Habitat quality, torpor expression and pathogen transmission in little brown bats (*Myotis lucifugus*)

By

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CHAPTER 1: GENERAL INTRODUCTION

Emerging infectious diseases of wildlife are a growing concern for conservation as human travel and trade increase movement of pathogens, and habitat loss or fragmentation affect host-pathogen dynamics (Daszak *et al.* 2000, Wilder *et al.* 2015). Introduced pathogens can have devastating effects on naïve host populations, in some cases driving host species to extinction and reducing overall biodiversity (Daszak *et al.* 2000, Smith *et al.* 2006, Mitchell *et al.* 2008). The impacts of a pathogen on a host population is a function both of pathogen traits (e.g., the basic reproductive number, R_0, Brunham *et al.* 1993), host physiology and behaviour (e.g., huddling) and the shared host-pathogen environment (e.g., resource patches like watering holes or oases, Anderson & May 1979, Scholthof 2007). Most host-pathogen systems are thought to be dependent on host density such that as a host population declines pathogen transmission will similarly decline (Anderson & May 1992). However, some pathogens may also persist independent of host density, as long at the frequency of interactions between conspecifics is still high (Anderson & May 1992). Extinction caused by a pathogen is more likely when transmission is frequency-dependent, rather than density-dependent, as is the case for many sexually transmitted pathogens or pathogens that can survive and, especially, reproduce in the environment without hosts present (de Castro & Bolker 2005).

Environmental factors can exert indirect and direct effects on host physiology and behaviour in ways that can affect host susceptibility and disease impacts. For some host-pathogen systems, weather conditions and temperature may affect susceptibility by compromising immune function (Raffel *et al.* 2006). For example, when exposed to low ambient temperatures (T_a) of about 5°C for extended periods (2-5 months), leopard frogs

(*Rana pipiens*) show decreased proliferation of both T-cell and B- cell lymphocytes, which normally allow animal immune systems to identify antigens and mount an adaptive immune response (Maniero & Carey 1997). The reduction in immune response resulting from environmental variation increases susceptibility of frogs to *Batrachochytrium dendrobatidis*, the fungal pathogen that causes the devastating disease chytridiomycosis (Raffel *et al*. 2006). Environmental variation can also affect pathogens in ways that might affect transmission. For example, *B. dendrobatidis* thrives in temperatures between 17° and 25°C but shows reduced growth and mortality above 28°C (Longcore *et al.* 1999).

The environment can also affect host behaviours. Chronic wasting disease, a prion disease affecting mule deer (*Odocoileus hemionus*, Miller *et al.* 2004), white-tailed deer (*Odocoileus virginianus*), and Rocky Mountain elk (*Cervus elaphus nelson*, Miller *et al.* 1998) is transmissible from an infected individual's saliva or feces to a susceptible ungulate (Mathiason *et al.* 2009, Tamguney *et al.* 2009). In areas where habitat quality is poor, large groups of cervids may aggregate in and around food patches which increases the likelihood of pathogen transmission both from infected individuals and substrates (Joly *et al.* 2006,Almberg *et al.* 2011). An understanding of the importance of host behaviour and physiology, and the importance of the environment for host-pathogen interactions can be crucial for management of wildlife disease (Scholthof 2007). For example, aggregation at supplemental feeding areas is suspected to have contributed to the increased transmission of tuberculosis in deer (Palmer *et al.* 2004), chronic wasting disease in deer and elk (*Cervus canadensis*, Williams *et al.* 2002), and brucellosis in elk and bison (*Bison bison*, Etter & Drew 2006). By discouraging and outlawing artificial feeders, wildlife managers were able to manipulate behaviour of host species and reduce

aggregation, thus reducing the risk of pathogen transmission and acquisition (Brown & Cooper 2006). Management strategies that are informed by host social behaviour and physiology can, therefore, be important for reducing impacts of infectious disease on wildlife populations.

A range of other host characteristics are also thought to influence host-pathogen dynamics, host susceptibility and disease impacts (Scholthof 2007). The density of host populations and aggregations, the lifespan/longevity of host individuals and sociality can all affect transmission throughout the population (Anderson & May 1992). For example, in Cape ground squirrels (*Xerus inauris*), females have smaller home ranges (Waterman 1995) and carry greater endoparasite loads than males, likely because they of their consistent exposure to conspecifics' fecal pellets in foraging areas (Hillegass *et al.* 2008). Males, who forage over large areas and nest in vacant burrows (Waterman 1995), are less likely to come in contact with infected feces (Hillegass *et al.* 2008). While a large body of theoretical research and epidemiological modeling exists (May & Anderson 1978, Anderson & May 1979, Barlow 1991, de Jong *et al.* 1995, Roberts 1996, McCallum *et al.* 2001), relatively few empirical studies have addressed how variation in host behaviour might affect pathogen transmission and proliferation within a population, and fewer still have conducted controlled experiments on the influence of host behaviour on host-pathogen dynamics.

Energy limitation caused by environmental constraints could force animals to shift their behaviour in ways that affect host-pathogen dynamics. Endothermic animals must expend energy to maintain body temperature (T_b) during cold exposure which is particularly challenging during periods of low food abundance (Lovegrove *et al.* 2001,

Mzilikazi & Lovegrove 2002, Geiser 2004). As a result, some endothermic species huddle with conspecifics during inclement weather (e.g., striped skunks, *Mephitis mephitis,* Gunson & Bjorge 1979), Siberian flying squirrels (*Pteromys volans*, Selonen *et al.* 2014), yellow-eyed penguins (*Megadyptes antipodes*, Seddon & Davis 1989), and white-backed mousebirds (*Colius colius,* McKechnie & Lovegrove 2001), which could increase their risk of pathogen transmission and acquisition.

Some endothermic animals are capable of heterothermy, or torpor, a state of reduced metabolic rate and T_b which conserves energy during periods of harsh weather conditions and low food availability. Many species of birds and mammals are heterothermic endotherms that can selectively enter torpor or longer-term hibernation to save energy (Willis *et al.* 2006; Canale & Henry 2011). In Richardson's ground squirrels (*Urocitellus richardsonii*), for example, hibernating for an 8-month winter can reduce energetic costs by nearly 90% compared to remaining normothermic (Wang 1979). In addition to energy savings, torpor may also help individuals avoid predation by minimizing the amount of time spent foraging and exposed to potential predators (Stawski & Geiser 2010). Subtropical insectivorous bats (*Nyctophilis bifax*) in good body condition enter torpor more often than individuals in poor condition, even during relatively high T_a and when food is abundant, possibly to reduce foraging requirements and avoid the risk of predation while foraging (Stawski & Geiser 2010). Predation avoidance as a side benefit of torpor expression suggests the additional possibility that increased use of torpor, and a concomitant reduction in activity, could also reduce the risk of acquiring pathogens and parasites. However, this potential indirect benefit of torpor has never been tested.

Although torpor expression reduces energetic costs and provides a number of additional benefits (Geiser & Brigham 2012), it also comes with associated costs (Humphries *et al.* 2003). The largest and best studied of these potential costs is delayed reproduction and offspring growth, and reproductive mammals tend to avoid torpor expression compared to non-reproductive conspecifics (Geiser 1996, Lausen & Barclay 2003, Dzal & Brigham 2013). Torpor delays parturition for pregnant females, and inhibits lactation, both of which may be especially detrimental for hibernators as offspring will have less time to grow and build fat stores for their first winter (Geiser 1996). Torpor also delays sperm production for males (Jagiello *et al*. 1992) and can slow healing rates from injury (Faure *et al.* 2009). To avoid these costs of torpor, in some situations and at certain times of year, many heterothermic endotherms will select warm microclimates, or huddle with conspecifics to avoid torpor and its associated costs.

Another host behaviour that can affect host-pathogen dynamics is sociality. Sociality, or the tendency of a species to live communally, has evolved in response to a suite of evolutionary pressures. Benefits of sociality include territorial control of resources (e.g., African lions, *Panthera leo*; Mosser & Packer 2009), protection from predation (e.g., meerkats, *Suricata suricatta*; Townsend *et al.* 2011), and ease of finding a mate (Hamilton, 1970). On the other hand, sociality can also be costly if it enhances the risk of acquiring pathogens or parasites (Altizer, *et al.* 2003). Highly social or colonial species are likely to have high contact rates with conspecifics and increase the potential for pathogen or parasite transmission such that less social individuals may benefit by avoiding group living (Alexander 1974). If pathogen risk is greater in large social groups,

there could be evolutionary pressure on group size, social interactions, and group composition (Côté & Poulin 1995, Alitzer *et al.* 2003).

Some socially transmitted diseases may be host-density-independent if transmission is a function of the frequency of interactions between host individuals. Frequency-dependent transmission is likely if infection occurs when hosts seek each other out, either for mating or social aggregations (Anderson & May 1991, Nunn *et al.* 2008, McCallum *et al.* 2009) or if the pathogen can survive in an environmental reservoir (Briggs *et al.* 2010). Frequency-dependent pathogens are particularly insidious and can drive their host populations to extinction because transmission still occurs at low host-population densities (Getz & Pickering 1983, Lockhart *et al.* 1996). Social interactions, therefore, reflect an evolutionary trade-off that can fluctuate depending on environmental conditions and pathogen prevalence within the host population (Silk 2007, Mitchell, *et al.* 2008).

Bats (Chiroptera) have potential as model organisms for studies of pathogen transmission among highly social, colonial hosts, and for understanding the role social behaviour and thermoregulation have on host-pathogen dynamics (Webber *et al.* 2016, 2017). Bats are heterothermic endotherms (Fenton & Barclay 1980) and exhibit an enormous range of group sizes and social systems ranging from completely solitary to colonies of millions of individuals (Webber *et al.* 2017). Understanding host-pathogen dynamics of bats has become especially urgent with the emergence of white-nose syndrome (WNS). Populations of hibernating bats in Canada and the United States are currently threatened by WNS, which is caused by the invasive fungal pathogen, *Pseudogymnoascus destructans* (Blehert *et al.* 2008, Frick *et al.* 2010). WNS is an

infectious skin disease that causes bats to rewarm from torpor too frequently during hibernation, and expend extra energy during winter torpor bouts, drastically depleting winter fat stores and resulting in mass mortalities at hibernation sites (Reeder *et al.* 2012, Warnecke *et al.* 2012, McGuire *et al*. 2017). Bats contract WNS through direct contact with infected individuals or substrates within hibernacula (Lorch *et al.* 2011). Since its introduction from Eurasia¸ where it is an endemic pathogen of multiple bat species (Wibbelt *et al.* 2010, Puechmaille *et al.* 2011b), *P. destructans* has caused tremendous declines of naïve populations of North American hosts (Frick *et al.* 2010, Frick *et al.* 2015). For example, Frick *et al.* (2015) found that, in some hibernacula, the disease has caused local extirpation, with mortality rates of 91%, on average, for little brown bats (*Myotis lucifugus*). As of January 2013, it was estimated that 5.7 to 6.7 million bats have been killed by WNS (U.S. Fish & Wildlife Service 2013) and this number almost certainly dramatically underestimates the impacts of WNS to date.

Little brown bats are one of the most studied and, until the emergence of WNS, were likely the most abundant bat species in North America (Harvey *et al.* 2011). During the months of May and June little brown bats form maternity colonies of up to hundreds of reproductive females and their pups, along with a few non-reproductive individuals. Little is known about male behaviour during the maternity season, but they are presumed to be mostly solitary throughout their range (Harvey *et al.* 2011). During the late summer and early fall, males and females congregate outside of hibernation sites, likely to mate and introduce juveniles to potential hibernacula (Hall & Brenner 1968, Agosta *et al.* 2005). Little brown bats hibernate in caves and mines during winter and are one of the species hardest hit by WNS (Harvey *et al.* 2011). Little brown bats, along with northern-

long eared (*Myotis septentrionalis*) and tricolored bats (*Perimyotis subflavus*), were recently listed as "endangered" by the Committee on the Statues of Endangered Wildlife in Canada under Schedule 1 of the Species at Risk Act in Canada (Government of Canada 2016).

To reduce the impacts of WNS, numerous chemical and biological management solutions have been designed and tested (Chaturvedi *et al.* 2011, Puechmaille *et al.* 2011a, Robbins *et al.* 2011, Raudabaugh & Miller 2013, Cornelison *et al.* 2014, Hoyt *et al.* 2015, O'Donoghue *et al.* 2015, Pannkuk *et al.* 2015, Dorville *et al. In prep.*) but no effective treatment protocols are currently available. At the habitat level, several mitigation techniques have been proposed including: culling of infected individuals (Langwig *et al.* 2015), creating warm micro-habitats within caves where bats can save on energetic costs during periodic arousals (Boyles & Willis 2010), and inhibiting *P. destructans* growth within caves by altering the microclimate (e.g. temperatures, humidity, Shelley *et al.* 2013, Grieneisen *et al.* 2015) or applying UV lighting (Palmer *et al.* 2018). However, these approaches would require continued interventions that may not be sustainable for the long-term (Kilpatrick 2006). Evolutionary rescue may provide a more viable solution for recovery of bat populations affected by WNS (Maslo & Feffernan 2015). Bat populations near the epicentre of WNS have begun stabilizing following their initial population crashes, albeit at dramatically reduced population sizes (Dobony *et al.* 2011, Langwig *et al.* 2012, 2017). This stabilization suggests the possibility of the evolution of resistance to (i.e., remaining uninfected by a pathogen), or tolerance to (i.e., harbouring the pathogen but limiting its impacts) the pathogen (Langwig *et al.* 2017). Survivors of WNS that are resistant or tolerant may pass these trait

on to their offspring increasing the frequency of these traits within populations (Maslo & Feffernan 2015, Carlson *et al.* 2016). If evolutionary rescue is a promising avenue for recovery of bat populations affected by WNS (Maslo & Feffernan 2015), then encouraging survival and reproduction of WNS survivors should be a critical priority for management (Donaldson *et al.* 2017).

Habitat enhancement can be a valuable conservation tool for threatened species, including those impacted by the effects of disease (Knaepkens *et al.* 2004, Savard & Robers 2007, Kapust *et al.* 2012, Wilcox & Willis 2016, Tripp *et al.* 2017). For example, prairie dogs (*Cynomys ludovicianus*) susceptible to plague (caused by the *Yersinia pestis* bacterium) benefited from reduced disease impacts after their burrows were treated with insecticide to kill fleas, the vector of the pathogen (Tripp *et al.* 2017). Habitat enhancement also improved the reproductive success of endangered European bullhead (*Cottus gobio*) that are experiencing population declines caused by habitat degradation (Knaepkens *et al.* 2004). For bats, enhancement of summer roosting habitat could be a valuable conservation tool if high quality roosting habitat can reduce thermoregulatory energy expenditure and/or torpor expression and increase healing rates and offspring growth rates. For species that regularly use anthropogenic structures (e.g., little brown bats), warm microclimates could be provided by insulating or painting roosts to retain solar heat or even by installing heating panels (Wilcox & Willis 2016). For species more dependent on natural roosts like tree hollows (e.g. *Myotis septentrionalis*), implementing habitat enhancement would be more challenging but evidence supporting this strategy would justify more effort to understand forest roost requirements of these species.

Habitat enhancement could be an effective management tool for WNS but could also be detrimental if it increases the potential for transmission of *P. destructans* or other naturally occurring pathogens and parasites of WNS-affected bats. Webber *et al*. (2016) showed that collapsing large, relatively fluid social networks of bats spread among many natural roosts, into a smaller number of more stable, large colonies can increase the risk of pathogen transmission and proliferation within bat colonies. Enhanced, relatively warm summer roosting habitat could also reduce torpor expression and, therefore, increase overall activity of individuals resulting in more opportunities for bats to contract pathogens and parasites from the environment or each other. Thus, while roosting habitat enhancement has potential to help bats recover from WNS (Maslo & Feffernan 2015, Wilcox & Willis 2015), it could be counterproductive if it increases the risk of transmission of *P. destructans* or other pathogens and parasites.

Objectives and Hypotheses

The objective of my thesis was to test the broad hypothesis that habitat quality, via its effects on torpor expression and aggregation, can influence transmission of wildlife pathogens. I also aimed to shed light on the possibility that enhancement of summer roosting habitat could reduce energetic costs of thermoregulation and, therefore, help survivors of WNS.

For my first data chapter (Chapter 2) I tested the hypothesis that high-quality (i.e., relatively warm), roosting habitat reduces reliance on torpor for individual bats, which, in turn, increases activity and the risk of pathogen acquisition from an infected substrate. I predicted that bats provided with high quality, warm roost boxes would: 1) reduce their

use of torpor during the active period at night, and spend more time actively investigating potential roosts; and 2) after an exposure period, exhibit higher intensity of infection with a proxy pathogen that I applied to substrates within the testing environment.

For my second data chapter (Chapter 3) I tested the hypothesis that limited high-quality roosting habitat increases passive aggregation for groups of bats and reduces their use of torpor which, in turn, increases the risk of pathogen acquisition. I predicted that bats provided with a relatively rare warm roost would: 1) form larger aggregations in the warm roost; 2) use less torpor; and 3) have a greater intensity of infection with a proxy pathogen that I applied to one randomly selected individual within their social group.

For both experiments I housed bats in outdoor flight enclosures and experimentally manipulated habitat quality by heating a subset of roost boxes in the enclosures. I then quantified the influence of roost temperature on expression of torpor and, for Chapter 3, aggregation size. I used non-toxic, ultraviolet (UV) fluorescent powder as a harmless proxy for a contact pathogen to assess the influence of torpor use and passive aggregation on pathogen transmission from substrates and among individuals.

Habitat quality may influence the transmission of wildlife pathogens via its effects on habitat selection, passive aggregation, and torpor expression. The findings from my study suggest that artificially heating roosts, along with protecting natural roosts with warm microclimates, could improve recovery rates and juvenile development of bats recovering from WNS. Understanding the impacts of environmental variation on the transmission of pathogens to their host species has implications for the design of conservation strategies and could enable managers to both target habitat necessary for

protection and find areas where habitat could be artificially improved to facilitate recovery of imperiled species.

Ethical statement

All procedures were conducted under Manitoba Conservation Wildlife Scientific Permit number WB16368 and approved by the University of Winnipeg Animal Care Committee. Although the nearest confirmed finding of *P. destructans* was over 200 km from my study site (northern Minnesota, www.whitenosesyndrome.org, 2016) at the time of my study, I still followed decontamination protocols for researchers outlined by the U.S. Fish and Wildlife to control the spread of *P. destructans* (www.whitenosesyndrome.org, 2016).

References

Agosta, S.J., Morton, D., Marsh, B.D., & Kuhn, K.M. 2005 Nightly, seasonal, and early patterns of bat activity at night roosts in the central Appalachians. *Journal of Mammalogy* **86,** 1210-1219.

Almberg, E.S., Cross, P.C., Johnson, C.J., Heisley, D.M., & Richards, B.J. 2011 Modeling routes of chronic wasting disease transmission: environmental prion persistence promotes deer population decline and extinction. *PLoS ONE* **6,** e19896.

Alexander, R.D. 1974 The evolution of social behavior. *Annual Review of Ecology and Systematics* **5,** 325-383.

Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., & Dobson, A.P., 2003 Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics* **34,** 517-547.

Anderson, R.M., & May, R.M. 1979 Population biology of infectious diseases: Part II *Nature* **280,** 455-461.

Anderson, R.M., & May, R.M. 1991 Infectious diseases of humans: dynamics and control. *University of Oxford Press,* New York.

Anderson, R.M., & May, R.M. 1992 Understanding the AIDS pandemic. *Scientific American* **266,** 58-66.

Barlow, N.D. 1991 A spatially aggregated disease/host model for bovine TB in New Zealand possum populations. *Journal of Applied Ecology* **28,** 777-793.

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., & Coleman, J.T.H., 2008 Bat white-nose syndrome: an emerging fungal pathogen? *Science* **323,** 227-235.

Boyles, J.G., & Willis, C.K.R. 2010 Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white-nose syndrome? *Frontiers in Ecology and the Environment.* **8,** 92-98.

Briggs, C.J., Knapp, R.A., & Vrendenburg, V.T. 2010 Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *PNAS* **107,** 9695-9700.

Brown, R.D., & Cooper, S.M. 2006 The nutritional, ecological, and ethical arguments against baiting and feeding white-tailed deer. *Wildlife Society Bulletin* **34,** 519-524.

Brunham, R.C., Plummer, F.A., & Stephens, R.S. 1993 Bacterial antigenic variation, host immune response, and pathogen-host coevolution. *Infection and Immunity* **61,** 2273-2276.

Canale, C.I., & Henry, P.Y. 2010 Energetic costs of the immune response and torpor use in a primate. *Functional Ecology* **25,** 557-565.

Carlson, S.M. Cunningham, C.J., & Westley, P.A.H. 2016 Evolutionary rescue in a changing world. *Trends in Ecology & Evolution* **29,** 521-530.

Chaturvedi, S., Rajkumar, S.S., Li, X., Hurteau, G.J., Shtutman, M., & Chaturvedi, V. 2011 Antifungal testing and high-throughput screening of compound library against *Geomyces destructans*, the etiologic agent of geomycosis (WNS) in bats. *PLoS ONE* **6,** 1-6.

Cornelison, C.T., Keel, M.K., Gabriel, K.T., Barlament, C.K., Tucker, T.A., Pierce, G.E., & Crow, S.A. 2014 A preliminary report on the contact-independent antagonism of *Pseudogymnoascus destructans* by Rhodococcus rhodochrous strain. DAP96253 *BMC Microbiology* **14,** 246.

Côté, I.M., & Poulin, R. 1995 Parasitism and group size in social animals. *Behavioural Ecology* **6,** 159-165.

Cryan, P.M., Meteyer, C.U., Boyles, J.G., & Blehert, D.S. 2013 White-nose syndrome in bats: illuminating the darkness. *BMC Biology* **11,** 47. (DOI:10.1186/1741-7007 PMID:23587401)

Daszak, P., Cunningham, A.A., & Hyatt, A.D. 2000 Emerging infectious diseases of wildlife- threats to biodiversity and Human Health. *Science* **287,** 443-448.

de Castro, F. & Bolker, B. 2005 Mechanisms of disease-induced extinction. *Ecological Letters* **8,** 117-126.

de Jong, M.C.M, Diekmann, O., & Heesterbeek, H. 1995 How does transmission of infection depend on population size? In *Epidemic Models: Their Structure and Relation to Data* (Mollison, D., ed.) pp. 84-94, Cambridge University Press

Dobony, C.A., Hicks, A.C., Langwig, K.E., von Linden, R.I., Okoniewski, J.C., & Rainbolt, R.E. 2011 Little brown myotis persist despite exposure to white-nose syndrome. *Journal of Fish and Wildlife Management* **2,** 190-195.

Donaldson, M.E., Davy, C.M., Willis, C.K.R., McBurney, S., Park, A., & Kyle, C.J. 2017 Profiling the immunome of little brown myotis provides a yardstick for measuring the genetic response to white-nose syndrome. *Evolutionary Applications* **10,** 1076-1090 (DOI:10.1111/eva.12514)

Dorville, N., Kunkel, E.L., Breit, A.M., Norquay, K.J.O., Dzal, Y.A., Fletcher, Q.E., Beekman, C., Shrivastav, A., Bollinger, T.K., Bennett, R.J., Overton, B., Turner, G., & Willis, C.K.R. *In prep.* Two potential treatments for white-nose syndrome in little brown bats (*Myotis lucifugus*).

Dzal, Y.A. & Brigham, R.M. 2013 The tradeoff between torpor use and reproduction in little brown bats (*Myotis lucifugus*). *Journal of Comparative Physiology B.* **183,** 279-288.

Etter, R.P. & Drew, M.L. 2006 Brucellosis in elk of eastern Idaho. *Journal of Wildlife Diseases* **42,** 271-278.

Faure, P.A., Re, D.E., Clare, E.L. 2009 Wound healing in the flight membranes of big brown bats. *Journal of Mammalogy* **90,** 1148-1156.

Fenton, M.B., & Barclay, R.M.R. 1980 *Myotis lucifugus*. *Mammalian Species* **142,** 1-8.

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M., & Kunz, T.H. 2010 An emerging disease causes regional population collapse of a common North American bat species. *Science* **329,** 679-682.

Frick, W.F., Puechmaille, S.J., Hoyt, J.R., Nickel, B.A., Langwig, K.E., Foster, J.T., Barlow, K.E., Bartonicka, T., Feller, D., Haarsma, A.J., Herzog, C., Horacek, I., van der Kooij, J., Bulkens, B., Petrov, B., Reynolds, R., Rodrigues, L, Stihler, C.W., Turner, G.G., Kilpatrick, A.M. 2015 Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography* **24,** 741-749.

Geiser, F. 1996 Daily torpor and energy savings in a subtropical blossom-bat *Syconycteris australis* (Megachiroptera). *Adaptations to the cold: tenth international hibernation meeting.*

Geiser, F. 2004 Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual review of physiology*. **66**, 239-274.

Geiser, F., & Brigham, R.M. 2012 The other functions of torpor. In: Ruf, T., Bieber, C., Arnold, W., Millesi, E. (eds.) Living in a Seasonal World. Springer, Berlin, Heidelberg.

George, D.B., Webb, C.T., Farnsworth, M.L., O'Shea, T.J., Bowen, R.A., Smith, D.L., Stanley, T.R., Ellison, L.E., & Rupprecht C.E., 2011 Host and viral ecology determine bat rabies seasonality and maintenance. *Proceedings of the National Academy of Science* **108,**10208-10213.

Getz, W.M., & Pickering, J. 1983 Epidemic models: thresholds and population regulation. *American Naturalist* **121,** 892-898.

Government of Canada. 2016. SARA Public Registry. January 15. http://www.registrelep-sararegistry.gc.ca.

Grieneisen, L.E., Brownlee-bouboulis, S.A., Johnson, J.S., & Reeder, D.M. 2015 Sex and hibernaculum temperature predict survivorship in white-nose syndrome affected little brown myotis (*Myotis lucifugus*). *Royal Society Open Science* **2,** 140470 (DOI: 10.1098/rsos.140470)

Gunson, J.R. & Bjorge, R.R. 1979 Winter denning of the striped skunk in Alberta. *Canadian Field-Naturalist* **93,** 252-258.

Hall, J.S., & Brenner, F.J. 1968 Summer netting of bats at a cave in Pennsylvania. *Journal of Mammalogy* **49,** 779-781.

Hamilton, W.D. 1970 Geometry for the selfish herd. *Journal of Theoretical Biology* **31,** 295-311.

Harvey, M.J., J.S. Altenbach, and T.L. Best. 2011. Bats of the United States and Canada. *The Johns Hopkins University Press*. Baltimore, US

Hillegass, M.A., Waterman, J.M. & Roth, J.D. 2008 The influence of sex and sociality on parasite loads in an African ground squirrel. *Behavioral Ecology* **19,** 1006-1011.

Hillegass, M.A., Waterman, J.M. & Roth, J.D. 2010 Parasite removal increases reproductive success in a social African ground squirrel. *Behavioral Ecology* **21,** 696-700.

Hoyt, J.R., Cheng, T.L., Langwig, K.E., Hee, M.M., Frick, W.F., Kilpatrick, A.M. 2015 Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS ONE,* (DOI:10.1371/journal.pone.0121329)

Humphries, H.H., Thomas, D.W., & Kramer, D.L. 2003 The role of energy availability in Mammalian Hibernation: A cost-benefit approach. University of Chicago Press.

Jagiello, G.M., Fang, J.S., Sung, W.K., Hembree, W.C., & Ducayen, M.B. 1992 Genetic characteristics of spermatogenesis in the Turkish hamster (*Mesocricetus brandti*) subjected to reduced temperature or light. *Journal of Thermal Biology* **17,** 175-184.

Joly, D.O., Samuel, M.D., Langenberg, J.A., Blanchong, J.A., Batha, C.A., Rolley, R.E., Keane, D.P., & Ribic, C.A. 2006 Spatial epidemiology of chronic wasting disease in Wisconsin white-tailed deer. *Journal of Wildlife Diseases* **42,** 578-588.

Kapust, H.Q.W., McAllister, K.R., & Hayes, M.P. 2012 Oregon spotted frog (*Rana*

pretiosa) response to enhancement of oviposition habitat degraded by invasive reed canary grass (*Phalaris arundinacea*). *Herpetological Conservation and Biology* **7,** 358-366.

Kilpatrick, A.M. 2006 Facilitating the evolution of resistance to avian malaria in Hawaiian birds. *Biological Conservation* **128,** 475-485.

Knaepkens, G., Bruyndoncx, L., Coeck, J., & Eens, M. 2004 Spawning habitat enhancement in the European bullhead (*Cottus gobio*), an endangered freshwater fish in degraded lowland rivers. *Biodiversity and Conservation* **13,** 2442-2452.

Knell, R.J., Begon, M., & Thompson, D.J. 1996 Transmission dynamics of *Bacillus thuringiensis* infecting *Plodia interpunctella*: a test of the mass action assumption with an insect pathogen. *Proceedings of the Royal Society of London B.* **263,** 75-81.

Langwig, K.E. Frick, W.F., Bried, J.T., Hicks, A.C., Kunz, T.H., & Kilpatrick, A.M. 2012 Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* **15,** 1050-1057.

Langwig, K.E., Voyles, J., Wilber, M.Q., Frick, W.F., Murray, K.A., Bolker, B.M., Collins, J.P., Cheng, T.L., Fisher, M.C., Hoyt, J.R., Linder, D.L., McCallum, H.I., Puschendorf, R., Rosenblum, E.B., Toothman, M., Willis, C.K.R., Briggs, C.J., & Kilpatrick, A.M. 2015 Context-dependent conservation responses to emerging wildlife diseases. *Frontiers in the Ecology and Environment* **13,** 195-202.

Langwig, K.E., Hoyt, J.F., Parise, K.L., Frick, W.F., Foster, J.T., & Kilpatrick, A.M. 2017 Resistance in persisting bat populations after white-nose syndrome invasion. *Philosophical Transactions of the Royal Society of London B, Biological Sciences* **372,** 20160044.

Lausen, C.L., & Barclay, R.M.R. 2003 Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. *Journal of Zoology*. **260,** 235-244.

Leroy, E.M., Epelboin, A., Mondonge, V., Pourrut, X., Gonzalez, J.P., Muyembe-Tamfum, J.J., & Formenty, P. 2009 Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector-borne and Zoonotic Diseases* **9,** 723-728.

Lockhart, A.B., Thrall, P.H. & Antonovics, J. 1996 Sexually transmitted disease in animals: ecological and evolutionary implications. *Biological Review* **71,** 415-471.

Longcore, J.E., Pessier, A.P., & Nichols, D.K. 1999 *Batrachochytrium dendrobatidis* gen.

et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91,** 219-227.

Lorch, J.M., Meteyer, C.U., Behr, M.J., Boyles, J.G., Cryan, P.M., Hicks, A.C., Ballmann, A.E., Coleman, J.T.H., Redell, D.N., Reeder, D.M., & Blehert, D.S. 2011 Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* **480,** 376-378.

Lovegrove, B.G., Raman, J, Perrin, M.R. 2001 Daily torpor in elephant shrews (Macroscelidea: *Elephantulus* spp.) in response to food deprivation. *Journal of Comparative Physiology B* **171,** 11-21.

Maniero, G.D. & Carey, C. 1997 Changes in selected aspects of immune function in the leopard frog, Rana pipiens, associated with exposure to cold. *Journal of Comparative Physiology B.* **167,** 256-263.

Maslo, B. & Fefferman, N.H. 2015 A case study of bats and white-nose syndrome demonstrating how to model population viability with evolutionary effects. *Conservation Biology* **29,** 1176-1185.

Mathiason, C.K., Hays, S.A., Powers, J., Hayes-Klug, J., Langenberg, J., Dahmes, S.J., Osborn, D.A., Miller, K.V., Warren, R.J., Mason, G.L., & Hoover, E.A. 2009 Infectious prions in pre-clinical deer and transmission of chronic wasting disease solely by environmental exposure. *PLoS ONE* **4,** e5916.

May, R.M., & Anderson, R.M. 1978 Regulation and stability of host-parasite interactions I. Regulatory processes. *Journal of Animal Ecology* **47,** 219-247.

McCallum, H., Jones, M., Hawkins, C., Hamede, R., Lachish, S., Sinn, D.L., Beeton, N., & Lazenby, B., 2009 Transmission of Tasmanian devil facial tumor disease may lead to disease-induced extinction. *Ecology* **90,** 3379-3392.

McGuire, L.P., Mayberry, H.W., & Willis, C.K.R. 2017 White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. *American Journal of Physiology* **313,** R680-R686.

McKechnie, A.E., & Lovegrove, B.G. 2001 Thermoregulation and the energetic significance of clustering behavior in the white-backed mousebird (*Colius colius*). *Physiological and Biochemical Zoology* **74,** 238-249.

Miller, M.W., Wild, M.A., & Williams, E.S. 1998 Epidemiology of chronic wasting disease in captive Rocky Mountain Elk. *Journal of Wildlife Diseases* **34,** 532-538.

Miller, M.W., Williams, E.S., Hobbs, N.T., & Wolfe, L.L. 2004 Environmental sources of prion transmission in mule deer. *Emergin Infectious Diseases,* **10,** 1003-1006.

Mitchell, K.M., Churcher, T.S., Garner, T.W.J. & Fisher, M.C. 2008 Persistence of the

emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction. *Proceedings of the Royal Society B.* **275,** 329-334.

Mosser, A., & Packer, C. 2009 Group territoriality and the benefits of sociality in the African lion, *Panthera leo*. *Animal Behavior* **78,** 359-370.

Mzilikazi, N., & Lovegrove, B.G. 2002 Reproductive activity influences thermoregulation and torpor in pouched mice, *Saccostomus campestris*. *Journal of Comparative Physiology B.* **172,** 7-16.

Nunn, C.L., Altizer, S., Jones, K.E, & Sechrest, W. 2003 Comparative tests of parasite species richness in primates. *American Naturalist* **162,** 597-614.

Nunn, C. L., & Altizer, S. 2006. Infectious diseases in primates: behavior, ecology and evolution. *Oxford University Press*, New York

O'Donoghue, A.J., Knudsen, G.M., Beekman, C., Perry, J.A., Johnson, A.D., DeRisi, J.L., Craik, C.S., & Bennett, R.J. 2015 Destructin-1 is a collagen-degrading endopeptidase secreted by *Pseudogymnoascus destrucans,* the causative agent of white-nose syndrome. *PNAS* **112,** 7478-7483.

Palmer, M.V., Waters, W.R., & Whipple, D.L. 2004 Shared feed as a means of deer-to-deer transmission of *Mycobacterium bovis*. *Journal of Wildlife Diseases* **40,** 87-91.

Palmer, J.M., Drees, K.P., Foster, J.T., & Lindner, D.L. 2018 Extreme sensitivity to ultraviolet light in the fungal pathogen causing white-nose syndrome of bats. *Nature communications* **9,** 25.

Pannkuk, E.L., Risch, T.S., & Savary, B.J. 2015 Isolation and identification of an extracellular subtilisin-like serine protease secreted by the bat pathogen *Pseudogymnoascus destructans. PLoS ONE* **10,** 1-18.

Puechmaille, S.J., Fuller, H., & Teeling, E.C. 2011 Effect of sample preservation methods on the viability of Geomyces destructans, the fungus associated with white-nose syndrome in bats. *Acta Chiropterologica* **13,** 217-221.

Puechmaille, S.J., Wibbelt, G., Korn, V., Fuller, H., Forget, F., Muhldorfer, K., Kurth, A., Bogdanowicz, W., Borel, C., Bosch, T., Cherezy, T., Drebet, M., Gorfol, T., Haarsma, A.J., Herhaus, F., Hallart, G., Hammer, M., Jungmann, C., Le Briss, Y., Lutsar, L, Masing, M., Mulkens, B., Passior, K., Starrach, M., Wojtaszewski, A., Zophel, U., and Teeling, E.C., 2011 Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. *PLoS ONE* **6:**e19167.

Raffel, T.R., Rohr, J.R., Kiesecker, J.M., & Hudson, P.J. 2006 Negative effects of changing temperature on amphibian immunity under field conditions. *Functional Ecology* **20,** 819-828.

Raudabaugh, D.B., & Miller, A.N. 2013 Nutritional capability of a substrate suitability for *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome. *PLoS ONE* **8,** 1-9.

Reeder, D.M., Frank, C.L., Turner, G.G., Meteyer, C.U., Kutra, A., Britzke, E.R., Vodzak, M.E., Darling, S.R., Stihler, C.W., Hicks, A.C., Roymon, J., Grieneisen, L.E., Brownlee, S.A., Muller, L.K., & Blehert, D.S. 2012 Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS ONE.* **7.**

Robbins, A., Tseng, F., Reynolds, R., Beck, E., Reeder, D. *et al.* 2011 Terbinafine dosage and safety in WNS infected *Myotis lucifugus*: correlation of survival, drug tissue levels, and toxic effects. White-nose syndrome symposium, Litle Rock, Arkansas May 17-19. Pp. 24.

Savard, J.P.L. & Robert, M. 2007 Use of nest boxes by goldeneyes in eastern North America. *The Wilson Journal of Ornithology* **191,** 28-34.

Scholthof, K.B.G. 2007 The disease triangle: pathogens, the environment and society. *Nature Reviews Microbiology* **5,** 152-156.

Seddon, P.J., & Davis, L.S. 1989 Nest-site selection by yellow-eyed penguins. *The Condor* **91,** 653-659.

Selonen, V., Hanski, I.K., & Wistbacka, R. 2014 Communal nesting is explained by subsequent mating rather than kinship or thermoregulation in the Siberian flying squirrel. *Behavioral Ecology and Sociobiology* **68,** 971-980.

Shelley, V., Kaiser, S., Shelley, E., Williams, T., Kramers, M., Haman, K., Keel, K., Barton, H.A. 2013 Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of white-nose syndrome. *Journal of Cave and Karst Studies* **75,** 1-10.

Silk, J.B. 2007 The adaptive value of sociality in mammalian groups. *Philosophical Transactions of the Royal Society B* **362,** 539-559.

Smith, K.F., Sax, D.F., & Lafferty, K.D. 2006 Evidence for the role of infectious disease in species extinction and endangerment. *Conservation Biology* **20,** 1349-1357.

Stawski, C., & Geiser, F. 2010 Fat and fed: frequent use of summer torpor in a subtropical bat. *Naturwissenschaften.* **97,** 29-35.

Tamguney, G., Miller, M.W., Wolfe, L.L., Sirochman, T.M., Glidden, D.V., Palmer, C., Lemus, A., DeArmond, S.J., & Prusiner, S.B. 2009 Asymptomatic deer excrete infectious prions in faeces. *Nature* **461,** 529-532.

Townsend, S.W., Zöttl, M. & Manser, M.B. 2011. All clear? Meerkats attend to contextual information in close calls to coordinate vigilance. *Behavioral Ecology and Sociobiology* **65,** 1927-1934.

Tripp, D.W., Rocke, T.E., Runge, J.P., Abbott, R.C., & Miller, M.W. 2017 Burrow dusting or oral vaccination prevents plague-associated prairie dog colony collapse. *EcoHealth* **14,** 451-462.

U.S. Fish & Wildlife Service 2012 North American bat death toll exceeds 5.5 million from white-nose syndrome. *U.S. Fish & Wildlife Service News Release*

Wang, L.C.H. 1979 Time patterns and metabolic rates of natural torpor in the Richardson's ground squirrel. *Canadian Journal of Zoology* **57,** 149-155.

Wang, L.F., Shi, Z., Zhang, S., Field, H., Daszak, P., & Eaton, B.T. 2006 Review of bats and SARS. *Emerging Infectious Diseases* **12,** 1834-1840.

Warnecke, L., Turner, J.M., Bollinger, T.K., Lorch, J.M., Misra, V., Cryan, P.M., Wibbelt, G., Blehert, D.S., & Willis, C.K.R. 2012 Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences*. **109,** 6999-7003.

Waterman, J.M. 1995 The social organization of the Cape ground squirrel (*Xerus inauris*; Rodentia: Sciuridae). *Ethology* **101,** 130-147.

Webber, Q.M.R., Brigham, R.M., Park, A.D., Gillam, E.H., O'Shea, T.J., & Willis, C.K.R. 2016 Social network characteristics and predicted pathogen transmission in summer colonies of female big brown bats (*Eptesicus fuscus*) *Behavioral Ecology and Sociobiology* **70,** 701-712.

Webber, Q.M.R. 2016 Personality, sociality and pathogen dynamics in little brown bats. Master of Science Thesis. University of Winnipeg, Winnipeg, Manitoba, Canada.

Webber, Q.M.R. & Fletcher, Q.E. 2017 Viral richness is positively related to group size, but not mating system, in bats. *EcoHealth* doi:10.1007/s10393-017-1276-3.

White-nose syndrome occurrence map – by year (2017). http://www.whitenosesyndrome.org/resoureces/map

Wibbelt, G., Kurth, A., Hellmann, D., Weishaar, M., Barlow, A., Veith, M., Pruger, J., Gorfol, T., Grosche, L., Bontadina, F., Zophel, U., Seidl, H.P., Cryan, P.M., and

Blehert, D.S., 2010 White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe *Emerging Infectious Diseases* **16,** 1237-1243.

Wilcox, A. & Willis, C.K.R. 2016 Energetic benefits of enhanced summer roosting habitat for little brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. *Conservation physiology* **4,** 1-12.

Williams, E.S., Miller, M.W., Kreeger, T.J., Kahn, R.H., & Thorne, E.T. 2002 Chronic wasting disease of deer and elk: A review with recommendations for management. *Journal of Wildlife Management* **66,** 551-563.

Willis, C.K.R., Brigham, R.M., & Geiser, F. 2006 Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften.* **93,** 80-83.

Wilson, E.O. 2000 Sociobiology: the new synthesis. Harvard University Press, Cambridge, Massachusetts

CHAPTER 2: EFFECTS OF ROOST TEMPERATURE ON TORPOR EXPRESSION AND PATHOGEN ACQUISITION IN SOLITARY LITTLE BROWN BATS

Abstract

Protection of habitat can improve survival and reproductive fitness of threatened and endangered wildlife, particularly if that habitat helps individuals maintain energy balance. Temperate bats are heterothermic and rely on torpor to save energy during winter hibernation and, to a lesser extent, during summer. However, torpor delays parturition and slows lactation for females, and inhibits sperm production for males, so reproductive bats should select warm roosts to help them avoid torpor. Torpor may also slow healing rates, which could have implications for bats that survive the winter with white-nose syndrome (WNS), a devastating fungal skin disease impacting hibernating North American bats. WNS survivors must emerge from hibernation and initiate reproduction while also healing from extensive wing damage caused by the disease. Warm roosting habitat could help WNS survivors avoid torpor to heal and reproduce more quickly, enhancing population recoveries. However, reduced torpor expression and increased activity and exploration could increase the chance of bats acquiring pathogens and parasites from substrates in their environment. I tested the hypotheses that warm roosting habitat: 1) reduces use of torpor by endangered little brown bats (*Myotis lucifugus*); but 2) increases the risk of pathogen acquisition from substrates in the environment. I captured bats from a fall swarm and housed individuals in outdoor flight enclosures equipped with either four heated or four unheated roost boxes. I quantified torpor expression using skin temperature dataloggers and used ultraviolet (UV) fluorescent

powder as a proxy pathogen which I applied to one of the four roost boxes in each tent. Bats provided with warm roosts used less torpor ($p<0.0001$), but the amount of time a bat spent in torpor ($p=0.26$), had no effect on intensity of infection with the proxy pathogen. My data highlight roost temperature as a driver of torpor expression in little brown bats but suggest that heated roosts will not speed rates of pathogen or parasite acquisition from the environment. This result supports the potential of enhancement of summer roosting habitat as a management strategy for WNS.

Introduction

Habitat quality can affect survival and reproductive fitness of wildlife (Nagar & Noordwijk 1992, Bryan & Bryant 1999). Consequently, habitat protection and enhancement have helped conserve declining or endangered species and populations (Knaepkens *et al.* 2004, Merz & Setka 2004, Savard & Roberts 2007, Kapust *et al.* 2012). Habitat enhancement involves modifying characteristics of a habitat to improve conservation outcomes for an individual species or overall biodiversity (Weller 1989). Improved population stability and recovery, the goal of habitat enhancement, can be achieved via direct effects of high quality habitat on individual growth rates (Pérez *et al.* 2008), body size and condition (Pérez *et al.* 2008, Ardia *et al.* 2009), and reproductive rates (Knaepkens *et al.* 2004, Merz & Setka 2004, Savard & Roberts 2007, Kapust *et al.* 2012). For example, Pérez *et al.* (2008) found that artificially heating the nest boxes of tree swallows (*Tachycineta bicolor*) improved body condition and feeding rates of incubating females. Nestlings in heated boxes also had improved body condition compared to those in unheated boxes. Increased nest temperatures may have reduced

energetic costs of thermoregulation for females, enabling them to invest more in incubation and provision of offspring (Bryan & Bryant 1999).

Roosting habitat enhancement could be an important conservation strategy for temperate-zone bats. Hibernating bats in North America are experiencing drastic population declines due to an invasive fungal pathogen, *Pseudogymnoascus destructans* and the resulting disease, white-nose syndrome (WNS) (Frick *et al.* 2010, Frick *et al.* 2015). Three species are now listed as endangered in Canada and one as threatened in the U.S. because of WNS (Government of Canada 2016). *P. destructans* infects the skin of hibernating bats during the winter causing increased evaporative water loss and metabolic rate during torpor and, ultimately, an increase in the frequency of arousals from torpor during hibernation, resulting in a rapid depletion of winter fat stores and, potentially starvation (Reeder, *et al.* 2012; Warnecke, *et al.* 2012, McGuire *et al*. 2017). The fungus is spread via direct contact with infected individuals or through contact with infected substrates. While *P. destructans* only grows at relatively cold temperatures characteristic of hibernation, conidia are thought to remain viable and transmissible within and among hibernacula, not only during winter but also during the spring maternity season and late-summer/fall "swarming" season (Ballmann *et al.* 2017). During fall swarming, before hibernation, bats visit the entrances of hibernacula in large numbers to mate promiscuously and possibly to investigate potential hibernation sites (Hall & Brenner 1968, Agosta *et al.* 2005). This behaviour results in high contact rates among bats creating potential for transmission of *P. destructans* (Dobony *et al*. 2011, Ballmann *et al*. 2017) and other contact pathogens and parasites (e.g., Webber *et al*. 2015). Bats also enter and exit hibernacula frequently during swarming, generating additional potential for

acquisition of parasites and pathogens like *P. destructans* from environmental substrates (Dobony *et al.* 2011, Langwig *et al.* 2015, Carpenter *et al.* 2016, Ballmann *et al.* 2017).

Heterothermic endotherms (i.e., mammals and birds), including bats, often use torpor to reduce metabolic rate and body temperature (T_b) and save energy when food is scarce or ambient temperatures (T_a) are low (Lyman *et al*. 1982, Humphries *et al*. 2003, Geiser 2004). The primary benefit of torpor is reduction in energy expenditure, but a range of other benefits could enhance survival and reproductive fitness (reviewed by Geiser & Brigham 2012). Arid-zone mammals and birds use torpor to reduce evaporative water loss and endure droughts. For example, stripe-faced dunnarts (*Sminthopsis macroura*) reduce evaporative water loss by 23 to 42% by entering torpor (Cooper *et al.* 2005). During pregnancy, torpor can delay parturition until favourable conditions for lactation (Racey & Swift 1981, Willis *et al.* 2006). Torpor may also help some heterotherms avoid predation. Stawski and Geiser (2010) found that Australian northern long-eared bats (*Nyctophilus bifax*) with larger fat reserves used longer and deeper torpor bouts than those with smaller reserves, suggesting that bats in good condition used torpor to reduce foraging requirements and, therefore, risk of predation. This finding suggests that reduced activity enabled by use of torpor could also reduce the risk of encountering and acquiring pathogens and parasites from the environment although, so far, this hypothesis has not been tested experimentally.

Despite its benefits, torpor is also associated with costs. The expression of torpor in free-living animals reflects an optimization balancing these costs and benefits (Humphries *et al.* 2003, Jonasson & Willis 2011, Czenze *et al.* 2017). Torpor slows gestation and inhibits lactation for reproductive females and inhibits sperm production for

males, so reproductive individuals tend to avoid torpor compared to non-reproductive conspecifics (Jagiello *et al.* 1992, Geiser 1996, Lausen & Barclay 2003, Dzal & Brigham 2013). Torpor may slow the last stages of juvenile development so young-of-the-year (YOY) animals might be expected to avoid torpor if possible. Torpor can also inhibit wound healing and tissue repair (Faure *et al.* 2009, Cryan *et al.* 2013). Like reproductive individuals and YOY, heterotherms recovering from injuries, or bats recovering from WNS in spring, may also benefit from remaining normothermic.

Torpor avoidance appears to influence roost and nest selection decisions of heterothermic endotherms (Holloway & Malcolm 2007, Coombs *et al.* 2010) presumably to help individuals reduce thermoregulatory energy costs. For instance, rodents select nesting sites like subterranean burrows that buffer against the surface environment (Begall *et al.* 2007), and flying squirrels select well-insulated tree cavities (Holloway & Malcolm 2007). In addition to helping individuals avoid torpor, warm microclimates can also speed growth rates of offspring (Tuttle 1976, McCarty & Winkler 1999). Benefits of warm roosts or nests for torpor avoidance and reproduction suggest that enhancements to roosting or nesting habitat, which reduce normothermic thermoregulatory costs during the reproductive season could be important for the conservation of heterothermic endotherms. This reduction in costs could be especially important for the few bats that survive the winter with WNS. Some WNS-affected individuals survive the disease and emerge from hibernation in spring (Maslo *et al.* 2015). However, these survivors mount an intense, energetically-expensive and, likely, maladaptive inflammatory response which causes immune-mediated tissue damage (Meteyer *et al.* 2012). They must, then, spend energy to heal tissues damaged by WNS and this subsequent immune response (Fuller *et*

al. 2011). For females, spring is also normally the time of reproductive investment but if fat reserves must be re-allocated to WNS recovery, this investment could negatively impact reproduction by survivors. Providing bats with relatively warm roosts that help them avoid torpor could facilitate faster healing and help bats reproduce earlier in summer, improving the potential for juveniles to survive their first winter (Wilcox & Willis 2016). By facilitating reproduction of WNS-survivors, roosting-habitat enhancement could also facilitate so-called 'evolutionary rescue', the evolution of WNS-resistance or tolerance traits in endangered bat populations (Willis & Wilcox 2014, Maslo *et al.* 2015, Maslo & Fefferman 2015).

Consistent with the habitat enhancement hypothesis, Wilcox & Willis (2016) used a captive colony of little brown bats to show that bats emerging from hibernation were significantly more likely to select an artificially heated bat house in their flight enclosure over an unheated control bat house. Moreover, bats recovering from WNS showed a dramatically stronger preference for the heated roost compared to healthy individuals. This preference suggests the possibility that artificially-heated roosts, as well as natural roosts that provide warm microclimates, could be beneficial for WNS survivors. On the other hand, one potential risk of roosting habitat enhancement could be increased risk of contracting parasites and pathogens, including *P. destructans*, if warm roosts reduce torpor expression and increase overall activity and exploration of the environment. Torpor is thought to reduce risk of predation for bats (Stawski & Geiser 2010) and could also reduce the chance of encountering and contracting pathogens and parasites from substrates in the environment.

I tested two hypotheses important for understanding the potential of roosting

habitat enhancement as a management tool for WNS. First, I tested whether warm roosting habitat reduces the expression of torpor by bats captured during the fall swarming season. I predicted that bats provided with heated roost boxes would use less torpor during the active period at night than bats provided with un-heated, control roost boxes because of improved potential to balance their energy budget. Second, I tested whether reduced reliance on torpor increases the risk of acquiring contact pathogens or parasites from substrates in their environment, presumably because of increased overall activity. I predicted that bats provided with heated roosts would exhibit higher prevalence and intensity of infection with a proxy pathogen (i.e., ultraviolet (UV) fluorescent powder) applied to substrates they would be likely to explore (i.e., potential roost sites) in their environment. Evidence that enhanced, warm roosting habitat can reduce torpor expression without increasing infection risk would support application of this management technique for imperiled populations and/or species of bats.

Methods

All procedures were conducted under Manitoba Conservation Wildlife Scientific Permit number WB16368 and approved by the University of Winnipeg Animal Care Committee. The nearest confirmed detection of *P. destructans* was >200 km from my study site at the time of my study (northern Minnesota, www.whitenosesyndrome.org 2016) but I still followed U.S. Fish and Wildlife Service decontamination protocols for researchers to control the spread of *P. destructans* (www.whitenosesyndrome.org, 2016).

I conducted 9 three-night trials between 17 August and 10 September 2016. On the first night of each trial I used a harp trap to capture 1 – 2 bats per night outside of St.

George Bat Cave near Fisher River First Nation, Manitoba, Canada (14U 613884 5700551). Since 2008, bats at this site have been marked with Passive Integrated Transponders (PIT tags) as part of a mark-recapture study and I used a handheld PIT tag scanner (EID-LID 573: Handheld reader, Electronic Identification Systems Inc., Brampton, ON, Canada) to identify recaptured bats and eliminate the chance of re-using bats for multiple trials in my experiment. Bats were then placed in cloth bags and within 2 h of capture were transported 13 km, by car, to a field laboratory near Lake St. George.

Once at the field laboratory, I sexed each bat and assessed reproductive status of females as "lactating" if nipples were bare and/or I could express milk, or "non-reproductive" if the hair had grown back around the nipples. I aged bats as adults (>1 year) or YOY (<1 year) based on the degree of ossification in the metacarpal-phalangeal joints of the fourth digit of the wing (Kunz & Anthony 1982). I measured length of the right forearm (± 0.1 mm) using digital calipers (100-300-8 EZ Cal, iGaging Precision Instruments, San Clemente, CA, USA) and mass (±0.1 g) using an electronic balance (FP 1000, Fuzion Diablo, Barcelona, Spain).

To quantify torpor expression, I recorded each bat's skin temperature (T_{sk}) using small temperature dataloggers (0.5°C resolution, iButtons, DS1922L, Maxim Integrated Products Inc., Dallas, TX, USA) modified to reduce their mass following Reeder, *et al*. (2012). Final miniaturized iButtons weighed ~1g. I programmed iButtons to record T_{sk} every 5 min and attached iButtons using non-toxic latex-based adhesive (294-Osto-bond, Montreal, Canada) to the dorsum between the scapulae. I did not trim the fur before attaching iButtons. This process likely increased ambient cooling of dataloggers relative to recording T_{sk} (see results) but, in my experience, skin attachment makes datalogger

removal much more difficult and would have increased stress for the animals. To remove iButtons at the end of each trial, I simply cut the fur between the glue and the skin, leaving a patch of slightly shorter fur. After processing, I placed bats in a 20 by 21 by 24 cm stainless steel mesh cage with *ad libitum* water and felt cloth to hang on until dusk the next day when their experimental trial began.

Beginning at sunset on the day following capture, I randomly assigned each bat to one of two outdoor flight enclosures (length: 4.3m, width: 4.3m, height: 2.0 m; Model #076-5460-2, Outbound Screen House, Outbound, Toronto, Canada). Each enclosure was outfitted with four custom-built single-chamber roost boxes (33 cm tall by 30 cm wide by 3 cm deep). Roosts were insulated with 3 cm thick Styrofoam insulation (Model # 547658, CodeBord Extruded Polystyrene Rigid Insulation, 3 cm thick, Foamular, Owens Corning Commercial Insulation, Toledo, OH, USA). Each roost box was open at the bottom, so bats could enter and exit freely and had a hinged front panel, allowing me to open the roost and remove bats at the end of each trial. The back of each roost was equipped with a layer of felt covered by window screening to provide footholds on which bats could hang. In one of the two enclosures (hereafter the control enclosure), roosts were not heated and roost temperature (T_{roost}) fluctuated with outside T_a. In the other enclosure (the heated enclosure), each roost box was equipped with a terrarium heating pad (Model # PT2035, Heat Wave Desert, Exo Terra, Montreal, Canada) positioned under the roosting felt/mesh. Heating pads were connected to outdoor temperature controllers (ETC-141000-000: NEMA 4X, Ranco ETC, Delphos, OH, USA) set to 32°C. I recorded T_{roost} and T_a adjacent to flight enclosures using unmodified iButtons set to record every 10 min. Heated roost boxes maintained significantly warmer T_{roost} (21.23 ± 1.79°C)

relative to control roosts (16.57 ± 1.89°C; Welch two sample T-test, t = 4.9, df =12.5, p = 0.0003, Figure 2.1)

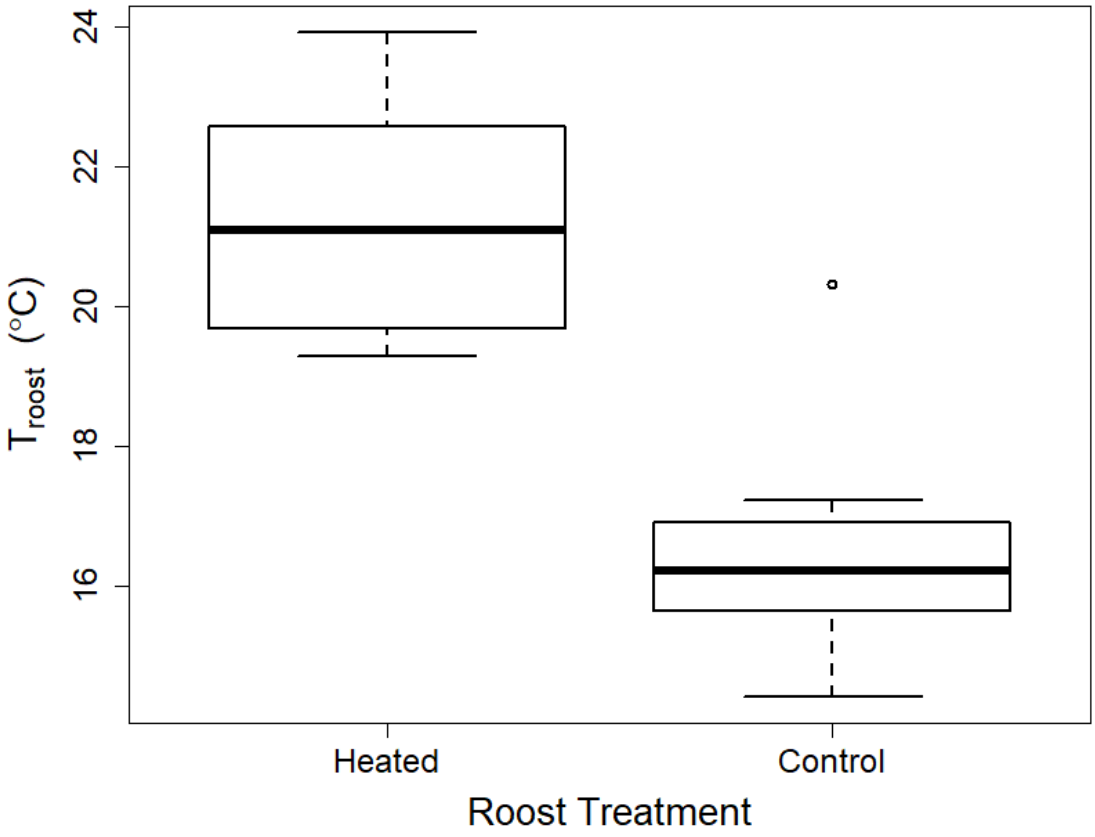

Figure 2.1: Boxplot of daily average T_{roost} in both heated and control roost boxes in heated and control flight enclosures. Centre lines represent median values, boxes represent the interquartile range, the upper whisker represents the third quartile plus 1.5 times the interquartile range, and the lower whisker represents the first quartile minus 1.5 times the interquartile range. Open circles represent outliers.

Water and mealworms, (larval *Tenebrio molitor*), gut-loaded with beta-carotene multivitamins (SRP 00300, Herptivite, CA, USA) and a nutrient supplement (Repashy Superfoods, Repashy Ventures Inc., CA, USA) were provided *ad libitum* on a small (71x51x77cm) folding table positioned in the middle of each enclosure.

Prior to introducing bats to either flight enclosure, I randomly selected one roost box in each enclosure for infection with my proxy contact pathogen, UV fluorescent powder (AX-18N, Signal Green, Day-Glo Color Corp. Cleveland, Ohio, USA). Webber (2016) found that transmission dynamics of this UV powder in this experimental setup were similar to those for real-world contact pathogens or parasites with negative binomial distributions for intensity of infection among hosts. Using a small paintbrush, I applied 100ml of UV powder to the mesh on the back panel of the selected "infected" roost. I applied the powder in a horizontal line, 2 cm wide, 30 cm long and 20 centimetres from the top of the roost. Thus, if a bat thoroughly investigated and/or chose to roost in the infected roost it would have crossed this infected zone and, in turn, become "infected" with the pathogen.

After I applied UV powder to the roosts, at dusk (range: 20:39 – 19:55) on the night of each experimental trial, I weighed each bat, hand-fed them both 10 mealworms and then released one individual into the control enclosure and the second bat into the heated enclosure. After 24 hours, I re-entered the flight enclosures, measured T_a and recorded the roost box in which each bat was found. I never observed bats outside of roost boxes during the day and, for my analyses, I assumed that bats roosted the entire day inside the roost in which I found them upon recapture. After removing each bat from the flight enclosure, I photographed the dorsal and ventral sides of its wing and tail

membranes to quantify the prevalence and intensity of infection with the proxy pathogen. I took photographs under a UV lamp (HQRP, Harrison, NJ, USA) with a digital camera (Digital Rebel XTi, Canon Inc., Oita, Japan) mounted on a tripod. I included a scaling item (i.e., U.S. penny, diameter = 19.05mm) in each photo to standardize image sizes. After photographing each bat, I weighed it and injected a uniquely coded PIT tag under the skin between the scapulae to identify recaptured individuals. I then released each bat at the capture site.

Methods for defining when endotherms are "in" or "out" of torpor have been a topic of debate among eco-physiologists (Barclay *et al.* 2001, Willis & Brigham 2003, Boyles *et al.* 2011, Brigham *et al.* 2011). One approach for comparing torpor among individuals is to calculate so-called degree-minutes of torpor expression by quantifying the area under a T_b or T_{sk} time-course below some torpor threshold (e.g., active temperature) (Barclay *et al.* 2001, Lausen & Barclay 2003). This approach has been criticized because it fails to account for Q_{10} effects (the rate of change in physiological reactions when T_b increases 10□) which result in larger energetic savings from initial, shallow reductions of T_b compared to smaller energetic savings accrued from reductions of the same magnitude at lower T_b (Willis & Brigham 2003, Brigham *et al.* 2011). However, for my study, defining the beginning and end of torpor bouts, or estimating energetic savings, was less important than quantifying overall differences in T_b among individuals and between treatments. Therefore, I used an area-under-the-curve approach, modified from Lausen and Barclay's (2003) degree-minute calculation. I first interpolated T_{sk} values for each 5 min interval between measurements by multiplying each measured T_{sk} value by five. I then summed all measured and interpolated T_{sk} values for each 24-

hour trial. In contrast to many studies which depend on some torpor threshold, (e.g., Barclay *et al.* 2001, Lausen & Barclay 2003) this calculation does not directly estimate expression of torpor but, instead, provides a relative index of the defense of high T_{sk} or, what I term, a normothermy index (NI). In other words, values of NI were higher for bats that spent less time in torpor and more time normothermic.

I used ImageJ (ImageJ v. 1.8.0_66, U.S. National Institutes of Health, Bethesda, Maryland, USA) to quantify the intensity of infection with UV powder in photographs of bats' wings. I first standardized images based on the scaling item in each photo, then outlined each wing to quantify total wing area (Fuller *et al.* 2011). I then summed the area covered with particles of UV powder and divided by total wing area. To facilitate analysis, for one bat that had no detectable UV powder on its wings, I replaced its value of zero with the lowest intensity value I determined for all other bats divided by 2, assuming that this lowest value represented my minimum limit of detection (Croghan & Egehy 2003). Intensity of infection fit a negative binomial distribution, so I used a logit transformation to achieve normality (see results). I then assessed the effect of degree minutes on infection intensity (Webber 2016).

I used R (version 3.2.3, GUI 1.51, R Development Core Team 2015) for all statistical analysis. To test whether bats in the heated enclosure were less likely to use torpor, I used a linear mixed effects model with NI as a response variable, daily average T_{roost} as a fixed effect, and trial number as a random effect to account for potential pseudo-replication of data across trial days. Neither sex nor mass was included in my models. Sex and body mass could affect torpor expression (Chapter 3) but, because of my sample size (n=15), I would not have had the statistical power to detect effects of sex or

mass. To test whether torpor expression influenced pathogen acquisition, I used a linear mixed effects model with intensity of infection (i.e., logit-transformed proportion of wing covered with UV powder) as the response variable, NI as a fixed effect and, again, trial day as a random effect. I examined residual plots to ensure all models met standard assumptions. I assessed significance at the α=0.05 level and all values are reported as the mean ± SD unless otherwise specified.

Results

Ambient temperature ranged from 10.0°C to 35.5°C throughout the study with average daily maxima of 24.1±6.2°C and minima of 13.8±1.8°C. Sunrise times ranged from 05:26 to 06:01, and sunset times from 19:03 and 19:51 (National Research Council Canada 2016). I ran trials on 15 individuals (n = 9 female, 6 male, Table 2.1). My T_{sk} measurements underestimated normothermic T_b because I did not trim the fur underneath dataloggers, but I was still able to record clear torpor/arousal cycles (Figure 2.2). T_{fur} was typically relatively low during the active phase at night but often increased rapidly when bats entered heated roosts in the morning (Figure 2.2).

I found a significant positive effect of average T_{roost} on NI (r^2=0.81, Table 2.2, Figure 2.3). Based on calculated parameter estimates, the model predicted a 603 degree-minute increase in NI for each 1°C increase in daily average T_{roost}. The NI incorporates both depth and duration of torpor so that a 603 degree-minute difference could represent a range of possible responses (e.g., an increase of T_b by 10°C for 60 minutes, 2°C for 300 minutes, etc.). However, the ultimate result was that higher values of T_{roost} were associated with higher values of T_{sk}.

Table 2.1: Summary of morphometric data and raw data for each animal used in analysis of torpor expression and infection intensity. *Original value of zero was assumed to be below the limit of detection so was changed to the lowest detection divided by 2 (see methods).

Trial	Treatment	Sex	Mass (g)	Intensity of Infection	Average T_{roost}	Average T_a	NI
1	Heated	Female	8.9	0.0061	23.9	20.7	44097.26
1	Control	Female	9.4	0.0002*	20.3	20.7	41915.47
2	Heated	Female	9.7	0.0946	21.4	17	44057.62
2	Control	Male	8.5	0.0041	16.6	17	39277.00
3	Heated	Female	9.4	0.0132	19.6	15.1	42191.63
3	Control	Male	11.3	0.0008	14.4	15.1	39859.21
4	Control	Female	8.5	0.0003	17.2	17.8	40240.57
5	Heated	Female	9	0.0119	23.8	18.8	44164.20
6	Heated	Female	8.4	0.0006	21.1	16	43787.62
6	Control	Male	8.6	0.0304	15.2	16	39768.35
7	Heated	Male	8.7	0.0232	21.1	17	43029.67
7	Control	Female	8	0.0026	16	17	39505.16
8	Heated	Male	12.1	0.0028	19.7	15.8	43214.98
8	Control	Male	12.2	0.0007	16.2	15.8	39467.08
9	Heated	Female	11.8	0.0036	19.3	13.5	43975.31

Table 2.2: Summary of linear mixed effects model assessing effects of average T_{roost} (with trial as a random effect, $p<0.0001$) on normothermy index of 15 little brown bats (n=6 male, 9 female) over 9 24-hour trials.

Variable	Coefficient	Standard Error	t-value	Degrees of Freedom	p-value
Average T_{roost}	603	78.9	7.64	5	<0.0001

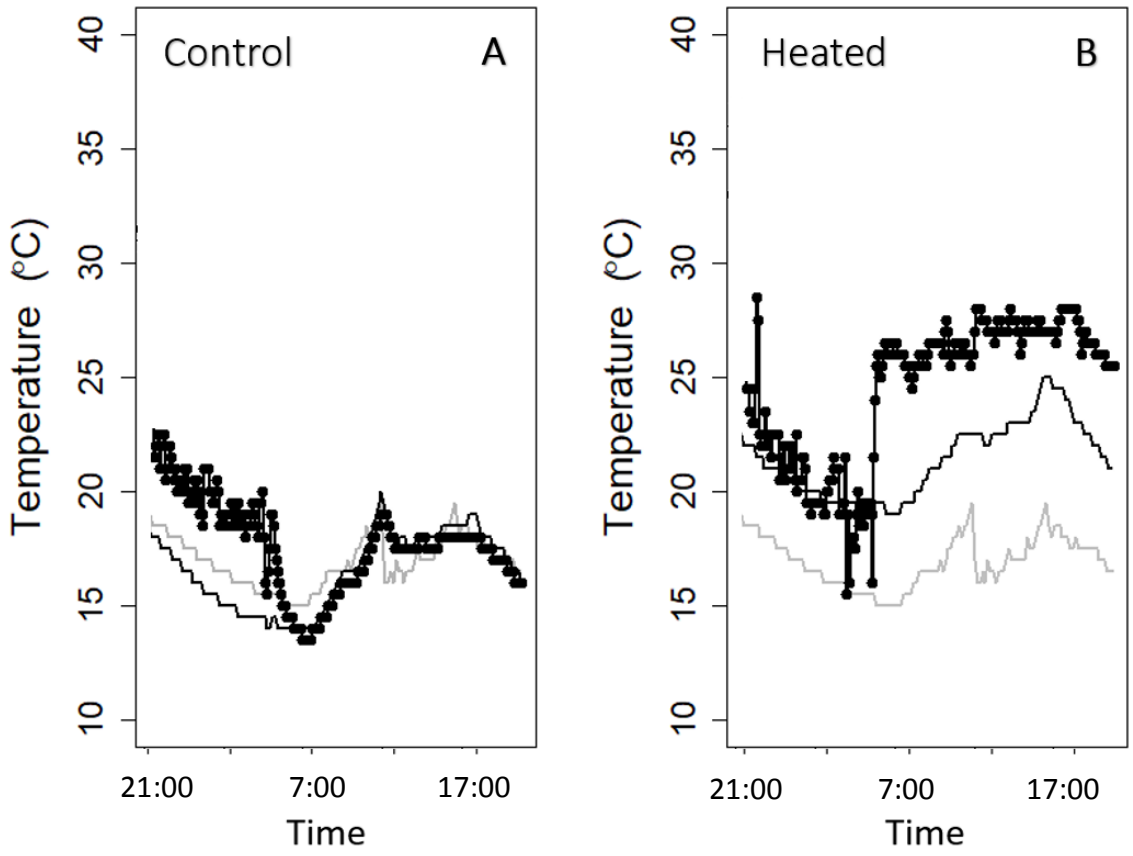

Figure 2.2 Representative time courses of T_{sk} (°C) (circles), T_{roost} (°C) (black solid line), and T_a (°C) (gray solid line) for **(A)** one bat in the control enclosure and **(B)** one bat in the heated enclosure during one of my 24-hour trials on 18-19 August 2016.

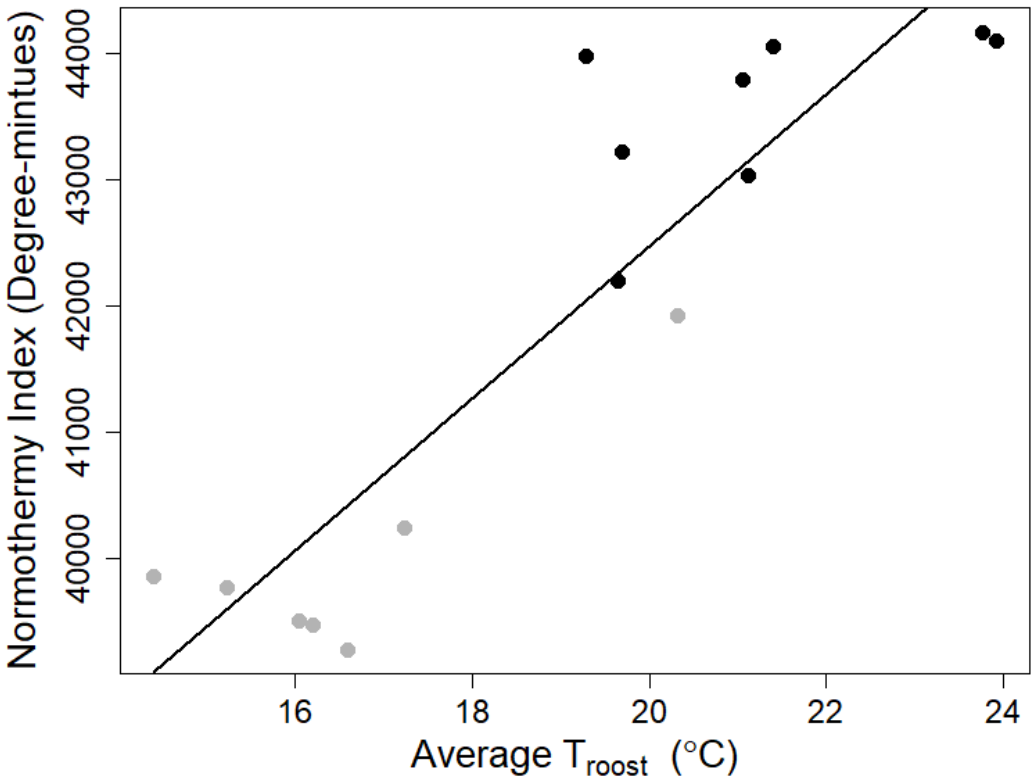

Figure 2.3: Scatter plot of the relationship between daily average T_{roost} (°C) and normothermy index for 15 little brown bats in heated (black circles) and control (gray circles) flight enclosures. Data points are coloured by T_{roost} treatments for illustration but heated vs. control treatment was not included in the model. A linear regression line is also included for illustration although data were analysed using a mixed effects model (see results).

Intensity of infection fit a negative binomial distribution (Figure 2.4A) but I achieved normality using a logit transformation (Shapiro-Wilk normality test, W= 0.97, p=0.92, Figure 2B). Intensity of infection averaged 1.50 ± 2.43% wing coverage and ranged from 9.46% to 0.03% coverage with one individual exhibiting no infection. I found no effect of individuals' NI values on logit-transformed intensity of infection (r^2=0.10, Table 2.3, Figure 2.5).

Table 2.3: Summary of linear mixed effects model assessing effects of normothermy index (with trial as a random effect, p=0.0001) on pathogen acquisition of 15 little brown bats (n=6 male, 9 female) over 9 24-hour trials.

Variable	Coefficient	Standard Error	t-value	Degrees of Freedom	p-value
NI	0.0003	0.0002	1.33	5	0.24

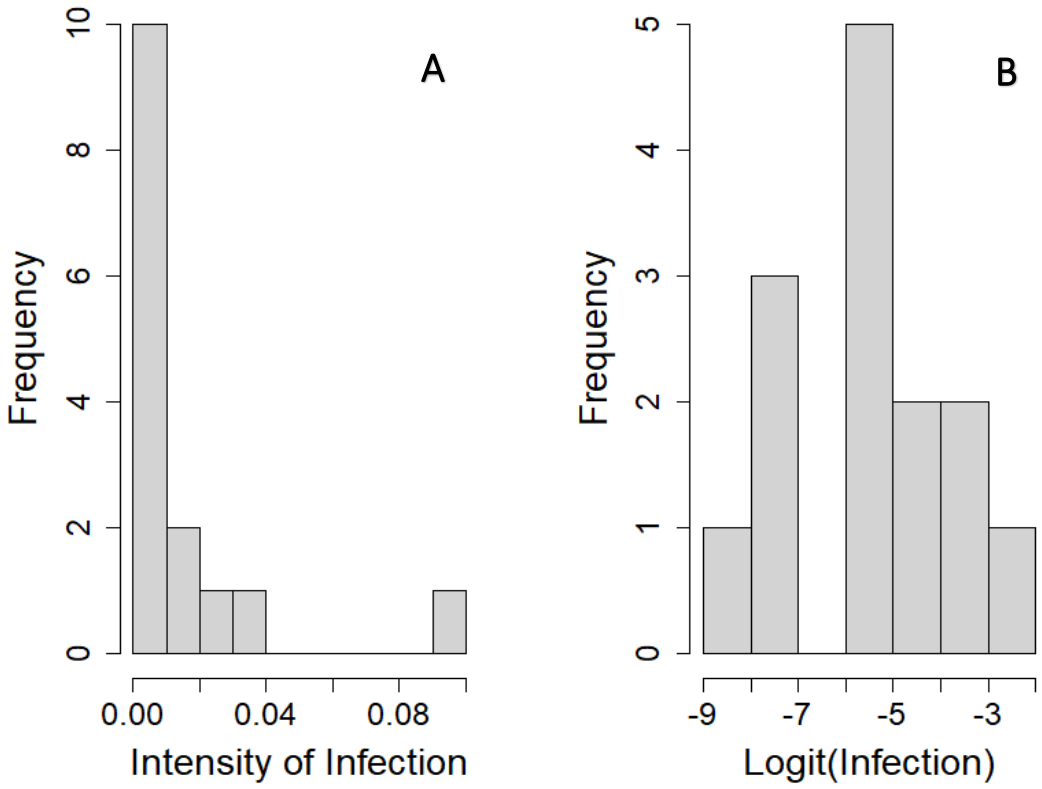

Figure 2.4: Histograms of **(A)** un-transformed intensity of infection (i.e., proportion of the wing covered with UV powder), and **(B)** logit-transformed intensity of infection for 15 little brown bats during environmental-substrate transmission infection experiment.

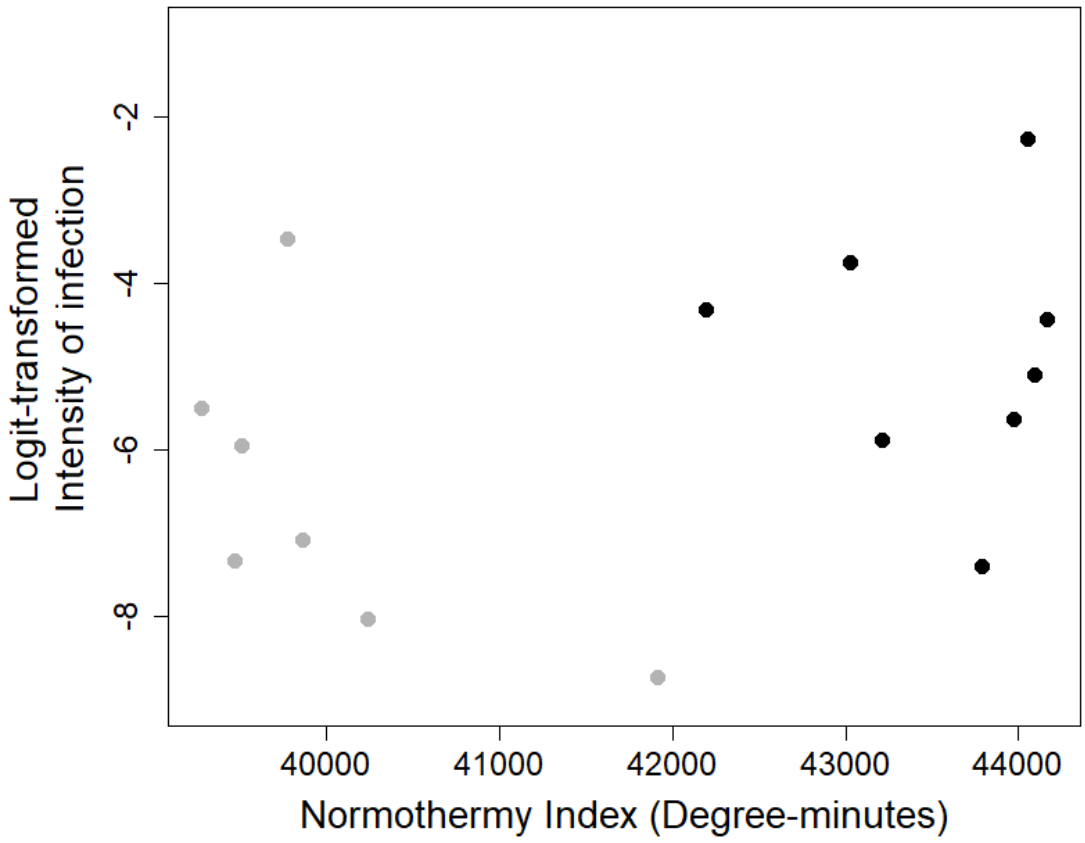

Figure 2.5: Scatter plot of the relationship between normothermy index and intensity of infection (proportion of wing covered by proxy pathogen) for 15 little brown bats in heated (black circles) and control (gray circles) flight enclosures. Data points are coloured by T_{roost} treatments for illustration but heated vs. control treatment was not included in the model (see results).

Discussion

I found support for my first hypothesis that T_{roost} influences torpor expression by little brown bats. Bats provided with warmer roosts maintained higher values of T_{sk} indicative of reduced torpor expression. Despite a pronounced effect of T_{roost} on use of torpor, however, I found no support for my second hypothesis that a reduction in torpor use increases the risk of acquiring contact pathogens or parasites from substrates in the environment. These results lend support to the potential of roosting habitat enhancement as a management tool for imperiled or endangered populations of temperate-zone bats.

White-nose syndrome causes increased torpid energy expenditure and frequent arousals in hibernating bats, leading to premature depletion of fat stores, and presumably starvation (Warnecke *et al.* 2012, Verant *et al.* 2014). Bats that survive the winter emerge from hibernation with severely reduced energy reserves and must begin the energetically-expensive task of healing while food is still scarce, and T_a is still cold (Wilcox & Willis 2016). Warm T_b is known to accelerate wound healing in heterotherms (e.g. Anderson & Roberts 1975, Faure *et al.* 2009) and bats healing from skin damage caused by WNS may select warm roost microclimates to increase healing rates while reducing energetic costs of thermoregulation (Wilcox & Willis 2016). Wilcox and Willis (2016) gave bats recovering from WNS access to both heated and unheated roost boxes, and the infected bats virtually always selected the artificially heated roost. Although bats in my study were all negative for WNS, my findings build on those of Wilcox and Willis (2016) by demonstrating that bats provided with relatively warm roosts reduce torpor expression which could be beneficial for individuals recovering from WNS. If bats can defend higher T_b at reduced energetic costs, this strategy could help them allocate more energy to

healing and/or reproduction. This benefit, in turn, could be important for helping foster evolutionary rescue of populations impacted by WNS. Bats that survive the winter with the disease may exhibit heritable behavioural and/or physiological traits that favoured their survival. Offspring born to those potentially resistant or tolerant individuals could inherit those favourable traits and help foster an evolutionary response to the disease (Gonzales *et al.* 2013, Maslo & Fefferman 2015, Maslo *et al.* 2015, Wilcox & Willis 2016). This result suggests that protecting existing summer habitats that provide warm roost microclimates, and potentially enhancing roosting habitats via artificially heating or other means to increase T_{roost}, could help support evolutionary rescue and population recovery.

One potential downside of roosting habitat enhancement is the potential to increase activity and exploration of the environment by bats and, therefore increase risk of encountering and contact pathogens and parasites from environmental reservoirs. Like Webber (2016), I found that transmission dynamics of UV fluorescent powder introduced to roosting substrates in a flight enclosure reliably mimicked patterns of acquisition for naturally occurring pathogens and parasites. Intensity of infection typically shows a negative binomial distribution for many pathogens and parasites (e.g., Shaw *et al.* 1998) and this pattern matches what Webber (2016) and I observed for UV powder. Despite this predicted pattern of infection, however, I found no evidence that warm roosts and/or reduced torpor expression, increased risk of infection for individuals. Although more work is needed, the fact that bats, especially individuals recovering from WNS, prefer artificially heated roosts (Wilcox & Willis 2016), that bats with heated roosts maintain higher T_b and express less torpor, and that reduced torpor expression does not appear to

increase risk of pathogen acquisition from environmental substrates, lend support to the habitat enhancement hypothesis that warm roosting microclimates could help bat populations recover from WNS and other threats.

Although my results support habitat enhancement as a management tool, there are limitations to my experiment that should be addressed before this management approach is implemented on a wide scale. For one, my study took place over one-month during the late-summer, early-fall swarming period so results may not apply to other times of year (e.g., spring WNS recovery and reproduction). Although large enough to detect large effects of heated roosts on torpor expression, my sample size was relatively small because there was only a very limited time window during the fall swarm period, during which I could conduct my experiment. This could have limited my ability to detect effects of torpor on pathogen transmission. According to my power analysis, my sample size would have had to be larger (37 bats, instead of my 15) in order to detect even a large effect of NI on pathogen acquisition. Based on an a priori power analysis, I recommend that future studies aim for sample sizes of 40-50 individuals in order to detect large effects, biologically meaningful effects if they exist. The timescale of each trial was also relatively short. Little brown bats do not survive well in captivity for long periods during the active season so to avoid impacts on this endangered species, I limited the duration of my experimental trials. It is possible that, over a longer time period, I would have observed an effect of T_{roost} and torpor expression on risk of pathogen acquisition. The spatial scale of my trials was also relatively small compared to the very large home ranges of little brown bats in my study region (Habrich *et al.* in prep.). This small scale could have led to unusual behaviour by bats in my study that reduced potential effects of

torpor expression on pathogen acquisition. On the other hand, enclosure size could have helped counteract potential effects of short-duration trials. Although my trials were brief, bats were housed in a relatively small space, increasing the chance that they would have encountered the infected substrate in their enclosure. Another factor which clearly differed in my experiment compared to the wild was provision of *ad libitum* food and water for all bats in my experiment. For free-ranging bats, warm roosts during periods of cold T_a may help reduce thermoregulatory costs of remaining normothermic but if very little food and only warm roosts are available, the inability to enter torpor could prevent bats from balancing their energy budgets. Support for the habitat enhancement hypothesis from this study and others (e.g., Wilcox & Willis 2016) suggest that similar experiments should be attempted on larger spatial and longer temporal scales with free-ranging bats.

Artificial heating of roost boxes is one habitat enhancement option for some bat species (e.g., little brown bats) which regularly use anthropogenic structures. However, other endangered bat species rarely use these kinds of structures (e.g., northern long-eared bats, *M. septentrionalis*). For these species, identifying, protecting and enhancing habitat features which naturally increase T_{roost} could be useful in terms of management. A meta-analysis conducted by Kalcounis-Ruppell *et al.* (2005) found that bats roosted in tall trees with greater diameter at breast height in stands with open canopy and high snag density. Features such as southern facing slopes and mixed age stands with abundant emergent trees already excavated may also provide warm microclimates. Brandenbark™ (Copperhead Environmental Consulting, Paint Lick, KY, USA), a new form of artificial roost made from polyurethane elastomeric Flex-Bark (Replications Unlimited, Hazelwood, MO, USA), may provide a more stable microclimate than natural roosts.

Brandenbark roosts have already been shown to buffer against cool T_a and their use has been confirmed for northern-long eared bats and Indiana bats (*M. sodalis*; Adams *et al.* 2015). The preservation and enhancement of high quality natural summer roosting habitat, and the creation of artificially enhanced habitat, could potentially be important management approaches for protection and conservation of bat species at risk.

I found that T_{roost} affects torpor expression of little brown bats. Bats with access to warmer roosts used less torpor than bats with only unheated control roosts. By using artificially warm roosts, bats presumably used less energy maintaining normothermia and could potentially invest more energy in healing from injury or disease and/or reproduction. This finding could, in turn help WNS-impacted populations recover and evolve resistance to, or tolerance of, WNS. While bats in warmer roosts used torpor less, they were not at greater risk of acquiring a contact pathogen from substrates in their environment compared to bats without access to warm roosts. Taken together, these results suggest that artificially heating bat houses and identifying, protecting and enhancing features of natural roosts which increase T_{roost}, could be an effective management approach for bat populations impacted by WNS and other threats.

References

Adams, J. Roby, P., Sewell, P., Schwierjohann, J., Gumbert, M., & Brandenburg, M. 2015 Success of Brandenbark, an artificial roost structure designed for use by Indiana Bats (*Myotis 57hermos57*). *Journal of the American Society of Mining and Reclamation* **4,** 1-15.

Agosta, S.J., Morton, D., Marsh, B.D., & Kuhn, K.M. 2005 Nightly, seasonal, and early patterns of bat activity at night roosts in the central Appalachians. *Journal of Mammalogy* **86,** 1210-1219.

Anderson, C.D., & Roberts, R.J. 1975 A comparison of the effects of temperature on wound healing in a tropical and temperate teleost. *Journal of Fish Biology.* **7,** 173-182.

Ardia, D.R., Pérez, J.H., Chad, E.K., Voss, M.A., & Clotfelter, E.D. 2009 Temperature and life history: experimental heating leads female tree swallows to modulate egg temperature and incubation behaviour. *Journal of Animal Ecology* **78,** 4-13.

Ballmann, A.E., Torkelson, M.R., Bohuski, E.A., Russell, R.E., & Blehert, D.S. Dispersal hazards of *Pseudogymnoascus destructans* by bats and human activity at hibernacula in summer. *Journal of Wildlife Diseases* **53,** 725-735.

Barclay, R.M.R., Lausen, C.L., & Hollis, L. 2001 What's hot and what's not: defining torpor in free-ranging birds and mammals. *Canadian Journal of Zoology.* **79,** 1885-1890.

Bondarenco, A., Körtner, & G., Geiser, F. 2013 Some like it cold: summer torpor by freetail bats in the Australian arid zone. *Journal of comparative physiology*. **183,** 1113-22.

Boyles, J.G., Smit, B., & McKechnie, A.E. 2011 Does use of the torpor cut-off method to analyze variation in body temperature cause more problems than it solves? *Journal of Thermal Biology.* **36,** 373-379.

Boyles, J.G., & Willis, C.K.R. 2010 Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white-nose syndrome? *Frontiers in Ecology and the Environment.* **8,** 92-98.

Brigham, R.M., Willis, C.K.R., Geiser, F., & Mzilikazi, N. 2011 Baby in the bathwater: Should we abandon the use of body temperature thresholds to quantify expression of torpor? *Journal of Thermal Biology.* **36,** 376-379.

Bryan, S.M., & Bryant, D.M. 1999 Heating nest-boxes reveals an energetic constraint on incubation behaviour in great tits, *Parus major*. *Proceedings of the Royal Society of London B.* **266,** 157-162.

Carpenter, G.M., Wilcox, E.V. Bernard, R.F., & Stiver, W.H., 2016 Detection of *Pseudogymnoascus destructans* on free-flying male bat captured during summer in the southeastern USA. *Journal of Wildlife Disease* **52,** 922-926.

Chruszvz, B.J., & Barclay, R.M.R. 2002 Thermoregulatory ecology of a solitary bat, *Myotis evotis*, roosting in rock crevices. *Functional ecology.* **16,** 18-26.

Cohen, J. 1988 Statistical power analysis. *Current directions in psychological science.* **1,** 98-101.

Cooper, C.E., McAllan, B.M., & Geiser, F. 2005 Effect of torpor on the water economy of an arid-zone marsupial, the stripe-faced dunnart (*Sminthopsis macroura*). *Journal of Comparative Physiology B* **175,** 323-328.

Csada, R.D., & Brigham, R.M. 1994 Reproduction constrains the use of daily torpor by free-ranging common poorwills (*Phalaenoptilus nuttalllii*) (Aves: Caprimulgidae). *Journal of Zoology, London.* **234,** 209-216.

Croghan, C. & Egeghy, P. 2003 Methods of dealing with values below the limit of detection using SAS. *Southern SAS User Group*

Cryan, P.M., Meteyer, C.U., Boyles, J.G., & Blehert, D.S. 2013 White-nose syndrome in bats: illuminating the darkness. *BMC Biology* **11,** 2-5.

Czenze, Z.J., Jonasson, K.A., & Willis, C.K.R. 2017 Thrifty females, frisky males: winter energetics of hibernating bats from a cold climate. *Physiological and Biochemical Zoology* **90,** 502-511.

Dobony, C.A., Hicks, A.C., Langwig, K.E., Von Linden, R.I., Okoniewski, J.C., & Rainbolt, R.E. 2011 Little brown myotis persist despite exposure to white-nose syndrome. *Journal of Fishery and Wildlife Management* **2,** 190-195.

Dzal, Y.A. & Brigham, R.M. 2013 The tradeoff between torpor use and reproduction in little brown bats (*Myotis lucifugus*). *Journal of Comparative Physiology B.* **183,** 279-288.

Faure, P.A., Re, D.E., & Clare, E.L. 2009 Wound healing in the flight membranes of big brown bats. *Journal of Mammalogy* **90,** 1148-1156.

Fuller, N.W., Reichard, J.D., Nabhan, M.L., Fellows, S.R., Pepin, L.C., & Kunz, T.H. 2011 Free-ranging little brown myotis (*Myotis lucifugus*) heal from wing damage associated with white-nose syndrome. *EcoHealth.* **8,** 154-162.

Geiser, F. 1996 Daily torpor and energy savings in a subtropical blossom-bat *Syconycteris australis* (Megachiroptera). *Adaptations to the cold: tenth international*

hibernation meeting.

Geiser, F., & Masters, P. 1994 Torpor in relation to reproduction in the Mulgara, *Dasycercus cristicauda* (Dasyuridae: Marsupialia). *Journal of Thermal Biology.* **19,** 33-40.

Geiser, F. 2004 Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual review of physiology* **66**, 239-274.

Geiser, F. & Brigham, M. 2012 The other functions of torpor. *Living in a Seasonal World.* Springer-Verlag Berlin Heidelberg

Giroud, S., Turbill, C., & Ruf, T. 2012 Torpor use and body mass gain during pre-hibernation in late-born juvenile garden dormice exposed to food shortage. *Living in a Seasonal World.* Springer-Verlag Berlin Heidelberg.

Gonzalez, A. Ronce, O., Ferriere, R., & Hochber, M.E. 2013 Evolutionary rescue: an emerging focus at the intersection between ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Science* **368,** 20120404.

Government of Canada. 2016. SARA Public Registry. January 15. http://www.registrelep-sararegistry.gc.ca.

Habrich, A., Moore, T., & Willis, C.K.R. *In prep*. Home range size in reproductive female little brown bats.

Hall, J.S., & Brenner, F.J. 1968 Summer netting of bats at a cave in Pennsylvania. *Journal of Mammalogy* **49,** 779-781.

Humphries, H.H., Thomas, D.W., & Kramer, D.L. 2003 The role of energy availability in Mammalian Hibernation: A cost-benefit approach. University of Chicago Press. Chicago, USA

Jonasson, K.A., & Willis, C.K.R. 2011 Changes in body condition of hibernating bats support the thrifty female hypothesis and predict consequences for populations with white-nose syndrome. *PloS ONE* **6,** e21061 (DOI: 10.1371/journal.pone.0021061)

Kalcounis-Ruppell, M.C., Psylakis, J.M., & Brigham, R.M. 2005 Tree roost selection by bats: an empirical synthesis using meta-analysis. *Wildlife Society Bulletin* **33,** 1123-1132.

Kapust, H.Q.W., McAllister, K.R., & Hayes, M.P. 2012 Oregon spotted frog (*Rana pretiosa*) response to enhancement of oviposition habitat degraded by invasive reed canary grass (*Phalaris arundinacea*). *Herpetological Conservation and Biology* **7,** 358-366.

Knaepkens, G., Bruyndoncx, L., Coeck, J. & Eens, M. 2004 Spawning habitat enhancement in the European bullhead (*Cottus gobio*), an endangered freshwater fish in degraded lowland rivers. *Biodiversity and Conservation* **13,** 2443-2452.

Kunz, T.H. & Anthony, E.L.P. 1982 Age estimation and post-natal growth in the bat *Myotis lucifugus*. *Journal of Mammalogy* **63,** 23-32.

Langwig, K.E., Frick, W.F., Reynolds, R., Parise, K.L, Drees, K.P., Hoyt, J.R., Cheng, T.L., Kunz, T.H., Foster, J.T., & Kilpatrick, A.M. 2015 Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proceedings of Biological Science* **282,** 201442335.

Lausen, C.L., & Barclay, R.M.R. 2003 Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. *Journal of Zoology*. **260,** 235-244.

Lewis, S.E., 1993 Effect of climatic variation on reproduction by pallid bats (*Antrozous pallidus*). *Canadian Journal of Zoology* **71,** 1429-1433.

Lovegrove, B.G., 2009 Modification and miniaturization of Thermochron iButtons for surgical implantation into small animals. *Journal of Comparative Physiology B* **179,** 451-458.

Lyman, C.P., Willis, J.S., Malan, A. & Want, L.C.H. 1982 Hibernation and Torpor in mammals and birds. New York: Academic Press.

Maslo, B. & Fefferman, N.H. 2015 A case study of bats and white-nose syndrome demonstrating how to model population viability with evolutionary effects. *Conservation Biology* **29,** 1176-1185.

Maslo, B., Valent, M., Gumbs, J.F., & Frick, W.F. 2015 Conservation implications of ameliorating survival of little brown bats with white-nose syndrome. *Ecological Applications.* **25,** 1832-1840.

McCarty, J.P., & Winkler, D.W. 1999 Relative importance of environmental variables in determining the growth of nestling tree swallows *Tachycineta bicolor*. *Ibis.* **141,** 286-296.

McGuire, L.P., Mayberry, H.W., & Willis, C.K.R. 2017 White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. *American Journal of Physiology* **313,** R680-R686.

McKechnie, A.E., & Lovegrove, B.G. 2001 Thermoregulation and the energetic significance of clustering behavior in the white-backed mousebird (*Colius colius*). *Physiological and Biochemical Zoology* **74,** 238-249.

McLean, J.A., & Speakman, J.R. 1999 Energy budgets of lactating and non-reproductive brown long-eared bats (*Plecotus 61hermos*) suggest females use compensation in lactation. *Functional Ecology.* **13,** 360-372.

Meteyer, C.U., Barber, D., & Mandl, J.N. 2012 Pathology in euthermic bats with white nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. *Virulence* **3,** 1-6.

Merz, J.E. & Setka, J.D. 2004 Evaluation of a spawning habitat enhancement site for Chinook Salmon in a regulated California river. *North American Journal of Fisheries Management*. **24,** 397-407.

Mzilikazi, N., & Lovegrove, B.G. 2002 Reproductive activity influences thermoregulation and torpor in pouched mice, *Saccostomus campestris. Journal of Comparative Physiology.* **172,** 7-16.

Nager, R.G. & Noordwijk, A.J.V. 1992 Energetic limitation in the egg-laying period of great tits. *Proceedings of the Royal Society of London* B **249,** 259-263.

National Research Council Canada, Government of Canada, 2016. Sunrise/sunset calculator (http://app.hia-iha.nrc-cnrc.gc.ca/cgi-bin/sun-soleil.pl)

Pérez, J.H., Ardia, D.R., Chad, E.K., & Clotfelter, E.D. 2008 Experimental heating reveals nest temperature affects nestling condition in tree swallows (*Tachycineta bicolor*). *Biology letters* **4,** 468-471.

Racey, P.A., & Swift, S.M. 1981 Variations in gestation length in a colony of pipistrelle bats (*Pipistrellus pipistrellus*) from year to year. *Journal of Reproduction and Fertility.* **61,** 123-129.

Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2016.

Reeder, D.M., Frank, C.L., Turner, G.G., Meteyer, C.U., Kutra, A., Britzke, E.R., Vodzak, M.E., Darling, S.R., Stihler, C.W., Hicks, A.C., Roymon, J., Grieneisen, L.E., Brownlee, S.A., Muller, L.K., & Blehert, D.S. 2012 Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PloS ONE.* **7.**

Reichard, J.D., & Kunz, T.H. 2009 White-nose syndrome inflicts lasting injuries to the wings of little brown bats (*Myotis lucifugus*). *Acta Chiropterologica* **11(2),** 457-464.

Savard, J.P.L., & Robert, M. 2007 Use of nest boxes by goldeneyes in eastern north America. *The Wilson Ornithological Society* **119,** 28-34.

Shaw, D.J., Grenfell, B.T., & Dobson A.P. 1998 Patterns of microparasite aggregation in wildlife host populations. *Parasitology.* **117,** 597-610.

Stawski, C., & Geiser, F. 2010 Fat and fed: frequent use of summer torpor in a subtropical bat. *Naturwissenschaften.* **97,** 29-35.

Stawski, C., Turbill, C., & Geiser, F. 2009 Hibernation by a free-ranging subtropical bat (*Nyctophilus bifax*). *Journal of comparative physiology.* **179,** 433-441.

Turbill, C. & Geiser, F. 2005 Thermal physiology of pregnant and lactating female and male long-eared bats, *Nyctophilus geoffroyi* and *N. gouldi*. *Journal of Comparative Physiology B.* **176,** 165-172.

Tuttle, M.D. 1976 Population ecology of the gray bat (*Myotis Grisescens*): factors influencing growth and survival of newly volant young. *Ecological Society of America.* **57,** 587-595.

Verant, M.L., Meteyer, C.U., Speakman, J.R., Cryan, P.M., Lorch, J.M., & Blehert, D.S. 2014 White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. *BMC Physiology.* **14,** 10.

Warnecke, L., Turner, J.M., Bollinger, T.K., Lorch, J.M., Misra, V., Cryan, P.M., Wibbelt, G., Blehert, D.S., & Willis, C.K.R. 2012 Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences*. **109,** 6999-7003.

Wang, Z., Liang, B., & Racey, P. 2008 Sperm storage, delayed ovulation, and menstruation of the female Rickett's big-footed bat (*Myotis ricketti*). *Zoological Studies.* **47,** 215-221.

Webber, Q.M.R., 2016 Personality, sociality, and pathogen dynamics in little brown bats. Master of Science Thesis, University of Winnipeg, Winnipeg, MB, Canada

Weller, M.W. 1989 Waterfowl management techniques for wetland enhancement, restoration, and creation useful in mitigation procedures. In Kusler, J.A., Kentula, M.E. eds, *Wetland Creation and Restoration: The Status of the Science, Vol 2, Perspectives.* Pp. 105-106. United States Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR, USA

Wilcox, A. & Willis, C.K.R. 2016 Energetic benefits of enhanced summer roosting habitat for little brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. *Conservation physiology* **4,** 1-12.

Willis, C.K.R., & Brigham, R.M. 2003 Defining torpor in free-ranging bats: experimental

evaluation of external temperature-sensitive radiotransmitters and the concept of active temperature. *Journal of Comparative Physiology B.* **173,** 379-389.

Willis, C.K.R., Brigham, R.M., & Geiser, F. 2006 Deep, prolonged torpor by pregnant, free-ranging bats. *Naturwissenschaften.* **93,** 80-83.

Willis, C.K.R., & Wilcox, A. 2014 Hormones and hibernation: possible links between hormone systems, winter energy balance and white-nose syndrome in bats. *Hormones and Behavior* **66,** 66-73.

Yom-Tov, Y., & Wright, J. 1993 Effect of heating nest boxes on egg laying in the Blue Tit (*Parus caeruleus*). *The Auk* **110,** 95-99.

CHAPTER 3: EFFECTS OF ROOST TEMPERATURE ON AGGREGATION AND TORPOR EXPRESSION BY GROUPS OF LITTLE BROWN BATS

Abstract

Habitat heterogeneity can lead to variation in aggregation patterns of conspecifics, which can result in costs or benefits for individuals. For endothermic animals, aggregation may increase access to high quality habitat or reduce energetic costs of thermoregulation but could also increase risk of pathogen or parasite acquisition. During the active season, female bats aggregate in warm roosts, huddle with conspecifics, and/or enter torpor (i.e., a state of reduced body temperature (T_b) and metabolism) to reduce thermoregulatory costs. Artificial roosts, such as bat houses, are often deployed as a management tool for bat populations. However, if bat houses increase aggregation beyond normal levels, they could increase pathogen or parasite transmission which could be especially problematic for species affected by white-nose syndrome (WNS). I used little brown bats (*Myotis lucifugus*) to test three hypotheses about effects of roost quality on aggregation, torpor expression and pathogen transmission: 1) limited, high quality, warm roosting habitat increases bat aggregation and 2) decreases torpor use; and 3) reduced torpor expression and increased aggregation increases pathogen transmission. I predicted that bats provided with access to a high quality, warm roost box would form larger groups, express shorter, shallower torpor bouts, and exhibit greater intensities of infection when a proxy pathogen was introduced to the group. To test these predictions, I housed groups of 10 bats in an outdoor flight enclosure and provided heated or unheated (control) roosts. I quantified roost occupation each day, assessed torpor expression using

temperature data-loggers attached to the skin, and used ultraviolet (UV) fluorescent powder as a proxy for a contact pathogen. Bats disproportionately aggregated in heated roosts ($p<0.0001$) and, after controlling for trial number, I found a significant effect of an interaction between roost temperature (T_{roost}) and aggregation ($p<0.0001$) on torpor use. T_{roost} had a stronger effect on torpor use ($p=0.0001$) for small groups than for medium ($p=0.81$) or large groups ($p=0.020$). However, pathogen transmission was not affected by either group size ($p=0.80$) or torpor expression ($p=0.68$). My findings suggest that roost boxes, artificially heated structures, and other warm microhabitats could help reduce energetic costs for bats affected by threats like WNS, without increasing transmission of contact pathogens or parasites. More work is needed to understand these relationships for larger group sizes over longer time scales.

Introduction

Animals tend to aggregate in areas of high quality habitat (Weir & Davison 1966, Johnson *et al.* 2000, Begall *et al.* 2007) which provide access to resources, protection from predators and, for endotherms, roost or nest sites that buffer against fluctuations in ambient temperature (T_a). For example, Eurasian badgers (*Meles meles*), a typically solitary species, will aggregate in areas with high food abundance when food distribution is patchy (Johnson *et al.* 2000), and the distribution of watering holes during dry periods influences the presence and abundance of many African mammals (Weir & Davison 1966). Small-bodied endotherms often select nest or den sites that minimize exposure to low T_a to decrease thermoregulatory costs, like subterranean burrows (Begall *et al.* 2007), or well-insulated tree cavities (Coombs *et al.* 2010).

Communally-living or colonial animals may also directly benefit from cooperative interactions with other conspecifics. Benefits can include increased overall reproductive success (Malcolm & Marten 1982, Burrows 1995, Roulin & Heeb 1999, Russell *et al.* 2002, Courchamp & MacDonald 2003, McGowan *et al.* 2003), improved foraging efficiency (Packer & Ruttan 1988, Wilkinson & Boughman 1998), information transfer about foraging habitat (Wilkinson 1992) or other nesting/roosting habitat (Chaverri *et al.* 2010), and reciprocal food sharing (Wilkinson 1985). The presence of multiple individuals in a den or roost can decrease thermoregulatory costs and water loss of individuals by raising T_a in the site by reducing the surface area of individuals exposed to ambient air or cold substrates (Arnold 1990, Hwang *et al.* 2006, Willis & Brigham 2007, Boyles *et al.* 2008, Boratynski *et al.* 2015). Social thermoregulation, or mutual warming, occurs when endotherms communally nest to reduce thermoenergetic costs associated with maintaining body temperatures (T_b) (Madison 1984, West & Dublin 1984, Koprowski 1998).

Many endotherms exhibit facultative heterothermy and use short bouts of torpor, periods of reduced T_b and metabolism, to save energy during times of low food or water availability and/or low T_a. For example, desert hedgehogs (*Paraechinus aethiopicus*) in Saudi Arabia frequently enter torpor bouts during spring and winter, occasionally for over 24 hours, and allow T_b to fall within 2-3°C of T_a. In this species, torpor likely reflects a response to the arid environment or the unpredictability of food resources (Boyles *et al.* 2017). Australian northern long-eared bats (*Nyctophilus bifax*) are also known to use daily torpor, even during periods of high food abundance and high T_a. Stawski and Geiser (2009) hypothesized that, because *N. bifax* roost in cryptic locations that are likely safe

from predators, the use of torpor allowed bats to reduce activity and the amount of time spent foraging, which could ultimately reduce their risk of predation. Thus, in addition to energy savings, the ability to express torpor may have allowed bats in good condition to reduce their risk from predation (Stawksi and Geiser 2009, Geiser and Brigham 2012).

Torpor can result in energy savings and a range of other benefits (Geiser & Brigham 2012), but it can also be detrimental. Humphries *et al*. (2003) showed that artificially supplementing eastern chipmunks (*Tamias striatus*) caused an increase in time spent normothermic during winter hibernation. Individuals supplemented with additional food were normothermic more than twice as frequently and defended higher temperatures (5°-10°C) during torpor bouts compared to non-supplemented conspecifics. These results show torpor may be avoided when it is not energetically necessary (e.g. periods of high food abundance), and that even during hibernation, animals may benefit from defending warmer T_b when possible. Costs of torpor for reproductive individuals are also well known. Pregnant and lactating mammals avoid torpor compared to non-reproductive conspecifics (Geiser 1996, Lausen & Barclay 2003, Dzal & Brigham 2013) because torpor slows fetal development (Hoying & Kunz 1998) and milk production (Wilde *et al.* 1999). Torpor may also slow the final stages of juvenile growth after independence, interrupt sperm development for males (Jagiello *et al.* 1992) and slow healing rates from injury (Cryan *et al.* 2013). Therefore, during some circumstances, individuals other than reproductive females may also benefit from avoidance of torpor expression.

During winter most temperate-zone bats use extended bouts of torpor, or hibernation, for weeks to months (Fenton & Barclay 1980, Jonasson & Willis 2012, Norquay & Willis 2014, Czenze *et al.* 2017). Hibernating bats in North America face

many threats including habitat loss (Kunz & Lumsden 2003), climate change (Humphries *et al.* 2002), industrial wind energy (Arnett *et al.* 2008), eviction from building colonies, and, most urgently, infectious disease (Frick *et al.* 2010, Langwig *et al.* 2012, Warnecke *et al.* 2012). North American bats are experiencing drastic population declines from the invasive fungal pathogen, *Pseudogymnoascus destructans*, which causes white-nose syndrome (WNS), an infectious skin disease. Bats with WNS rewarm from torpor too frequently during hibernation and deplete their winter fats stores prematurely, resulting in mass mortalities at hibernation sites (Reeder *et al.* 2012, Warnecke *et al.* 2012). The fungus is spread from infected individuals and substrates to uninfected individuals via direct contact (Lorch *et al.* 2011), and transmission may be affected by the degree of aggregation by hosts (Wilder *et al*. 2011, Langwig *et al*. 2012).

Most proposed management approaches for WNS involve actions that would be implemented in winter to reduce fungal loads on bats or substrates in hibernacula, and/or reduce disease severity and improve winter survival of bats (e.g., Boyles & Willis 2010, Cheng *et al*. 2016, Cornelison *et al*. 2014, Palmer *et al.* 2018). However, any such intervention would be extremely challenging from a logistic perspective and so-called 'evolutionary rescue' may be the best hope for recovery of afflicted populations (Maslo & Feffernan 2015). Some colonies near the original, 2007 outbreak of the disease in the northeastern U.S. are beginning to stabilize at 5-30% of their original population size (Dobony *et al.* 2011, Langwig *et al.* 2012, Frick *et al.* 2015). This stabilization suggests the possibility that surviving individuals possess heritable traits and genotypes that, in the face of an intense selective sweep, have increased in frequency within remnant populations (Willis & Wilcox 2014, Langwig *et al.* 2017). If WNS survivors can pass on

adaptive traits to their offspring, this could result in evolutionary rescue of populations (Maslo & Feffernan 2015, Carlson *et al.* 2016, Donaldson *et al.* 2017). Survivors of WNS emerge from hibernation in the spring but face the energetically expensive process of healing and reproduction. Roosting habitat that helps these survivors reduce torpor expression, may help speed recovery (Faure *et al.* 2009, Cryan *et al.* 2013) and increase reproductive success of survivors (Bryan & Bryant 1999) which could enhance the potential of evolutionary rescue and population recoveries. Protection and enhancement of spring and summer habitats could be just as, if not more important for population recoveries than treatments or other winter management actions (Wilcox & Willis 2016).

Some bat species impacted by WNS will regularly use anthropogenic structures and artificial habitats, like bat boxes (Kunz 1982). Wilcox and Willis (2016) used one of these species, the little brown bat (*Myotis lucifugus*), to test the habitat enhancement hypothesis that active-season roosting habitat could be important for helping bats recover from WNS. They provided a heated roost box, warmed to near-thermoneutral roost temperature (T_{roost}), and a control roost box without heating (T_{roost} = 18°C) to each of two groups of bats (i.e., recovering from WNS versus control) in spring, immediately following hibernation. Both groups preferentially chose the heated bat box in their respective enclosures, but the WNS group was dramatically more likely to use the heated roost box compared to control bats. Wilcox and Willis (2016) used an energetic model to show that roosts maintaining thermoneutral T_{roost} most or all of the time would allow an average bat, defending normothermia, to reduce daily energy expenditure by 75 – 81% under typical spring conditions in central Canada. If enhanced roosting habitat reduces the energy required to defend high T_b and the expression of torpor, this approach could

help bats recover from WNS in spring, reproduce earlier summer, and better prepare for hibernation in fall. However, to date no study has quantified torpor expression of bats provided with artificially enhanced roosting habitat.

One potential risk of using enhanced summer roosting habitat is disruption of social networks and increased aggregations of bats beyond natural colony sizes. Increasing social group size or increasing connections among more individuals in a social network, can increase pathogen or parasite transmission for bats and other animals (Ryder *et al.* 2007, Nunn *et al*. 2015, Webber *et al.* 2017). Many bat species exhibit fission/fusion social dynamics, with a large social group splitting into multiple subgroups on a day-to-day basis, often in multiple roost structures within the same local area (Kerth & König 1999, Willis and Brigham 2004, Kerth *et al.* 2011). However, as bats switch roosts over time, individuals from each sub-group split apart and fuse with other individuals, maintaining cohesion within a large colony despite roosting in relatively small sub-groups. This behaviour is thought to help individual bats maintain a wide range of potential roosting partners for social thermoregulation while reducing overall loads of pathogens and parasites within the colony (Kerth & König 1999, Willis & Brigham 2004, Kerth 2008). Webber *et al.* (2016) used epidemiological models based on social network data from colonies of big brown bats (*Eptesicus fuscus*) to show that collapsing a dispersed fission/fusion network of small subgroups into a smaller number of roosting aggregations with more individuals in the same roost structure, increased the potential for pathogen transmission and loads within the overall colony. If roosting habitat enhancement causes a similar pattern of increased aggregation for bats recovering from WNS, it could potentially increase transmission of *P. destructans* and/or other parasites

and pathogens which could, in turn, be counter-productive for conservation and management.

I used an enclosure experiment with little brown bats to test three related hypotheses about potential costs and benefits of artificial habitat as a management tool for bats. First, I tested whether high quality, warm roosting habitat increases passive aggregation by groups of bats. I predicted that, if groups of bats were provided with limited, artificially heated roosting habitat, the sizes of their aggregations would increase in these warm roosts. Second, I tested whether limited, warm roosting habitat, and/or greater aggregation sizes, influenced thermoregulation. I predicted that bats roosting in warmer roosts and/or larger groups would use torpor less than those in cold roosts and small groups. Finally, I tested whether aggregation and/or torpor expression would increase risk of pathogen acquisition. I predicted that bats roosting in larger aggregations, and using less torpor, would exhibit increased intensity and prevalence of infection following introduction of a proxy pathogen (i.e., ultraviolet (UV) fluorescent powder) via one infected individual within the group.

Methods

All procedures were conducted under a Manitoba Conservation Wildlife Scientific Permit number WB16368 and approved by the University of Winnipeg's Animal Care Committee. I followed WNS decontamination protocols outlined by the U.S. Fish and Wildlife Service (www.whitenosesyndrome.org, 2016) to slow the spread of *P. destructans*, although at the time of the study, the nearest confirmed case of WNS was 200km from my study site (northern Minnesota, www.whitenosesyndrome.org, 2016).

From 17 August through 10 September 2016 I conducted 11 enclosure trials, each lasting 24 hours. On the first night of each trial, I captured 10 little brown bats outside of a known hibernaculum in central Manitoba, near Fisher River First Nation (14U 613884 570051) using a harp trap. I confirmed species and sex and scanned each bat for a passive transponder (PIT tag) to rule out recaptures, at the capture site, and then placed bats in small cloth bags within a stainless-steel metal cage (20cm x 20cm x 20cm). I then transported the bats 13 km by car to a field laboratory at the Manitoba Sustainable Development, Forestry Branch Bunkhouse. At the field lab, I weighed each bat and measured forearm length to quantify body condition. Bats that weighed less than 7.5 grams at any point of the trial, or bats that lost 5% or more of their body mass within 24 hours were released early at the capture site and not used in the study.

I used temperature data-loggers (0.5°C resolution, iButtons, DS1922L, Maxim Integrated Products Inc., Dallas, TX, USA) to quantify torpor expression. I miniaturized data-loggers following Reeder *et al*. (2012) by removing the metal casings and trimming the circuit board to reduce mass. I then coated each iButton with Plasti dip (Plasti-dip Aerosol spray, Blaine, MN, USA) and labelled each with a unique number, allowing me to use them to identify individual bats. I programmed the iButtons to record temperature every 5 minutes and attached one to each bat between the scapula using non-toxic surgical adhesive (294-Osto-bond, Montreal, Canada). Bats were held in captivity for less than 48 hours, so I glued the iButtons to the fur, rather than directly to the skin, to facilitate removal prior to release (Chapter 2). At the end of each trial, I removed iButtons by clipping the fur and downloaded T_{fur} data using One wire software (Maxim Integrated, San Jose, California, USA).

For each 24-hour trial, I housed bats in an outdoor flight enclosure, large enough for bats to fly freely and select from among multiple roosts (length: 4.3m, width: 4.3m, height: 2.0 m; Model #076-5460-2, Outbound Screen House, Outbound, Toronto, Canada). The flight enclosure included a small table that was large enough for bats to land on which was provisioned with a dish of water and a dish of 100 mealworms (provided *ad libitum,* 10 per bat) gut-loaded with beta-carotene multivitamins (SRP 00300, Herptivite, CA, USA) and a nutrient supplement (Repashy Superfoods, Repashy Ventures Inc., CA, USA) and water). I set up four roost boxes (33cm x 30cm x 3cm), one in each corner of the enclosure. Roost boxes were open at the bottom to allow bats to freely enter and exit. One of the roost boxes was insulated (Model # 547658, CodeBord Extruded Polystyrene Rigid Insulation, 2cm thick, Foamular, Owens Corning Commercial Insulation, Toledo, OH, USA) and heated using an outdoor thermostat (ETC-141000-000, Ranco ETC, NEMA 4X, Delphos, OH, USA) and heating mat (Model # PT2035, Heat Wave Desert, Exo Terra, Montreal, Canada) adhered to the back-interior wall of the roost box while the three remaining roosts were un-heated (hereafter referred to as "control"). The thermostat was set to 30°C and I recorded T_{roost} inside each roost box using an un-modified iButton set to record every 10 min. Due to fluctuations in outside T_a, the actual T_{roost} varied from 13.0 to 32.0°C compared to 9.5 to 28.0°C in the un-heated control roosts. I quantified daily average T_{roost} as the mean of all 144 10-min temperature readings for each 24-h experimental trial. Heated roost boxes were significantly warmer (22.2±1.54°C) compared to control roost boxes (16.51±1.61°C, Welch two sample T-test, t=13.3, df=24.1, p=<0.0001, Figure 3.1). Thus, my

experimental setups allowed me to provide bats with multiple roost selection options while simulating a rare, high quality roost among a number of lower quality roosts.

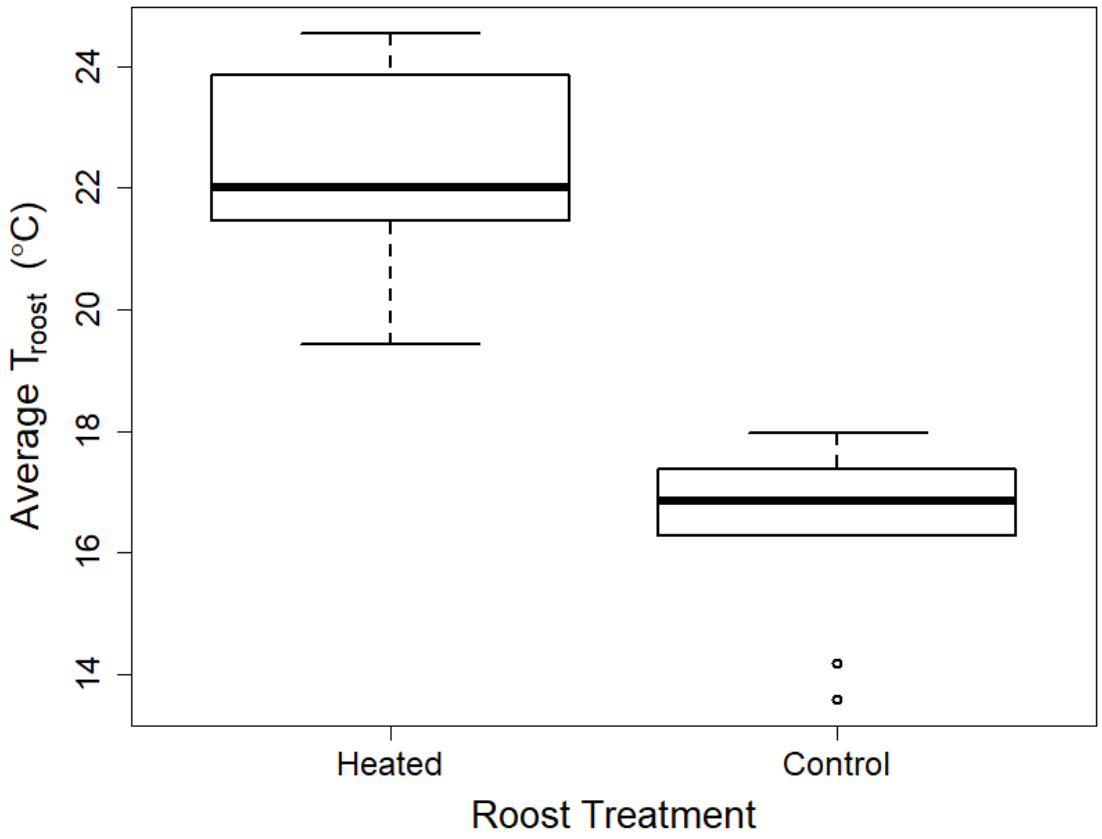

Figure 3.1: Boxplots of daily average T_{roost} in both artificially heated and control roost boxes over the course of 11 24-hour trials ($p<0.0001$). Centre lines represent median values, boxes represent the interquartile range, the upper whiskers represent the third quartile plus 1.5 times the interquartile range, and the lower whiskers represent the first quartile minus 1.5 times the interquartile range. Open circles represent outliers.

To quantify pathogen transmission, I used a non-toxic ultraviolet (UV) fluorescent powder (AX-18N, Signal Green, Day-Glo Color Corp. Cleveland, Ohio, USA) to "infect" one randomly selected individual from the 10 bats captured for each trial. On the second night after capture I used a 5 mm paint brush to apply a 40 mg dose of UV powder to the dorsal side of each wing of the "infected" individual. I then immediately released the nine other "un-infected" bats into the flight enclosure with the "infected" individual. That individual was then free to interact with, and potentially transmit the proxy pathogen to, the other nine bats in the enclosure for the next 24 hours.

Twenty-four hours after introducing the proxy pathogen, I re-entered the enclosure to record T_{roost} for each box, group size in each box, and the location of each individual. I then recaptured each bat and photographed the dorsal and ventral wing membranes under UV light (395nM Blacklight, Ledwholesalers Inc., Hayward, CA) using a digital camera (Digital Rebel Xti, Canon Inc., Oita, Japan). I weighed each bat and any individuals that were within 90% of their original starting weight were implanted with a uniquely coded passive transponder (PIT tag, Trovan Ltd. ID 100-01 Douglas, UK) under the skin between the scapulae. This PIT tag, along with trimmed fur on the dorsum, would have allowed me to identify, and exclude recaptured individuals from subsequent trials although I never recaptured any bats over the course of the study. At the end of each trial, I released all bats at the original capture site.

After each trial, all surfaces of the enclosure were sanitized and cleaned for residual UV powder with a 10% bleach solution and all roost boxes were soaked in 10% bleach solutions (sodium hypochlorite, The Clorox Company, Oakland, CA) for 20

minutes. The enclosure and roost boxes were allowed to air dry for 20 hours before being used in the next trial.

Quantifying when animals are "in" versus "out of" torpor is controversial (Barclay *et al.* 2001, Willis & Brigham 2003, Boyles *et al.* 2011, Brigham *et al.* 2011). One approach is to quantify "degree-minutes," or the area under the curve for a time course of T_{sk} below some T_{sk} threshold assumed to reflect the boundary between torpor and normothermia (Barclay *et al.* 2001, Lausen & Barclay 2003). Degree-minutes has limitations (Willis & Brigham 2003, Brigham *et al.* 2011, see Chapter 2) but for my study, I was interested only in quantifying relative variation in torpor use among individuals. Therefore, I used a method similar to that of Lausen & Barclay (2003) and calculated the area between T_{sk} and 0° for each bat over the 24-hour trial. I first interpolated the temperature between 5-minute readings by multiplying each T_{sk} reading by five. I then simply summed these measured and interpolated T_{sk} values for each individual over the course of each trial. I used these values as, what I term, a relative Index of Normothermy (hereafter NI) to compare torpor expression by individuals in my study (Barclay *et al.* 2001, Lausen & Barclay 2003, Chapter 2). Low values of NI reflect individuals that expression deep and/or long bouts of torpor.

I quantified intensity of infection with UV powder using ImageJ (ImageJ v. 1.8.0_66, U.S. National Institutes of Health, Bethesda, Maryland, USA). I scaled images using a standardized object (i.e. U.S. penny, diameter = 19.05mm) and quantified the total area of each bat's wing and the area covered with proxy pathogen (Fuller *et al.* 2011). I then calculated intensity of infection as the proportion of wing covered with UV powder (Webber 2016, Fuller *et al.* 2011). For bats without detectable infections (n = 3), I

replaced the values of zero with the lowest non-zero proportion I found for other bats divided by two, assuming that my lowest detected value approximated a minimum limit of detection (Croghan & Egehy 2003).

I used R (version 3.2.3, GUI 1.51, R Development Core Team 2015) for all statistical analyses. To determine the effect of roost box type (i.e., heated or control) on aggregation I used a Chi-squared test with expected values calculated based on the null assumption that bats would select roosts at random (i.e., a probability of being found in any box = 0.25). To assess the effect of average T_{roost} on aggregation, I used a generalized linear model (GLM, family=binomial) with proportion of bats as my response variable and average T_{roost} as my predictor variables. Numbers of bats fluctuated slightly among trials (i.e., from n = 6 to 10 bats per trial) because of premature releases due to mass loss and two escape events so I used proportion rather than number of bats in each roost box, as my response variable for this analysis and used a logit transformation to achieve normality.

To understand factors influencing torpor expression, I used a linear mixed effects (LME) model with Restricted Maximum Likelihood (REML) with each individual's NI value as the response variable and average daily T_{roost}, group size (i.e., the number of bats found in the individual's roost box), mass, sex, and an interaction between T_{roost} and group size as predictor variables. Despite the differences in number of bats used for each trial, I used number of bats in a given roost box, instead of proportion of bats, as the predictor variable for this analysis based on the assumption that absolute number of bats would be the more important driver of social thermoregulation. I included an interaction between T_{roost} and group size based on the assumption that effects of group size on torpor

expression could vary with T_{roost}. I did not include T_a outside of roost boxes in my analysis because T_a significantly affected T_{roost} for both heated and control roost boxes (LME with trial as a random effect ($p<0.001$), heated roosts: $t=11.4$, $df=9$, $p<0.0001$, $r^2=0.93$; unheated roosts: $t=13.7$, $df=9$, $p<0.0001$, $r^2=0.93$, Figure 3.2). I also included individual trial number as a random effect to account for pseudoreplication (Dingemanse & Dochtermann 2013).

To understand how group size and torpor expression affected pathogen transmission, I used a LME with REML to test for effects of NI, group size (i.e., number of bats found in the individual's roost box), and sex on each individual's intensity of infection with UV powder. I again included trial as a random effect to account for pseudoreplication and used number instead of proportion of bats based on the assumption that, as for torpor expression, the number of roost-mates was more likely to influence pathogen transmission. Intensity of infection fit a negative binomial distribution (see results) so I used a logit-transformation to achieve normality. I examined residuals to ensure that all models met LME assumptions. All values are reported as the mean ± SD and I assessed significance at $\alpha=0.05$.

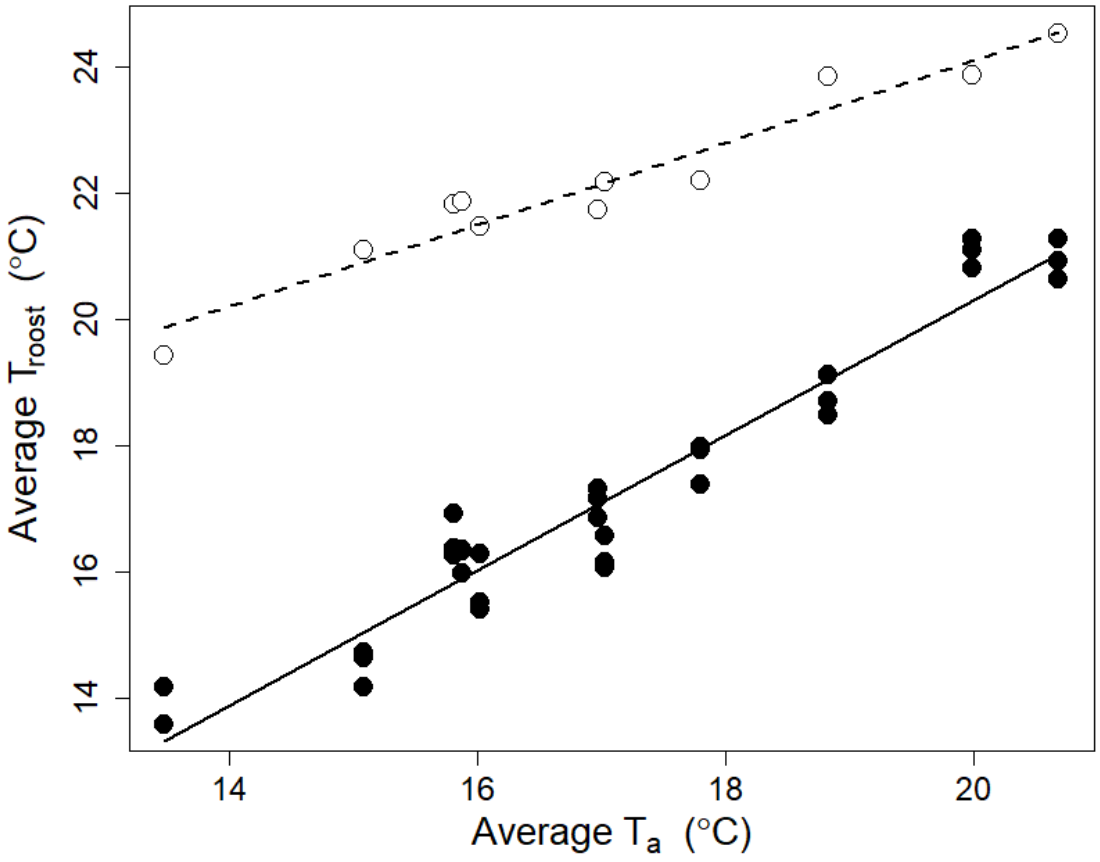

Figure 3.2: Scatter plot of the relationship between average daily T_a (°C) and average daily T_{roost} (°C). Open circles represent heated roosts and black circles represent control roosts. Linear regression lines are also included for illustration although data were analyzed linear mixed effects models including trial as a random effect (see methods).

Results

Ambient temperature ranged from 10.0°C to 35.5°C throughout the study with average daily maxima of 24.1±6.2°C and minima of 13.8±1.8°C. Sunrise times ranged from 05:26 to 06:01, and sunset times from 19:03 and 19:51 (National Research Council Canada 2016). I caught 46 female and 22 male little brown bats over the course of 11 capture events. For all but one of the 11 trials, most bats roosted in the heated roost box (Figure 3.3). On average, 76.5±2.2% of bats roosted in the heated box each day over the control boxes, which is significantly greater than the expected 25% (X^2=169.6, d.f.=1, $p<0.0001$; Figure 3.3). The effect of average T_{roost} on proportion of bats in each roost approached significance (r^2=0.79, z=1.8, p=0.06, Figure 3.4) after controlling for trial as a random effect (p=0.01).

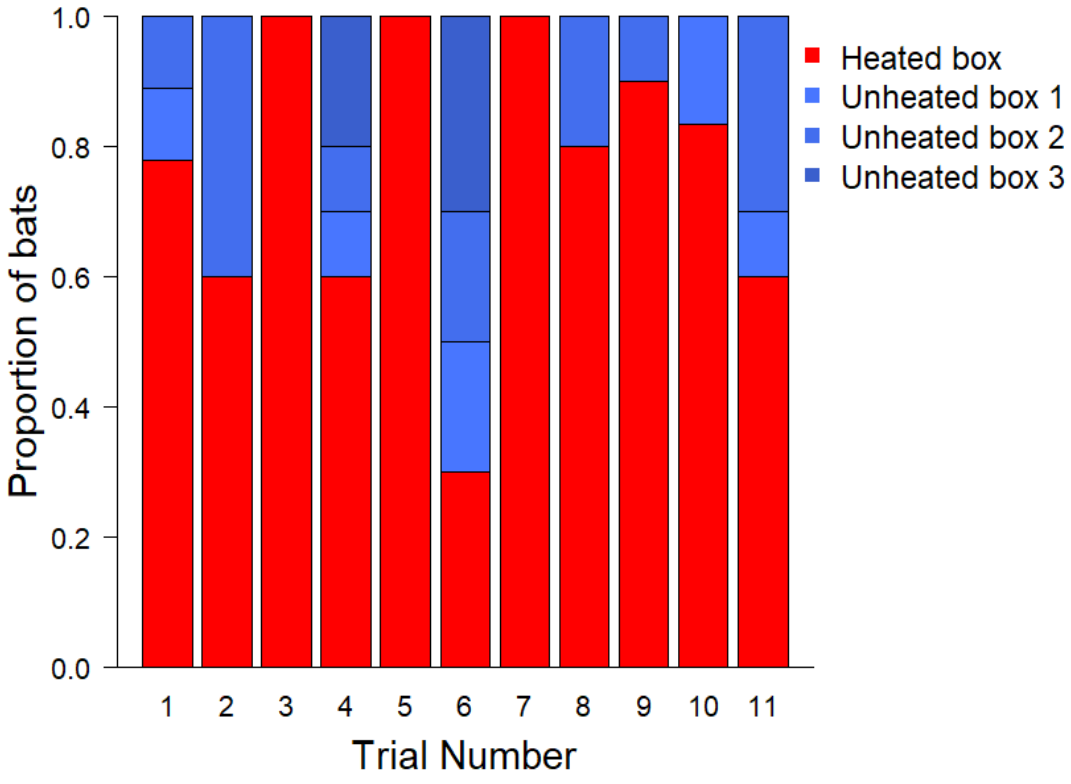

Figure 3.3: Proportions of bats roosting in one heated versus three un-heated control roost boxes over the course of 11 24-h trials.

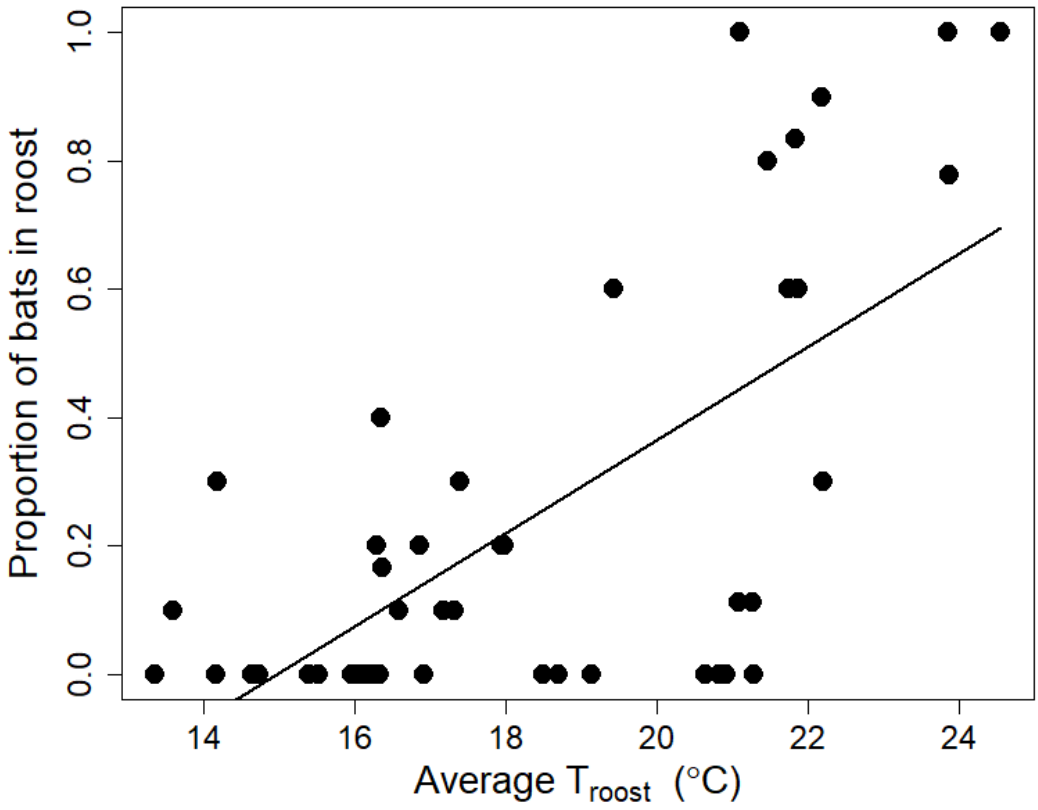

Figure 3.4: Scatter plot of the relationship between the proportion of bats in a roost and daily average T_{roost} (°C). A linear regression line is also included for illustration although data were analyzed using a generalized linear model (family=binomial, see results).

Values of NI ranged from 46400 degree-minutes (i.e., the lowest level of torpor expression) to 37710 degree-minutes with an average of 43390±2144.417 degree-minutes. NI was significantly affected by sex, with females defending higher NI than males, and an interaction between group size and T_{roost} (r^2=0.88, Table 3.1). Group size fit a tri-modal distribution clearly dividing into small (1-3 bats), medium (4-6 bats), and large (7-10 bats) group sizes (Figure 3.5) so to understand this interaction I analysed the relationship between NI and T_{roost} for each of the three group sizes independently. For small groups T_{roost} significantly affected NI such that a 1°C increase in average T_{roost} increased an individual's NI by 802 degree-minutes (r^2=0.95, Table 3.2). The NI incorporates both the depth and duration of torpor bouts into one unit so that an 802 degree-minute increase could represent many different responses (e.g., a 3°C increase in T_{fur} lasting about 4.5 hours or a 14°C increase in T_{fur} lasting about 1 hour, Table 3.2). There was no effect of sex on NI for small groups but, interestingly, the effect of mass approached significance (Table 3.2) with higher values of NI for lighter bats. Average T_{roost} also significantly affected individual NI for bats in large groups such that a 394 degree-minute increase in NI could represent many possible responses (e.g. a 1°C increase in T_{fur} lasting 6.5 h or a 3°C increase lasing about 2 hours, r^2=0.47, Table 3.3). In large groups there was a significant sex effect with females defending NIs 857 degree-minutes warmer, on average, than males (Table 3.3). There was no effect of body mass on NI for individuals in large groups. None of the predictor variables significantly affected NI for medium-sized groups (r^2=0.06, Table 3.4)

Table 3.1: Summary of linear mixed effects model assessing effects of group size, average T_{roost}, their interaction term, sex, and mass (with trial as a random effect, $p<0.0001$) on normothermy index of 68 little brown bats (n= 22 male, 45 female) over 11 24-hour trials.

Variable	Coefficient	Standard Error	t-value	Degrees of Freedom	p-value
Group size	1493	286	5.23	53	<0.0001
Average T_{roost}	930	85.9	10.8	53	<0.0001
Group size: Average T_{roost}	-66.5	13.4	-4.96	53	<0.0001
Sex	-169	217	-2.17	53	0.035
Mass	120	62.3	1.93	53	0.059

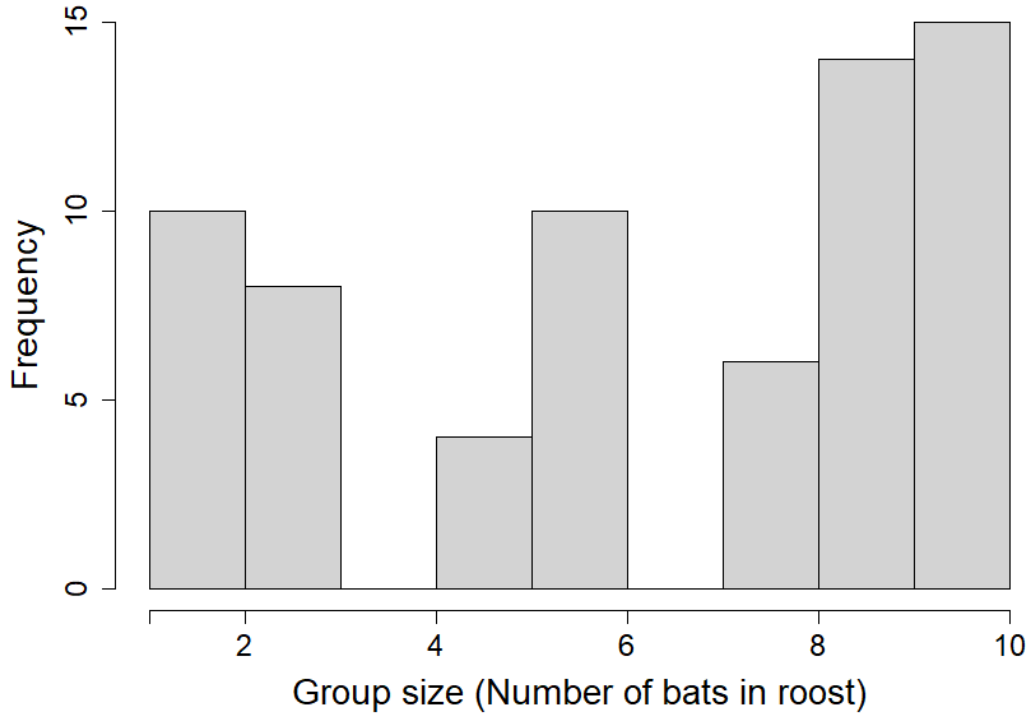

Figure 3.5: Histogram of the group size (number of bats in roost) in which each of the 68 little brown bats chose to roost over 11 24-hour trials (see results).

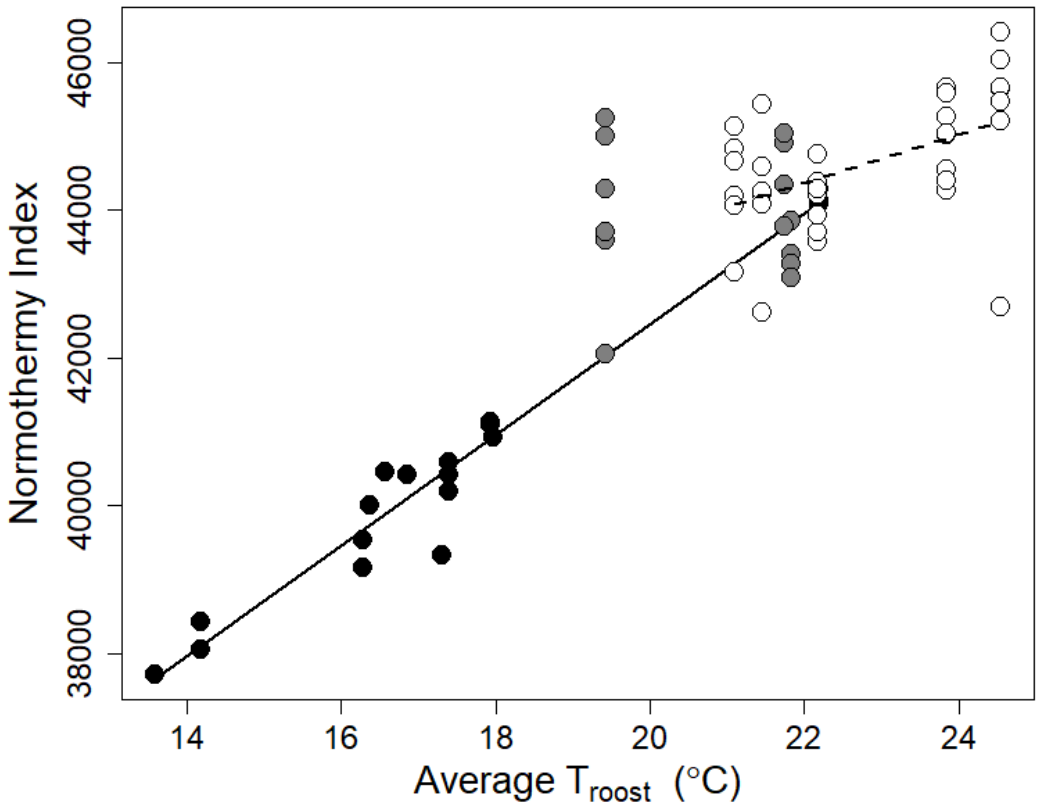

Figure 3.6: Scatter plot showing the effect of average T_{roost} on normothermy index. Group size showed a tri-modal pattern (see Figure 3.4) and is represented here by black circles for groups of 1-4 individuals, dark gray circles for medium-sized groups of 4-7 individuals, and white circles for large groups of 8-10 individuals. Relationships between groups size and NI were significant for small and large but not medium-sized groups (see results).

Table 3.2: Summary of linear mixed effects model assessing effects of average T_{roost}, sex, and mass (with trial as a random effect, $p<0.0001$) on normothermy index of 18 little brown bats (n= 8 males, 10 females) that roosted in small-sized groups (1-3 individuals).

Variable	Coefficient	Standard Error	t-value	Degrees of Freedom	p-value
Average T_{roost}	802	47.0	18.1	9	<0.0001
Sex	-153	169	-0.90	9	0.39
Mass	-177	82.4	2.15	9	0.06

Table 3.3: Summary of linear mixed effects model assessing effects of average T_{roost}, sex, and mass (with trial as a random effect, $p<0.0001$) on normothermy index of 35 little brown bats (n= 7 males, 28 females) that roosted in large-sized groups (7-10 individuals).

Variable	Coefficient	Standard Error	t-value	Degrees of Freedom	p-value
Average T_{roost}	394	86.1	4.58	3	0.020
Sex	-1006	294	-3.43	28	0.002
Mass	89.6	120	0.74	28	0.46

Table 3.4: Summary of linear mixed effects model assessing effects of average T_{roost}, sex, and mass (with trial number as a random effect, $p<0.0001$) on nomothermy index of 14 little brown bats (n= 7 male, 7 female) that roosted in medium-sized groups (4-6 individuals).

Variable	Coefficient	Standard Error	t-value	Degrees of Freedom	p-value
Average T_{roost}	-104	344	-0.303	1	0.81
Sex	-117	599	-0.196	9	0.85
Mass	-126	167	-0.756	9	0.47

Three individuals had no proxy pathogen on their wings while all others had at least some infection for an overall prevalence of 95.5%. The greatest intensity of infection (proportion of wing covered by UV powder) was 11.8% with an average of 0.96±0.02%. Infection intensity showed a negative binomial distribution with few individuals having high infection intensities and most individuals having low infection intensities (Figure 3.7a), but I achieved a normal distribution using a logit transformation (Figure 3.7b, Shapiro-Wilk normality test, W=0.98, p=0.45). None of NI, group size or sex had any effect on infection intensity (r^2=0.06, Table 3.5, Figure 3.8 and 3.9).

Table 3.5: Summary of linear mixed effects model assessing effects of group size, normothermy index, their interaction term, and sex (with trial as a random effect) on the logit-transformed intensity of infection of 68 little brown bats over 11 24-hour trials.

Variable	t-value	Degrees of Freedom	p-value
Group size	0.255	54	0.800
NI	-0.409	54	0.684
Interaction between group size and NI	-0.208	54	0.836
Sex	-1.606	54	0.114

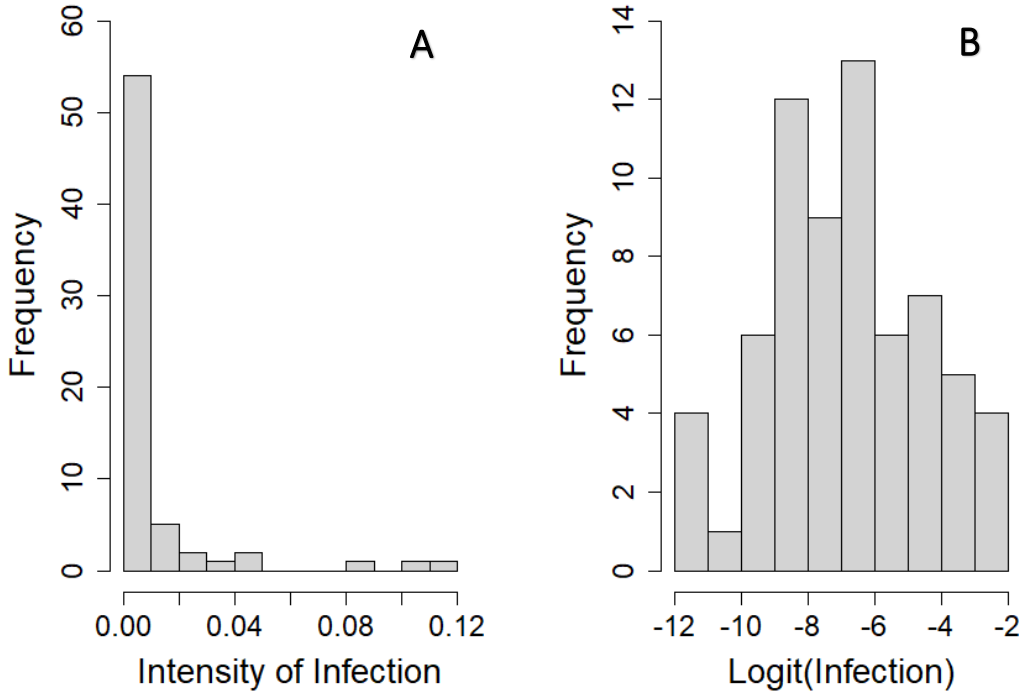

Figure: 3.7: Histograms of **(A)** un-transformed intensities of infection (i.e., proportion of the wing covered with UV powder), and **(B)** Histogram of logit-transformed intensity of infection showing normal distribution.

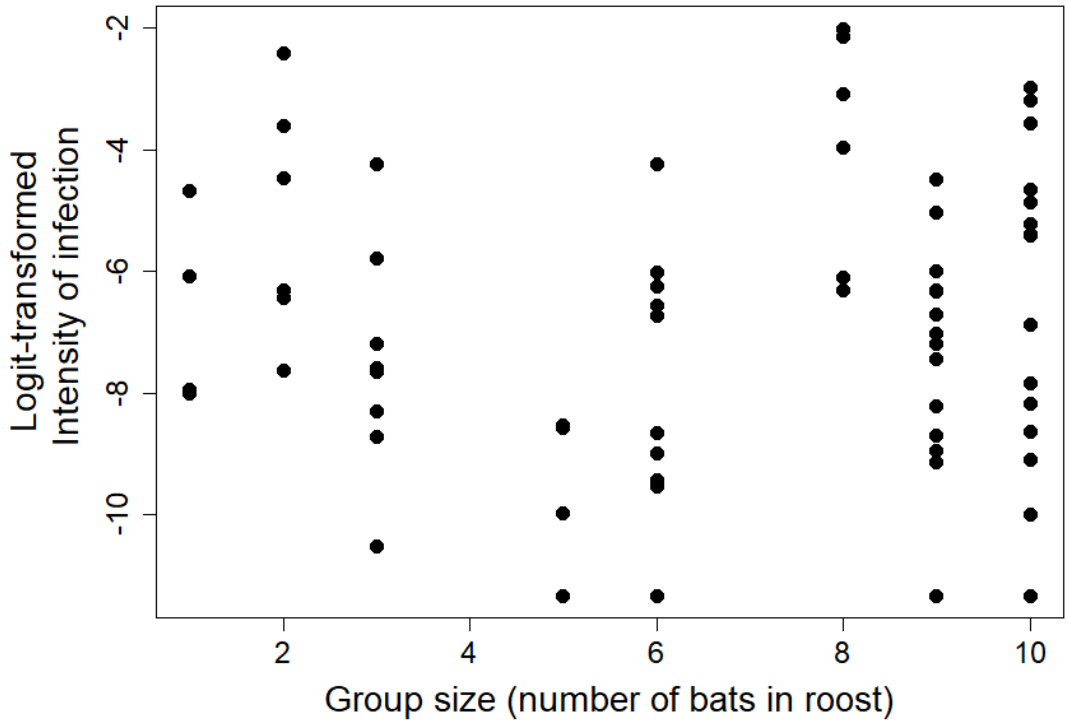

Figure 3.8: Scatter plot of the relationship between logit-transformed intensity of infection for each individual and the size of the group in which the bat was found (p=0.80).

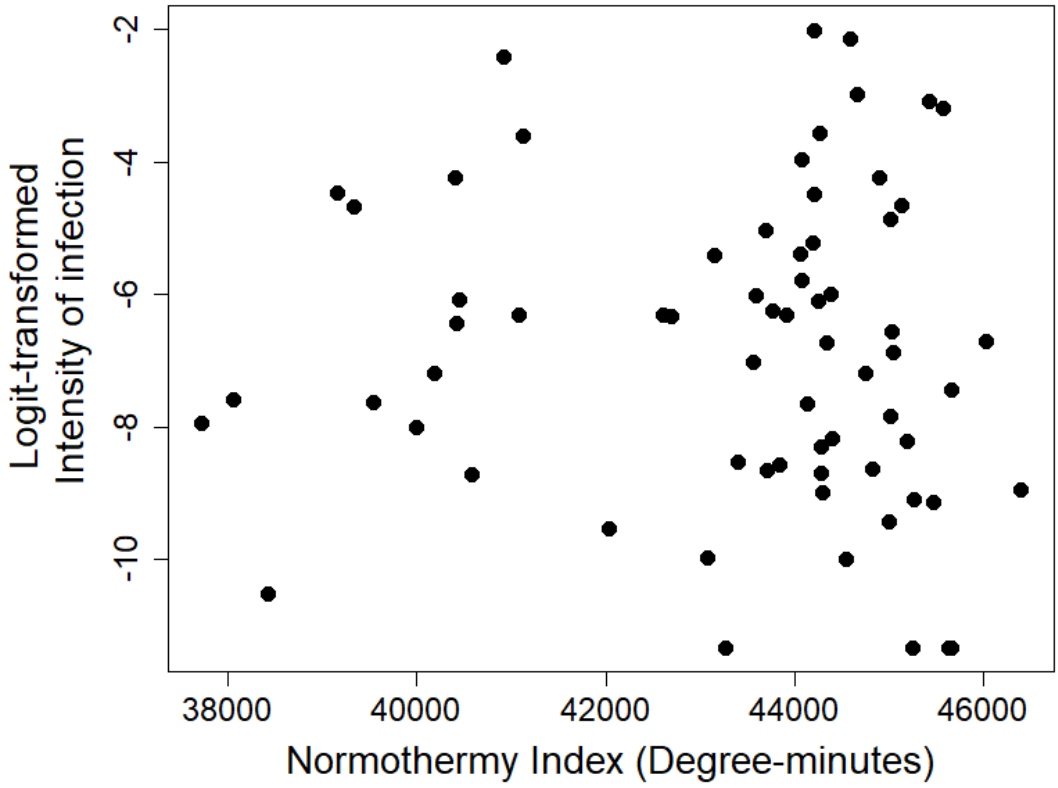

Figure 3.9: Scatter plot of the relationship between logit-transformed intensity of infection for each individual and it's NI (p=0.68).

Discussion

My results provide the first experimental evidence that warm roosting habitat affects both aggregation sizes and torpor expression in a colonial, temperate-zone bat. However, I found no evidence that either aggregation size or torpor expression influenced intensity of infection with a proxy pathogen. Taken together, these results lend support to Wilcox and Willis' (2016) habitat enhancement hypothesis that providing bats with relatively warm roosting habitat during the active season could help them reduce torpor expression and potentially improve rates of healing and recovery from WNS, as well as speed rates of reproduction and offspring growth.

Consistent with my first hypothesis, bats were disproportionately more likely to roost in the one roost box in their enclosure that was heated compared to un-heated control roost boxes. There was also a trend for a positive effect of T_{roost} on aggregation size that approached significance. Willis and Brigham (2007) found a similar positive relationship between group size and T_{roost} for big brown bats (*Eptesicus fuscus*) but in their study, group size appeared to drive T_{roost} and not the other way around (i.e., more bats in a given tree hollow caused warmer T_{roost}). Because my heated boxes were artificially heated and were consistently warmer than control boxes throughout all trials, independent of group size, bats likely had little to no effect on T_{roost}.

In support of my second hypothesis, bats that roosted in warmer roosts and with more conspecifics used less torpor. Larger numbers of bats tended to aggregate in heated roosts and those bats employed less torpor. In small groups, T_{roost} had a strong effect on NI which suggests that bats inclined to use torpor may select colder roosts and avoid large groups of conspecifics. There was also a trend ($p=0.06$) for higher NI in lighter bats

than heavier bats in small groups in my experiment. Stawski and Geiser (2010) also found similar trends, in that bats in better body condition used more torpor than those in poor condition. They hypothesized that this pattern may be because bats are employing torpor as a means of reducing activity to avoid predation, not strictly as an energy-saving mechanism. There was also a positive effect of T_{roost} on NI for bats in large groups, but this effect was less pronounced than for small groups suggesting that a combination of passive aggregation in warm roosts, plus reduced heat loss from social thermoregulation, allows bats in larger groups to avoid torpor expression (Figure 3.6). Interestingly, for large groups, there was also an effect of sex on torpor expression with females defending higher NI than males. If anything, I expected that males should have defended higher T_{fur} than females at the time of year of my study because of the importance of warm T_b for sperm production. One possibility is that females, which are highly social during summer, spent more time out of torpor while in large groups in my experiment because of behaviours associated with social interactions between conspecifics.

Communal roosting can theoretically increase pathogen transmission (Alexander 1974). Although T_{roost} affected bat aggregation size and torpor expression, I found no support for my third hypothesis that group size or torpor affect pathogen transmission in this species, despite the fact that transmission dynamics of my proxy pathogen approximated that for most natural pathogens with most individuals exhibiting low intensity of infection and only a few individuals being heavily infected (e.g., Shaw *et al*. 2016). At least at this small group size of a maximum of 10 bats, and over a 24-hour time scale, neither aggregation nor torpor expression affected pathogen transmission. This pattern could reflect the influence of host traits other than aggregation and torpor

expression. Hillegass *et al.* (2008) found similar results in Cape ground squirrels in that group size had no effect on ectoparasite or endoparasite loads, but instead sex was a more important determinant of parasite loads. Males had 3 times as many ectoparasites as females, while females had 3 times as many endoparasites. Hillegass *et al.* (2008) suggested that this sex difference may be caused by sexual selection, in that females have smaller home ranges (greater likelihood of coming into contact with endoparasites) and allogroom more frequently (reducing ectoparasite load) compared to their male conspecifics that have greater ectoparasite loads possibly because of high androgen levels and lower grooming times. However, I did not find an effect of sex on pathogen acquisition in my study. Parasite transmission may also vary with differences in the mobility of the host and the mode of parasite transmission (Patterson & Ruckstuhl 2013). A meta-analysis conducted by Patterson and Ruckstuhl (2013) found that group size does have a significant positive effect on pathogen transmission overall, but when focusing only on mobile parasites, pathogen load is negatively correlated with group size and when investigating only mobile hosts, group size does not affect pathogen load either. In the case of my study, the proxy pathogen was transmitted via direct contact and could be considered density dependent, so in larger groups pathogen load should have been greater.

Using captive bats allowed me to control for a range of variables, and also recapture individuals to assess intensity of infection after a consistent time interval. However, captivity and my experimental design exposed bats to conditions that would differ, potentially dramatically, from those experienced by free-ranging bats. For one, bats in my study were provided food *ad libitum*, and the available food was never

completely consumed. Thus, bats may have been in positive energy balance more often than would occur in the wild, which could have influenced roost preferences and torpor expression. If bats had limited food availability they might have been motivated to conserve energy, select colder roosts and express torpor (e.g., Lovegrove *et al.* 2001, Mzilikazi & Lovegrove 2002). On the other hand, T_a during my study rarely fell below 11 °C during the first few hours after sunset (when most bat foraging activity occurs). Given the strong relationship between T_a and flying insect availability, free-ranging bats would probably only rarely have been limited by food availability on most of the nights when I conducted my experiment. Manipulating T_a and food availability in future studies could provide additional insight into factors affecting bats' thermoregulatory behaviours in the wild.

In addition to *ad libitum* food, the spatial and temporal scale of my experiment was small relative to natural home range size for little brown bats which could have influenced my ability to quantify pathogen dynamics. The results of my power analysis show that in order to detect a large effect of NI, group size, sex, and mass on pathogen acquisition, my sample size would have had to be 131 bats, compared to my 68 individuals. Based on an a priori power analysis, I recommend future studies conduct 15-20 trials of 10 bats per trial, in order to detect large, biologically significant results. However, because of the life history of bats, I would not have been able to capture bats earlier in the summer or later in the fall for my experiment. Each trial had a maximum of 10 bats and the median roosting group size I observed was 8 individuals per roost box, which is relatively small compared to maternity colony sizes (Davis & Hitchcock 1965, Barclay & Cash 1985, Kalcounis & Brigham 1994, although smaller group sizes could be

more likely during other times of year). This behaviour may have reduced the potential for interactions among bats in my study and caused me to underestimate pathogen transmission relative to natural colonies. Bats inside each roost were considered to be part of one group, but did not necessarily cuddle in a tightly packed group. On the other hand, the small spatial scale available to bats in my experiment could have increased the likelihood of interactions between conspecifics, in turn increasing the chance of pathogen transmission. In this case, high contact rates in my flight enclosure may have effectively swamped any signal of torpor expression or aggregation size on pathogen transmission. If this was the case, however, I would have expected very high intensities of infection for most bats and which is not what I observed. The negative binomial distribution of infection intensity I observed is consistent with patterns seen for most pathogens and contact parasites in nature and suggests that transmission dynamics of my proxy pathogen approximated those of a natural pathogen (Shaw *et al.* 2016). Pathogen loads could have also been altered as bats groomed off the powder as it was observed in their feces. Although such studies would be challenging, I recommend future attempts to use larger group sizes of species that are more tractable in captivity (e.g., big brown bats) or free-ranging bats roosting in different contexts (e.g., rock crevices vs. buildings, Lausen & Barclay 2006). Studies of free-ranging bats could potentially quantify prevalence and/or loads of naturally occurring pathogens like the rocky mountain coronavirus in big brown bats (Dominguez *et al.* 2007, Misra *et al.* 2009) or the *Myotis* coronavirus (Misra *et al.* 2009) which appear to chronically infect colonial bats with little pathology and no evidence of risk for humans. Using observational studies of existing viruses, combined with experiments like mine, could help improve understanding of the influence of

roosting aggregation and torpor expression on pathogen dynamics in the wild.

My study provides further evidence that summer habitat enhancement could be a valuable management tool for conservation of bat species at risk from WNS and other threats. For some bat species that regularly use anthropogenic structures (e.g., little brown bats), artificial heating of roost boxes could be a viable management option. Other endangered species, however, are less likely not use anthropogenic structures and, instead, rely much more heavily on tree hollows and other roosts in forests (e.g., northern long-eared bats). For these species, identifying and protecting habitat features that naturally increase T_{roost} may also be important for management. Older, larger trees on south-facing slopes may provide relatively warm microclimates and my results suggest that other habitat features which enhance warm microclimates should be identified and preserved. Protecting and enhancing summer roosting habitat could be a viable management option for conserving bats species at risk.

Taken together, my results lend support to the habitat enhancement hypothesis, that managing bat habitats in ways that increase availability of warm roosts, could be beneficial for helping bat populations recover from WNS and other threats (Wilcox and Willis 2016). Reducing torpor expression could help bats heal and recover more quickly from WNS in spring (Chapter 2, Faure *et al.* 2009). After recovery, warm roosts may also improve reproductive success of WNS survivors by speeding fetal growth, enhancing lactation and growth rates of juveniles (e.g. McCarty & Winkler 1999), and enhancing sperm production for males in summer and fall (Jagiello *et al.* 1992). Although bats in my study were all WNS-negative, like control bats studied by Wilcox and Willis (2016), they still showed a strong preference for warm roosts and spent more time at warmer T_b when

roosting in warm structures. In addition, I found no evidence for an increase in pathogen or parasite transmission for bats using less torpor in heated roosts, which is one potential downside of habitat enhancement. More work is needed with free-ranging bats, but so far, habitat enhancement appears to have promise as a potential management strategy for bats imperiled by WNS and other threats.

References

Alexander, R.D. 1974 The evolution of social behavior. *Annual Review of Ecology and Systematics* **5,** 325-383.

Angeli, N.F., DiRenzo, G.V., Cunha, A., & Lips, K.R. 2015 Effects of density on spatial aggregation and habitat associations of the glass frog (*Espadarana (Centrolene) prosoblepon)*. *Journal of Herpetology*

Arnold, W. 1990 The evolution of marmot sociality: II. Costs and benefits of joint hibernation. *Behavioral Ecology and Sociobiology* **27,** 239-246.

Barclay, R.M.R., & Cash, K.J. 1985 A non-commensal maternity roost of the little brown bat (*Myotis lucifugus*). *Journal of Mammalogy* **66,** 782-783.

Barclay, R.M.R., Lausen, C.L., & Hollis, L. 2001 What's hot and what's not: defining torpor in free-ranging birds and mammals. *Canadian Journal of Zoology.* **79,** 1885-1890.

Begall, S., Burda, H., & Schleich, E. 2007 Subterranean rodents: news from underground. Springer, Berlin Heidelberg.

Berger, L., Speare, R., Daszak, P., Green, D.E., Cunningham, A.A., Goggin, C.L., Slocomb, R., Ragan, M.A., Hyatt, A.D., McDonald, K.R., Hines, H.B., Lips, K.R., Marantelli, G., Parkes, H. 1998 Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Science USA* **95,** 9031-9036.

Boratynski, J.S., Willis, C.K.R. Jefimow Malgorzata, & Wojciechowski, M.S. 2015 Huddling reduces evaporative water loss in torpid Natterer's bats, *Myotis nattereri*. *Comparative Biochemistry and Physiology, Part A.* **179,** 125-132.

Boyles, J.G., & Willis, C.K.R. 2010 Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white-nose syndrome? *Frontiers in Ecology and the Environment.* **8,** 92-98.

Boyles, J.G., Smit, B., & McKechnie, A.E. 2011 Does use of the torpor cut-off method to analyze variation in body temperature cause more problems than it solves? *Journal of Thermal Biology.* **36,** 373-379.

Boyles, J.G., Bennett, N.C., Mohammed, O.B., & Alagaili, A.N. 2017 Torpor patterns in desert hedgehogs (*Paraechinus aethiopicus*) represent another new point along a thermoregulatory continuum. *Physiological and Biochemical Zoology.* **90,** 445-452.

Briggs, C.J., Knapp, R.A., & Vrendenburg, V.T. 2010 Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *PNAS* **107,** 9695-9700.

Brigham, R.M., Willis, C.K.R., Geiser, F., & Mzilikazi, N. 2011 Baby in the bathwater: Should we abandon the use of body temperature thresholds to quantify expression of torpor? *Journal of Thermal Biology.* **36,** 376-379.

Chaverri, G. Gillam, E.H., & Vonhof, M.J. 2010 Social calls used by a leaf-roosting bat to signal location. *Biology Letters* **6,** 441-444.

Cheng, T.L., Mayberry, H., McGuire, L.P., Hoyt, J.R., Langwig, K.E., Nguyen, H., Parise, K.L., Foster, J.T., Willis, C.K.R., Kilpatrick, A.M.K., & Frick, W.F. 2016 Efficacy of a probiotic bacterium to treat bats affected by the disease white-nose syndrome. *Journal of Applied Ecology* **54,** 701-708.

Cohen, J. 1988 Statistical power analysis. *Current directions in psychological science.* **1,** 98-101.

Coombs, A.B. Bowman, J., Garroway, C.J. 2010 Thermal properties of tree cavities during winter in a northern hardwood forest. *Journal of Wildlife Management* **74,** 1875-1881.

Cornelison, C.T., Keel, M.K., Gabriel, K.T., Barlament, C.K., Tucker, T.A., Pierce, G.E., & Crow, S.A. 2014 A preliminary report on the contact-independent antagonism of *Pseudogymnoascus destructans* by Rhodococcus rhodochrous strain. DAP96253 *BMC Microbiology* **14,** 246.

Côté, I.M., & Poulin, R. 1995 Parasitism and group size in social animals. *Behavioural Ecology* **6,** 159-165.

Courchamp, F. & Macdonald, D.W. 2001 Crucial importance of pack size in the African wild dog *Lycaon pictus*. *Animal Conservation* **4,** 169-171.

Croghan, C. & Egeghy, P. 2003 Methods of dealing with values below the limit of detection using SAS. *Southern SAS User Group*

Cryan, P. M., Meteyer, C.U., Boyles, J.G., & Blehert, D.S. 2013 White-nose syndrome in bats: illuminating the darkness. *BMC Biology* **11,** 2-5.

Czenze, Z.J., Jonasson, K.A., & Willis, C.K.R. 2017 Thrifty females, frisky males: winter energetics of bats from a cold climate. *Physiological and Biochemical Zoology* **90,** 502-511.

Davis, W.H., & Hitchcock, H.B. 1965 Biology and migration of the bat, *Myotis lucifugus*, in New England. *Journal of Mammalogy* **46,** 296-313.

Dingemanse, N.J., & Dochtermann, N.A. 2013 Quantifying individual variation in behaviour: mixed-effect modelling approaches. *Journal of Animal Ecology* **82,** 39-54.

Dittmann, L. & Schausberger, P. 2017 Adaptive aggregation by spider mites under predation risk. *Scientific Reports* **7**, 10609. DOI:10.1038/s41598-017-10819-8

Dominguez, S.R., O'Shea, T.J., Oko, L.M., & Holmes, K.V. 2007 Detection of group 1 coronaviruses in bats in North America. *Emerging Infectious Diseases* **13,** 1295-1300.

Edelman, A.J., & Koprowski, J.L. 2007 Communal nesting in asocial Abert's squirrels: The role of social thermoregulation and breeding strategy. *Ethology* **113,** 147-154.

Ebensperger, L.A. 2001 A review of the evolutionary causes of rodent group-living. *Acta Theriologica* **46,** 115-144.

Faure, P.A., Re, D.E., Clare, E.L. 2009 Wound healing in the flight membranes of big brown bats. *Journal of Mammalogy* **90,** 1148-1156.

Fenton, M.B., & Barclay, R.M.R. 1980 Myotis lucifugus. *Mammalian Species* **142,** 1-8.

Foster, W.A. & Treherne, J.E. 1981 Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. *Nature* **293,** 466-467.

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M., & Kunz, T.H. 2010 An emerging disease causes regional population collapse of a common North American bat species. *Science* **329,** 679-682.

Fuller, N.W., Reichard, J.D., Nabhan, M.L., Fellows, S.R., Pepin, & L.C., Kunz, T.H. 2011 Free-ranging little brown myotis (*Myotis lucifugus*) heal from wing damage associated with white-nose syndrome. *EcoHealth.* **8,** 154-162.

Henzi, S.P., Lycett, J.E., & Piper, S.E. 1997 Fission and troop size in a mountain baboon population. *Animal Behavior* **53,** 525-535.

Hillegass, M.A., Waterman, J.M. & Roth, J.D. 2008 The influence of sex and sociality on parasite loads in an African ground squirrel. *Behavioral Ecology* **19,** 1006-1011.

Hoying, K.M., & Kunz, T.H. 1998 Variation in size at birth and post-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). *Journal of Zoology* **245,** 15-27.

Humphries, M.M., Speakman, J.R., & Thomas, D.W. 2002 Climate-mediated energetic constraints on the distribution of hibernating mammals. *Nature* **418,** 3313-316.

Humphries, M.M., Kramer, D.L., & Thomas, D.W. 2003 The role of energy availability in mammalian hibernation: an experimental test in free-ranging eastern chipmunks. *Physiological and Biochemical Zoology* **76,** 165-179.

Hwang, Y.T., Lariviere, S. & Messier, F. 2006 Energetic consequences and ecological significance of heterothermy and social thermoregulation in striped skunks (*Mephitis mephitis*). *Physiological and Biochemical Zoology* **80,** 138-145.

Jagiello, G.M., Fang, J.S., Sung, W.K., Hembree, W.C., & Ducayen, M.B. 1992 Genetic characteristics of spermatogenesis in the Turkish hamster (*Mesocricetus brandti*) subjected to reduced temperature or light. *Journal of Thermal Biology* **17,** 175-184.

Johnson, D.D.P., MacDonald, D.W., & Dickman, A.J. 2000 An analysis and review of models of the sociobiology of the Mustelidae. *Mammal Review* **30,** 171-196.

Jonasson, K.A., & Willis, C.K.R. 2012 Hibernation energetics of little brown bats. *Journal of Experimental Biology* **215,** 2141-2149.

Kalcounis, M.C. & Brigham, R.M. 1994 Impact of predation risk on emergence by little brown bats, *Myotis lucifugus* (Chiroptera: Vespertilionidae), from a maternity colony. *Ethology* **98,** 201-209.

Kerth, G. 2008 Causes and consequences of sociality in bats. *BioScience* **58,** 737-746.

Kerth, G., & König, B. 1999 Fission, fusion, and non-random associations in female Bechstein's bats (*Myotis bechsteinii*). *Behavior,* **136,** 1187-1202.

Kerth, G., Perony, N., Schweitzer, F. 2011 Bats are able to maintain long-term social relationships despite the high fission-fusion dynamics of their groups. *Proceedings of the Royal Society B* **278,** 2761-2767.

Koprowski, J.L. 1998 Conflict between the sexes: a review of social and mating systems of the tree squirrels. In: Ecology and Evolutionary Biology of Tree Squirrels. (Steele, M.A., Merritt, J.F., & Zegers, D.A., eds) pp. 33-41. Martinsville, VA: Virginia Museum of Natural History Special Publication 6.

Kunz, T.H. 1982 Roosting Ecology of Bats. In: *Ecology of Bats* (eds. Kunz T.H.) Boston, MA, Springer.

Kunz, T.H., Lumsden, L.F. 2003 Ecology of cavity and foliage roosting bats. In *Bat Ecology* (eds. Kunz, T.H., Fenton, M.B.) pp.1-89. Chicago, IL, University of Chicago Press.

Langwig, K.E., Frick, W.F., Bried, J.T., Hicks, A.C., Kilpatrick, A.M. 2012 Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* **15,** 1050-1057.

Lausen, C.L., & Barclay, R.M.R. 2003 Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. *Journal of Zoology*.**260,** 235-244.

Lausen C.L., & Barclay, R.M.R. 2006 Benefits of living in a building: big brown bats (*Eptesicus fuscus*) in rocks versus buildings. *Journal of Mammalogy* **87,** 362-370.

Lingle, S. 2001 Anti-predator strategies and grouping patterns in white-tailed deer and mule deer. *Ethology.* **107,** 295-314.

Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J., Carey, C., Livo, L., Pessier, A.P., & Collins, J.P. 2006 Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *PNAS* **103,** 3165-3170.

Longcore, J.E., Pessier, A.P., & Nichols, D.K. 1999 *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. *Mycologia* **91,** 219-227.

Lovegrove, B.G., Raman, J, Perrin, M.R. 2001 Daily torpor in elephant shrews (Macroscelidea: *Elephantulus* spp.) in response to food deprivation. *Journal of Comparative Physiology B* **171,** 11-21.

Madison, D.M. 1984 Group nesting and its ecological and evolutionary significance in overwintering microtine rodents. In: Winter Ecology of Small Mammals (eds. Merritt, J.F.) pp. 267-274. Pittsburgh, PA, Carnegie Museum of Natural History Special Publication 10.

Malcolm, J.R. & Marten, K. 1982 Natural selection and the communal rearing of pus in African wild dogs (*Lycaon pictus*). *Behavioural Ecology and Sociobiology* **10,** 1-13.

Maslo, B., Valent, M., Gumbs, J.F., & Frick, W.F. 2015 Conservation implications of ameliorating survival of little brown bats with white-nose syndrome. *Ecological Applications.* **25,** 1832-1840.

McCarty, J.P. & Winkler, D.W. 1999 Relative importance of environmental variables in determining the growth of nestling tree swallows (*Tachycineta bicolor*). *Ibis* **141,** 286-296.

McGowan, A., Hatchwell, B.J. & Woodburn, R.J.W. 2003 The effect of helping behaviour on the survival of juvenile and adult long-tailed tits *Aegithalos caudatus. Journal of Animal Ecology.* **72,** 491-499.

Misra, V., Dumonceaux, T., Dubois, J., Willis, C.K.R., Nadin-Davis, S., Severini, A., Wandeler, A., Lindsay, R., & Artsob, H. 2009 Detection of polyoma and corona viruses in bats of Canada. *Journal of General Virology* **90,** 2015-2022.

Mzilikazi, N., & Lovegrove, B.G. 2002 Reproductive activity influences thermoregulation and torpor in pouched mice, *Saccostomus campestris*. *Journal of Comparative Physiology B.* **172,** 7-16.

Norquay, K.J.O. & Willis, C.K.R. 2014 Hibernation phenology of *Myotis lucifugus. Journal of Zoology (London)* **294,** 85-92.

Nunn, C.L., Jordán, F., McCabe, C.M., Verdolin, J.L., & Fewell, J.H. 2015 Infectious disease and group size: more than just a numbers game. *Philosophical transactions B* **370,** 20140111.

Packer, C., & Ruttan, L. 1988 The evolution of cooperative hunting. *The American Naturalist* **132,** 159-198.

Palmer, J.M., Drees, K.P., Foster, J.T., & Lindner, D.L. 2018 Extreme sensitivity to ultraviolet light in the fungal pathogen causing white-nose syndrome of bats. *Nature communications* **9,** 25.

Patterson, J.E.H., & Ruckstuhl, K.E. 2013 Parasite infection and host group size: a meta-analytical review. *Parasitology* **140,** 803-813.

Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016.

Reeder, D.M., Frank, C.L., Turner, G.G., Meteyer, C.U., Kurta, A., Britzke, E.R., Vodzak, M.E., Darling, S.R., Stihler, C.W., Hicks, A.C., Jacob, R., Grieneisen, LE., Brownlee, S.A., Muller, L.K., & Blehert, D.S. 2012 Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS ONE.* **7,** e38920.

Roulin, A. & Heeb, P. 1999 The immunological function of allosuckling. *Ecology Letters.* **2,** 319-324.

Ryder, J.J., Miller, M.R., White, A., Knell, R.J. & Boots, M. 2007 Host-parasite population dynamics under combined frequency- and density-dependent transmission. *Oikos* **116,** 2017-2026.

Russell, A.F., Clutton-Brock, T.H., Brotherton, P.N.M., Sharpe, L.L., McIlrath, G.M., Dalerum, F.D., Cameron, E.Z., & Barnard, J.A. 2002 Factors affecting pup growth and survival in co-operatively breeding meerkats *Suricata suricatta. Journal of Animal Ecology.* **71,** 700-709.

Shaw, D., Grenfell, B., & Dobson, A. 1998 Patterns of microparasite aggregation in wildlife host populations. *Parasitology* **117,** 597-610.

Silk, J.B., Alberts, S.C., & Altmann, J. 2003 Social bonds of female baboons enhance infant survival. *Science* **302,** 1231-1235.

Silk, J.B. 2007 The adaptive value of sociality in mammalian groups. *Philosophical Transactions of the Royal Society B* **362,** 539-559.

Stawski, C., & Geiser, F. 2010 Fat and fed: frequent use of summer torpor in a subtropical bat. *Naturwissenschaften.* **97,** 29-35.

Treisman, M. 1975 Predation and the evolution of gregariousness. I. Models for concealment and evasion. *Animal Behaviour* **23,** 779-800.

Turner, G.F., & Pitcher, T.J. 1986 Attack Abatement: A model for group protection by combined avoidance and dilution. *The American Naturalist* **128,** 228-240.

Warnecke, L, Turner, J.M., Bollinger, T.K., Lorch, J.M., Misra, V., Cryan, P.M., Wibbelt, G., Blehert, D.S., & Willis, C.K.R. 2012 Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *PNAS.* **109,** 6999-7003.

Webber, Q.M.R. 2016 Personality, sociality and pathogen dynamics in little brown bats. Master of Science Thesis. University of Winnipeg, Winnipeg, Manitoba, Canada.

Webber, Q.M.R. & Fletcher, Q.E. 2017 Viral richness is positively related to group size, but not mating system, in bats. *EcoHealth* doi:10.1007/s10393-017-1276-3.

Weir, J. & Davison, E. 1966 Daily occurrence of African game animals at water holes during dry weather. *Zoologica Africana* **1,** 353-368.

West, S.D., & Dublin, H.T. 1984 Behavioral strategies f small mammals under winter conditions: solitary or social? In: *Winter Ecology of Small Mammals* (Merritt, J.F., ed.) pp. 267-274. Pittsburgh, PA, Carnegie Museum of Natural History Special Publication 10,

White-nose syndrome occurrence map – by year (2017). http://www.whitenosesyndrome.org/resoureces/map

Wilcox, A. & Willis, C.K.R. 2016 Energetic benefits of enhanced summer roosting habitat for little brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. *Conservation physiology* **4,** 1-12.

Wilde, C.J., Knight, C.R., & Racey, P.A. 1999 Influence of torpor on milk protein

composition and secretion in lactating bats. *Journal of Experimental Zoology* **28,** 35-41.

Wilder, A.P., Frick, W.F., Langwig, K.E., & Kunz, T.H. 2011 Risk factors associated with mortality from white-nose syndrome among hibernating bat colonies. *Biology Letters* **7,** 950-953.

Wilkinson, G.S. 1995 Information transfer at evening bat colonies. *Animal Behaviour* **44,** 501-518.

Wilkinson, G.S. 1985 The social organization of the common vampire bat: I. Pattern and cause of association. *Behavioral Ecology and Sociobiology* **17,** 111-121.

Wilkinson, G.S., & Boughman, J.W. 1998 Social calls coordinate foraging in greater spear-nosed bats. *Animal Behaviour* **55,** 337-350.

Willis, C.K.R., & Brigham, R.M. 2003 Defining torpor in free-ranging bats: experimental evaluation of external temperature-sensitive radiotransmitters and the concept of active temperature. *Journal of Comparative Physiology B.* **173,** 379-389.

Willis, C.K.R., & Brigham, R.M. 2004 Roost switching, roost sharing and social cohesion: forest dwelling big brown bats, *Eptesicus fuscus,* conform to the fission-fusion model. *Animal Behaviour* **3,** 495-505.

Willis, C.K.R., & Brigham, R.M. 2007 Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behavioural Ecology and Sociobiology.* **62,** 97-108.

CHAPTER 4: GENERAL CONCLUSIONS

Pathogens of wildlife can affect host survival and reproductive success, which may lead to species declines or even extinctions and loss of overall biodiversity (Daszak *et al.* 2000, Smith *et al.* 2006, Mitchell *et al.* 2008). Host behaviour, like aggregation, can affect an individual's overall pathogen load. Larger aggregations of hosts should have more interactions, which could result in greater pathogen transmission (Altizer *et al.* 2003). High quality habitat, when surrounded by lower-quality habitat, can cause aggregations of conspecifics. These aggregations can bring about many benefits, (e.g., increased predator vigilance and decreased thermoregulatory costs, however, they could also bring about detrimental costs (i.e. greater likelihood of acquiring pathogens.

White-nose syndrome (WNS) has caused drastic population crashes, resulting in regional declines and anticipated regional or global extinction of several species of hibernating bats (Frick *et al.* 2010, Langwig *et al.* 2012). Providing bats with warm microclimates, either by preserving or enhancing existing habitat or by creating artificial habitat, may help survivors recovering from WNS heal more quickly in spring and reproduce faster in summer (Wilcox & Willis 2016). Therefore, warm summer roosts could help populations recover from WNS as long as aggregation at these warm roosts do not increase transmission of *P. destructans* or other harmful pathogens. I tested the over-arching hypothesis that bats prefer roosting in warm microclimates, which enables them to reduce the energetic cost of defending warm body temperatures (T_b) without entering torpor. I also investigated whether the lesser use of torpor may affect their likelihood of acquiring pathogens if it caused them to come into contact with more infected individuals or substrates. My research showed that bats preferred warm roosts and that warm roosts

reduced their use of torpor (potentially allowing them allocate energy to other processes like healing or growth), with no evidence of increased rates of pathogen acquisition.

In Chapter 2, I examined the relationship between habitat quality and torpor use and tested how torpor use could affect the likelihood of acquiring substrate-transmitted pathogen. I tested whether warm roosting habitat would reduce the amount of torpor used by bats. I predicted that bats that had access to warm, artificially-heated roost boxes would employ less torpor than bats with access to only un-heated control roost boxes because of the energetic benefits gained from warm microclimates. Second, tested whether the reduced torpor use would increase the likelihood of acquiring a contact pathogen from an infected substrate, likely because of increased activity. I predicted that bats provided with warm roosts would show greater prevalence and intensity of infection.

I found a positive relationship between habitat quality and torpor expression with individuals using less torpor in warm roosts. At least on the small spatial and temporal scale of my experiment, I found no effect of torpor expression or roost temperature in intensity of infection with ultraviolet fluorescent powder applied to substrates in the experimental environment. From a management perspective, these results suggest that providing artificial high-quality roosts may help survivors of WNS recover and reproduce after emerging from hibernation without increased risk of acquiring pathogens or parasites from infected substrates.

In Chapter 3, I examined the relationship between habitat quality and aggregation, and how they affect torpor expression. I then examined how aggregation and torpor expression affect pathogen transmission. First, I tested whether limited, warm roosts would increase passive aggregation by groups of bats. I predicted that if groups were

given access to limited, warm roosting habitat, that the group size of aggregations would increase. Second, I tested whether limited, warm roosting habitat and/or large aggregations would affect thermoregulation. I predicted that bats in warm roosts and/or with larger groups would use less torpor than those in colder roosts or in smaller groups. Third, I tested whether aggregation and/or torpor use would increase the risk of pathogen acquisition. I predicted that bats roosting in larger groups and/or using less torpor would have greater prevalence and intensity of infection after an infected individual was introduced.

I found that bats disproportionately chose to aggregate in warm roosts. I also found a negative relationship between habitat quality (T_{roost}), group size, and the interaction between T_{roost} and group size, and the normothermy index of bats. As group size increased, the effect of T_{roost} on torpor use decreased. Overall, the greater T_{roost} and the larger the aggregation, the less torpor the bat used, which also follows the predictions of the social aggregation hypothesis. Endotherms that social thermoregulate may benefit from huddling and warm microclimates to the point of reducing the need to enter torpor.

My research has important implications for conservation and wildlife management. My results suggest that bats currently recovering from WNS, and which use anthropogenic structures may benefit from heated roosts, to reduce the cost of maintaining higher T_b, allowing them to invest more into healing, reproduction, and offspring development. Enhancement to forest roosting habitat could also be important for species which do not use artificial structures and my results suggest that significant effort should be undertaken to understand habitat features of forest roosts that might increase roost temperatures. If habitat enhancement can help animals reduce

thermoenergetic costs, then it could help them to invest more energy into recovering from disease, reproducing, and raising young, leading to lower death rates and greater population growth rates. Encouraging the survival and reproduction of WNS survivors should be a conservation priority as evolutionary rescue may be the most viable management option to avoid extinction of bats affected by WNS.

References

Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A., & Dobson, A.P., 2003 Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annual Review of Ecology, Evolution, and Systematics* **34,** 517-547.

Daszak, P., Cunningham, A.A., & Hyatt, A.D. 2000 Emerging infectious diseases of wildlife- threats to biodiversity and Human Health. *Science* **287,** 443-448

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G.G., Butchkoski, C.M., & Kunz, T.H. 2010 An emerging disease causes regional population collapse of a common North American bat species *Science* **329,** 679-682.

Langwig, K.E. Frick, W.F., Bried, J.T., Hicks, A.C., Kunz, T.H., & Kilpatrick, A.M. 2012 Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome *Ecology Letters* **15,** 1050-1057.

Mitchell, K.M., Churcher, T.S., Garner, T.W.J. & Fisher, M.C. 2008 Persistence of the emerging pathogen *Batrachochytrium dendrobatidis* outside the amphibian host greatly increases the probability of host extinction *Proceedings of the Royal Society B.* **275,** 329-334.

Smith, K.F., Sax, D.F., & Lafferty, K.D. 2006 Evidence for the role of infectious disease in species extinction and endangerment *Conservation Biology* **20,** 1349-1357

Wilcox, A. & Willis, C.K.R. 2016 Energetic benefits of enhanced summer roosting habitat for little brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. *Conservation physiology* **4,** 1-12.