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## Energetics of Migratory Bats during Stopover: A Test of the Torpor-Assisted Migration Hypothesis

Dylan E. Baloun, *The University of Western Ontario*

Supervisor: Dr. Christopher G. Guglielmo, *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

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## ABSTRACT

The torpor-assisted migration hypothesis posits that during stopover bats regulate time in torpor facultatively so that daily energy expenditure is independent of ambient roosting temperature. However, direct measurements of total roosting energy expenditure in relation to ambient and body temperature are lacking. I captured migratory silver-haired bats (*Lasionycteris noctivagans*) at Long Point, Ontario in spring and fall 2016. I used quantitative magnetic resonance analysis to measure full-day energy expenditure of bats roosting at 10, 17 and 25°C and assessed the effects of migration season, body mass, sex and age on energy use. I found that daily energy expenditure was independent of roosting temperature, and that this was achieved by flexible torpor use. Body mass, season, sex and age all affected torpor use and energy expenditure. This study provides support for predictions of the torpor-assisted migration hypothesis and furthers our understanding of energy management at stopovers for migratory bats species.

Key Words: body composition, Chiroptera, *Lasionycteris noctivagans*, migration, quantitative magnetic resonance, stopover, thermal regulation, torpor-assisted migration hypothesis, torpor

## CO-AUTHOR STATEMENT

A version of this thesis will be submitted to Journal of Experimental Biology with Christopher G. Guglielmo as co-author. Dr. Guglielmo was directly involved in experimental design, provided equipment, assisted in data interpretation, and provided crucial editorial feedback.

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# TABLE OF CONTENTS

ABSTRACT .....	I
CO-AUTHOR STATEMENT.....	II
ACKNOWLEDGEMENTS .....	III
TABLE OF CONTENTS.....	V
LIST OF TABLES.....	VII
LIST OF FIGURES.....	VIII
LIST OF APPENDICES .....	X
INTRODUCTION.....	1
Staying resident and active .....	2
Staying and hibernating .....	3
Non-energetic costs of torpor.....	8
Escape through migration.....	11
Bat migration.....	13
The intersection of torpor and migration in bats .....	15
Spring migration and reproduction.....	17
METHODS .....	19
Study site and species.....	19
Energy and torpor quantification.....	20
Statistical analyses.....	24
RESULTS .....	26
Energy expenditure during roosting.....	27
Torpor use .....	30
DISCUSSION .....	34
Body mass, torpor, and energetics at stopover.....	34
Energy budgets for migrating bats.....	41
Preferred roost temperatures and the energetics of a thermal neutral zone.....	46

CONCLUSION .....	49
REFERENCES.....	51
APPENDIX A.....	71
APPENDIX B.....	72
CURRICULUM VITAE.....	73

## LIST OF TABLES

<b>Table 1:</b> Body mass, dry fat mass, and wet lean mass (g) of adult (Ad) and juvenile (Juv) silver-haired bats ( <i>Lasionycteris noctivagans</i> ) during stopover at Long Point, Ontario in spring and fall 2016 before entering a daytime stopover temperature treatment.....	26
<b>Table 2:</b> Torpor use ( $A_{\text{Torpor}}$ ; mean $\pm$ SD) of silver-haired bats exposed to one of three temperature treatments (cold, intermediate or warm) during a 12-hour daytime stopover, from both spring (1 Apr. - 3 June, 2016) and fall (15 Aug. – 30 Sept., 2016) migratory seasons.....	31



## LIST OF FIGURES

- Figure 1:** Example quantification of torpor use ( $A_{\text{Torpor}}$ ) from skin temperature ( $^{\circ}\text{C}$ ;  $T_{\text{sk}}$ ; thick black points) of a silver-haired bat (*Lasionycteris noctivagans*) during a daytime stopover roosting at  $25^{\circ}\text{C}$ .....24
- Figure 2:** Body composition, body mass and energy expenditure of female and male silver-haired bats from both the spring (white boxes) and fall (grey boxes). **A)** Dry fat mass (g), **B)** wet lean mass (g), quantified using quantitative magnetic resonance. **C)** body mass of female and male bats taken before exposure to temperature treatment. **D,E,F)** Fat mass loss, lean mass loss, and total energy expended during a 12-hour daytime stopover at one of three temperature treatments.....27
- Figure 3:** **A) & B)** Total energy use (kJ) by silver-haired bats during a full daytime stopover from both migratory seasons, at one of three 12-hour temperature treatments (cold, intermediate, or warm). **C)** Total energy use during a full daytime stopover and body mass (g; natural log transformed) for females in both seasons (spring: black; fall: grey). **D)** Total energy use (kJ) during a full daytime stopover and body mass (g; natural log transformed) of young-of-the-year (juvenile: black) and older than one-year (adult: grey) male silver-haired bats during fall migration. **E&F)** Comparing the effects of body mass (natural log transformed) on  $A_{\text{Torpor}}$  (square-root transformed) from 12-hour daytime stopovers between temperature treatments: cold (black square), intermediate (dark grey circle), and warm (light grey triangle), for spring (**E**) and fall (**F**) silver-haired bats.....32

**Figure 4:** Ambient temperature traces from Long Point Bird Observatory, Long Point, ON, from both spring (15 April – 3 June, 2016; left panel) and fall (15 August – 30 September, 2016; right panel) sampled every 30 seconds.....37

## LIST OF APPENDICES

APPENDIX A: Ethics Approval.....	71
APPENDIX B: Permits.....	72
CURRICULUM VITAE.....	73

## INTRODUCTION

Living at high latitudes can be energetically and physiologically challenging due to seasonal changes in the environment. Seasonal challenges that animals have to adapt to include changes in photoperiod, temperature and resource abundance (Dawson et al., 2001; Duquette, 1988; Humphries et al., 2002). Climatic conditions change seasonally from high ambient temperatures ( $T_a$ ) during the summer, to below freezing during the winter months which can make up the majority of a year (~8 months) at the highest northern latitudes where animals are found. During the winter, food can be extremely limited as all trophic levels are affected by decreases in light availability and  $T_a$ . Seasonal variation in climate, photoperiod, and resource abundance are not restricted solely to high latitudes. Low latitudes experience seasonal changes that are often driven by periods of high rainfall (i.e., monsoon season; Mares and Ernest, 1995). Although there is seasonal variation at both high and low latitudes some of the most dramatic differences (i.e., photoperiod, temperature) are observed in high latitudes. But, like in all systems that have been studied, there are many adaptations and survival strategies that have evolved to meet the challenges of high latitude ecosystems.

There are three main life-history strategies that have evolved in endothermic homeotherms (mammals and birds) to survive the long periods of low  $T_a$  and the resource scarcity of winter: 1) “active and eating” or continuous homeothermy, 2) hibernation, and 3) migration. Each strategy has its own set of limitations and restrictions that are apparent in both the morphology and physiology of the species from each group. There is also inter- and intraspecific physiological variation within each survival strategy, and

occasionally we find species that employ parts of several strategies to survive ecological hardships in unusual ways.

Staying resident and active

The “active and eating” life-history strategy describes animals that remain resident and maintain a high body temperature ( $T_b$ ) through metabolic expenditure that does not vary greatly from a euthermic or homeothermic level, even during winter when thermoregulatory costs are greatest (Brigham and Geiser, 2012; Geiser, 2004). Winter resident species remain at northern latitudes for the duration of the year, and are therefore forced to eat, seemingly constantly (especially for small species; see below) to balance their energy budgets throughout the winter period. American red squirrels (*Tamiasciurus hudsonicus*) remain active, defending a territory and a food hoard on which they will feed on throughout the winter (Brigham and Geiser, 2012; Larviée et al., 2010). Other examples of resident species that remain active and maintain high  $T_b$  for the duration of the winter are ungulate species (i.e., deer, moose, etc.) and many, but not all, higher trophic-level predators (i.e., fox, coyote, wolf, etc.). Some small homeotherms also remain resident and active throughout the winter by using food sources that remain available throughout the winter, such as black-capped chickadees (*Poecile atricapillusi*), several ptarmigan, voles, lemming, and mouse species (Andrzejewski, 1975; Díaz and Alonso, 2003; Irving et al., 1967). Homeotherms rely on insulation from fur or thick plumage (feathers), to some degree, to reduce heat loss to the environment as well as stored fat as a source of energy in between feeding opportunities (for a review see: Staples, 2016). Larger species (i.e., ungulates) have a larger capacity to store fat (increasing the overall amount of fat on reserve) and generally have a lower cost of

thermoregulating when remaining euthermic (when taking into account mass-specific metabolic rates; McNab, 1982). In general, the obligate winter-active, homeothermic species weigh more than 10 kg, with some exceptions, such as several bear species that weigh more than >80 kg and do not remain homeothermic throughout the winter (Bruce et al., 1990; Geiser, 2004; Oeltgen et al., 1989; Riek and Geiser, 2013). Some species, such as the black-capped chickadee, cache food throughout their home range for future use similarly to red squirrels, but in smaller amounts in any one place (Sherry, 1984). However, despite their small body size and ability to cache and retrieve stored food, there is some evidence that black-capped chickadees adjust their metabolism to decrease energy expenditure during cold winter night temperatures (Chaplin, 1976). Several bear species (>80kg) that also appear to have the physiological capacity to suppress their metabolism to reduce thermoregulatory costs (Bruce et al., 1990; Oeltgen et al., 1989; Sahdo et al., 2013). Just like the interspecific variation in body size/mass in species that remain active and eating throughout the winter, there is similar variation within species that are able to adjust their metabolism to reduce energy costs (and reduce the need to forage) throughout the winter.

#### Staying and hibernating

Not all species that occupy northern latitudes year-round do so by maintaining euthermia (a high, tightly regulated  $T_b$  that allows activity and bodily functions, that varies among species; Geiser, 2004) and foraging to balance energy budgets throughout the winter.

These sedentary species follow a “stay and hibernate” life-history strategy where they remain in northern habitats but do not stay active or forage throughout the winter.

Although there are several relatively small species (i.e., American red squirrel) that

remain euthermic throughout the winter, many smaller species have seemingly adapted to take advantage of their lower thermal inertia and increased surface area to volume ratio to allow their  $T_b$  to drop with  $T_a$ . The reduction of  $T_b$  is unlike the passive loss that occurs in hypothermia, but rather is controlled by metabolic suppression and is reversible. “Stay and hibernate” species have adapted flexible control of their metabolism which allows them to adjust their metabolic set points according to  $T_a$  or resource abundance (Brown et al., 2007; Geiser, 2004; Kutschke et al., 2013; Staples, 2016). Species that are adapted to be physiologically capable of such metabolic suppression (which results in a drop in body temperature;  $T_b$ ) are able to enter a state referred to as “torpor”. Species that are able to adjust their metabolism actively are also referred to as “heterotherms”. Due to the typically long winter periods hibernating species have also adapted to enter torpor for long periods of time (often 1-2 weeks) to maximize energy savings and endure an entire winter season without feeding. Based on respirometry studies, the primary metabolic fuel used during torpor is endogenous fat (Frank, 1992; Speakman and Rowland, 1999).

Hibernation is made up of long periods (i.e., days to weeks) of torpor and low  $T_b$  interspersed with arousals where metabolic rate increases and  $T_b$  returns to euthermic levels, only to return to a torpid state after a short (relative to torpor bout) period (Heller et al., 1993). During a torpor bout, metabolic rate drops dramatically allowing individuals to save energy that normally would be spent thermoregulating (Geiser, 2004; Hock, 1951). Hibernators, which include many mammals and one known bird species, are relatively small (~10-1000g), with some exceptions like black and brown bear species (>80 kg), as smaller species have relatively large surface area to volume ratios (increased loss of heat per unit of mass; for review: Geiser, 2004 and Staples, 2016).

A torpor bout consists of three phases: 1) a controlled suppression of metabolism and decrease in  $T_b$ , 2) a period of reduced, relatively stable metabolism and  $T_b$ , conforming closely to  $T_a$  (the degree of conformation is dependent on body size; Rieck and Geiser, 2013) and, 3) an increase in metabolism and rise in  $T_b$  back to euthermic levels – the arousal period. The most energetically expensive phase of a torpor bout, regardless of its depth and duration, is the arousal phase, which can account for up to ~70-80% of the total energy spent during the bout (Thomas et al., 1990; Wang, 1978).

There are several mechanisms or processes that could be responsible for metabolic suppression in heterotherms (for review: Staples 2016). It is possible that multiple signalling cascades cooperate simultaneously to trigger metabolic suppression and arousal into and out of a torpid state. However, the idea of multiple signalling cascades cooperating simultaneously is a complex and general statement. A more specific proposed mechanism of metabolic suppression is that there is a temperature sensitive protein or endogenous opiate that acts as a biochemical “hibernation induction trigger” (HIT; Bruce et al., 1990; Dawe and Spurrier, 1969; for review: Staples 2016). This has been tested by infusing blood from hibernating individuals into “summer condition” individuals (a state where an animal would not normally enter torpor), that is placed in a hibernating environment (i.e., darkness and low  $T_a$ ). If the HIT is in circulation of an individual in a torpid state the summer condition individual will respond to it physiologically when presented with a temperature challenge and enter torpor. There have been several papers on different species that suggest the presence of a HIT, but there has been minimal success with respect to isolating blood borne HIT (Bruce et al., 1990; Dawe and Spurrier, 1969; for review: Staples 2016). Several signalling systems that have



been explored include:  $\delta$ -opioids, (Oeltgen et al., 1982), fibroblast growth factor 21 (FGF21; for review see Melvin and Andrews, 2009; Nelson et al., 2013), H<sub>2</sub>S (Revsbach et al., 2014), and thioredoxin interacting protein (Txnip; Hand et al., 2013). Although these studies have found changes in blood metabolites and several signalling systems that match cycles of hibernation, further research is needed to tease apart the role of each compound within each signalling system and the possible interactions between signalling systems in the suppression of metabolism. However, it is also important to note that hibernation occurs with a multitude of physiological and environmental changes, (i.e., changes in reproductive status, photoperiod, T<sub>a</sub>) and fasting (either due to resource scarcity or behavioural change), that could all have cumulative effects making it difficult to pin down one exact mechanism (for review: Staples 2016).

Suppression of mitochondrial metabolism is an important process prior to torpor entry as the mitochondrial electron transport system is responsible for a large amount of the endogenous heat produced by endotherms (Jansky, 1973; for review: Staples 2016). However, if an individual is not balancing endogenous heat production with dissipation to the environment, at a basal level, it will be required to transition from passive thermoregulatory methods (i.e., thermoregulatory behaviours such as sun basking, huddling with conspecifics, etc.) to recruiting active thermogenic processes to increase heat production at the cost of energy (Heldmaier et al., 1990; Hohtola, 2004; Jansky, 1973). Shivering, or the rapid repetitive contraction of skeletal muscles, is an active thermoregulatory process used by several taxa of vertebrate endotherms (Heldmaier et al., 1990; Hohtola, 2004). Although muscle fibres are moving, it is the skeletal muscle mitochondria that are responsible for heat production. Mammals have also adapted to

actively produce heat by non-shivering means through the action of uncoupling protein 1 (UCP1), which is found in large quantities inside mitochondria of brown adipose tissue (BAT; Jansky, 1973; Klingenspor and Fromme, 2012). The mitochondria of BAT are unique in that no other mitochondria have UCP1 (Jansky, 1973; Klingenspor and Fromme, 2012). UCP1 “uncouples” the flow of protons across the inner membranes of mitochondria from ATP production, creating a futile cycle that results in pure heat production (Jansky, 1973; Klingenspor and Fromme, 2012). It is not known to what extent either of the active methods of producing heat is “more efficient” or “effective”, or what trade-offs are made and at what time transitioning should or does occur between them. To summarize, both shivering and non-shivering thermogenesis appear to be involved in endogenous heat production during arousal from torpor.

Studies of the time spent in a controlled, metabolically suppressed state have focused on how animals are able to maintain suppression. Heller et al. (1993) proposed the hypothesis that the lower  $T_b$  suppressed the circadian clock (influencing sleep cycles) to reduce its function and lengthen the period of time in torpor before arousal. This made sense considering the proportionally inverse relationship between bout length and hibernating  $T_b$  (Twente and Twente, 1965a,b). However, by measuring the rhythmicity and amplitude of  $T_b$  of golden-mantled ground squirrels (*Callospermophilus lateralis*) hibernating at different  $T_a$ , cycles in  $T_b$  (that were used as the proxy for circadian rhythm), were not slowed, thus suggesting differences in bout length were related to  $T_b$  and  $T_a$  (Grahn et al., 1993; Heller et al., 1993; Körtner and Geiser, 2000).

### Non-energetic costs of torpor

Torpor and hibernation have clear advantages in energy-limited environments, but animals do not use torpor whenever it is possible for them. There are several hypotheses as to why individuals would remain euthermic even though they have the physiological capacity to enter torpor, and they likely apply to the third and most expensive phase of torpor, the arousal period. Ultimately, arousing from a torpor bout is not as costly as it would be if the individual remained euthermic for the entire period of time. Considering the physiological state of a torpid individual, there are several activities that are not possible, or are drastically reduced when at a lower  $T_b$ .

Based on electroencephalogram studies (electrical activity of the brain; EEG), of thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*), torpid individuals do not show rapid eye movement (REM) sleep if brain temperatures are below 27°C, which  $T_b$  can be lowered well below 27°C (Walker et al., 1977, 1981). EEG and electromyography (electrical activity or nerve function in muscles; EMG) recordings from hibernating individuals, show that they are sleep deprived or running a “sleep debt” (Daan et al., 1991; Trachsel et al., 1991). Heller and Glotzbach (1985) proposed that hibernation could have evolved through the increased selection for decreases in  $T_b$  found during non-rapid eye movement (NREM) sleep (Heller and Glotzbach, 1985).

There are also consequences of torpor for immune system function and response (Prendergast et al., 2002; Sahdo et al., 2013). Circulating leukocytes decrease during hibernation in thirteen-lined ground squirrels to approximately 10% of pre-hibernal levels, and they are not replaced until arousal from torpor (Bouma et al., 2013). Similarly, torpor reduces antibody production (Sidky and Auerbach, 1968), and suppresses DNA

synthesis in and proliferation of lymphocytes (Manasek et al., 1965; Sieckmann et al., 2014). A more recent study exploring the response of the immune system to torpor use, however, found adaptations to maintain immune protection in the respiratory system; a system whose function is reduced, but is still active during hibernation, and open to infection by foreign bodies (Bohr et al., 2014).

Torpor use, especially at low  $T_a$ , can increase the risk of mortality by predation and natural disasters. Although many cryptic species have adapted to remain still when faced with predators, torpor using species (despite having adapted cryptic coloration and/or burrowing behaviours) can be more or less “sitting ducks”, as they are unable to engage in “fight or flight” in their defense until their  $T_b$  increases closer to euthermic levels. Individuals using torpor are not only exposed to predation, but also natural disasters such as wildfires (Nowack et al., 2017). At cold  $T_b$ , bodily function and therefore reaction to stimuli could be hindered. Some studies have found small amounts of slow locomotion possible at  $T_b$  as low as 14.8-17.9°C, which is likely an adaptation to escape detrimental circumstances (i.e., predation: Warnecke et al., 2008; Warnecke and Geiser, 2010; and or wild fire: Nowack et al., 2016; Rojas et al., 2012).

Body mass also significantly affects an individual's “decision” to enter torpor, as well as for how long and how deep to suppress metabolism (French, 1985; Murie and Boag, 1984; Sheriff et al., 2013; Zervanos et al., 2014). The effect of body mass on torpor use is not to be mistaken for effects of body size, but is an effect of body composition or the size of endogenous fat stores. Pre-hibernal individuals increase their fat deposition, which can lead to enormous body mass changes (Murie and Boag, 1984; Sheriff et al., 2013; Zervanos et al., 2014). The fat deposition is often affected by sex, with females

depositing more fat than males (Murie and Boag, 1984; Sheriff et al., 2013; Zervanos et al., 2014) which is likely an adaptation to manage carry-over effects on energy stores for future reproduction.

Torpor can be used in shorter increments (i.e., hours), and in some species can be important in daily energy management. The acute benefit of daily torpor use is saving thermogenic energy, but secondarily, these “savings” decrease the intake-output energy differential, increasing the rate of fat deposition in individuals prior to migration and/or hibernation (Blem, 1980; McNab, 1982; Murie and Boag, 1984; Sheriff et al., 2013; Zervanos et al., 2014). Other than the total time spent in torpor, torpid metabolic rates are higher during short term or daily torpor use than hibernation (Ruf and Geiser, 2015). The rate at which some species are able to deposit fat can be rapid; *Myotis thysanodes*, a migrating bat, can increase its fat stores nine-fold in 11 days (Ewing et al., 1970). This rapid and intense fat deposition period is likely associated more closely with hibernation than migration as hibernation occurs over a longer period of time and individuals are not able to replenish fat stores during the winter (Fleming and Eby, 2003; Murie and Boag, 1984; Sheriff et al., 2013; Zervanos et al., 2014;).

Although torpor use can reduce mobility acutely, there is no impact or overall connection between the overall mobility of an animal not using torpor and the decreased mobility while at torpor  $T_b$ . Many species with the appropriate morphological and physiological adaptations that promote rapid movement over the landscape employ migratory life-history strategies.

### Escape through migration

Seasonal migration is the movement from one habitat to another as part of a life-history strategy used to escape habitats and periods of resource scarcity (Dingle, 1996, 2014; Fleming and Eby, 2003). Migratory behaviours can be observed in a plethora of taxa that occupy aquatic, terrestrial, and aerial ecosystems (cetacean: Kshatriya and Blake, 1988; fish: Hansen et al., 1993; insect: Wassenaar and Hobson, 1998; bird: Alerstam and Lindström, 1990; bat: McGuire et al., 2012; terrestrial: Duquette, 1988). The distance each species migrates can vary both among and within species, but all are associated with seasonal cycles in resource abundance and reproduction. Flying migrants have different physiological adaptations and energy budgets given the expense of flight compared to cursorial or aquatic migrants (Guglielmo, 2010; McClelland, 2004; Schimdt-Nielsen, 1975). Flight is more energy efficient than cursorial locomotion per unit of distance, but not as efficient as swimming, however, the higher speeds available to flying species than aquatic species means that flying allows for the traversing of large distances for less energy (Alexander, 2005; Butler, 2016; Schimdt-Nielsen, 1972). Many insect species have evolved long-distance aerial migrations, but these may be carried out over several generations. Birds and bats are able to make long-distance migrations of thousands of kilometers within the annual cycle, and many complete numerous migrations throughout their lifetimes (Holland et al., 2006; Stefanescu et al., 2012). Despite the convergent evolution of flight, the dynamics of energy expenditure and locomotion for aerially migrating insects are different compared to relatively large aerial endotherms (i.e., bird or bat species; for a review of insect thermoregulation: May, 1979; Miller, 1964; Schimdt-Nielsen, 1972).

The adaptation of flight has allowed for relatively quick, long distance (>500 km) movements of birds and bats across the landscape. For species living in northern latitudes, the evolution of migratory movement patterns means they can escape before the onset of winter to reach suitable hibernation sites or wintering grounds, and return when habitat and food become abundant in the following spring. Migratory bats can be classified into 'regional' and 'long-distance' migrants based on their distance travelled. Regional migrants travel moderate distances between summer and wintering grounds (~100-500 km), which is often associated with species that migrate to hibernation sites close to their breeding grounds (Fleming and Eby, 2003). Long-distance migrants, however, travel >500-1000 km between summer and wintering grounds, and are often morphologically specialized for long-distance flight (Fleming and Eby, 2003; Norberg and Rayner, 1987; Savile, 1956). Migratory bats, like birds, generally have high aspect ratio wings (long and thin), high wing loading, and pointed wing tips, all of which are morphological specializations for efficient long distance flight (Norberg and Rayner, 1987; Savile, 1956). Some long-distance migratory species, such as Mexican free-tailed bat (*Tadarida brasiliensis*) and European robin (*Erithacus rubecula*) are considered partial migrants as not all individuals or populations migrate (Biebach, 1983; Cockrum, 1969).

Many flying migrant species are small and can only carry enough fuel for a portion of their migratory journey. The primary fuel for migratory flight is endogenous fat although protein can play an important role as a fuel to support key metabolic pathways and provide water (Gerson and Guglielmo, 2011; Jenni and Jenni-Eiermann, 1998; McWilliams et al., 2004; Ramenofsky, 1990). Migrants replenish their fat stores

between flights at stopover sites. Stopovers can last several hours to weeks depending on weather and stopover habitat quality (Barclay et al., 1988; Dossman et al., 2016; Eikenaar and Bairlein, 2014; McGuire et al., 2012; Wikelski et al., 2003). During stopover, birds spend energy foraging intensely, staying alert for predators, preening, and in particular thermoregulating at euthermic  $T_b$ , which can increase or decrease according to  $T_a$ . Counter-intuitively, for birds, stopovers can be the most energetically expensive part of a migratory journey. Total energy costs accrued through stopover activities (especially from thermoregulation) can be twice the energy expended during migratory flights (Wikelski et al., 2003).

#### Bat migration

Bats (order Chiroptera) show perhaps the most widespread and the greatest capacity to use torpor of all mammals. This highly diverse order is comprised by the only true flying mammals, with the majority of bat species being nocturnal. Their physiological capacity to use torpor is likely due to the, on average, small body sizes (that result in low thermal inertia) of bats, that allow them to easily dissipate body heat as they suppress metabolic rates and conform to  $T_a$  (Geiser, 2004; Ruf and Geiser, 2015). North American bat species have been observed using torpor in a number of situations from winter hibernation, to daily torpor use, and even to wait out periods of inclement weather like spring storms, including during migratory stopover (McGuire et al., 2012, 2014; Willis et al., 2016). Species such as little brown bats (*Myotis lucifugus*) or big brown bats (*Eptesicus fuscus*) hibernate throughout the winter, while eastern red bats (*Lasiurus borealis*), hoary bats (*Lasiurus cinereus*), and silver-haired bats (*Lasionycteris noctivagans*) migrate south to escape winter and return north to reproductive grounds the



following spring season (Cryan, 2003; Fleming and Eby, 2003; McGuire et al., 2012; Willis et al., 2006). It is not known how far each migratory bat species travels seasonally, but some studies suggest wintering grounds could be from southern USA and Central America while northern reproductive and summer grounds can into Canadian latitudes (e.g.,  $\sim 54^{\circ}\text{N}$ ; Cryan, 2003). It is also not known whether or not migratory bats hibernate throughout the winter, how much variation in time spent hibernating that might occur, or if they migrate far enough south that climatic conditions are not conducive to hibernation.

Migratory patterns of bat species are broadly similar in many ways to migration of birds. In general, temperate bats fly south in the fall, to escape the resource scarce winter months after a summer of growth (for juvenile bats) and foraging to build fat stores. Nearing the end of the summer, before migrating south, migratory bats mate, and females store sperm throughout the fall migration and the subsequent winter. In the following spring, bats migrate north with pregnant females preceding males (Jonasson 2017). Due to the elusive nature of bats, and the, until recently, weight limitations of tracking technology, it is difficult to know how far there bats migrate, or how many stopovers are necessary, given the unknown speed of migration. Migrating bats are forced to stopover each daytime period and bats may face different energetic challenges between spring and fall migratory seasons (Dzal et al., 2009; Jonasson, 2017; McGuire et al., 2012).

The winter destinations of many bats are not well understood, as there have been little to no observations of long-distance migratory species during the winter. It is possible that species that migrate in and out of Canadian latitudes are partial migrants, where some populations remain in the United States, giving fall migrants potential options to hibernate near or with sedentary conspecifics, or to continue south to remain

euthermic and active throughout the winter (Cryan, 2003; Fleming and Eby, 2003). Recently, a hoary bat (a migratory species) was tracked in California moving >1000km, as a round trip movement to a hibernation site (Weller et al., 2016). Although an individual migratory bat was observed exhibiting medium-distance flights (one-way) and two flightless weeks where the individual used torpor, this bat did not leave the mountain areas of California, or enter any higher latitude ecosystems. This could be described as a medium range migratory movement, but it could also be considered a sedentary individual similar to other hibernating species of bats like little brown bats, or gray bat (*Myotis grisescens*) that have been observed travelling ~400-500 km to hibernation sites (Norquay et al., 2013; Tuttle, 1976). Despite the lack of direct evidence for the destination of populations of temperate migrating bats, it is agreed that migration allows them to escape harsh seasonal climatic changes, and or to avoid periods of low resource (insect) abundance (Fleming and Eby, 2003).

#### *The intersection of torpor and migration in bats*

The relationships between critical aspects of bat ecology such as heterothermy and migration are largely unstudied. Migration is energetically expensive for bats, both during flight and stopover, and may be time constrained due to nocturnal behaviours, all of which contribute to the selection for traits to increase the efficiency of migration (McGuire and Guglielmo, 2009, 2012; Wikelski et al., 2003). Migratory bats use torpor during mandatory daytime stopovers in both spring and fall seasons to save energy that would otherwise be spent defending euthermia (Cryan and Wolf, 2003; Jonasson, 2017; McGuire et al., 2012, 2014). However, our understanding of how bats budget their energy

during daytime migratory stopovers and how torpor use affects these energy budgets, is still incomplete.

Fleming and Eby (2003) made three predictions regarding fuel use and migration for bats, all of which assume that migratory bats will hibernate for at least some period during the winter: 1) some individuals will need to refuel before hibernation upon reaching wintering grounds, 2) bats experience selection for short migration distances to conserve fat for hibernation, and 3) bats refuel during stopovers to maintain the size of fat stores for hibernation, or at least for surviving winter. However, McGuire et al. (2012) found that most fall migrating silver-haired bats stopped over for less than 24 hours, and did not appear to refuel. McGuire et al. (2014) then showed that migrating silver-haired bats use torpor extensively and facultatively during fall stopovers. Based on metabolic rate measurements (from respirometry) and body temperature data collected from free ranging bats (by telemetry), McGuire et al. (2012, 2014) suggested that torpor allowed for estimated energy savings as high as 91% during a daytime stopover. Based on the potential for high energy savings, the authors predicted that due to the facultative use of torpor, energy use should be independent of ambient roosting temperature during daytime stopovers. They then proposed the torpor-assisted migration hypothesis, which posits that migrating bats may not need to refuel at stopover due to torpor use and predicts that stopover energy use will be independent of  $T_a$ . Considering estimates of flight distances and times of silver-haired bats, McGuire et al. (2012) suggested that fall migrant bats stopping in southern Ontario, Canada, had enough fat to fly for ~9 nights (not including stopovers), enough to reach their proposed wintering area. This would mean that migratory bats may be removed from pressure to refuel en route.

McGuire et al. (2012 and 2014) were unable to tell what stage of migration the silver-haired bats were at (either early or late in migration) upon capture in southern Ontario and also did not suggest that bats do not change behaviours later in migration upon approaching or arrival at wintering grounds from potentially early migration stopover behaviour. Regardless of the stage of migration, torpor use during daytime stopovers could still be beneficial in terms of energy savings, increased rates of fat deposition, or by reducing the need to forage at all thus reducing the time spent migrating, at all stages of migration. However, the amount of energy used during stopover has not been directly quantified, nor have the estimates made by McGuire et al. (2012) been validated by direct quantifications of energy use during stopovers.

#### Spring migration and reproduction

Jonasson (2017) used similar methods as McGuire et al. (2014) to observe free ranging bats during spring migration. Jonasson (2017) found that bats spent more time in torpor in the spring than the fall, which was attributed to the cooler ambient roosting temperatures in spring. However, female bats spent less time in torpor than males during spring migration, and qualitative evidence suggested that mass differences between sexes were driving torpor use, and in hand, energy use during daytime stopovers. The sex differences observed in the spring (and not the fall) make sense given the delayed fertilization and pregnancy strategy of temperate bats (Cryan and Wolf, 2003; Dzal and Brigham, 2013; Hood and Smith, 1989; Solick and Barclay, 2006; Stawski, 2010; Turbill and Geiser, 2006). Bats occupying temperate latitudes only give birth in the summer months (Oxberry, 1979) reflecting the timing of peak insect abundance (Barclay, 1988; Druecker, 1972; Taylor, 1963). In late summer/early fall, migrating *Lasionycteris* and

*Lasiurus* species collected from wind turbine mortality searches, have been found to be reproductive ready in that spermatozoa were present and several stages of ovarian follicles were observed, suggesting that mating occurs prior to or during fall migration (Cryan et al., 2012). Female hibernating temperate species produce follicles that survive in the ovary, throughout hibernation, and sperm survives in the uterus until the emergence from hibernation when ovulation and fertilization occur (Wimsatt, 1944a,b; Wimsatt and Parks, 1966). Spermatogenesis occurs during the summer for male temperate species and mating occurs generally in the fall, forcing spermatozoa to survive within the uterus of a female bat for up to seven months until ovulation and fertilization (Racey, 1979; Racey, 1982; Wimsatt, 1969). Racey (1982) suggests that pre-hibernal fattening could be associated with natural estrus in temperate bats.

Bats are placental mammals and are reliant on their mother after parturition until they become volant. Torpor use means a reduction in heart rate, reduction in circulation to and from the foetus, which, therefore could hinder foetal development (Cryan and Wolf, 2003; Currie et al., 2014; Rauch and Beatty, 1975). Several studies have shown differential torpor use for reproductive migratory bats, with pregnant females refraining from torpor use, hypothetically to safeguard foetal development (Cryan and Wolf, 2003; Currie et al., 2014; Rauch and Beatty, 1975; Willis et al., 2006). Other studies have shown that torpor use delays parturition as well as lactation for bat species (Racey, 1973; Racey and Swift, 1981; Turbill and Geiser, 2006; Wilde et al., 1999), which suggests that bats balance trade-offs between the energetic savings and the rate of foetal development.

Previous research demonstrates that migrating bats save energy during daytime stopovers by using torpor. However, little is known about the dynamics of energy

expenditure during daytime stopover, how reproductive costs affect energy budgets for female bats, or how the intersection between the decision to use torpor and reproductive costs influence these budgets. No study to date has directly quantified energy use during daytime stopover for migratory bats, nor has energy use been quantified while controlling for migratory season. My objectives were to 1) directly quantify energy use during full daytime stopover for migrating silver-haired bats and 2) test a key prediction of the torpor-assisted migration hypothesis that energy use during daytime stopovers is independent of roosting  $T_a$  across migratory seasons. I hypothesized that reproductive life history differences will affect torpor use and energy use during daytime stopovers for migrating bats. I predicted that energy expenditure would be independent of roosting  $T_a$  in both migratory seasons but differ between sexes due to additional costs of foetal development for female bats in spring. I also predicted that bats, regardless of sex and season, will adjust the depth and/or duration of torpor in response to variation in  $T_a$ .

## METHODS

### Study site and species

All methods were approved by Western University's Animal Care Committee (2010-020; Appendix A) and Ontario Ministry of Natural Resources (Wildlife Scientific Collector's Authorization Permit: 1082592; Appendix B). I caught silver-haired bats (*Lasionycteris noctivagans*; Le Conte, 1831) at Long Point Bird Observatory (Long Point, Ontario, Canada), a well known stopover site for migratory birds and bats (Dzal et al., 2009). Bats were caught using mist nets (3 – 10m in height x 3 – 12 m long, 0.5m above ground) from 1 April to 3 June (spring migration) and 15 August to 30 September (fall migration) 2016 to sample an entire migratory year. Nets were opened approximately 30 minutes

before sunset and were closed 30 minutes after sunrise. Nets were closed during periods of inclement weather (e.g., high winds and rain) or when temperature was below 3°C. All handling and processing of bats was performed in a mobile laboratory (Field Laboratory for Integrative Ecological Research - FLIER). Sex was determined by genital inspection, age (juvenile or adult in fall only) by observing the degree of epiphyseal ossification of the second metacarpal joint in the fourth digit (Kunz and Anthony, 1982), and mass was measured ( $\pm 0.01$  g; CS200; OHAUS, Parsippany, New Jersey). We also palpated the torso of female bats upon capture to assess whether any bats were noticeably pregnant (Jonasson, 2017). Although I was not able to detect pregnancies this way, it was likely caused by the early stage of gestation. Up to 10 bats per night were held in light cotton bags from the time of capture until the next morning to be used in torpor experiments. To prevent pathogen transfer between bats, bags were used once and washed using scent free detergent.

#### Energy and torpor quantification

I measured daytime energy expenditure and torpor use by hanging the cloth bags containing bats in a temperature controlled cabinet ( $\pm 0.2^\circ\text{C}$ ; model PTC-1 with PELT-5 temperature controller; Sable Systems, Las Vegas, NV, USA) for approximately 12 hours, from 07:00 to 19:00 (spring: mean  $t_{\text{start}} = 07:08 \pm 19.4$  min; mean  $t_{\text{stop}} = 19:40 \pm 20.8$  min; mean trial duration = 12.22 h  $\pm 8.6$  min; fall: mean  $t_{\text{start}} = 07:45 \pm 45.3$  min; mean  $t_{\text{stop}} = 19:35 \pm 176.4$  min; mean trial duration = 12.25 h  $\pm 7.9$  min), to mimic a natural daytime stopover in both the spring and the fall. There were three temperature treatments: cold ( $9.39 \pm 0.64^\circ\text{C}$ ), intermediate ( $16.95 \pm 0.2^\circ\text{C}$ ), and warm ( $25.09 \pm 0.1^\circ\text{C}$ ). Bats were released after sunset at the capture site. Treatment temperatures were

selected to span a naturally occurring range of temperatures in both seasons that were within the capability of the equipment used. Treatment temperatures were also used to control for any potential effects of acclimatization due to naturally occurring  $T_a$  across arrival dates between migratory seasons (i.e., bats being captured 15 April:  $T_a = \sim 4^\circ\text{C}$  and 15 Sept.:  $T_a = \sim 14^\circ\text{C}$ ). Treatment temperatures were measured every 30 seconds throughout the duration of a trial using a Temperature/RH smart sensor cable attachment on a HOBO Micro Station ( $\pm 0.01^\circ\text{C}$ ; S-THB-M002, H21-002, Onset Computer Corporation, Bourne, MA, USA).

I weighed bats immediately before entry and upon exit from the temperature cabinet and body composition was quantified using quantitative magnetic resonance (QMR; EchoMRI-B; Echo Medical Systems, Houston, Texas, USA). QMR is a non-invasive technique for determining fat and lean mass of small species that does not require anesthesia ( $\pm 2\%$  wet lean mass (g) and  $\pm 10\%$  dry fat mass; (McGuire and Guglielmo, 2010). Changes in dry fat and wet lean mass were converted to kJ using conversions: dry fat mass (g)\*39.6 kJ/g and wet lean mass (g)\*5.3 kJ/g, and summed to calculate total energy expenditure (Gerson and Guglielmo, 2011).

Skin temperature ( $T_{sk}$ ) is considered an accurate surrogate measure of core body temperature ( $T_b$ ) (Audet and Thomas, 1996; Barclay et al., 1996; Jonasson, 2017; McGuire et al., 2014; McKechnie et al., 2007; Willis and Brigham, 2005; Willis et al., 2006). To record  $T_{sk}$  and assess torpor use throughout the temperature treatments, bats were outfitted with temperature sensitive radio-transmitters (0.3g;  $\pm 0.1^\circ\text{C}$ ; BD-2XT, Holohil Systems Ltd., Carp, ON, CAN) immediately after the QMR scan at capture. A small amount of hair was trimmed from the intrascapular region and the transmitter was

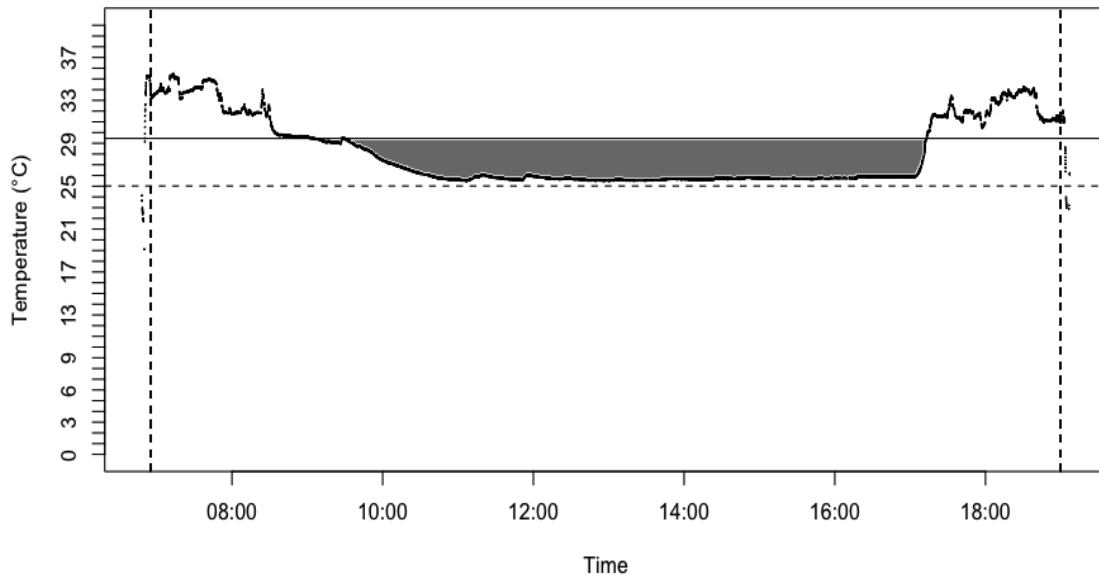


attached using a non-toxic latex glue (Osto-Bond, M.O.C., Montreal, CAN). Each transmitter produced radio pulses that were shorter than 23 ms, on a different VHF radio frequency within 0.048 MHz of the tag frequency, 3dB above background noise, and the pulse frequency varied with temperature. Raw transmitter signals (indicating  $T_{sk}$ ) were received by a custom built Sensorgnome receiver ([www.sensorgnome.org](http://www.sensorgnome.org)) which sampled each active transmitter for repeated ~4-8 second intervals throughout the day. Transmitters were removed after a treatment using a small amount of an adhesive solvent (Remove, Smith & Nephew, USA) and reused on other bats. The bats' fur that surrounded the transmitter was then washed of all solvent with a dilute biodegradable soap solution and dried thoroughly before the final QMR scan.

Filtering and conversion of raw transmitter pulse frequency data to  $T_b$  were calculated using methods outlined by Jonasson (2017). The raw transmitter signals were filtered for background and harmonic noise, using the *hampel* function in R (package: *pracma*; v 3.3.2), then converted from pulse frequency to  $T_{sk}$  ( $^{\circ}\text{C}$ ) by applying the pulse frequency data to calibration curve from the respective transmitter provided by the manufacturer. Calibration curves provided by the manufacturer were deemed accurate after placing each transmitter in a precision water bath ( $\pm 0.1^{\circ}\text{C}$ ; Lauda Eco, LAUDA-Brinkmann, Delran, New Jersey, USA) at  $5^{\circ}\text{C}$  increments from  $5\text{-}45^{\circ}\text{C}$  for five minutes (or until pulse frequency readings were stable), and finding that the frequency values did not differ between both calibration curves, prior to use on bats. After the conclusion of all experiments a second water bath calibration was completed using the same method as above with all transmitter pulse frequencies drifting a maximum of 8% from the initial calibration.

To quantify torpor use we used R to produce plots of  $T_{sk}$  by time (sec) as time course images (.png files) and processed them using ImageJ (version 1.49v, National Institute of Health, USA; for a visual example of the quantification of torpor see figure 1). I set a conservative torpor threshold for each bat, following the methods of previous torpor studies, using an equation that takes the lower limit of a 99% confidence interval of reported a normothermic mean for silver-haired bats ( $34^{\circ}\text{C}$ ; Jonasson, 2017; McGuire et al., 2014) and subtracts  $3^{\circ}\text{C}$  (to take into account differences in transmitter attachment) and each bat's body mass into account (equation 4 – Willis, 2007; Jonasson, 2017; McGuire et al., 2014). I used the 'area' tool to calculate the area between the torpor threshold and  $T_{sk}$  at any given time ( $A_{\text{Torpor}}$ ), for a minimum of 15 min (Jonasson, 2017; McGuire et al., 2014), during a treatment. By quantifying the area under the threshold, I was able to take into account some of the variability in  $T_{sk}$  in response to  $T_a$  as well as the duration of each torpor bout an individual entered. Individuals that use longer (duration), deeper (a greater difference between euthermic and torpid  $T_{sk}$ ), torpor therefore would have a larger  $A_{\text{Torpor}}$  value. I acknowledge the limits of using  $A_{\text{Torpor}}$  values to describe torpor behaviours, especially for individuals that entered shallow torpor for a long duration, and individuals that entered deep torpor for a short duration, but making this level of differentiation is not within the scope of this project or the questions I aimed to address. However, by using  $A_{\text{Torpor}}$  values instead of 'time spent in torpor' in combination with an 'average depth of torpor', I was able to simultaneously take into account differences in torpor use within treatments and energy used by any partial arousals (i.e., increases in  $T_{sk}$  that don't cross the torpor threshold). I did not use one of the recent techniques proposed for classifying torpor use (heterothermy index: Boyles et al., 2016 or

non-stationary waveform analysis: Levesque et al., 2017) that did not rely on an arbitrary torpor threshold or “cut-off”, to allow our data to be comparable to previous studies working in this study system (Jonasson, 2017; McGuire et al., 2014).



**Figure 1:** Example quantification of torpor use ( $A_{\text{Torpor}}$ ) from skin temperature ( $^{\circ}\text{C}$ ;  $T_{\text{sk}}$ ; thick black points) of a silver-haired bat (*Lasionycteris noctivagans*) during a daytime stopover roosting at  $25^{\circ}\text{C}$  (horizontal dashed line). The torpor threshold (horizontal solid black line) is calculated based on the lower limit of a 99% confidence interval around the mean of the euthermic  $T_{\text{sk}}$ .  $A_{\text{Torpor}}$  is represented by the dark grey shaded area under the torpor threshold and above the  $T_{\text{sk}}$  trace.

### Statistical analyses

All statistical analyses were run in R (version 3.3.2, R Core Development Team, 2016). Linear models (analysis of variance: ANOVA and analysis of co-variance: ANCOVA) were used to analyze the effects of season, sex, body mass, age, on  $A_{\text{Torpor}}$  and  $\text{En}_{\text{Total}}$

(Zuur et al., 2009). I used backwards-step-elimination to remove non-significantly contributing terms from models ( $\alpha=0.05$ ) until only significant terms remained (Zuur et al., 2009). In the case of significant interaction terms (between two or more predictor variables) we reanalyzed the data by creating new linear models (ANOVA or ANCOVA) by sub-setting or splitting the interaction term (Zuur et al., 2009). After splitting interaction terms, I included all predictor variables from the original linear model, including all possible interactions between the remaining predictor variables. For comparing means of two groups independently of other factors, I used Student's t-tests (Zuur et al., 2009). General additive models (GAM) were used to take into account non-linear relationships, specifically in models that included temperature and torpor use (*mgcv* function: R; Zuur et al., 2009). Akaike's information criterion (AIC) were used to compare linear and non-linear models that included the same variables to determine the model of best fit for the given data in question. A model is "preferred" or, better fits a dataset, when it has the lowest AIC value, by at least 2 points, between the models in question (Welham et al., 2015). All results were reported using Type III sum of squares using the *drop1* function in R to report values taking into account a randomized order of terms included in each linear model. The distribution of all variables were assessed using Shapiro-Wilks test (*shapiro.test* function: R) and *qqplot*'s when assessing normality of residuals from linear models. Variables that were not normally distributed were transformed (i.e., natural log or square root) to satisfy the assumption of normality within linear models. Mass was transformed using a natural log ('ln(mass)' from hereafter) and  $A_{\text{Torpor}}$  was square-root transformed (' $A_{\text{Torpor}}$ ' from hereafter) to meet the assumption of normality.

## RESULTS

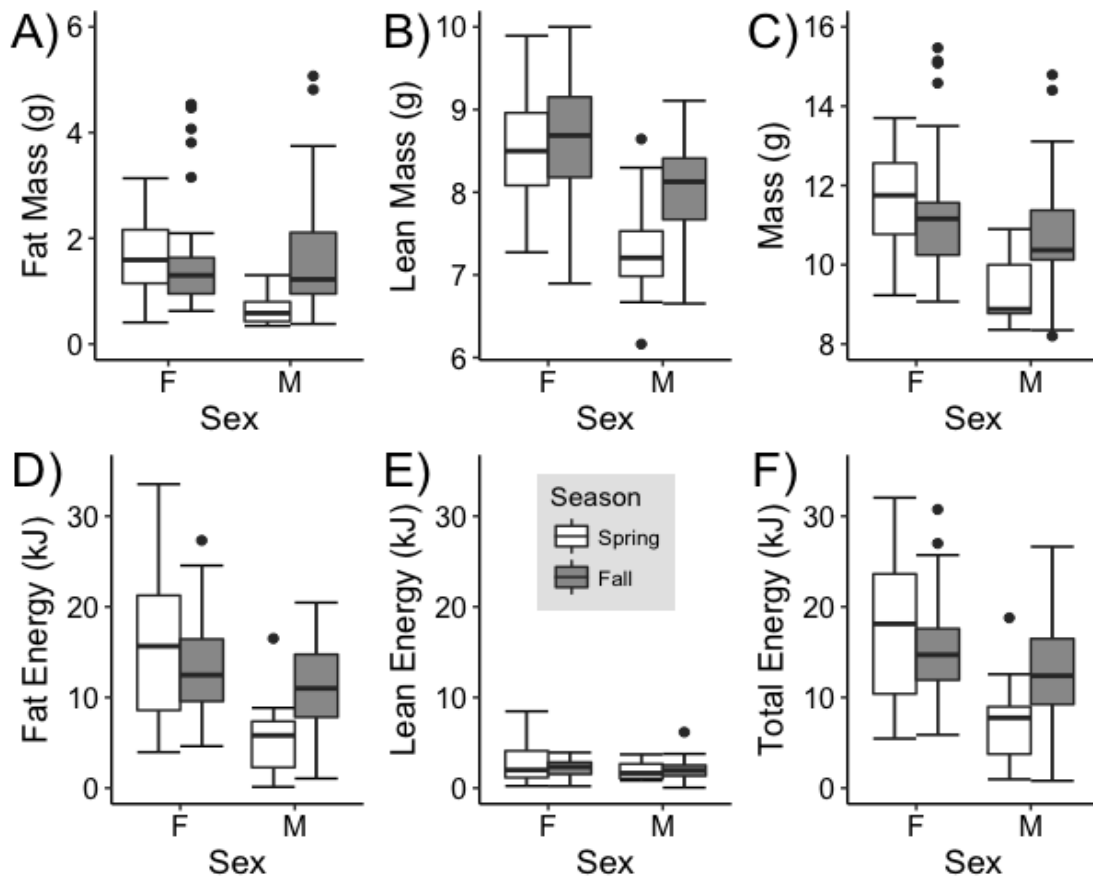
Body composition during a daytime stopover varied with season, sex, body mass, and ambient temperature (Table 1). There were differences in body mass ( $F_{5,143} = 10.46$ ;  $P < 0.0001$ ), fat ( $F_{5,134} = 8.83$ ;  $P < 0.0001$ ), and lean mass ( $F_{5,134} = 9.78$ ;  $P < 0.0001$ ) of silver-haired bats between seasons, sexes, and age classes upon arrival at stopover. In general females had greater body mass, fat and lean mass than males in the spring. Males were lighter and more lean compared to both spring females and fall bats of both sex and age classes. Spring females did not differ greatly in body composition and mass from fall bats. There were also minimal differences observed in body composition and body mass between bats in the fall.

**Table 1:** Body mass, dry fat mass, and wet lean mass (g) of adult (Ad) and juvenile (Juv) silver-haired bats (*Lasionycteris noctivagans*) during stopover at Long Point, Ontario in spring and fall 2016 before entering a daytime stopover temperature treatment. No juvenile bats were found during spring migration. Shared letters indicate no significant difference at  $\alpha = 0.05$  by ANOVA followed by a Tukey's multiple comparison test.

Sex	Season	Age	N	Mass (g)	Fat (g)	Lean (g)
Female	Spring	Ad	48	11.59 ± 1.29 <sup>a</sup>	1.58 ± 0.64 <sup>a</sup>	8.54 ± 0.65 <sup>a,b</sup>
		Ad	23	11.03 ± 1.39 <sup>a</sup>	1.88 ± 1.22 <sup>a</sup>	8.72 ± 0.75 <sup>a</sup>
	Juv	12	10.71 ± 0.70 <sup>a,b</sup>	1.09 ± 0.24 <sup>a,b</sup>	8.36 ± 0.61 <sup>a,b,c</sup>	
Male	Spring	Ad	12	9.24 ± 1.00 <sup>c</sup>	0.67 ± 0.31 <sup>b</sup>	7.34 ± 0.75 <sup>d</sup>
	Fall	Ad	31	11.58 ± 1.68 <sup>a,b</sup>	1.88 ± 1.16 <sup>a</sup>	8.12 ± 0.57 <sup>b</sup>
		Juv	14	10.02 ± 0.86 <sup>b,c</sup>	1.04 ± 0.28 <sup>a,b</sup>	7.92 ± 0.57 <sup>c,d</sup>

Energy expenditure during roosting

Combining energy expenditure (based on QMR data) of bats from both seasons and all three temperature treatments, female bats, on average, used around 39% more energy than males during a daytime stopover (females:  $16.64 \pm 6.25$  kJ; males:  $10.19 \pm 4.58$  kJ;  $t = 4.91$ ,  $df = 134.08$ ,  $P < 0.0001$ ). During spring migration, females used approximately 58% more energy than males (females:  $18.16 \pm 7.63$  kJ; males:  $7.56 \pm 3.90$  kJ;  $t = 5.75$ ,  $df = 27.39$ ,  $P < 0.0001$ ; Fig. 2). During fall migration, female bats used around 18% more energy than males (female:  $15.11 \pm 4.87$  kJ; male:  $12.83 \pm 5.25$  kJ;  $t = 2.16$ ,  $df = 72.60$ ,  $P = 0.034$ ; Fig. 2).



**Figure 2:** Body composition, body mass and energy expenditure of female and male silver-haired bats from both the spring (white boxes) and fall (grey boxes). **A)** Dry fat mass (g), **B)** wet lean mass (g), quantified using quantitative magnetic resonance. **C)** body mass of female and male bats taken before exposure to temperature treatment. **D,E,F)** Fat, lean, and total energy expended (kJ) during a 12-hour daytime stopover at one of three temperature treatments (cold, intermediate, or warm). **F)** Total energy expended is the summation of fat and lean energy expended based on substrate to energy conversions provided by Gerson and Guglielmo (2011; Dry fat mass: 39.6 kJ/g; wet lean mass: 5.3 kJ/g).

To determine if energy use during daytime migratory stopover is independent of ambient roosting temperature across both fall and spring migratory seasons, I created linear models to test for effects of predictor variables on energy use and to address significant effects of two and or three-way interactions between predictor variables on  $En_{Total}$ . I carried out a similar analysis but replaced  $En_{Total}$  with  $A_{Torpor}$ , to assess the effects of the same predictor variables on torpor use.

The initial global energy model included  $En_{Total}$  as the response variable, season,  $\ln(\text{mass})$ , sex, temperature, and all two-way, three-way and four-way interactions between predictor variables. The three-way interaction between season, sex and  $\ln(\text{mass})$  significantly predicted  $En_{Total}$  ( $F_{1,131} = 8.43$ ;  $P = 0.004$ ). There was also a significant effect of the temperature and season interaction ( $F_{1,129} = 4.77$ ,  $P = 0.03$ ), while all other two and three-way interaction terms including ‘temperature’, did not affect  $En_{Total}$  ( $P > 0.05$ ).

To address the three-way interaction between season, sex and  $\ln(\text{mass})$ , I divided the global model into two female-only, season-controlling, models (one for spring and one for fall females) and two male-only, season-controlling, models (one for spring and one for fall males), where I included  $\text{En}_{\text{Total}}$  as the response variable,  $\ln(\text{mass})$ , temperature, and the two-way interaction between mass and temperature as predictor variables. Age, and all interactions with the other predictor variables (stated above), were also included in the fall models.

For spring-females, there was a positive relationship between  $\ln(\text{mass})$  and  $\text{En}_{\text{Total}}$  ( $F_{1,46}=55.52$ ;  $P<0.0001$ ) with heavier female bats using more energy (Eqn:  $y = 53.20x - 111.96$ ;  $R^2 = 0.54$ ;  $F_{1,46} = 55.52$ ; Fig. 3C). There was no effect of the interaction between, or the main effects of,  $\ln(\text{mass})$  and temperature ( $P>0.05$ ). For fall-females, temperature,  $\ln(\text{mass})$ , age, and all of the two- and three-way interactions between the predictor variables did not predict  $\text{En}_{\text{Total}}$  ( $P>0.05$ ). When compared to spring females, there was considerably more variation in  $\text{En}_{\text{Total}}$  as body mass increased in fall, and the maximum body mass found in the fall was larger than the spring. The largest females captured in the fall drove the lack of relationship between  $\text{En}_{\text{Total}}$  and body mass. Thus, in spring body mass was a strong predictor of roosting energy expenditure, and in both seasons  $T_a$  was not important.

For spring-males, there was no effect of  $\ln(\text{mass})$ , temperature or their interaction ( $P>0.05$ ) on  $\text{En}_{\text{Total}}$ . For fall-males, the age\* $\ln(\text{mass})$  interaction was significant ( $F_{1,41}=11.44$ ,  $P= 0.002$ ). There was no effect, however, of all other two and three-way interactions or main effects of temperature,  $\ln$  mass and age ( $P>0.05$ ) on  $\text{En}_{\text{Total}}$  for fall males. The interaction between  $\ln(\text{mass})$  and age is likely produced by the difference in



mean body mass between adult and juvenile males in the fall, and in both season  $T_a$  was not important.

To address the interaction between age and mass for fall-males, I separated the ages and compared the effects of mass on  $En_{Total}$ . Adult males had larger masses in the fall than juveniles ( $t = -2.95$ ,  $df = 34.41$ ,  $P = 0.006$ ). However,  $En_{Total}$  did not respond the same to  $\ln$  mass between age categories. Figure 2D shows the positive relationship between  $En_{Total}$  and mass for juvenile males (Eqn.  $y = 60.22x - 124.26$ ;  $F_{1,12} = 16.24$ ;  $P = 0.002$ ;  $R^2 = 0.54$ ), and the lack of relationship between mass and  $En_{Total}$  for adult male bats in the fall.

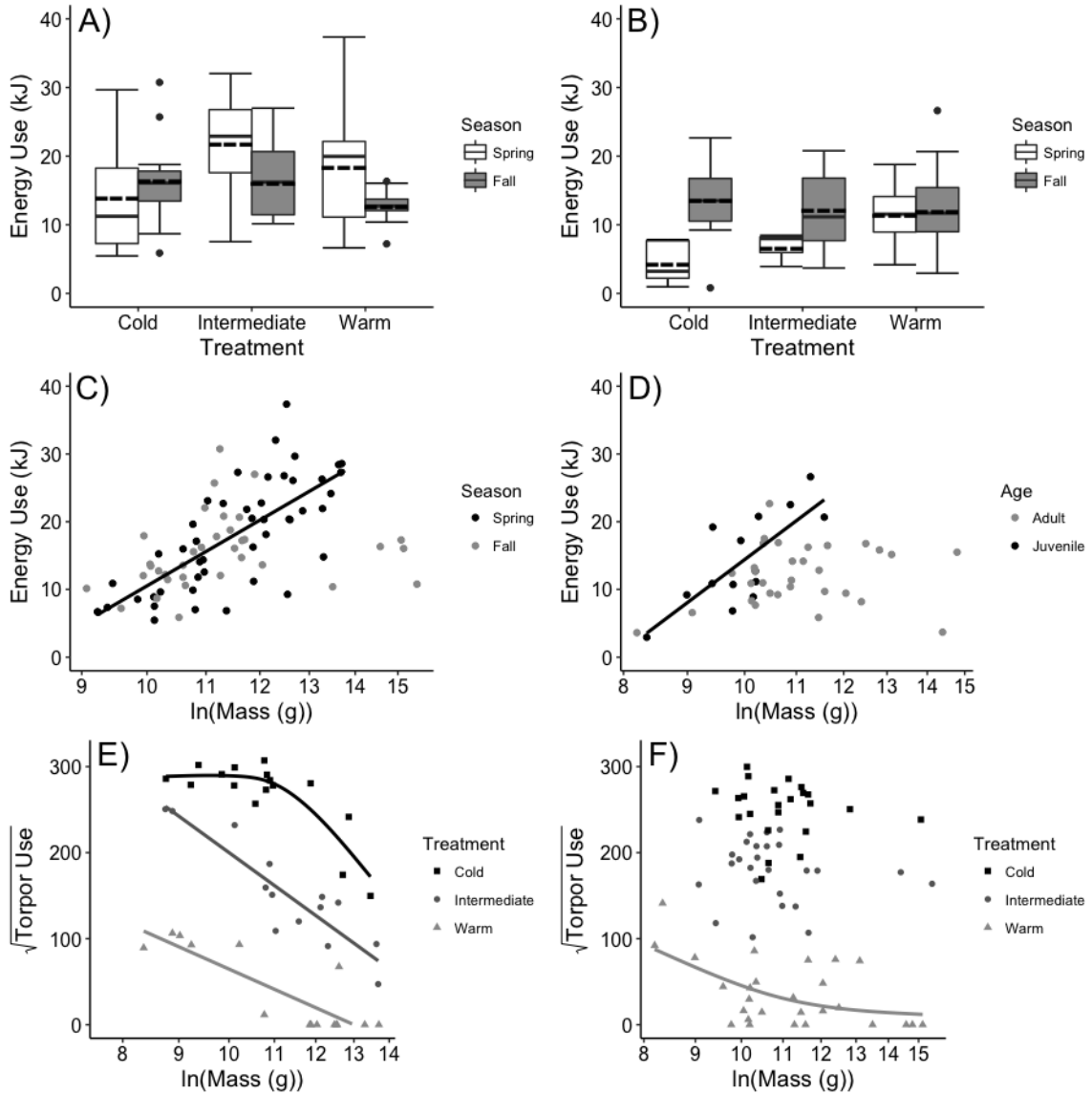
#### Torpor use

From the global 'torpor-use' model, the interaction between  $\ln(\text{mass})$  and season as well as temperature significantly predicted  $A_{Torpor}$  ( $F_{1,117} = 17.65$ ,  $P < 0.0001$ ;  $F_{1,117} = 851.64$ ,  $P < 0.0001$ , respectively). There was no effect of any of the other interaction terms between predictor variables or sex on  $A_{Torpor}$  ( $P > 0.05$ ). Thus, the body mass upon arrival at stopover affects torpor use differently between seasons and there is a negative relationship between torpor use and temperature (Table 2).

**Table 2:** Torpor use ( $A_{\text{Torpor}}$ ; mean  $\pm$  SD) of silver-haired bats exposed to one of three temperature treatments (cold, intermediate or warm) during a 12-hour daytime stopover, from both spring (1 Apr. - 3 June, 2016) and fall (15 Aug. – 30 Sept., 2016) migratory seasons. For more detail regarding the calculation and quantification of torpor use see the methods section.

	Cold		Intermediate		Warm	
	Female ( $A_{\text{Torpor}}$ )	Male ( $A_{\text{Torpor}}$ )	Female ( $A_{\text{Torpor}}$ )	Male ( $A_{\text{Torpor}}$ )	Female ( $A_{\text{Torpor}}$ )	Male ( $A_{\text{Torpor}}$ )
<b>Spring</b>	72401	76179	19834	49976	1833	1014 $\pm$
	$\pm$ 22374	$\pm$ 8820	$\pm$ 14339	$\pm$ 21279	$\pm$ 3440	1778
	(n=13)	(n=3)	(n=11)	(n=3)	(n=12)	(n=3)
<b>Fall</b>	69157	61965	31445	35531	1205	3322
	$\pm$ 15693	$\pm$ 17455	$\pm$ 11823	$\pm$ 13349	$\pm$ 2403	$\pm$ 4968
	(n=11)	(n=13)	(n=13)	(n=13)	(n=9)	(n=18)

After separating seasons and combining sexes, there were negative relationships between  $\ln(\text{mass})$  and temperature on  $A_{\text{Torpor}}$  in the spring at all temperatures, and for bats roosting at warm temperatures in the fall. For spring bats, there were significant negative effects of  $\ln(\text{mass})$  ( $F_{1,41} = 86.95$ ;  $P < 0.0001$ ) and increasing temperature ( $F_{2,41} = 215.47$ ;  $P < 0.0001$ ) on  $A_{\text{Torpor}}$ , where heavier bats used less torpor and bats roosting at warmer temperatures used less torpor (Fig. 3E). For fall bats, there was also a significant negative relationship between temperature ( $F_{2,73} = 256.55$ ;  $P < 0.0001$ ) and  $\ln(\text{mass})$  ( $F_{1,73} = 8.11$ ;  $P < 0.006$ ) on  $A_{\text{Torpor}}$ , where bats roosting at cold temperatures used the more torpor than bats roosting at warm temperatures. However, when bats from each treatment temperature were analyzed separately there was only an effect of body mass at warm temperatures, where heavier bats used less torpor than lighter bats (Fig. 3F).



**Figure 3:** **A) & B)** Total energy use (kJ) by silver-haired bats during a full daytime stopover (~12 hours) from either the spring (white filled boxes) or fall (grey filled boxes) migration season, at one of three temperature treatments (cold, intermediate, or warm). Energy use is independent of  $T_a$  in both season for both female (**A**) and male (**B**) bats. **C)** Total energy use (kJ) during a full daytime stopover and body mass (g; natural log transformed) for females in both seasons (spring: black; fall: grey). The black trend line represents the positive relationship between energy use and ln(mass) within spring-

females (Eqn:  $y = 53.20x - 111.96$ ;  $R^2 = 0.54$ ;  $F_{1,46} = 55.52$ ;  $P < 0.0001$ ). **D**) Total energy use (kJ) during a full daytime stopover and body mass (g; natural log transformed) of young-of-the-year (juvenile: black) and older than one-year (adult: grey) male silver-haired bats during fall migration. The black trend line represents the positive relationship between energy use and  $\ln(\text{mass})$  within the juvenile male bats (Eqn.  $y = 60.22x - 124.26$ ;  $F_{1,12} = 16.24$ ;  $P = 0.002$ ;  $R^2 = 0.54$ ). There is no relationship between energy use and body mass for adult male bats. **E&F**) Comparing the effects of body mass ( $\ln(\text{mass})$ ) on  $A_{\text{Torpor}}$  (square-root transformed) from 12-hour daytime stopovers between temperature treatments: cold (black square), intermediate (dark grey circle), and warm (light grey triangle), for spring (**E**) and fall (**F**) silver-haired bats. **E**) A non-linear line (black) calculated from a GAM that describes the relationship between  $A_{\text{Torpor}}$  and  $\ln(\text{mass})$  for bats roosting at cold temperatures ( $\text{edf} = 1.91$ ,  $\text{Ref. df} = 1.99$ ,  $F = 20.77$ ,  $P < 0.0001$ ,  $R^2 = 0.78$ ). The negative relationships between  $A_{\text{Torpor}}$  and  $\ln(\text{mass})$  for both intermediate and warm treatments are described by the dark (Eqn:  $y = 1172.04 - 420.21x$ ;  $F_{1,9} = 16.34$ ,  $P = 0.003$ ,  $R^2 = 0.61$ ) and light grey trend lines (Eqn:  $y = 623.40 - 243.12x$ ;  $F_{1,10} = 10.59$ ,  $P = 0.009$ ,  $R^2 = 0.47$ ). **F**) A non-linear line (light grey) calculated from a general additive model that describes the relationship between  $A_{\text{Torpor}}$  and  $\ln(\text{mass})$  for bats roosting at warm temperatures ( $\text{edf} = 2.84$ ,  $\text{Ref. df} = 2.98$ ,  $F = 7.94$ ,  $P < 0.001$ ,  $R^2 = 0.45$ ).

## DISCUSSION

This study is the first to directly quantify energy expenditure of migrating bats during full-length daytime stopovers from both spring and fall migration. Overall, I found support for the prediction that, regardless of sex and season, energy use during a daytime stopover for migratory bats is independent of ambient roosting temperature as proposed by the torpor-assisted migration hypothesis (Fig. 3A&B; McGuire et al., 2014). Heavier spring females and fall juvenile male bats used more energy than lighter individuals within their respective sex and migratory season. Body mass was also found to predict torpor use where heavier spring individuals, regardless of sex, and fall individuals roosting at warm temperatures, used less torpor. I also confirmed the finding that during stopover, migratory bats use torpor facultatively, using more torpor when roosting at cold  $T_a$ , but I did not find the predicted decrease in torpor use by spring females with rising temperature (Fig. 3E&F).

### *Body mass, torpor, and energetics at stopover*

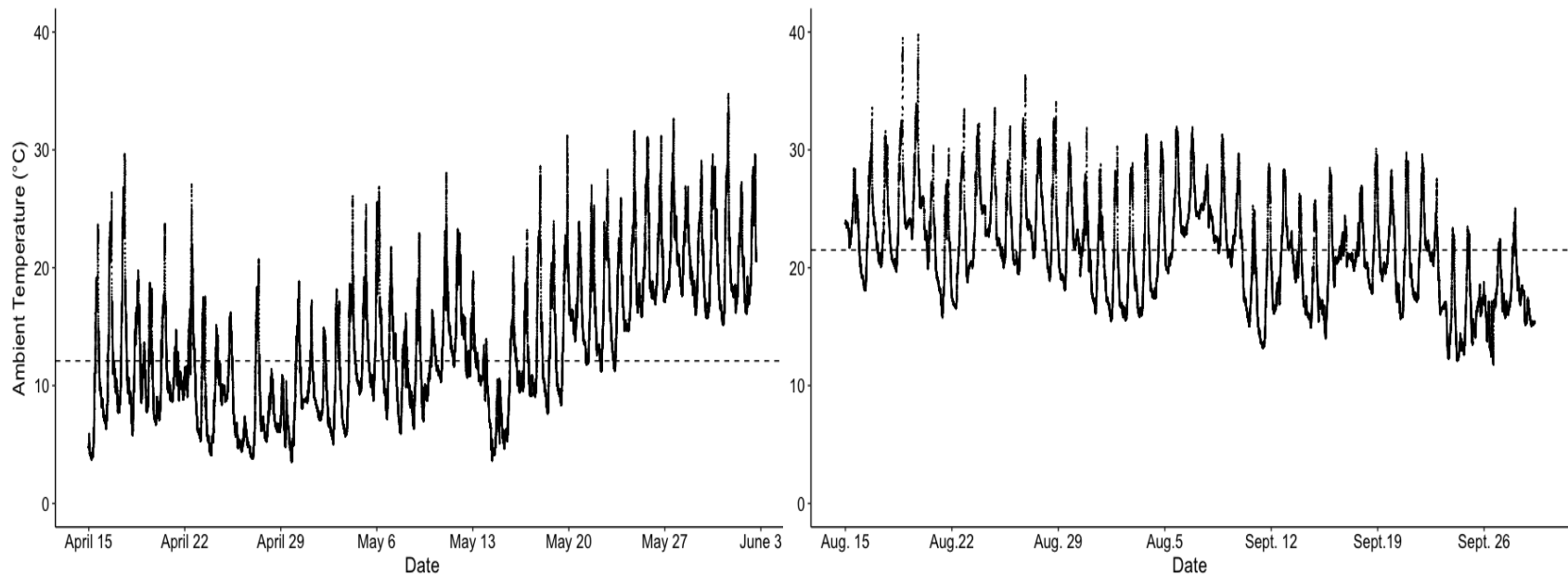
Female bats tended to use more energy in the spring than in the fall, despite having comparable body masses in the two seasons. Spring females also tended to show larger individual variation in energy use than fall females, and males from both seasons, which can be explained by considering the variation in, and the effect of body mass on energy use (mass range: 7.69-13.77 g; Fig. 3C&F). The effects of variation in body mass upon arrival at stopover, the departure dates or conditions of previous stopover sites, migration speeds, and different stages of pregnancy, all require further testing to determine the totality of their impacts on energy budgets for migratory bats.

There were differences in energy use between sexes in the spring, with females using more energy at the cold temperatures than males and tending to do so at all temperatures. The difference in energy use between sexes agrees with findings and predictions proposed by Jonasson (2017). Using radio-telemetry of free-ranging bats, Jonasson (2017) found female bats to use less torpor than males, suggesting that this was an adaptation to maintain the rate of foetal development, which has been observed several times in bats (Cryan and Wolf, 2003; Dzal and Brigham, 2013; Geiser, 1996; Lausen and Barclay, 2003; Willis et al., 2006). The difference between these previous studies, Jonasson (2017) and this study, is that they did not look at migratory bats during stopover (but rather maternity sites) or failed to specify the role of the particular capture habitat with respect to migration or reproduction. If pregnant females were using less torpor they should use more energy than males in spring, and perhaps more appropriately, more energy than non-pregnant females in the fall (Cryan and Wolf, 2003; Grinevitch et al., 1995; Hamilton and Barclay, 1994). But, this does not appear to be the case for this study, as I did not find a statistical difference in energy use between spring and fall females, or torpor use between seasons or sexes. There was some deviation in torpor use between spring and fall females at intermediate roosting temperatures, with females using less torpor in the spring than the fall, but the linear models did not demonstrate treatment temperature to be a significant predictor of energy use.

I found significant negative relationships between body mass and torpor use for spring migrants, regardless of sex at all three treatment temperatures, and for bats held in the warm treatment in the fall. The variation in energy use of bats with intermediate body masses in the fall could be explained by the variation in age. Although age did not

significantly predict energy use, which one might predict if juvenile bats were not as physiologically developed and therefore would not respond similarly to  $T_a$  as adults. It could be that our age classification system is too coarse to truly assess physiological capacity at different stages of development for juvenile bats. Creating a more sensitive age classification system might explain some of the variation in torpor use for fall bats. Considering the scope of this study and the higher average environmental  $T_a$  in the fall than the spring (Fig. 4; Spring: mean:  $12.1 \pm 4.7^\circ\text{C}$ ; range:  $3.6\text{-}34.7^\circ\text{C}$ ; Fall: mean:  $21.5 \pm 4.3^\circ\text{C}$ ; range:  $11.8 - 39.8^\circ\text{C}$ ), however, I hypothesize that fall bats are not as acclimatized to respond to cold temperature challenges as spring bats. It also could be that if I had caught more individuals with especially high body masses, that the negative trends between torpor use, body mass and  $T_a$ , might persist. To best assess the effects of season (or stage of reproduction) and body mass on energy and torpor use during stopover, an experiment should be designed to control for mass of bats (especially between sexes) as well as take place throughout the entire of the spring migratory season to monitor the use of torpor throughout pregnancy.

Similar to McGuire et al. (2014), I found that there were no sex differences in torpor use during fall migration (Fig. 3E&F). Due to the larger sample size in my study than McGuire et al. (2014), I also found an effect of age when analyzing energy use by male bats in the fall. The difference in energy use between juvenile and adult males is again driven by body mass (Fig. 3D). There is likely selection pressure for juvenile males to fatten quickly and acquire ample fat stores so they are able to participate in the fall migration, and or to potentially increase the rate of development and allow mating in their first year. Thus, there are reasons to believe that migration could be experienced different



**Figure 4:** Ambient temperature traces from Long Point Bird Observatory, Long Point, ON, from both spring (15 April – 3 June, 2016; left panel) and fall (15 August – 30 September, 2016; right panel) sampled every 30 seconds. Dashed lines represent mean  $T_a$  (spring: 12.1°C; fall: 21.5°C) and juvenile bats.



between adults and juvenile bats. Adults may have experience, or be more physiologically developed to thermoregulate efficiently, but from my data, it appears that juveniles are just as prepared as adults of similar mass to handle stopover and thermoregulation (see Woodrey and Moore, 1997 for discussion on migratory experience). The only recorded difference between adult and juvenile bats in this study was body mass, which is possibly related to the time allotted for each age class to acquire fat during the summer. Adult male bats have more time and are able to allocate more resources to depositing fat, whereas some resources will be put to growth and development for juvenile bats. It appears that migration costs are related to body mass, and so there should be pressure for bats to grow quickly, develop efficient hunting skills quickly to maximize the short fattening period, fatten quickly while minimizing energy expenditure (which would increase the rate of fat deposition; Ewing et al., 1970), and select food items that maximize nutrient deposition.

The effect of body mass on energy use is not necessarily surprising when one considers the lack of dimorphism in body size between sexes for silver-haired bats (Jonasson, 2017; McGuire et al., 2014). Differences in body mass are due to differences in body composition (i.e., fat and or lean mass), and could support the hypothesis that larger fat stores are being selected for within migratory bats. Jonasson (2017) and this study found body composition differences with spring females having more fat than males upon arrival at stopover in spring (Table 1), fitting with the prediction that females that are pregnant or are preparing for pregnancy will have sufficient fat stores for the costs of foetal development and lactation.

Fall migrant silver-haired bats have been found to have an average of 19.3% body fat (range: 11.9-28.7%; McGuire et al., 2012), however, I found the averages to be lower (females: 14.0%, males: 15.1%). Spring migrants had 8.9% body fat (Jonasson, 2017) while, I found 10.5% (females: 13.6%, males; 7.3%). The amount of body fat in migrating bats, regardless of season and sex, is much lower than the 50% and 60% found in male and female (respectively) ground squirrels, or approximately 40% body fat seen in pre-hibernal bats (little brown bats: Kunz et al., 1998; ground squirrels: Sheriff et al., 2013). Pre-migratory birds also increase fat stores during migration and may be a more realistic comparison for migrating bats as ground squirrels are larger and use their fat stores to fuel hibernation in burrows. Migratory semipalmated sandpipers (*Calidris pusilla*) have been found to have 30-39% body fat prior to migrating in the fall (Page and Middleton, 1972). Due to their small body size, my study provides an example of the physical constraints on the capacity to store fat for bats. Flying species need to increase lean mass proportionally to fat deposition to balance power production (by increasing the size of flight muscles) and the increasing energy expenditure during flight per unit of mass (Lindström and Alerstam, 1992). Counter intuitively, smaller bats and birds have larger power margins (the difference between the maximum flight power they can produce and how much power they need to fly given their body mass) and greater non-stop flight distances per unit of fuel than large bats and birds (Hedenström and Alerstam, 1992; Klaassen, 1996; Norberg and Rayner, 1987). Thus, it is unlikely that the reduced capacity to store fat is related to restrictions in power production by flight muscles (within reasonable limits), or to pressures selecting for larger maximum fat stores for migrating silver-haired bats. Bats have large power margins to counter fluctuations in

body mass or “total load” while flying (Norberg and Rayner, 1987). Bats carry their young during pre-volant development while foraging or carrying young to switch roosts, and body mass often fluctuates on a daily basis and vastly in preparation for hibernation (for review: Norberg and Rayner, 1987).

Alternatively, migratory bats may maintain a low percent body fat (relatively to pre-hibernal bats) to facilitate increased maneuverability for foraging while at stopover or en route. By maintaining a relatively lower body mass their wing loading decreases, which increases the ability to make rapid and tight turns that may be necessary for catching insects during flight (Norberg and Rayner, 1987). The constraint on the size of endogenous fat stores due to reductions in maneuverability may explain why we see such thermal plasticity and efficient energy use strategies (i.e., using torpor during stopover) in temperate zone bat species. Insect availability might be a crucial exogenous energy source for fueling flight and re-fueling endogenous energy stores, as well as be a key characteristic of a suitable stopover site for migrating bats.

Migratory bats may need to spend less energy to cover migratory distances (on account of their smaller, more efficient morphology for flight), thus removing pressures of maximizing the size of fat stores. However, given the other metabolic constraints of being relatively small animals (i.e., high surface area to volume ratio, increased thermal conduction, etc.) and the variable  $T_a$  migrating bats can experience throughout their journey (Fig. 3), their efficient morphology does not undercut the importance of having relatively stable and adequate fat stores through migration (discussed below – *Energy budgets for migrating bats*).

Despite the similarities between migrating bats and birds there are many differences, such as morphology, but other differences, such as physiological differences (some of which were discussed in the introduction; i.e., torpor, etc.) are less overt. A less obvious difference between bats and birds is the dramatic difference in percent body fat upon arrival at the same stopover (Long Point, ON) between bats and birds. Bats have significantly higher percent body fat than comparably sized migratory songbirds, which is consistent in both the spring and the fall migratory seasons (yellow-rumped warbler (*Setophaga coronata*): spring: 3.2%, fall: 0.5%; western palm warbler (*Setophaga palmarum*): spring: 2.3%, fall: 2%; house wren (*Troglodytes aedon*): spring: 1.0%, fall: 1.1%; Kennedy, 2012). The differences in fat loads upon arrival at stopover (and in-hand, migratory strategies) between migrating bats and birds may be adaptations to differences in life-histories and behaviour. Although many bird species migrate at night to take advantage of better flying conditions, bats are exclusively nocturnal which means that they have to stopover more frequently (opposed to song birds stopping when fat stores are depleted). The difference in fat load upon arrival at stopover only highlights then the importance of Long Point as a stopover site in general and potentially being a crucial habitat for migrating birds.

#### Energy budgets for migrating bats

By directly quantifying the energy used during stopover in both seasons at different  $T_a$  we are a step closer to determining the daily expenditure made by migrating bats. Other previous studies have also predicted energy use by bats during migration. McGuire et al. (2014) estimated that migrating silver-haired bats used fat at a rate of 0.98 mg/g/h (range: 0.3 - 2.0 mg/g/h), during daytime stopovers. If one takes into account the mean mass of

bats in the study (10.6 g) and total for 12 hours (the length of a daytime stopover), bats would be predicted to use an estimated 0.12 g of fat (range: 0.038 - 0.25 g of fat). Although the maximum estimate of 0.25 g of fat per daytime stopover is lower than the mean 0.28 g of fat per daytime stopover (this study), there was no difference in overall body mass (10.6 g and 10.7 g) between McGuire et al. (2014) and this study (more fat available = more fat used; this study), nor were bats tested at dramatically different  $T_a$  (McGuire et al., 2014: ~15-27°C; this study: ~9-25°C). There was much greater variation in fat mass used during daytime stopover with the maximum fat use being 70% greater than the maximum fat use estimated from McGuire et al. (2014). Besides the difference in sample size between McGuire et al. (2014) and this study (McGuire et al. (2014): 24; this study: 123) the inter-annual variation in energy use could be attributed to an underestimation of energy use by McGuire et al. (2014). McGuire et al. (2014) estimated energy use based on respirometry and field  $T_b$  data, whereas energy use was directly quantified in this study. Alternatively, the bats measured in this study could have behaved differently than they would have if they had been roosting in the wild like in McGuire et al. (2014). An effect of the artificial roosting environment on energy use behaviour is unlikely considering bats were handled in both studies, and kept in the same temperature-controlled cabinet for both respirometry (McGuire et al. 2014) and my roosting  $T_a$  treatments.

Tuttle (1976) estimated total migratory energy expenditure of the short distance migrant, gray bat (~15g) is 1.5-4 g of fat during their migration to their hibernation grounds (stopovers and migratory flight included). For gray bats, the migratory distance is estimated to be <500 km (between maternity and hibernation sites; Tuttle, 1976) but no

estimates have been made regarding how long this journey takes, or the number of stopovers required. However, during the migration from maternity to hibernation sites, Tuttle (1976) estimated that gray bats lose 0.5 g per 100 km of flight (for bats traveling ~450 km). McGuire et al. (2012) ran flight simulation models to estimate energy expenditure and potential “time until empty” (referring to fat stores), distances by migrating silver-haired bats (based on flight speeds of migrating silver-haired bats as they flew through a telemetry array in southern Ontario and basal metabolic rate (BMR) from allometric curves from Speakman and Thomas, 2003). McGuire et al. (2012), suggests that ~13 g silver-haired bats could cover 250-300 km per night and travel at least 1393 km (range: 1393-4135 km; roughly at least the distance from Long Point to the Gulf of Mexico) over 5-6 nights with the largest individuals (>13 g) still having a portion of stored fat remaining.

If we consider that the average amount of fat used by migratory silver-haired bats of both sexes in both migratory seasons during one 12-hour daytime stopover is  $0.28 \pm 0.14$  g, (range: 0.027 - 0.85 g), the estimated cost of 0.5 g of fat per 100 km of the distance (Tuttle, 1976), and the estimated the migration speed of 6 nights (with 5 daytime stopovers) to cover 1400 km, silver-haired bats would require 8.4 g of fat. This would require a minimum of 10 hours of flight at ~23.5 km/h, which is within their capacity, and may even be a conservative flight speed for silver-haired bats (Jonasson, 2017).

Although gray bats are of similar body mass as silver-haired bats, they are morphologically different in terms of wing shape and aspect ratio, which may change the estimated costs of flight (Tuttle, 1976; Norberg and Rayner, 1987; Savile, 1956).

Estimates by Tuttle (1976) did not take into account any fat accumulation prior to or post

migratory flight and capture. Theoretically, migratory costs of 8.4 g of fat for silver-haired bats is double the highest estimated costs for gray bats (4 g) while covering almost four times the distance. Migrating silver-haired bats have not been observed in the wild with more than 4 g of body fat, let alone 8 g required by these calculations to complete a migratory journey. Migration simulations from McGuire et al. (2012) suggest that migrating silver-haired bats complete the 1400 km journey on approximately 2.2 g of body fat (calculated using reported mean body mass of 11.5 g and 19% body fat). The large discrepancy between fat use estimations between McGuire et al. (2012) and this study could be attributed to the inclusion of weather variables and or the lack of inclusion of direct measurements of energy use during flight in computer simulations.

It is likely that migratory bats are supplementing their energy budgets by foraging at some point during migration. If the fat requirements are greater than predicted by McGuire et al. (2012), it is also likely that supplementing energy budgets by foraging also removes bats from the need to evolve a capacity to carry the total amount of fat that will be required for an entire migratory journey.

Regardless of differences in fat or energy use during stopover, no study has directly quantified energy use for flying migratory bats to date. Wikelski et al. (2003) calculated that stopovers make up 2/3 of total energy expended by migratory birds, however, the Swainson's and hermit thrush (*Catharus ustulatus* and *Catharus guttatus*, respectively) used in their study were not heterothermic. I predict that flight costs make up a larger proportion of migratory costs for bats as they are able to use torpor facultatively to counter costs of thermoregulation and will make up a larger portion of the energy budget for migrating bats than birds.

It is possible that bats are able to save enough energy using torpor during daytime stopovers that they do not need to forage – another prediction of the torpor-assisted migration hypothesis (McGuire et al., 2014). Jonasson (2017) as well as Voigt et al. (2012), however, proposed that bats forage opportunistically during spring stopover. The large variation in energy and torpor use for migrating bats, especially for spring-females (Fig. 2F and Fig. 3A&E), could be due to increased foraging, as proposed by Jonasson (2017) and found in Matheson et al. (2010) in hibernating little brown bats. Recent foraging has been shown to delay entry into torpor which would have decreased  $A_{\text{Torpor}}$  values for the bat we observed for spring-females (Matheson et al., 2010). Although insect abundance has been shown to increase with  $T_a$ , it could theoretically increase with date (i.e., date of capture) during the spring, assuming the collinear response of insect abundance to spring date and  $T_a$  (Fig. 4, Taylor, 1963; Baloun *unpublished*).

Due to the higher  $T_a$  in the fall and in-hand increase in abundance of insects, both sexes could forage equally and reduce any effects of feeding on energy use. If insect abundance is more consistent and high in the fall, and females are not pregnant, then energy expenditure for both sexes could be similar. In the fall, there was no difference in energy use between sexes across roosting  $T_a$ . If females eat more in the spring, it could explain why their energy expenditure is higher than that of males. Several studies have used breath  $\text{CO}_2$   $^{13}\text{C}$ -isotope analyses to determine feeding status and fuel use of bats at capture, and this method can allow us to determine if individuals are feeding during stopover and the possible effects of feeding on energy management during daytime stopovers (Podlesak et al., 2005; Voigt et al., 2008a,b). Stable  $^{13}\text{C}$ -isotope analysis of breath  $\text{CO}_2$  uses the natural difference in the ratio of heavy and light C atoms, ( $^{13}\text{C}/^{12}\text{C}$ )



that are found in different metabolic substrates (i.e., lipids, protein, carbohydrates) to determine what fuel is being metabolized at the time of sampling (Perkins and Speakman, 2001). A breath CO<sub>2</sub> sample that is <sup>13</sup>C-depleted would suggest the metabolism of lipids at the time of sampling (Hatch et al., 2002). A breath CO<sub>2</sub> sample that is <sup>13</sup>C-enriched, would suggest the metabolism of protein or carbohydrates (Hatch et al., 2002; Voigt and Speakman, 2007). Previous studies using this technique with insect-eating migratory bats showed that, when insects were available, bats metabolized insect protein over endogenous lipids (Voigt et al., 2012). I collected breath CO<sub>2</sub> samples from bats upon capture during both migratory seasons and, a preliminary analysis of <sup>13</sup>C-isotope values of the breath CO<sub>2</sub> suggests that bats forage during spring stopovers, and foraging increases with date of capture (Baloun *unpublished*). Although it is likely that bats are foraging during stopover, based on energy expenditure estimates as well as preliminary breath CO<sub>2</sub> isotope analyses, the effects of foraging at stopover as well as the availability of insects across seasons should be assessed to fully evaluate the energy budget of migrating bats and their ecology at stopover sites.

#### *Preferred roost temperatures and the energetics of a thermal neutral zone*

My data suggest that from an energy expenditure stand-point, there is no “preferred” roosting T<sub>a</sub> for migrating bats, however, there are downsides to torpor use that could be important. The preferred T<sub>a</sub> for migratory bats, and especially for pregnant females, would be any T<sub>a</sub> that eliminates the need to enter torpor (i.e., >25°C; this of course assumes that rapid foetal development is preferred). The preferred roosting T<sub>a</sub> then would be any T<sub>a</sub> that allowed bats to maintain a stable metabolic rate that is high enough to promote euthermy, but low enough to not incur other fitness costs of cooling. This range

of  $T_a$  is referred to as the thermal neutral zone (TNZ). Further studies are needed to determine the effects of torpor use on foetal development and fitness at early stages of pregnancy during spring migration, as well as to test energy use at warmer temperatures (i.e.  $>25^\circ\text{C}$ ) that are in, and or above the TNZ for silver-haired bats.

The TNZ can vary within and among species, and it has not been calculated for silver-haired bats. If we use the TNZ reported by Willis et al. (2005) for the comparably sized big brown bat, I found that  $25^\circ\text{C}$  (my warmest roosting treatment  $T_a$ ), is close to the lower critical temperature (the lowest  $T_a$  where an individual would not be required to use more energy to remain euthermic) of  $26.7^\circ\text{C}$ . Willis et al. (2005) included a disclaimer, however, that big brown bats are a sedentary (relatively) hibernating species, and considering the life-history differences between silver-haired and big brown bats, it might be inappropriate to use the reported TNZ. However, the TNZ for big brown bats ( $26.7\text{-}35^\circ\text{C}$ ), could act as a starting point when discussing the full range of  $T_a$  that energy use for roosting silver-haired bats could be independent of during stopover, or in other words what an optimal roosting  $T_a$  could be, from an energy saving perspective (Willis et al., 2005). If we consider my energy use results and the upper critical limit reported for big brown bat TNZ, energy use by silver-haired bats could be independent of roosting  $T_a$  from  $\sim 10$  to  $35^\circ\text{C}$ . This range is ecologically relevant (although, the full range is not likely to be experienced in the same migratory season) for migrants stopping over at Long Point Bird Observatory. In the context of a “preferred” roosting temperature, further studies are needed to determine the TNZ of silver-haired bats as well as the temperatures of stopover roosts. Direct measurements aside, it would make sense for pregnant females to select roosts with the highest available temperature to reduce the

amount of torpor used and promote foetal development. For male migrants, however, we might expect the opposite based on basic torpor and energy use generalizations – a selection of cooler roosts  $T_a$  to minimize energy use. But, from this study, we should predict that males would use comparable amounts of energy regardless of roosting  $T_a$ .

In the context of metabolic rates during daytime stopovers for bats, McGuire et al. (2014) present separate regressions for euthermic and torpid metabolic rates (TMR) across  $T_a$  that intersect at  $27^\circ\text{C}$  (the highest treatment temperature used in the study). Considering the intersecting torpid and euthermic metabolic rate regression lines and the independence of energy use across temperatures, we can consider the metabolic rate for bats at  $27^\circ\text{C}$  while resting and roosting to be the BMR. This suggests that bats are facultatively using torpor to buffer thermogenic energy expenditure to remain at a time-averaged BMR throughout daytime stopovers despite variation in  $T_a$ . Although bats are using torpor during daytime stopovers, they are remaining at BMR, which is greater than the very low TMR that has been observed during hibernation (as seen in: Geiser, 1988, Wilz and Heldmaier, 2000; Song et al., 1997; Geiser and Brigham, 2000). MR reduction to low levels is crucial for winter survival for hibernating species as they are not able to arouse and forage to supplement energy budgets like bats during stopover (Geiser, 2004). The availability of insects for migrating bats at stopover (and potentially throughout the migratory journey), might relieve pressure for bats to suppress metabolism to hibernation level TMR or they may not be physiologically primed to readily reach such levels of metabolic suppression outside of the winter season.

## CONCLUSION

My study tested a key prediction made by the torpor-assisted migration hypothesis that energy use is independent of roosting  $T_a$  in bats and assessed the effects of migratory season, sex, and age on energy and torpor use during full-length, daytime stopovers by migratory bats. I found that energy use is independent of roosting  $T_a$  and that body mass predicts energy use for spring females and fall male juveniles. I also found that heavier individuals use less torpor during spring stopovers, however, body mass only predicted torpor use for fall bats roosting at warm  $T_a$ .

The use of torpor and the apparent ability to “top-up” fuel stores by feeding in small increments periodically throughout migration are adaptations that, in general, separate bat migration from bird migration. These adaptations appear to alter energy budgets for bats and explain why we see such high percent body fat upon arrival at stopover in bats, how they have physiologically adapted to this “daily” stopover strategy (assuming that nocturnality evolved before migratory flight), and how they can traverse large distances within a similar seasonal context as migrating birds.

This study has furthered the field of migration energetics and our understanding of energy management during daytime stopovers of migratory bats. It also has brought to light a number of directions that future migratory research should emphasize in order to help complete our understanding of migratory energetics and physiology. Future studies should examine the relationship between body mass and energy use by controlling for body mass between sexes to isolate any potential effects of pregnancy on energy use during stopover as well as determine the readiness of pregnant females to enter torpor and the impacts of using torpor at various stages of foetal development. Although I found

minimal effects of age on energy use during stopover, a more fine-scale aging technique would allow for a more in depth analysis of energy use by bats during the first year of life and how age might affect energy management strategies during a bat's first migration. In terms of more specific physiological directions, validation studies should be done to determine the range of the TNZ to infer preferential roosting  $T_a$  for silver-haired bats, and the costs of migratory flight should be directly quantified to clarify estimated total migration costs. Lastly, assessing the abundance and timing of insects at stopover, the role of exogenous energy sources in energy management strategies, and identifying key habitats that promote insect availability as well as suitable stopover habitats, will fill in several key gaps in our knowledge with respect to the energetics, management, and conservation of migrating bats and associated ecosystems.

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# APPENDIX A

## Ethics Approval

**Chris Guglielmo**

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**From:** [REDACTED]  
**Sent:** March-28-14 12:36 PM  
**To:** [REDACTED]  
**Cc:** [REDACTED]  
**Subject:** esinus Notification - New Animal Use Protocol is APPROVED2010-020:5

**AUP Number:** 2010-020  
**PI Name:** Guglielmo, Christopher  
**AUP Title:** Physiological Ecology Of Migratory Birds And Bats

**Approval Date:** 03/28/2014

**Official Notice of Animal Use Subcommittee (AUS) Approval:** Your new Animal Use Protocol (AUP) entitled "Physiological Ecology Of Migratory Birds And Bats

" has been APPROVED by the Animal Use Subcommittee of the University Council on Animal Care. This approval, although valid for four years, and is subject to annual Protocol Renewal.2010-020:5

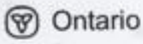
1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Copeman, Laura  
on behalf of the Animal Use Subcommittee  
University Council on Animal Care

# APPENDIX B

## Permit



Ministry of Natural Resources  
Ministère des Richesses naturelles

### Wildlife Scientific Collector's Authorization

### Autorisation pour faire la collecte scientifique d'animaux sauvages

Authorization No. / N° d'autorisation: 1082592

Local Reference No. / N° de référence local: 2016

Issuer Account No. / N° de compte du délivreur de permis: 10003042

This authorization is issued under Section 39 of the Fish and Wildlife Conservation Act, 1997 to:  
 Cette autorisation est délivrée en vertu de l'article 39 de la Loi sur la protection du poisson et de la faune de 1997 à:

Name of Authorization holder / Nom du titulaire de l'autorisation	Last Name / Nom de famille <b>Mr. Guglielmo</b>	First Name / Prénom <b>Christopher</b>	Middle Name / Second Prénom <b>G</b>
Name of Business/Organization/Affiliation (if applicable) / Nom de l'entreprise/l'organisme/l'affiliation (le cas échéant)	<b>Department of Biology, Western University</b>		
Street address of Authorization holder / Adresse postale du titulaire de l'autorisation	[Redacted]		

This authorization permits the above-named person(s) to:  
 Cette autorisation permet à la personne nommée ci-haut de:

Capture wildlife of the species and sex, in the numbers, and in the area set out below.  
 Capturer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous.

Keep game wildlife or specially protected wildlife in captivity for the purposes of education or science.  
 Garder des animaux sauvages spécialement protégés et du gibier sauvage en captivité à des fins éducatives et scientifiques.

Release the captured wildlife in the area of capture, if the captured wildlife is not to be removed from that area.  
 Rendre en liberté les animaux sauvages capturés dans la zone de capture si les animaux capturés ne doivent pas être enlevés de cette zone.

OR / OU

Capture and kill wildlife of the species and sex, in the numbers, and in the area set out below.  
 Capturer et tuer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous.

Species / Espèces	Sex / Sexe	Numbers / Nombre	Location / Endroit
Silver-Haired Bat		200	Long Point Bird Observatory, Long Point
Hoary Bat		30	Long Point Bird Observatory, Long Point
Eastern Red Bat		30	Long Point Bird Observatory, Long Point

Yes/Oui  Additional list attached / Liste additionnelle ci-jointe

Authorization Date / Date d'autorisation	Effective Date / Date d'entrée en vigueur (YYYY-MM-DD)	Expiry Date / Date d'expiration (YYYY-MM-DD)
	2016-03-30	2016-10-31

Authorization conditions: This authorization is subject to the conditions contained in Schedule A, if included. / Cette autorisation doit respecter les conditions de l'annexe A si celle-ci est jointe.

Conditions de l'autorisation:  Yes/Oui  No/Non Schedule A included. / Annexe A ci-jointe

Authorized by (name only) / Autorisé par (seulement le nom) <b>Cam McCauley</b>	Date of Issue / Date de délivrance (YYYY-MM-DD) <b>2016-03-30</b>
Signature of [Redacted]	Date (YYYY-MM-DD) <b>2016-03-30</b>

Personal information provided on this form is subject to the Access to Information Act and the Privacy Act. / Les renseignements personnels dans ce formulaire sont soumis conformément à la Loi sur la protection des renseignements personnels et de la vie privée.

FW2016 (04/00)

## CURRICULUM VITAE

### Dylan E. Baloun

#### Academic Training

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M.Sc. *University of Western Ontario, Canada* **Current Position**

“Energetics of Stopover for Bat Migration: A Test of the Torpor-Assisted Migration Hypothesis”

Advisor: Dr. Christopher G. Guglielmo

B. Sc. (Honours) Biology, *University of Winnipeg, Canada* June 2015

“The Effects of Recent Feeding on Plasma Metabolites in Little Brown Bats (*Myotis lucifugus*)”

Advisor: Dr. Craig K. R. Willis

#### Scholarships, Awards, and Bursaries

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University of Saskatchewan’s Dean’s Scholarship (\$22000 x 3) - 2017

Michael Locke Graduate Travel Bursary (\$500) – 2017

NSERC *Science Exposed* – Research photo contest- Top 20, 2017

University of Winnipeg Student Association Travel Grant (\$250) - 2014

University of Winnipeg Student Association Travel Grant (\$180) - 2013

UW Special Entrance Scholarship (\$800) – 2010

Otto & Florence Mueller General Proficiency Scholarship (\$200) – 2010

Scurfield Family Scholarship (\$1000) – 2010

#### Service and Outreach

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-Society of Biology Graduate Students Sustainability Representative

-Chair and Founder of Biology Graduate Student Sustainability Committee

-Community and Classroom Science Outreach Presenter and Volunteer

-Biology Graduate Research Forum Organizing Committee

-49<sup>th</sup> Intl. Prairie University Biology Symposium Planning Committee

#### Peer-reviewed Publications

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- (3) Menzies A.K., Webber Q.M.R., **Baloun D.E.**, McGuire L.P., Muise K.A., Cote D., Tinkler S. and Willis C.K.R. (2016). Metabolic rate, colony size and latitude, but not phylogeny, affect rewarming rates of bats. **Physiology and Behaviour**. 164:361-368.

- (2) Turner J.M., Warnecke L., Wilcox A., **Baloun D.E.**, Bollinger T.K., Misra V., Willis C.K.R. (2015). Conspecific disturbance contributes to changes in hibernation patterns of bats affected by white-nose syndrome. **Physiology and Behaviour**. 140:71-78.
- (1) Norquay, K.J.O., Menzies, A.K., McKibbin, C.S., Timonin, M.E., **Baloun, D.E.**, and Willis, C.K.R. (2010). Silver-haired bats (*Lasionycteris noctivagans*) found ensnared on burdock (*Arctium minus*). **Northwestern Naturalist**. 91:339-342.

## Selected Conference Presentations

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- (7) **56<sup>th</sup> Canadian Society of Zoologists Meeting**, Winnipeg, MB  
“Energetics of Stopover: A Test of the Torpor-Assisted Hypothesis” – **Dylan Baloun** and Christopher G. Guglielmo\*
- (6) **Society of Integrative and Comparative Biology**, New Orleans, LA  
“Energetics of Migratory Bats during Stopover: A Test of the Torpor-Assisted Migration” – **Dylan Baloun** and Christopher G. Guglielmo
- (5) **45<sup>th</sup> North American Symposium on Bat Research**, Monterey, CA  
“Metabolic rate, colony size and latitude, but not phylogeny, affect rewarming rates of bats”- Allyson Menzies, **Dylan Baloun\***, Quinn Webber, Liam McGuire, Damien Cote, Craig Willis
- (4) **49<sup>th</sup> Intl. Prairie University Biology Symposium**, Winnipeg, MB  
“The Effects of Flight and Feeding on Plasma Metabolites in *Myotis lucifugus*” – **Dylan Baloun\***, Quinn Webber, Liam McGuire, Anuraag Shrivastav, Craig Willis
- (3) **44<sup>th</sup> North American Symposium on Bat Research**, Albany, NY  
“Using Plasma Metabolite Analyses to Understand Refueling Differences and Pre-Hibernation Fueling in *Myotis lucifugus*” - **Dylan Baloun\***, Quinn Webber, Liam McGuire, Anuraag Shrivastav and Craig Willis
- (2) **Annual Meeting of The Wildlife Society**, Pittsburgh, PA  
“How WNS Kills Bats: Physiological and Behavioral Mechanisms of a Conservation Crisis” - Willis, C.K.R.\*, L.P. McGuire, J.M. Turner, L. Warnecke, H. Mayberry, A. Wilcox, N.W. Fuller, S. Bohn and **D. Baloun**
- (1) **16<sup>th</sup> International Bat Research Conference and 43<sup>rd</sup> North American Symposium on Bat Research**,  
*San Jose, Costa Rica*  
“Changes in Roosting Behaviour of Hibernating *Myotis lucifugus* Innoculated with *Pseudogymnoascus destructans*” - **Dylan Baloun\***, Lisa Warnecke, James Turner, Alana Wilcox, Trent Bollinger, Vikram Misra and Craig Willis