Using Behaviour to Understand Conservation and Management Strategies for Threatened Little Brown Bats (*Myotis lucifugus*)

By

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CHAPTER 1
GENERAL INTRODUCTION

Wildlife conservation aims to preserve species abundance and diversity, while protecting wildlife habitat from anthropogenic impacts and environmental change (Lacy 2010; Soulé 1985). Wildlife conservation often focuses on small populations isolated in fragmented or imperilled habitat, or populations facing rapid declines. Caughley (1994) formalized a dichotomy in wildlife conservation with two population models. The small population paradigm addresses risks unique to small populations (e.g., inbreeding depression) and maintenance of long-term viability to reduce extinction risk, while the declining population paradigm focuses on detection and prevention of factors influencing declines (Caughley 1994; Young 2000). If factors influencing decline can be mitigated, restoration efforts can target the underlying causes of decline to enhance population growth (Young 2000).

Strategies for conservation of both small and declining populations can be divided into two broad categories (Lacy 2010). *Ex situ* conservation involves the preservation of species through breeding programs and captive housing, often as a response to dangerously low population levels with high risk of extinction (Lacy 2010). In comparison, *in situ* conservation aims to preserve species where they occur in the wild (Lacy 2010). *In situ* conservation efforts may involve habitat manipulation or restoration to improve habitat quality in ways that promote population growth (Lacy 2010). Although these two conservation methods depend on very different approaches and expertise, they may be used concurrently for highly endangered populations or species facing urgent conservation threats.

In situations where threats to wildlife populations are extremely severe, *ex situ* conservation may reduce the risk of extinction (Zippel et al. 2011). However, as *ex situ* conservation often requires that populations be maintained in unnatural environments (e.g., captivity), this method can be extremely labour-intensive and expensive (Pessier and Mendelson 2010; Zippel et al. 2010). For instance, $5.3 million over 10 years was allocated to the Calgary Zoo's Centre for Conservation Research to establish a captive breeding and rearing program to combat declines of the greater sage grouse (*Centrocercus urophasianus*; Calgary Zoo 2014). Part of this expense reflects the need to house large numbers of individual animals to maintain genetic diversity (Kunz et al. 2010; Zippel et al. 2011). In the Elliot's pheasant (*Syrmaticus ellioti*), for example, haplotype diversity was over 300x greater in wild versus captive individuals (Jiang et al. 2005). Repeated introduction of wild caught individuals may be needed to maintain diversity (Frankham and Loebel 1992), but this practice carries its own risks, especially from pathogen introduction (Zippel et al. 2010). Introduction of Cape platanna (*Xenopus gilli*) into a captive amphibian breeding facility in 1991 resulted in the mortality of 23 endangered captive Majorcan midwife toads (*Alytes muletensis*) from chytridiomycosis, an amphibian fungal disease (Walker et al. 2008). Strict protocols for decontamination and quarantine must be maintained in order to limit the risk of contamination and spread of pathogens in captive assurance programs (Zippel et al. 2010).

Ex situ conservation programs are usually based in facilities with expertise in captive housing of wild animals, such as zoos and aquariums, which can facilitate public access to wildlife. These interactions between the public and conservationists can provide unique opportunities to improve awareness and raise funds that may not be available to *in*

situ wildlife conservation programs. Public education programs encourage visitors to learn about endangered species, conserve habitat, and identify and potentially help to minimize risks that could contribute to species declines. For example, organizations, including the U.S. Fish and Wildlife Service, Bat Conservation International, and Organization For Bat Conservation, partnered to create a series of online webinars and webcasts, collectively termed BatsLIVE, to educate the public about bats and the conservation threats they face (BatsLIVE 2014). Education and awareness programs often run concurrently with funding campaigns. The Year of the Frog campaign to raise public awareness about chytridiomycosis helped zoos and aquariums raise significant funds for conservation (Year of the Frog 2008; Zippel et al. 2010). An additional benefit of *ex situ* conservation and captive assurance is the potential to provide animals for research that would otherwise need to be collected from wild populations (Zippel et al. 2010). This can be critical for answering questions about mechanism behind declines and answer questions about how to reduce impacts on populations.

Captive assurance populations have been established to address severe population declines for many endangered species (Earnhardt et al. 2014; U.S. Fish and Wildlife Service 2013), but these efforts will always come with challenges and often these challenges stem from differences in animal behaviour. For example, in situations where social animals are housed collectively behavioural tendencies and social dynamics could influence acclimation to captivity and success of a captive assurance program (Thompson 1993). Dominant and risk-taking individuals tend to be bold and aggressive (Dingemanse and de Goede 2004; Verbeek et al. 1999) which, in the wild, can result in greater access to resources and larger territories. This could influence feeding interactions in captivity,

with dominant or bold individuals monopolizing access to food at the expense of subordinates (Ceacero et al. 2012; Heg et al. 2011; Thompson 1993). Understanding the influence of behavioural tendencies and dominance structure is critical for ensuring the success of *ex situ* conservation efforts and could be important for guiding feeding and housing protocols.

In situ conservation occurs while endangered species continue to occupy their natural range in the wild, but this requires that the cause of declines be identified, that habitat be protected, and that strategies to facilitate stabilization and/or recovery of populations can be known (e.g., Lacy 2010). It is important to understand habitat quality, as well as processes that underlie selection of habitats and may influence distribution and abundance. For instance, density-dependence processes can influence reproductive capacity and survival. In white rhinoceros (*Ceratotherium simum*) negative density-dependence population growth influences recruitment of calves decreased with an increase in density within fenced conservation boundaries, while low-density population females have calves at younger ages (Rachlow and Berger 1998). On the other hand, understanding positive density-dependence is important for highly social or colonial animals, which benefit from relatively high density of conspecifics. For instance, some species, like little brown bats (*Myotis lucifugus*), congregate in large groups, which can increase energy savings, reduce evaporative water loss, and increase survival (Gilbert et al. 2010). In cooperative breeding species, like the African wild dog (*Lycaon pictus*), small group size may reduce the capacity for pup care and limit individuals available for cooperative hunting (Courchamp et al. 2002). So, social species could be susceptible to Allee effects (i.e., a decrease in individual fitness at low population sizes), especially

when populations decline and capacity for beneficial behaviours (e.g., colonial roosting; Gregory et al. 2010). In this way, large social groups are likely to increase probability for survival for colonial species and should be considered when developing management plans.

Maintenance of species in their natural habitat can benefit endangered populations and simplify conservation management practices. In some cases the cost of *in situ* conservation is minimal in comparison to *ex situ* programs (German Advisory Council on Global Change 2001; Leader-Williams 1990). For instance, although costs will vary for different taxa, the cost of housing rhinoceros in zoos is nearly 50x more expensive than maintaining habitat in a national park (German Advisory Council on Global Change 2001; Leader-Williams 1990). Establishment of protected areas can reduce habitat destruction and disturbance, allowing species to maintain normal social behaviours observed in the wild (Lacy 2010), while also facilitating ecological research and monitoring to assure population health (Linklater 2003).

In situ conservation in the form of protection of high quality habitat can have positive impacts on recovery of endangered populations, but this strategy can also be coupled with habitat enhancement to improve reproductive success and survival. For example, many endothermic species face energetic constraints during reproduction and poor weather. Warm microclimates are known to improve conditions for successful gestation and offspring growth (Dawson et al. 2005; Eeva et al. 2002; McCarty and Winkler 1999; Tuttle 1976). In cavity-nesting birds, the use of artificial heating increases the time allocated to incubation and can result in enhanced survival of offspring (Bryan and Bryant 1999; Nager and Noordwijk 1992; Schifferli 1973). Warm microclimates may

also facilitate healing (Andersen and Roberts 1975; Prendergast et al. 2002) and could be beneficial for recovery from disease. Thus, habitat enhancements that target the microclimate in habitats used by endangered species could help to slow declines and recover populations in the face of habitat loss and disease.

With over 1,200 species, bats account for over 20% of mammalian species and they face a diverse range of conservation threats. Deforestation and habitat degradation have impacted numerous species (Brosset et al. 1996; Shinnaka et al. 2007). In Guiana, species richness declined over 60% after deforestation, with 27 rare species of bats being completely eliminated from deforested sites (Brosset et al. 1996). Wind energy facilities are often viewed as environmentally friendly alternatives for energy production, but high mortality of migratory insectivorous bats is often observed at these facilities (Barclay et al. 2007; Jameson and Willis 2012). Modern wind facilities are taller than at historic developments resulting in an increase in the number of collisions (Barclay et al. 2007). In fact, in 2012 alone wind energy facilities resulted in an estimated 600,000 individuals killed in North America (Arnett and Baerwald 2013; Hayes 2013). Forestry and industrial developments have reduced forest cover, changing flight patterns, and increasing mortality due to motor vehicle collisions (Russell et al. 2008). A highway development in Pennsylvania, U.S.A., resulted in mortality of numerous species of bats, including the U.S. federally endangered Indiana bat (*Myotis sodalis*), in part because bats crossed the highway closer to traffic where forest canopy was low (Russell et al. 2008). Some bats may be subject to direct persecution (Mahmood-ul-Hassan et al. 2011) and some tropical species even face hunting pressure (Craig et al. 1994; Mickleburgh et al. 2009; Wiles et al. 1997). Over 180,000 bats were shipped to the Mariana Islands from Palau for

commercial use and food between 1975-1994 (Wiles et al. 1997). Although this is not an exhaustive list of threats facing bats, the resulting mortality has driven a need for conservation and management strategies to combat declines.

Since 2007, throughout eastern North America, white-nose syndrome (WNS) has caused one of the fastest declines of bats ever observed. WNS is an infectious disease of hibernating bats caused by the cold-tolerant fungus *Pseudogymnoascus destructans* (*Pd*). Multiple species have been affected, but little brown bats are among the hardest hit (Frick et al. 2010a; Langwig et al. 2013; White-Nose Syndrome.org 2014a). Cutaneous infection with *Pd* causes erosion of epidermis and connective tissue in exposed skin (Gargas et al. 2009; Lorch et al. 2011; Meteyer et al. 2009; Warnecke et al. 2012) that can lead to disruption of water and electrolyte balance, altered thermoregulatory behaviour, and often severe wing damage that could impact flight (Cryan et al. 2010; Cryan et al. 2013; Fuller et al. 2011; Warnecke et al. 2013; Willis et al. 2011; Zook 2005). Though the causative mechanism behind mortality is not fully understood, evidence suggests that hypotonic dehydration due to wing damage may result in an increased arousal frequency during hibernation leading to premature depletion of fat stores (Cryan et al. 2010; Cryan et al. 2013; Willis et al. 2011; Warnecke et al. 2013). No proven method to control WNS has been identified for use in the wild, though a range of compounds and interventions are being assessed (e.g., Cornelison et al. 2014). As treatment options remain in development, studies of other potential *in situ* and *ex situ* conservation strategies that could assist survivorship and improve recovery of populations from WNS are vital.

Conservation threats have greatly impacted many species of bats and prompted an urgent need to understand methods that could be used to mitigate declines. Many bat species can be difficult to maintain in a captive setting, while others (e.g., big brown bats (*Eptesicus fuscus*)) acclimate readily to captivity (Lollar 2010). Behavioural tendencies and social dynamics could influence acclimation to captivity, but we know little about the implications of behaviour for captive housing. Understanding the influence of behavioural tendencies and social behaviour on feeding and mass gain could improve our ability to maintain bats in stable body condition during the active season and allow obligate-hibernators like the little brown bat to accumulate sufficient fat during autumn to enable captive hibernation.

Improved understanding of behaviour could also enhance potential *in situ* conservation efforts. Bats select roosts with microclimates that improve energy savings and reproductive success (Lausen and Barclay 2003). Throughout the active season bats will readily employ torpor to save energy, but this can delay parturition (Lewis 1993) and lactation (Wilde et al. 1995). Torpor is also known to inhibit energetically expensive immune responses in other hibernators (Prendergast et al. 2002) and, thus, could negatively impact recovery from WNS in the spring for the minority of bats that survive the disease. Habitat modification, like artificial heating of roost structures, which targets thermoregulatory cost, could help bats sustain normothermic body temperatures (T_b; i.e., when T_b is regulated at high levels; Geiser 2013) for reproduction and recovery from WNS. This approach to *in situ* conservation could be particularly important if some bats exhibit heritable traits that facilitate survival from WNS (Menzies 2014).

The broad objective of my thesis is to use behaviour to improve understanding of the potential for both *ex situ* and *in situ* conservation strategies for temperate insectivorous bats. In Chapter 2, I examine whether the individual differences in behaviour could affect captive management (i.e., *ex situ* conservation) of little brown bats. Specifically, I report results of an experiment testing the hypothesis that behavioural tendencies that appear to reflect personality of little brown bats (e.g., activity/locomotion and anxiety/grooming) could influence feeding, body condition, and pre-hibernation mass gain for healthy and WNS-affected bats held in captivity. I also report results that show that captive little brown bats may exhibit a dominance hierarchy that influences feeding behaviour and body condition. In Chapter 3, I report results of a behavioural experiment and a bioenergetic model designed to test the potential of habitat enhancement targeting thermoregulation as an *in situ* conservation strategy. Specifically, I tested the hypothesis that little brown bats would preferentially select an artificially-heated bat house in order to reduce energy expenditure during the active season and compared the preferences of healthy and WNS-affected bats. I then modeled the energy expenditure of a hypothetical bat roosting in either artificially-heated or natural roosts to test whether artificial heating could provide biologically significant energy savings. Testing these hypotheses advances our understanding of the potential of both *in situ* and *ex situ* conservation strategies for management of threatened bat populations.

CHAPTER 2

THE EFFECT OF INDIVIDUAL BEHAVIOURAL TENDENCIES ON FEEDING AND BODY CONDITION IN A CAPTIVE COLONY OF LITTLE BROWN BATS (*MYOTIS LUCIFUGUS*)

ABSTRACT

Captive assurance populations can help preserve populations in the face of rapid declines that cannot be mitigated in the wild. Individual behavioural tendencies or personality may affect how easily different individuals acclimate to captivity and ultimately influence the success of captive assurance efforts. Temperate bat species face a range of conservation threats including habitat loss and degradation, mortality from wind energy facilities, and white-nose syndrome (WNS), a fungal disease causing the most rapid declines of mammals ever observed. I tested the hypothesis that behavioural tendencies associated with animal personality and the shy-bold continuum affect feeding behaviour in healthy and WNS-affected captive little brown bats (*Myotis lucifugus*), one of the species most affected by WNS. I predicted that, in comparison to shy individuals, bold, active bats would spend more time feeding, feed more frequently, arrive earlier to the food dish each night, and gain more mass in captivity than shy individuals. Bats were captured from the wild in spring and held in the laboratory in one of two flight cages, isolating healthy and WNS-affected individuals. I quantified behavioural tendencies using a hole-board test and recorded feeding behaviour. Every two days during the feeding experiment, I recorded body mass to quantify pre-hibernation mass accumulation. I found that more active WNS-affected bats fed more frequently and that grooming in the hole-board test (which may reflect anxiety) was correlated with body mass at capture

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(i.e., at the end of the preceding hibernation season). I also found that individuals from both healthy and WNS-affected groups arrived at the feeding dish in approximately the same order each night, which could reflect a dominance hierarchy. However, I found no evidence that order of arrival at the food dish affected feeding or mass accumulation. My results suggest that behavioural tendencies during the active season may impact captive maintenance of bats for conservation, particularly those affected by disease. More work is required to understand the role of dominance interactions on the potential success of captive assurance populations.

INTRODUCTION

Ex situ conservation involves removing wildlife from their natural environments to preserve species facing rapid declines and imminent extirpation or extinction due to threats that cannot be managed in the wild (Lacy 2010; Zippel et al. 2011). Captive assurance populations and captive breeding programs have been used successfully to conserve a number of endangered species, such as the Mauritius kestrel (*Falco punctatus*) and Guam rail (*Gallirallus owstoni*; Snyder et al. 1996). Declines of black-footed ferret (*Mustela nigripes*) populations in the United States, resulting from habitat loss and decreases in prey species (e.g., prairie dog (*Cynomys* spp.), were mitigated by the establishment of captive facilities which held nearly 300 individuals in 2013 (U.S. Fish and Wildlife Service 2013). Captive assurance populations have also been critical for species reintroductions, including black-footed ferrets and Puerto Rican parrots (*Amazona vittata*; Earnhardt et al. 2014).

In captive populations, or situations in which individuals must be housed

collectively, understanding behavioural tendencies could shed light on the potential of different species and/or individuals to acclimate to captivity. Species variation to captivity has been recorded across a variety of mammals and birds (Chamove et al. 1988; Mettke 1995; Mason 2010). Small-bodied primate species respond to the presence of humans with greater increases in activity than large-bodied primates (Chamove et al. 1988). Acclimation to captivity may also be affected by the physiological stress responses. High levels of glucocorticoids are observed in birds exhibiting feather-plucking behaviour (Owen and Lane 2011) with species varying in the intensity of feather-plucking (Mettke 1995; Mason 2010), suggesting that some species are more prone to stress-induced behaviour in captivity. Novel environments, proximity to humans, and animal handling can induce fear and escape responses, but environmental enrichment (e.g., providing toys) and early and gradual acclimation to handlers can reduce stress response (Seibert and Sung 2010; Fox and Millam 2004; Meehan et al. 2004). Reactions to novel environments and other stressors associated with captivity could influence the potential for different species or individuals to acclimate and survive in captive assurance populations.

Understanding the behavioural tendencies of individuals, or animal personality, may be particularly important for successfully acclimating animals to captivity. Personality refers to behavioural traits that remain consistent between individuals across time and situations. These traits are typically differentiated into a number of axes and include exploration and avoidance, shyness and boldness, and activity (Réale et al. 2007). Many behavioural tendencies are heritable (Careau et al. 2011), which suggests that maintenance of behavioural diversity in captivity may help to maintain genetic diversity.

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However, maintaining behavioural diversity may be challenging if captivity exerts artificial selection pressure favouring rapid adaptation to novel environments.

Population social structure and dominance-subordinate relationships could also affect an individual's acclimation to captivity and the success of captive assurance populations. Dominance refers to the status of an individual in contests for resources, territory, or status and can affect feeding behaviour in social environments (Barrette and Vandal 1986; Drews 1983). Therefore, assessing the behaviour of individuals housed collectively can reveal important information about the potential influence of social interactions on captive populations. For instance, Ceacero et al. (2012) demonstrated that dominant female red deer (*Cervus elaphus*) spent more time feeding during the first hour after food was provided. Behavioural tendencies linked to personality may also correlate with dominance (Dingemanse and de Goede 2004; Verbeek et al. 1999) and these tendencies can impact feeding behaviour. In barnacle geese (*Branta leucopsis*), bold individuals tend to search for food themselves and spend more time feeding than shy individuals (Kurvers et al. 2010). Dominance may also be associated with aggression (Verbeek et al. 1999) and, in some species, dominant individuals may target aggression at newly introduced individuals, excluding them from the group (Thompson 1993). Thus, population social structure and dominant-subordinate relationships may influence how animals acclimate to captivity and should influence procedures for the maintenance of animals in captive assurance populations.

Given that individual behaviour influences feeding, behavioural tendencies and social structure could impact energy acquisition and, in turn, affect the body condition of individuals in captive assurance populations. In cichlid fish (*Neolamprologus pulcher*),

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bold females grew faster in length than shy individuals because of higher feeding rates (Heg et al. 2011). Anxiety may also influence feeding behaviour and body condition. Japanese quail (*Coturnix japonica*) bred for high body mass tend to have lower plasma corticosterone levels, show reduced fear response, and approach novel objects sooner than individuals bred for low body mass (Jones et al. 1997). Koivula et al. (1995) found that dominant willow tits (*Poecile montanus*) gained more mass during the day than subordinate birds, but when food was supplemented, subordinates gained more mass with no effect on dominant individuals. A similar change occurred in great tits (*Parus major*) after dominant individuals were relocated to another flock (Lange and Leimar 2004). For captive maintenance programs, understanding how behavioural tendencies affect feeding and growth could be critical for designing a feeding regime, determining how much food to provide, and deciding which individuals to house together.

Many species of insect-eating bats are difficult to maintain in captivity (Lollar 2010), but face a range of urgent threats including loss of habitat (Kunz and Lumsden 2003), impacts from industrial development (e.g., mortality at wind turbines; Arnett and Baerwald 2013; Hayes 2013; Jameson and Willis 2012), and, most recently in North America, the infectious disease white-nose syndrome (WNS; Cryan et al. 2010; Warnecke et al. 2012). The variety of threats to bat populations, combined with current uncertainty about methods for keeping them in captivity, make it important to explore factors influencing the potential application of captive assurance for conservation of bats. Little brown bats (*Myotis lucifugus*) face an urgent conservation threat from WNS and represent a good model species for evaluating impacts of behavioural tendencies on captive maintenance. Little brown bats are a well-studied species and recent work has

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quantified individual variation in behavioural tendencies that appear to reflect personality (Menzies et al. 2013; Webber et al. 2014). Understanding how behavioural tendencies and patterns of interaction within social groups affect feeding behaviour and body condition in captivity could improve our potential to use captive assurance as a conservation strategy for bats.

To better understand individual behaviour and social structure in captive bats, I studied a captive colony of healthy and WNS-affected little brown bats. I used an open field test combined with a behavioural experiment to test the hypothesis that behavioural tendencies associated with the shy-bold continuum affect feeding behaviour and rate of pre-hibernation fat accumulation. I predicted that bold individuals would spend more time feeding and feed more frequently than shy individuals during pre-hibernation in late summer, resulting in greater mass gain. I also tested the hypothesis that captive little brown bats would exhibit signs of social structure that is linked to individual behavioural tendencies and has implications for feeding behaviour and body condition. I predicted that bats would arrive at their food dish in roughly the same order each evening, which can reflect a dominant-subordinate social structure (King et al. 2008), and that individuals exhibiting bold behavioural tendencies would arrive earliest at the food dish, feed for longer and more frequently, and gain mass most quickly.

METHODS

All procedures were conducted under Manitoba Conservation and Ontario Ministry of Natural Resources permits and were approved by the University of Winnipeg Animal Care Committee. Between 28 April and 5 May 2013, 32 adult male little brown

bats (*WNS+*) were captured from two WNS-positive abandoned mines less than 90 km apart near Renfrew, Ontario (45.4717°N, 76.6831°W) and Gatineau, Québec (45.4833°N, 75.6500°W), Canada. Of these individuals, most were used for an experiment to understand wing-healing following WNS, but eight were available for this study. Mass was measured using a portable electronic balance (Ohaus Corporation, CS200, Pine Brook, New Jersey, U.S.A.) and forearm length was measured using digital calipers (Mastercraft, digital caliper, Vonore, Tennessee, U.S.A.) at the site of capture. Bats were outfitted with temperature dataloggers (DS1922L-F5 Thermochron iButton, Maxim, San Jose, California, U.S.A., modified to reduce mass following Lovegrove 2009 and Reeder et al. 2012) attached in the interscapular region using a latex-based adhesive (Osto-Bond, Montreal Ostomy Centre, Vaudreuil-Dorion, Québec, Canada). A unique symbol was marked on the exterior surface of each iButton to differentiate individual bats. Bats were held in cloth bags inside hepa-filtered animal carriers that were stored in a temperature-controlled cabinet to encourage hibernation during transport 2,128 km by car to the University of Winnipeg. Upon arrival bats were weighed again and banded with a uniquely numbered, lipped aluminum forearm band (Porzana Limited, East Sussex, U.K.).

On 28 May 2013, 39 male little brown bats (*WNS-*) were captured from a WNS-negative cave in central Manitoba, Canada (53.2083°N, 99.3000°W) as controls for the wing-healing study, and eight of these bats were available for my study. Bats were banded and placed in cloth bags. Each bag was suspended in a plug-in ventilated cooler, which was transported 432 km by car to the University of Winnipeg. Upon arrival, all

morphometric measurements were obtained and dataloggers attached as described for
WNS+ bats. Bats were housed until the beginning of hibernation on 11 October 2013.

Temperature and relative humidity (RH) in the holding room were controlled at
18°C and 60% RH, and light was set to maintain a natural photoperiod (light:dark =
11:13 hours) with a graduated lights-on to lights-off transition. Both groups of bats (i.e.,
WNS+ and *WNS-*) were housed in custom-built, aluminum flight cages 2.24 m^2 long x
1.01 m wide x 2.42 m high. Each flight cage was equipped with two custom-built, single-
chambered bat houses (44 cm x 6.3 cm x 60 cm) placed side-by-side at the one end of
each flight cage. The back of one bat house was lined with an electrical heating coil
(Exoterra Temperature Heating Cable, 12 V, Rolf C. Hagen Group, Mansfield,
Massachusetts, U.S.A.) controlled using an electronic temperature controller set to 30°C
(Nema 4x Electrical Temperature Control, Ranco, Invensys, U.K.). This temperature was
selected to provide a T_a close to the thermoneutral zone (i.e., 32°C; Humphries et al.
2002, 2005; Speakman and Thomas 2003; Stones and Wiebers 1967) allowing bats to
express shallow torpor to save energy if they seek it. In the *WNS+* flight cage the heated
bat house was mounted on 7 May 2013, but, because the second bat house was not yet
ready at the time I had to collect the bats, the unheated bat house was not installed until
21 May 2013. In the *WNS-* flight cage both the heated and unheated bat houses were
installed on 21 May 2013.

Throughout captivity, bats were provided mealworms (*Tenebrio molitor*) and
water *ad libitum*. Mealworm substrate (i.e., cream of wheat, powdered milk, natural
wheat bran) was supplemented with calcium, mineral, and vitamin powder. On 27 June
2013 I attempted to supplement the mealworm diet with crickets for 2 nights, but feeding

dramatically declined during this period so I returned to a mealworm-only diet. I applied

Selamectin (30 μL, Revolution, Zoetis, Florham Park, New Jersey, U.S.A.), a topical

parasiticide, to the interscapular region on 7 June 2013 once bats had adjusted to

captivity. To more easily identify individuals and monitor activity, bats were tagged with

passive transponders (PIT tags) beginning on 26 June 2013 and finishing on 08 July

2013. Bats were collected from inside bat houses for weighing approximately every two

days (every 1.9 ± 1.60 days for *WNS+* bats; every 2.3 ± 1.27 days for *WNS-* bats).

To help ensure the bats remained normothermic during behavioural trials

(Matheson et al. 2010) bats were hand-fed mealworms prior to testing. Tests were

conducted during the natural foraging period in a dark room between 19:00 and 22:00 hrs

CDT on 4 July 2013 for *WNS-* bats and on 11 July 2013 for *WNS+* bats. Behaviours were

recorded for 10 minutes with an infrared video camera (SONY Nightshot HandyCam

HDR-CX150 HD Camcorder, Toronto, Ontario, Canada) mounted on a tripod. Infrared

lights (Wildlife Engineering, IRLamp6, LaCrosse, Wisconsin, U.S.A.) were used to

provide additional light.

I use the term behavioural tendencies rather than personality because, although

the traits I quantified have been shown to be repeatable over a 24-hour period in

captivity, long-term repeatability has not yet been confirmed (Menzies et al. 2013,

Webber et al. 2014). I followed Menzies et al. (2013) to quantify behavioural tendencies.

I used a hole-board apparatus constructed from a 57 cm long by 14 cm wide by 42 high

plastic storage container with a Plexiglas® top (Figure 2.1). The test apparatus was hung

vertically with the long axis parallel to the floor, to provide an environment mimicking

the surface of a roost which a bat would crawl. An entrance opening (14.5 x 14.5 cm) was

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drilled into the bottom and center of the long axis and covered with a square of Plexiglas®

to serve as a door. Two pieces of fishing line were attached to the top corners of the door

allowing it to be opened and closed with minimal disturbance to the bat being tested. A

Plexiglas® tube (16 cm long x 8 cm diameter) was attached to the outside of the test

apparatus to act as a start box. Inside the chamber four circular holes (2 cm deep x 3 cm

diameter) were drilled to simulate roost openings that bats might investigate in the wild

(Figure 2.1). Two holes were positioned close to the center of the chamber (holes 1 and 2

were 15 cm from left or right wall of the test apparatus; Figure 2.1) and two were placed

near to the corners of the chamber (holes 3 and 4 were 5.5 cm from the left or right wall;

Figure 2.1). The back of the chamber was lined with plastic window screening to allow

climbing. At the start of each trial, a bat was placed in the start box and allowed to enter

the test apparatus. If the bat did not enter the chamber I gently nudged it into the testing

chamber and sealed the door so that bats could not return to the start box. I used a

stopwatch to record how long it took bats to enter the test apparatus.

Immediately after each trial I measured body temperature (T_b) using a

thermocouple probe inserted 3 mm into the rectum (TC-2000 Thermocouple Meter, Sable

Systems International, Las Vegas, Nevada, U.S.A.) to confirm bats were not torpid (i.e.,

$T_b > 30°C$; Menzies 2010) and then recorded body mass. I also counted fecal pellets in

the test after each trial as defecation in a novel environment test can reflect anxiety in

rodents (Hall 1934; Hare 1998). The chamber was washed thoroughly with hot water

between trials to limit eliminate chemical olfactory cues that might have influenced

behaviour. To prevent the spread of *Pd*, *WNS+* and *WNS-* bats were run in separate but

identical test chambers.
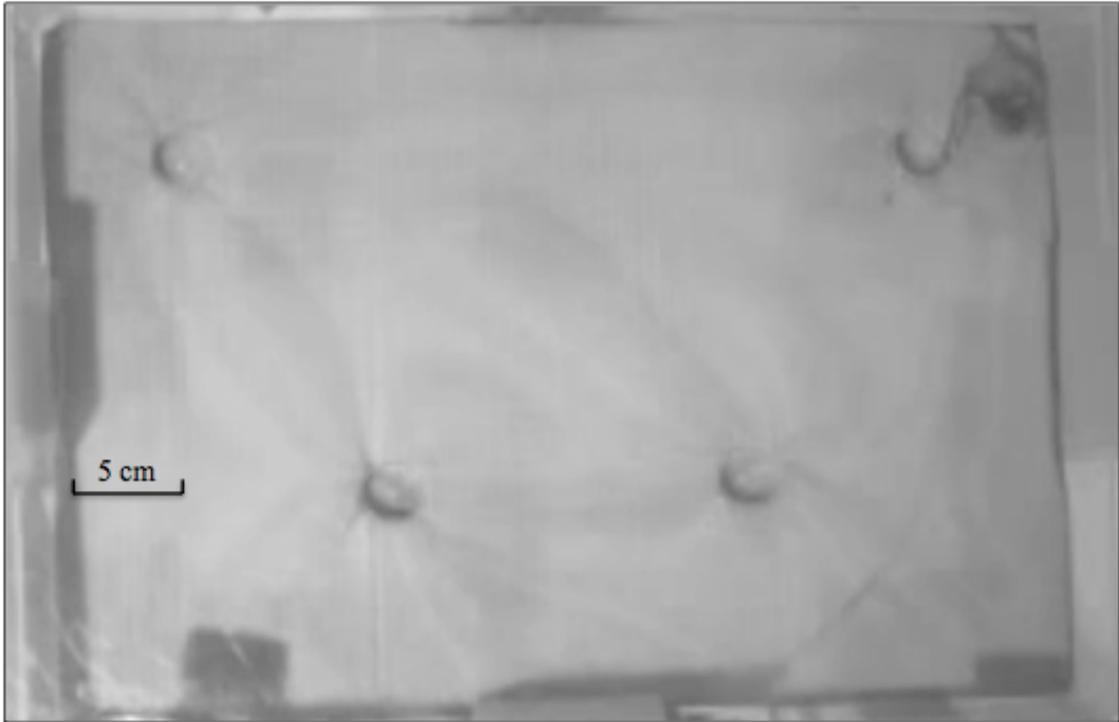
Figure 2.1. Screen capture from a video recording of a little brown bat in the hole-board test.

I scored videos for behaviours thought to reflect personality in bats (Menzies 2013; Webber et al. 2014). These included the latency to, and duration and frequency of grooming (i.e., contact of the hind foot, wing, and/or thumb with the head, or body to Scratch; Hofstede and Fenton 2005), locomotion (i.e.,movements indicated by motion of the forelimbs and the thumbs breaking contact with the surface of the chamber), echolocation/scanning (i.e., back-and-forth movements of the head without any other form of locomotion), flight attempts, and exploration of the holes in the hole-board test apparatus. I superimposed a horizontal and a vertical line, intersecting in the center of the image on each video to quantify the frequency of line crosses as an additional index of activity (i.e., the number of times an individual crossed one of the grid lines). For a few videos the camera battery failed before the 10 minute test was completed. Therefore, I scored only the first 8.5 minutes (i.e., the maximum recording time I obtained) of all videos. For any behaviour that did not occur during a given trial, I set the duration as the maximum duration of the test (i.e., 8.5 minutes; Budaev 1997).

I conducted a feeding experiment over five nights from 30 August to 3 September 2013. Bats were separated by infection status (i.e., *WNS+* and *WNS-*) and relocated to nylon mesh enclosures (41.9 x 41.9 x 77.5 cm; modified Flexarium, ExoTerra) in a temperature-controlled room set at 18°C. Photoperiod was maintained at levels observed in late August (light:dark = 17:7 hours). Each night bats were provided with 50.0 g of mealworms and water. A loop antenna (Dorset single coil antenna, 30.5 x 24.75 x 10.2 cm, Aalten, Netherlands) was positioned over the food dish and sealed along the edges so that bats had to pass through the loop to access the food. When a bat entered the food dish its unique PIT-tag code was recorded and time-stamped by a decoder/datalogger

(Dorset stationary decoder, Lid650N v727, Aalten, Netherlands). I later calculated the order of arrival at the dish, the frequency of visits to the dish, and the duration of each visit, except for one *WNS-* bat which was removed from the analysis as he remained near the loop antenna and was continuously recorded throughout the night.

All statistical analyses were conducted in R (version 2.14.1; R Core Development Team 2011). I analyzed behavioural tendencies for each group (i.e., *WNS-* and *WNS+*) independently because they were always housed in separate cages and could have behaved differently due to population differences or infection status. Normally principal component analysis is used to reduce the number of measured behavioural variables into smaller components (Menzies et al. 2013; Webber et al. 2014), but as large sample sizes are required to obtain reliable results (O'Rourke and Hatcher 2013) I was unable to apply this procedure. As an alternative, I determined the variance inflation factors (VIF; Zuur et al. 2009a) to test for multicollinearity among behaviour variables and reduce the number of variables used in my analysis. Variables with the highest VIF were sequentially dropped from my analysis until all VIF values were below 3 (Zuur et al. 2009a). Most of the variables I scored were strongly correlated, which left only three variables for subsequent analysis: durations of locomotion, grooming, and echolocation. These were the same behavioural tendencies that Menzies et al. (2013) and Webber et al. (2014) found best-explained personality variation in little brown bats.

I tested for an effect of the behavioural traits on feeding behaviour using a general linear model. I included the average duration or frequency of visits to the food dish recorded over the five days of the experiment as the response variable and the behavioural tendencies in the hole-board test. I ran separate analyses for each of the three

behavioural tendencies (e.g., duration of locomotion, grooming, and echolocation). I did not correct for multiple comparisons using Bonferroni-adjusted alpha levels because this method is overly conservative with small sample sizes (Narum 2006; de Muth 2006; Norman and Streiner 2008).

I ranked the order of arrival at the food dish for all 8 individuals in each group for the five nights of the feeding experiment. I used a two-way intraclass correlation to determine if the order of arrival at the food dish was repeatable over the five-day experiment. I also calculated the average arrival rank for each individual over the five days and then used a generalized linear model to determine if order of arrival was related to each of the individual behavioural variables I measured in the hole-board test. My data were not normally distributed, so I specified a Poisson distribution with a log link function based on preliminary tests selecting for the optimal distribution using the Akaike Information Criterion (AIC; Zuur et al. 2009a, 2009b).

I used a general linear model with average duration or frequency of visits to the food dish measured over five days as the response variable and the average order of arrival at the food dish for each individual as the predictor variable to determine if the order of arrival at the food dish influenced feeding behaviour.

I used three different measures of body mass to examine the effect of behavioural tendencies on changes in body condition throughout captivity: (1) mass at the time of capture in spring, (2) mass on the day bats began hibernation at the end of my study on 10 October 2013, and (3) the change in mass from capture to hibernation. I used a general linear model with mass as the response variable and behavioural variables as predictors with forearm length for each individual included as a covariate to control for body size. I

also conducted this analysis using average order of arrival at the food dish as a predictor

variable to determine if order of arrival was related to body condition.

RESULTS

For *WNS-* bats there was no effect of the duration of locomotion in the hole-board

test on duration of feeding ($F_{1,5} = 0.06$, $p = 0.82$). Likewise, there was no effect of

duration of grooming ($F_{1,5} = 0.004$, $p = 0.95$) or duration of echolocation ($F_{1,5} = 0.52$,

$p = 0.50$) on duration of feeding. For *WNS+* bats, there was no effect of duration of

locomotion ($F_{1,6} = 1.32$, $p = 0.29$), duration of grooming ($F_{1,6} = 1.34$, $p = 0.29$), or

duration of echolocation ($F_{1,6} = 1.47$, $p = 0.27$) on duration of feeding.

Results were similar for frequency of feeding. For *WNS-* bats there was no effect

of duration of locomotion ($F_{1,5} = 0.002$, $p = 0.97$), duration of grooming ($F_{1,5} = 0.07$,

$p = 0.80$), or duration of echolocation ($F_{1,5} = 0.34$, $p = 0.58$) in the hole-board test on

frequency of feeding. Similarly, for *WNS+* bats, the duration of grooming ($F_{1,6} = 0.008$,

$p = 0.93$) and duration of echolocation ($F_{1,6} = 0.68$, $p = 0.44$) were not related to the

frequency of feeding, but the duration of locomotion ($F_{1,6} = 14.34$, $p = 0.009$; $r^2 = 0.71$;

Figure 2.2) was positively correlated with the frequency of feeding.

Order of arrival at the food dish was highly repeatable for both *WNS-*

($F_{6,26.9} = 12.00$, $p < 0.001$) and *WNS+* ($F_{7,29.7} = 3.46$, $p < 0.001$) bats (Table 2.2). For

WNS- bats, order of arrival was not related to behavioural responses in the hole-board test

including the duration of locomotion ($Z_{6,3} = 1.33$, $p = 0.18$; Figure 2.3), grooming

($Z_{6,3} = 0.61$, $p = 0.54$), or echolocation ($Z_{6,3} = 1.81$, $p = 0.07$). Results were similar for

WNS+ bats where order of arrival to the food dish was not related to the duration of

locomotion ($Z_{7,4} = -1.05$, p = 0.29; Figure 2.3), grooming ($Z_{7,4} = -0.39$, p = 0.70), or echolocation ($Z_{7,4} = -1.32$, p = 0.19).

For *WNS-* bats there was no relationship between the order of arrival at the food dish and duration of feeding ($F_{1,5} = 0.09$, p = 0.78) or the frequency of feeding ($F_{1,5} = 0.81$, p = 0.41). Similarly, for *WNS+* bats, order of arrival was not related to either the duration ($F_{1,6} = 0.26$, p = 0.63) or frequency of feeding ($F_{1,6} = 0.38$, p = 0.56).

Behavioural tendencies recorded during the hole-board test were not related to mass at the time of capture in *WNS-* bats ($F_{4,2} = 0.37$, p = 0.82), but there was a positive relationship between the duration of grooming recorded in the test and mass at the time of capture the preceding spring for *WNS+* bats ($F_{1,6} = 6.92$, p = 0.04; $r^2 = 0.54$; Figure 2.4). Behavioural tendencies were not related to mass at the onset of hibernation after the summer in captivity ($F_{4,2} = 0.17$, p = 0.94) or the change in body mass from capture to hibernation ($F_{4,2} = 0.15$, p = 0.94) in *WNS-* bats. Similarly, for *WNS+* bats there was no relationship between behavioural tendencies and body mass at hibernation onset ($F_{4,3} = 0.81$, p = 0.60) or the change in mass from capture to hibernation ($F_{4,3} = 0.38$, p = 0.81).

Order of arrival at the food dish was not related to mass at the time of capture ($F_{2,4} = 1.17$, p = 0.40), mass at hibernation onset ($F_{2,4} = 1.36$, p = 0.35), or the change in mass between capture and hibernation ($F_{2,4} = 1.42$, p = 0.34) for *WNS-* bats. Likewise, for *WNS+* bats, the order of arrival was not related to mass at capture ($F_{2,5} = 0.15$, p = 0.87), hibernation ($F_{2,5} = 1.39$, p = 0.33), or the change in mass between capture and hibernation ($F_{2,5} = 1.20$, p = 0.38).

Table 2.1. Summary of intraclass correlation results testing for repeatability in the order of arrival to the food dish in healthy (n=7) and white-nose syndrome affected (n=8) bats. ICC represents intraclass correlation coefficient.

Subject	ICC	Degrees of Freedom	F	P
WNS-	0.923	6, 26.9	12	<0.001
WNS+	0.731	7, 29.7	3.46	0.007
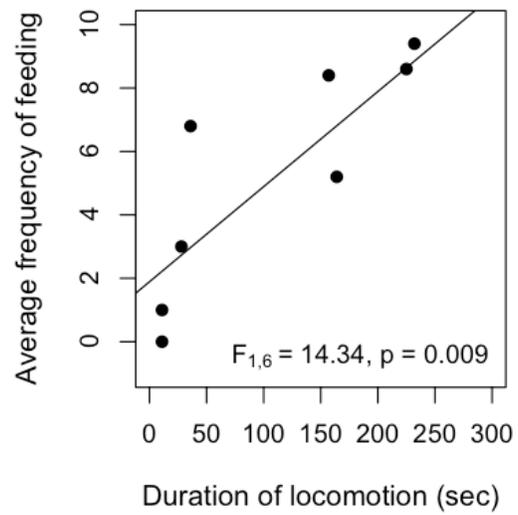
Figure 2.2. The effect of locomotion (sec) recorded during the hole-board test on the average frequency of feeding for white-nose syndrome affected little brown bats.
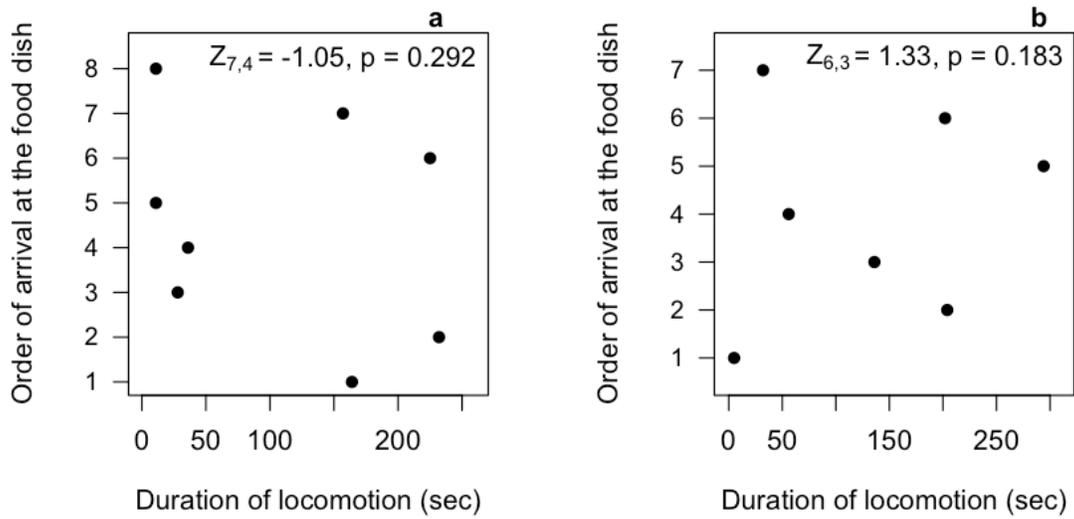
Figure 2.3. The effect of locomotion (sec) recorded during the hole-board test on the order of arrival at the food dish for (a) white-nose syndrome affected and (b) healthy little brown bats.
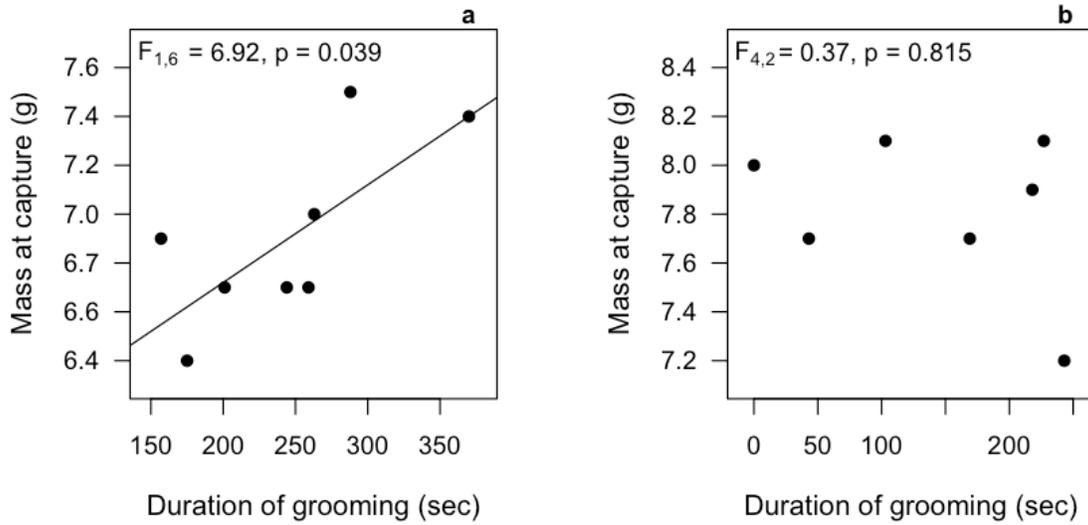
Figure 2.4. The relationship between mass at time of capture in the spring (g) and the duration of grooming (sec) recorded in a hole-board test later in the summer on (a) white-nose syndrome affected and (b) healthy little brown bats.

DISCUSSION

Behavioural tendencies have the potential to influence acclimation to captivity, particularly by affecting access to food resources. Boldness, dominance, and other behavioural traits can affect feeding and body condition in other taxa (Ceacero et al. 2012; Heg et al. 2011) and I found evidence that suggests these relationships for a captive colony of little brown bats. For *WNS+* bats I found that the duration of locomotion in the hole-board test positively correlated with the frequency of feeding in a subsequent feeding experiment and I found a relationship between grooming during the hole-board test (which reflects anxiety; Kalueff and Tuohimaa 2005) and body mass at the time of capture earlier in the spring. However, counter to my hypothesis, individuals that spent more time grooming were heavier at capture, which could reflect a relationship between behavioural tendencies and hibernation energetics. In addition to this, I found that the order of arrival at the food dish was highly consistent throughout my feeding experiment, but this was not related to behavioural tendencies, feeding behaviour, or mass accumulation.

The behavioural tendencies did not correlate with the duration or frequency of feeding. Explorative and anxious behaviours (e.g., echolocation and grooming; Kalueff and Tuohimaa 2005; Menzies et al. 2013; Webbet et al. 2014) often influence feeding (Heg et al. 2011; Jones et al. 1997), but it is possible that the consistent placement of the feeding dish within the enclosure could have limited the need to explore the environment and/or anxiety in searching for food. Locomotion was positively correlated with the frequency of visits to the food dish, but only in *WNS+* bats. In some cases,

Table 2.2. Potential effects of behavioural tendencies and social structure on feeding and

mass accumulation in a captive colony of little brown bats.

Hypothesis	Predictions	Results
- Behavioural tendencies associated with the shy-bold continuum affect the duration and frequency of feeding	- Bold bats will spend more time feeding and feed more frequently than shy individuals	- No effect of behavioural tendencies on the duration of feeding - Duration of locomotion was positively related to the frequency of feeding in *WNS+* bats
- Captive bats exhibit signs of a social structure - Order of arrival at the food dish relates to behavioural tendencies associated with the bold-shy continuum	- Bats will arrive in a consistent order to the feeding dish - Bold bats will arrive earliest to the feeding dish	- Bats from both *WNS-* and *WNS+* groups arrived in the same order to the food dish - Order of arrival at the food dish was not related to behavioural tendencies
- Order of arrival at the food dish affects the duration and frequency of feeding	- Bats that arrive earliest to the food dish will spend more time feeding and feed more frequently than individuals that arrive later to the food dish	- Order of arrival at the food dish did not affect the duration or frequency of feeding
- Behavioural tendencies associated with the shy-bold continuum affect mass during post-hibernation in spring, during pre-hibernation, and mass accumulation in captivity	- Bold bats will be heavier during post-hibernation and pre-hibernation, and will gain more mass in captivity than shy individuals	- No effect of behavioural tendencies on pre-hibernation mass and mass gain in captivity - Duration of grooming was positively related to mass during post-hibernation in *WNS+* bats
- Order of arrival at the food dish affects mass during post-hibernation in spring, during pre-hibernation, and mass accumulation in captivity	- Bats that arrive earliest to the food dish will be heavier during post-hibernation and pre-hibernation, and will gain more mass in captivity than individual that arrive later to the food dish	- No effect of the order of arrival at the food dish on post-hibernation or pre-hibernation mass and mass gain in captivity

locomotion is associated with boldness and aggression in personality studies (Bell 2005).

If this was the case in my study, my results could reflect active and bold individuals

defending the food dish when other bats approached in an attempt to monopolize food

resources. However, I did not quantify level of aggression between individuals during the

feeding study. Another possibility is that active bats may have had a greater frequency of

contact with the antenna, therefore increasing the number of recorded PIT-tag codes.

Active bats would have entered the food dish or bent over the opening of the antenna then

quickly withdrew and continued activity. Regardless, this result was only observed in

WNS+ bats, suggesting the behavioural tendencies may influence feeding in bats affected

by *Pd* and should be considered in developing captive housing assignments. To examine

the effect of WNS on behavioural tendencies and feeding, I recommend that behavioural

tendencies (e.g., aggression) be quantified during recovery and that behavioural

interactions be observed around a common food source.

I did not find a relationship between the behavioural tendencies and body

condition during pre-hibernation or mass accumulation in captivity. Though behavioural

tendencies often correlate with mass accumulation (Koivula et al. 1995; Lange and

Leimar 2004), *ad libitum* feeding in captivity may have reduced variation in mass

between individuals and thus limited my ability to detect a relationship between

behavioural tendencies and body condition. However, I did find evidence that grooming

behaviour in the open field test was related to mass at the time of capture in *WNS+* bats.

Grooming behaviour in rodents reflects anxiety that can be energetically expensive

(Careau et al. 2008; Kalueff and Tuohimaa 2005) and I predicted that anxious bats would

exhibit reduced body condition compared to less anxious individuals. However, bats that

groomed the most in my study were heavier at the time of capture in spring at the end of

hibernation. This suggests that the most anxious individuals might either accumulate

larger energy reserves during fall or spend their reserves less rapidly during hibernation

by using longer torpor bouts or shorter arousals. Interestingly, I only observed this effect

for *WNS+* bats. This could reflect the possibility that *WNS+* bats that groomed more in

the hole-board test also groomed more during hibernation, which may have helped reduce

Pd infection and thus minimize energy expenditure until spring. Thus, shy behavioural

tendencies could reduce overall energy expenditure for bats with WNS during the winter

and potentially improve survival in the face of disease.

Order of arrival at the food dish during my feeding trials was highly repeatable for

both *WNS-* and *WNS+* bats, which is consistent with, though not definitive evidence of,

little brown bats show a dominant-subordinate social structure. Limited data are available

on dominance hierarchies in bats especially when it comes to feeding behaviour.

Dominance relationships in feeding have only been confirmed for the common vampire

bat (*Desmodus rotundus*), where hierarchies result from competition over food (Schmidt

and van de Flierdt 1973; Wilkinson 1986). Kilgour et al. (2013) suggested that dominant

individuals might monopolize optimal thermal niches within roosts restricting access by

subordinates. Though this type of dominance interaction has been observed in other taxa

(Calf et al. 2002; Ekman and Askenmo 1984), it has not yet been directly observed in

bats. Despite the potential for dominance structure in captive little brown bats, order of

arrival at the food dish was not related to the duration or frequency of feeding in either

group. In rhesus macaques (*Macaca mulatta*) dominance status does not influence food

intake (Deutsch and Lee 1991), suggesting that social structure may not influence feeding

behaviour in some species or during *ad libitum* feeding, as in my study. Therefore more work is needed to understand social structure in captive little brown bats, particularly with regards to feeding behaviour. An alternative approach to assess dominant-subordinate relationships might be to observe controlled dyadic interactions in laboratory settings (Grainger et al. 2014), then examine feeding behaviour to better understand social dynamics of both captive and free-ranging bats.

Despite evidence of a consistent feeding order in captive little brown bats, behavioural tendencies and mass were not related to the order of arrival at the food dish. Some of these behavioural tendencies are associated with dominance in other species (Garland et al. 1990; Perry et al. 2004), but it is possible that factors other than the behavioural tendencies I measured could have influenced order of feeding. In addition to behavioural tendencies or dominance, order of feeding can be affected by experience (Pillot et al. 2010; Reebs 2000), social affiliation (King et al. 2011), or motivation (e.g., hunger or thirst; Fischhoff et al. 2007). For instance, in chacma baboons (*Papio ursinus*) departure order from sleeping sites was associated with social relationships between individuals, with closely associated individuals departing as a group (King et al. 2011). Variation in feeding behaviour caused by factors other than dominance could have influenced order of arrival at the food dish and/or obscured a potential relationship with behavioural tendencies and mass. Therefore, I recommend that future experimental studies test the effect of varying combinations of dominant and submissive individuals in a given group to understand the influence of social structure on behaviour and body condition in captivity.

Experimental design may have also contributed to the lack of relationship between behavioural tendencies, order of feeding, and feeding behaviour. Some studies assessing dominance, or related behavioural tendencies, restrict food availability prior to experimental trials to ensure subjects are motivated (Mottley and Giraldeau 2000, Herborn et al. 2010). I provided *ad libitum* food daily for two reasons: first, little brown bats can be difficult to maintain in captivity and I wanted to increase the chance that all individuals could obtain enough food to maintain body mass, and second, because this species is more likely to enter torpor quickly following food restriction (Matheson et al. 2010). However, this approach could have reduced variation in motivation and/or limited the likelihood of agonistic interactions during feeding trials if bats anticipated the consistent availability of food. So, again, I recommend additional experiments quantifying pairwise interactions with limit food availability during feeding trials to access food resources, as this was not possible for my study. Behavioural traits can also change after prolonged captivity (Butler et al. 2006; Dingemanse and de Goede 2004; Herborn et al. 2010; Verbeek et al. 1999). Behavioural tendencies measured in the hole-board test were taken within two months of captivity and if captivity influences behavioural traits in little brown bats, this could explain why I did not detect correlations previously observed for other species. A small sample size may also have limited my ability to detect relationships among variables. Studies in Wistar rats (*Rattus norvegicus*) show that relationships between behavioural traits (e.g., locomotion and defecation) may be undetectable with small sample sizes (<20; Archer 1973). However, despite the potential influence of captivity on behaviour and a small sample size, I still detected similar patterns of behavioural tendencies as those previously described for larger

samples of wild-caught bats (Menzies et al. 2013, Webber et al. 2014), suggesting that, if relationships between behavioural tendencies, feeding behaviour, and body condition exist for little brown bats, these relationships may be relatively weak.

Little brown bats face an urgent threat from WNS and other insectivorous bats face a range of conservation threats. Although captive assurance populations of insectivorous bats may not be practical for all species or circumstances, understanding factors that might influence the success of these programs is still important to ensure they can be implemented if necessary. Many bat species, like little brown bats, are highly social and will likely require colonial housing to thrive in captivity (Bat World Sanctuary 2010). Understanding how individual and social behaviours are influenced by captivity will provide crucial insight for captive assurance. I found evidence to suggest that behavioural tendencies commonly associated with personality in bats and other taxa influenced feeding behaviour and body condition. I found that locomotion affected the frequency of feeding and that a behavioural trait associated with anxiety was related to body mass at end of hibernation, suggesting a link between behavioural tendencies and hibernation energetics (at least for WNS-affected bats). I also found that the order of feeding across individuals was highly repeatable over multiple nights. My results suggest that dominance interactions and disease status influence foraging behaviour in captive bats. More work is needed to better understand the social dynamics of hibernating, insectivorous bats in captivity.

CHAPTER 3

HEATED BAT HOUSES AS A CONSERVATION TOOL IN ADDRESSING WHITE-NOSE SYNDROME IN LITTLE BROWN BATS (*MYOTIS LUCIFUGUS*)

ABSTRACT

Habitat modification can improve management outcomes for imperilled wildlife populations. Insectivorous bats in North America face a range of conservation threats including habitat loss and the fungal disease white-nose syndrome (WNS). Bats face energetic constraints during spring and form maternity colonies for thermoregulation, often in artificial bat houses. Enhancing habitat to reduce thermoregulatory costs could increase survival, fitness, and population growth. Exposure to warm environments after hibernation could also enhance recovery from WNS. I tested the hypothesis that artificial heating of bat roosts could assist with management of threatened bat populations. I evaluated three specific predictions: 1) after hibernation, captive bats are more likely to select a roost maintained at a temperature near their thermoneutral zone compared to a colder roost; 2) bats that are recovering from WNS at the end of hibernation show a stronger preference for heated roosts compared to healthy bats; and 3) heated roosts result in biologically significant energy savings for bats compared to natural roosts or unheated bat houses. I housed healthy and WNS-affected little brown bats (*Myotis lucifugus*) in flight cages equipped with heated and unheated bat houses and quantified the presence of bats in each bat house approximately every two days over two months. I then used a series of bioenergetic models to quantify thermoregulatory costs for bats in both heated and unheated roosts under a number of scenarios. I found that both healthy and WNS-

affected little brown bats preferentially selected heated bat houses, but that WNS-affected

bats were much less likely to use an unheated bat house than healthy individuals. My

model revealed up to 71.5% reduction in energy expenditure in an artificially-heated

roost and energetic benefits were greatest when roost temperature was allowed to cool at

night to facilitate short torpor bouts. My results emphasize the importance of

microclimate for roost preferences of bats and suggest the potential for temperature-

controlled bat houses as a management tool for imperilled bat populations.

INTRODUCTION

Habitat quality can have dramatic implications for survival, reproduction, and

fitness of wildlife (Bryan and Bryant 1999; Nager and Noordwijk 1992; Yom-Tov and

Wright 1993). Protection of high quality habitat can be especially important for

conservation and recovery of threatened species or populations (Kapust et al. 2012;

Knaepkens et al. 2004; Savard 2007). Habitat enhancement represents the process by

which an environment is modified to improve conditions for conservation of individual

species or overall biodiversity (Weller 1989). For instance, European bullhead (*Cottus

gobio*) have benefitted from artificial structures mimicking spawning substrates

(Knaepkens et al. 2004) and removal of invasive reed canarygrass (*Phalaris

arundinacea*) enhanced oviposition in Oregon spotted frogs (*Rana pretiosa*; Kapust et al.

2012). Habitat modification and enhancement can be used to target many aspects of the

biology of threatened species including support for nesting or egg laying (Hamerstrom et

al. 1973; Johnson 1967; Kapust et al. 2012; Knaepkens et al. 2004; Savard 2007),

increased growth rates and individual size (Riley and Fausch 1995), and reduced

competition with invasive species (Grarock et al. 2013, 2014). Ultimately these effects can lead to increased survival, enhanced population growth rates, and improved management outcomes in terms of population stability and recovery (Riley and Fausch 1995).

For endothermic species (i.e., mammals and birds), enhancing habitat to reduce thermoregulatory costs has potential as a management tool for threatened populations (e.g., Boyes and Willis 2010; Bryan and Bryant 1999; Nager and Noordwijk 1992; Yom-Tov and Wright 1993). Warm microclimates can be especially important for successful gestation and improved offspring growth (Dawson et al. 2005; Eeva et al. 2002; McCarty and Winkler 1999; Tuttle 1976). Yom-Tov and Wright (1993) found that, when nest boxes were heated 6°C above ambient temperature (T_a) Eurasian blue tits (*Parus caeruleus*) had an energy savings of ~3.21 kJ over 7 hours and interruption in egg laying was reduced. Similarly, in great tits (*Parus major*) a 3.4°C increase above T_a for 11 hours saved birds approximately 7 kJ, increased the time allocated to incubation, and allowed birds to lay larger eggs (Bryan and Bryant 1999; Nager and Noordwijk 1992). These changes, resulting from microclimate enhancement, can improve reproductive fitness, enhance survival of chicks (Nager and Noordwijk 1992; Schifferli 1973), and facilitate species conservation.

Bats are one group of endothermic animals facing a range of conservation threats including habitat loss (Kunz and Lumsden 2003), climate change (Humphries et al. 2002), industrial development (Hayes 2013; Arnett and Baerwald 2013), and disease (Boyles and Willis 2010; Cryan et al. 2010; Warnecke et al. 2012). These threats make bats a candidate taxon for a habitat enhancement approach to management. Bats spend

most of their lives roosting and roost microclimate is thought to play a key role in individual survival and reproductive success (e.g., Fenton and Barclay 1980; Foster and Kurta 1999; Hamilton and Barclay 1994; Kunz and Lumsden 2003; Lausen and Barclay 2003). In the temperate zone, roost temperature (T_{roost}) and T_a will often fall below thermoneutrality (Willis and Brigham 2005) and many bat species employ torpor (i.e., reduced body temperature (T_b) and metabolism) to reduce energetic costs (Willis et al. 2006). However, torpor can delay parturition (Lewis 1993) and inhibit lactation (Wilde et al. 1995) in female bats, and is known to inhibit spermatogenesis in male ground squirrels (Barnes et al. 1986). Therefore, while bats are known to employ some torpor during the reproductive season, they may select roosts with characteristics that help them reduce their reliance on torpor expression (Brigham and Fenton 1986; Chruszcz and Barclay 2002; Hamilton and Barclay 1994; Lausen and Barclay 2003). Pregnant big brown bats (*Eptesicus fuscus*) select roosts that are cold during the morning when they enter torpor, but warm up rapidly during the day (Hamilton and Barclay 1994; Lausen and Barclay 2003). Lactating females tend to select warm, thermally stable roosts to facilitate milk production and rapid juvenile development (Lausen and Barclay 2003). Males, on the other hand, may select colder roosts to facilitate use of torpor and energy savings (Hamilton and Barclay 1994). The dependence of bats, especially reproductive females, on warm roost microclimate suggests that habitat enhancements that increase availability of warm microclimates could benefit individual survival, reproduction, and population growth.

Roost microclimate may also impact survival from disease. White-nose syndrome (WNS) is an infectious disease of hibernating bats, caused by the fungus

Pseudogymnoascus destructans (*Pd;* Lorch et al. 2011; Warnecke et al. 2012). WNS has resulted in unprecedented declines of bats across eastern North America and is spreading across the continent (U.S. Fish and Wildlife Service 2012). Mechanisms underlying mortality are still not fully understood, but affected bats are emaciated as a result of an increase in arousal frequency and energy expenditure during hibernation (Warnecke et al. 2012). Despite rapid declines, some bats survive (Dobony et al. 2011) and could have heritable traits that may be important for population recovery (Menzies 2014, Willis and Wilcox 2014). Unfortunately, individuals that survive the winter exhibit a rapid reversal of immune suppression in spring that appears to result in immune reconstitution inflammatory syndrome (IRIS), a condition characterized by a sudden inflammatory response leading to deterioration in physiological condition and possibly mortality (Meteyer et al. 2012). Healing after infection with WNS and damage caused by this massive inflammatory response is likely energetically expensive (Fuller et al. 2011; Lee 2006). Thus, providing warm microclimates for bats could help survivors recover more quickly in spring and initiate reproduction earlier in the summer. If survivors exhibit heritable traits that help them survive the winter with WNS (Menzies 2014; Willis and Wilcox 2014), this approach could facilitate the evolution of "survival traits" in populations threatened by WNS.

I used a combination of behavioural experiments with a captive colony of little brown bats (*Myotis lucifugus*) and a series of bioenergetic models to test the hypothesis that artificial heating of bat roosts could assist with management of bat populations imperilled by WNS and habitat loss. I evaluated three specific predictions: (1) during the post-hibernation period, captive bats are more likely to select a roost maintained at a

temperature near their thermoneutral zone than they are to select a colder roost; (2) bats that are recovering from WNS at the end of hibernation show a stronger preference for a heated roost compared to healthy bats; and (3) artificially-heated bat houses deployed in the field could result in biologically-significant energy savings for bats compared to natural roosts.

METHODS

Preferential selection for heated bat houses

All procedures were conducted under Manitoba Conservation and Ontario Ministry of Natural Resources permits and were approved by the University of Winnipeg Animal Care Committee. Between 28 April and 5 May 2013, 32 adult male little brown bats (*WNS+*) were captured from two WNS-positive mines less than 90 km apart near Renfrew, Ontario (45.4717°N, 76.6831°W) and Gatineau, Québec (45.4833°N, 75.6500°W), Canada. Of these individuals, 11 were available for use in this study. Eight of these individuals were also used for experiments reported in Chapter 2, but the remaining three declined in body condition and were euthanized. At the time of capture, bats were weighed (Ohaus Corporation, CS200 portable scale, Pine Brook, New Jersey, U.S.A.), measured using digital calipers (Mastercraft, digital caliper, Vonore, Tennessee, U.S.A.), and outfitted with temperature dataloggers (DS1922L-F5 Thermochron iButton, Maxim, San Jose, California, U.S.A., modified to reduce mass following Lovegrove 2009 and Reeder et al. 2012) attached in the interscapular region using a latex-based adhesive (Osto-Bond, Montreal Ostomy Centre, Vaudreuil-Dorion, Québec, Canada). A unique symbol was marked on the exterior surface of each iButton to allow identification of

individual bats. Bats held in cloth bags inside hepa-filtered animal carriers stored in a temperature-controlled cabinet to facilitate long-distance travel 2,128 km to the University of Winnipeg. Bats were later banded with a uniquely numbered, lipped aluminum forearm band (Porzana Limited, East Sussex, U.K.).

On 28 May 2013, 39 male little brown bats (*WNS-*) were captured from a WNS-negative cave in central Manitoba, Canada (53.2083°N, 99.3000°W). Of these individuals, 26 were available for my study, with eight of these individuals also being used in experiments in Chapter 2. Bats were tagged and placed in cloth bags, suspended in a plug-in ventilated cooler, and transported 432 km to the University of Winnipeg. All morphometric measurements were obtained as described for *WNS+* bats. Bats from both the *WNS-* and *WNS+* groups were housed over the summer until the beginning of hibernation on 11 October 2013 when they were transferred to another study.

Temperature and humidity in the holding room were controlled at 18°C and 60% relative humidity, and lights were set to maintain a natural photoperiod (light:dark = 11:13 hours) with a graduated lights-on to lights-off transition. *WNS+* and *WNS-* bats were housed in separate but identical flight cages 2.24 m^2 long x 1.01 m wide x 2.42 m high. Each flight cage was equipped with two custom-built single-chambered bat houses (44 cm x 6.3 cm x 60 cm). The back of one bat house was lined with an electrical heating coil (Exoterra Temperature Heating Cable, 12 V, Rolf C. Hagen Group, Mansfield, Massachusetts, U.S.A.) controlled using an electronic temperature controller set to 30°C (Nema 4x Electrical Temperature Control, Ranco, Invensys, U.K.), just below the lower critical temperature (i.e., 32°C; Humphries et al. 2002, 2005; Speakman and Thomas 2003; Stones and Wiebers 1967) that I predicted would reduce normothermic (i.e., when

T_b is regulated at high levels; Geiser 2013) energy expenditure but also allow bats to use shallow torpor to save energy if needed. In the *WNS+* flight cage the heated bat house was mounted on 7 May 2013 and was available to bats as soon as they arrived at the laboratory. The unheated bat house was not yet available when the bats were collected, so it could not be provided until 21 May 2013. In the *WNS-* flight cage both the heated and unheated bat houses were installed on 21 May 2013 and were available as soon as the bats were released into the flight cage.

Throughout captivity, bats were provided mealworms (*Tenebrio molitor*) and water *ad libitum*. Mealworm substrate (i.e., cream of wheat, powdered milk, natural wheat bran) was supplemented with calcium, mineral, and vitamin powder. On 27 June 2013 I attempted to supplement the mealworm diet with crickets for 2 nights, but feeding dramatically declined during this period so I returned to a mealworm-only diet. I applied Selamectin (Revolution, Zoetis, Florham Park, New Jersey, U.S.A.; 30 μL), a topical parasiticide, to the interscapular region on 7 June 2013 after most bats had adjusted to captivity and were eating well. To more easily identify individuals and monitor activity in flight cages, bats were tagged with passive transponders (PIT tags) between 26 June 2013 and 08 July 2013. Bats were collected from inside bat houses for weighing about every two days (1.9 ± 1.60 days for *WNS+* bats; 2.3 ± 1.27 days for *WNS-* bats). Between 9 June and 31 July 2013, while collecting bats for these weighing sessions, I recorded the number of bats in each type of bat house in each flight cage.

Some species of insectivorous bats, such as little brown bats, are particularly difficult to maintain in the laboratory during the active season and do not adjust well to captivity (Lollar 2010). Between 3 May 2013 and 28 July 2013, some *WNS+* (n=2) and

WNS- bats (n=17) bats exhibited abnormal behaviour including impaired and weakened gait, inability to fly, lack of feeding, and a decline in body mass. Bats exhibiting these symptoms were isolated in smaller nylon mesh cages (20.3 cm x 20.9 cm x 24.1 cm) to provide them easier access to food and water, and to facilitate monitoring. If their condition continued to decline, as indicated by a loss of >5% body mass, they were anesthetised using isoflurane in oxygen (5%) and humanely euthanized via CO_2 asphyxiation. I was not able to determine the cause of this abnormal behaviour. Pathological examination showed no evidence of bacterial or viral infection and did not exhibit signs of fatty liver syndrome, which can occur in captive bats as a combined result of excess food consumption and stress, and could have caused the kinds of behavioural changes I observed (Barnard 2009; Bat World Sanctuary 2005). In addition to this unidentified problem, between 23 July and 31 July 2013 two bats were isolated for lower abdominal swelling that turned out to be a bacterial infection caused by *Proteus morganii*. One *WNS+* bat had to be euthanized and all remaining bats were treated with the antibiotics enrofloxacin (0.1 mL/day) and cefazolin (0.1 mL/day) for two weeks. These challenges with captive management led to fluctuations in the number of bats in each flight cage as individuals were moved between flight cage and isolation, and a gradual decline in the total numbers of bats in each flight cage as bats were removed from the study. By the end of the study, eight bats remained in each group. As a result of these fluctuating numbers of bats, I quantified roosting preferences as the proportion of bats in each cage found roosting in the heated versus unheated houses during the times when I captured bats for weighing.

Energetic Models

I ran a series of bioenergetics models in Microsoft Excel for Mac (version 14.4.6) to quantify the potential energy savings provided by artificially-heated bat houses. Pregnant female bats select maternity roosts with T_as that help to reduce energy expenditure, increase time spent normothermic, and avoid, but not eliminate, torpor expression (e.g., Barclay 1982, Dzal and Brigham 2013). Female bats appear to select roosts that facilitate torpor in the early morning when T_{roost} is lowest and then gradually rewarm throughout the day, often reaching values of T_{roost} well above outside T_a, to help maintain normothermia (Dzal and Brigham 2013).

I relied largely on equations from Humphries et al. (2002, 2005; Table 3.1) for parameter values in my model and calculated all metabolic rates (MR) as mass-specific oxygen consumption ($\dot{V}_{O_2}$, ml O_2 g^{-1} hr^{-1}). I first calculated normothermic energy expenditure (E_{norm}) using:

$$E_{norm} = RMR + (T_{lc} - T_a) \, C_{norm} \qquad\qquad 1.$$

Where RMR is the resting metabolic rate, T_{lc} is the lower critical temperature of the thermoneutral zone, T_a is ambient temperature, and C_{norm} is thermal conductance at normothermia (Humphries et al. 2002; Humphries et al. 2005). I assumed that the decline in T_b from T_{norm} to T_{tor} cost 67.2% of the cost of warming following Thomas et al. (1990, Equation 4). During torpor, MR and T_b decline to a minimum set-point temperature ($T_{tor-min}$) at which metabolic heat production is required to defend torpid T_b (Geiser 2004; Geiser 2013; Humphries et al. 2002). Therefore, I used two equations to determine energy expenditure during torpor depending on whether T_a was lower or higher

than $T_{tor-min}$:

$$\text{When } T_a > T_{tor-min}, \; E_{tor} = TMR_{min}Q_{10}^{(T_a - T_{tor-min})/10} \qquad \qquad 2.$$

$$\text{When } T_a \leq T_{tor-min}, \; E_{tor} = TMR_{min} + (T_{tor-min} - T_a) \, C_t \qquad \qquad 3.$$

Where Q_{10} is the change in torpid metabolic rate (TMR) over a 10°C change in T_a, and C_t is thermal conductance below $T_{tor-min}$ (Humphries et al. 2002; Humphries et al. 2005). I calculated metabolic costs of active arousals from torpor as the energy required to increase T_b from T_{tor} to T_{norm} based on McKechnie and Wolf's (2004) equation, along with the specific heat capacity of the tissues, S (0.131 ml O_2/g/°C; Humphries et al. 2002; Humphries et al. 2005) and mass (McKechnie and Wolf 2004). Therefore, I calculated the cost of arousals from torpor as:

$$E_{ar} = S \, (T_{norm} - T_{tor-min}) \, M_b + D_{rewarm} \, (TMR + (RMR - TMR) \, / \, 2) \qquad \qquad 4.$$

Note that the second term in Equation 4 quantifies the rate of rewarming as a linear function following McKechnie and Wolf (2004), with D_{rewarm} as the duration of rewarming. To estimate TMR for equation 4, I used McKechnie and Wolf's (2004) equation:

$$TMR = TMR_{min} + C_{tor} \, (T_{tor-min} - T_a) \qquad \qquad 5.$$

Table 3.1. Parameter values used in my bioenergetic models to quantify

thermoregulatory costs in heated and unheated roosts of little brown bats.

Parameter	Value	Reference
Mass	8.47 g	Jonasson and Willis 2011
Basal metabolic rate (BMR)	1.43 ml O_2/g/hr	Barclay and Harder 2003
Resting metabolic rate (RMR)	2.6 ml O_2/g/hr	Stones and Wiebers 1967; Humphries et al. 2002, 2005
Minimum torpid metabolic rate (TMR_{min})	0.03 ml O_2/g/hr	Hock 1951; Humphries et al. 2002, 2005
Normothermic temperature (T_{norm})	35°C	Thomas et al. 1990; Humphries et al. 2002, 2005
Lower critical temperature (T_{lc})	32°C	Stones and Wiebers 1967; Humphries et al. 2002, 2005
Minimum temperature during torpor ($T_{tor-min}$)	2°C	Hock 1951; Humphries et al. 2002, 2005
Normothermic conductance (C_{nor})	0.2638 ml O_2/g/°C	Stones and Wiebers 1967; Humphries et al. 2002, 2005
Torpor conductance (C_{tor})	0.055 ml O_2/g/°C	Hock 1951; Humphries et al. 2002, 2005
Q_{10}	$1.6 + 0.26T_a - 0.006\ T_a^2$	Hock 1951; Humphries et al. 2002, 2005
Specific heat capacity of tissue	0.131 ml O_2/g/°C	Thomas et al. 1990; Humphries et al. 2002, 2005

The metabolic cost of a passive arousal (i.e., when T_a or solar radiation would have warmed a roost) was calculated as 1.04 times basal metabolic rate (BMR; Willis et al. 2004).

To convert values of $\dot{V}_{O_2}$ into SI units of heat production or energy expenditure (J g^{-1} hr^{-1}), I used Equation 3 from Campbell et al. (2000), which accounts for differences in the catabolism of lipids (L), carbohydrates (C), and proteins (P) in the diet:

$$\text{Heat production} = (17.71P + 20.93C + 19.55L)\ \dot{V}_{O_2} \qquad\qquad 6.$$

Little brown bats are generalist insectivores and eat a wide variety of flying insects (Clare et al. 2014), so I used the average composition of flying insects found in a typical diet for little brown bats by Kurta et al. (1989) as proportions for conversion. On average, the digestible fraction of flying insects is 71.2% protein, 18.4% fat, and 8.8 % carbohydrate (Kurta et al. 1989). To calculate whole-animal MR, I used the average mass of little brown bats captured in central Manitoba in early spring (i.e., 8.47 g; Jonasson and Willis 2011) before converting all values of heat production to kilojoules.

In my study area, insectivorous bats emerge from hibernation in late April (Norquay and Willis 2014) at a time when they likely have to rely on torpor to reduce energy expenditure and balance their energy budgets, despite the fact torpor will delay gestation and parturition (Lewis 1993). To understand how roost microclimate might influence energy expenditure for reproductive females (and survivors of WNS) emerging in early spring (April-May), I quantified daily energy expenditure under four roost-microclimate scenarios approximating natural conditions, or microclimate manipulations

that wildlife managers could potentially employ in the field: (1) outside T_a (i.e., assuming that $T_{roost} = T_a$); (2) T_{roost} reflecting conditions in a typical maternity roost (Lausen and Barclay 2006); (3) an artificially-heated bat house in which T_a is cycled on and off to reduce thermoregulatory costs during periods when reproductive bats are typically normothermic, while allowing some torpor expression to reduce energetic costs when food is scarce; and (4) a heated bat house with T_{roost} maintained within the thermoneutral zone (TNZ; i.e., 32°C; Fenton and Barclay 1980; Studier and O'Farrell 1976).

For Roost Scenario 1, I used maximum and minimum daily T_a recorded from April to May at the meteorological station in Hodgson, Manitoba (51.1109°N, 97.2754°W) over a 10-year period from 1996 – 2005. This site is the closest weather station to Lake St. George Caves Ecological Reserve, the location of the largest little brown bat hibernaculum in central Canada. I used these T_a values, and equations 1-4, to calculate hourly energy expenditure during normothermia, cooling, steady-state torpor, and rewarming for each day in April – May. For each day, I assumed that the T_a experienced by bats during normothermia was the daily maximum T_a recorded at Hodgson, and that the T_a experienced during torpor was the minimum daily T_a. I determined the approximate duration of each phase of torpor and arousal based on values from the literature. On average, a pregnant female little brown bat spends 133 minutes day^{-1} in torpor (Dzal and Brigham 2013), so for roost scenarios where bats were exposed to temperature below the TNZ (i.e., Roost Scenarios 1-3) I assumed that torpor bouts lasted 133 minutes. The duration of active rewarming was calculated using the published rewarming rate for little brown bats (0.8°C/min; Willis 2008) and the difference between torpid and normothermic T_b following:

When $T_a > T_{tor-min}$, minutes to rewarm = $T_{norm} - T_a$ / 0.8   7.

When $T_a \leq T_{tor-min}$, minutes to rewarm = $T_{norm} - T_{tor-min}$ / 0.8   8.

This duration was then multiplied by the metabolic cost of warming. Therefore, my

model assumed that pregnant female little brown bats remained normothermic for the

remaining time (i.e., the time remaining after accounting for torpor, warming,

and cooling).

For the other three roost scenarios, I used the same approach, but varied the

temperature inputs into the calculations. For Roost Scenario 2 (i.e., a typical maternity

colony), I calculated the average difference between values for T_{roost} reported in the

literature for building or rock crevice roosts of big brown bats versus T_a recorded outside

those roosts (4.3°C ± 5.65°C; Lausen and Barclay 2006). I then added this value to the

maximum and minimum daily T_a recorded at Hodgson. As with Roost Scenario 1 (i.e.,

roosting at T_a), I assumed that bats rewarmed from torpor actively (i.e., using metabolic

heat production) and could not exploit passive rewarming.

For Roost Scenario 3, I calculated daily energy expenditure for bats roosting in a

bat house that was heated most the time, but allowed to cool for part of the night to

facilitate short, energy-saving torpor bouts similar to those observed for free-ranging bats

(Dzal and Brigham 2013). I set the daytime maximum T_{roost} in the heated bat house as

32°C, which is at the lower end of the TNZ for little brown bats (Fenton and Barclay

1980; Studier and O'Farrell 1976), and used the daily minimum T_a from the Hodgson

meteorological station as the minimum T_{roost}. This model assumed that bats could rewarm from torpor passively as T_{roost} in the heated bat house was raised back to 32°C in the morning.

For Roost Scenario 4, I held T_{roost} constant at 32°C within the TNZ so that energy expenditure during roosting was equal to BMR and normothermic T_b could be maintained with no additional thermoregulatory energy expenditure (Fenton and Barclay 1980; Studier and O'Farrell 1976).

Statistical Analyses

All analyses were conducted in R (version 2.14.1; R Core Development Team 2011). To test whether bats were more likely to occupy artificially-heated or unheated bat houses within each treatment group (i.e., *WNS-* and *WNS+*), I used a 2x2 contingency table quantifying the number of paired observation days when at least one bat was present or absent in either the heated or unheated bat house. I then used McNemar's test with Yate's correction for continuity to test the hypothesis that the number of observation days with presence in the heated bat house was greater than that of the unheated bat house.

To test whether there was a difference in the use of the heated or unheated bat house between *WNS-* and *WNS+* bats, I used a 2x2 contingency table, this time quantifying the presence and absence of at least one bat in the heated and unheated bat house for each group. I then used a Fisher's exact test to test the hypothesis that group (i.e., *WNS+* and *WNS-*) affected the presence and absence of bats for each bat house. The bats arrived in captivity at different times and the bat houses were introduced at different

times, so I used only observations from 20 June to 23 July 2013 for this analysis when

both groups were present in the flight cages simultaneously with access to both

bat houses.

To compare the energetic implications of my four roost scenarios, I first

calculated the average daily energy expenditure for April and May of each year (i.e.,

from 1996-2005). Thus, I calculated one value of average daily energy expenditure for

April 1996, one value for May 1996, one value for April 1997, and so on. I then used an

ANOVA for each month with average daily energy expenditure as the response variable,

Roost Scenario (i.e., 1-4) as the predictor and year as the experimental unit. I used

Tukey's honest significance test for post-hoc analysis. Significance was assessed at the

$p<0.05$ level and all values are reported as the mean $\pm$ SD.

RESULTS

Bats occasionally roosted outside of the bat houses, hanging on the aluminum

mesh of the flight cages, but the vast majority of individuals roosted in the bat houses

throughout their time in captivity. Healthy bats exhibited a clear preference for the heated

bat house (Figure 3.1) and on average $82.6 \pm 16.1\%$ of individuals were observed

roosting in the heated box on my observation days (Figure 3.1). The number of

observation days on which at least one *WNS-* bat was observed in the heated bat house

was greater than the number of observation days when at least one bat was observed in

the unheated bat house (McNemar's $X^2_1 = 9.09$, $p = 0.003$). This preference was even

stronger for *WNS+* bats (Figure 3.2), with bats almost always observed in the heated bat

house. On average, $95.2 \pm 21.8\%$ of bats were observed roosting in the heated box on my

observation days (Fig. 3.2) and *WNS+* bats were almost never observed in the unheated

bat house (McNemar's $X^2_1 = 15.42$, p < 0.001). *WNS+* bats were significantly less likely

to use the unheated bat house. At least one bat was observed in the heated bat house on

an equal number of observation days for the *WNS+* and *WNS-* groups (p = 1.0; Figure

3.3a), whereas *WNS+* bats were much less likely than their healthy counterparts to use

the unheated bat house (p <0.001; Figure 3.3b).

Based on my energetic model there was a significant effect of roosting scenario

on energy expenditure during April ($F_{3,36} = 412.1$, p < 0.001; Figure 3.4) and all scenarios

differed significantly from each other except for Scenarios 3 and 4 (Figure 3.4, 3.5).

Energy expenditure was reduced by as much as 71.5% in the heated roost (Scenario 3),

compared to roosting in a typical maternity colony. Similarly, during May there was also

a significant effect of roosting scenario on energy expenditure ($F_{3,36} = 471.3$, p < 0.001;

Figure 3.4) and all scenarios differed significantly, except for Scenarios 3 and 4 (Figure

3.4, 3.5). Energy expenditure was as much as 63.5% lower in the heated roost (Scenario

3), compared to roosting in a typical maternity colony.
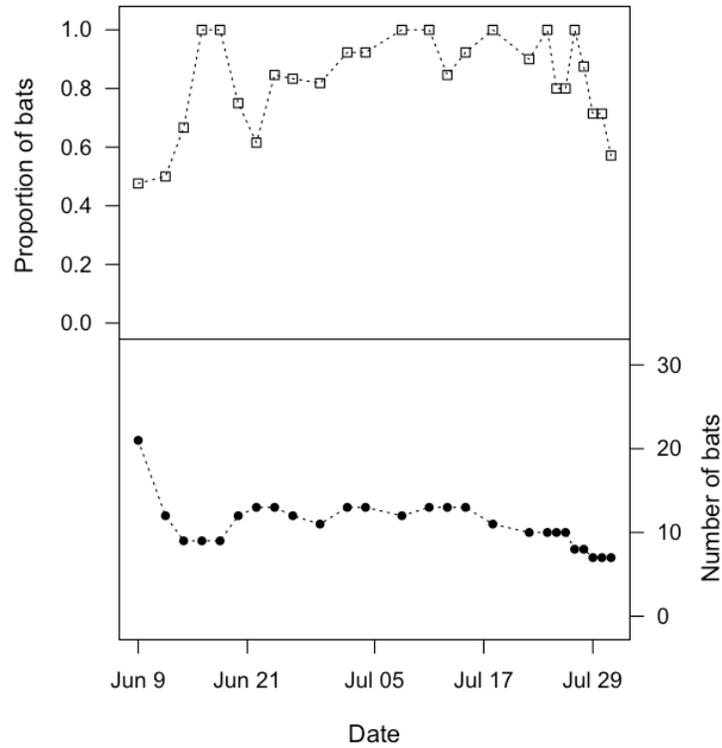
Figure 3.1. The proportion of bats from the *WNS-* flight cage, roosting in the heated bat house over the two-month sampling period for healthy bats (top panel). Some bats had to be removed from the experiment over time (see methods), so also shown is the number of bats remaining in the flight cage on each sampling day (bottom panel).
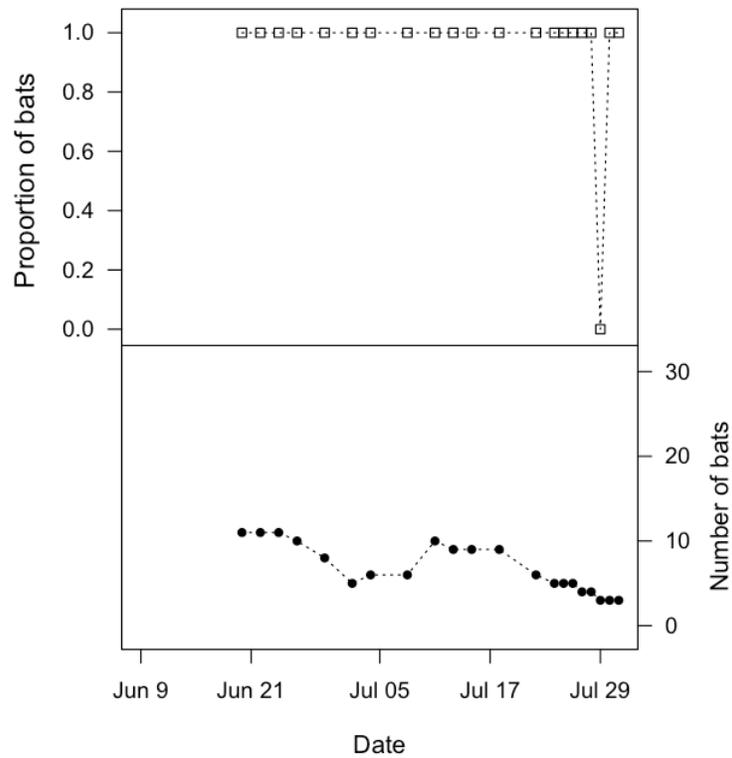
Figure 3.2. The proportion of bats from the *WNS+* flight cage, roosting in the heated bat house over the two-month sampling period (top panel). Some bats had to be removed from the experiment over time (see methods), so also shown is the number of bats remaining in the flight cage on each sampling day (bottom panel).
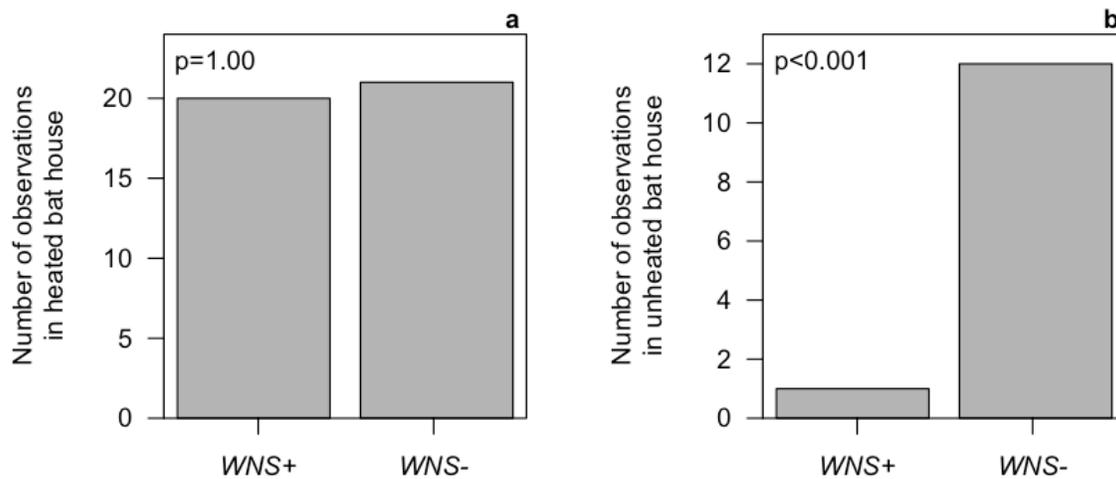
Figure 3.3. The number of observations in which at least one bat was observed in either the heated (a) or unheated (b) bat house for bats infected with *Pseudogymnoascus destructans* (*WNS+*) and uninfected bats (*WNS-*). $N = 21$ observation days.
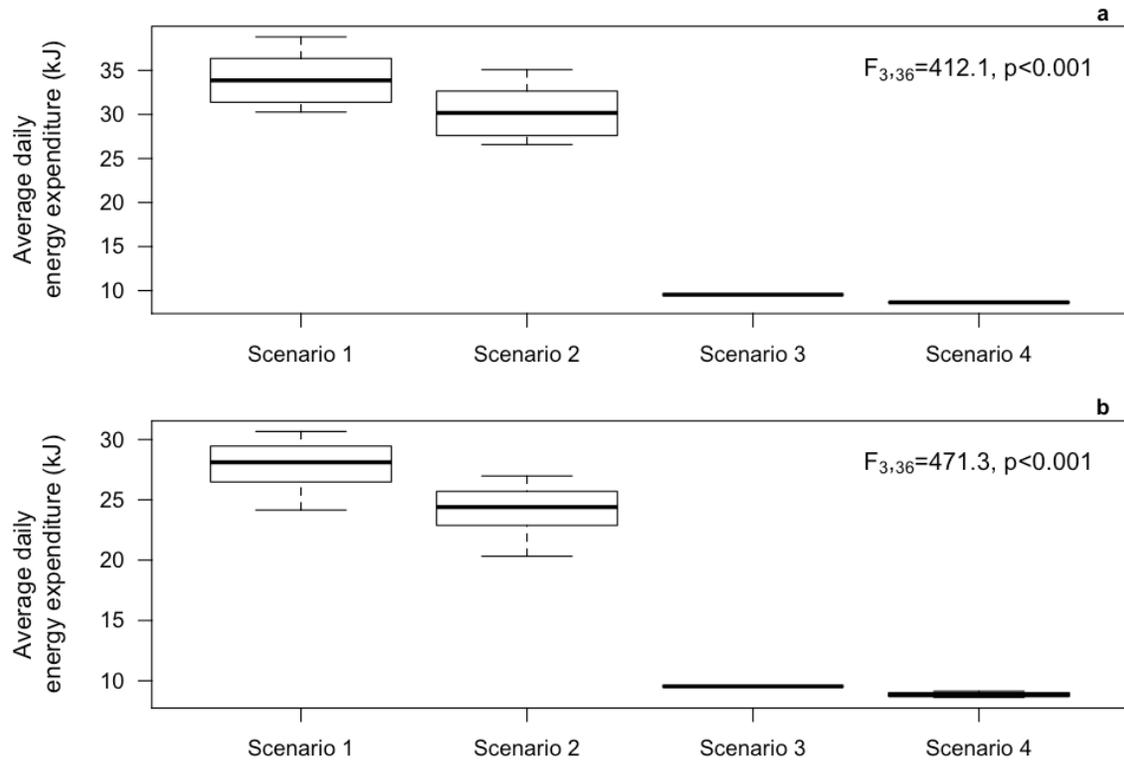
Figure 3.4. Average daily thermoregulatory energy expenditure (kJ) in (a) April and (b) May for a hypothetical 8.42 g little brown bat (*Myotis lucifugus*) roosting at ambient temperature (Scenario 1), at a temperature typical for a temperate bat maternity roost (Scenario 2), in a heated bat house with T_a cycled to allow some torpor expression (T_a cycled; Scenario 3), and a heated bat house maintained within the thermoneutral zone (32°C; Scenario 4).
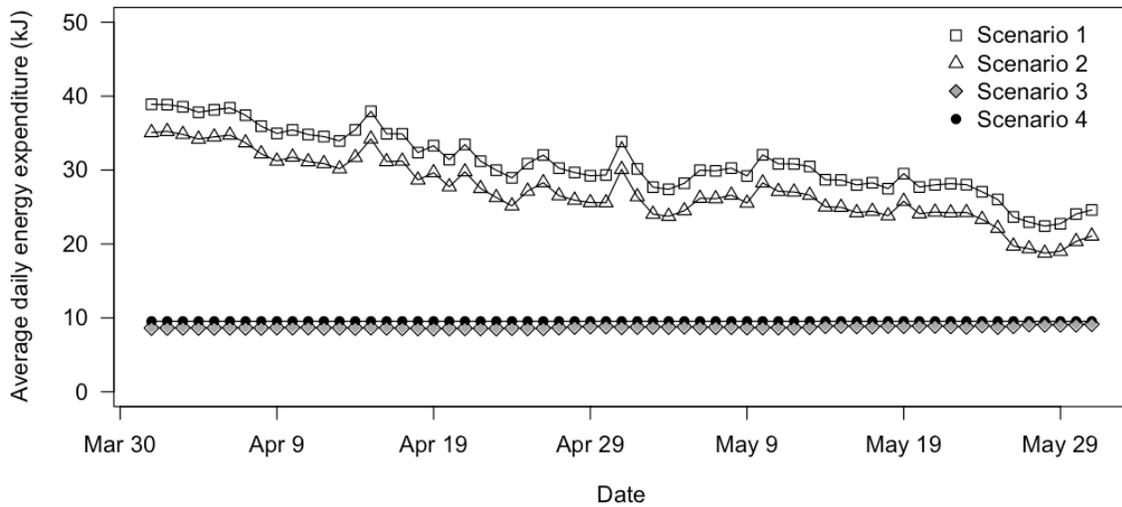
Figure 3.5. Predicted values of daily thermoregulatory energy expenditure (kJ) averaged over 10 years from 1996-2005 during spring for a hypothetical 8.42 g little brown bat (*Myotis lucifugus*) roosting at ambient temperature (Scenario 1), at a temperature typical for a temperate bat maternity roost (Scenario 2), in a heated bat house with programmed daily fluctuation in temperature to allow some torpor expression (T_a cycled; Scenario 3), and a heated bat house maintained within the thermoneutral zone (32°C; Scenario 4).

DISCUSSION

My results support the potential of roosting habitat enhancement as a management tool for threatened, temperate-zone bats in general, and as a response to WNS in particular. I found strong evidence that both *WNS+* and *WNS-* bats preferred roosting in an artificially-heated bat house and that *WNS+* bats, recovering from infection with *Pd*, were less likely to select the unheated bat house than healthy individuals. My model suggests that heated bat houses could provide dramatic thermoregulatory energy savings during conditions typical of the post-hibernation period in central Canada, compared to energy expenditure in natural roosts. Taken together, my results suggest that heated bat houses could help speed gestation and parturition following hibernation and improve recovery and survival from WNS.

Habitat modification in the form of artificial heating of localized areas (e.g., nests) has been used to address ecological questions and has the potential to be used for conservation. Heated nest boxes used by cavity-nesting birds increase reproductive fitness by reducing the energetic cost of incubation and improving offspring survival (Bryan and Bryant 1999; Nager and Noordwijk 1992). This suggests that, during times of energy limitation such as reproduction, animals should select microclimates that help to minimize energy expenditure. I found that male little brown bats from both *WNS+* and *WNS-* groups preferentially roosted in the heated bat house (Figure 3.1, 3.2, 3.3a), suggesting that by selecting microclimates within the TNZ male bats may improve conditions for spermatogenesis (Speakman and Thomas 2003). Though my study did not test for the preferential selection of heated bat houses by females, heated microclimates could reduce the energetic demand of gestation and lactation in female bats, and aid in

offspring growth (Lausen and Barclay 2003; Speakman and Thomas 2003). Therefore, my results suggest that heated bat houses could reduce energy expenditure and have the capacity to enhance reproductive fitness.

Warm roost microclimates could also improve conditions for recovery from WNS. WNS results in an increased arousal frequency during hibernation that prematurely reduces fat stores (Warnecke et al. 2012), which means affected bats will emerge with minimum energy reserves in spring and then mount an immune response to infection while food resources are still relatively scarce. Immune activation and healing can significantly increase energy costs (Eraud et al. 2005; Martin et al. 2003). Little brown bats that survive WNS also experience an extremely elevated immune system response to infection in spring (e.g., IRIS; Meteyer et al. 2012) that could further deplete critically low fat stores. Artificial heating of roosts may reduce energy expenditure for WNS survivors and increase healing rates, as warm microclimates are known to improve healing (Andersen and Roberts 1975). In my study, *WNS+* bats selected heated bat houses on almost 100% of observation days (Figure 3.2, 3.3a) and occupied the unheated bat house on fewer occasions than *WNS-* bats (Figure 3.3b). This suggests that heated bat houses reduce energetic demand for *WNS+* bats, allowing them to maintain normothermia and improve body condition.

The timing of emergence from hibernation in bats appears to be influenced by trade-offs between pressure on females to initiate gestation as early as possible, and risks and energetic costs associated with cold and potentially inclement weather during spring (Norquay and Willis 2014). Successful gestation and pregnancy in female bats are tightly linked with available fat stores, so it is not surprising that adult female little brown bats

have been shown to have better body condition in late hibernation than adult males and young of the year (Jonasson and Willis 2011), and that females in the best body condition emerge earliest in spring (Norquay and Willis 2014). Early gestation and parturition also improve annual survival for newborn pups with individuals born earliest in spring most likely to survive their first winter (Frick et al. 2010b). Bats affected by WNS emerge with limited fat stores that could delay embryonic implantation and gestation, thereby reducing pup survival. Artificial heating of roosts could improve conditions for pregnant female bats, particularly those affected by WNS. My results show that heated bat houses could dramatically reduce energy expenditure by as much as 71.5% compared to maintaining normothermia in a natural roost. If this is the case in the wild, heated bat houses may provide a method to mitigate declines in WNS-affected bats by improving adult condition (i.e., minimizing depletion of fat stores) and, in turn, adult and juvenile survival. I recommend that studies investigate preferences for, and energetic benefits of, heated bat houses for females of temperate bat species in the wild and determine the impact artificial heating has on gestation, juvenile development, and survival.

This study was the first attempt at long-term maintenance of a colony of little brown bats during the active season. Insectivorous bats can be difficult to maintain in captivity (Lollar 2010) and the decline in numbers of bats I observed may have been related to stress or insufficient nutrients in the diet. Stress responses are known to affect behaviour in captivity (Morgan and Thromborg 2007) and could have impacted individual and social behaviours (e.g., sick bats may select cooler environments to facilitate torpor and reduce energy expenditure). This is unlikely to have affected my results because I removed bats from my experiment if they showed abnormal behaviour

(i.e., bats exhibiting abnormal behaviour were isolated from the colony), but future tests of roost microclimate preferences with free-ranging bats could provide additional insight.

The timing of availability for each type of bat house in my experiment could also have influenced my results. *WNS+* bats had a 2-week exposure to the heated house on its own, whereas *WNS-* bats were introduced to both heated and unheated bat houses at the same time. Thus, the more pronounced preference for the heated bat house I observed for *WNS+* bats could have reflected a longer period of acclimation to this box in their flight cage. However, I argue that this is unlikely as free-ranging bats routinely switch between multiple roost sites in the wild, as frequently as every 1-2 days (Willis and Brigham 2004). Therefore, if the *WNS+* bats in my study were motivated to use a cooler environment, they could have readily switched to the unheated bat house shortly after it was provided. Indeed, all *WNS+* individuals switched to the unheated bat house on 29 July 2013 for one night, before switching back. This suggests that bats investigated both roosting options but still preferred the heated bat house. Abrupt environmental changes (e.g., T_a, barometric pressure, RH) and disruption to the colony could have given incentive to the bats to switch bat houses for a single day, but I found no evidence of such disturbance. Therefore, in my view, it is unlikely that differences in the time each bat house was available had a major impact on my results.

My study on preferential selection for heated bat houses was conducted solely on male bats and does not account for an increased tendency to select cooler microclimates during food shortages (Kerth et al. 2001). Female bats commonly select warm T_as to enhance reproductive capacity (Lausen and Barclay 2003), but during periods of limited food availability pregnant female Bechstein's bats (*Myotis bechsteinii*) selected cooler

microclimates to facilitate torpor (Kerth et al. 2001). Nonetheless, evidence suggests that reproductive females should select warm microclimates given adequate foraging opportunities. In the same study of Bechstein's bats, cooler microclimates resulted in delayed parturition and lower reproductive success, so it is likely that warm microclimates that improve conditions for gestation would have been selected if food were accessible (Kerth et al. 2001; Lausen and Barclay 2003). Further, presumably to reduce energy expenditure, bats are known to select roosts within their thermoneutral zone (Lourenço and Palmeirim 2004). So, though my analysis was conducted on male bats, it is likely that female bats would show a similar preference for warm microclimates, assuming foraging opportunities are present. To examine this, I suggest that future studies quantify preferential selection for heated microclimates and energy savings in wild-caught female bats. In this manner bats could be housed in an outdoor, temporary captive facility with heated and unheated bat houses and preferential selection could be determined by altering which bat houses are warmed.

Few published data exist on the energetics of thermoregulation or reproduction for bats in early spring just after hibernation, so I had to make a number of assumptions that could have influenced my energetics models. However, results of my models were consistent with published estimates of energy expenditure from free-ranging bats. My model predicted that basal plus thermoregulatory energy costs for a pregnant female little brown bat in a naturally occurring roost would amount to 26.6 kJ day^{-1} on average. Kurta et al. (1989) found that pregnant female little brown bats expended about 29.9 kJ day^{-1} in late spring/early summer. Kurta et al.'s (1989) energetic estimates included the additional costs of foraging flight (i.e., about 4.15 kJ h^{-1}), but were also obtained during a warmer

time of year and in a warmer climate (New Hampshire and Massachusetts U.S.A.). For simplicity, I did not include foraging time and costs of foraging flight in my model, but if bats foraged for 2-3 hours per night this would have added only about $8 - 12.5$ kJ day^{-1} onto my estimates of daily energy expenditure. Cooler weather in my study would have augmented energy expenditure as bats would increase MR to defend the established T_b (i.e., during normothermia or at $T_{tor-min}$; Speakman and Thomas 2003). In this way, the energy expenditure predicted in my study would be higher than values determined at warmer temperatures, so would likely approximate (or exceed if accounting for foraging costs) those in Kurta et al. (1989). Therefore, this suggests that the results of my model reasonably approximate energetic costs of different roosting conditions in central Canada.

Despite the potential energetic benefit of heated bat houses, artificially-warmed microclimates may also increase fat store depletion. Bats emerging from hibernation could face reduced foraging opportunities during early spring, as some insect species do not peak in abundance until late spring when T_as warm (i.e., May-June; McElligott and Galloway 1991). Without adequate chances for feeding, bats would not be able to replenish fat stores and would be required to operate on minimal energy availability, thus limiting the capacity to maintain the elevated energetic requirements of normothermia. In order to reduce the potential for bats to use heated bat houses when torpor use would be more beneficial, I recommend that a T_a threshold be defined so that artificial heating may only be employed (i.e., heating turned on) when energy savings occur. It is also possible that for WNS-affected bats, close proximity within bat houses may increase transmission of disease (Langwig et al. 2012; Lorch et al. 2011). Though, even if new infections happen after emergence from hibernation, it is unlikely that substantial physiological

disruption or mortality would occur as *Pd* requires low T_as for growth (i.e., optimal temperature is 4°C; Gargas et al. 2009) and mortality from WNS is not apparent until months after infection (i.e., 120 days; Lorch et al. 2011). Nonetheless, monitoring should occur at heated bat houses in order to limit transmission of disease and bat houses should be disinfected between years.

Bat houses are often described as one of the leading ways to help improve habitat and enhance survivorship from many threats to bats, including WNS (Organization For Bat Conservation 2014; White-Nose Syndrome.org 2014b). In the United States approximately 25,000 bat houses are sold every year (R. Mies personal communication) and, with increased public awareness about WNS, my findings suggest a possible benefit of public engagement in conservation. Maternity colonies of bats often occur in man-made structures (Fenton and Barclay 1980; Lausen and Barclay 2006) or in established bat houses on private or public property. Heated bat houses running on AC or solar power could be mounted near existing colonies to increase the chance for reproductively active and/or WNS-affected bats to use these warmer microclimates. Heated bat houses could also be placed outside known hibernacula to allow bats to use these refugia before migrating to summer roosts, although this might require additional resources to provide power. Alternatively, structures that retain solar heat and passively warm (i.e., passive solar heating building designs) could provide warmer T_{roost} to benefit bats during cool spring weather.

I found that both *WNS-* and *WNS+* bats preferentially selected heated bat houses and that *WNS+* bats selected unheated bat houses on fewer occasions than *WNS-* individuals. My bioenergetic models suggested that thermoregulatory benefits likely

explain this preference with bats gaining a significant energetic benefit in an artificially-

heated roost compared to roosting in a natural site. Taken together these results suggest

that heated bat houses could be useful as a management tool for free-ranging bats by

improving conditions for gestation, parturition, and offspring development, and by

enhancing the rate of recovery in bats that survive the winter with WNS. However, before

this strategy is attempted in the field, more data are needed on effects of T_{roost} on

gestation, pup development, and food requirements, as well as healing and survival

from WNS.

CHAPTER 4

GENERAL CONCLUSIONS

Wildife conservation aims to maintain species diversity and abundance often in light of species declines. Although not mutually exclusive, *ex situ* (e.g., captive assurance) and *in situ* (e.g., field-based conservation) conservation provide a range of tactics that can potentially mitigate declines of threatened species by enhancing survival and reproduction. *Ex situ* conservation focuses on maintaining wildlife in captive assurance and breeding programs and will typically be most useful in situations where populations face rapid and severe declines (Zippel et al. 2010). *In situ* conservation tactics target free-living wildlife within their natural range often combining both species-focused measures and habitat protection (Lacy 2010). *In situ* approaches may be most successful when the threat to the population can readily be identified and mediated, as in the case of habitat loss or degradation with subsequent habitat modification to mitigate impacts (Zippel et al. 2010). Combining both conservation methods to promote population recovery could provide a kind of insurance policy to ensure populations or species can persist while habitat can be restored or enhanced, or threats can be mitigated.

For threatened species of insectivorous bats, captive assurance populations may have some potential as a last resort in cases of severe declines. Understanding the potential of captive assurance has become especially important for bats in light of white-nose syndrome (WNS), an invasive fungal disease causing dramatic mortality. Some species affected by WNS can readily be maintained throughout the year (e.g., big brown bats (*Eptesicus fuscus*)), but the hardest hit species have only been successfully housed in large numbers during hibernation over the winter (e.g., little brown bats (*Myotis*

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lucifugus); Warnecke et al. 2012). Understanding variation in behaviour following introduction to captivity could be valuable for developing approaches for captive assurance.

For Chapter 2, I studied a captive colony of healthy and WNS-affected little brown bats to test the hypothesis that behavioural tendencies associated with the shy-bold continuum of animal personality (Réale et al. 2007) affect feeding behaviour and rate of pre-hibernation fat accumulation in captivity. I also tested whether captive bats exhibit signs of a dominance hierarchy and whether subordinate-dominant tendencies might influence feeding behaviour and mass accumulation. I found evidence that locomotion affected the frequency of feeding in *WNS+* bats and that grooming tendency (which is thought to reflect anxiety in rodents and bats; Kalueff and Tuohimaa 2005) was positively correlated with post-hibernation body mass in *WNS+* bats, suggesting that anxious bats may exhibit an energetically conservative pattern of hibernation (Chapter 2). I also found preliminary evidence of a dominance hierarchy as bats arrived at the feeding dish in a consistent order each night. My results suggest that certain behavioural tendencies may influence feeding and body condition in captivity, but more work is needed to determine how behaviour and dominance influence captive housing of hibernating bats. Kilgour et al. (2013) suggested that dominance interactions could influence thermoregulation with dominant individuals excluding subordinates from optimal roosting positions. If this is the case in little brown bats, dominance interactions could have implications for acclimation to captivity. I recommend that future studies characterize possible dominance hierarchies via interaction tests between pairs of individuals and observe interactions in captivity to assess social behaviour, particularly

those associated with feeding. In light of dramatic declines in population sizes caused by WNS, understanding behavioural variation of bats in captivity and the influence of behaviour on acclimation to captivity is important for conservation and management of hibernating bats.

Captive assurance can serve as a last resort to prevent extinctions but, for most populations and situations, *in situ* conservation approaches will usually be preferable and will almost always be essential for managing impacts of conservation threats in the wild. Habitat modification or enhancement can help stabilize and grow threatened populations in the face of a range of conservation threats (Ehler 1998; van den Bosch and Telford 1964). Small-bodied mammals like bats require warm temperatures for gestation and lactation, but may be faced with temperatures below their thermoneutral zone (TNZ) during much of the active season (Lausen and Barclay 2006). Habitat modifications that reduce thermoregulatory costs during the active season could speed reproduction and enhance adult and juvenile survival (Frick et al. 2010b). Warm roost microclimates might also help bats that survive the winter with WNS balance their energy budgets and/or heal more quickly. WNS-affected bats emerge from hibernation when it is still relatively cold, immediately mount an immune response that is energetically costly (Meteyer et al. 2012; Moore et al. 2013), and then begin to heal from wing damage caused by the fungus (Fuller et al. 2011). Reducing thermoregulatory costs during this period could help more bats survive this period of energetic limitation and help survivors reproduce more quickly.

In Chapter 3, I tested the potential of artificially-heated bat houses as a management tool for insectivorous bats threatened by habitat loss and WNS. I found that

both healthy and WNS-affected bats preferentially selected heated bat houses for roosting and that WNS-affected bats selected unheated bat houses on fewer occasions than healthy individuals. I used a bioenergetics model to show that energy expenditure would be dramatically reduced for bats using a heated bat house compared to roosting in a typical maternity colony following emergence from hibernation in the spring (Chapter 3). These results suggest that artificial heating of roosts may reduce energy expenditure and improve conditions for gestation and pup development. Given the large market that exists for bat houses in North America, heated bat houses running on AC power adjacent to known colonies could improve the chance that bats would find and use the heated roosts. Alternatively, other types of structures that enhance solar heat gain passively and provide warmer spring temperatures could also be useful. I recommend future research aimed at better quantifying microclimate preferences of free-ranging bats and experimentally testing the influence of bat house microclimates on recovery from WNS, gestation rates, pup development, and annual survival.

My project provides new knowledge on behaviour of a bat species facing dramatic declines from WNS and provides a preliminary test of one method to potentially address those declines. My work also contributes to a small but growing literature on the housing of bats in captivity and provides new information enhancing our understanding of behavioural interactions within social groups of bat species. Understanding behavioural variation is important for identifying factors that could affect successful *ex situ* or *in situ* management approaches and will assist development of conservation and management plans to address alarming population declines of North American bats.

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