave and that it can

provide important information to bats about food availability (e.g., Paige 1995, Turbill 2008). Winter activity in bats is correlated with falling barometric pressure (Turbill 2008) and 98.6 kPa is almost identical to the pressure that corresponded with the highest activity by both flying insects and tri-coloured bats (*Perimyotis subflavus*) (Paige 1995). Paige (1995) argued that these bats were energetically stressed and used barometric pressure as a signal of insect abundance. This energetically inexpensive method would allow bats to avoid foraging on periods of cold temperature, high pressure, and low insect activity without leaving the hibernaculum. Although I found no effect of barometric pressure on emergence, all females I observed emerging left the hibernaculum on nights when pressure fell below 98.6 kPa. A reasonable hypothesis is that little brown bats use barometric pressure as a cue to time their emergence on nights when the possibility of insects is highest. The fact I failed to find a significant effect of pressure could reflect a small sample size of female emergence times (N=5).

For other hibernators, intraspecific variation in emergence date can be affected by external cues like winter severity, snow cover, snow depth, food availability, and air temperature (Murie and Harris 1982; Inouye et al. 2000; Arlettaz et al 2001; Schorr et al 2009; Blumstein 2009; Sheriff et al. 2011). Lucan et al. (2013) found that, over the past 20 years, Daubenton's bats (*Myotis daubentonii*) have returned to their summer roosts following hibernation earlier during warmer springs suggesting that spring temperature influences hibernation phenology. For 27 April, when the first bats emerged in my study, the average temperature for Grand Rapids over the past five years has fluctuated from -0.8 °C to 10.9 °C, a range of 11.7 °C (National Climate Data and Information Archive, 2013). During this same five-year period, temperatures on 16 June, when the last of the

bats emerged, fluctuated from 15.2°C to 20.5°C, a range of only 5.3°C (National Climate Data and Information Archive, 2013). Norquay (2013) found that a few bats emerged in mid-March 2012 when unseasonably high temperatures reached 25°C. High spring temperatures may have a positive impact on hibernating bats as warmer weather could allow them to emerge earlier and increase their active season. However, high variation in early spring temperatures could have implications for hibernating bats, especially reproductive females. If females' emergence date is not plastic they may not be able to take advantage of warmer years and/or fall victim to colder ones. Over the past 20 years progressively later snowmelts has delayed the emergence time of resident Columbian ground squirrels by almost 0.5 days per year (Lane et al. 2012). Animals exposed to shorter active seasons have responded via within-individual phenotypic plasticity of emergence date but this population's fitness has been steadily declining over the past two decades suggesting that climate change in northern ecosystems may not always be beneficial (Lane et al. 2012).

This study has implications for understanding white-nose syndrome, the fungal disease caused by *Pseudogymnoascus destructans* (*Pd*), which is devastating populations of hibernating bats in eastern North America. Premature depletion of hibernation fat reserves, due to increased arousal frequency, is thought to be the primary cause of mortality from WNS (Warnecke et al. 2012, Reeder et al. 2012). Differences in hibernation patterns and phenology suggest that females and males may experience differential mortality with the arrival of WNS (Jonasson and Willis 2011). Bats in northern Manitoba may be able to better withstand the extra *Pd*-induced arousals compared to southern bats. A recent energetic model suggests that, because they

accumulate much larger fat stores bats from Manitoba can withstand an extra 12 arousals or 74 days of additional hibernation compared to bats from Vermont (Norquay 2013).

This may mean that northern bats and especially females in good condition emerging early may experience lower rates of mortality from WNS, although they may deplete fat reserves normally required for reproduction.

This study confirms that little brown bats abandon a circadian pattern to arousal during the coldest parts of the winter. However, they resume this pattern in late winter and synchronize arousals with conspecifics more often during late hibernation which could enhance energy savings via social rewarming. Little brown bats in more southerly ranges may benefit from longer active seasons brought on by climate change. My data confirm Norquay's (2013) observations that females in the best condition during the winter emerge earliest suggesting during mild winters and that these individuals especially may have a higher survival rate. If larger females are emerging earliest and this trait is heritable this population may be buffered against WNS-related mortality.

Understanding intersexual differences in hibernation patterns as well as differences in climactic effects between populations is especially important for conservation. When considering conservation action plans special attention should be given to the specific winter conditions hibernators must deal with and their susceptibility and vulnerability to impacts like WNS for bats, and climate change for hibernators in general.

CHAPTER 4: THRIFTY FEMALES, FRISKY MALES: WINTER ENERGETICS OF BATS IN A COLD CLIMATE

Introduction

Seasonal variation in climate and environmental conditions strongly influence the energy budgets of animals (McNab 2002). Winter is a time of both low ambient temperature (T_a) and food availability in temperate areas, which makes it more difficult for endotherms to balance the energy budget. Species living in these areas have evolved a range of behavioural and physiological adaptations to contend with unfavourable 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). This reduction in MR confers enormous energy savings and allows hibernators to survive periods as long as eight months on a small store of food or fat (Lyman 1982; Wilkinson and South 2002; Geiser 2004; Stawski and Geiser 2010; Norquay 2013).

Despite the energetic benefits, long-term expression of torpor is not without costs. Costs may include respiratory acidosis as blood CO_2 increases (Elvert and Heldmaier 2000; Heldmaier et al. 2004), accumulations of metabolic wastes, and dehydration stress due to the inability to urinate or drink (Thomas and Cloutier 1992; Thomas and Geiser 1997). Additionally, the immune system of hibernators is depressed which could make them more susceptible to pathogens (Prendergast et al. 2002; Luis and Hudson 2006; Bouma et al. 2010). Presumably to counteract these physiological costs all hibernators interrupt their torpor bouts with brief periodic arousals to normothermic T_b (Geiser 2004). Although arousals are brief, due to the high metabolic cost of thermoregulation during

normothermia at a 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 animals should perform the longest and deepest torpor bouts possible to maximize their energy savings has been challenged by the torpor optimization hypothesis (Humphries et al. 2003a; 2003b; Munro et al. 2005; Boyles et al. 2007; Wojciechowski et al. 2007; Landry-Cuerrier et al. 2008; Jonasson and Willis 2011; 2012). This hypothesis predicts that, to optimize their energy reserves, hibernators should balance the energetic savings of performing long torpor bouts against their associated physiological costs (Humphries et al. 2003a). In other words, individuals with excess energy reserves should mitigate the physiological costs of torpor by reducing the duration of their torpor bouts. Studies on food-caching species like eastern chipmunks (*Tamias striatus*) and Richardson's ground squirrels (*Urocitellus richardsonii*) support the torpor optimization hypothesis (Munro et al. 2005; Michener and Locklear 1990). To test whether an increase in energy reserves reduces expression of torpor Munro et al. (2005) supplemented the food caches of some individual eastern chipmunks. Supplemented individuals reduced torpor bout duration compared to controls (Munro et al. 2005). Furthermore, free-ranging eastern chipmunks hibernating during years of high food abundance dramatically reduced their torpor expression compared to those in low food years (Landry-Cuerrier et al. 2008).

Fat-storing hibernators appear to behave similarly to food-caching hibernators when they are energetically constrained (Wojciechowski et al. 2007; Boyles et al. 2007). The T_a a hibernator experiences is an important factor influencing torpor behaviour. Torpid MR (TMR) is influenced by T_a and individuals hibernating in colder microclimates spend less

energy than those in warmer microclimates (Geiser 2004). The torpor optimization hypothesis predicts that animals in poor condition with “less fat to lose” should choose colder microclimates. Consistent with their hypothesis, Big brown bats (*Eptesicus fuscus*) with experimentally-constrained fatty acid stores, and therefore energy reserves, preferentially chose colder microclimates (Boyles et al. 2007). Moreover, heavier and presumably fatter free ranging little brown bats (*Myotis lucifugus*) preferentially chose to hibernate in warmer microclimates (Boyles et al. 2007).

The torpor optimization hypothesis also predicts that individuals using energy reserves for reproduction after hibernation will increase their use of torpor during winter (Humphries et al. 2003a, Jonasson and Willis 2011). In some species females emerge later than males (Michener and Locklear 1990). Early-emerging males must balance the benefits of access to mating territories, defense of females from other males, and early spermatogenesis with the costs of exposure to lingering winter weather (Barnes et al. 1986; Buck and Barnes 2003; Hwang et al. 2007). For male Richardson’s ground squirrels over-winter energy reserves are critical to reproductive success as they compete immediately after emergence from hibernation for reproductive females (Michener and Locklear 1990). Females of this species are not so energetically constrained during hibernation and deplete their energy reserves more quickly than more conservative males (Michener and Locklear 1990).

Little brown bats exhibit intersexual differences in the timing of reproductive investment. Immediately before emergence both sexes aggregate in large groups around the entrance to hibernacula where they mate promiscuously (Kunz et al. 1998). The males’ primary sexual investment occurs during swarming and into early hibernation

while they undergo spermatogenesis (Thomas et al. 1979). Conversely, females' sexual investment comes after hibernation immediately after emergence. Female little brown bats store sperm in their reproductive tract and delay fertilization until weather and food availability become more favourable in spring (Wimsatt 1944; 1945). Therefore, it is critical for females to emerge from hibernation in good body condition to fuel pregnancy and allow parturition as soon as possible (Kunz et al. 1998).

In keeping with this hypothesis and, like male Richardson's ground squirrels, female little brown bats were more "thrifty" with their fat reserves during hibernation than males (Jonasson and Willis 2011). Jonasson and Willis (2011) found that male bats, not under the extra energetic burden of pregnancy, presumably used more of their winter energy reserves to mitigate physiological costs of prolonged torpor. Male bats may also have been spending more energy during their arousals by mating with torpid females (Thomas et al. 1979). However, in a subsequent study, patterns of body temperature during hibernation, recorded using temperature radio-telemetry, did not support the torpor optimization hypothesis, and no effect of sex or body condition index (BCI) on torpor bout or arousal duration was found (Jonasson and Willis 2012). The absence of difference in skin temperature (T_{sk}) patterns of male and female little brown bats in Jonasson and Willis' (2012) study could reflect a small sample size (N=3-11 individuals per demographic). Thus, it would be useful to revisit the thrifty female hypothesis with a larger sample of free-ranging bats.

The torpor optimization hypothesis also predicts differences in hibernation behaviour for adult vs. young-of-the-year (YOY) hibernators. In general YOY born earlier in the spring have a longer active season to develop and accumulate fat for their

first hibernation. In bats, a longer active season is critically important for overwinter survival (Frick, Reynolds and Kunz 2010) and in ground squirrels short active seasons are associated with higher mortality (Lane et al. 2012). Historically juvenile bats captured during hibernation have been in lower body condition than their adult counterparts (Schowalter 1980). Young-of-the-year males are sexually immature in their first year whereas a small proportion of YOY females do reach sexual maturity before hibernation (Carter 1970; Gustafson and Shemesh 1976). This suggests that female YOY may face similar reproductive energetic constraints as adults combined with an even smaller fat reserve. If so, the torpor optimization hypothesis predicts that YOY females should increase their expression of torpor even more than adults. YOY males, on the other hand, could behave similarly to adult males or, if their reserves are relatively small, hibernate conservatively like adult females.

In cold climates little brown bats may hibernate for up to eight months (Norquay 2013) and in these extremes budgeting energy reserves becomes even more critical for survival. Little brown bats hibernate across the continent in mines and caves and the northern extent of their hibernacula occurs exclusively in Canada and Alaska (Humphries et al. 2002). The complete reliance of little brown bats' on their fat store during the winter, combined with large group sizes, intersexual differences in hibernation behaviour, and relative ease of capture make them an attractive model organism for understanding hibernation strategies.

My objective was to provide additional tests of Humphries et al.'s (2003a) torpor optimization hypothesis, and Jonasson and Willis's (2012) thrifty female hypothesis, which predict that arousal and/or torpor bout duration should vary with sex, age, and BCI.

I combined T_{sk} data collected by Jonasson and Willis (2012) with two years of new data from the same study area. I used temperature telemetry to record T_{sk} of free-ranging bats combined with a model of metabolic rate during different thermoregulatory phases, to quantify the energetic costs of torpor and arousals. I predicted that: 1) Bats in better body condition with larger fat reserves would exhibit a high-energy hibernation pattern, performing longer arousals and shorter torpor bouts in order to offset physiological costs of torpor; 2) Females would exhibit a low-energy hibernation pattern with shorter arousals and longer torpor bouts than males because of strong selection pressure on them to emerge from hibernation with a fat reserve large enough to support pregnancy; and 3) YOY would exhibit an extremely low-energy hibernation pattern with longer torpor bouts and shorter arousals than adults in order 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 caves north of the town of Grand Rapids, in central Manitoba Canada ($53^{\circ} 30' N$; $99^{\circ} 24' W$). I 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. The region has extensive limestone karst topography and several caves are known little brown bat hibernacula including the two which served as my study sites, Firecamp Cave and Dale's Cave. Firecamp Cave is

accessed by a sinkhole entrance approximately 50cm in diameter and consists of a bell-shaped chamber about 7m deep. Between 30 and 60 bats over-winter in Firecamp Cave from mid-September until late-May (J. Dubois and C.K.R. Willis, unpublished data). Bats in this cave all hibernate in one tight cluster within a crack 1m long by 20cm deep, on the ceiling of the main chamber. Dale's Cave is a limestone cave accessed by a 3m deep sinkhole and consists of a bell-shaped chamber (approximately 7m in length, 5m in breadth with a 4m ceiling) that slopes away from the entrance. Approximately 80 to 200 bats over-winter 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, Texas, USA) 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 two hours in Dale's and Firecamp. In 2013 I also placed data loggers in Dale's Cave (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Cape Cod, Massachusetts, USA) which had greater precision ($\pm 0.2^\circ\text{C}$) than iButtons. All dataloggers were positioned in areas known to be used by bats to ensure recording of microclimates used by the bats.

To minimize disturbance to bats during hibernation each cave was only entered once and the duration of the visit was less than 120 minutes. Bats were captured by hand on 29 November 2009 (Jonasson and Willis 2012), 20 February 2012, and 24 January 2013, sexed, weighed to the nearest 0.01g (Durascale-100, MyWeigh, ON, Canada) and their forearm length was measured to the nearest 0.05mm. Young of the year were identified based on un-fused 3rd digit metacarpal-phalangeal joints, and tapered rather than "knobby" joints following Davis and Hitchcock (1965). In the northern study area

YOY can be readily identified during swarming and even into hibernation (Jonasson and Willis 2011; 2012; Czenze et al. 2013). This distinction may be difficult or impossible in other parts of this species' range.

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). Skin temperature was recorded by attaching temperature-sensitive radio-transmitters (0.80-0.90g, BD-2NT; Holohil Systems Ltd, Carp, Ontario) using a non-toxic, latex-based adhesive (Osto-Bond, Montreal Ostomy, Vaudreuil, Quebec, Canada) after clipping a small ($<1\text{cm}^2$) patch of fur between the shoulders. Bats from all three winters weighed, on average $9.73 \pm 1.2\text{g}$, respectively, and transmitters represented between 6.7 and 10.4% of body mass. This is greater than the 5% guideline suggested by Aldridge and Brigham (1988) but similar to the size which Jonasson and Willis (2012) demonstrated had no negative impact on body condition in hibernating little brown bats. I calibrated transmitters prior to use in a water bath at 5°C increments from 0.3°C to 45°C against a thermometer traceable to the National Institute of Standards and Technology.

In 2009-2010 Jonasson and Willis deployed a datalogging receiver (Lotek SRX400; Lotek Engineering Inc., Newmarket, ON, Canada) outside Dale's cave and an array of four 30cm omnidirectional whip antennas were used inside the cave to record T_{sk} every 10 min. During 2012 and 2013, I used a similar datalogging receiver (Lotek SRX600; Lotek Engineering Inc., Newmarket, Ontario, Canada) powered by two solar panels and connected to one 30cm omnidirectional whip antenna in 2012 and three 4-element yagi antennas in 2013. Receivers were programmed to record T_{sk} data every 10 min.

I followed Jonasson and Willis (2012) to define the phases of torpor and arousal.

Large differences between cave T_a and normothermic T_{sk} led to obvious arousals in the T_{sk} trace. Torpor bouts were defined as periods of reduced stable T_{sk} between the obvious warming and cooling phases associated with each arousal (Fig. 4.1). Skin temperature measured using Holohil transmitters occasionally appears to increase slightly and gradually after initial entry into a torpor bout (e.g., Jonasson and Willis 2012, Czenze et al. 2013) but I could readily identify this artefact in my T_{sk} traces and it did not affect my 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 cave's 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). This normothermic period was readily defined 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. I excluded data from the first 24 hours after releasing the bats to avoid the influence of disturbance on my results. Some bats also exhibited what Jonasson and Willis (2012) referred to as heterothermic arousals. During a heterothermic arousal an individual allows T_b to fall to an intermediate level after the initial rewarming phase which it defends before returning to higher T_b associated with typical arousals. I defined heterothermic arousals following Jonasson and Willis (2012) as those during which the T_{sk} of an individual fell below 25°C for at least 20 minutes (i.e., two T_{sk} recordings).

All analyses were conducted in R version 2.10.1 (R Development Core Team 2009) and values are reported as the mean \pm SD with N equal to the number of animals and n equal to the number of events/arousals. I assessed significance at the $P < 0.05$ level. To assess potential differences in torpor and arousal duration while controlling for the fact

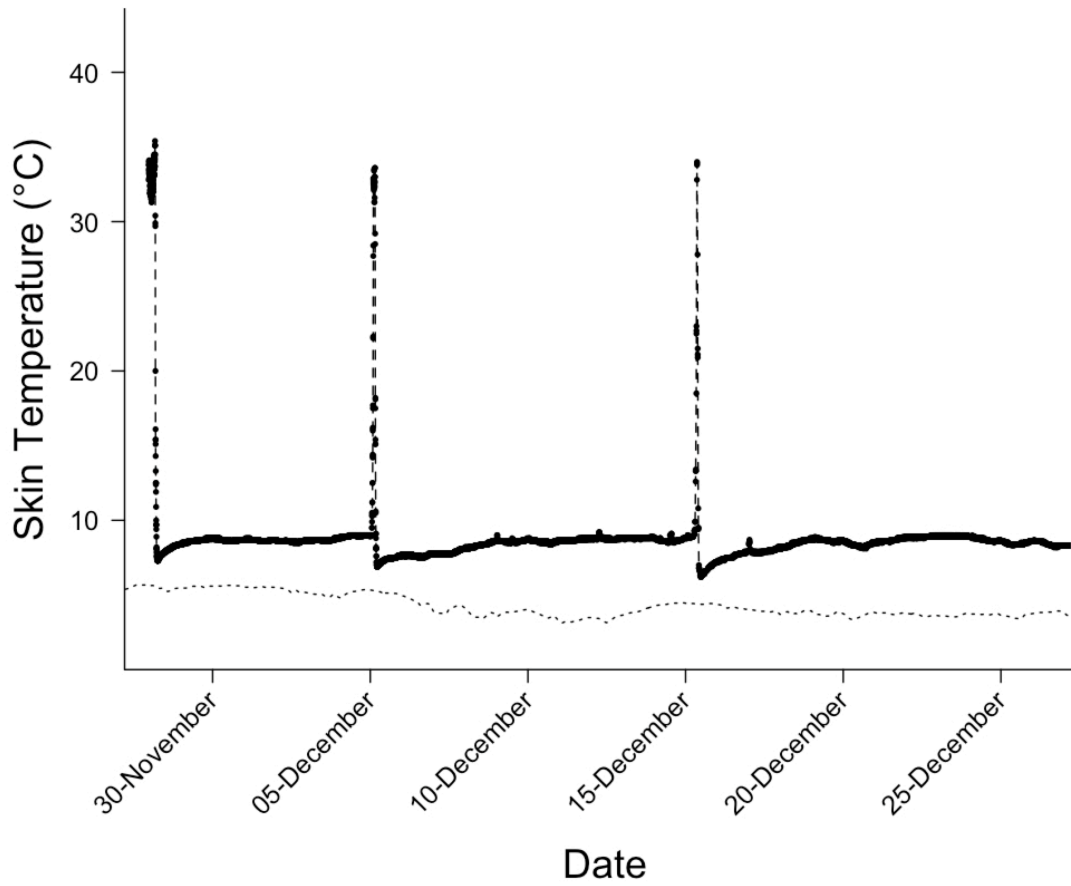


Figure 4.1. Example skin temperature trace of an adult male little brown bat in Dale's cave, central Manitoba during the winter of 2009-2010. Dotted line represents cave T_a

that data were recorded over three winters in two different caves I used linear mixed models with a conservative random effect structure (i.e., individual nested within cave nested within year). I quantified the hibernation period as the number of days since 15 September; this is the average recorded immergence date of little brown bats in this area from 2011 (Norquay 2013). I conducted model selection by comparing nested models using maximum likelihood tests until only significant variables remained. I examined whether sex, age, BCI, date, and cave T_a affected torpor bout duration, arousal duration and estimated energy expenditure. To convert the duration of arousals and torpor bouts into an energetic currency (i.e., kilojoules, kJ), I used a model first presented by Thomas et al. (1990) which Jonasson and Willis (2012) demonstrated most accurately predicted energy expenditure of hibernating little brown bats. To calculate torpid energy expenditure, I assumed a torpid metabolic rate of $0.02 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$. I assumed that the warming phase of arousals cost $2.71 \text{ J g}^{-1} \text{ }^\circ\text{C}^{-1}$, the homeothermic phase cost $8.29 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$, and the cooling phase cost 67.2% of the cost of the warming (Wang 1978; Thomas et al. 1990). As hibernating bats in good condition rely almost exclusively on fat metabolism, for all calculations I assumed $1 \text{ ml O}_2 = 20.1 \text{ J}$ as a conversion factor for energetic equivalence (Thomas et al. 1990).

Results

During three winter field seasons from 2009–2013, 3247 bat-days of T_{sk} data were recorded from 63 individuals (Table 4.1, 11 Adult Females, 11 YOY Females, 35 Adult Males, and 6 YOY males). Bats exhibited long torpor bouts averaging 16.2 ± 11.4 days (range=0.02–60.25 days) interspersed with brief periodic arousals averaging 233 ± 88

Table 1. Summary of hibernation patterns and morphometric data for hibernating little brown bats in Dale's (D) and Firecamp (F) caves in Central Manitoba. TBD refers to torpor bout duration, and AD refers to arousal duration.

Year	Cave	Capture Date	Bat Days	No. of Individuals	Mean Mass (g) at Capture	Mean BCI (g/mm) at Capture	No. of Torpor Bouts	No. of Arousals	Mean TBD (Days)	Mean AD (min)
2009	D	29-Nov	1511	21	10.23 ± 0.92	0.27 ± 0.02	54	64	13.1 ± 10.8	260 ± 94
2012	F	20-Feb	459	15	9.66 ± 1.6	0.26 ± 0.04	42	29	15.3 ± 8.9	198 ± 66
2013	D	24-Jan	1277	27	9.44 ± 1.1	0.25 ± 0.02	104	46	20.07 ± 14.6	190 ± 90

minutes (range=64–475 minutes). During this time 200 torpor bouts and 139 arousals were recorded.

Body condition was not significantly different between age classes ($P=0.50$, $N=63$ bats) but females were in better condition than males ($P<0.001$, $N=63$). Torpor bout duration was not affected by sex, date, or BCI. However, the interaction between age class and cave T_a did affect torpor bout duration (Table 4.2, Fig. 4.2, $P=0.005$, $N=62$, $n=158$ bouts). Torpor bouts of adults were longer and, as cave T_a increased slightly (from 2.2°C to 3.4°C) toward the spring, adult bats spent less time torpid while YOY spent more time torpid. Furthermore, I found maximum torpor bouts of adults were longer than YOY ($P=0.03$, $N=63$, $n=63$) and as the winter period progressed maximum torpor bout duration increased ($P=0.02$, $N=63$, $n=63$)

None of age class, cave T_a , or date affected duration of arousals. However, BCI was a significant predictor of arousal duration with bats with higher BCI exhibiting longer arousals ($P=0.036$, $N=52$ bats, $n=98$ arousals). There was also a significant sex effect with arousals by male bats lasting 22% longer on average than those of females (Fig. 4.3, $P=0.008$, $N=52$ bats, $n=98$ arousals). Longer arousals by males did not result in greater energy expenditure based on the model I used and there were no effects of age class, sex, T_a or date on energy expenditure during an arousal. However, BCI was a significant predictor of energy expenditure during arousals with bats in better condition spending more energy (Fig. 4.4, $P<0.001$, $N=52$ bats, $n=98$ arousals). I also analyzed the energy spent during the warming and homeothermic phases and, again, BCI was a significant predictor of both (warming: $P<0.001$, homeothermy: $P=0.004$, $N=52$ bats, $n=98$ arousals) while date was a significant predictor of energy expenditure during the

Table 4.2. Significant predictors (i.e., age class, cave temperature (cave T_a), and the interaction between the two) of torpor bout duration (TBD) in hibernating little brown bats from Dale's and Firecamp caves in Central Manitoba. 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.

Predictor Variable	t-value	P-value	No. of Individuals	No. of Torpor Bouts
Age	-3.23	0.002	62	158
T_{cave}	-2.38	0.019	62	158
Age: T_{cave}	2.85	0.005	62	158

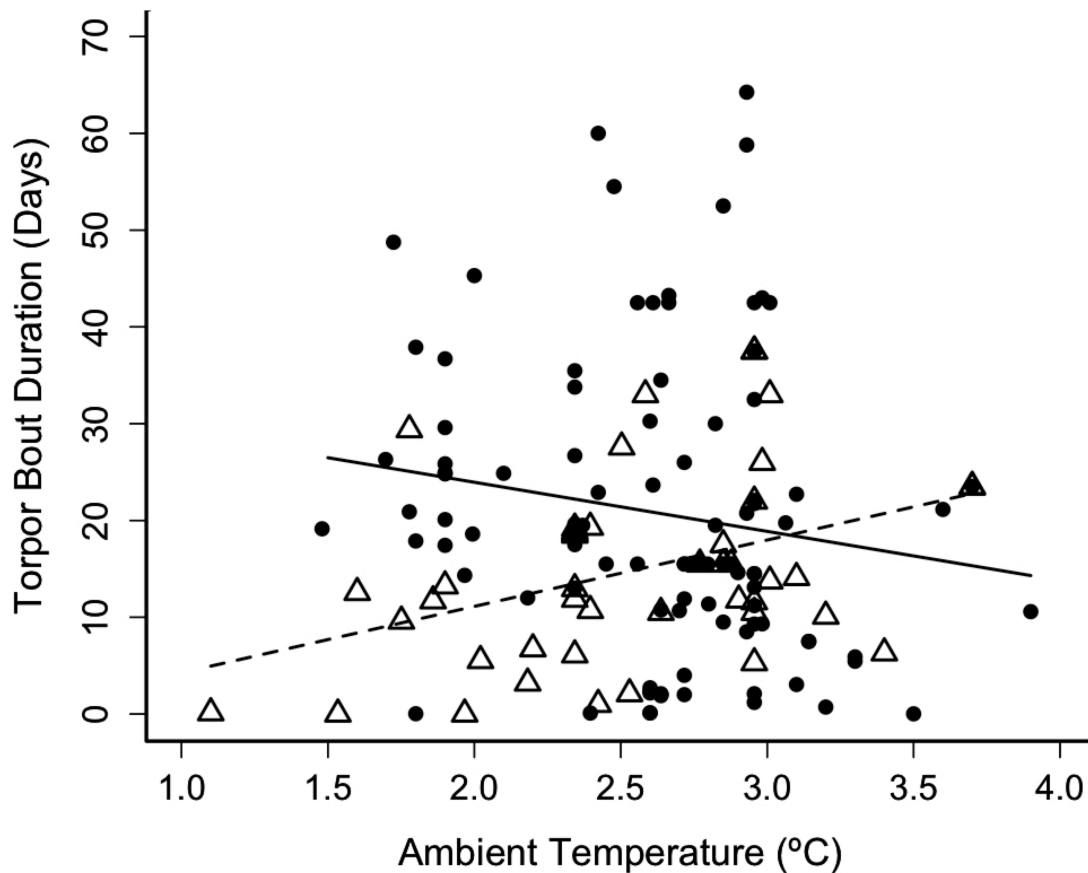


Figure 4.2. Effect of ambient temperature in the hibernaculum (cave T_a) on torpor bout duration of hibernating little brown bats in Dale's and Firecamp caves in Central Manitoba. A linear mixed model was used to analyze the data ($P=0.019$, $t=2.85$, $N=62$ bats, $n=158$ bouts) but ordinary least squares (OLS) regression lines are shown here to illustrate the relationships. Filled circles and solid regression line represent adults (OLS $r^2=0.02$). Empty triangles and dotted regression line represent young of the year (YOY) (OLS $r^2=0.15$).

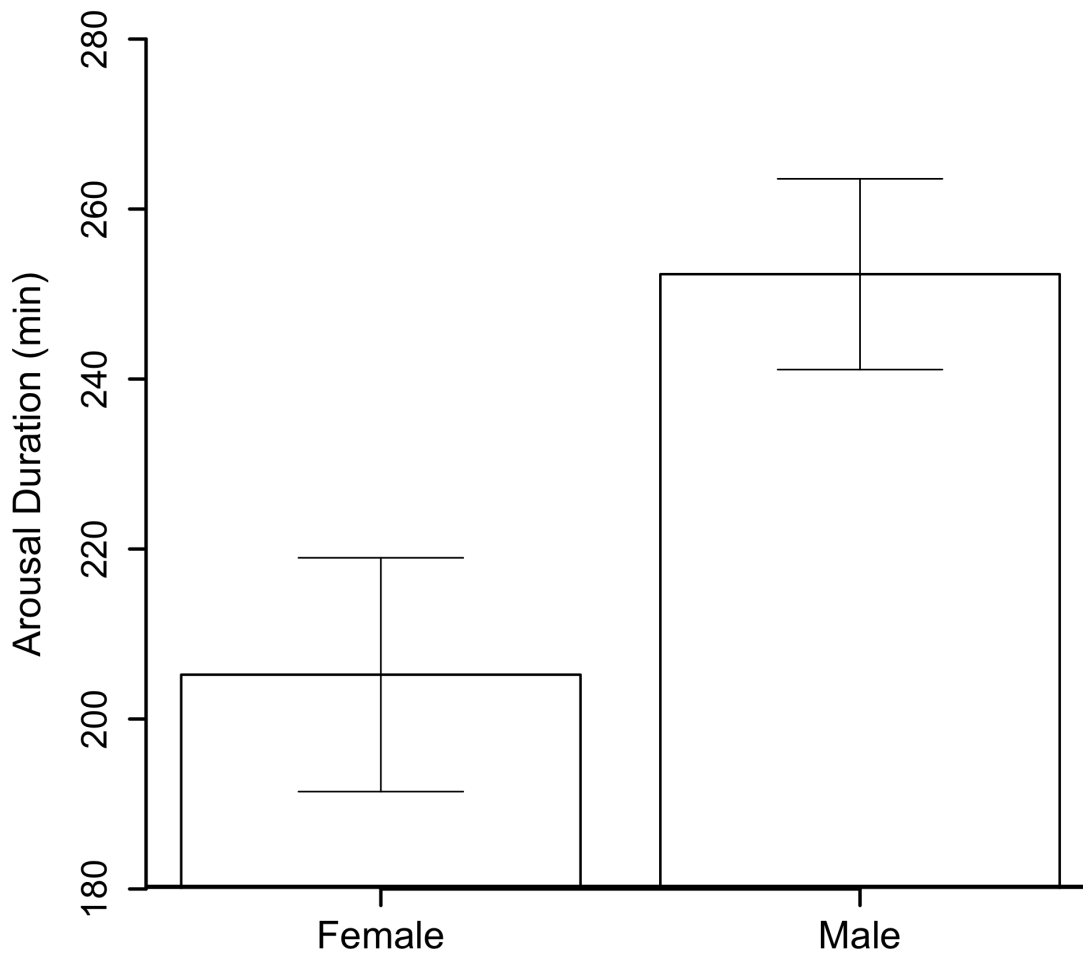


Figure 4.3. Arousal duration (minutes) of hibernating male and female little brown bats + standard error in Dale's and Firecamp caves in Central Manitoba. ($P=0.008$, $t=3.58$, $N=52$ bats, $n=98$ arousals).

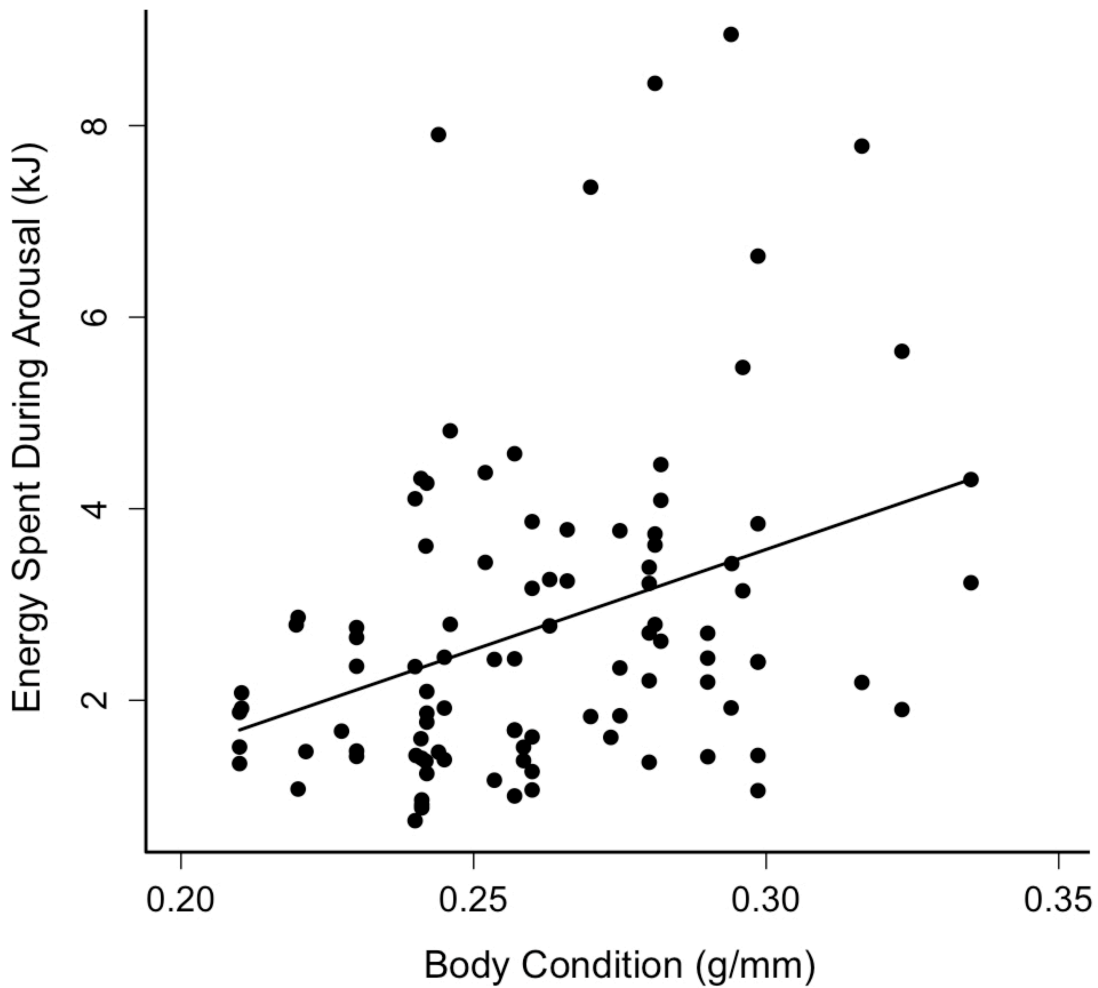


Figure 4.4. Relationship between body condition (g/mm) at capture and calculated energy expenditure during arousals (kJ) for hibernating little brown bats from Dale's and Firecamp caves in Central Manitoba. A linear mixed model was used to analyze the data ($P < 0.001$, $t = 3.57$, $N = 52$ bats, $n = 98$ arousals) but the ordinary least squares (OLS) regression line is shown here to illustrate the relationships ($r^2 = 0.13$).

homeothermic phase. Energy expenditure during the homeothermic phase of arousals declined as hibernation progressed (Fig. 4.5, $P=0.049$, $N=52$ bats, $n=98$ arousals).

The majority of the arousals I recorded were plateau shaped, with obvious warming, homeothermic, and cooling phases (Fig. 4.6a). However 30% (33/110) of arousals were heterothermic during which individuals defended T_{sk} below 25°C for at least 10 minutes, that clearly reflected shallow torpor, in the midst of an arousal (Fig. 4.6b). I used mixed models to test for a difference between males and females in the proportion of arousals which included a bout of heterothermy. I also compared the proportion of individuals which exhibited at least one heterothermic arousal for males vs. females. I found that the proportion of heterothermic arousals was higher for females than males (Fig. 4.7a, $P<0.01$, $N=53$, $n=110$). Furthermore, the proportion of individual females using at least one heterothermic arousal was higher than for males (Fig. 4.7b, $P=0.004$, $N=53$).

Discussion

This study lends support to the torpor optimization hypothesis but suggests that adjustments in arousal duration are more important than adjustments in torpor bout duration for balancing physiological costs of torpor against its energetic benefits and for adjusting energy expenditure during hibernation to conserve energy for spring reproduction. The analysis supported my first prediction that bats with high BCI should exhibit greater energy expenditure than bats with lower BCI. This is consistent with previous work on yellow-bellied marmots (*Marmota flaviventris*) which showed that individuals with larger fat reserves spent more energy than smaller conspecifics (French

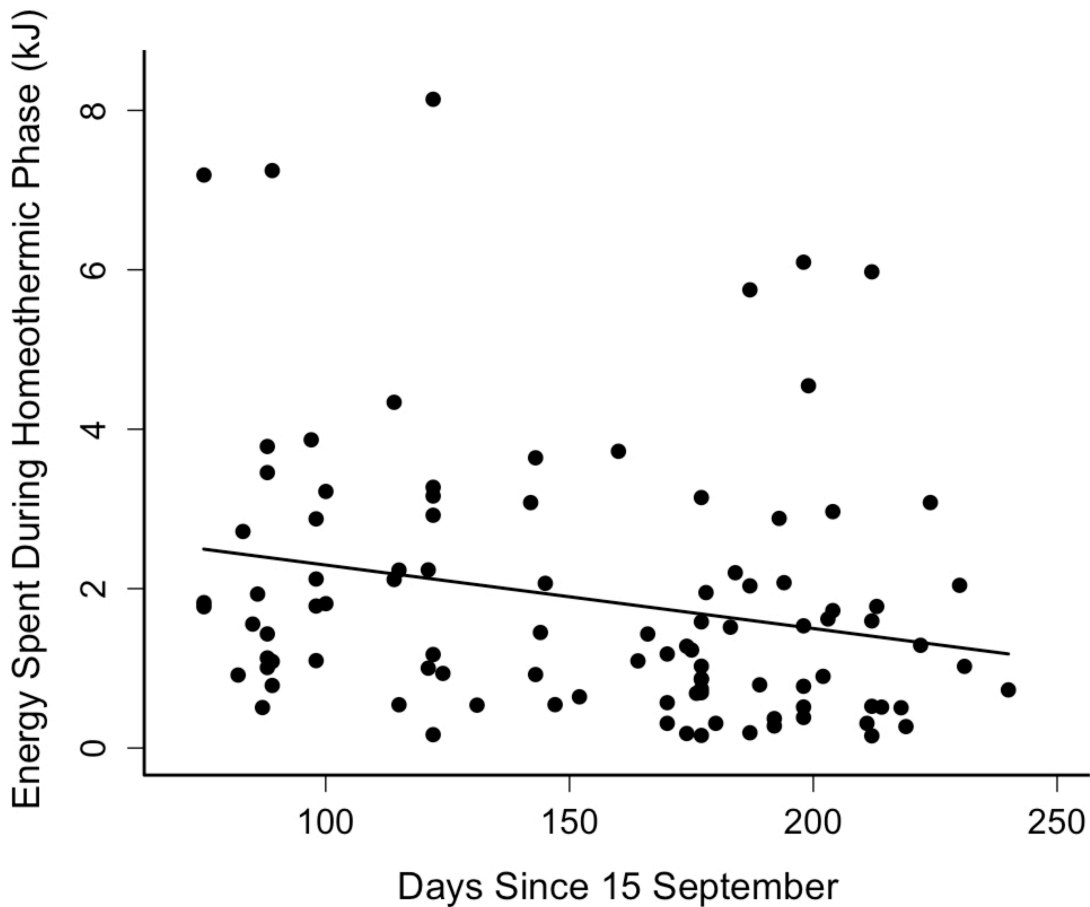


Figure 4.5. Relationship between the time in hibernation (i.e., the number of days since 15 September) and energy expenditure during the homeothermic phase (kJ) for arousals by hibernating little brown bats from Dale's and Firecamp caves in Central Manitoba. A linear mixed model was used to analyze the data ($P=0.049$, $t=-2.02$, $N=52$ bats, $n=98$ arousals) but ordinary least squares (OLS) regression line is shown here to illustrate the relationships ($r^2=0.06$).

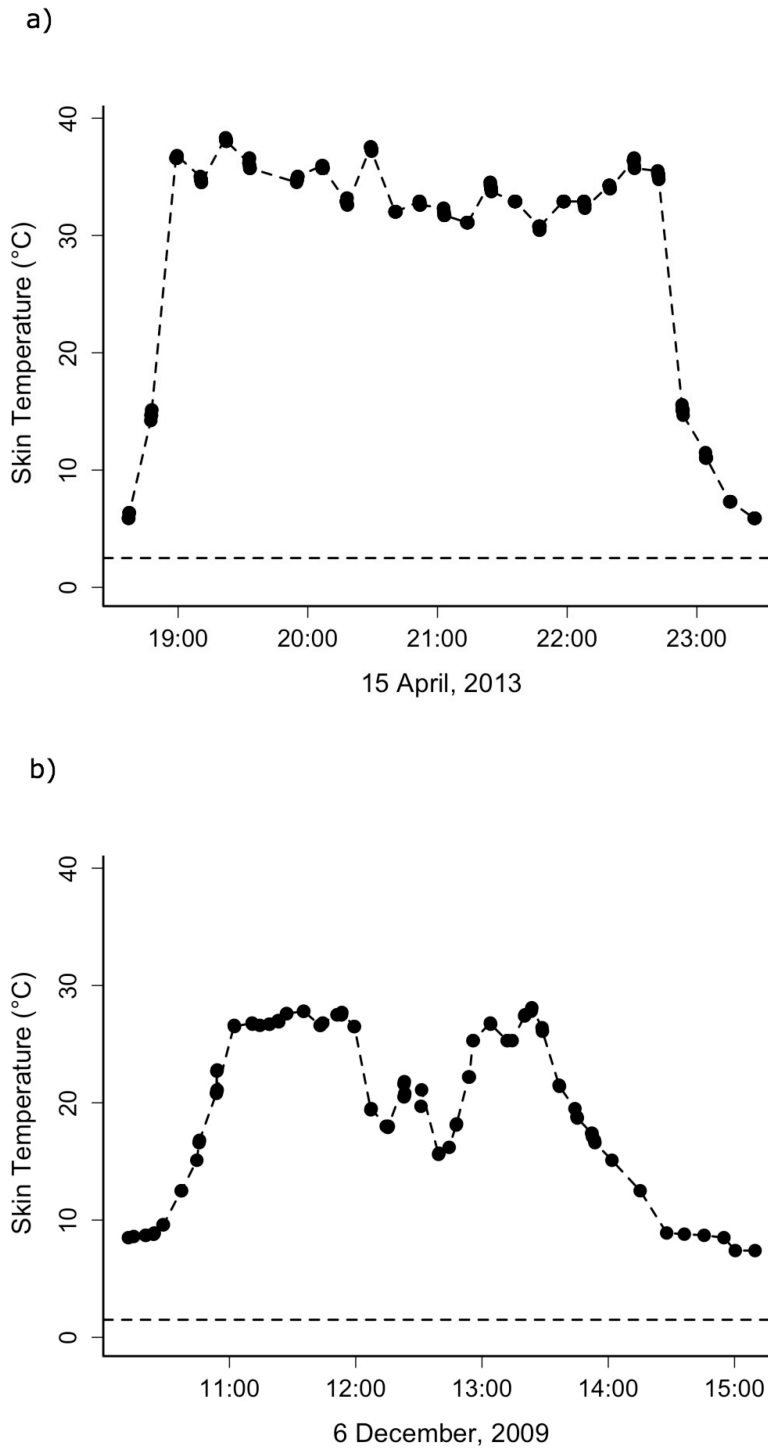


Figure 4.6. Example of T_{sk} during a plateau arousal on 15 April, 2013 (a); and (b) heterothermic arousal on 6 December, 2009 by hibernating adult male little brown bats in Dale's in Central Manitoba. Lower dashed line represents cave T_a .

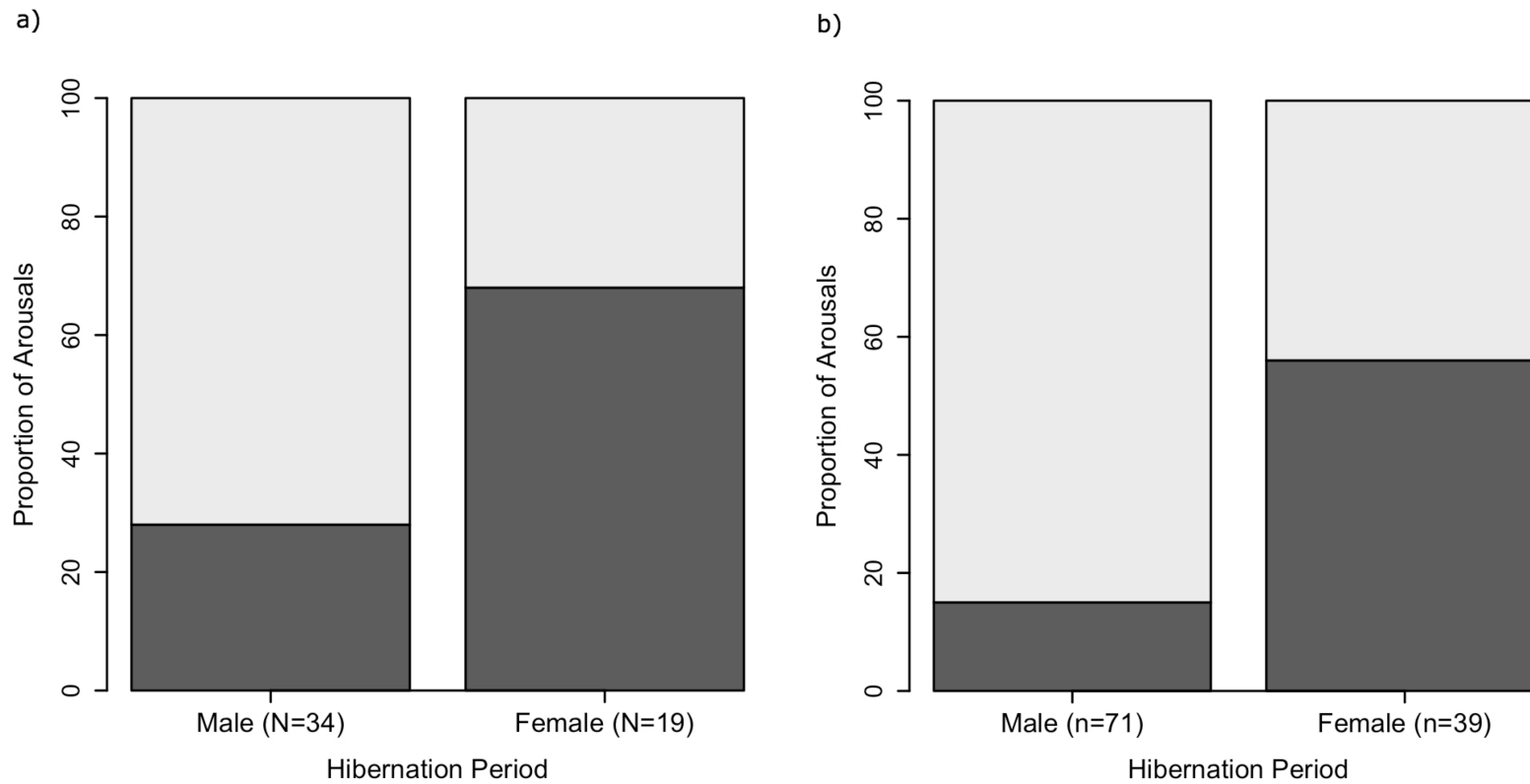


Figure 4.7. Proportion of individuals exhibiting at least one heterothermic arousal (dark grey) (male=0.28, N=34 bats; female=0.68, N=19 bats, $P=0.004$, $Z=-2.86$) for hibernating little brown bats in Dale's and Firecamp caves in Central Manitoba (a); and (b) Proportion of shallow (dark grey) vs. plateau arousals (light grey) (male=0.15, N=34 bats, n=71 arousals, female=0.56, N=19 bats, n=39 arousals, $P<0.001$, $Z=-3.75$).

1985; French 1990). Bats in good body condition spent their extra energy not by reducing the duration of torpor bouts but by increasing arousal duration, specifically during the warming and homeothermic phases. These differences in duration translated into changes in energy expenditure based on Thomas et al.'s (1990) model. These findings are consistent with the torpor optimization hypothesis if longer, more energetically expensive arousals allow hibernators to better pay off the physiological debts accrued during torpor. This may also be especially important for male reproductive success, if individuals in the best condition are able to spend more time normothermic they could potentially engage in more mating attempts and increase their reproductive success when compared to individuals in poorer condition. Individual bats exhibited shorter arousals, spent less energy during the homeothermic period, and had longer maximum torpor bouts as the winter progressed. This is also consistent with the optimization hypothesis, as individuals have likely exhausted the majority of their energy reserves by late hibernation and need to maximize their energy savings. As the end of hibernation nears, the energetic cost of maintaining a long homeothermic period likely begins to outweigh benefit of mitigating negative physiological consequences of torpor.

Contrary to my prediction that females should exhibit longer torpor bouts than males, I found no effect of sex on either duration of torpor bouts or energy expenditure during torpor. Several studies of hibernating bats have reported a similar lack of sex effect (Park et al. 2000; Dunbar and Tomasi 2006; Hope and Jones 2010; Halsall et al. 2012, Jonasson and Willis 2012), although most of these have had relatively low statistical power. Despite a relatively large sample size I cannot exclude the possibility that the large between-individual variability in torpor bout duration masked a potential

sex effect. However, my analysis suggests that the arousal period is a more important driver of between-sexes differences in the overwinter energy budgets of bats.

Although adult females did not spend longer in torpor, consistent with the thrifty female hypothesis, they did exhibit shorter arousals compared to adult males. Sex effects on arousal duration were 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). This suggests that the relatively slow decline in body condition of females compared to males during hibernation observed by Jonasson and Willis (2011) is more strongly influenced by adjustments of arousal duration rather than torpor bout duration. It may be that males, not under the same energetic constraints as females, use their energy stores during arousals to more fully mitigate the negative physiological consequences of long torpor bouts. Longer arousals may also reflect mating attempts by males during winter (Racey 1972, Thomas et al. 1979).

Although arousals by females were shorter than those of males, surprisingly, when I calculated the energetic costs of arousal using Thomas et al.'s (1990) model, I found no effect of sex on arousal energy expenditure. This finding may be a consequence of differences in body mass between males and females, combined with an artefact of the energetic model I used. The calculation incorporates the body mass of individuals to calculate their energy expenditure during warming and homeothermy. Therefore, larger females ($11.1 \pm 1\text{g}$) spending the same amount of time as males ($9.4 \pm 0.7\text{g}$) during an arousal would spend more energy. The model also assumes that MR during arousal (i.e., $8.29\text{ml O}_2 \text{g}^{-1}\text{h}^{-1}$ Thomas 1990) is constant regardless of the T_b the individual is defending, which is clearly an over-simplification. An individual defending a T_b of 35°C

would, according to Thomas et al.'s (1990) model, spend the same amount of energy as an individual defending 25°C during the same time period. If all arousals were plateau-shaped with stable T_{sk} , this would be a reasonable assumption but, as reported by Jonasson and Willis (2012), I found that 30% of arousals were not plateau-shaped. Instead, some individuals defended a reduced T_b in the midst of their homeothermic phase and females were significantly more likely to exhibit these heterothermic arousals than males. Thus, in addition to shorter arousals, female bats may use heterothermic arousals to reduce their energy expenditure even further compared to males and assumptions of Thomas et al.'s (1990) model may have led me to overestimate their energy expenditure. As suggested by Jonasson and Willis (2012) more work is needed to better quantify the energy expenditure of bats during different phases of torpor and arousal throughout hibernation.

I found no support for my third prediction, that YOY should have longer torpor bouts than adults. In fact, I observed the opposite pattern with YOY bats exhibiting shorter torpor bouts than adults. The effect of age class on torpor bout duration during hibernation is not consistent across species. There have been no effects detected for some species (Park et al. 2000; Munro et al. 2005), longer bouts by juveniles for some species (French 1990; Young 1990; Michener 1992), and shorter bouts by juveniles for others (Armitage et al. 2003; Landry-Cuerrier et al. 2008). Torpor bout duration was shorter in juvenile yellow-bellied marmots compared to adults and Armitage et al. (2003) attributed this to reduced insulation and higher thermal conductance. Increased juvenile heat loss led to higher TMR and O_2 consumption resulting in more energy spent during torpor bouts compared to adults (Armitage et al. 2003). Increased TMR and O_2 consumption

would also lead to an increase in the buildup of metabolic wastes which should require juveniles to arouse more often to excrete these wastes. Disparity between adult and juvenile torpid O_2 consumption has been observed in Daubenton's bats (*Myotis daubentonii*) with juveniles exhibiting 2.75 times higher TMR than adults (Kokurewicz and Speakman 2006). Increased TMR by YOY could explain the shorter torpor bouts, and maximum torpor bouts I observed for this demographic in my study. It may be that, although YOY bats are spending less time torpid than adults, they are using more energy during their torpor bouts, resulting in the shorter bouts.

When I considered YOY alone, surprisingly, I found that individuals with the lowest BCI had the shortest torpor bouts. This is not consistent with the torpor optimization hypothesis and has also been observed for Natterer's bats in the United Kingdom (Hope and Jones 2012). One possibility is that YOY bats in worse condition are more susceptible to disturbance than adults or YOY in better condition. In general, YOY could be more sensitive to disturbances by colony mates in hibernacula compared to adult bats which have learned (or been selected) to ignore potential sources of disturbance. In some cases naïve YOY roost closer to hibernaculum entrances making them easier targets for predation (Kokurewicz 2004). Poor body condition is associated with shorter torpor bouts in some U.K. bat species presumably to help these individuals exploit as many mild nights and potential feeding opportunities as possible to increase their fat reserves (Hope and Jones 2012). In my colder study site, this type of winter-feeding is impossible for bats and individuals synchronize with huddle mates, presumably to take advantage of social rewarming (Czenze et al. 2013; Chapter 2; 3). In this northern environment one possibility is that YOY in the worst condition take advantage of warming huddle mates as

often as possible to save energy and this drives their shorter torpor bout duration. However, I found the proportion of arousals overlapping with a conspecific ($Z=-0.17$, $P=0.86$) and the proportion of individuals rewarming with a conspecific ($Z=-0.61$, $P=0.54$) did not differ between age classes. This result may stem from the disparity in sample size between age classes (YOY: $N=15$, $n=32$; adult: $N=42$, $n=91$), and I recommend further research, where applicable, put an emphasis on distinguishing between age classes.

Although the variation in cave T_a throughout the study was very small (1.1 to 4.0°C) compared to variation in outside T_a (-39.8 to 26.8°C), cave T_a was a significant predictor of torpor bout duration for both adult and YOY bats. However, the direction of this effect differed between age classes. Adults decreased their torpor bout duration as cave T_a increased, while YOY increased torpor bout duration. Similar to the adult bats in my study, European ground squirrels (*Spermophilus citellus*) exposed to different temperature treatments exhibited shorter torpor bouts at higher temperatures (Nemeth et al. 2009) and Golden-mantled ground squirrels (*Callopermophilus lateralis*) exhibited the same pattern with decreased torpor duration when T_a increased from 2 to 8°C (Geiser and Kenagy 1988). These authors hypothesized that the animals experience a reduction in neural sensitivity to metabolite buildup at lower temperatures leading to their longer torpor bouts. Additionally the buildup of these metabolites increased more slowly at lower T_b and therefore needed to be expelled less often, leading to longer torpor bouts. The slow accumulation of, and decreased sensitivity to, these metabolic wastes could lead to longer torpor bouts by adults at lower cave T_a .

The pattern of increased torpor bout duration with increased cave T_a in YOY is

more puzzling but could reflect the cold climate at my northern study site. In more southern populations of little brown bats, YOY are indistinguishable from adults by their first winter. This is not the case in northern Manitoba and YOY bats are distinguishable even during hibernation (Jonasson and Willis 2011; 2012; Czenze et al. 2013) which likely reflects their shorter growing season. If YOY are not fully developed by their first winter they may be less able to tolerate colder cave T_a chosen by their adult counterparts. For all hibernating animals there is a lower critical temperature (T_{crit}) below which energy savings cease and metabolic costs increase because individuals must defend a minimum T_b . For adult little brown bats T_{crit} is thought to occur around 0.5°C (Hock 1951). There is little information on the difference of T_{crit} between adults and subadults, but it is possible that inexperienced YOY bats have an elevated T_{crit} compared to adults. At low T_a YOY may, therefore, need to spend more energy defending their T_b than adults. This would result in a more rapid accumulation of metabolic wastes, and more rapid water loss, compared to adults, and require more frequent arousals. This suggests that, if YOY have access to multiple microclimates during hibernation they should choose warmer temperatures closer to their T_{crit} . I recommend future studies using open-flow respirometry and/or behavioural tests of thermal preferences to better understand differences in this critical aspect of hibernation behaviour.

The threat of white-nose syndrome, the fungal infection caused by *Pseudogymnoascus destructans* (*Pd*), is an important concern when studying hibernation in little brown bats because this species is among the most susceptible (Frick et al. 2010; Langwig et al. 2012). Increased arousal frequency and subsequent depletion of fat reserves are thought to be the primary causes of mortality from WNS (Warnecke et al.

2012; Reeder et al. 2012). Little brown bats in central Manitoba face extreme energetic constraint during an eight month winter and, as such, are ideal for addressing questions about hibernation energy balance. These bats are unusual in that, unlike many other hibernating species and even their southern counterparts, they have no ability to augment their fat reserves during the winter. My results support Jonasson and Willis's (2011) hypothesis that adult female bats, especially those with high BCI, may be buffered against WNS-related mortality. The combination of hibernating in the coldest regions of caves, arousing for short periods of time and/or using heterothermic arousals could allow females to spend less energy and lose less fat over the winter. Conversely, my results suggest YOY females may be most susceptible to WNS-related mortality, especially if they are reproductive in their first year. Young of the year females in poor condition are likely to choose warmer microclimates and exhibit shorter torpor bouts, which lead to higher over-winter energy expenditure. Bats in Manitoba enter hibernation with dramatically larger fat reserves than southern bats (Norquay 2013) and this between population difference could also allow bats from this study area to experience lower mortality rates than some more southern populations.

This study shows that energy reserves, sex and age influence patterns of torpor, arousal, and energy expenditure during hibernation. My data provide an explanation for Jonasson and Willis's (2011) observation that female body mass declines more slowly than that of males during hibernation, specifically that females exhibit energetically conservative arousals compared to males, despite no difference in torpor bout duration. My results also support the torpor optimization hypothesis as bats in better condition spent more energy during hibernation, again as a result of increasing arousal duration

rather than reduced torpor bout duration. This pattern may allow males with high BCI to increase their reproductive fitness by providing them with increased mating opportunities during hibernation. Although torpor bout duration did not vary between the sexes, it did vary by age class. This could reflect differences in TMR and T_{crit} between adult and YOY and highlights the importance of, when possible, distinguishing between age classes in studies of hibernation. Since this study was performed on free-ranging bats I was not able to measure metabolic rate at any phase of the torpor-arousal cycle. Thus, for future studies I recommend use of open-flow respirometry to better calculate TMR of different sexes and age classes over a range of T_a .

CHAPTER 5: GENERAL CONCLUSIONS

Hibernation is a critical aspect of many temperate animals' annual cycle. During this period individuals drastically reduce body temperature and metabolic rate to survive on energy reserves. Hibernators must balance the energetic savings resulting from torpor against physiological costs (e.g., dehydration and metabolic wastes) associated with long bouts of reduced metabolism (Humphries et al 2003a). Individuals arouse to a normothermic body temperature briefly and regularly, presumably to expel wastes, restore water balance and pay off the debts accrued during torpor (Geiser 2004). Factors influencing the timing of these arousals are poorly understood. Therefore, my objective was to improve understanding of factors influencing these arousal patterns of little brown bats (*Myotis lucifugus*) hibernating in central Manitoba, in a study site with the coldest and longest winters where hibernation has been studied for any bat species.

For Chapter 2, I found that bats in central Manitoba do not synchronize their arousals with sunset during mid- to late-winter. This is unlike bats in the United Kingdom (U.K.) which maintain a circadian pattern to their arousals throughout winter, arousing within 120 min of sunset (Hope and Jones 2012). The U.K. bats hibernate in mild climates where ambient temperature (T_a) regularly exceeds 10°C and flying insects are available. Central Manitoba experiences much harsher winters than the U.K. and, from 1 November to 1 May mean T_a rarely exceeds freezing. In this environment there is no opportunity for occasional winter-feeding and therefore no energetic benefit of maintaining a circadian pattern to their arousals. Instead, my data show that these cold-

climate bats synchronize their arousals with conspecifics, possibly as a means of minimizing energy expenditure through social rewarming.

For Chapter 3, I revisited circadian and social patterns of arousals. Specifically I tested whether the asynchrony between arousals and sunset continued from late winter into early spring and whether individuals were more likely to arouse with conspecifics during this period. As for Chapter 2, during late winter when temperature is too cold for flying insects there was no circadian pattern to arousals. However, during the early spring when almost 20% of nights were warm enough to support flying insects, bats resumed a circadian pattern to arousal and, like hibernating bats from the U.K. rewarmed within about 120 min of sunset. During this period bats also doubled their propensity to arouse with conspecifics compared with late winter. Thus, in early spring, when fat reserves are dwindling, bats were more likely to arouse at a time of day when there was at least some chance of acquiring food and they were also more likely to take advantage of social rewarming. This could be critical to overwinter survival for individual bats. By synchronizing arousals with sunset and with conspecifics, bats' may be able to gain significant energetic benefits.

I also examined factors influencing emergence from hibernation for Chapter 3. I found that the emergence phenology was affected by both bat-specific factors and environmental factors. Similar to a recent study from this region (Norquay 2013), I found a strong effect of sex on emergence timing and females emerged earlier compared to males. Females face very different energetic pressures than males during early spring and presumably emerge earlier in order to balance the reproductive benefit of giving birth early in the active season with costs of enduring lingering winter weather. I also found

that body condition at capture (during winter) affected female (but not male) emergence timing. Females in the best body condition, and thus best able to cope with potentially inclement spring weather, emerged first. Although the effect was not significant for males the pattern appeared to be reversed. The male in the best body condition remained in hibernation longest while the individual in the worst condition emerged first. Males in the best condition should remain in hibernation the longest to minimize their exposure to sources of extrinsic mortality. I did find that cave T_a affected male emergence date. The gradual increase in cave T_a that occurred in my study site during spring may be a good proxy for outside ambient temperature and this may be a cue used by males to time their emergence. None of the environmental variables I measured predicted female emergence time. However, I found that every occurrence of female emergence coincided with a significant drop in barometric pressure. Increased insect activity can occur with falling barometric pressure, as this environmental variable is associated with passing weather fronts. Although temperature and other environmental variables fluctuate very little on a day-to-day basis, inside the caves where I worked barometric pressure may better reflect conditions outside the cave. Thus, this could be a one cue used by females to time their emergence to improve the chance of encountering flying insects (Paige 1995).

In Chapter 4, I examined a range of factors influencing torpor/arousal patterns throughout hibernation and revisited the thrifty female hypothesis proposed by Jonasson and Willis (2011). I found differences in arousal duration (but not torpor bout duration) between the sexes and differences in torpor bout duration (but not arousal duration) between adults and young of the year (YOY). In general, after controlling for age, male bats had longer arousals than females and this may reflect mating attempts during

hibernation and/or better mitigation of physiological costs of torpor. Shorter arousals by females may also reflect their thrifty strategy to save energy during hibernation and emerge with enough fat to support pregnancy. Interestingly when I converted the arousal duration into an energetic currency this sex effect disappeared. This could reflect limitations of my energetic model combined with the occurrence of heterothermic arousals, during which hibernating bats will defend a body temperature well above ambient temperature but below normothermic levels (Jonasson and Willis 2012). I found that females were 2-3 times more likely to express heterothermic arousals. This disparity likely caused me to overestimate the energy expenditure of females.

I also found that, at low cave temperatures adult bats performed longer torpor bouts than at higher temperatures but, surprisingly, observed the opposite pattern in YOY. Bats in their first winter exhibited longer torpor bouts as cave temperature increased gradually during late winter. This could reflect differences in lower critical temperature between adults and YOY. Warmer cave temperatures will lead to higher adult energy expenditure/torpid metabolic rate, in turn leading to shorter torpor bouts as bats arouse to expel metabolic wastes. Young-of-the-year may not be able to defend as low a body temperature as adults and may, therefore, seek out the warmest possible microclimates resulting in higher metabolic rate and thus shorter torpor bouts. It would be useful for future studies to quantify body temperature and energy expenditure over the entire hibernation period. I was limited in my study due to the battery life of my transmitters and was only able to record a portion of the hibernation period. I found differences in hibernation behaviour between mid-and late-hibernation and it is reasonable to assume that differences also exist between early-and mid-hibernation.

Further research should also examine whether the longer male arousals I recorded do indeed reflect mating attempts. Mating attempts are likely more common during early hibernation when fat reserves are still relatively large (Racey 1972; Thomas et al. 1979). Furthermore, future research should examine whether male body conditions influences winter mating attempts by males.

Although I found pronounced differences in emergence timing of males and females, future research should also aim to better characterize these differences using larger sample sizes and more detailed environmental recordings. I found evidence that bats are responding to environmental conditions and future field research should aim to quantify variables like air pressure inside caves, airflow in and out of caves and signals associated with spring melting (i.e., acoustic or tactile stimulation from water dripping inside caves) to better understand factors influencing emergence. Laboratory research using respirometry to better quantify energy expenditure of clustered and solitary roosting bats during all phases of hibernation would also be extremely valuable. More detailed metabolic measurements, combined with body temperature data collected over the entire winter would allow for improved modeling of the energetics of hibernation and a better understanding of hibernation behaviour in bats and other species.

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