



SYMPOSIUM

Trade-offs Influencing the Physiological Ecology of Hibernation in Temperate-Zone Bats

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Synopsis Seasonality of temperature and food availability can lead to trade-offs between the benefits of immediate reproduction and costs associated with mortality risk from starvation, inclement weather, or predation. Hibernating mammals exhibit an enormous seasonal shift in physiology and behavior and provide a useful system to examine the effect of this trade-off on key events in the annual cycle. Most of what we understand about the ecological energetics and phenology of hibernation comes from studies of rodent hibernators such as ground squirrels, chipmunks, and dormice. Temperate-zone, insectivorous bats, however, provide another useful model system to examine trade-offs influencing seasonal change within individuals. Here, I review recent studies from my laboratory on little brown bats (*Myotis lucifugus*) from central Canada to understand the interplay between capacity for energy storage, energy expenditure during hibernation, and the timing of key events in the annual cycle of hibernating mammals. These studies have relied on measurements of body condition to assess energetic status, biologging of skin temperature using temperature telemetry, and use of passive transponders (i.e., PIT tags) to quantify emergence timing. In general, these studies suggest that, in part due to constraints associated with flight, bats exhibit unique, or at least unusual, adaptations for extreme energy savings during winter. The results also support the optimization hypothesis that current energetic status and future energy requirements influence energy expenditure during hibernation and the timing of emergence from hibernation in spring. Taken together, this work provides insight into the influence of reproductive timing and energy availability on hibernation behavior and physiology. It also has implications for understanding responses of bat populations to anthropogenic impacts like climate change and white-nose syndrome.

Introduction

A key concept of life-history theory is a trade-off between energetic costs and mortality risk resulting from immediate reproduction, versus the potential success of future reproductive attempts (e.g., Stearns 1992). Variation in selection pressures associated with this trade-off underlies the classic continuum of life history traits with some species exhibiting “fast” life-histories characterized by high growth rates, early age at first reproduction, high reproductive rates and short lifespan while other species mature slowly, reproduce infrequently and live many years (Stearns 1992; Barclay and Harder 2003). Among animals, body size tends to correlate strongly

with this continuum such that, with a few interesting exceptions, slow life histories occur in large-bodied species, while small animals live fast and die young (Stearns 1992; Barclay and Harder 2003). Understanding the influence of the life-history continuum on variation in the timing of events in the annual cycle for different species, and how environmental change might influence the timing of these events, is useful for understanding life history evolution and for conserving species at risk from human-caused environmental change.

In the temperate world, seasonality of ambient temperature (T_a) and food availability can exert great influence on the trade-off between current versus

future reproduction and lead to substantial seasonal physiological and behavioral changes within individual animals. Although many birds migrate to avoid winter, migration is rare among all but relatively large-bodied mammals (but see [Fleming and Eby 2003](#)). Instead many mammals remain close to their breeding home-range, dramatically delay reproduction, and spend long periods in prolonged hibernation ([Geiser 2004, 2013](#)). These species maintain long-term energy balance by abandoning high and constant body temperatures (T_b) and entering prolonged bouts of energy-saving torpor. During torpor, metabolic heat production is down-regulated and T_b is allowed to fall to a reduced set-point ([Geiser 2004, 2013](#)). Many temperate-zone species can express torpor during summer and winter but, for hibernators, winter torpor expression is especially pronounced with torpor bouts lasting days, weeks, or even months (e.g., [Geiser 2004, 2013](#); [Ruf and Geiser 2015](#)).

Much of our understanding of how trade-offs influence the ecological energetics and phenology (i.e., timing in the annual cycle) of hibernation comes from studies of rodents, especially ground squirrels, chipmunks, and dormice, and several key patterns have emerged. First, a trade-off between physiological costs of prolonged torpor versus its energy-saving benefits appears to drive an optimization of torpor expression and energy expenditure during winter. Expression of deep torpor is associated with costs including accumulation of metabolic wastes, water loss that must be replenished, inhibition of the restorative functions of sleep, and inhibition of immune system function ([Daan et al. 1991](#); [Thomas and Geiser 1997](#); [Elvert and Heldmaier 2000](#); [Ben-Hamo et al. 2013](#)). As a result of these costs, all mammalian hibernators spend a large fraction of their winter energy budget on brief but crucial periodic arousals to normal T_b . Although they represent a tiny fraction of the time budget for individuals, arousals can account for up to 90% of overwinter energy expenditure ([Wang 1978](#); [Thomas et al. 1990](#); [Geiser 2004](#)). In a classic experiment on eastern chipmunks (*Tamias striatus*), [Humphries et al. \(2003\)](#) showed that experimental supplementation of energy reserves for chipmunks reduced their expression of torpor bouts during winter, presumably to help them avoid these physiological costs. Chipmunks with supplemented food hoards aroused more often and expressed shallower torpor than non-supplemented individuals ([Humphries et al. 2003](#)). Observations consistent with this pattern of optimization have also been reported for fat-storing hibernators (e.g., [Boyles et al. 2007](#); see below).

A second trade-off, also illustrated first for rodent hibernators, appears to exist between one ecological cost of hibernation (i.e., missed reproductive opportunities) and one benefit of hibernation (i.e., avoidance of mortality risks from predation, starvation, or inclement weather outside a protected hibernaculum). For example, male and non-reproductive female Richardson's ground squirrels (*Urocitellus richardsonii*) appear to avoid active-season predation risk by fattening and entering hibernation during mid-summer when food is still abundant, the weather is warm, and reproductive females are still rearing offspring ([Michener 1978, 1979, 1998](#)). On the other hand, during early spring, when the weather can be highly unfavorable, male ground squirrels emerge from hibernation much earlier than females and juveniles, to defend territories and begin spermatogenesis ([Michener 1983](#)). This pattern occurs in other rodent and some marsupial hibernators ([Körtner and Geiser 1998](#); [Blumstein 2009](#)). Females, which make the bulk of their reproductive investment during summer, remain in hibernation longer than males but also face a trade-off between the benefit of early emergence to initiate reproduction and the cost of exposure to inclement conditions and food scarcity in spring ([Michener 1979](#); [Inouye et al. 2000](#); [Lane et al. 2011](#)). Thus, differences in the timing of reproductive investment for males (i.e., in early spring) and females (i.e., spring and summer), combined with mortality risks associated with activity, appear to strongly influence patterns of immergence into, and emergence from hibernation. For another rodent hibernator, [Bieber et al. \(2014\)](#) found that, consistent with torpor optimization, edible dormice (*Glis glis*) with the largest fat reserves used shorter torpor bouts and longer arousals than individuals with smaller reserves but that there was no effect of energy reserves on the duration of hibernation. In other words, the fattest dormice remained in hibernation longer than necessary based on energetics alone suggesting a tradeoff between costs of hibernation and benefits of mortality avoidance from inactivity. A number of studies have found that individuals in better body condition, with more fat reserves, tend to express more frequent or longer/deeper torpor bouts than those in poor body condition, again, likely because the inactivity and reduced foraging activity enabled by torpor expression reduces mortality risk (e.g., [Stawski and Geiser 2010](#); [Vuarin et al. 2013](#); [Nowak et al. 2017](#)).

Bats represent a potentially useful taxon for comparison to better studied rodents in order to advance understanding of the influence of reproductive timing and reproductive investment on the ecological

energetics, physiology, and phenology of hibernation. Many bat species exhibit pronounced heterothermy and rely on torpor expression in both winter and summer (Stawski et al. 2013). In the temperate zones, most bat species hibernate for prolonged periods lasting up to 8 months, surviving on only a few grams of stored fat (e.g., Norquay and Willis 2014). Hibernating bats are good models for testing hypotheses about energetic constraints in different taxa, optimization of the costs and benefits of torpor, and the inter-play between reproductive timing and hibernation phenology because of similarities, but also pronounced difference, from rodent hibernators. For example, in temperate bats, sex differences in the seasonal timing of reproductive investment are flipped relative to rodents (Fig. 1). Spermatogenesis for male bats occurs in summer, well after spring emergence, and males store sperm in the cauda epididymis until a period known as fall swarming (Gustafson 1979; Hosken et al. 1998). During fall swarming bats of both sexes gather at the entrances of caves, mines, or other hibernation sites each night, for up to 2 months before hibernation begins. They mate promiscuously during swarming and, to some extent, during hibernation itself (Thomas et al. 1979). Females then store sperm over the winter and, if they are in good condition in spring, they ovulate, fertilization occurs, and gestation begins (Wimsatt and Kallen 1957; Buchanan 1987). Thus, in contrast to ground squirrels, males invest in reproduction during summer, fall, and even into winter, while females invest in reproduction during spring and early summer (Fig. 1). Like male ground squirrels then, female bats should face selection pressure to optimize emergence date to reduce mortality risk from starvation or bad weather, but increase the potential for rapid offspring growth, which is known to affect survival of young-of-the-year (YOY) through their first winter (e.g., Frick et al. 2010).

Understanding the energetics of hibernation in bats has become increasingly important with the emergence of white-nose syndrome (WNS) in North America. WNS represents an unusual disease in that it target bats specifically during hibernation. The fungal pathogen *Pseudogymnoascus destructans*, which occurs widely throughout Europe and Asia and has likely co-evolved with palearctic bats, cannot grow at eutherian mammalian T_b and, instead, appears highly adapted for growth in the skin of torpid bats (Puechmaille et al. 2011; Verant et al. 2012; Warnecke et al. 2012; Leopardi et al. 2015; Hoyt et al. 2016). Infection of the skin with *P. destructans* leads to an increase in metabolic rate

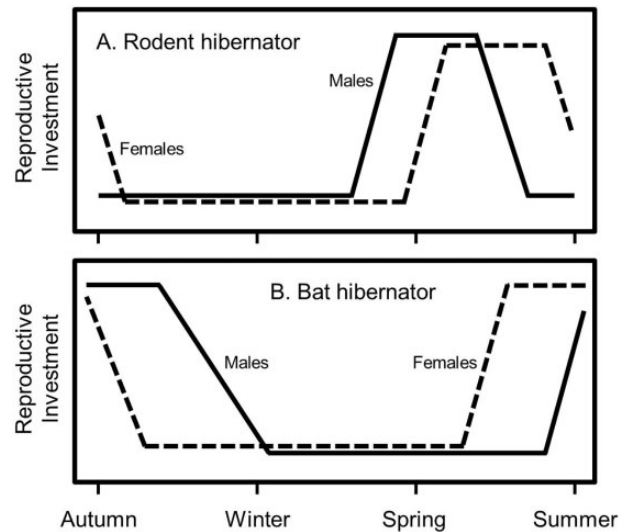


Fig. 1 Schematic time course showing hypothetical patterns of reproductive investment for (A) male and female rodent hibernators (e.g., ground squirrels) and (B) male and female bats (e.g., little brown bats) throughout the annual cycle. Male and female ground squirrels both make their investment in reproduction during spring but males emerge first to undergo spermatogenesis and defend territories. In bats, the ability to store sperm generates a pronounced mismatch in this timing, with males spending most of their reproductive effort in late summer, autumn, and early winter while females store sperm and depend on fat accumulated in autumn to initiate reproduction in early spring. Note, the schematic is intended to illustrate the timing of investment and the magnitude of reproductive investment is not to scale.

and evaporative water loss during torpor, as well as an increase in the frequency of periodic arousals by bats during hibernation, effects that appear to culminate in premature depletion of fat reserves, early emergence from hibernation, and starvation (Reeder et al. 2012; Warnecke et al. 2012; L. P. McGuire et al., manuscript under revision). To date, the disease has killed millions of North American bats and caused massive population declines in what may be the fastest decline of wild mammals ever observed (Frick et al. 2015). Given its influence on energy expenditure during hibernation, the introduction of *P. destructans* to North America represents a dramatic anthropogenic environmental change that could be imposing strong selection for traits associated with the ecological energetics and phenology of hibernation in bats (Frick et al. 2015). Thus, understanding these aspects of hibernation biology of healthy temperate bats has become an important priority for wildlife conservation.

Recent work in my laboratory suggests that energetic constraints faced by hibernating bats, and the hibernation behavior and physiology of bats, differ in important ways from better-studied rodent

hibernators. However, similar trade-offs between costs and benefits driving expression of torpor, and costs and benefits driving hibernation phenology, appear to influence energy expenditure and patterns of torpor expression during winter, as well as differences in emergence phenology between males and females. Here I review recent studies, primarily from my group, using little brown bats (*Myotis lucifugus*), one of the species hardest hit by WNS, to address predictions generated by three hypotheses about similarities to, and differences from, better-studied rodent hibernators. Taken together, these studies support the conclusion that little brown bats are unusual, compared with rodents, in terms of their ability for pronounced energy conservation during winter, but are similar in terms of the influence of reproductive timing on their storage of energy before hibernation, and patterns of energy expenditure throughout winter until spring emergence. I first review work testing whether bats exhibit unique adaptations to help them deal with pronounced energetic constraints that may be exclusive to hibernating bats in part because of their reliance on flight. Next I discuss studies testing the optimization hypothesis that torpor expression and energy expenditure by bats during winter reflects a balance between physiological costs and energetic/reproductive benefits of prolonged torpor. Finally, I discuss our work testing whether trade-offs between energy conservation, avoidance of spring mortality, and pressure for rapid offspring development, influence the phenology of emergence from hibernation. Unlike other taxa addressed in this symposium and special issue, for bats our understanding of neuroendocrine mechanisms associated with such trade-offs is in its infancy (e.g., see Willis and Wilcox 2014). Thus, I focus primarily on behavioral and whole-animal physiological data, but, to conclude I suggest several endocrine pathways which might be fruitful for future investigation.

Methods for studying hibernation energetics and phenology

For the past 10 years, my research group has undertaken studies of hibernating little brown bats in a study area throughout central Canada from near The Pas, Manitoba (53.8255°N, 101.2476°W) in the west to Thunder Bay, Ontario (48.3809°N, 89.2477°W) in the east. The study area is characterized by winters that are among the longest and coldest in which studies of bats have ever been conducted. All research was approved under relevant Manitoba and Ontario permits, and by the

University of Winnipeg Animal Care Committee. Little brown bats in the region hibernate in limestone caves and abandoned mines in numbers ranging from a few tens to more than 10,000 individuals per site. Many of these hibernacula are found in flat terrain in boreal forest, in relatively small caves extending 5–15 m underground, with small entrance openings (50 cm² to 2 m²) which funnel immersing and emerging bats. These features make them well suited to study bats using two technologies that can provide an excellent window on hibernation energetics and phenology.

To understand patterns of torpor expression and thermoregulatory energy expenditure during winter, we have used small temperature-sensitive radio-transmitters (BD-2NT, Holohil Systems Ltd, Carp, Ontario, Canada) affixed to the skin between the scapula using latex-based, non-toxic adhesive (Osto-bond, Montreal Ostomy, Vaudreuil, Quebec, Canada) after trimming a small, 1 cm² patch of fur (Jonasson and Willis 2012; Czenze et al. 2013; Czenze and Willis 2015; Czenze et al. 2017). These transmitters encode temperature in their pulse rate in a predictable and calibrated relationship and we record pulse rates using datalogging radio-telemetry receivers (SRX400 or SRX600, Lotek Engineering Inc., Newmarket, Ontario, Canada) housed outside caves, and powered by solar panels and deep-cycle batteries. Receivers are connected to omnidirectional or directional yagi antennas placed inside hibernacula close to locations used by roosting bats during hibernation. We have recorded T_a inside and outside hibernacula using wax-coated (to provide protection from high humidity and to attenuate ultrasonic noise, Willis et al. 2009) temperature dataloggers (DS1922L, iButtons, Maxim Integrated Products, Dallas, TX, USA).

The second technology we have used, that has been useful for understanding hibernation phenology, relies on radio-frequency identification tags, also known as passive transponder (PIT) tags, combined with PIT-tag antenna/dataloggers mounted at hibernaculum entrances (e.g., Norquay and Willis 2014; Czenze and Willis 2015). We have primarily captured bats for tagging at hibernacula during fall swarms using harp traps although we have also tagged some bats captured at summer maternity roosts. We have recorded body mass and forearm length for calculation of body condition index (BCI), age (YOY or adult based on ossification of phalangeal–metacarpal joints) and, from some individuals, obtained measurements of behavioral traits (i.e., personality), ectoparasite loads (e.g., Webber et al. 2015) and/or blood samples for plasma

metabolite or hormone analyses (e.g., [McGuire et al. 2016](#)). Prior to release, after obtaining these measurements, we have implanted PIT tags (ID100-01, Trovan Ltd, Douglas, UK) sub-cutaneously between the shoulders. These individually-coded tags are then recorded by remote datalogger/decoders (LID650, Trovan Ltd) connected to 475 by 400 mm panel antennas (ANT612, Trovan Ltd) or custom-built 50 cm loop antennas. Antennas are mounted in the entrances to hibernacula and the small cave openings funnel bats in close proximity to antennas allowing us to build encounter histories for individuals while also logging the timing of immergence into, and emergence from, hibernation. Since 2008 we have tagged over 13,000 individual bats across the 1,200 km wide study area. Using antennas deployed at 7–8 hibernacula (depending on the year) we have re-detected more than 30% of these individuals entering and/or emerging from hibernation across the study area.

In addition to field studies using temperature telemetry and PIT tags, we have also conducted laboratory experiments as part of an effort to understand and develop management strategies for WNS in bats (e.g., [Warnecke et al. 2012](#); [Turner et al. 2015](#)). For these experiments we have housed bats in nylon mesh cages within temperature and humidity controlled incubators held at cold but not freezing T_a and high, near saturation humidity to match conditions inside natural hibernacula. We have used temperature dataloggers (iButtons modified as described by [Reeder et al. 2012](#)) to record T_{sk} , and motion-activated, infrared (IR) video surveillance cameras to record the occurrence of arousals and behavior during arousals ([Wilcox et al. 2014](#); [Turner et al. 2015](#); [Bohn et al. 2016](#)). Bats are sensitive to disturbance during winter so video surveillance allows us to monitor behavior without causing unnecessary arousals and premature fat depletion.

Unique energetic constraints and adaptations of hibernating bats

As the only mammals capable of powered flight, bats are highly successful globally, and are second only to rodents in their diversity among mammals. For temperate-zone hibernating species, however, the ability to fly generates a particular set of constraints likely to affect hibernation energetics. First, bats are limited in their ability to accumulate energy reserves. Unlike many rodent hibernators like chipmunks (e.g., [Humphries et al. 2003](#)), they are not able to store food and must, therefore, rely entirely on stored fat for winter survival ([Stawski et al. 2013](#)). They must also maintain the ability to fly and, therefore, are

limited in the amount of fat they can accumulate. In addition to a limited capacity for fat storage, bats likely also face unique constraints influencing energy expenditure. For one, again in contrast to rodents, they are not able to dig a protected burrow or build an insulated nest, both of which effectively reduce thermal conductance. Instead, many hibernating bats roost in open-air hibernacula in caves, mines, or rock crevices where free and forced convection, as well as conduction with cold substrates in the hibernaculum, likely increase heat loss relative to a rodent hibernator in an enclosed and protected nest or burrow. Also unlike rodents, the flight membranes of bats represent a large surface that is highly vascularized yet un-furred, increasing both heat loss during arousals, as well as water loss during torpor and arousal. If water loss is a potential driver of arousal during hibernation ([Thomas et al. 1990](#)), increased water loss across the flight membranes during torpor could lead to higher overall rates of energy expenditure during winter relative to rodents.

Work from our study system, and from laboratory studies with European bats, suggests that several unique or at least unusual behavioral and physiological adaptations of bats help them maintain over-winter energy balance and differentiate them from rodent hibernators. First, some bat species appear to express extremely long torpor bouts lasting many weeks. [Czenze et al. \(2017\)](#) routinely recorded torpor bouts lasting more than a month, with the longest recorded bout (of 200 torpor bouts by 63 individuals over three winters) lasting more than 60 days. These likely represent an underestimate of maximum torpor bout duration because of the short battery life of the small temperature sensitive transmitters that hibernating little brown bats can carry. Studies of other hibernating bat species suggest that extremely long bouts exceeding 60 days are relatively common for bats ([Ruf and Geiser 2015](#)).

In addition to long torpor bouts, little brown bats from central Canada also appear to adjust characteristics of their arousals to reduce energetic costs. [Jonasson and Willis \(2012\)](#) and [Czenze et al. \(2017\)](#) observed multiple occurrences of what [Jonasson and Willis \(2012\)](#) termed “heterothermic arousals”. During these arousals, bats initially rewarmed normally to a euthermic T_{sk} but, shortly after rewarming, they re-entered shallow torpor but defended a T_{sk} well above levels typical of deep, thermoconforming torpor. At first [Jonasson and Willis \(2012\)](#) hypothesized that these patterns reflected battery failure or partial detachment of transmitters during arousals but two lines of evidence suggest that heterothermic arousals were not an artefact. First, they occurred often, representing 30% of 110 arousals over three winters and, for a given

individual, heterothermic arousals were often followed, several weeks later, by subsequent normal arousals (Jonasson and Willis 2012; Czenze et al. 2017). Second, sex differences in the use of heterothermic arousals are consistent with predictions of the torpor optimization hypothesis (see below). This suggests that, in some situations, bats can save energy using heterothermic arousals while still repaying at least some of the physiological debt that accrues during torpor.

In addition to using heterothermic arousals, hibernating bats may also avoid rewarming altogether if an arousal is physiologically unnecessary. Mayberry et al. (manuscript under revision) used IR video recordings of captive bats, combined with T_{sk} measurements obtained using temperature dataloggers, to document multiple observations of what they termed “cold arousals” during hibernation. During cold arousals, bats were capable of crawling around their cages when T_b was virtually identical to that during steady-state torpor, albeit with markedly reduced behavioral performance compared with during euthermic arousals (H. W. Mayberry et al., manuscript under revision). These cold arousals have also been observed for European bats by Bartonička et al. (2016). Cold arousals may be especially beneficial if an individual bat is dislodged from its cluster by a colony-mate during hibernation but has recently expressed a normal arousal and has, therefore, recently restored the physiological imbalance resulting from the preceding torpor bout. A cold arousal could allow an individual to climb back up the wall of the hibernaculum to a safe roosting position without spending energy unnecessarily. Some of Mayberry et al.’s (manuscript under revision) observations of deeply torpid bats at the water dish in their cages also suggests the possibility that bats could drink to restore water balance during cold arousals although more observations are needed to test this hypothesis. Interestingly, Mayberry et al. (manuscript under revision) also observed that the frequency of cold arousals increased as bats approached the end of hibernation which, as for heterothermic arousals, is consistent with the optimization hypothesis (see below).

Another marked difference between bats and most other hibernators is their reliance on clustering with other individuals, often in aggregations numbering thousands of individuals. Bats are the most gregarious hibernators and this behavior may help them deal with energetic and hygric constraints resulting from their large open-air hibernacula, combined with large un-furred areas of exposed skin. Boratyński et al. (2015) found that clusters of European Natterer’s bats (*Myotis nattereri*) had lower

rates of water loss during torpor than solitary individuals which has direct implications for water balance but also indirect implications for energy expenditure because slower rates of water loss could allow bats to prolong their torpor bouts (Thomas et al. 1990). Clustering could also serve to reduce energetic costs of arousals by reducing individual heat loss as both captive and free-ranging bats show evidence of synchronized rewarming (Czenze et al. 2013; Czenze and Willis 2015; Turner et al. 2015). Arousing at the same time as conspecifics could allow individuals in the same cluster to share arousal costs and reduce overall energy expenditure. Interestingly, however, little brown bats do not appear to perfectly synchronize arousals with each other, which would need to occur for all individuals to benefit equally from social rewarming. Instead arousals of individuals in a given group occur one after another, in so-called “arousal cascades” (Czenze et al. 2013; Czenze and Willis 2015; Turner et al. 2015). This could mean that arousing later in a cascade results in greater energy savings than arousing early because other individuals in the cluster have already provided heat to facilitate passive rewarming. This leaves open the possibility that arousal cascades reflect disturbance of some bats by others which could be maladaptive if disturbance causes individuals to arouse more often than they should to maintain energy balance (Turner et al. 2015). Alternatively, it also raises the possibility of an interesting evolutionary gamesmanship occurring within clusters of bats such that long torpor bouts, and resistance to the normal triggers for arousal, are favored. Under this scenario, bats that can endure torpor the longest and avoid being the first in a given cluster to warm up could benefit from reduced energetic costs. Interestingly both Czenze and Willis 2015 and Turner et al. (2015) observed that arousals became more synchronized in time as spring approached, suggesting that either bats were more susceptible to disturbance or more likely to exploit social thermoregulation as their fat reserves declined. Regardless of the motive, clustering seems to be one of the unusual adaptations of bats allowing them to address unique energetic constraints.

Thrifty females and winter energy expenditure

The torpor optimization hypothesis predicts that bats which can afford to spend energy reserves should spend these reserves avoiding expression of torpor and its associated costs, while bats with limited reserves should rely more heavily on torpor

(Humphries et al. 2003). Boyles et al. (2007) reported both observational and experimental support for this hypothesis for little brown bats in Ohio, USA. They observed that bats in relatively poor condition, with smaller energy reserves, selected relatively cold microclimates within their hibernacula presumably to increase depth and duration of torpor bouts. In an elegant experiment, they also limited energy availability for bats in the laboratory by injecting mercaptoacetate (MA), which prevents mitochondrial oxidation of fatty acids and, therefore, mimics energy limitation (Boyles et al. 2007). Consistent with the optimization hypothesis, bats injected with MA showed a strong preference for cold microclimates relative to sham-injected controls and this change in microhabitat selection would have favored deeper and more prolonged torpor (Boyles et al. 2007).

Our results from central Canada, comparing energy expenditure, torpor expression and arousal patterns between male and female bats, are also consistent with torpor optimization. Jonasson and Willis (2011) proposed the “thrifty female hypothesis” that female bats should be more conservative with their winter energy reserves than males throughout winter because they depend on fat stores obtained prior to hibernation to initiate and fuel gestation in spring. Several lines of evidence support this hypothesis. First, Jonasson and Willis (2011) found that female bats accumulated larger fat reserves than males in fall but that they used these reserves more slowly, at a rate of about 14.9 mg of fat per day (averaged over 211 days between swarming and late hibernation), compared with males which used about 17.1 mg of fat per day. Over the course of 240 days of hibernation, Jonasson and Willis (2011) calculated that females used 3.58 g of fat while males used 4.1 g despite the fact that females began winter with a larger fat reserve than males. For perspective, this difference in fat expenditure between males and females is pronounced in the context of hibernation, amounting to nearly 13% of total energy expenditure over a 240 day winter, but would represent a small amount of energy for a little brown bat during the active season. Kurta et al. (1989) used doubly-labeled water to show that a 7.9 g free-ranging, lactating female little brown bat should spend 41.2 kJ day⁻¹ on average. Assuming an energetic equivalence of 39.3 kJ per gram of fat (Schmidt-Nielsen 1997), the 0.52 g difference in fat expenditure between males and females throughout hibernation, then, would amount to only about half a day’s worth of total energy expenditure for a lactating female during summer.

Jonasson and Willis (2012) used temperature telemetry to understand how torpor and arousal might

affect patterns of energy expenditure but, given individual variation in torpor expression and a relatively small sample size, they were not able to detect significant differences between sexes. Czenze et al. (2017) combined data from Jonasson and Willis (2012) with 2 years of additional measurements and did detect significant effects consistent with the thrifty female hypothesis and torpor optimization. First, Czenze et al. (2017) found that females did not express longer torpor bouts than males but that their arousals were 22% shorter than those of males which resulted in significant differences in calculated energy expenditure. Females were also significantly more likely to express the energy-saving heterothermic arousals first observed by Jonasson and Willis (2012, see above). More generally, after controlling for sex, there was no effect of energy reserves (estimated based on BCI) on torpor bout duration but, again, Czenze et al. (2017) detected a significant relationship between arousal duration and energy reserves with bats in the best condition expressing the longest arousals.

Taken together, these results from my laboratory suggest that little brown bats adjust patterns of arousal, but not necessarily torpor bout duration, to optimize energy expenditure over the winter. Female bats, which depend on stored fat to support spring reproduction, may be forced to repay less of the physiological debt that accrues during torpor bouts. Males remain euthermic for longer during arousals possibly to sleep, excrete metabolic wastes, and/or drink thus helping to more fully restore homeostasis (Czenze et al. 2017). Males may also remain warm longer to search for potential mates and to mate with torpid or euthermic females (Thomas et al. 1979; Czenze et al. 2017). In general, little brown bats appear to adjust patterns of winter energy expenditure based on their current energetic status (i.e., fat reserves) but also their future energy requirements (i.e., spring reproduction for females).

Fall fat reserves and spring emergence

In addition to affecting energy expenditure during hibernation, differences in the timing of reproductive investment for male and female bats also have implications for hibernation phenology. The timing of entry into, and emergence from, hibernation in some rodent hibernators appears to be influenced by a trade-off between risks of active season mortality versus the benefits of reproductive opportunities (Michener 1979; Inouye et al. 2000; Lane et al. 2011). Our data for little brown bats suggest similar trade-offs, at least in part, although with different patterns

reflecting difference in the annual cycle of bats versus rodent hibernators.

Norquay and Willis (2014) and Czenze and Willis (2015) used data from PIT-tagged bats to test for effects of sex and body condition (i.e., an index of energy reserves) on spring emergence phenology. Based on patterns observed in the annual cycle of ground squirrels, these studies predicted that female bats should emerge from hibernation earlier than males to initiate reproduction as early in the season as possible and allow offspring to reach adult size quickly. They also predicted that females with the largest energy reserves should emerge first, presumably because they could use these reserves as insurance against the risk of mortality due to low food availability and inclement spring weather (Norquay and Willis 2014; Czenze and Willis 2015). These studies also predicted that males should remain in hibernation longer than females and that the fattest males should hibernate the longest and use stored energy to reduced active-season mortality risk (Norquay and Willis 2014; Czenze and Willis 2015). These studies revealed the predicted pattern for females but not for males. Both studies found that bats trickled out of their hibernacula over about a 6-week period beginning in mid-April and lasting until early June. Females emerged during a narrower window of time and about 20 days earlier than males on average. Moreover, females in the best condition at initial capture during either fall swarming (Norquay and Willis 2014) or mid-hibernation (Czenze and Willis 2015) emerged earliest in the spring. Interestingly, however, neither study detected an effect of fat reserves at initial capture on emergence date in males despite the prediction that fat males should use stored energy to avoid active season mortality in spring.

One explanation for the lack of influence of fat reserves on male emergence timing is that predation rates on bats may be relatively low compared with those for rodent hibernators. This could relax selection pressure on male bats to use stored fat to remain in hibernation into the summer. Therefore, males may simply wait long enough to emerge until ambient conditions are generally favorable but relatively weak selection for prolonged hibernation compared with ground squirrels means that any influence of body condition is small and difficult to detect.

Interestingly, Czenze and Willis (2015) found evidence that both males and females used environmental cues to precisely time their emergence from hibernation. They found no evidence that even large day-to-day changes in outside T_a caused enough of a signal in T_a within hibernacula for bats to detect and

adjust emergence. However, they found evidence that abrupt changes in barometric pressure (BP), which will equilibrate quickly inside small-volume caves like those in our study system, did provide a reliable cue for emergence. All female bats emerged on nights following a pronounced drop in BP consistent with the arrival of a warm front. The earliest emerging males (i.e., those emerging when ambient conditions could still be unfavorable) also emerged after falling BP events consistent with warm fronts. This further suggests that bats time their emergence phenology in part to avoid inclement conditions and high spring mortality risk.

Conclusions

Little brown bats in central Canada provide an interesting opportunity to evaluate the links between reproductive trade-offs, hibernation energetics, and hibernation phenology. In general the studies I review here support the hypothesis that bats face greater energetic constraints than rodent hibernators during winter, likely due in part to their reliance on flight combined with dependence on fat stores, inability to burrow and inability to store food. Bats appear to have evolved adaptations for extreme energy savings such as long torpor bouts, heterothermic arousals, cold arousals, and clustering in large groups. Our results also support Humphries et al.'s (2003) optimization hypothesis that bats adjust energy expenditure based on both their current energetic status (i.e., fat reserves) and future energy requirements (i.e., for reproduction in females). A tradeoff between benefits of early reproduction and active season mortality risk appears to influence the timing of emergence from hibernation, especially in female bats for which there appears to be an influence of fall or mid-winter energy reserves on emergence timing.

These results have implications for understanding the potential of bats to respond to human impacts like climate change or WNS. Intuitively, it seems reasonable to assume that a warming climate might be beneficial for hibernating mammals because it could reduce winter duration and relax energetic constraints associated with winter. However, one significant impact of a changing climate is reduced predictability of environmental conditions, and implications of this effect have been observed for at least one hibernator. In Columbian ground squirrels (*Urocitellus columbianus*) reduced predictability of spring snowstorms due to climate change, has led to negative impacts on reproductive fitness of individuals with consequences for population growth (Lane et al. 2012). A similar effect could occur for

hibernating bats, especially for females which emerge early at a time when the chance of inclement weather is already high.

WNS represents a more abrupt anthropogenic impact than climate change for little brown bats and several other bat species, with millions of bats killed in less than a decade and three species now listed as federally endangered in Canada because of WNS (Frick et al. 2015). Despite high mortality rates, some individuals, even heavily impacted species, appear to be persisting in the WNS-affected region (Langwig et al. 2017). Strong links between WNS and hibernation energetics raise the possibility that traits important for hibernation energy balance help underlie this persistence due to strong selection by WNS. For example, if fall fat reserves and/or emergence phenology are heritable in bats, as they appear to be in at least one other hibernator (Lane et al. 2011), WNS could lead to evolution for increased fat accumulation in fall (Willis and Wilcox 2014; Frick et al. 2015). Thus, conservation measures that enhance the ability of individuals with these favored traits to survive and reproduce in summer could be especially important for the conservation of affected bats (Frick et al. 2017).

Future studies should use the tools of quantitative genetics to quantify heritability of traits associated with hibernation energetics and phenology in bats so we can better understand the evolutionary potential of these traits. Work aimed at understanding neuroendocrine mechanisms underlying hibernation energetics and phenology would also be useful for understanding how these traits might evolve in response to environmental change. Although relatively little is known about these mechanisms, Willis and Wilcox (2014) suggested a few potential pathways that would be interesting to investigate in the context of WNS including the role of leptin signaling for fat accumulation in fall and energy expenditure in winter, the role of melatonin as an influence on seasonal and circadian rhythms, and the role of glucocorticoid stress hormones for influencing arousal patterns and sensitivity to disturbance during hibernation. Studies of these pathways in the context of environmental change would be useful for understanding the influence of trade-offs as drivers of hibernation energetics and phenology, and for addressing conservation challenges for insect-eating bats in the temperate world.

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