

# **The physiological ecology of preparing for and recovering from hibernation in temperate insectivorous bats**

by

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## **Author's Declaration**

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

# Abstract

Temperate insectivorous bats that hibernate must both prepare for and recover from hibernation during times of the year with low resource abundance and low ambient temperatures, resulting in energetic bottlenecks in both seasons. Assessing the energetic challenges of both seasons is important not only for an integrated comprehension of the scope of the annual cycle of hibernating bats, but also important for understanding the effects of white-nose syndrome (WNS) outside of hibernation. In this thesis, I focus on the seasons directly before and after hibernation to demonstrate the energetic demand of both seasons, and how WNS may exacerbate energetic challenges. In Chapter 2, I review literature concerning the post-emergence season, which presents a large knowledge gap in current understanding of the annual cycle of bats. I discuss the possible energetic challenges that bats may face during this season, and present possible challenges of WNS and climate change. In Chapter 3, I use morphometric and plasma metabolite data to analyze how hibernation preparation has changed in a region that has been affected by WNS for over a decade. Post-WNS, adult bats gained more mass before hibernation, and subadult bats, which previously lost mass, instead slightly increased body mass throughout the season. However, plasma triglyceride concentrations did not indicate any intense foraging throughout the season, despite documentation of elevated plasma triglycerides before the introduction of WNS. The results of this study were consistent with an adaptive response to WNS and may indicate changes in behavior and physiology that may result in reproductive trade-offs. Combined, my thesis emphasizes the significance of the pre- and post-hibernation seasons as energetic bottlenecks in the annual cycle, and the importance of considering the

effects of WNS outside of the actual hibernation season, opening a variety of avenues for future research in the costs and challenges of hibernation.

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# Table of Contents

<b>Author's Declaration .....</b>	<b>ii</b>
<b>Abstract.....</b>	<b>iii</b>
<b>Acknowledgements .....</b>	<b>v</b>
<b>List of Figures.....</b>	<b>viii</b>
<b>List of Tables .....</b>	<b>x</b>
<b>Chapter 1: Physiological Ecology, Ecological Energetics, and Wildlife Disease.....</b>	<b>1</b>
<b>1.1 Physiological Ecology.....</b>	<b>1</b>
<b>1.2 Ecological Energetics of Temperate Bats.....</b>	<b>2</b>
<b>1.3 Thesis Structure .....</b>	<b>5</b>
<b>Chapter 2: The energetic bottleneck faced by temperate hibernating bats throughout the early spring.....</b>	<b>8</b>
<b>2.1 Overview .....</b>	<b>8</b>
<b>2.2 Environment and Energy .....</b>	<b>8</b>
<i>2.2.1 Environmental variability .....</i>	<i>9</i>
<i>2.2.2 Energy availability.....</i>	<i>11</i>
<i>2.2.3 Energetic challenges of cold weather .....</i>	<i>12</i>
<i>2.2.4 Hibernation .....</i>	<i>14</i>
<b>2.3. Ecological Energetics and Hibernating Bats .....</b>	<b>17</b>
<i>2.3.1 Hibernating bats and environmental variation.....</i>	<i>17</i>
<i>2.3.2 Energetic bottlenecks .....</i>	<i>17</i>
<i>2.3.3 Sex differences .....</i>	<i>19</i>
<i>2.3.4 Post-hibernation season environmental constraints .....</i>	<i>22</i>
<b>2.4 Emerging Threats and Challenges .....</b>	<b>26</b>
<i>2.4.1 White-nose syndrome .....</i>	<i>26</i>
<i>2.4.2 Climate change .....</i>	<i>30</i>
<b>2.5. Future Directions .....</b>	<b>33</b>
<b>2.6 Conclusions.....</b>	<b>34</b>
<b>2.7 References.....</b>	<b>38</b>

<b>Chapter 3: Ten years after the arrival of white-nose syndrome, swarming bats develop larger fat stores but show no evidence of hyperphagia .....</b>	<b>54</b>
<b>3.1 Overview .....</b>	<b>54</b>
<b>3.2 Introduction.....</b>	<b>55</b>
<b>3.3 Methods.....</b>	<b>60</b>
<i>3.3.1 Study site .....</i>	<i>60</i>
<i>3.3.2 Bat capture and data collection .....</i>	<i>60</i>
<i>3.3.3 Foraging analysis .....</i>	<i>61</i>
<i>3.3.4 Weather conditions .....</i>	<i>62</i>
<i>3.3.5 Statistical analysis.....</i>	<i>63</i>
<b>3.4 Results .....</b>	<b>63</b>
<i>3.4.1 Body mass .....</i>	<i>63</i>
<i>3.4.2 Plasma metabolites .....</i>	<i>68</i>
<i>3.4.3 Weather conditions .....</i>	<i>69</i>
<b>3.5 Discussion.....</b>	<b>70</b>
<b>3.6 References .....</b>	<b>75</b>
<b>Chapter 4: Synthesis, Conclusions, and Future Directions .....</b>	<b>85</b>
<b>References.....</b>	<b>91</b>

## List of Figures

**Box 2.1:** A case study in early spring environmental conditions in northwestern Ontario

**Figure 2.1:** Summary of what is understood about the post-hibernation season of temperate insectivorous bats. Some information is available in the categories of Staging and Migration, Foraging, Thermoregulation, and WNS Recovery and Carryover. However, many knowledge gaps also exist within these categories that present exciting avenues for future research.

**Figure 3.1.** Size-corrected body mass of little brown bats (*Myotis lucifugus*) during the swarming period. Adults (●, solid line) gained mass, and subadults (○) slightly gained mass. Subadult males (dashed line) were generally lighter than subadult females (dotted line) but gained mass in a similar manner.

**Figure 3.2.** Comparison of body mass of adult little brown bats (*Myotis lucifugus*) during swarming in 2007 and 2021. Body mass did not differ in the early period, suggesting bats arrived at the swarming site in similar condition in both years. However, adults gained more mass in 2021 than adults in 2007, as indicated by a period by year interaction. Data are presented as mean and standard error, with sample sizes within each bar.

**Figure 3.3.** Comparison of body mass in subadult little brown bats (*Myotis lucifugus*) in 2007 and 2021 in the early and late periods of swarming. There was no difference in body mass during the early period, suggesting that subadults arrived at the swarming site in similar condition in both years. However, by the late period of swarming, bats weighed more in 2021 than in 2007. In



fact, subadult bats gained mass in 2021, whereas in 2007 they lost mass. Data are presented as mean and standard error, with sample sizes within each bar.

**Figure 3.4.** Plasma triglyceride concentrations across the swarming season in both adult (●) and subadult (○) bats. Plasma triglyceride concentrations were consistently low in 2021, not varying over time throughout the period of the study.

**Figure 3.5:** Mean nocturnal temperature (a) and cumulative weekly precipitation (b) from 2006 to 2021, with the two focal study years in light grey. There was no difference in either temperature or precipitation between the two focal years. Precipitation did not vary among years.

## List of Tables

**Table 3.1:** Sample size of *Myotis lucifugus* captured during pre-hibernation swarming at an abandoned mine in southeastern Ontario. All bats were included in analysis of body mass with a subset of those (indicated in parentheses) used for plasma metabolite analysis.

# **Chapter 1**

## **Physiological Ecology, Ecological Energetics, and Wildlife Disease**

### **1.1 Physiological Ecology**

The ways in which animals achieve energetic balance in the face of environmental constraints is a major aspect of the field of physiological ecology (Bennett, 1987).

Environmental adaptations allow animals to live in a range of different environments that pose varied energetic challenges. However, there is a limit to the degree of environmental energetic constraint an animal can withstand (McEwen and Wingfield, 2003; Tomlinson et al., 2014).

Environments are rarely stable and exert a variety of stressors on animals over periods of seconds, minutes, hours, days, and seasons. When animals face extreme or combined energetic constraints, the resulting energetic stress can have fitness consequences (McEwen and Wingfield, 2003; Romero et al., 2009). Furthermore, intrinsic demands vary among species, and species differ in the manner that they interact with the environment. Therefore, environmental stressors vary among species and do not affect all animals equally (Tomlinson et al., 2014). The overarching field of physiological ecology, and specifically the subdiscipline of ecological energetics, offers insight into how animals respond to environmental challenges, including acute and chronic stressors (Cooke et al., 2013). Physiological ecology offers an integrative approach to organismal biology, building on the basis of animal ecology, which focuses on the interaction between animals and their environments.

When considering energetic challenges, certain traits or characteristics are important for understanding animals that face greater energetic constraints. For example, small-bodied

endotherms rapidly lose heat to their environment due to the high volume to surface area ratio, and high mass-specific metabolic rates (Tattersall et al., 2012). Highly mobile animals, particularly flying animals, have high exercise costs and interact with their landscape on a broad scale, potentially encountering wide variations in environment (López-González et al., 2015). Temperate animals experience dramatic seasonal variation in environmental conditions, experiencing severe winter weather conditions every year. Animals with ephemeral food sources (e.g., insects, fruits) experience periods of low resource abundance at certain times of the year. Reproductive costs are high for most animals, but reproductive costs are especially high for mammals, particularly lactating females (e.g., Dzal and Brigham, 2013). As flying, small-bodied, insectivorous mammals, temperate bats exemplify all of these traits and are adapted to meet the resulting energetic challenges (Speakman and Thomas, 2003). As such, temperate insectivorous bats are an exemplary system for assessing ecological energetics and environmental constraints (Willis, 2017).

## **1.2 Ecological Energetics of Temperate Bats**

Bats are the second most speciose order of mammals and can be found worldwide outside of polar regions. While bat species richness is highest in tropical regions, many species live in high-latitude regions that experience extended periods of harsh winter conditions (e.g., Norquay and Willis, 2014; Meyer et al., 2016). All temperate bat species are insectivorous, and experience reduced or limited prey availability in the winter. The energetic cost of prolonged exposure to cold weather with little prey abundance means that bats have little capacity for cold tolerance and must avoid the energetic stress of winter by either hibernating or migrating (Auteri, 2022). Some species migrate long distances to warmer regions to spend the winter (e.g., *Lasiurus*

*borealis*, *Lasiurus cinereus*, *Lasionycteris noctivagans*; Fleming, 2019), while many hibernating species (e.g., *Myotis lucifugus*, *Myotis sodalis*, *Eptesicus fuscus*) undertake shorter regional migrations to hibernacula (Fenton, 1969; Neubaum et al., 2006; Norquay et al., 2013; Krauel et al., 2018). Before immersing into (entering) hibernation, bats accumulate large fat stores that act as the primary source of energy throughout the winter (Kunz et al., 1998; Stawski et al., 2014). Bats spend most of hibernation in torpor (reduced body temperature and metabolic rate), and periodically arouse to normal body temperature, during which they drink, excrete waste, and perform other necessary functions (Speakman and Thomas, 2003). In the relatively cold environment of a hibernaculum, warming from torpid to euthermic body temperature is energetically costly, and arousals may constitute approximately 90% of the energy used throughout hibernation (Thomas et al., 1990).

Hibernation energetics have received increasing attention since the introduction of white-nose syndrome (WNS) to North America. In 2006, the first signs of the causal agent of WNS were documented in New York state (Blehert et al., 2009). White-nose syndrome is caused by *Pseudogymnoascus destructans*, a psychrophilic (“cold-loving”) fungus that grows along the nose, ears, and wing membranes of bats (Lorch et al., 2011). Throughout hibernation, WNS induces more frequent arousals and causes bats to expend their fat stores more quickly, effectively starving them to death (Reeder et al., 2012). Despite massive population declines after the pathogen was initially introduced, small populations remain in regions where the disease has been present for more than a decade (e.g., Dobony et al., 2011; Kurta et al., 2020). Ecological energetics are an essential framework in understanding how remnant populations persist. While hibernation energetics is an important aspect of understanding how WNS survival is possible, recent studies (e.g., Fuller et al., 2020) suggest that WNS may have energetic

consequences outside of the hibernation season. In the first years of exposure to *P. destructans* there is a very high mortality rate, but not all infected bats are killed by the disease and bats in regions where the disease has become endemic now experience WNS every winter (Langwig et al., 2015a).

The periods directly before and after hibernation have long been understood by biologists as examples of energetic bottlenecks, or periods when the resources an animal needs are not matched by the environment (Humphries et al., 2003). From a fitness perspective, bats exemplify the importance of maintaining a positive energy balance throughout the pre- and post-hibernation season, as fat stores deposited in the autumn are important for both winter survival and reproductive success in the spring (Kunz et al., 1998). Bats emerge from hibernation earlier than peak insect abundance in high-latitude regions (Meyer et al., 2016), so fat stores deposited prior to hibernation continue to be important sources of energy in the spring (Norquay and Willis, 2014). Bats rely on residual fat stores when conditions do not permit foraging, and nightly temperatures can regularly drop to freezing (Kunz et al., 1998; Besler and Broders, 2019; Hranac et al., 2021). In terms of WNS, the pre-emergence and post-emergence seasons may determine, to some degree, whether bats survive the disease or not. Pre-hibernation fat stores deposited in autumn appear related to WNS survival because bats with more fat have the energy to survive increased arousal frequency (Cheng et al., 2019). In addition to the already energetically challenging nature of the pre- and post-hibernation periods, WNS increases resource demand, requiring more fattening in the autumn despite low insect abundance. Post-hibernation, bats can recover from WNS, but at high energetic cost that involves tissue reparation and may involve longer bouts of euthermia (Fuller et al., 2020). However, the effects of WNS outside of

hibernation have remained relatively understudied. As a result, there may be many gaps in our understanding of the full annual cycle of WNS.

### **1.3 Thesis Structure**

In my thesis, I assessed the physiological ecology of temperate hibernating bats during the early spring and autumn swarming season. By approaching the autumn and spring with a physiological framework, we can assess what energetic challenges may be exacerbated due to WNS. My thesis consists of 4 chapters, including this introductory chapter. Chapters 2 and 3 were conceived and prepared as separate manuscripts for publication. Chapter 2 has been prepared following the format of the journal *Biological Reviews* and Chapter 3 has been prepared following the format of the *Canadian Journal of Zoology*. Chapter 4 presents a synthesis of the ideas in the thesis and is not intended for independent publication.

In Chapter 2 of the thesis (“The energetic bottleneck faced by temperate hibernating bats throughout the early spring”) I review the literature of the energetic challenges faced by bats early in the active season. There is a paucity of studies that focus on bats directly after they emerge from (exit) hibernation. My review seeks to compile what is already known, describe likely responses to the energetic challenges of the early spring based on previous knowledge, and highlight important knowledge gaps. Initially, my plan for Chapter 2 had been to assess the foraging physiology of free-living bats during the immediate post-hibernation period in a region recently affected by white-nose syndrome. Little is known about the physiological demands of recovering from hibernation and how bats acquire energy in the early spring, though one possibility is that they forage intensely on warmer nights. However, due to COVID-19 restrictions, we were unable to travel to our study site of Kenora, Ontario, in 2021. While writing

a review paper was a contingency plan, it provides an opportunity to describe and explore the post-hibernation energetic bottleneck and highlight possibilities for future studies focusing on the physiology of bats in the early spring more thoroughly.

In Chapter 3 (“Ten years after the arrival of white-nose syndrome, swarming bats develop larger fat stores but show no evidence of hyperphagia”) I focus on the foraging and fattening physiology of bats prior to hibernation in a region that had been confirmed as WNS-positive for over a decade. An intuitive way of assessing the effects of WNS on a population is to compare behaviour and physiology before and after the arrival of the disease. I was presented with just such an exciting opportunity. In 2007, McGuire et al., (2009) conducted fieldwork at an abandoned mine in eastern Ontario, collecting data on both body mass and plasma metabolites. Body mass is a good method for assessing the size of fat stores and plasma triglyceride concentration provides an indicator recent feeding intensity (Jenni-Eiermann and Jenni, 1994; McGuire et al., 2018). I hypothesized that bats enter hibernation with larger fat stores due to selective pressure from WNS. By comparing data I gathered in 2021 to data collected in 2007, I tested the prediction that prior to hibernation *M. lucifugus* would have larger fat stores (greater body mass), and that depositing larger fat stores would be accomplished by hyperphagic foraging, indicated either by plasma triglyceride concentration that remained elevated for longer (longer period of hyperphagia), or reached greater concentration (greater intensity of hyperphagia), than in 2007.

In Chapter 4 (“Synthesis, Conclusions, and Future Directions”) I synthesize the ideas and results that emerged throughout my time conducting this research. The concept of energetic bottlenecks is an important framing device throughout my thesis. For relatively long-lived animals, no part of the annual cycle is truly independent from others (Marra et al., 2015), and



energetically strenuous seasons can have far-reaching consequences. Temperate bats are adapted for seasonal energetic bottlenecks, but when these energetic challenges are exacerbated by disease, the resulting fitness consequences can have negative population-scale effects. Yet, there is now ample evidence that some bats can survive WNS, and some remnant populations even show signs of stability (Maslo et al., 2015). Understanding how bats survive WNS, during the period of hibernation and subsequent periods of the annual cycle is integral for understanding the challenges faced by bats most affected by WNS, and the opportunities for conservation efforts and policies to support and facilitate population recovery.

## **Chapter 2**

### **The energetic bottleneck faced by temperate hibernating bats throughout the early spring**

#### **2.1 Overview**

The annual cycle of temperate hibernating bats is generally separated into the hibernation period and active season. The pre-hibernation (swarming) period has been extensively studied (at least for some species), but the post-hibernation period has received considerably less attention, particularly as it relates to North American bats and their recovery from white-nose syndrome (WNS). With energetically stressful environmental conditions and low resource availability, the post-hibernation season represents a strong energetic bottleneck that can have persistent effects (e.g., poor health) on subsequent seasons, which may result in reproductive or fitness consequences. Research during the post-hibernation period can provide insight into how bats cope with severe energetic challenges, and how WNS affects bats beyond the hibernation season. In this chapter I provide a review of important background concepts and focus on what is known about the post-hibernation season, important knowledge gaps, and emerging threats to bats in this period.

#### **2.2 Environment and Energy**

Much of the field of physiological ecology focuses on the energetic constraints imposed on animals by their environments, and how these constraints may change through space and time (Bennett, 1987). Energetic constraints from the environment are prominent drivers of animal evolution. The energetic intake of an animal should match energetic demands (whether current or

future), but environmental challenges can disrupt energetic balance (Tomlinson et al., 2014). Energetic resources are finite, and the rate at which an animal can acquire resources is limited by how quickly they can find, handle, digest, and absorb food (Weiner, 1992). Thus, physiological and behavioural traits can reflect the energetic constraints imposed on animals by their environment. However, despite habitat-specific adaptations, all animals experience limitations in energy availability throughout their lives. When energetic balance cannot be maintained, animals face energetic stress (McEwen & Wingfield, 2003). The effects of the energetic stress that come from especially strenuous challenges may have long-lasting effects (O'Connor et al., 2014). An understudied period in any part of the life of an animal may obfuscate the effects of a particular stressor.

### *2.2.1 Environmental variability*

The environment is rarely stable but varies in both space and time. Spatial variation is obvious in variation among habitats (e.g., arid regions, high altitudes) but there is also considerable variation in environmental conditions across microhabitats. Environmental constraints that affect energy balance and limiting resources are important drivers of adaptation. Spatial variation can drive phenotypic plasticity (particularly in reproduction; Burgess et al., 2011) and the adoption of bet-hedging strategies in spatially heterogeneous habitats (Morrongiello et al., 2012). Environments also vary temporally (e.g., hourly, weekly, and annually) and in many cases there are predictable changes in environment, including daily and seasonal variation. Environmental conditions, including humidity, visibility, and temperature vary throughout the day, resulting in different pulses of species activity within a span of just twenty-four hours (Daan, 1981). Changes in activity throughout the day are a part of a daily

routine in response to a “scheduled” event. The behaviours of animals also follow predictable environmental changes throughout the year (McNamara & Houston, 2008), such as changes in weather between seasons. Animals experience various types of stressors throughout the year, and a full annual cycle approach is critical in fully understanding the implications of these stressors (Marra et al., 2015).

In addition to predictable environmental change, animals must also respond to unexpected or unpredictable environmental changes. Changes in weather conditions are among the most common examples of unpredictable changes that animals may encounter. Behavioral changes are one way animals can respond to, and in some situations take advantage of, unpredictable weather. For example, animals that are inactive during midday to avoid heat stress may become active at midday if the sky is cloudy, and temperatures are consequently lower (e.g., lizards; Beuchat, 1989). Animals can also prepare for unexpected energetically challenging conditions by maintaining endogenous fat reserves (McEwen & Wingfield, 2003). However, there is a limit to the unexpected stressors that animals can tolerate (McEwen & Wingfield, 2003). Extreme unexpected events, such as flooding after a rainstorm or widespread damage due to a forest fire, cause energetic stress beyond what animals are prepared for and can have disastrous effects at both individual and population levels.

Similar to the spatial scale of environmental variation (from local microclimates to global climate patterns), the energetic constraints of unexpected or unpredictable environmental conditions can vary at different temporal scales. Unpredictable changes can last hours, days, a season, or several years. The intensity and duration of these changes affects how animals may be able to respond to the resulting energetic constraints (Romme et al., 1998). For more long-lasting

challenges, animals may exhibit plastic responses, such as changing diet in response to shortages in a preferred food source (e.g., owls, Korpimäki et al., 1990; bats, Kaupas and Barclay, 2018).

### *2.2.2 Energy availability*

Resources scale with time and space, and resource abundance within a wide, macrohabitat context is often predictive of environmental constraints for many species (Tomlinson et al., 2014). At this wider scale, we can assess the effects a general trend in environment can have on animals. Many animals engage in energetically demanding activities around general periods when resources are most abundant. For example, many animals time reproduction in the spring and summer to coincide with advancing phenology and peak resource abundance (e.g., hamsters, Franceschini-Zink and Millesi, 2008; bats, Frick et al., 2010). Similarly, animals may undertake energetically expensive maintenance during more resource-abundant times of the year, or in more resource-abundant areas (e.g., birds molting old feathers, Visser et al., 2006).

Animals face challenges maintaining energetic balance when endogenous demands increase, when environmental resources are limited, and when environmental demands are elevated. Resource availability often follows an annual pattern but does not always align with the resource needs of animals. When animals require more energy, such as during reproduction, resource needs increase. Similarly, environmental conditions can increase energetic demands, such as physiological costs of thermoregulation in extremely hot or cold environments. If resource availability is sufficient, animals can maintain energy balance in the face of increased energetic demands, whether endogenous or environmentally driven. However, when the

resources needed by an animal are not matched by the resources available in the environment, animals face an energetic bottleneck (Humphries et al., 2003).

Energetic bottlenecks often arise due to temporal and/or spatial variability. For example, warmer stream temperatures induce greater energetic costs on steelhead trout (*Oncorhynchus mykiss*), accentuating the effects of food shortages which limit growth rate (Myrvold and Kennedy, 2015). While steelhead may normally live in colder streams that facilitate growth, temperature variation in different streams across space can increase the amount of energy needed for steelhead to grow (Myrvold and Kennedy, 2015). Regional differences can also account for spatial variation in energetic bottlenecks. High-latitude little brown bats (*Myotis lucifugus*) must deposit larger fat stores than their southern conspecifics, despite shorter active seasons and colder nightly temperatures (Norquay and Willis, 2014; McGuire et al., 2016). Temporal variation often has predictable effects, but unpredictable environmental changes can also occur. For example, Ural Owls (*Strix uralensis*) feed predominately on voles, which have population fluctuations from year to year. In years of low vole abundance, owls maintain energy intake by consuming more diverse diets (Korpimäki et al., 1990). Unpredictable environmental changes may also occur over short-term or acute timescales. For example, migratory hoary bats (*Lasiurus cinereus*) sometimes encounter late-spring snowstorms upon arrival to the breeding grounds. In response, hoary bats use torpor to save energy and possibly delay parturition so that offspring are born when conditions have improved (Willis et al., 2006).

### 2.2.3 Energetic challenges of cold weather

Seasonal changes in weather are among the most well-understood examples of energetic bottlenecks. Winter often imposes intense energetic stress on a spatial and temporal scale. In

temperate regions, winter tends to increase thermoregulatory costs during a time of the year with generally low resource abundance, necessitating adaptations and strategies to withstand the costs of winter. There are three strategies animals use to survive through the winter: resistance, migration, and torpor (Williams et al., 2015). Animals that resist cold weather remain active, buffered by cold-weather adaptations (e.g., growth of an undercoat during the winter). Animals that depart the region and move to locations with more favourable environmental conditions are considered seasonal migrants (Dingle and Drake, 2007). Torpor refers to a controlled reduction in body temperature and metabolic rate by an animal (Geiser, 2013). Though generally viewed as three distinct categories (resistance *or* migration *or* torpor), the wide diversity in how animals survive winter suggests these strategies may not be mutually exclusive (Auteri, 2022).

Adaptations for cold-weather survival exist within a spectrum of tolerance and avoidance strategies as in the case of short- and long-distance migrants, facultative and obligate hibernators, or animals that alternate between periods of torpor and activity. Understanding variation in cold weather survival strategies is integral to developing a more comprehensive framework of characterizing how animals respond to cold weather. A more comprehensive framework also opens possibilities for more accurate and nuanced studies on the physiological challenges animals face in cold weather, instead of focussing on challenges associated with a singular cold-weather survival strategy.

Small-bodied endotherms exemplify the spectrum of cold weather survival strategies. Endothermy offers a degree of cold weather resistance, but within limits (Auteri, 2022). Small endotherms proportionately lose more heat than larger endotherms, due to their higher mass-specific metabolic rate and high surface area to volume ratio (Tattersall et al., 2012). Despite this, small endothermic species can live in high latitude regions with long and harsh winters

through variety of cold-weather survival strategies (Canale and Henry, 2010). Many species of birds and bats migrate to avoid the energetic stress of high latitude winters (e.g., Norris and Taylor, 2006; McGuire et al., 2014; Jonasson and Guglielmo, 2016; López-Calderón et al., 2019). Small mammals that do not travel long distances can use some combination of torpor and resistance to survive the winter, using torpor to offset energetic costs when inactive (Lynch et al., 1978). Other species are almost completely inactive throughout the winter and hibernate to avoid the season altogether.

#### *2.2.4 Hibernation*

Hibernation, like migration, is a strategy used to avoid most of the energetic costs of winter. Many mammals hibernate to avoid the winter by entering a prolonged state of torpor (Geiser, 2013). Hibernating animals rely on endogenous fat stores, food stores, or both for energy throughout hibernation (Geiser, 2013). Though hibernation is spent predominantly in torpor, arousal from torpor (periodic warming to normal body temperature) incurs a substantial energetic cost, accounting for up to 90% of energy used throughout the season (Thomas et al., 1990; Speakman and Thomas, 2003). Periodic arousals are energetically expensive due to the thermogenic and thermoregulatory costs of warming the body to normothermic temperatures and defending normothermic body temperature in the cold environment of a hibernaculum (Thomas et al., 1990). Arousals may occur for a variety of reasons, though necessary behaviors such as drinking, sleeping, and excretion of metabolic waste have been proposed as reasons for arousals (Geiser, 2004; Geiser, 2011).

Hibernating at cooler temperatures reduces metabolic rate and frequency of arousals (Humphries et al., 2003). However, at a certain minimum temperature, metabolic rate increases



to avoid freezing (Boyles et al., 2020). More recent evidence suggests that the goal of hibernation is likely not energy minimization, but rather an optimization of trade-offs between energy savings and consequences of deep and prolonged torpor (Humphries et al., 2003; Boyles et al., 2007; Boyles et al., 2022). Torpor has a variety of costs that can be detrimental for an animal, so animals must balance those costs with the benefits of energy savings (Boyles et al., 2020). For example, torpor at lower temperatures is associated with telomere shortening from oxidative damage, which can affect aging (senescence) and survival (Nowack et al., 2019). The trade-offs between the costs and benefits of torpor are a major aspect of understanding the expression of hibernation (Boyles et al., 2020).

Preparing for hibernation is also energetically challenging. Regardless of how an animal avoids starvation throughout the winter (food cache or fat stores), the combined cost of acquiring declining resources and increasing thermoregulatory costs as temperatures drop creates an energetic bottleneck in the autumn (Humphries et al., 2003). Studies in bats (Kunz et al., 1998; McGuire et al., 2009) and ground squirrels (Sheriff et al., 2013) have focused on how fat-storing animals are able to gain mass despite declining resources. Pre-hibernation mass gain strategies (e.g., heterothermy, hyperphagia) represent adaptations in response to expected temporal variation in environmental conditions affecting resource availability.

In the early spring, animals emerge from hibernation and face similar constraints as in the late autumn. However, many animals additionally face the added energetic challenge of reproduction which often occurs shortly after spring emergence. In an extreme case, male arctic ground squirrels (*Urocitellus parryii*) rely on fat throughout hibernation but use food stores to regain mass during a pre-emergence period of euthermia (Buck and Barnes, 1999). Emerging up to a month before females, male ground squirrels establish breeding territories when there may

still be up to 100% snow cover and very few resources and lose most of their pre-emergent mass in the process (Buck and Barnes, 1999). While emergence times are not as early in other species, reproductive pressure is nevertheless a prominent factor in the phenology of hibernators. Garden dormice (*Eliomys quercinus*) born later in spring fail to develop the same levels of fat stores before hibernation as earlier born mice (Stumpfel et al., 2017). Common hamsters (*Cricetus cricetus*) have two litters each season, having more pups in their first litter if conditions permit (Franceschini-Zink and Millesi, 2008). A delay in emergence of even two weeks results in poorer female condition at emergence and gives offspring less time to develop, to the detriment of reproductive success and fitness (Hufnagl et al., 2011). Similarly, little brown bats (*Myotis lucifugus*) rely on remaining fat stores from hibernation for reproductive success in the spring (Kunz et al., 1998).

Studies of bats can advance current understanding of the effects of reproductive pressure on hibernation phenology (Willis, 2017). Selection for emerging in good condition results in adoption of conservative energy use throughout hibernation, and bats are an exemplary taxon for this phenomenon (Stawski et al., 2014). While the energetic challenges of the hibernation (and the pre-hibernation) season for bats have received close attention, the period directly following hibernation emergence has remained comparatively understudied. Early spring environmental conditions can be highly variable, characterized particularly by fluctuating temperatures and unexpected weather events (e.g., snowstorms). Understanding environmental constraints of the early spring and the corresponding adaptations of temperate bats can identify knowledge gaps and important opportunities for future study.

## 2.3. Ecological Energetics and Hibernating Bats

### 2.3.1 Hibernating bats and environmental variation

Discussions of bat hibernation energetics must consider spatial variation, as changes in latitude affect the depth and duration of the energetic stress of winter, and the corresponding expression of hibernation (Hranac et al., 2021). Many hibernating species with widespread ranges (e.g., *M. lucifugus*, *Eptesicus fuscus*) vary substantially in how long they hibernate. At lower latitudes, winter conditions are milder and, in some regions permit bats to emerge and feed throughout the winter (Bernard et al., 2021), while bats at higher latitudes may not exit their hibernacula at all (Czenze and Willis, 2015; but see Hays et al., 1992; Klug-Baerwald et al., 2016). Prolonged activity in cold conditions is not viable at high latitudes. Like other small endotherms, bats lose heat quickly, and compensating for heat loss is a substantial energetic challenge (Stawski et al., 2014). Bats do not cache insects to consume during the winter, therefore in regions where regular winter foraging is not possible bats must develop fat stores that will supply them energy throughout hibernation (Speakman and Thomas, 2003). Body condition of bats at spring emergence is therefore dependent on how bats manage fat stores throughout the winter, and how they can take advantage of environmental cues to determine the timing of spring emergence.

### 2.3.2 Energetic bottlenecks

When bats emerge in the spring, low temperatures and low resource availability force bats to continue relying on endogenous fat stores for energy. The fat stores bats deposit in the autumn must be sufficient for fuelling hibernation, but for some portion of the early spring period as well. However, during the autumn bats experience an energetic bottleneck (Humphries

et al., 2003) as aerial insect abundance declines as the autumn continues, but bats need to deposit large fat stores relative to their body size (Stawski et al., 2014).

Prior to hibernation, many bat species gather at hibernacula in a behaviour known as swarming, during which mating occurs (Fenton 1969). Studies of bats preparing for hibernation have primarily been conducted at swarming sites (e.g., Fenton 1969; McGuire et al., 2009; Gallant and Broders, 2015). When preparing for hibernation bats can use hyperphagia (increased feeding) to quickly gain mass (McGuire et al., 2009; Suba et al., 2010), though this is not consistent across regions. At high latitudes, bats may not show signs of hyperphagia at swarming sites, despite gaining more mass, and it is possible that foraging occurs at more insect-abundant sites (McGuire et al., 2016). Bats may also select cooler roosts and use torpor during the autumn to further reduce energetic output, facilitating rapid mass gain amidst declining insect populations (Speakman and Rowland, 1999).

To ensure they have some remaining fat to cope with the energetic challenges of the cold ambient temperatures and limited prey availability they experience in the early spring, bats must manage their energy use throughout hibernation. When bats emerge from hibernation, there is likely a low abundance of insect prey which may be variable and unpredictable as weather conditions vary (**Box 2.1**; Meyer et al. 2016). Bat emergence may be related to barometric pressure, as changes in barometric pressure can indicate incoming warm fronts and thus more favourable conditions (Paige, 1995; Berkova and Zukal, 2010; Czenze and Willis, 2015). Increasing temperatures also drive emergence (e.g., Pettit and O’Keefe, 2017), but bats in regions with extremely long winters may emerge before snow has finished melting completely (**Box 2.1**). Likely associated with the greater energetic challenge of longer winters and colder temperatures farther north, higher-latitude bats appear to be more sensitive to environmental cues

which would indicate nights warm enough to permit foraging (Meyer et al., 2016). Endogenous factors such as individual body condition may also affect emergence time (Norquay and Willis, 2014; Czenze et al., 2017) with fatter bats which can withstand harsher post-hibernation conditions generally emerging earlier than bats in poor condition. Regardless of the underlying factors that drive emergence from hibernation, bats at this time of year face the challenge of both relatively low insect abundance and cold nightly temperatures.

### 2.3.3 Sex differences

An important factor in spring emergence is reproductive pressure, which results in important differences between sexes (Willis, 2017). Most small mammals (e.g., ground squirrels) reproduce during the spring, and males and females invest energy into reproduction at roughly the same time (Michener, 1983; Michener, 1998; Buck and Barnes, 1999). However, most temperate hibernating bats mate in the autumn but give birth in the spring (Wimsatt, 1944). While some bat species (e.g., *Plecotus auritus*) may mate during the spring and fall (Furmankiewicz et al., 2013), the timing of energetic investment for bats is largely distinguished by sex, putting more pressure on males and females at different times of the year. Male bats face the most energetic pressure during pre-hibernation swarming as they try to maximize the number of copulations. Males are usually heavier before swarming begins, relying more on these energy stores during the mating period (Burns and Broders, 2015; Kohyt et al., 2016). Males also enter hibernation later than females, either to maximize mating opportunities or to regain mass lost during swarming (Norquay and Willis, 2014). With their season of reproductive investment over with, male bats have no strong selective pressure to emerge early in spring. Once male bats enter hibernation, they remain in hibernacula until spring conditions are most suitable for emergence

(i.e., warmer ambient temperatures and consequently more reliable availability of flying insect prey). Males can hibernate for up to a month longer than females (Norquay and Willis, 2014), and when they emerge, male bats usually spend their active season solitary or in small groups (Fenton and Barclay, 1980).

In contrast to males, the female annual cycle is driven by reproductive costs incurred in the spring. Females face strong selective pressure to give birth early in the active season (Balzer et al., 2022), requiring females to initiate pregnancy when resources are limited. Therefore, females in the post-hibernation period rely on fat stores deposited prior to hibernation (Jonasson and Willis, 2012). Females conserve fat throughout hibernation by adopting more energetically conservative behaviours (e.g., shorter arousals, increased frequency of heterothermic arousals; Czenze et al., 2017). The phenomenon of “thriftiness” in female bats is especially pronounced at higher latitudes with longer winters and where spring conditions are more energetically challenging (Czenze and Willis, 2015). Females usually emerge earlier than males, and at a heavier mass (Norquay and Willis, 2014; Czenze and Willis, 2015).

As with many other mammals (e.g., Franceschini-Zink and Millesi, 2008), later emergence can have negative fitness consequences, such as reduced likelihood of a successful pregnancy or reduced offspring survival (Ransome and McOwat, 1994; Frick et al., 2010; Norquay and Willis, 2014). Later parturition gives first-year bats less time to complete somatic development and to develop foraging skills, which means that later-born bats will be less efficient at depositing fat stores before hibernation (Frick et al., 2010). The torpid metabolic rate of first-year bats can be as much as 2.75x greater than adults, and they gain mass at a slower rate throughout the pre-hibernation season (Kokurewicz and Speakman, 2006). Even when first-year bats consume as much food as adults, they may lose body mass through the swarming period due to

limitations in foraging skill and torpor use (McGuire et al., 2009). Thus, there is strong selective pressure for females to emerge from hibernation as early as possible, but the risk of spring weather conditions such as a snowstorm or delayed spring can also potentially lead to reproductive failure for that year (Balzer et al., 2022).

#### *2.2.4 Spring emergence*

There is little information surrounding the conditions bats face upon initial emergence from hibernation. Some bat species move around hibernacula after emergence for a short time, a behaviour known as staging (Roby et al., 2019). Depending on the species and region, staging may be associated with post-hibernation recovery of fat stores (Lacki et al., 2015; Roby et al., 2019). However, few studies have investigated staging, leaving many questions and hypotheses. Lower-latitude bats emerge throughout hibernation to feed (e.g., Turbill and Geiser, 2008; Bernard et al., 2021), so foraging immediately following staging may just be a product of higher temperatures.

Most hibernating bats migrate from summer roosts (maternity colonies for females) to hibernacula in the autumn, and then back to summer roosts in the spring (Fenton and Barclay, 1980; Grinevitch et al., 1995; Lučan et al., 2013). Hibernating bats generally follow a “star-like” radiation in their spring migration, following no real pattern (Hutterer et al., 2005). Migration distance is variable, but >100 km is not uncommon (Norquay et al., 2013). There is little information on the actual energetic cost of these migrations. Given the ease at which bats can move over a long distance compared to a similarly sized terrestrial mammal, it may be that bats can migrate these distances in only a few days, foraging when possible (McGuire et al., 2012; Roby et al., 2019).

#### *2.3.4 Post-hibernation season environmental constraints*

Throughout the post hibernation season, much of the activity of bats is determined by whether there is a potential energetic benefit of foraging. While bats may emerge briefly to drink, foraging takes more time and energy, especially when prey is scarce and harder to locate earlier in the year (Speakman and Thomas, 2003). The use of torpor may buffer bats from the energetic challenges of inclement spring weather, in response to much the same stressors as the autumn—low temperatures and low resource abundance. While torpor use during the spring has remained understudied, the stressors of the early spring are intuitive. Torpor is likely an important aspect of cold weather survival post-hibernation, for northern latitude bats particularly where conditions experienced upon emergence are a major energetic constraint.

Torpor delays fetal development in bats (Racey and Swift, 1981), so a major trade-off in the spring season is using torpor at the cost of later parturition (Willis, 2017). However, females are not pregnant when they emerge from hibernation, and bats at maternity colonies in the early spring are likely not far enough along in their pregnancy that the pregnancy is a major energetic cost (Fenton and Barclay, 1980; Besler and Broders, 2019). Much like throughout hibernation, female bats in the early spring may have a more frugal approach to energy use. Torpor is used more regularly in early stages of pregnancy, suggesting torpor use throughout the post-hibernation season may in part be how bats survive an otherwise energetically stressful period of the year (Dzal and Brigham, 2013; Besler and Broders, 2019).

Early spring foraging success is related to the combination of predictable improvement in environmental conditions and unpredictable fluctuations on short-term time scales (**Box 2.1**). As spring progresses, nocturnal temperatures generally increase, providing a trend of improving foraging conditions and reduced thermoregulatory demands. However, weather conditions may



vary from night to night and sudden and unexpected poor weather conditions may preclude foraging. Bats may take advantage of warm nights to feed but early spring nights during which bats can achieve positive energy balance may be inconsistent. Therefore, bats may need to feed intensely on warmer nights before returning to their roosts and using torpor (**Box 2.1**).

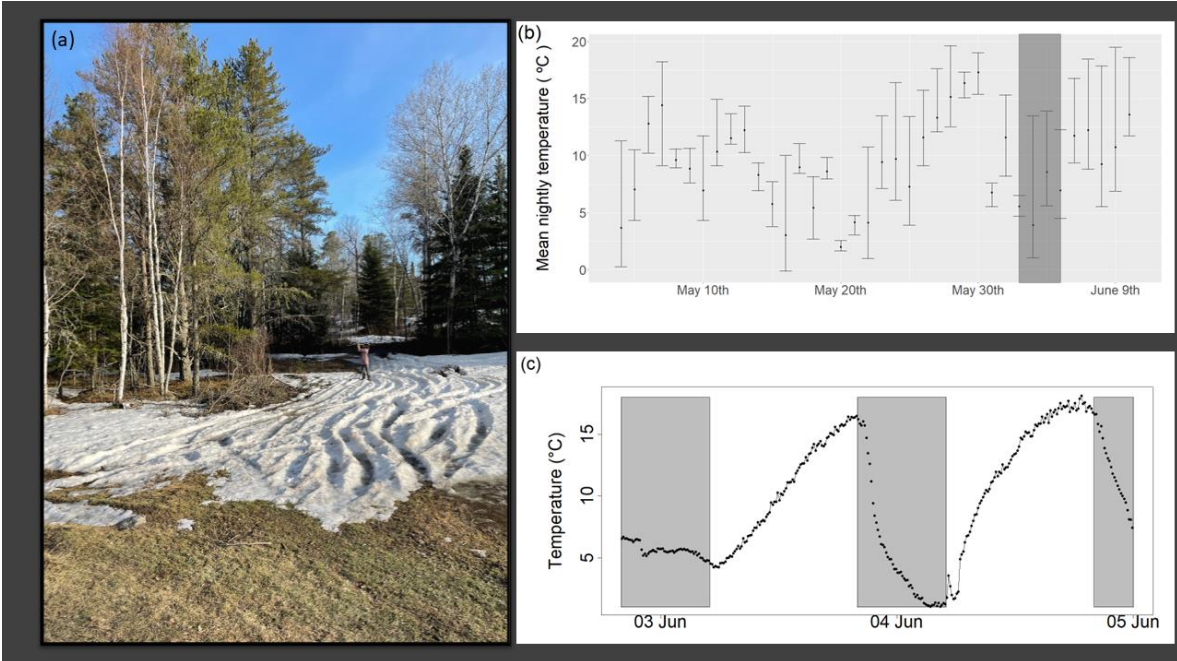
Hyperphagia in the spring has not been documented, but it may be one way in which bats can maximize energy intake when foraging opportunities are infrequent and unpredictable. As foraging conditions gradually improve, and as bats recover energy stores that were depleted over hibernation, bats may gradually reduce the frequency and intensity of hyperphagic foraging as nights get warmer and prey availability increases (**Box 2.1**).

Bats have been documented to switch their dietary niche during more energetically stressful periods of the year, during which aerial insect abundance is lower (Burles et al., 2008; Kaupas and Barclay, 2018; Salvarina et al., 2018). Non-aerial arthropods can remain active longer in colder weather conditions, and dietary changes are seen in regions that experience low temperatures throughout the pre-hibernation period (Maucieri and Barclay, 2021). Bats may use a similar strategy post-hibernation, where temperatures can drop to nearly freezing some nights (**Box 2.1**). Bats also have short windows for foraging, with temperatures that quickly drop within a few hours (**Box 2.1**). High-latitude bats face a similar challenge, and by consuming more energy dense prey (i.e., spiders), bats can effectively spend less time foraging but increase their energy intake compared to southern conspecifics (Boyles et al., 2016). Bats in the spring may take advantage of their short foraging time similarly. While dietary niche shifts in aerial hawkers have only been observed in association with extreme energetic challenges before hibernation, it may be that similar shifts occur during the spring as well, in response to very similar conditions.

Changes in diet, hyperphagia, and torpor can be used in combination to drive positive energy balance throughout the post-hibernation period. Surviving cold weather does not need to be linked to one method (Auteri, 2022) and in the case of the early spring, some combination of tolerance (foraging when possible) and avoidance (torpor) is likely. Similar to pre-hibernation fattening, the most effective strategies for surviving the post-hibernation season likely depend on both region and species. The early spring is a more stressful season in colder regions, where low temperatures likely preclude foraging on many nights and promotes hyperphagic foraging when conditions are favourable. Lower-latitude species may not use hyperphagia in the spring if insect abundances are higher earlier into the post-hibernation season, because nightly temperatures are both higher and more consistently warm from night to night.

### **Box 2.1: A case study in early spring environmental conditions in northwestern Ontario**

At higher latitudes, spring conditions impose an energetic bottleneck on bats that have recently emerged from hibernation. Here we present observations from ongoing fieldwork in northwestern Ontario, Canada (49°47", 94°26"). There was still snow and ice that had not yet melted when the first bats of the season were caught (May 7<sup>th</sup>; Figures 1 and 2). Figure 2 illustrates the ranges of temperatures that occur throughout the night across the study period. Weather conditions were extremely variable, with near freezing temperatures occurring even weeks after the first observations of bats (e.g., night of May 20<sup>th</sup> and May 21<sup>st</sup>). Temperature can vary dramatically over just a few nights, exemplified by the nights of May 4 – 7, with mean nightly temperature from <5°C to nearly 15°C. The days highlighted in grey in Figure 2 (June 3<sup>rd</sup> to June 5<sup>th</sup>) demonstrate a wide range of temperatures that can occur within a night. Figure 3 focuses on the daily temperature fluctuation on these days, with the highlighted regions specifying 20:00 to 05:00 for each night. As a general approximation, 10°C is often used as a threshold below which foraging activity is greatly reduced due to reduced activity of flying insect prey (e.g., Czenze et al., 2013). Considering this threshold, the night of June 3<sup>rd</sup> was unlikely to have been suitable for foraging. However, the subsequent nights may have permitted brief periods of foraging earlier in the evening.



## 2.4 Emerging Threats and Challenges

Over evolutionary time bats have adapted to the energetic challenges they experience throughout the year. Bats maintain some amount of excess capacity, which enables them to cope with short-term and low intensity disruptions of expected energetic challenges (Kunz et al., 1998; Norquay and Willis, 2014; Hranac et al., 2021). However, bats are long-lived and may require many generations to respond to changes in environmental conditions and associated energetic challenges. Rapid and novel energetic threats pose challenges for hibernating bats. Two specific emerging threats are white-nose syndrome and climate change.

### 2.4.1 White-nose syndrome

White-nose syndrome (WNS) has led to the widespread decline of bat populations since its introduction to North America (Dzal et al., 2011; Ford et al., 2011; Reynolds et al., 2016). White-nose syndrome is caused by a psychrophilic (cold-loving) pathogenic fungus, *Pseudogymnoascus destructans* (Lorch et al., 2011). From its first documentation in New York state (Blehert et al., 2009), WNS has spread to 8 Canadian provinces and 38 American states in just under two decades (reviewed in Hoyt et al., 2021; United States Fish and Wildlife Service 2022). Bats affected by WNS experience both higher torpid metabolic rates and evaporative water loss throughout the hibernation period (McGuire et al., 2017). As a result, bats experience increased arousals throughout hibernation, eventually leading to death (Reeder et al., 2012).

Despite initial concerns of regional extinctions (Frick et al., 2010), bat populations persist post-WNS (Dobony et al., 2011; Kurta et al., 2020), and appear to stabilize after *initial P. destructans* exposure causes mass mortality (Maslo et al., 2015; Langwig et al., 2017; Frick et al., 2017). However, the fungus is environmentally persistent (Hoyt et al., 2015) resulting in

repeated and annual exposure to *P. destructans* at hibernation sites, with infection prevalence reaching 100% by the end of the winter (Langwig et al., 2015). After emergence, WNS-affected bats go through a recovery period. White-nose syndrome recovery in the post-hibernation period is accompanied with inflammatory immune responses directly following spring emergence (Hoyt et al., 2021). In a study on captive bats, Fuller et al., (2020) observed decreased pathogen loads one week after hibernation, and an intense healing phase 8 – 15 days after hibernation. How wild bats heal from WNS in the post-hibernation period, however, remains unstudied, and the environmental challenges faced by wild bats may make their healing a longer, more difficult process.

While it is intuitive to consider bats that survive the hibernation period as WNS survivors, the effects of WNS persist beyond hibernation (Davy et al., 2017). In the post hibernation-season, bats continue to face the effects of WNS, and the energetic cost of recovering from the disease (Fuller et al., 2011; Fuller et al., 2020). Bats with damaged wings may be less efficient at foraging (Voigt, 2013). Given the environmental challenges of the early spring, reduced foraging efficiency under conditions that are already energetically challenging may shift the energetic trade-off of foraging towards sick bats remaining torpid under marginal environmental conditions that could permit feeding in healthy bats. The early spring has limited food availability which, compared to captive studies that provide food *ad libitum*, makes healing from WNS a more difficult process. Bats likely use torpor more in the earliest stage of WNS recovery because they have limited energy stores following hibernation (Fuller et al., 2020). However, as the healing and recovery process proceeds, there is likely to be a period when bats do not use torpor as often while they heal and regrow damaged tissue (Fuller et al., 2020). Tissue regeneration cannot occur when bats are torpid and is one of the reasons for why defending a

euthermic body temperature is necessary for WNS recovery (Fuller et al., 2020). The cost of euthermia for a prolonged period in the post-hibernation season, when ambient temperatures are far below normothermia for hibernating bats, could be fatal for bats without sufficient energy to compensate for the cost of healing. However, this phenomenon has not been studied in wild bats, and it is possible that both the timing and duration of such periods of relative homeothermy may differ from those observed in captivity. Torpor is critical for surviving the energetic bottleneck of early spring and trade-offs between the energy cost of thermoregulation and immune function may result in sufficient energetic stress leading to death. The additional energetic stressor of WNS suggests another trade-off of the post-hibernation season, between the energetic savings of torpor and necessity for healing.

While few studies exist documenting WNS effects on reproduction, the potential effects have received some attention. Healing wing tissue and the associated requirement of euthermia are energy-intensive (Fuller et al., 2011). The energetic demand of intense disease recovery likely precludes pregnancy, suggesting some trade-offs between healing and delayed pregnancy. The necessity for healing prior to reproduction suggests that in the annual cycle of bats, the post-hibernation period includes a WNS recovery period, and then a reproductive period once the infection has been cleared (Davy et al., 2017).

Even if females make a complete recovery from WNS and give birth later in the season, there may be carryover effects related to the chronic stress imposed by infection and recovery. Cortisol levels in claws collected from dead bats suggest that WNS recovery induces chronic stress on bats (Davy et al., 2017). Chronic stress has been associated with impaired reproductive success and compromised immunocompetence in many species, such as brown trout (Pickering and Pottinger, 1989), multiple tropical bat species (Seltmann et al., 2017), meerkats (Young et

al., 2006), and mountain goats (Dulude-de Broin et al., 2020). Models of population recovery often fail to integrate carry-over effects from WNS recovery and could even overestimate the time in which populations can recover (Davy et al., 2017). As populations have been reduced to a small fraction of the pre-WNS population, understanding factors related to reproductive success among survivors is critical for understanding population recovery trajectories and guiding management actions, particularly in combination with other stressors such as variable weather conditions or habitat loss.

At the present time, there is no conclusive evidence for adaptive responses to WNS, though there are many theories that explain why bat populations persist. Intense selective pressure can result in selective sweeps that result in evolutionary rescue (Wilson et al., 2017). In the case of WNS, it may be that the disease has selected for adaptive genetic variants that were already present in the population (i.e., soft selective sweep; Gignoux-Wolfsohn et al., 2021). The “fat bat” hypothesis suggests bats are now observed with larger pre-hibernation fat stores to compensate for the increased energetic demand of hibernation with WNS (Cheng et al., 2019). However, this pattern is not observed everywhere; following the arrival of WNS in high-latitude populations there was no change observed in the amount of pre-hibernation fat stores compared to pre-WNS, likely because pre-hibernation body mass already approaches an upper ceiling beyond which flight ability may be compromised (CKR Willis, unpublished data). Likewise, lower-latitude bats may not increase mass prior to hibernation if they can forage throughout the winter to supplement their fat stores, adopting an income hibernation strategy rather than a capital hibernation strategy (*sensu* Drent and Daan 1980). Whether depositing more fat prior to hibernation, or foraging more regularly throughout hibernation, both strategies would result in

bats emerging from hibernation in better body condition, and better suited to deal with the energetic challenges of the immediate post-hibernation period.

It is also important to consider regional variation in host-pathogen dynamics, and implications for persistence of remnant populations (Grimaudo et al., 2022). If high-latitude bats cannot buffer against WNS by increasing pre-hibernation fat stores, torpor expression may be altered to use less energy throughout the winter. Male bats could, for example, begin using heterothermic arousals like females, while females arouse less frequently. The expression of hibernation represents the net result of trade-offs related to several factors (Boyles et al., 2020), which now include trade-offs related to WNS and altered strategies related to surviving the post-hibernation energetic bottleneck. Saving energy throughout the winter incurs non-energetic costs, which may be higher for high-latitude bats. Bats increasing body mass prior to hibernation may experience trade-offs with reproduction if additional foraging reduces time available for mating. In a promiscuous mating system, males are under far greater pressure to mate with as many females as possible, and therefore this trade-off may be male biased (Gallant and Broders, 2015; McGuire et al., 2016). During the spring, high-latitude female bats face a more substantial reproductive trade-off as well, having both a shorter active season and more intense WNS healing phase than lower-latitude bats (Hranac et al., 2021). Though commonly thought of as a disease of hibernation, the impacts that WNS has on early spring energetics requires further investigation.

#### *2.4.2 Climate change*

Increasing global temperatures are one important aspect of climate change in many regions, resulting in longer and more intense summer weather that can have negative affects on



animals, with many local extinctions having already occurred due to climate change-related events (Wiens et al., 2016). For bats, much of early spring survival is dependent on balancing energy amongst cold weather, limited resources, and withholding earlier reproduction due to poor environmental conditions. However, such a trade-off may be lessened with increasing global temperatures. Climate modelling indicates that increasing temperatures increases reproductive success in some species, aided by earlier emergence from hibernation (and later entry in autumn) and thus a longer growing season for offspring (Barbier et al., 2022; Munding et al., 2022). If global climate change indeed results in warmer conditions and earlier spring phenology, bats may have time to recover from WNS and still have sufficient time for reproduction. Given the possible stress effects of recovery on reproduction, earlier reproduction could also give pups that suffer the effects of a difficult pregnancy more time to develop before their first hibernation.

Unpredictable temperatures are another facet of climate change that could affect early spring activity, such as sudden warm fronts. Temperature changes are unlikely to affect bats during hibernation as surface temperature fluctuations are not readily detectable from within hibernacula (Czenze et al., 2017). In the early spring, however, such warm fronts may provide bats with more opportunities to forage. Such periods of foraging may also be more productive due to higher insect abundance. Sudden changes in temperature can especially be of assistance to bats with WNS, which may be able to heal faster either by foraging or using warmer temperatures to facilitate their healing periods.

Changes in precipitation are another aspect of climate change, and high latitudes are expected to experience increases in yearly precipitation (Dore, 2005). While precipitation is beneficial for reproductive females (Frick et al., 2010), precipitation in the early spring can have

deleterious effects on bats. Early spring is prone to a variety of precipitation events that induce energetic stress on bats, particularly rainfall and snowstorms (Willis et al., 2006). Precipitation limits the foraging ability of bats during the early spring (Zahn et al., 2007) and increases the thermoregulatory cost of flight (Voigt et al., 2011). When foraging opportunities in the early spring are limited, increasingly frequent and increasingly extreme precipitation events further exacerbate the environmental constraints of the post-hibernation season.

With increasing temperatures, insects tend to emerge, and peak, earlier in the year. For many long-distance migratory species, this results in a phenological mismatch, or a period within which two species do not have synchronized annual cycles (Visser et al., 2006). A common example is migratory birds arriving to their summer habitats after resources have peaked (Both et al., 2006). Long-distance migrants have no reliable cues that provide information about the environmental conditions at their distant breeding grounds. However, hibernating bats likely would not be affected by phenological mismatch in this way. Though bats can migrate long distances to hibernacula, migration distances are probably not far enough for surface weather conditions to differ dramatically between the area surrounding the hibernacula and the summer roosts. Hibernating bats can likely remain in relative synchrony with their insect prey and be robust to phenological mismatches in a way long-distance migratory species would not be.

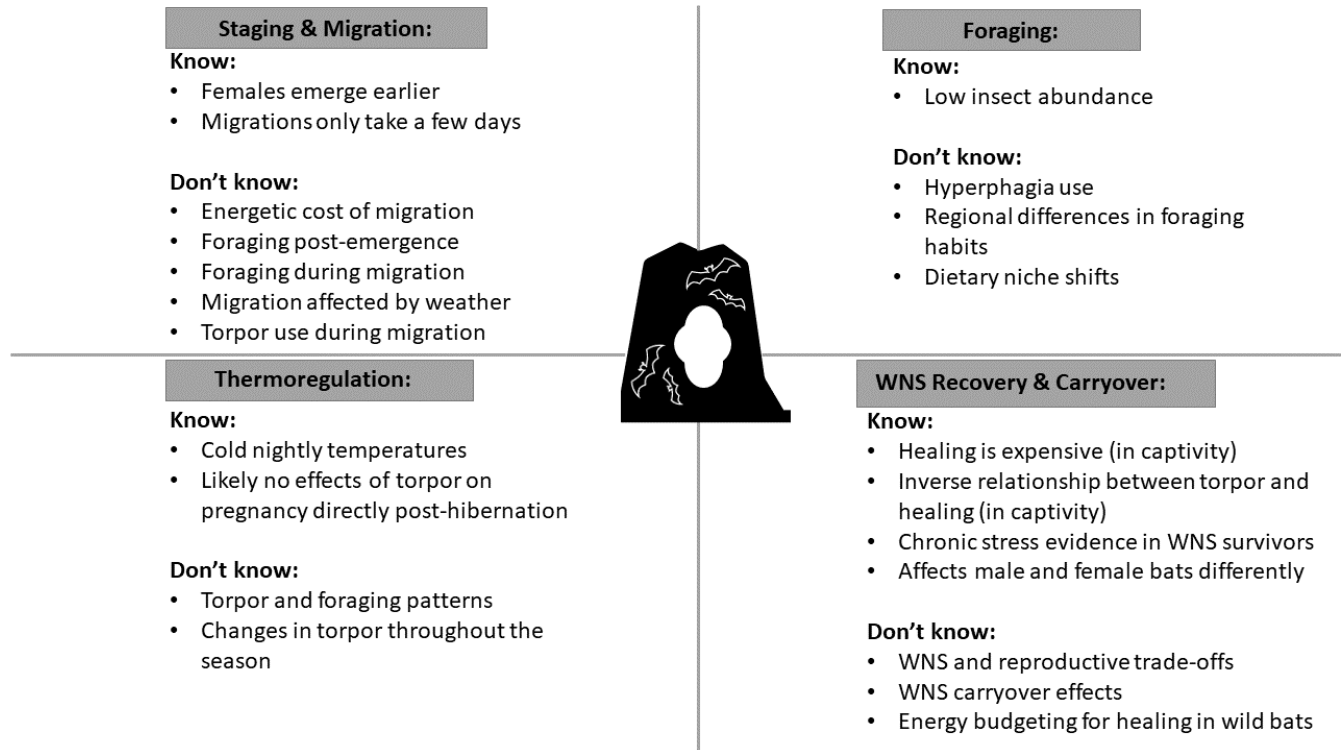
While the overall trend of climate change is towards warmer temperatures, variation among years and regional variation may result in periods of relatively cooler weather. Longer, or later, winters and colder springs can delay the onset of pregnancy and increase thermoregulatory costs during the post-emergence period. Temperature changes, particularly at low latitudes that are subject to extreme lows in temperatures because of climate change (Cohen et al., 2021), may also increase the energetic cost of winter for hibernating bats. More frequent extreme colds can

limit hibernation foraging opportunities and result in lower-latitude bats emerging in poorer condition or result in changes in pre-hibernation fattening.

## **2.5. Future Directions**

In this review paper I have summarized the available literature to understand the energetic bottleneck experienced by hibernating bats when they emerge in spring. In some areas (e.g., emergence timing factors, torpor use in cold weather, WNS recovery) there is a robust body of literature available, but in others there is scant empirical evidence. It is difficult to assess the entirety of an annual cycle without also considering the carry-over effects of other seasons (O'Connor et al., 2014). Non-reproductive seasons are often overlooked when considering annual cycles (Marra et al., 2015), but the effects of those seasons give a more detailed scope of the challenges animals face throughout the year. There is increasing evidence to suggest that the energetic challenges of the post-hibernation season can have long-lasting effects (e.g., Davy et al., 2017), which means that a detailed comprehension of the post-hibernation season is critical for having an integrative, holistic understanding of the seasonal and annual energetic challenges of hibernating bats.

The wide array of knowledge gaps surrounding the post-hibernation season offer exciting opportunities for elucidating not only the ecology of early spring, but the specific energetic challenges and carryover effects on later seasons (**Figure 2.1**). Combined with the effects of WNS and climate change, we can understand not only the specific energetic challenges of the early spring, but how these challenges differ by region and time period as well.



**Figure 2.1:** Summary of what is understood about the post-hibernation season of temperate insectivorous bats. Some information is available in the categories of Staging and Migration, Foraging, Thermoregulation, and WNS Recovery and Carryover. However, many knowledge gaps also exist within these categories that present exciting avenues for future research.

## 2.6 Conclusions

The following list summarizes aspects of the post-hibernation season that are well understood, and where there are important knowledge gaps. While many energetic challenges are intuitive, a lack of empirical data leaves many questions met with only speculation (**Figure 2.1**).

- 1) Female bats emerge from hibernation before males and travel to maternity roosts.

Females at higher latitudes are heavier when they emerge compared to their more southern conspecifics. There appears to be no consistent driver of emergence, though

temperature and barometric pressure both have been shown to cue bat emergence. Bats generally emerge before insects peak, so prey may be low in abundance, although it is unknown whether low prey abundance is a limiting factor for foraging bats. After emerging, bats travel to summer roosts, which may take a few days.

- 2) Throughout the early spring, bats use torpor to offset the cost of maintaining homeothermy in cold temperatures. While the use of torpor may conflict with reproductive success, bats are likely not pregnant in the early spring and the use of torpor likely only becomes potentially problematic later in the post-hibernation period. Bats likely supplement energy savings from torpor by foraging, when possible (i.e., warmer nights), potentially to the extent of hyperphagia.
- 3) Bats must recover from WNS in the early spring. The energetic cost of WNS recovery likely exacerbates the energetic challenges of the early spring. Compared to captive studies, recovery costs of wild bats are likely exacerbated by the energetic constraints of the post-hibernation period. With less available food and increased reliance on torpor, WNS recovery may come at a greater cost for wild bats and result in important trade-offs with reproductive success, particularly if there are complications from the chronic physiological stress imposed by WNS. Recovery may be further compromised if WNS negatively affects foraging ability.
- 4) Climate change likely has mixed effects on bats in the early spring, both within and among different regions. Increasing temperatures may result in earlier emergence, a longer active season, and a shorter hibernation season, thus reducing the negative impacts of WNS and providing more time for healing upon spring emergence. As hibernators, bats are more robust to possible phenological mismatch. However, increased precipitation

could limit foraging opportunities, which limits energetic input throughout the early spring.

- 5) Many knowledge gaps exist concerning various aspects of the early spring season for bats. It is unknown what bats do directly after emergence and there are no studies of the immediate post-hibernation period. How much bats eat both before migrating, and *en route*, is also unknown, though difficult to ascertain. How are these migrations affected by weather? Do they take longer than a few days if conditions are poor, and if so, what is the consequence of this?
- 6) Another knowledge gap is understanding which temperatures allow bats to forage throughout the early spring, and to what intensity. Bats use hyperphagia in the autumn, though this degree of hyperphagia may vary with latitude. Is early spring foraging characterized by opportunistic foraging? And if so, how does that vary regionally or across latitudes?
- 7) How do bats use torpor in the early spring to manage energy budgets, particularly on nights which may be favourable for foraging? Furthermore, how are thermoregulatory strategies related to recovery from WNS? Do bats use torpor for a longer period due to low resource abundance? Do wild bats experience extended periods of euthermia associated with healing, as observed in captive animals? What are the energetic implications of healing in free-living bats? Are bats able to offset healing costs through some combination of heterothermy and foraging? What effects does this recovery period have on reproduction?
- 8) Effective conservation strategies must include all periods of the annual cycle. To fully understand the extent of damage caused by WNS, we need to understand its effects

outside of hibernation. Additionally, we need to consider that WNS is not purely a disease of hibernation and has energetic costs and fitness consequences that may affect a bat until it enters hibernation the next year. With no clear method for preventing infection, or reducing the severity of disease during hibernation, it may be particularly beneficial to focus conservation efforts on supporting remnant populations, with particular consideration of the energetic challenges of the post-hibernation period.

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## Chapter 3

### Ten years after the arrival of white-nose syndrome, swarming bats develop larger fat stores but show no evidence of hyperphagia

#### 3.1 Overview

White-nose syndrome (WNS) has devastated temperate hibernating bat populations across North America, but many remnant populations persist. Larger fat stores are associated with an increased likelihood of survival during hibernation and may indicate an adaptive response to WNS. We hypothesized that WNS has selected for bats that build larger fat stores for hibernation and predicted that during pre-hibernation swarming bats would gain more mass and forage more intensely compared to the period before WNS arrived in North America. In 2021 we recorded body mass of bats during pre-hibernation swarming, and used plasma triglyceride analysis to quantify foraging intensity, repeating the methods of a study conducted in 2007 prior to WNS. We conducted our study at the same study site (an abandoned mine in eastern Ontario, Canada) and on the same calendar dates as the previous study. From 10 August to 16 September, we collected morphometric data on 572 little brown bats (*Myotis lucifugus*) and collected blood samples from a subset of 96 of those bats. Compared to the 2007 study, adults gained more mass prior to hibernation. In the pre-WNS study subadult bats lost mass through swarming, but we observed slight mass gain through the study period. Thus, both adults and subadults have increased body mass deposition compared to the pre-WNS study. While our results are consistent with other studies that have documented increased fat storage after *P. destructans* exposure, our study also attempted to ascertain the underlying mechanism for increased fat stores post-WNS. Increased foraging prior to hibernation is an intuitive mechanism for pre-hibernation fat store



deposition. However, despite our findings of increased mass gain post-WNS, plasma triglyceride analysis did not indicate more intense periods of feeding. This may suggest that changes in pre-hibernation behaviour or physiology may explain how bats can develop larger fat stores.

### **3.2 Introduction**

In temperate regions, winter is often accompanied by annual minimums in both ambient temperature and resource availability, posing a major environmental constraint on energy availability (Humphries et al., 2003; Auteri, 2022). Winter is especially difficult for small endotherms, which maintain body temperature through endogenous heat production but more easily lose heat to their environment than larger endotherms due to their high surface area to volume ratio (Tattersall et al., 2012). Species that rely on seasonal resources for food (e.g., insects) that decline in winter especially have difficulty matching the thermoregulatory costs of winter. For this reason, many small endotherms with seasonal diets use either torpor (controlled reduction of body temperature and metabolic rate) or migration to avoid the costs of winter, though these strategies are not necessarily mutually exclusive and can also occur with some degree of cold-weather resistance (Auteri, 2022).

With an insectivorous diet and small body size, temperate bats must use avoidance strategies throughout the winter (Speakman and Thomas, 2003; Willis et al., 2017). Some species migrate to avoid the winter (e.g., McGuire et al., 2014; Jonasson and Guglielmo, 2019), while others hibernate (e.g., Fenton and Barclay, 1980; Speakman and Rowland, 1999; Bernard et al., 2021). Bats that hibernate throughout the winter may go months without foraging, relying on endogenous fat stores for energy (Thomas et al., 1990). Bats reach their heaviest mass of the year in the late summer and early autumn, before they immerse into (enter) hibernation (Kunz et

al., 1998; Lacki et al., 2015). The pre-immersion period is characterized by declining temperature (increased thermoregulatory cost) and declining aerial insect abundance (reduced energy availability), resulting in an energetic bottleneck throughout the pre-hibernation period (Humphries et al., 2003). To build fat stores, bats may use torpor to reduce energy expended for thermoregulation and thus reducing the amount of dietary energy used for current energetic demand and depositing more fat for future energy demands (Speakman and Rowland, 1999; McGuire et al., 2009a). Bats may also forage hyperphagically (increased feeding intensity) during the pre-hibernation season (McGuire et al., 2009a; Suba et al., 2010).

Increased feeding and reduced activity are adaptations associated with the predictable decline in resource availability in late summer and early fall. However, if energetic requirements are unexpectedly increased, or energetic availability decreased, the resulting energetic stress can have population-scale effects. Wildlife disease often is a cause of disruptions in the energetic balance of animals, capable of exacerbating pre-existing challenges (McEwen and Wingfield, 2003). In 2006, the first instance of white-nose syndrome (WNS) was documented in North America during a routine cave survey in New York state (Blehert et al., 2009). After initial discovery of the disease, WNS spread throughout much of the northeastern United States and parts of Canada, and as of 2022, has reached eight Canadian provinces and thirty-eight American states (Hoyt et al., 2021; US Fish and Wildlife Service, 2022). White-nose syndrome is caused by the psychrophilic (“cold-loving”) fungus *Pseudogymnoascus destructans* (Lorch et al., 2011), which causes physiological disruptions throughout hibernation (Wilcox et al., 2014; Verant et al., 2014; McGuire et al., 2017) that lead to the premature depletion of fat stores (Reeder et al., 2012). White-nose syndrome has resulted in the death of millions of bats, pushing once common species (e.g., *Myotis lucifugus*, *Myotis septentrionalis*) into threatened or endangered species

status. Three species (*M. lucifugus*, *M. septentrionalis*, *Perimyotis subflavus*) have received federal endangered species status in Canada, while *M. septentrionalis* and *P. subflavus* are currently listed as threatened but have been proposed for endangered species status in the United States (US Fish and Wildlife Service, 2022). Population declines can affect macroecological patterns, including changes in the relative abundance of bat species (disproportionate declines of susceptible species) and changes in diversity of local assemblages (Frick et al., 2015; O’Keefe et al., 2019).

Despite widespread and dramatic population declines (>90% population decline in many cases; Frick et al., 2017), remnant populations of bats persist in areas that have been affected by the disease for more than a decade (Dobony et al., 2011; Kurta et al., 2020). White-nose syndrome is now endemic in many regions, with fungal infections reaching 100% prevalence by the end of the winter (Langwig et al., 2015). Though initial exposure to *P. destructans* (i.e., the first few years since the arrival of the pathogen at a new site) results in mass mortality, populations begin to stabilize after repeated exposure, with some populations even showing signs of recovery (Maslo et al., 2015). Adaptive responses to wildlife disease can occur within a very short time frame (Altizer et al., 2003), particularly if a disease is both highly transmissible and has a high mortality rate (e.g., Epstein et al., 2016). Recent evidence suggests that WNS-affected bat populations have experienced soft selective sweeps that have permitted continued survival (Gignoux-Wolfsohn et al., 2021). A notable trait that appears consistent with WNS survival is the deposition of larger fat stores prior to hibernation (Cheng et al., 2019).

Throughout hibernation, bats arouse periodically (return to euthermic body temperature in between bouts of torpor), and these arousals account for up to 90% of the winter energy budget (Thomas et al., 1990). Arousals are energetically costly due to the cost of warming from

torpid body temperature to euthermic body temperature and defending euthermic body temperature in the cold conditions of the hibernaculum (Thomas et al., 1990; Speakman and Thomas, 2003). Thus, hibernation energetics are driven largely by arousal frequency, and bats that arouse too frequently die after prematurely depleting their fat stores. While the exact reason for increased arousals from WNS is not well-understood, there is theoretical and empirical evidence to suggest an increased rate of evaporative water loss may be related to arousal frequency (Cryan et al., 2010; Willis et al., 2011; McGuire et al., 2017). Entering hibernation with larger fat stores may offset the costs of more frequent arousals, allowing bats to survive until spring emergence (Cheng et al., 2019). In cases where traits that are associated with increased survival and fitness are identified (e.g., increased fat storage), the widespread observation of such traits may indicate adaptive responses.

Comparisons of patterns in areas within which WNS is now endemic to patterns observed prior to the introduction of WNS can offer an opportunity for understanding population-level impacts of WNS. In 2007, McGuire et al., (2009a) conducted fieldwork at an abandoned mine in eastern Ontario, focusing on patterns in foraging and fattening prior to hibernation. During the pre-hibernation swarming period, adult bats gained mass while subadult bats (young of the year) lost mass. Swarming was characterized by a biphasic pattern of foraging, with hyperphagic foraging (see plasma triglyceride analysis below) earlier in the season that dropped in the latter half of the season, coincident with the onset of reproductive behaviours.

Assessing foraging intensity provides complementary information beyond observations of fat accumulation. Plasma triglyceride analysis is a method that has been widely used for this purpose (Jenni-Eierman and Jenni, 1994; Whittier and Mason, 1996; Guglielmo et al., 2005). Plasma triglyceride concentration is elevated following feeding, with increased concentrations

observed in as little as 15 minutes from feeding and remaining elevated for a period of several hours (Jenni-Eiermann and Jenni, 1994). For example, in a study of post-lactating female *M. lucifugus* fasted bats had plasma triglyceride concentration of  $0.03 \pm 0.01$  mmol/L, compared with  $0.84 \pm 0.10$  mmol/L on a summer night with good weather, and  $0.22 \pm 0.05$  mmol/L on a rainy summer night when bats would have had limited foraging opportunities (McGuire et al. 2009b). During pre-hibernation fattening, plasma triglyceride concentrations as high as 3 mmol/L clearly indicate hyperphagia relative to normal summer foraging levels (McGuire et al. 2009a). Therefore, plasma triglycerides give a snapshot of feeding intensity in the minutes to hours prior to capture (Jenni-Eiermann and Jenni, 1994; McGuire et al., 2009b).

Our research objective was to assess changes in pre-hibernation foraging and fattening in a population of *M. lucifugus* that has been exposed to *P. destructans* for many years. Specifically, we sought to determine if there is evidence to support the contention that bat populations exposed to WNS show an adaptive response in the form of increased pre-hibernation fat stores. To assess changes in pre-hibernation foraging and fattening following the arrival of WNS, we repeated the methods of McGuire et al., (2009a). The site had not been WNS-positive at the time of the original study but was confirmed WNS-positive in 2010 (US Fish and Wildlife Service, 2022). As of 2021, bats in the region had been affected by WNS for over a decade (US Fish and Wildlife Service, 2022). We performed our study over the same calendar dates, at the same site, and collected morphometric data and blood samples using the same methodology. We assessed pre-hibernation fattening with morphometric data and assessed foraging by collecting blood samples for plasma triglyceride analysis. We predicted that bats would gain more mass in 2021 than 2007, in both adult and sub-adult age classes. We also predicted that the increase in mass gain would be the result of increased hyperphagia, expecting either increased intensity of

hyperphagia (greater plasma triglyceride concentrations than observed pre-WNS), or a longer hyperphagic period than observed in 2007, or a combination of both. Finally, we compared the weather (temperature and precipitation) across the time periods spanning both studies to assess whether differences between the two study years may be associated interannual weather differences.

### **3.3 Methods**

#### *3.3.1 Study site*

We conducted our study at an abandoned mine in Renfrew County, Ontario (see Fenton [1969] for details). This site has been the subject of studies related to bat hibernation for several decades (e.g., Fenton 1969, Barclay et al., 1979, Brigham, 1987, McGuire et al., 2009a). In 2007, prior to the introduction of WNS to the region, McGuire et al., (2009a) captured bats from 9 August to 13 September, recording sex, age, capture time (minutes after sunset), forearm length and body mass, and collecting blood samples from a subset of captured individuals. We repeated those same methods from 10 August to 16 September 2021.

#### *3.3.2 Bat capture and data collection*

We placed a harp trap at the main entrance to the mine, and continuously monitored the trap when capturing bats to collect blood samples. When collecting morphometric data, we checked the trap every 10-15 minutes. We recorded sex and classified bats as either adult or subadult based primarily on the fusion of metacarpal-phalangeal joints (Kunz and Parsons, 2009). We measured forearm length ( $\pm 0.01$  mm) as a measure of body size, and recorded body mass ( $\pm 0.01$  g) using a digital scale (OHAUS, Parsippany, New Jersey).

### *3.3.3 Foraging analysis*

We documented pre-hibernation foraging intensity using plasma triglyceride analysis. Methods for collecting blood samples and analyzing plasma triglyceride concentration followed McGuire et al., (2009a). To avoid potential confounding effects of capture stress, all blood samples were collected within 10 min of capture, and we recorded bleed time (time from capture to completion of blood collection). Though stress responses are evident in plasma in as little as two minutes (Cyr et al., 2007; Edwards et al., 2021), there is no evidence of plasma metabolites being affected by stress effects in under ten minutes (Widmaier and Kunz, 1993). We punctured the interfemoral vein with a 27-gauge needle and collected blood with a heparinized capillary tube. We applied pressure after sample collection was complete, which was sufficient for most animals, and used a clotting agent (KwikStop; MiracleCorp) in the rare cases of persistent bleeding. All individuals were held for a minimum period of 10 minutes of observation before release. We did not observe any adverse effects of blood sampling and all bats flew away in apparent good condition.

We centrifuged blood samples for 5 min at 3384g (RK-17414-70; Cole-Parmer Canada, Quebec) and transferred the plasma into a cryotube secured with an O-ring. All plasma samples were frozen on site at liquid nitrogen temperatures in a cryoshipper (55709-228, VWR International, Mississauga, Ontario). We measured plasma triglyceride concentration using a commercial endpoint assay kit (TR0100, Sigma-Aldrich Canada, Oakville, Ontario) modified to work with 5µL of plasma (Guglielmo et al., 2005; McGuire et al., 2009b). We analyzed each sample in duplicate, volume permitting. For duplicate samples, we determined the coefficient of variation (CV) between the two samples. If the CV was > 15%, and there was sufficient plasma

available, we ran a third replicate, and selected the pair of replicates with the lowest CV. We excluded all samples with  $CV > 15\%$  and difference of triglyceride concentration  $> 0.1 \text{ mmol/L}$  between samples. Sample volume was limiting for some bats, only permitting a single sample to be analyzed. We conducted our analysis with and without single samples and found no qualitative difference in the interpretation of the results, therefore we include these samples in the results presented here. We used mean bleed time (8.2 min) for 6 bats that had no bleed time recorded. We calculated triglyceride concentration (triolein equivalents) by subtracting free glycerol from total glycerol following lipase hydrolysis.

#### *3.3.4 Weather conditions*

We retrieved historical weather (temperature and precipitation from 2006 - 2021; station 6106001; Environment Canada) to compare environmental conditions spanning the period of the McGuire et al., (2009a) study and our current research. The meteorological station chosen was about 130 km away, but previous analysis by McGuire et al., (2009a) showed that there was no significant difference in the weather conditions between the field site and location of the weather station. For dates from August 15 to September 30 each year, we calculated nighttime temperature as the mean of hourly temperature measurements made from 21:00 to 04:00 and calculated weekly cumulative precipitation (mm). We considered differences in precipitation between the 2006 and 2007 and 2020 and 2021 as well, to account for any potential carry-over effects from the prior years that could have resulted in different body conditions between our focal years.



### 3.3.5 Statistical analysis

All statistical analyses were performed in R Studio (R Core Team, 2022). Using sequential linear modeling, we assessed the effects of body size (forearm length), sex, date, age, and all two-way interactions on the body mass of bats. For analysis, date was considered as the number of days after August 1. In a separate analysis, we defined early (first ten days of the study) and late (last ten days of the study) periods to highlight mass changes between the beginning and end of our study. We assessed the difference between sexes, periods (early and late), and years (2007 and 2021) with separate ANOVAs for adults and subadults. We analyzed triglyceride data ( $\log_{10} [\text{triglyceride} + 0.01]$  transformed for normality) with sequential linear modeling to test the effects of age, sex, forearm length, body mass, bleed time, capture time, and date. Finally, we tested whether either 2007 or 2021 were exceptional years for weather in separate models. We used one-way ANOVA to test for differences in weekly precipitation, and an used an ANCOVA to test for differences in mean nightly temperature among years and used Tukey's post-hoc tests to identify pairwise differences among years.

## 3.4 Results

### 3.4.1 Body mass

Over a period of 37 days, we captured 572 little brown bats (**Table 3.1**). Adult and subadult bats gained mass at different rates (age: date interaction;  $F_{1,562} = 45.4$ ,  $p < 0.0001$ ) therefore we analyzed the two age groups separately. Controlling for body size (forearm length;  $F_{1,280} = 16.7$ ,  $p < 0.0001$ ), adults gained mass throughout the swarming season ( $F_{1,280} = 181.0$ ,  $p < 0.0001$ ; **Figure 3.1**), with no difference in body mass ( $F_{1,279} = 0.55$ ,  $p = 0.47$ ) or rate of mass gain between sexes (sex:date interaction;  $F_{1,278} = 2.2$ ,  $p = 0.14$ ). Similarly, controlling for body size

(forearm length;  $F_{1,282} = 10.7$ ,  $p < 0.0001$ ), subadults gained mass throughout the swarming season ( $F_{1,282} = 2.4$ ,  $p = 0.01$ ; **Figure 3.1**), and while subadult males generally weighed less than subadult females ( $F_{1,282} = 8.6$ ,  $p < 0.0001$ ) there was no difference in the rate of mass gain between the sexes (sex:date interaction;  $F_{1,281} = 0.58$ ,  $p = 0.22$ ). Adults gained mass at a rate of  $0.06 \pm 0.005$  g d<sup>-1</sup> compared to  $0.01 \pm 0.004$  g d<sup>-1</sup> for subadults.

Table 3.1: Sample size of *Myotis lucifugus* captured during pre-hibernation swarming at an abandoned mine in southeastern Ontario. All bats were included in analysis of body mass with a subset of those (indicated in parentheses) used for plasma metabolite analysis. Distribution of sex and age classes was fairly even for morphometric analysis, though metabolite analysis was heavily adult male-skewed.

	Male	Female
Adult	156 (48)	127 (11)
Subadult	157 (19)	132 (18)

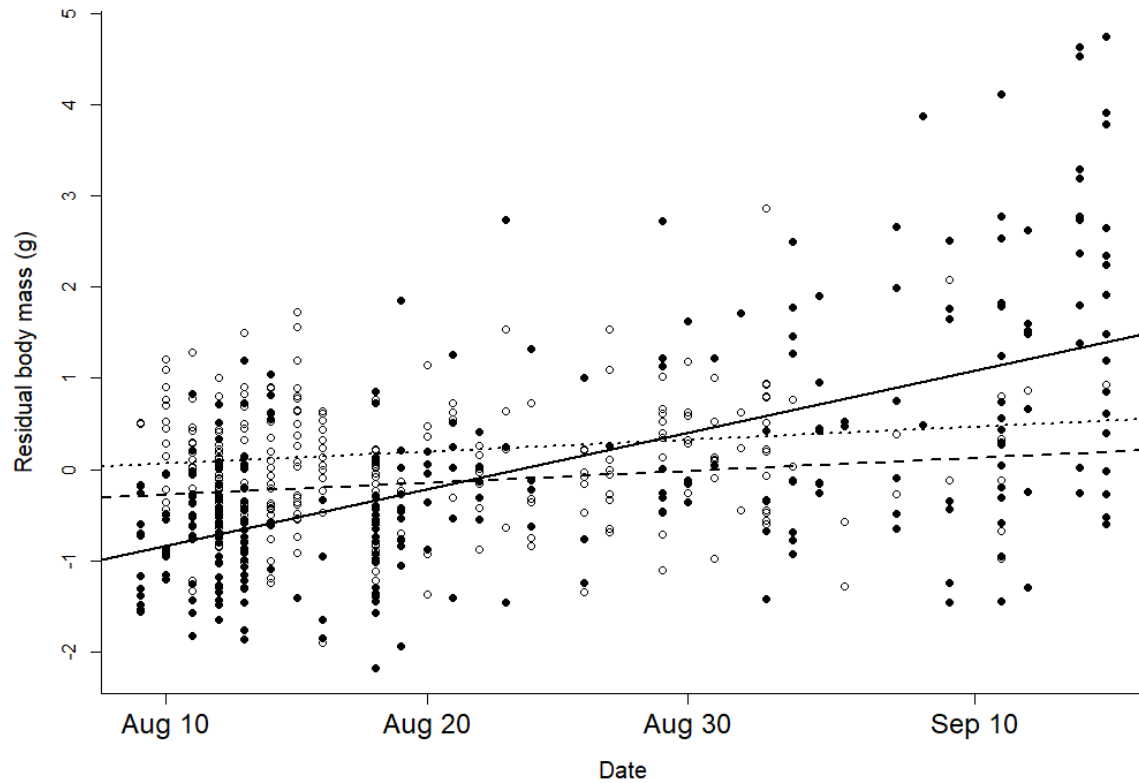


Figure 3.1. Size-corrected body mass of little brown bats (*Myotis lucifugus*) during the swarming period. Adults (●, solid line) gained mass, and subadults (○) slightly gained mass. Subadult males (dashed line) were generally lighter than subadult females (dotted line) but gained mass in a similar manner.

In the early period, adult body mass was the same in 2021 and 2007 ( $F_{1,179} = 1.25$ ,  $p = 0.88$ ), however, by the late period adults in 2021 weighed  $1.5 \pm 0.30$  g more than they did in 2007 ( $F_{1,104} = 24.2$ ,  $p < 0.0001$ ; **Figure 3.2**). Similarly, after accounting for greater body mass in subadult females than subadult males ( $F_{1,197} = 24.6$ ,  $p < 0.0001$ ) there was no difference in the early period body mass for subadult bats ( $F_{1,197} = 2.9$ ,  $p = 0.09$ ; **Figure 3.3**). However, by the

late period there was no difference in body mass between sexes ( $F_{1,19}$ ,  $p = 0.22$ ) and bats in 2021 weighed  $1.07 \pm 0.30$ g more than they did in 2007 ( $F_{1,20}$ ,  $= 13.1$ ,  $p = 0.002$ ).

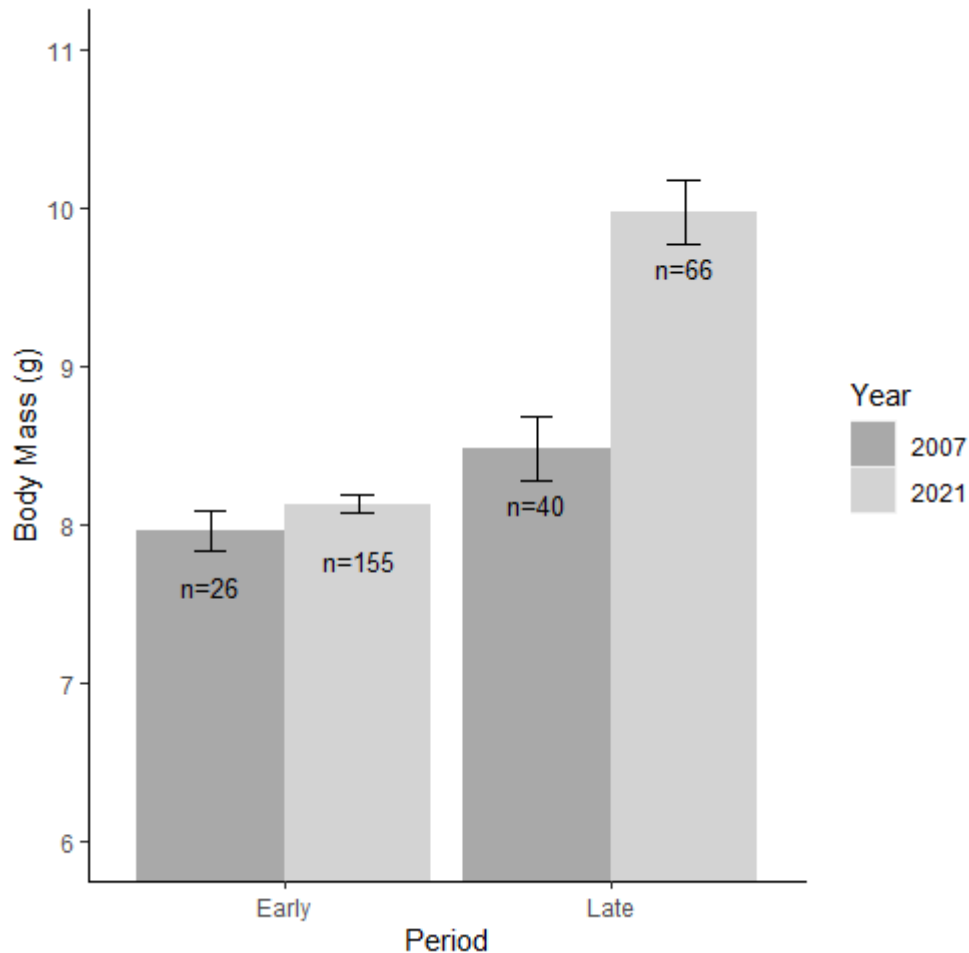


Figure 3.2. Comparison of body mass of adult little brown bats (*Myotis lucifugus*) during swarming in 2007 and 2021. Body mass did not differ in the early period, suggesting bats arrived at the swarming site in similar condition in both years. However, adults gained more mass in 2021 than adults in 2007, as indicated by a period by year interaction. Data are presented as mean and standard error, with sample sizes within each bar.

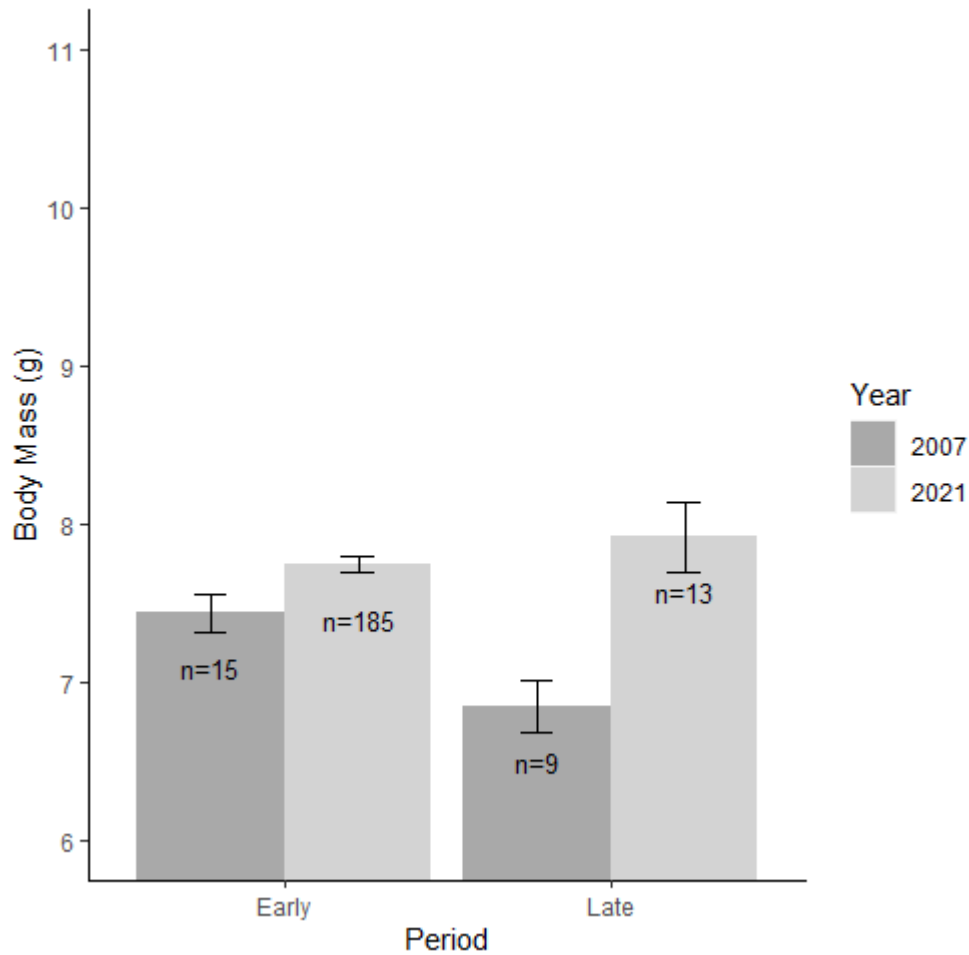


Figure 3.3. Comparison of body mass in subadult little brown bats (*Myotis lucifugus*) in 2007 and 2021 in the early and late periods of swarming. There was no difference in body mass during the early period, suggesting that subadults arrived to the swarming site in similar condition in both years. However, by the late period of swarming, bats weighed more in 2021 than in 2007. In fact, subadult bats gained mass in 2021, whereas in 2007 they lost mass. Data are presented as mean and standard error, with sample sizes within each bar.

### 3.4.2 Plasma metabolites

We collected blood samples from 96 bats (**Table 3.1**). We found no effect of forearm length, body mass, sex, date, or bleed time (all  $p > 0.05$ ) on triglyceride concentration, but there was a sex by age interaction ( $F_{1,85} = 4.72$ ,  $p = 0.03$ ). Therefore, we split the analysis by age, which revealed that triglyceride concentration decreased through the night for subadults ( $F_{1,35} = 6.58$ ,  $p = 0.01$ ) but there was no effect of capture time for adults ( $F_{1,57} = 2.2$ ,  $p = 0.14$ ). However, the parameter estimate for subadult capture time was  $-0.001$ , and therefore the effects of capture time were likely negligible.

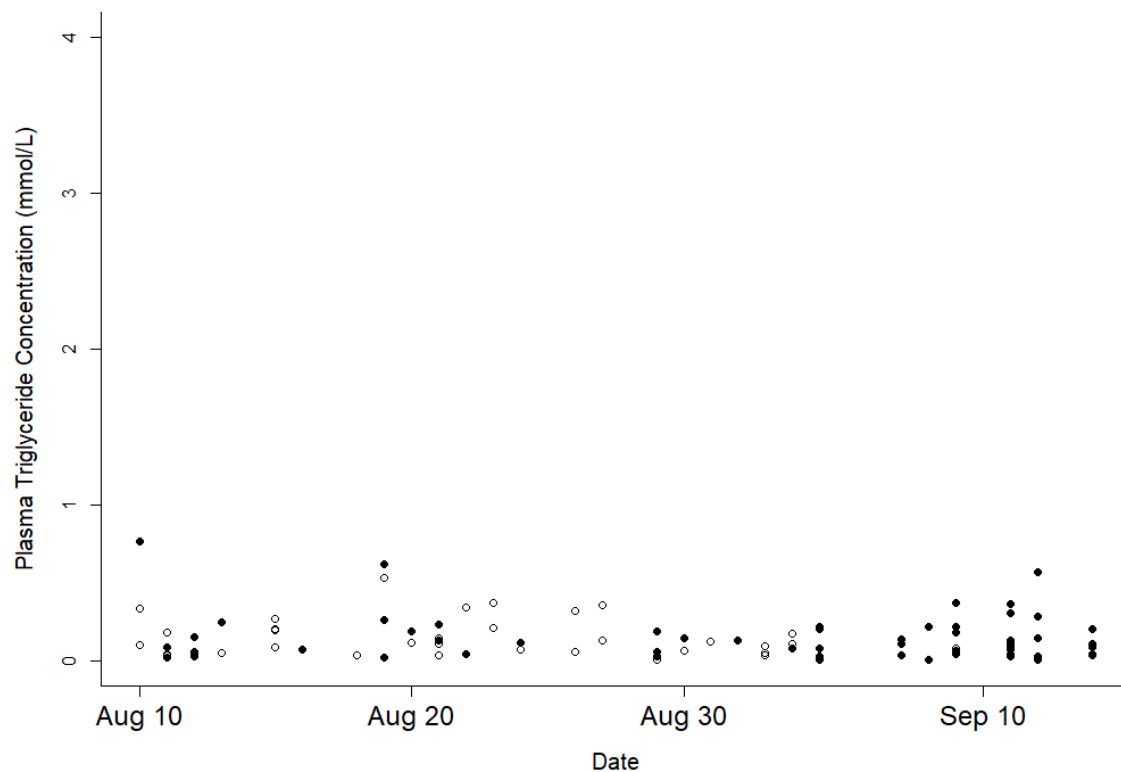


Figure 3.4. Plasma triglyceride concentrations across the swarming season in both adult (●) and subadult (○) bats. Plasma triglyceride concentrations were consistently low in 2021, not varying over time throughout the period of the study.

### 3.4.3 Weather conditions

Our initial analysis revealed a difference in the effect of date among years (date:year interaction;  $F_{15,715} = 2.27$ ,  $p = 0.003$ ) due to an unseasonably warm spell in late September 2017. Given that 2017 was not one of the years that we were specifically interested in, we removed this year from our analysis. Among the remaining years, there was a consistent decline in temperature through the season ( $F_{1,684} = 253.9$ ,  $p < 0.001$ ). Nocturnal temperature differed among years ( $F_{14,684} = 3.1$ ,  $p < 0.001$ ; **Figure 3.5a**). There was no difference in nocturnal temperature between our two focal years (Tukey's post-hoc  $p = 0.78$ ). We also considered differences between our focal years and the preceding years in case of possible carryover effects. There was no difference between 2006 and 2007 (Tukey's post-hoc  $p = 0.99$ ) but nocturnal temperatures were warmer in 2021 than 2020 (Tukey's post-hoc  $p = 0.01$ ). Weekly precipitation did not vary among years ( $F_{15,113} = 0.52$ ,  $p = 0.92$ ; **Figure 3.5b**).

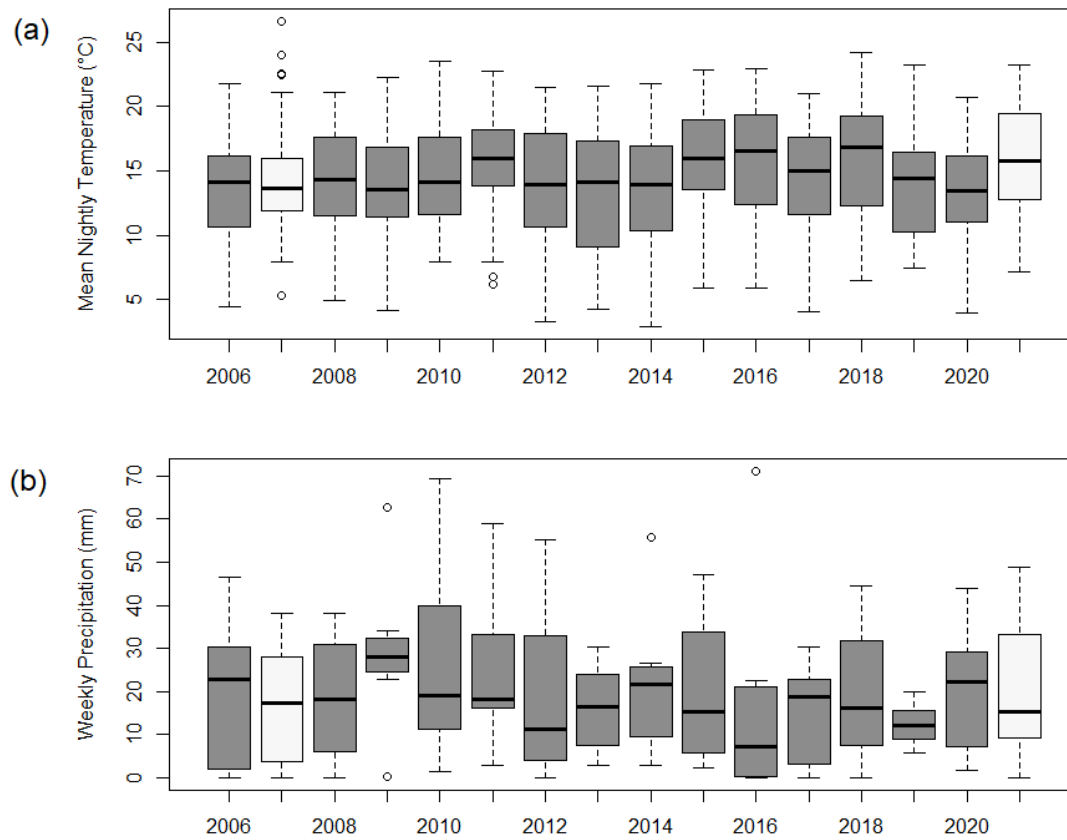


Figure 3.5: Mean nocturnal temperature (a) and cumulative weekly precipitation (b) from 2006 to 2021, with the two focal study years in light grey. There was no difference in either temperature or precipitation between the two focal years. Precipitation did not vary among years.

### 3.5 Discussion

Consistent with our hypothesis, after a decade of WNS bats were heavier in our 2021 study compared to the pre-WNS study in 2007 (**Figure 3.2, 3.3**). However, contrary to our predictions, the observed increase in body mass was not accompanied with any evidence of hyperphagia. We observed consistently low plasma triglyceride concentrations throughout the



season, similar to the plasma triglyceride concentrations observed for fasted bats or bats with limited foraging opportunities on rainy nights (McGuire et al. 2009b) and notably lower than the plasma triglyceride concentrations observed during pre-hibernation hyperphagia prior to WNS (McGuire et al. 2009a). We also analyzed differences in weather between 2021 and 2007 to determine if climactic factors may explain any of the changes documented throughout our study. Precipitation did not vary among years, and we found no evidence for temperature differences between 2007 and 2021, or 2006 and 2007 (**Figure 3.5**). The year of our study was warmer than its preceding year (**Figure 3.5**), which could indicate possible carry-over effects if 2020 was a particularly cold year, and bats spent much of their active season in poorer condition and had to compensate for their low mass throughout swarming. However, because bats arrived at the swarming site in 2021 in a similar condition to bats in 2007 (**Figure 3.2, 3.3**), it is unlikely that the differences between 2020 and 2021 explain the change in mass documented throughout our study.

Although we found increased body mass for both adults and subadults, the effect for subadults was particularly notable. McGuire et al. (2009a) observed that subadults lost mass throughout the swarming period, but in 2021 they slightly gained mass during swarming (**Figure 3.1, 3.3**). Mass gain is difficult for subadult bats, generally because of higher energetic demands (Kokurewicz and Speakman, 2006; McGuire et al., 2009a). Declining insect abundance throughout the season and increasing thermoregulatory costs makes mass loss more likely to occur in subadults than adults (Suba et al., 2010; Kohyt et al., 2016). Furthermore, temperate bats are less likely to survive their first winter if born later in the year, likely because they cannot deposit large enough fat stores prior to hibernation (Frick et al., 2010). The energetic cost of WNS recovery may also require a post-hibernation recovery period, which can delay parturition

dates for females (Davy et al., 2017; Fuller et al., 2020). If this is the case, then first-year bats are being born later into the spring but are still gaining more mass in less time than before WNS was introduced.

The pattern of triglyceride concentration we observed (consistently low throughout the swarming period) was quite different from the previous study conducted prior to WNS (McGuire et al, 2009a). The triglyceride concentrations of our study ( $0.14 \pm 0.01$  mmol/L) resemble those of *M. lucifugus* with low foraging success. Prior to WNS, there was a period of hyperphagia early in the swarming season coupled with mass gain throughout. Despite consistently low plasma triglyceride concentrations, bats gained substantial body mass in 2021, exceeding the body mass gain observed prior to WNS. A similar pattern was observed by McGuire et al. (2016) in central Manitoba, where bats hibernate for up to 8 – 9 months (Norquay and Willis, 2014), and require larger fat stores than Ontario bats. Despite greater observed mass gain, swarming bats in Manitoba had consistently low plasma triglyceride concentrations ( $0.30 \pm 0.03$  mmol/L) compared to the biphasic pattern observed in Ontario, with hyperphagic concentrations as high as 3 mmol/L (McGuire et al., 2009a). The arrival of WNS at our study site in Ontario may have resulted in changes in pre-hibernation fattening, leading bats to use similar fattening strategies as Manitoba bats to maximize mass gain prior to hibernation. Where bats in Manitoba require large fat stores to survive a longer winter, bats at our study site may now require larger fat stores to survive the energetic demands of WNS.

Torpor is very likely involved in how bats are able to deposit fat stores prior to hibernation, effectively by allowing bats to reduce daily energy expenditure and therefore deposit more nutrients from foraging as fat for later use. During the pre-hibernation season, bats can maximize mass gain after foraging by selecting cooler roosts and using torpor (Speakman

and Rowland, 1999; McGuire et al., 2009a; Suba et al., 2010). Post-WNS, bats may use torpor and cooler day roosts more often than before the disease was introduced. This may be especially prevalent in subadults, which before WNS experienced a decrease in mass during the swarming season. Subadult bats tend to emerge later than adult bats, allowing more time to develop fat stores. Productive ecosystems are also important for mass gain in energetically constrained seasons (Dechmann et al., 2014), so it may be that bats continue to forage hyperphagically in preparation for hibernation, but that this foraging now occurs at other, more insect-dense sites. Plasma triglycerides can remain elevated in the bloodstream for several hours after feeding (Jenni-Eiermann and Jenni, 1994), so it is unlikely that any of the bats we sampled were feeding within proximity of the swarming site. This suggests that bats may not forage intensely on nights when they visit the swarming site, in contrast to the pattern observed prior to WNS.

Mating is an important function of swarming in bats (Thomas et al., 1979; Furmankiewicz et al., 2013; Kohyt et al., 2016; Lacki et al., 2015). In a promiscuous mating system, male bats seek to mate with as many females as possible, but the number of copulation events may be less important for females. Males make their greatest energetic investment in reproduction throughout the swarming period, compared with females that primarily incur reproductive costs during the summer pregnancy and lactation periods (Willis, 2017). If bats increasingly spend time foraging at sites away from swarming sites or spend more time in torpor, males may face a trade-off between reproduction and surviving WNS. For female bats, this reproductive trade-off with hibernation preparation is likely less prominent. Females invest most of their energy into reproduction in the spring, and do not spend as much time at swarming sites as males (Burns and Broders, 2015), generally emerging sooner than males in the pre-hibernation season (Norquay and Willis, 2014). Swarming may play a role in gene flow between

populations as well (Kerth et al., 2003; Norquay et al., 2013), and altered mating strategies or less energy allocated to travel between sites may affect genetic diversity.

Inter-individual variation may affect patterns observed in our mass and triglyceride data. For example, the heavier bats captured towards the end of the season may have only just arrived at the swarming site, having delayed arrival until mid-September, while bats in poorer condition arrived earlier. It could also be that certain bats deposit their fat stores at different times, either before or after swarming, and our results simply reflect these two strategies. It could be as well that individuals arriving earlier in the season dedicate more energy to swarming than bats that arrive later and maintain a lower body mass because of this. The low triglycerides found in this study may also reflect foraging strategies specific to individuals. It is unlikely, however, that potential inter-individual variation would explain the change in mass throughout our study period as anything but pre-hibernation fattening, as many studies focusing on the same species have documented patterns in mass gain consistent with ours (Kunz et al., 1998; McGuire et al., 2009a; McGuire et al., 2016; Lacki et al., 2015; Cheng et al., 2019).

Future work focusing on post-WNS swarming physiology and behaviours should focus on understanding the mechanism by which bats are able to build larger fat stores within the same time period, but without foraging as intensely at swarming sites. The highly mobile nature of bats makes tracking individuals throughout the swarming season difficult, but automated telemetry (e.g., Garroway and Broders, 2007) offers methods with which nightly bat visitation to hibernacula can be quantified. Automated telemetry has been used to assess the frequency with which, and for how long, bats visit hibernacula (e.g., Gallant and Broders, 2015). Given the amount of time spent at hibernacula by female bats and male bats in good body condition ( $61 \pm 31$  minutes, Fenton, 1969), it may be that bats spend more time throughout the swarming period

foraging at other locations. If bats visit swarming sites and then leave to forage for the remainder of the night, this may explain the low plasma triglycerides found in our study.

If remnant populations of bats persist because of larger pre-hibernation fat stores, it highlights a shift in physiology and behaviour after the introduction of WNS. Given the apparent selective pressure of WNS, the genetic diversity of populations may shift if fat deposition has a genetic and heritable component, since bats that deposit larger fat stores pre-hibernation are more likely to survive, and hence have higher fitness. The expression of hyperphagia at swarming sites may become restricted to specific latitudes, as more populations of bats adapt to more energetically stressful winters. While our study only focuses on one site, there is clear evidence to support variation in pre-hibernation swarming physiology among populations with different winter challenges (e.g., Kunz et al., 1998; Lacki et al., 2015; McGuire et al., 2016). Regional variation in environment is an important aspect of understanding how remnant populations of bats persist (Grimaudo et al., 2022). While increased fat stores are a plausible explanation, and our results are consistent with other studies (e.g., Cheng et al., 2019), other mechanisms may be responsible for how bats survive WNS. It is also important to consider that increased fat stores are just one potential trait associated with surviving WNS. White-nose syndrome may nevertheless affect the pre-hibernation foraging and fattening physiology of entire regions, and future comparative studies may be able to elucidate regional adaptations to the disease.

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## **Chapter 4**

### **Synthesis, Conclusions, and Future Directions**

Animals are adapted for periods of energetic constraints imposed by variable environmental conditions (McEwen and Wingfield, 2003). For example, animals carry fat reserves that enable them to survive sudden drops in food availability (e.g., poor weather conditions that prevent foraging) with larger fat reserves typical of animals that inhabit highly variable environments (Fischer et al., 2010; McEwen and Wingfield, 2010). Such adaptations allow animals to maintain “stability through change” in their environment, a concept known as allostasis (McEwen and Wingfield, 2003; McEwen and Wingfield, 2010). Environmental challenges cause changes in behavior and/or physiology, and the cumulative effects of these challenges make up the allostatic load (McEwen and Wingfield, 2003). As challenges mount, particularly unexpected ones, allostatic load increases to the point of allostatic overload, a state where animals cannot meet their energetic demands (McEwen and Wingfield, 2003, Romero et al., 2009).

Throughout energetic bottlenecks, animals are on a knife’s edge against allostatic overload. For hibernating bats in autumn, declining resources and increasing thermoregulatory costs necessitate the use of energy-saving strategies to facilitate fat deposition with limited energy intake (e.g., Speakman and Rowland, 1999). In Chapter 2, I discussed how bats emerging from hibernation similarly have high thermoregulatory requirements despite often low food availability, requiring fat deposited in autumn to survive the early parts of the active season. Considering the allostatic load of bats during the shoulder seasons of hibernation may be an

effective framework for understanding the effects of white-nose syndrome (WNS) outside of hibernation.

The different timing of reproductive investment for male (spermatogenesis and copulation in autumn) and female (pregnancy and lactation in spring and summer) bats results in differences in the allostatic load experienced by each sex (Dzal and Brigham, 2013; Gallant and Broders, 2015). For male bats, the increased pressure to enter hibernation with large fat stores may preclude more frequent visits to swarming sites, instead forcing males to spend some nights feeding at insect-dense sites (McGuire et al., 2016). Thus, males may experience a fitness trade-off between reproduction and survival. Females may be less constrained in autumn, but face increasing allostatic load in the spring (Fuller et al., 2020). The cost of recovery from white-nose syndrome, and accompanying chronic stress, may preclude or delay pregnancy (Davy et al., 2017). Early parturition date is an important predictor of first-year survival, emphasizing the consequences of WNS-related delays in pregnancy (Frick et al., 2010). Additionally, in Chapter 3 I found that subadults, like adults, now deposit more fat during swarming, and the time required to do so may put additional pressure on female bats to reproduce sooner. Females may now face increased selection pressure for early pregnancy to maximize fitness through survival of their offspring, while at the same time facing delayed pregnancy and fitness trade-offs associated with spring healing and their own survival (Fuller et al., 2011; Fuller et al., 2020). In this way, WNS may result in fitness consequences for both male and female bats, but at different points in the year. In both sexes, increased costs associated with WNS may result in elevated allostatic load associated with reproductive failure, and potentially allostatic overload if they are unable to survive.



Subadult bats may experience a massive change in allostatic load throughout their active season, making them more susceptible to allostatic overload. Subadult bats face different energetic challenges from adult bats (Kokurewicz and Speakman, 2006). In preparing for their first hibernation, subadult bats both face the energetic challenges of somatic growth and fat deposition (McGuire et al., 2009). The energetic cost of the swarming season was substantial before WNS, enough that subadult bats lost mass throughout the season (McGuire et al., 2009). In Chapter 3 I found that after over a decade of being affected by WNS, both adult and subadult bats are heavier than they were before WNS. The effects of climate change are complex, and while warmer weather may offer lower thermoregulatory costs and more foraging opportunities, the effects of severe weather can also cause energetic challenges that may affect survival (Frick et al., 2010). The combined effects of climate change and white-nose syndrome throughout a period of low resource abundance make allostatic overload more likely for subadult bats. Given the energetic challenges of the post-emergence season, younger bats may be less likely to survive WNS recovery, which could shift the age structure of remnant bat populations as young-of-the-year bats die more often. This may result in remnant population recovery taking more time, even if adult females can successfully give birth after recovering from WNS.

There is evidence that WNS has resulted in a selective sweep that allows remnant populations of bats to persist (Gignoux-Wolfsohn et al., 2021). Certain beneficial genotypes associated with WNS survival could result in evolutionary rescue (and possibly are), reducing the likelihood of extinction in affected species (Maslo and Fefferman, 2015). Consistent with this, some remnant populations now experience increasing WNS survival rates (Maslo et al., 2015). High mortality rates are generally observed in the second year of WNS at a site (Langwig et al., 2015b), but many sites (especially sites with large pre-WNS population) experience

population growth as early as the third year from initial exposure (Maslo and Fefferman, 2015). Differences in the energetic challenges for populations at the leading edge of the spreading disease and populations in endemic regions have received little research attention but are important for discussions of how WNS may continue to affect bat populations, how populations may look in the future, and how the energetic challenges of bats may change. Adaptations in response to stressors are major ways in which animals can respond to present and future stressors and offset allostatic load when under energetic stress (McEwen and Wingfield, 2003). If adaptation to WNS is rapid enough, the energetic costs of the pre- and post-hibernation season may decrease as bats become better at resisting or coping with the disease.

White-nose syndrome conservation efforts should consider the types of factors that influence the energetic challenges of bats. The post-hibernation season exemplifies one intuitive challenge that has a likely solution. One of the largest energetic constraints of the season is that bats recovering from WNS must remain euthermic to recover, but during a time when cool ambient temperatures preclude sustained periods of euthermia (Fuller et al., 2020). Heated bat houses (lined with heating coils and powered by electricity) appear to assist WNS recovery and post-hibernation survival (Wilcox and Willis, 2016). Bats that roost in heated bat boxes can remain euthermic throughout their healing phase, without spending excess energy. In this way, bats can recover from WNS faster, and females can reproduce sooner into the spring. Ongoing research also supports the importance of heated bat boxes for post-hibernation bats recovering from WNS. In northwestern Ontario, bats appear to preferentially roost in heated bat boxes, and are present in heated bat boxes several weeks before bats in are present in non-heated boxes (**Box 2.1**). Many bats in northwestern Ontario that did not roost in a heated bat box also seemed to prefer chimneys or other manmade structures, further suggesting heat sources are important for

post-hibernation bats in colder regions specifically. However, not all species use bat boxes, and even those that do also roost in a variety of locations, such as tree snags (Grinevitch et al., 1995), foliage (Veilleux et al., 2003), buildings, or other anthropogenic structures (Lausen and Barclay, 2006). Maintenance of natural structures in high quality natural habitat can provide bats with more roosting locations and productive foraging areas. Male bats typically do not roost with females in maternity colonies, so efforts that focus on general habitat preservation would benefit all bats affected by WNS.

Conservation actions also should focus on the energetic challenges of the pre- and post-hibernation seasons, working to offset allostatic load in both. Preserving habitats such as wetlands can provide post-hibernation bats in the spring with more prey availability. Additionally, providing artificial roosts such as heated bat boxes or artificial tree roosts (Noteman, 1993) can be especially beneficial for bats affected by WNS that need warmer ambient temperatures to recover. In the pre-hibernation season, the energetic requirement is high enough that bats face a trade-off between foraging and swarming. Therefore, much like in the spring, productive habitats that provide high prey availability (such as wetlands) could assist bats in pre-hibernation fat deposition (Cheng et al., 2019). Focusing on habitats particularly close to hibernacula may also offset some of the reproductive challenges experienced by males during the swarming season. Artificial foraging habitats have been proposed as a possible way to increase pre-hibernation fat stores in swarming bats. For example, the Fat Bat Project is a coordinated effort by Bat Conservation International to set up UV lighting around hibernacula to attract more insects to the area (Anklam, 2021).

Many discussions about wildlife disease focus on how the disease can be treated. White-nose syndrome has no feasible treatment or method for containing its spread, so one of the most

feasible methods for assisting bats affected by WNS is reducing the allostatic load imposed on bats by WNS. This should involve comparing the effect of WNS on bats of different ages and sexes, different species, in different regions, different seasons, and different time periods relative to the arrival of WNS. By considering the energetic challenges bats face before, during, and after hibernation, we can develop conservation strategies and policies to support remnant populations and facilitate population-level recovery from this devastating wildlife disease.

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