DISSERTATION

ECOPHYSIOLOGICAL AND BEHAVIORAL DETERMINANTS OF NICHE RANGE IN HIBERNATING BATS AFFECTED BY WHITE NOSE SYNDROME

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Spring 2022

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ABSTRACT

ECOPHYSIOLOGICAL AND BEHAVIORAL DETERMINANTS OF NICHE RANGE IN HIBERNATING BATS AFFECTED BY WHITE NOSE SYNDROME

The restrictions of a fundamental niche range, physiological conditions under which an organism can persist, becomes increasingly important as populations are subjected to extreme climatic conditions. Hibernating animals are annually subjected to such extremes. For example, insectivorous bats will survive months without caloric intake in winter by lowering body temperature to near freezing to mitigate loss of energy through heat transfer and water through evaporation. However, there is strong overlap between the fundamental niche of hibernating bats and that of the keratinolytic fungus, *Pseudogymnoascus destructans* (*Pd*). As a result of *Pd* growth disrupting wing membranes, hibernating bats are forced to enact frequent energetically costly arousals that can result in starvation and mortality. The resulting disease, white nose syndrome (WNS), has resulted in mass die offs of millions of hibernating bats across North America since *Pd* introduction. However, there is significant inter- and intraspecific variation in host responses, and the realized niche for bat hibernation may be wider and more variable than previously theorized, making host responses difficult to predict. Ecophysiological models predict torpor arousal and hibernation survival with WNS as a function of microclimates, but they are largely dependent on laboratory-based experiments measuring metabolic parameters like metabolic rate and evaporative water loss that are likely subject to intraspecific local variation. We require a better understanding of the physiological, environmental, and behavioral drivers of successful bat hibernation in natural systems with and without *Pd* so we can improve risk assessment and guide management strategies for populations affected by WNS. To better understand how torpor arousal is dependent on experienced microclimates, we attached temperature and humidity data loggers to free-ranging *Eptesicus fuscus* to record microclimates and arousal frequency throughout hibernation. Fitting this data to ecophys-

iological models describing torpor, we found that while ecophysiological models provide adequate boundaries to biological capabilities for arousal, stochasticity inherent in natural systems can lead to earlier and more frequent arousal than models suggest. To determine how hibernation roosting niche is constrained in spatiotemporally variable hibernacula, we measured microclimates throughout a hibernaculum where *Myotis lucifugus* populations have thrived despite regional WNS-related mass mortality. Using hierarchical modeling to predict spatiotemporal underground microclimates based on above-ground conditions, we find that hibernation roosts are likely established early in the hibernation season at microsites that are locally stable within a given hibernaculum chamber, but not necessarily the most stable across the hibernaculum. This suggests that *M. lucifugus* are capable of a more flexible niche space than previously theorized, which may assist in WNS survival. Lastly, we use approximate Bayesian computation to test different hypotheses for how bats survive WNS in this hibernaculum, using ecophysiological models and longitudinal microclimate data to compare local adaptation, microclimate selection, clustering, and grooming strategies. While grooming removal of *Pd* load appears to be essential to describe observed population survival, we find evidence of all four hypotheses contributing to biologically realistic survival. Ultimately, the indirect fundamental niche range contraction due to *Pd* disrupting physiological host processes is mitigated by a combination of adaptation and conspecific facilitation expanding realized niche range. Our work represents advancements in novel technological and modeling advancements that allow evaluation of niche range in free-living populations. The results of this study suggest that there are populations with exaptations that facilitate WNS survival, but that alteration of environmental conditions in other hibernacula could lead to a change in niche space outside the range for which residents are locally adapted. Our findings help to inform and guide assessment of at-risk species and inform potential management strategies by considering the significant individual- and population-level variation in local adaptation and microclimate use that can impact WNS survival.

ACKNOWLEDGEMENTS

I would like to begin by thanking the institutions and individuals who made this work financially possible and logistically feasible. My projects were funded in part by Cooperative Agreement #G16AC00343 between Colorado State University and USGS for Collins Science Center, Ecosystems Mission Area, National Park Service (NPS), U.S. Fish and Wildlife Service (FWS), and Morris Animal Foundation grant #D16ZO-408. Thanks to J. Coleman, C. Kocer, and J. Reichard at FWS, C. Hopkins and B. Reichert at USGS, and S. Baker, M. Evans, G. Jones, M. Davison, S. Fullmer, E. Graves, D. Licht, C. Mather, E. Valencia, and M. Verant at Fort Laramie National Historical Park (FOLA) and NPS for site access, advice, and logistical support, as well as Ted Weller for helping to develop the project and the volunteers who assisted in fieldwork, especially Richard Clark and Angette Pastuszek. I thank the New York Department of Environmental Conservation (NYDEC) for allowing access to the study hibernaculum, and the numerous NYDEC personnel and volunteers who assisted with data logger placement and recovery, especially Carl Herzog, Casey Pendergast, and Melanie Berger. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. *[Because this manuscript has not yet been approved for publication by the U.S. Geological Survey (USGS), it does not represent any official USGS finding or policy.]*

I have benefitted from the mentorship and kindness of many individuals since starting at Colorado State University. In particular, thank you to Kevin Castle for encouraging this budding wildlife veterinarian, Shweta Bansal for welcoming me to the East Coast with an academic home away from home, and my committee members David Hayman and Kate Huyvaert for providing helpful feedback, guidance, and mentorship. I am hugely grateful for Paul Cryan's mentorship and willingness to let me helm this project with him. His positive can-do attitude made the long nights mixing cement in dingy hotel bathrooms a much more fun experience.

A big thank you goes out to all of the friends I made through the graduate program, including David Markman, Whitney Beck, Alison Post, Rick Trippe, Craig Marshall, Kim Dolphin, Brady,

Xoco Shinbrot, Rob Griffin-Nolan, and the many members of the Webb lab who have provided invaluable feedback and support over the years: Clint Leach, Lindsay Beck-Johnson, Tanya Dewey, Clif McKee, Clay Hallman, Erin Gorsich, Ryan Miller, Tatiana Xifara, E Schlatter, Gabe Gellner, Mark Wilbur, Deedra Murrieta, Katie Owers, Brooke Berger, Kendra Gilbertson, Rachel Oidtman, Brandon Simony, Sam Smith, and Catherine Herzog.

I will forever be hugely grateful for my advisor, Colleen Webb, who bravely took the chance to accept a quantitative ecology graduate student whose previous training in mathematics and ecology was minimal to none. You have always been an exemplary mentor, providing guidance and friendship with patience and kindness. I still marvel at my luck for stumbling into your lab, and look forward to our continued interactions because like many Webb lab members, I see no reason to really leave.

None of this would have been possible without the love and support of my partner, Kate Huebner. You always push me to be the best I can, while also making sure we take the time to find new adventures. Your spirit, passion, and drive are inspirational, and I am remarkably grateful to have you by my side through all our journeys.

Finally, last and least (in size), Taegan. You made finishing this dissertation infinitely more difficult, but you gave me infinitely more drive to complete and continue to make life infinitely more fun.

DEDICATION

To Kate. Let this mark the beginning of many more adventures to come.

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Chapter 1

Introduction

We generally regard an organism's niche as being comprised of physiological requirements for survival (fundamental niche) and biotic interactions changing niche range (realized niche) (Hutchinson, 1957). The realized niche concept was famously demonstrated by experiments involving interspecific competition excluding species growth in areas where they would otherwise thrive (Connell, 1961). Less commonly do we consider biotic interactions that could potentially expand niche range (Bruno *et al.*, 2003). When niche range is constrained by external stressors, it is worth considering how populations persist by maintaining or expanding their niche range to overcome potential extirpation. The concepts of niche range constriction and expansion can be difficult to demonstrate in natural systems due to an overwhelming number of potential niche axes and inherent stochasticity. However, we can limit this overwhelming dimensionality by examining natural systems wherein external influences are limited and stability is more the rule than the exception, such as subterranean systems (Perry, 2013; Mammola *et al.*, 2019b). Due to limited influences external to the system, the issue of white nose syndrome (WNS), a disease caused by the fungus *Pseudogymnoascus destructans* (*Pd*) infecting insectivorous bats (Blehert *et al.*, 2009; Frick *et al.*, 2010), is an ideal scenario for us to examine how host niche range can exist outside of overlapping pathogen niche to improve survival outcomes.

WNS emerged in 2006, causing mass mortality in a hibernaculum in upstate New York, and has since spread across North America (Blehert *et al.*, 2009). *Pd* was likely introduced from continental Eurasia and spread at least in part by anthropogenic activity (Reynolds & Barton, 2013; Drees *et al.*, 2017). Inhabiting cavern walls and persisting for years, *Pd* is often assumed to be pervasive throughout a hibernaculum when it is introduced (Verant *et al.*, 2012). There is significant variation in inter- and intra-specific host response to *Pd*, but depending on the population, mortality rates can be as high as 100% in some hibernacula (Frick *et al.*, 2010). As a result, millions of bats have died, and species like *Myotis lucifugus*, once one of the most widespread and populace mammals in

North America, are now experiencing local extirpation and looming endangerment (O'Shea *et al.*, 2016; Cheng *et al.*, 2021). This is a problem not only because *Pd*'s generality allows infections of dozens of different bat species, but also because these bats provide important ecosystem services. Estimates suggest that insectivorous bats save United States agricultural practices billions of dollars worth of pest removal by consuming common crop pests (Boyles *et al.*, 2011). The interlaced impacts on animals, ecosystems, and humans make WNS a One Health issue (Zinsstag *et al.*, 2011), suggesting the need for an integrated multidisciplinary approach to establish holistic management solutions.

To develop effective management solutions, we need to better understand the biotic and abiotic factors that lead to variation in host responses and outcomes. More simply put: why do some bats die while others appears unaffected? Studies suggest that WNS is largely a problem of niche overlap between *Pd* and bat hosts (Langwig *et al.*, 2012; Hayman *et al.*, 2016). *Pd* is a psycrophilic keratinolytic fungus, growing on skin and digesting proteins in relatively cold temperatures (Verant *et al.*, 2012). Then, when bats lower body metabolism in torpor and body temperature drops to near ambient temperature (Twente, 1955; Davis & Reite, 1967), bat hosts essentially become petri dishes for *Pd* growth (Lorch *et al.*, 2011). As such, if bats hibernate in temperature and humidity (henceforth "microclimates") that are ideal for *Pd* growth, the more likely they are to succumb to WNS. When bats are badly affected, the disease manifests as increased arousal from hibernation (Reeder *et al.*, 2012). These arousals are energetically costly; one study suggests that *M. lucifugus* arousal from torpor may cost as much energy as 67 days spent in torpor (Thomas *et al.*, 1990). As a result, when bats arouse too frequently, they die of starvation or cold exposure as they leave the hibernaculum midwinter to seek out insect food sources that are seasonally absent (Reeder *et al.*, 2012).

There are multiple hypotheses for why bats arouse from torpor, but two prominent mechanistic models parsimoniously describe arousal frequency both with and without *Pd* as functions of ambient temperature and humidity (Hayman *et al.*, 2016; Haase *et al.*, 2019a). They suggest that arousal is triggered when limits to energy or water loss are reached, processes that are accelerated

by *Pd* disruption of skin membranes, and that mortality is likely when bat physiological requirements for hibernation strongly overlap with *Pd*'s physiological requirements for growth (Hayman *et al.*, 2016). While these models have success in describing WNS mortality patterns in a limited number of populations, there are limits to their widespread application for risk assessment and management purposes.

First, most of what we know about torpor physiology has been developed under experimental laboratory measurements (Hock, 1951; Hanus, 1959; Speakman *et al.*, 1991; Haase *et al.*, 2019a). The physiological traits that these models portray as parameters are not easily measured, requiring advanced equipment and significant time to develop accurate results and enclosing bats in controlled environments to make precise measurements (Lighton & Halsey, 2011). This removes the processes from important environmental context, diminishing relevance to natural systems, and trying to reproduce such experiments for the many species of interest while accounting for population-specific variation in traits is intractable. We require means to feasibly estimate important physiological parameters within their environmental context in a way that is specific to populations of interest.

Second, these models have been applied and tested under the common assumption that hibernating bats seek out “optimal" stable conditions for hibernation (Elliott & Clawson, 2001; Tuttle & Kennedy, 2011), such that averages of temperature and humidity are sufficient to describe experienced microclimates (Clawson *et al.*, 1980; Nagel & Nagel, 1991; Langwig *et al.*, 2012). While these assumptions may hold true for some bat populations (Haase *et al.*, 2019a), other recent studies show significant microclimate variation in roosting sites used for hibernation (Boyles *et al.*, 2017; Ryan *et al.*, 2019). Longitudinal hibernation microclimate data throughout hibernacula are difficult to gather due to electrical equipment malfunction from prolonged exposure to high humidity (Kurta *et al.*, 1990; Verant *et al.*, 2012; Boyles *et al.*, 2017), but it may be important to capture mid-hibernation microclimate shifts and extremes that could significantly alter energetic expenses and WNS outcomes. Thus, we require a better understanding of the conditions under which hibernation roosts are established, and how those conditions can change over time and impact survival.

Third, we need to begin incorporating these hypotheses into a broader environmental context that accounts for natural system complexity. By testing these hypotheses in conjunction with other hypotheses that may contribute to WNS survival, we can compare the driving forces that impact outcomes. In addition to microclimate use (Hayman *et al.*, 2016; Haase *et al.*, 2019a), other behaviors, such as clustering (Langwig *et al.*, 2012; Hayman *et al.*, 2017) and grooming (Brownlee-Bouboulis & Reeder, 2013) have been implicated in affecting WNS outcomes individually, but not in combination. Given that these behaviors all could have implications in physiological processes, it makes sense to examine them within a common framework.

In this dissertation, I aim to define the hibernating bat niche space that is relevant to populations at risk of WNS. To do so, I integrate unprecedentedly detailed microclimate data and novel modeling approaches to isolate biologically relevant processes and interactions in a complex natural system (Restif *et al.*, 2012) without invasive handling or disturbance of hibernacula during energetically critical periods (Speakman *et al.*, 1991). Each chapter helps to define bat niche space by developing our understanding of ecophysiological interactions between bat hosts and their detailed hibernation microclimate environments. In Chapter 2, I study a population of *Eptesicus fuscus* that has not yet been exposed to *Pd* to develop baseline understandings of the limits to normal torpor. In Chapters 3 and 4, I consider a New York hibernaculum that has become a refuge for *M. lucifugus* hibernation. Despite regional population die offs, this hibernaculum has not experienced significant WNS-related mortality, which makes it an excellent case study for how bats use available microclimates to survive WNS.

Chapter 2 establishes the importance of variance in torpor behavior and metabolic traits in free-ranging individuals as compared to experimentally-derived observations. I use unique data of microclimates used paired with torpor bout lengths for the entire hibernation period in *E. fuscus*. By using these data to inform a Bayesian hierarchical ensemble model, I am able to simultaneously determine which environmental cues are driving arousal frequency and estimate metabolic trait values. By limiting our niche investigation to the individual's experienced environmental con-

ditions, I can evaluate how expected arousal frequency can change with environmental use and how natural system stochasticity affects hibernation niche space.

In Chapter 3 I consider the driving factors for roost establishment in a hibernaculum with spatiotemporally variable microclimates. By examining an enclosed subterranean system in its entirety, I am able to infer conditions that draw bats to common hibernation microsites, as well as the conditions that drive bats away from unused sites. Hibernaculum microclimate availability is sure to change over time as climate change results in warmer surface temperatures, so I developed a hierarchical model wherein surface temperature is used to predict underground temperatures throughout the hibernaculum, which is in turn used to inform roost establishment. In so doing, I develop a predictive model of spatial hibernation niche as a function of climatic changes influencing microclimate availability.

While Chapter 3 establishes the spatial niche of experienced microclimates, Chapter 4 investigates how those microclimates interconnect with other potential behavioral and physiological drivers of WNS survival. While *Pd* constricts host niche space, hosts may be able to counteract this by using niche space outside of *Pd* niche range, *Pd* mitigation to retain niche space, or conspecfic facilitation to expand niche space. In order to learn how bat hosts are interacting physiologically with their environment to allow survival, I develop a suite of models representing different combinations of behavioral and physiological hypotheses for why this population is so robust. Comparing these, I find evidence that no one mechanism can represent the system in a biologically realistic way, but due to physiological interactions between mechanisms, a combination can represent natural system observations.

In summary, we build our representation of free-ranging hibernating bat niche space by developing our expectations of physiological consequences of environment on bat torpor in sequentially larger capacities. First, we relate the microclimates that individuals experience to torpor length expectations (Chapter 2). Then, we examine the larger hibernaculum space to compare microclimates that bats do and do not use for hibernation, as well as how these microclimates can change with climatic shifts (Chapter 3). Following this, we examine the interactions between environment, host

physiology, pathogen physiology, and host behavior within the context of the larger hibernaculum (Chapter 4). In so doing, we further advance our understanding of how niche space can come to be defined in free-ranging populations, how pathogens can exploit this niche space, and how hosts can counteract that exploitation.

Chapter 2

Ecophysiological models describe biological limits to hibernating bat behavior

2.1 Introduction

Torpor, the lowering of metabolic rate to reduce energy expenditure, is a common life history strategy found in a wide diversity of mammalian and avian clades (Ruf & Geiser, 2015). Torpor is sometimes seen as a strategy of survival; populations capable of mitigating energy requirements during extreme conditions of stress or scarcity may be more capable of avoiding extirpation (Geiser & Turbill, 2009; Nowack *et al.*, 2017). Whether to bypass times of scarcity or to avoid time periods of active predation, some animals are capable of lowering energy use to an impressively scant one percent of euthermic basal metabolic rate (Geiser & Ruf, 1995). Torpor strategies generally fall into two distinct categories: daily torpor, which represents often periodic shifts in metabolic rate within a twenty-four hour period, and hibernation, which is marked by a series of elongated torpor bouts that can last as long as days, weeks, or even months under the right conditions (Geiser, 2004). Because of the greater physiological demands of longer torpor, seasonal hibernators may be at increased risk of extirpation due to anthropogenic changes, such as range constriction associated with climate change or mortality from human-introduced diseases (Humphries *et al.*, 2002; Blehert *et al.*, 2009; Frick *et al.*, 2010; Sherwin *et al.*, 2013; Nowack *et al.*, 2017). It is important that we understand the physiological trade-offs of balancing energetic constraints with anthropogenic pressures in elongated torpor (Humphries *et al.*, 2003), so that we can improve our expectations of population survival in the face of changing environments.

We can further examine how hibernation survival can be impacted by anthropogenic change by considering hibernating bats. In temperate climates, many insectivorous bat species hibernate through cold winter months to avoid food scarcity, rather than migrating to warmer temperatures

(Humphries *et al.*, 2002). These heterothermic endotherms practice astounding feats of metabolic control. In bouts of torpor, they lower body temperature to be closer to ambient temperature, with some species dropping to near freezing to mitigate energetic loss through heat exchange (Davis & Reite, 1967). However, if ambient temperature drops below the lowest body temperature that hibernators can maintain, energetic costs increase (Hock, 1951; Hanus, 1959), and even in ideal conditions, animals cannot maintain torpor indefinitely. Whether to hunt scant winter insects (Avery, 1985), replenish water stores (Thomas & Geiser, 1997), or in response to external stimuli (Speakman *et al.*, 1991) or an internal "biological alarm clock" (Twente & Twente, 1987), bats, like many hibernating species, must arouse periodically mid-hibernation, increasing body temperature to euthermic conditions that allow normal activity. One estimate for *Myotis lucifugus* suggests several hours of arousal is equivalent to as much as 67 days of hibernation in terms of energy use (Thomas *et al.*, 1990). This is similar to non-bat extreme hibernators. Despite spending more than 90% of hibernation in deep torpor, *Urocitellus richardsonii* spend only 17% of their total hibernation energy budget on torpor, compared to 19% used for the act of arousal. (Wang, 1979). Thus, arousal is a major source of energy store depletion during hibernation (Thomas, 1995). Understanding why and how frequently hibernators arouse from torpor mid-hibernation can help us predict the conditions under which a bat has enough energy to survive hibernation through winter.

The need to characterize mechanisms driving bat torpor in particular has become urgent since humans introduced the fungus *Pseudogymnoascus destructans* (*Pd*) to naive hibernating bat populations (Reynolds & Barton, 2013). The resulting disease, white-nose syndrome (WNS), can cause up to 100% mortality in some affected populations (Frick *et al.*, 2010; Cheng *et al.*, 2021). In response, researchers have developed multiple models to assess population mortality risk based on torpor duration as a function of microclimates, local ambient environmental conditions (e.g., temperature or humidity), that can vary spatially and temporally throughout hibernacula (Langwig *et al.*, 2012; Hayman *et al.*, 2016; Haase *et al.*, 2019a). WNS is caused by the keratinolytic fungus *Pseudogymnoascus destructans* (*Pd*) growing on hibernating bat skin in cool, wet microclimates

(Blehert *et al.*, 2009; Verant *et al.*, 2012). Infection can increase arousal frequency, potentially leading to starvation (Reeder *et al.*, 2012), but the mechanism behind this increased frequency is yet unknown. Proposed hypotheses include dehydration, energy loss, thermoregulatory disruption, circulatory or respiratory disruption, and immunological responses (Cryan *et al.*, 2010; Puechmaille *et al.*, 2011; Reeder *et al.*, 2012; Cryan *et al.*, 2013), though not all of these mechanisms are relevant to describing torpor bout duration in absence of disease. Two prominent models describe separate parsimonious hypotheses for bat torpor bout duration both with and without WNS (Hayman *et al.*, 2016; Haase *et al.*, 2019a). They represent the hypotheses using broadly relevant mechanistic equations linking torpor arousal frequency with environmental conditions and host and pathogen traits. The first model (henceforth the "energetics model") makes arousal frequency dependent on temperature-driven metabolism, with the rate of energy loss driving arousal (Hayman *et al.*, 2016). The second model (henceforth the "hydration model") describes arousal frequency as a function of evaporative water loss; when bats lose a certain percentage of body weight to evaporation, they arouse to rehydrate (Haase *et al.*, 2019a). In both, environmentally-dependent fungal growth accelerates these processes, increasing arousal frequency. Bats hibernating in microclimates where they survive uninfected but exhaust energy stores with *Pd* are using 'ecogological traps' (Battin, 2004; Leach *et al.*, 2016). Bats hibernating in microclimates that leave them with excess available energy after hibernation are using 'survival space.' Both models hold promise for predicting microclimate-based bat hibernation activity (Hayman *et al.*, 2016; Haase *et al.*, 2019a), but neither have been validated using field data of natural torpor bouts in free-ranging bats. To further develop predictions of torpor bout duration in natural systems, we require methodology that accounts for multiple arousal mechanisms and data representing natural torpor behavior and corresponding microclimates.

In order to gain better understanding of the physiological trade-offs present in extended torpor, we must bridge the gap between experimentally-derived information and natural observations, which can be challenging. Most of what we understand about torpor bout duration has been developed under laboratory conditions in controlled environments (Hock, 1951; Hanus, 1959; Speak-

man *et al.*, 1991; Armitage *et al.*, 2003; Armitage & Woods, 2003; Karpovich *et al.*, 2009; Haase *et al.*, 2019a). Parameters controlling model expectations, such as thermal conductance, metabolic rate, and evaporative water loss rate, are by necessity measured in enclosed spaces (e.g., respirometry chambers) under standardized environmental conditions to improve measurement precision (Lighton & Halsey, 2011). There have been few attempts to measure metabolic output in free-ranging hibernators directly (Wang, 1979; Kurta *et al.*, 1989a,b, 1990; Stenvinkel *et al.*, 2013). Controlled laboratory experiments improve our understanding of torpor physiology and are valuable in formulating and testing hypotheses (McGuire *et al.*, 2017), but there are barriers to applying these studies to natural systems and free-ranging animal behavior. For example, big brown bats (*Eptesicus fuscus*) are one of the most widespread mammals in North America, and studies have demonstrated significant gradients of morphological and natural history variation (Burnett, 1983; Neubaum *et al.*, 2007; Dunbar & Brigham, 2010; O'Shea *et al.*, 2011). Estimated values for physiological parameters derived from laboratory conditions do not account for population-specific variation in these parameters or individual-level variation in behavioral choices. Furthermore, natural systems are stochastic, with changing external influences difficult to predict or simulate in artificial settings. This stochasticity complicates direct inference from laboratory-developed model estimations to naturally occurring field observations. Thus, when applying ecophysiological models to field observations we should use methods that account for this increased variation in a meaningful way (Restif *et al.*, 2012).

Field-gathered observations of natural systems are often more limited than experimentally-derived data. Challenges to collecting observations of natural torpor bout duration and microclimate use make field validation of ecophysiological models difficult to achieve. For example, difficulty of accessing hibernacula (Olson *et al.*, 2011; Wainwright & Reynolds, 2013), intermittently moving individuals (Whitaker Jr & Rissler, 1992), changing environmental conditions relevant to ecophysiology (Boyles *et al.*, 2017), and humid environments that degrade electronic equipment (Kurta *et al.*, 1990; Verant *et al.*, 2018) all work against researchers studying free-ranging animal behavior. Furthermore, direct observation of activities and environmental conditions mid-

hibernation requires humans entering hibernacula, which can result in arousal and further depletion of essential energy stores (Speakman *et al.*, 1991), a major concern for WNS-susceptible populations. However, challenge breeds ingenuity, and bat researchers specifically have developed a number of methods for studying bats that increasingly take these difficulties into account (Castle *et al.*, 2015). As a result of these scientific advances, we can attach miniaturized data loggers that record environmental conditions to bats for entire hibernation seasons and longer, yielding data on microclimate use and torpor bout duration in free-ranging bats.

Here we bridge the laboratory-field gap for understanding physiological trade-offs of hibernating species by reparameterizing models describing observed torpor bout duration in free-ranging *E. fuscus* as a function of hibernation microclimates measured using novel technology. We use parameter sensitivity analysis to guide parameter estimation via a Bayesian hierarchical ensemble model that tests the relative importance of the energetics and hydration mechanisms under heterogeneous environmental conditions. With the reparameterized model, we predict torpor bout duration in a more relevant way for free-ranging populations. We then recontextualize our findings by applying our new parameters to the models investigating WNS mortality to inform risk assessment of a population not yet exposed to *Pd*. Our approach creates a better understanding of the limits of ecophysiological models' predictive capabilities while significantly reducing prediction variance by accounting for individual variation. Our findings inform us on how microclimates influence the mechanisms driving torpor behavior, and by extension survival of winter hibernation, informing risk assessment of populations susceptible to anthropogenic changes like WNS.

2.2 Materials and Methods

2.2.1 Data collection and interpretation

We expand on previous studies that attached temperature data loggers to bats to approximate hibernation activity (Jonasson & Willis, 2012; Reeder *et al.*, 2012) by incorporating humidity, the next most vital microclimate aspect suspected to drive arousal (Cryan *et al.*, 2010; Willis *et al.*,

2011; Cryan *et al.*, 2013; McGuire *et al.*, 2021b), and measuring microclimate through the entire hibernation season. Data logger development and methodology is discussed further in Appendix A.1. In short, we attached miniaturized data loggers recording hourly temperature and relative humidity (RH) to bats using a mattress suture technique developed by Castle *et al.* (2015) (Figure 2.1.a). We used a monofilament absorbable suture such that suture would eventually degrade and data loggers detach from unrecoverable bats.

We chose to study a summer colony of adult female *E. fuscus* because they are capable of robust hibernation under a wide range of microclimates with or without WNS (Hayman *et al.*, 2016). The colony had no known *Pd* exposure. We captured bats in mist nets as they emerged from a human-built structure in Goshen County, Wyoming, and stored them in cloth bags until we attached data loggers under anesthesia on site. We recorded body mass, forearm length, sex, and reproductive status, and a physical exam was performed by veterinarians prior to anesthesia. Following anesthesia, bats were released when flight-capable, typically within fifteen minutes. Initial captures occurred in Summer 2018, and because hibernation location was unknown, recapture attempts occurred at the same site in late Spring and Summer of 2019. We cancelled subsequent recapture attempts due to the SARS-CoV-2 pandemic. We released 100 bats with data loggers in 2018 and recaptured 3 bats in 2019. It is unknown whether low recapture rates were due to WNS-related mortality, low annual roost fidelity, suture failure and device loss, or device-related mortality. However, there was no noted significant pathology for any recaptured individuals in this study or others using similar methods (Castle *et al.*, 2015). Upon recapture, we removed data loggers without anesthesia. Bat handling and data logger attachment protocols were approved by the USGS Fort Collins Science Center Institutional Animal Care and Use Committee (FORT-IACUC_2017-06) and Wyoming Game and Fish Department (permits 33-1190_2018 and 33-1190_2019).

We interpreted data logger output to describe relevant microclimate measurements and define periods of torpor and euthermia. Our data loggers recorded a combination of skin and ambient temperature, which we assume in torpor to be equal to ambient temperature (T_a). In small mammals, skin temperature can be used to approximate body temperature (T_b) (Audet & Thomas,

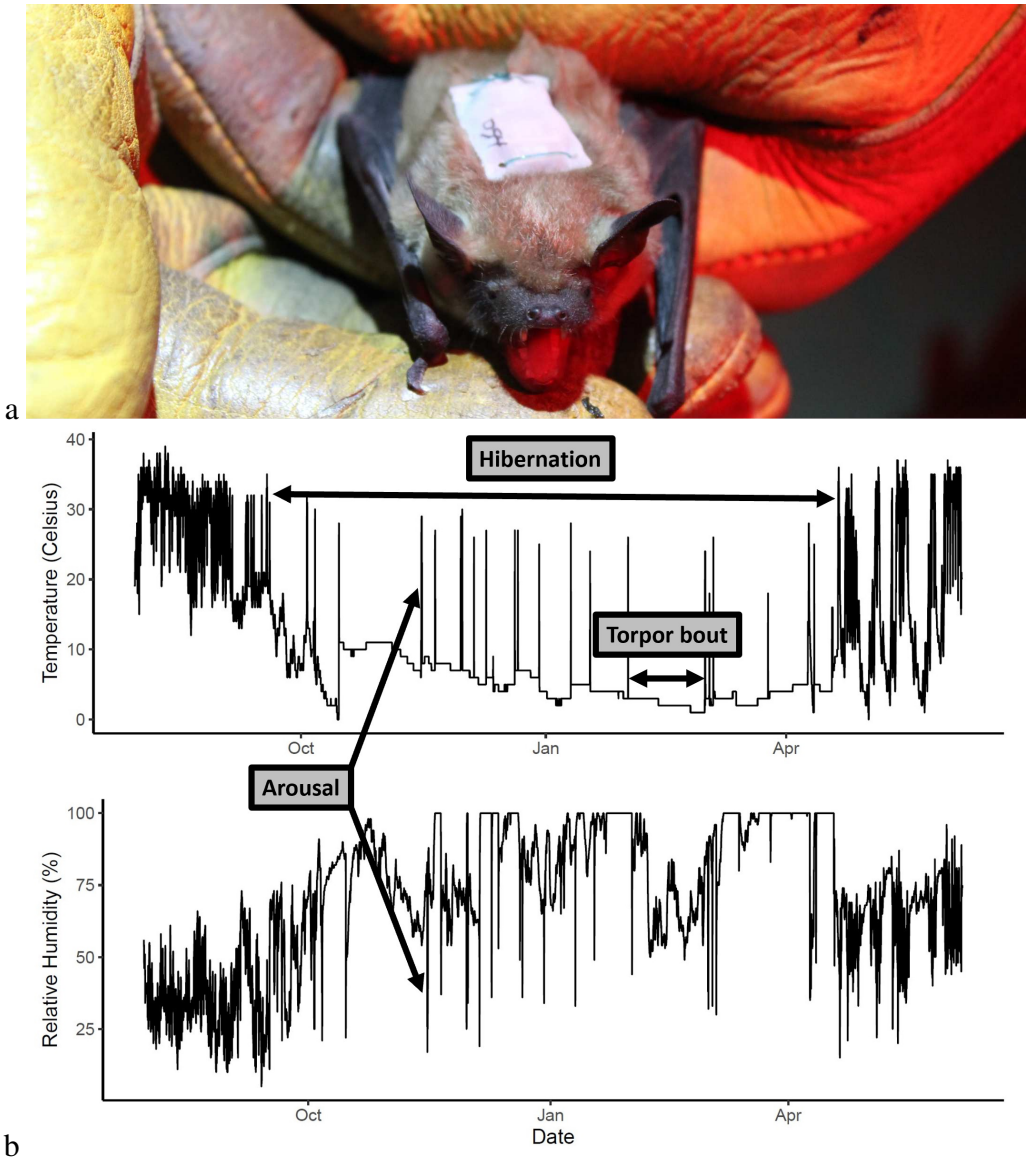


Figure 2.1: (a) Big brown bat (*Eptesicus fuscus*) with a temperature/humidity data logger attached using a mattress suture. (b) Example of recorded temperature (top) and relative humidity (bottom) data from a single bat. We mark examples of hibernation, torpor, and arousal as they pertain to our data interpretation. The beginning and end of hibernation are marked by multiple consecutive days of consistently low body temperature with no daily arousal to feed. Hibernation is split into distinct torpor bouts, extended periods of low body temperature, by periods of arousal wherein body temperatures rise to euthermic levels, recorded by our data loggers as a rise in skin temperature. Arousal results in an artificial sudden drop in recorded relative humidity due to relative humidity being calculated by loggers as the percent ratio of measured water vapor pressure over maximum potential saturated vapor pressure, which increases with temperature.

1997; Barclay *et al.*, 1996; Willis & Brigham, 2003). Thus, above the bat's minimum maintained body temperature ($T_{tor,min}$), we assume that in torpid bats, body temperature is equal to recorded temperature is equal to ambient temperature, similar to previous validations of the energetics and hydration models (Hayman *et al.*, 2016; Haase *et al.*, 2019a). When ambient temperature drops below the $T_{tor,min}$ threshold, bats expend energy to maintain body temperature at $T_{tor,min}$ (Hayman *et al.*, 2016; Haase *et al.*, 2019a). We use sudden changes in recorded temperature to delineate arousal, euthermia, cooling, and torpor (Figure 2.1.b) (Jonasson & Willis, 2012). Consistently low temperatures over an extended period represent torpor; torpor bouts are interrupted by sudden increases in temperature associated with increased skin temperature during arousal. When recorded temperature increases suddenly by 5 or more degrees, we interpret this as a warming event and mark the previous hour as the beginning of arousal (Jonasson & Willis, 2012). Similarly, we mark the beginning of the cooling period as the hour before the last greater than five degree drop in temperature occurs following euthermia. Euthermia is the time period between warming and cooling, and torpor is the time period between cooling and warming.

Data loggers also recorded hourly RH, which we converted to water vapor pressure (WVP) using ambient temperature to define saturated vapor pressure and multiplying by the percent RH (Equation 2.1) (Campbell & Norman, 2012; Haase *et al.*, 2019a). Sudden downward spikes in RH coinciding with arousal periods (Figure 2.1.c) are data loggers spuriously converting increased skin temperature and measured WVP to reported RH, so only values recorded during torpor were used to reflect ambient WVP (WVP_a). Per the hydration model, we assume that bat skin WVP is equal to saturated vapor pressure for the bat's body temperature (Campbell & Norman, 2012; Haase *et al.*, 2019a). The WVP deficit (ΔWVP) is then calculated as skin WVP minus ambient WVP. We used each torpor bout's mean T_a and WVP_a as input for the energetics and hydration models.

$$WVP_a = 0.611e^{\frac{17.503*T}{T+240.97}} * RH \tag{2.1}$$

2.2.2 Model structure and parameter estimation

Standard procedure for parameterizing an initial biological model is to use values derived from relevant literature, e.g., Hayman *et al.* (2016). When we used the logger-recorded torpor microclimates as input for the energetics and hydration models, literature-based parameterizations did not accurately describe *E. fuscus* torpor bout duration variation, with no significant correlation between observed torpor bout durations and model predictions (see Appendix Figure A.1). Recognizing that the model structures were validated for different species and populations than our study population, whereas regionally specific variation in metabolic functions exists (Dunbar & Brigham, 2010), we fit the models while allowing for variation in parameters to which torpor bout duration is sensitive. Thus, for each ecophysiological model, we performed partial rank correlation coefficient (PRCC) parameter sensitivity analysis using Latin hypercube sampling (Marino *et al.*, 2008) with torpor bout duration as the dependent variable to determine which parameters to estimate. Given that we were working with a complex non-linear model containing known correlation between some parameters (e.g., body mass and metabolic rate (Kleiber *et al.*, 1932)), we estimated only the most sensitive parameters to avoid identifiability problems.

To estimate population- and individual-specific values and variation in these parameters, we used a Bayesian hierarchical ensemble model (Figure 2) that calculates torpor bout duration for both ecophysiological models (Equations 2.2 and 2.3 for the energetics and hydration models, respectively, see Table 2.1). We used initial 2018 capture mass to represent body mass (M_{body}). To identify the relative importance of the energetics and hydration hypotheses under a range of microclimates, we estimated time in torpor (t_{torpor}) as a weighted average of the energetics and hydration models, where the percent contribution of each (π for energetics, $1 - \pi$ for hydration) is dependent on environmental factors (Equation 2.4). Temperature data were centered and scaled in Equation 2.4 for ease of fit. We add time to cool from euthermic to torpid body temperature (t_{cool}, Equation 2.5) and time to warm to euthermic temperature (t_{warm}, Equation 2.6) (Haase *et al.*, 2019b) to t_{torpor} to estimate the maximum potential time for a bout of torpor in a given microclimate (t_{limit}, Equation 2.7).

Table 2.1: Parameters for the energetics and hydration models for big brown bats (*Eptesicus fuscus*), their literature-based values, and distributions used for estimation.

Parameter	Definition and units	Value	Prior Distribution	Reference
$T_{tor,min}$	Minimum body temperature maintained in torpor (°C)	3.5	Uniform(0.5, 5)	(Hanus, 1959; Hayman *et al.*, 2016)
TMR_{min}	Torpid metabolic rate (ml O_2 g^{-1} $hour^{-1}$)	0.028	Gamma($\mu = 0.03, \sigma = 0.05$)	(Hanus, 1959; Hayman *et al.*, 2016)
C_t	Torpid conductance (ml O_2 g^{-1} $°C^{-1}$)	0.055	Uniform(0.01, 0.2)	(Hock, 1951; Hayman *et al.*, 2016)
C_{eu}	Euthermic conductance (ml O_2 g^{-1} $°C^{-1}$)	0.2		(Halsall *et al.*, 2012; Haase *et al.*, 2019b)
S	Tissue-specific heat capacity (ml O_2 g^{-1} $°C^{-1}$)	0.131		(Thomas *et al.*, 1990; Hayman *et al.*, 2016)
Q_{10}	Change in torpor metabolism	$1.6 + 0.26T_a - 0.006T_a^2$		(Hock, 1951)
$t_{tor,max}$	Maximum torpor duration (hours)	792	Uniform(maximum observed, 1000)	(Brack Jr & Twente, 1985)
M_{body}	Body mass (g)			Measured in this study
SA_{body}	Body surface area (cm^2)	$10 * M_{body}^{2/3}$		(Gouma *et al.*, 2012)
$percent_{fat}$	Percent of M_{body} that is fat tissue	0.3		(Hayman *et al.*, 2016)
SA_{wing}	Wing surface area (cm^2)	$SA_{body} * \frac{19.68}{39.36}$		Calculated as the wing/body ratio reported in (Haase *et al.*, 2019a)
$rEWL_{body}$	Rate of cutaneous evaporative water loss from the body (mg hr^{-1} ΔWVP^{-1} cm^{-2})	0.027	Gamma($\mu = 0.1, \sigma = 0.1$)	see Appendix A.2 (Klüg-Baerwald & Brigham, 2017; Haase *et al.*, 2019a)
$rEWL_{wing}$	Rate of cutaneous evaporative water loss from the wings (mg hr^{-1} ΔWVP^{-1} cm^{-2})	$rEWL_{body} * \frac{0.1}{0.33}$		Calculated as a similar wing/body ratio to (Haase *et al.*, 2019a)
T_{eu}	Euthermic body temperature (°C)	37		(Halsall *et al.*, 2012)
WR	Warming rate (°C $hour^{-1}$)	90		(Halsall *et al.*, 2012)

$$t_{tor,energetics} = \begin{cases} t_{tor,max}/Q_{10}^{\left(\frac{T_a - T_{tor,min}}{10}\right)} & T_a > T_{tor,min} \\ \frac{t_{tor,max}}{1+(T_{tor,min}-T_a)*\left(\frac{C_t}{TMR_{min}}\right)} & T_a \leq T_{tor,min} \end{cases} \tag{2.2}$$

$$\begin{aligned} EWL_{\text{cutaneous}} &= (SA_{body} * rEWL_{body} + SA_{wing} * rEWL_{wing})\Delta WVP \\ t_{tor,WVP} &= \frac{0.027 * M_{body}(1 - percent_{fat}) * 1000}{EWL_{\text{cutaneous}} + \frac{TMR_{min} * M_{body}}{0.2095 * 0.3 * 1000} * \frac{\Delta WVP}{0.46152(273.15+T_a)}} \\ t_{tor,hydration} &= \min(t_{tor,energetics}, t_{tor,WVP}) \end{aligned} \tag{2.3}$$

$$\begin{aligned} \beta_k &\sim \text{Normal}(0, 1) \\ \pi &= \text{logit}^{-1}(\beta_0 + \beta_1 T_{a,scaled} + \beta_2 \Delta WVP + \beta_3 T_{a,scaled} \Delta WVP) \\ t_{torpor} &= \pi t_{tor,energetics} + (1 - \pi) t_{tor,hydration} \end{aligned} \tag{2.4}$$

$$t_{cool} = \frac{\log(T_{eu} - T_{tor})}{C_{eu} * M_{body}^{0.67} * \frac{\log(T_{eu} - T_a)}{S * M_{body}}} \tag{2.5}$$

$$t_{warm} = (T_{eu} - T_a)/WR \tag{2.6}$$

$$t_{limit} = t_{cool} + t_{torpor} + t_{warm} \tag{2.7}$$

For parameter estimation, we used published literature estimates as informative priors and allowed for biologically meaningful variation by confining values to within an order of magnitude of the literature estimate (Table 2.1). We drew population values from uniform distributions, and then used this population value as a mean for a Gamma distribution to estimate individual-specific values. This enhances our understanding of what regional variation might differentiate our population from those previously sampled while also accounting for individual variation.

Allowing variation in parameterization of the ecophysiological models, we still found that fitting the models directly to torpor bout duration data yielded poor fits ignoring large portions of variance in the observed data (see Appendix Figure A.2). Thus, we let ecophysiological models define potential boundaries of behavior by using the model output from Equations 2.2-2.7 as an upper limit to torpor bout duration. Actual torpor duration (t_{bout}) was determined as a percentage (ρ) of t_{limit}. For a given microclimate, ρ was drawn from a Beta distribution with a mean that is partially dependent on environmental conditions, similar to π (Equation 2.8). We used a Gamma distribution to define and predict t_{bout}, matching mean and variance moments to the shape and rate parameters (Equation 2.9). This prevents negative values for t_{bout} and allows unexpectedly long outlier bout lengths to exist above t_{limit}. Table 2.1 provides definitions, values, and resources for all equation parameters.

$$\begin{aligned} \mu_\rho &= \gamma_0 + \gamma_1 T_a + \gamma_2 \Delta WVP + \gamma_3 T_a \Delta WVP \\ \sigma_\rho &\sim \text{Uniform}(0, 0.5) \\ \rho &\sim \text{Beta}\left(\frac{\mu_\rho^2 - \mu_\rho^3 - \mu_\rho \sigma_\rho^2}{\sigma_\rho^2}, \frac{\mu_\rho - 2\mu_\rho^2 + \mu_\rho^3 - \sigma_\rho^2 + \mu_\rho \sigma_\rho^2}{\sigma_\rho^2}\right) \end{aligned} \tag{2.8}$$

$$\begin{aligned} \sigma_i &\sim \text{Uniform}(0, 50) \\ t_{bout,n,i} &\sim \text{Gamma}\left(\frac{(\rho * t_{limit})^2}{\sigma_i^2}, \frac{(\rho * t_{limit})}{\sigma_i^2}\right) \end{aligned} \tag{2.9}$$

2.2.3 Estimating hibernation time with and without *Pd*

Given the large amount of variation we could introduced by estimating t_{bout} as a percentage of t_{limit}, as well as the fact that torpor bouts are being modeled as independent events, we wanted to ensure that the summation of our torpor bouts would result in reasonable approximations of hibernation duration. Thus, we used our predictions to estimate total hibernation time in two ways: first as a quality control by comparing predictions with observed hibernation for individual bats,

second as a risk assessment to evaluate potential survival space. For quality control purposes, we estimated hibernation time by summing the ith bat's predicted n_i bout lengths with n_i+1 euthermic period lengths drawn from a Gamma distribution with the same mean and standard deviation as our measured euthermic periods ($\mu_{eu} = 1.6$ hours, $\sigma_{eu} = 2.1$ hours). We can then compare to our measured hibernation times for bats given their known hibernation microclimates and number of bouts.

As a risk assessment to estimate the potential for mortality when *Pd* is introduced to this population, we evaluated the potential for hibernation across a range of microclimates. For a given ambient temperature and RH, we estimated a bat's expected potential hibernation time using the same equations and process as the model(s) selected by our ensemble function, but substituting in our population's estimated parameters. We compared expected hibernation times without and with *Pd* growth. We identified the microclimates that match with observed individual torpor bouts to evaluate chosen microclimates for long-term hibernation use. We then estimated the percent loss in survival space as ecological traps emerge, i.e. the percent of used microclimates where bats survive uninfected but die when *Pd* is introduced.

All work was custom-coded in R (R Core Team, 2021) using the rjags (Plummer, 2021), tidyverse (Wickham *et al.*, 2019), and lubridate (Grolemund & Wickham, 2011) packages. Sensitivity analysis was performed using the lhs (Carnell, 2021), fitur (Roh, 2018), and sensitivity (Iooss *et al.*, 2021) packages. Images were prepared using the cowplot (Wilke, 2020), reshape2 (Wickham, 2007), and ghibli (Henderson, 2020) packages. Data and reproducible code have been stored in a public repository.

2.3 Results

We recaptured three bats with a total 66 independent torpor bouts: 26 from Bat 1, 21 from Bat 2, and 19 from Bat 3. We found via PRCC sensitivity analysis (Figure 2.2) that the most important traits influencing bout duration are torpor conductance (C_t), rate of evaporative water loss from the body ($rEWL_{body}$), torpid metabolic rate (TMR_{min}), the minimum body temperature the bat

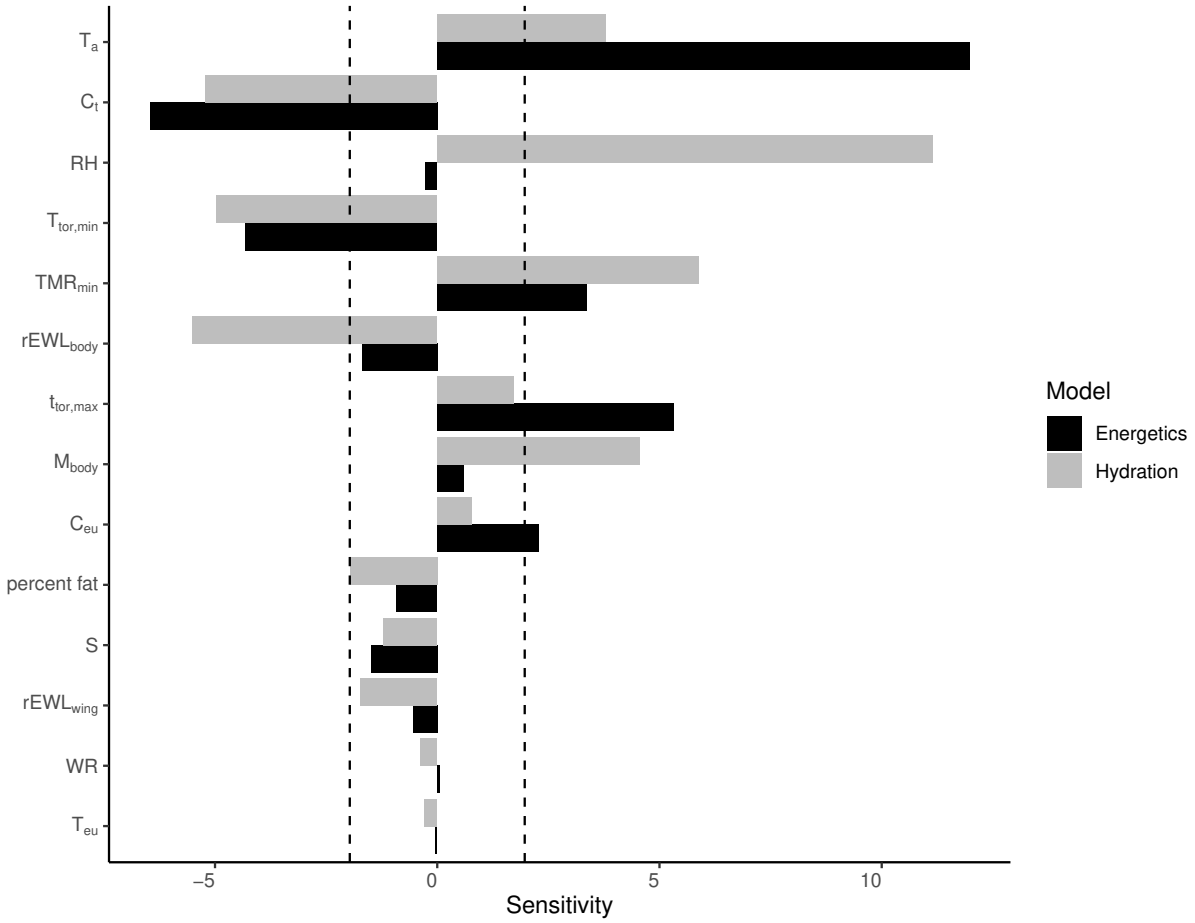


Figure 2.2: Partial rank correlation coefficient parameter sensitivity analysis results for the energetics and hydration models with torpor bout duration as the output. Sensitivity denotes a monotonic correlational relationship between the given parameter and torpor bout duration, where directionality of sensitivity and correlation are the same. Note that temperature (T_a) and relative humidity (RH) are covariates, not parameters, but they were included in analysis to ensure that we take into account the potential for parameter sensitivity to change under different microclimates, as opposed to testing under one static microclimate.

will maintain in torpor ($T_{tor,min}$), and maximum possible torpor length ($t_{tor,max}$). The first four parameters relate to metabolic processes in torpor that have been estimated via experimental measurements for populations of interest (Haase *et al.*, 2019a). Using previously published values to establish biologically relevant priors (Table 2.1), we estimate the five sensitive parameters (Figure 2.3.a). Parameter values are consistent across bats within the population, and literature-based values were generally within the credible interval of our posterior estimations, but our population likely differs from populations previously measured. Given the high sensitivity of the model to these parameters, it is important that our estimates be specific for our population of interest. Thus,

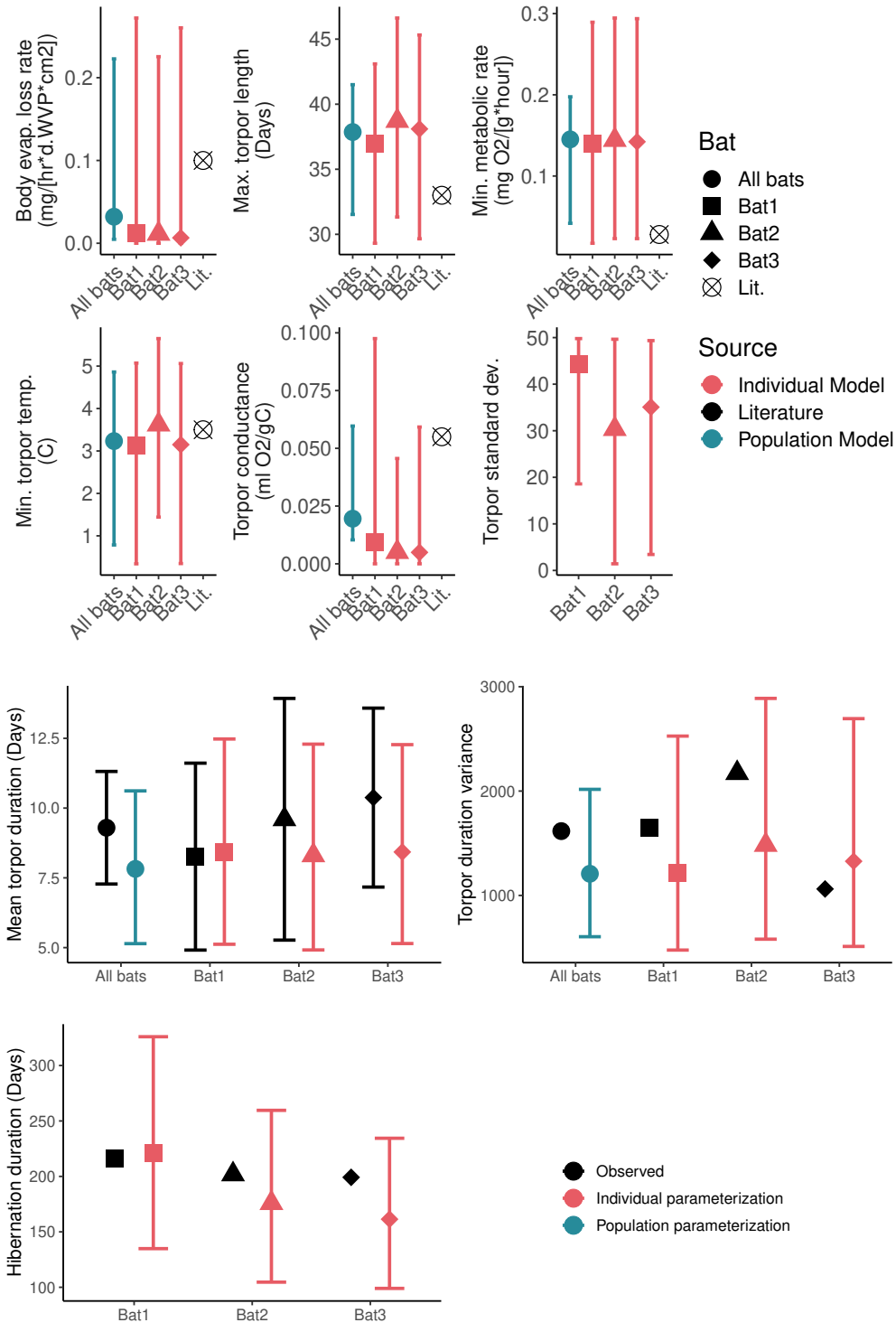


Figure 2.3: Bayesian hierarchical model output for (a) estimated parameters and (b) mean and variance moment and hibernation duration. Error bars represent 95% credible intervals for model output and 95% confidence interval for observed data.

on average, our bats exhibit increased values of $t_{tor,max}$ and TMR_{min} and decreased values of C_t compared to previous reports; these changes reflect a capability for increased torpor duration at colder temperatures (Equation 2.2). We also find that our bats have a lower rate of evaporative water loss than reported for *M. lucifugus* (Haase *et al.*, 2019a), which we might expect given regional variation allowing for low rates of evaporative water loss in dry locations like our study site (Cryan *et al.*, 2010; Klüg-Baerwald & Brigham, 2017).

Allowing variance in our population's parameterization, we can define a population-specific physiological limit that bounds bat behavior (Figure 2.4). Our reparameterization captures variance in torpor duration that is not described by the energetics or hydration models using published parameters (black lines in Figure 2.4.a and 2.4.b, respectively) (see Appendix Figure A.2). There is a large amount of variance in predicted bout length for conditions that allow longer hibernation, which reflects the potential for a bat to wake up for unpredictable reasons prior to reaching its physiological limits in torpor. We see that the originally parameterized temperature model is similar to our model at higher temperatures, and could have worked similarly as an upper limit in its own right. However, as ambient temperature drops below $T_{tor,min}$, bats are capable of considerably longer torpor bouts than theorized under the original parameterization. These longer torpor bouts at low temperatures reflect the maximum potential bout duration in the population-level parameterization. Bat-specific individual parameterizations treat these data points with greater uncertainty, suggesting that bats could potentially hold a torpor bout in cold temperatures for longer than our subjects exhibit.

Our results strongly favor the energetics model, with π equal to near 1 above five degrees (Figure 2.4.c). The hydration model is partially incorporated at lower temperatures, though the temperature model is more than 50 percent favored even in the coldest microclimates observed. Thus, we find support for the hypothesis that, at least in this healthy *E. fuscus* population, bat torpor duration is driven more by temperature than humidity. This is likely a result of this study's bats hibernating in much drier conditions than what the hydration model would predict. Some of the longest torpor bouts occur in 55 to 80% RH, while the hydration model favors longer torpor

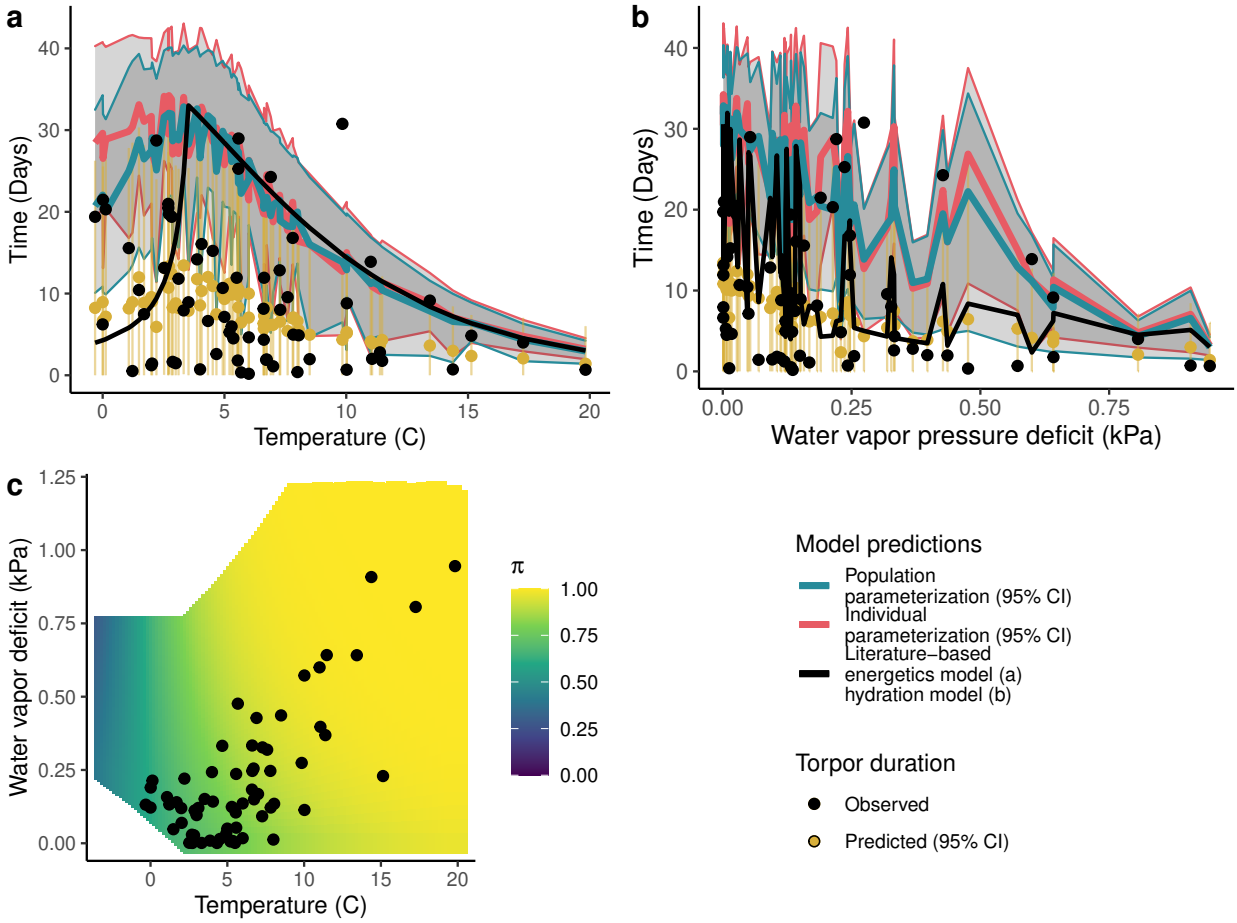


Figure 2.4: Model predictions for torpor bout duration as a function of (a) temperature and (b) water vapor pressure using ecophysiological models as biological limits to potential behavior. Ecophysiological model boundary predictions are shown separately for the population-level parameterization (teal, μ_θ in Appendix Figure A.3) and individual-level parameterizations (red, θ in Appendix Figure A.3). Torpor bout duration predictions are in gold, with variances that span the majority of area under the ecophysiological boundary curve. (c) The relative weight of the energetics versus hydration models for a given microclimate, with $\pi = 1$ favoring the energetics model (black line in panel a) and $\pi = 0$ favoring the hydration model (black line in panel b). Black dots represent observed torpor bout durations as measured by data loggers.

bouts in conditions of near saturated vapor pressure (Haase *et al.*, 2019a). That the hydration model only begins to be influential at lower temperatures is in keeping with previous findings that evaporative water loss better predicts torpor duration at lower temperatures (Thomas & Cloutier, 1992).

As a result of fitting our model to a physiology-defined limit, we can capture the distribution of torpor bout durations (Figure 2.3.b), but we have difficulty fitting the exact temporal sequence of torpor bout durations. It is notable that our model's prediction of torpor duration's upper limits accurately matches approximately a dozen observed torpor bouts wherein bats are presumably in torpor until physiology dictates arousal (Figure 2.4.a,b). In addition, we captured important general features of temporal progression. Given each bout's environmental conditions, our model was able to predict total hibernation duration for each bat (Figure 2.3.b). Having validated that the distribution of microclimates used can predict overall hibernation outcomes, we can begin to examine how microclimates help or hurt winter survival.

We use our population-specific parameterization to estimate changes in survival space with *Pd*'s introduction. We estimate survival using the energetics model equations estimating grams of fat used in hibernation (Hayman *et al.*, 2016), inserting our parameters in place of published values. In order to conceptualize survival space, we consider whether a bat with finite energy (grams of fat) can survive hibernation through a 4780 hour winter (our shortest observed winter length) under a given static microclimate. We calculate survival across ranges of temperature and RH, presenting the 95% credible interval limits and mean time until a bat runs out of energy stores (Figure 2.5). We overlay this with points that represent microclimates used for each bout duration measured, reflecting survival potential if a bat were to hibernate in that microclimate repeatedly, with size scaled by the actual length of torpor. As expected, in the absence of *Pd*, bats generally spend the majority of their hibernation, including the longest torpor bouts, in microclimates promoting survival to the end of hibernation (Figure 2.5). On the low end of the credible interval, we find only a very narrow range of microclimates wherein our bats survive, suggesting that individuals unfit for hibernation (e.g., low fat stores) will have difficulty surviving outside of precise conditions.

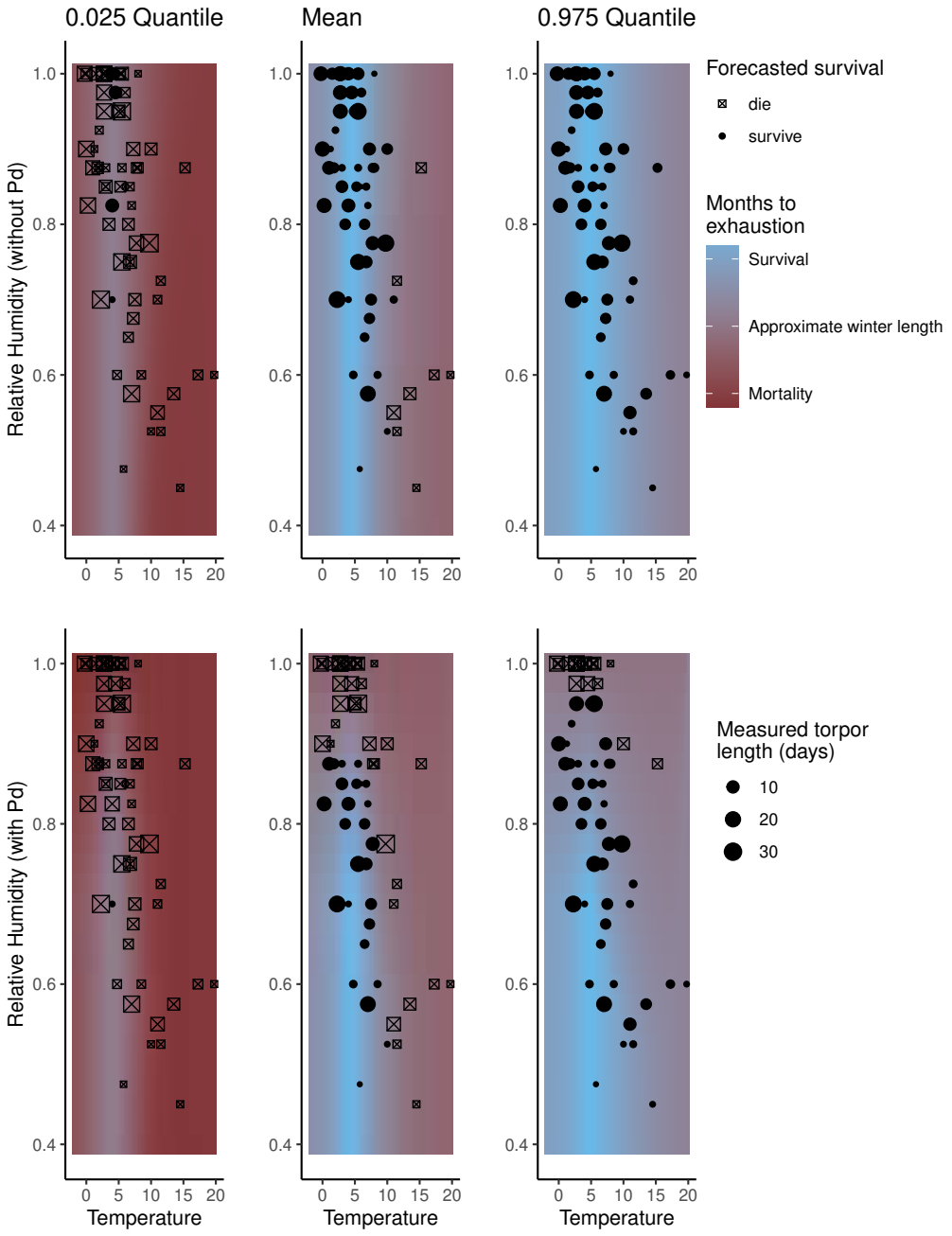


Figure 2.5: Survival expectations across a range of microclimates indicating survival space and potential ecological traps. For a given temperature and relative humidity, we use the population-level parameterization to estimate the time until bats exhaust energy stores if hibernating in that microclimate indefinitely. We compare this to hibernation expectations (approximate winter length = 4780 hours) and indicate for our observed microclimates whether an average individual would survive (circle) or die of starvation (crossed square) in the given microclimate. We deem microclimates where individuals could survive as survival spaces. Microclimates where individuals could survive without *Pd* (top row) but die if *Pd* is introduced (bottom row) are ecological traps. We perform this analysis across iterations of our Bayesian model chains to generate a 95% credible interval for survival expectations. The first column is the credible interval low end, the third column is the credible interval high end, and the middle column is the mean survival expectation.

Conversely, on the high end of the credible interval, survival is expected in all microclimates investigated, suggesting that particularly well-conditioned individuals should have little trouble surviving winter.

With the introduction of *Pd*, there are frequently used microclimate ranges that would result in likely mortality. *E. fuscus* that hibernate with *Pd* in greater than 90% RH are at significant risk of developing WNS (Figure 2.5). There is also loss of survival space in lower humidity at warmer temperatures, though bats that hibernate in lower humidity at colder temperatures appear to be still capable of survival. Importantly, there are multiple long torpor bouts that occur within microclimates that convert from survival to mortality with *Pd* introduction, suggesting a strong potential for these otherwise favorable conditions to be ecological traps in the presence of *Pd*. On average, 88% of observed torpor microclimates are suitable for long-term use without infection, but when *Pd* is introduced, this reduces to 44%. Thus, half of the observed survival space used by these bats is eliminated. Even in the best-case scenario at the upper end of the credible interval, we observe a 28% loss of previously viable microclimates for hibernation, indicating that while we do not expect population extirpation in a single hibernation season, significant population losses in response to *Pd* introduction are likely in this population of *E. fuscus*.

Discussion

Torpor is a strategy employed by many species to survive cold weather by lowering metabolic rate such that body temperature approaches environmental temperature, minimizing heat loss and energy use. However, torpor cannot be maintained indefinitely, requiring periodic arousal mid-hibernation. This can leave hibernators sensitive to anthropogenic disturbances, such as the pathogenic fungus *Pd*, which significantly increases bat arousal frequency, leading to starvation (Reeder *et al.*, 2012). Since the emergence of WNS, the need to develop ecophysiological models that describe hibernation behavior has become an urgent undertaking. Multiple mechanistic models of torpor bout duration, each describing a distinct hypothesis for arousal timing, have been developed and validated for a few of the many species affected by WNS (Hayman *et al.*, 2016; Haase *et al.*,

2019a). Given dozens of species exposed to *Pd* and significant intraspecific variation in morphology, metabolism, and behavior relevant to hibernation (Burnett, 1983; Neubaum *et al.*, 2007; Cryan *et al.*, 2010; Dunbar & Brigham, 2010; O'Shea *et al.*, 2011; Hayman *et al.*, 2017; Klüg-Baerwald & Brigham, 2017), there is concern that models developed based on literature-derived parameterizations or measured under laboratory conditions might not represent free-ranging populations. To address this, we used sensitivity analysis to specify significant parameters for predicting torpor bout duration and estimated these in a Bayesian hierarchical ensemble framework, while testing two hypotheses for arousal frequency. We define population-specific parameterizations that inform on natural history, redefine ecophysiological models as limits of torpor capability rather than direct predictors of torpor behavior, and identify environmental conditions under which each hypothesis influences torpor duration outcomes.

We find that *E. fuscus* is capable of longer torpor bouts at low temperatures than literature-based models theorize (Figure 2.4.a). To accommodate these observations, our model estimated torpor conductance (C_t) as low and minimum metabolic rate in torpor (TMR_{min}) high compared to literature values (Figure 2.2). These covarying parameters are present in the temperature model as a ratio that regulates the curve's slope at temperatures less than $T_{tor,min}$. By estimating a smaller conductance to metabolic rate ratio, we observe a flatter plateau rather than the sharp peak observed in the temperature model's published parameterization (Figure 2.4.a). This suggests that perhaps our *E. fuscus* population is particularly good at mitigating heat loss or maintaining metabolic rate in colder temperatures. Additionally, we find that the reparameterized rate of evaporative water loss ($rEWL_{body}$) is decreased compared to literature values, suggesting an increased capacity for water retention. There are demonstrated differences in capacity for water retention across *E. fuscus* populations (Klüg-Baerwald & Brigham, 2017), so it makes sense that our population living in the relatively dry plains of eastern Wyoming should be adapted to reduce evaporative water loss. By allowing parameter variation to let the model adhere to our natural observations, we can make inference on metabolic parameters without intensive experimentation. When more detailed risk assessments are required (e.g., endangered species), we can reduce cost and intensive handling time

by guiding measurement of parameters that are most likely to impact torpor duration expectations (e.g., conductance, torpid metabolic rate).

We establish that ecophysiological models are more useful in describing potential limits to behavior than predicting behavior directly. Torpor duration in natural systems does not necessarily equate to what is observed in a stable laboratory environment. There are a number of external influences that can be difficult to account for in the lab, including but not limited to microclimate heterogeneity, auditory disturbance from other animals in the environment, or tactile disturbance, such as might be experienced when a neighboring bat arouses (Speakman *et al.*, 1991). Any of these disturbances, but especially tactile disturbance, could considerably shorten observed torpor bout length in an unpredictable manner (Speakman *et al.*, 1991). Therefore, ecophysiological models of torpor bout length can describe the biological limits of which hibernators are capable, and that in a natural environment, disturbances may prevent them from achieving this limit. Accounting for this source of natural variation can significantly change model outcomes and the relative importance of different processes. Literature-based models attempting to describe observed torpor durations generally overestimated torpor bout duration, particularly in microclimate ranges favorable for long-term torpor (Figure 2.4.a,b). Overestimation of torpor duration results in increased expectations for hibernation capability and survival potential, which could result in misclassification of at-risk bat populations as safe from WNS. However, literature-based models also underestimated torpor duration in cold and dry microclimates (Figure 2.4.a,b), which could counterbalance overestimation. Care should be taken to ensure that model functional form adheres to data presentation, and applying models as biological limits helps to eliminate these sources of over- and underestimation.

To better understand the drivers of arousal from torpor, we used an ensemble modeling approach to test the weighted importance of two hypotheses under different microclimates. We found that the energetics model was dominant in all observed microclimates used for hibernation, but the hydration model becomes increasingly influential as temperatures decrease below 5^o C (Figure 2.4.c). This suggests that ambient temperature is a stronger driver of arousal than am-

bient humidity in natural systems, despite experimental evidence supporting humidity as a driver of arousal (Ben-Hamo *et al.*, 2012, 2013). Hayman *et al.* (2016) assert that the energetics model phenomenologically considers evaporative water loss in that there is a correlation between temperature and humidity. Efforts by Haase *et al.* (2019a) expand on the energetics model, explicitly modeling evaporative water loss simultaneously as an alternative mechanism for arousal and taking the minimum torpor duration of the two, resulting in the hydration model (Equation 2.3). Then, the evaporative water loss portion of the hydration model takes effect when expected torpor from hydration is shorter than that expected from energetics, which would increasingly occur in temperatures below $T_{tor,min}$. This is because below this minimum threshold, skin WVP, defined as saturation at body temperature, will consistently be higher than ambient WVP, which is limited by ambient temperature (Equation 2.1), such that as temperature drops there is an increasing WVP deficit even at 100% RH (Figure 2.4.c). Thus, we believe that the hydration hypothesis will be increasingly important for populations with higher minimum body temperatures held in torpor or small-bodied species with higher surface area-to-mass ratios, though this hypothesis requires further experimentation to confirm.

Our analysis of the drivers and estimation of torpor duration has clear implications for bats facing WNS, with an average of 50% of survival space converting to ecological traps with the introduction of *Pd* (Figure 2.5). While some mortality may be expected, there are a variety of potential behavioral and evolutionary responses to mitigate WNS mortality. These may include changes in increased sociality and coordination of arousal behavior (i.e., social thermoregulation) (Hayman *et al.*, 2017), shifts in hibernation microclimates used to ensure longer torpor bout length (Johnson *et al.*, 2016), or increases in gathered fat stores to more easily last through the winter (Cheng *et al.*, 2019). Alternatively, populations may not have the adaptive capacity to escape ecological traps (Hopkins *et al.*, 2021). By observing the outbreak and spread of WNS in real time, there has been opportunity to consider the evolutionary responses of bats and the impacts on population survival (Maslo & Fefferman, 2015; Gignoux-Wolfsohn *et al.*, 2021). We propose that changes in survival space could be used to estimate strength of selection across a spatiotemporal landscape.

Incorporating *Pd* selection strength with heritability of survival-related traits in a framework to evaluate population survival via evolutionary rescue (Golas *et al.*, 2021) could further improve risk assessment of affected populations.

Our work represents a shift in understanding of how we can apply ecophysiological models to natural disease systems. We demonstrate methods to derive insight on energetics without the need for repeated intensive animal handling or disturbance of sensitive natural systems during periods critical to survival. Using unprecedentedly complete microclimate data of full hibernation from only three individuals, we are able to estimate population-specific metabolic parameters that are more useful than previously established literature-based values. We believe we have learned so much from so few individuals in this study by embracing the approach of model-guided field work (Restif *et al.*, 2012), which holds promise for garnering similarly important information from endangered populations. With more robust recapture rates, perhaps in more accessible colonies in both winter and summer to allow for potential collection of mortality-related information, we could incorporate mark-recapture statistics to our hierarchical model to better evaluate overall population survival. We hope that this work is the beginning of a line of investigation to improve survival predictions and risk assessment across many species of bats threatened by WNS, as well as other hibernators threatened by changing environments, and that in learning more about hibernation dynamics we can develop novel methods to help conserve biodiversity.

Chapter 3

Predicting hibernating bat roost establishment in spatiotemporally complex hibernacula

3.1 Introduction

Realized niche space theory suggests that organisms exist within an “n-dimensional" space that is restricted by biotic and abiotic interactions (Hutchinson, 1957). Controlled experiments can demonstrate important drivers of niche partitioning (Connell, 1961), but the complexity and stochastic variability of natural systems creates an extreme dimensionality that can make it difficult to identify which drivers are significant. Naturally isolated ecosystems may create an opportunity to more easily define niche space. For example, subterranean environments are generally relatively stable, with limited temperature fluctuation and more often than not consistently saturated water vapor pressure in the air (Mammola *et al.*, 2019b). These underground ecosystems provide shelter for a surprisingly wide range of biodiversity and adaptive strategies. The stable conditions and general lack of outside influence results in unique locally adapted populations that can become dependent on their particular environment (Sánchez-Fernández *et al.*, 2021), representing a clear example of adaptation to fit a particular niche space. However, this niche dependence can result in inability to respond to sudden environmental changes, such as anthropogenic disturbances (Mammola *et al.*, 2019a; Pallarés *et al.*, 2020; Castaño-Sánchez *et al.*, 2020; Sánchez-Fernández *et al.*, 2021). One group of animals that are seasonally dependent on underground conditions and of great interest to people for their widespread biodiversity and ecosystem services are insectivorous hibernating bats (Boyles *et al.*, 2011).

Hibernating bats are assumed to seek out ideal stable conditions for hibernation (Elliott & Clawson, 2001; Tuttle & Kennedy, 2011), but roosting conditions within a single hibernaculum can vary widely (Boyles *et al.*, 2017). These bats may be physiologically limited to specific condi-

tions for hibernation, but underground temperature and humidity are notoriously difficult to measure over long periods due to costly equipment failure (Verant *et al.*, 2012; Boyles *et al.*, 2017), and too-frequent disturbance of human intrusion can be detrimental to sensitive flora and fauna (Thomas, 1995). We can use bats to explore the concept of niche dependency by characterizing chosen hibernation conditions and identifying the drivers behind why bats roost where they do. With data of hibernation conditions proving a limiting factor, we can benefit from predictive modeling to describe underground conditions as a function of above-ground climatic changes. Doing so can reduce reliance on difficult and costly measurements while simultaneously gaining understanding of how subterranean environments can change according to above-ground influences. Thus, hibernating bats provide opportunity to define realized niche space in natural systems with minimal intrusion, and predict how that niche space changes spatiotemporally due to outside influences. Investigating hibernating bat niche space has become increasingly important since the emergence of white nose syndrome (WNS), a disease that has led to widespread bat mortality (Blehert *et al.*, 2009; Frick *et al.*, 2010). *Pseudogymnoascus destructans* (*Pd*), the keratinolytic fungus causing WNS, overlaps in niche space with bats in torpor, creating a new biotic niche dimension that restricts bat physiological capabilities, constraining their realized niche. To predict bat survival capabilities in a hibernaculum, we need to better understand the dimensional axes that constrain the hibernation roost niche in presence of *Pd*.

Defining these axes can be difficult because roost establishment of hibernating bats across underground hibernacula with spatiotemporally variable local environmental conditions (e.g. temperature and humidity, henceforth "microclimates") can be highly heterogeneous. Bat choice of specific within-hibernaculum locations (henceforth "microsites") for roosting likely results from some combination of bat traits and available microclimates (Twente, 1955). This is because hibernating cave-dwelling bats are heterothermic endotherms, lowering body metabolism to reduce body temperature to more closely approximate colder ambient conditions. This reduces energetic costs of maintaining warmth and mitigating fat depletion for months when insect food sources are unavailable (Davis & Reite, 1967; Humphries *et al.*, 2002). Microclimates determine bat metabolism and

evaporative water loss in hibernation, and a given microclimate can lead to survival or starvation and dehydration depending on the individual's physiological needs (Thomas *et al.*, 1990; Thomas & Cloutier, 1992). This combination of bat physiological traits and microclimate use is exploited by *Pd*. When bats hibernate in niche space overlapping *Pd*'s physiological needs, it results in fungal growth that increases the frequency of energetically costly bat arousal, potentially leading to starvation and mortality (Reeder *et al.*, 2012). Anthropogenic disturbance like climate change affects hibernation microclimates (Humphries *et al.*, 2002; Sherwin *et al.*, 2013), which in turn affects *Pd* growth and bat traits (Langwig *et al.*, 2012; Hayman *et al.*, 2016; Haase *et al.*, 2019a). In addition to physiological traits, we need to consider the social aspects of hibernatory behavior, as bat clustering has the potential to influence hibernation roost choice as well as bat response to *Pd* (Clawson *et al.*, 1980; Langwig *et al.*, 2012). Thus, we need a more basic science understanding of site selection and bat success under different microclimates to understand the natural history of how bats survive hibernation in the wild as well as how they respond to *Pd* presence and microclimate availability changing as a function of above-ground climate change.

A comprehensive understanding of changes to the realized niche, as defined through roost selection, will combine knowledge of bat physiological traits, such as metabolic rate and evaporative water loss, as they relate to microclimates to result in energetically successful hibernation (Hayman *et al.*, 2016; Haase *et al.*, 2019a). For decades researchers have developed laboratory experiments to measure physiological traits for populations of interest (Hock, 1951; Hanus, 1959; Speakman *et al.*, 1991; Haase *et al.*, 2019a), and these efforts can be used in conjunction with field research and mechanistic modeling to predict bat hibernation outcomes under a range of environmental conditions (Hayman *et al.*, 2016; Haase *et al.*, 2019a; Golas *et al.*, in review). However, hibernaculum microclimates are not always easily characterized, and attempts to measure variation in available microclimates underground are often fraught with difficulty due to electrical equipment malfunction and poor quality of retrieved data (Kurta *et al.*, 1990; Verant *et al.*, 2012; Boyles *et al.*, 2017). Many studies bypass detailed evaluation of spatiotemporal microclimate variation by relying on singular or averaged measurements to draw inference (Clawson *et al.*, 1980; Nagel & Nagel,

1991; Langwig *et al.*, 2012), which may be a valid assumption in particularly stable environments (Haase *et al.*, 2019a). However, even in the most stable underground microclimates, temperature is expected to correlate to mean annual surface temperature (MAST) (Perry, 2013; McClure *et al.*, 2020), which we expect to vary year to year, especially as climate change increases global temperatures (Loarie *et al.*, 2009). In addition, hibernaculum structure (e.g. size, shape, elevation, number of entrances) can change airflow in ways that affect available microclimates for roosting (Perry, 2013). Very few studies attempt to characterize the available microclimates throughout the hibernaculum for the entire winter period, but those that do are able to draw important inference on bat behavior related to microclimate variability (Elliott & Clawson, 2001; Boyles *et al.*, 2017; Ryan *et al.*, 2019). However, at this time we are not aware of any studies that characterize the entirety of hibernaculum microclimates available to bats, or describe those microclimates as a function of above-ground changes. To demonstrate how bats select hibernaculum microsites under variable conditions, we require methods that predict available underground microclimates and relate them to changing external conditions. By describing spatiotemporal microclimate variation as a function of above ground conditions rather than reducing our conceptualization of roost conditions to annual average microclimates or maximally stable MAST conditions, we can make novel inference in where and why bat roosts develop in addition to how changes above ground and anthropogenic disturbances can affect underground roost suitability.

Because of assumptions of stable hibernation microsites, evaluations of bat physiology in response to WNS often use climatic averages rather than longitudinal microclimate data (Hayman *et al.*, 2016). This results in simplified representations of niche space that may over-emphasize the niche overlap between bats and *Pd*. There is significant variation in host response to WNS that could be explained by bats maintaining a wider niche space through spatiotemporal microclimate variation. For example, WNS can cause as much as 100% mortality in *Myotis lucifugus* hibernacula, and resulted in mass die offs in the northeast United States when it was first introduced (Frick *et al.*, 2010; Cheng *et al.*, 2021). However, one hibernaculum home to over 30,000 overwintering *M. lucifugus* in New York is unique within this region in that through the epidemic emergence of

Pd, the population overwintering there has not declined significantly (Cheng *et al.*, 2019). This may be a result of unique subterranean system structure resulting in microclimate profiles or variability of microclimates that give *M. lucifugus* range to hibernate safely outside of *Pd* niche space. Given that bats have been consistently found roosting in the same locations without significant population decline despite *Pd* presence, we propose that this hibernaculum is uniquely useful for developing methods and investigating questions regarding hibernaculum roost establishment.

We aim to describe the realized niche of *M. lucifugus* in a complex spatiotemporally variable hibernaculum in terms of microsite selection for hibernation. We recorded microclimates throughout hibernation in roosting and non-roosting sites across the hibernaculum. We then used a Bayesian hierarchical modeling approach to simultaneously predict underground microclimates and within-hibernaculum site occupancy as a function of above ground conditions. Although hibernation roost establishment is influenced by microclimate profiles, sociality, and microsite stability, we find that sociality and early hibernation temperature profiles are primary drivers of hibernation niche space. In so doing, we improve our understanding of the factors that drive bat behavior and how an intricate underground environment can change with above-ground climate. We demonstrate prediction of hibernation niche range under a climate change scenario and discuss how our model can be further developed as a tool for conservation and management of an important refuge for otherwise-devastated bat populations.

3.2 Materials and Methods

3.2.1 System and data description

Our study hibernaculum is a cavernous subterranean abandoned mine in New York state. The exact name and location are not disclosed because in addition to common bat species like *M. lucifugus* and *Eptesicus fuscus*, endangered *Myotis leibii* and *Myotis sodalis* overwinter there. The hibernaculum is regularly monitored with midwinter surveys every other year to count its particularly large and stable *M. sodalis* population, but our study focuses on *M. lucifugus* because they are better studied in terms of their physiological traits and hibernation requirements (Thomas *et al.*,

1990; Jonasson & Willis, 2012; Hayman *et al.*, 2016; Haase *et al.*, 2019a). In 1993 and 2013 the hibernaculum's *M. lucifugus* population was censused, representing pre- and post-*Pd* introduction, which occurred in approximately 2009. These censuses did not reveal any significant decline in *M. lucifugus* in response to *Pd* (Cheng *et al.*, 2019). We assign our study microsites as being established hibernation roosts or not based on midwinter survey observations. Each roost microsite is found within one of three larger roosting areas in the hibernaculum.

Structurally, the hibernaculum can be divided into three distinct chambers: the large open "Upper chamber" that is closest to surface level, the similarly expansive "Lower chamber" that is connected to the Upper chamber by multiple tunnels through walls of stone, and the relatively narrow "Side chamber" that branches off the Upper chamber with very minimal airflow (Figure 3.1). Each of these chambers contains a general area where *M. lucifugus* are commonly found roosting during midwinter surveys and areas where they are not commonly found. While the Upper chamber has multiple surface openings, there are two that appear significant to airflow and available microclimates (Figure 3.1). The "warm entrance", which is higher in elevation, allows warmer air in summer months to passively diffuse into the mine, cooling and sinking as it contacts the walls until the local MAST-related analog is reached. The "cold entrance", lower in elevation than the warm entrance but angled to be higher elevation than the mine chambers within, allows cold air to flow into the mine in winter months. This cold air sinks below the MAST-related temperature air, further cooling the mine when temperatures are cold outside. The resulting airflow creates temperature and humidity gradients throughout the hibernaculum that represent a combination of variable surface-level conditions and the more consistent hibernaculum wall temperatures derived from MAST.

To characterize available microclimates throughout the hibernaculum, we deployed a total of 117 modified DS1923-F5# Hygrochron iButton temperature/relative humidity data loggers throughout the hibernaculum. Prior to deployment, loggers were modified with intent to prevent humidity-related equipment failure common with long-term deployment of electronics in underground ecosystems (Kurta *et al.*, 1990; Verant *et al.*, 2012; Boyles *et al.*, 2017). Modifications included remov-

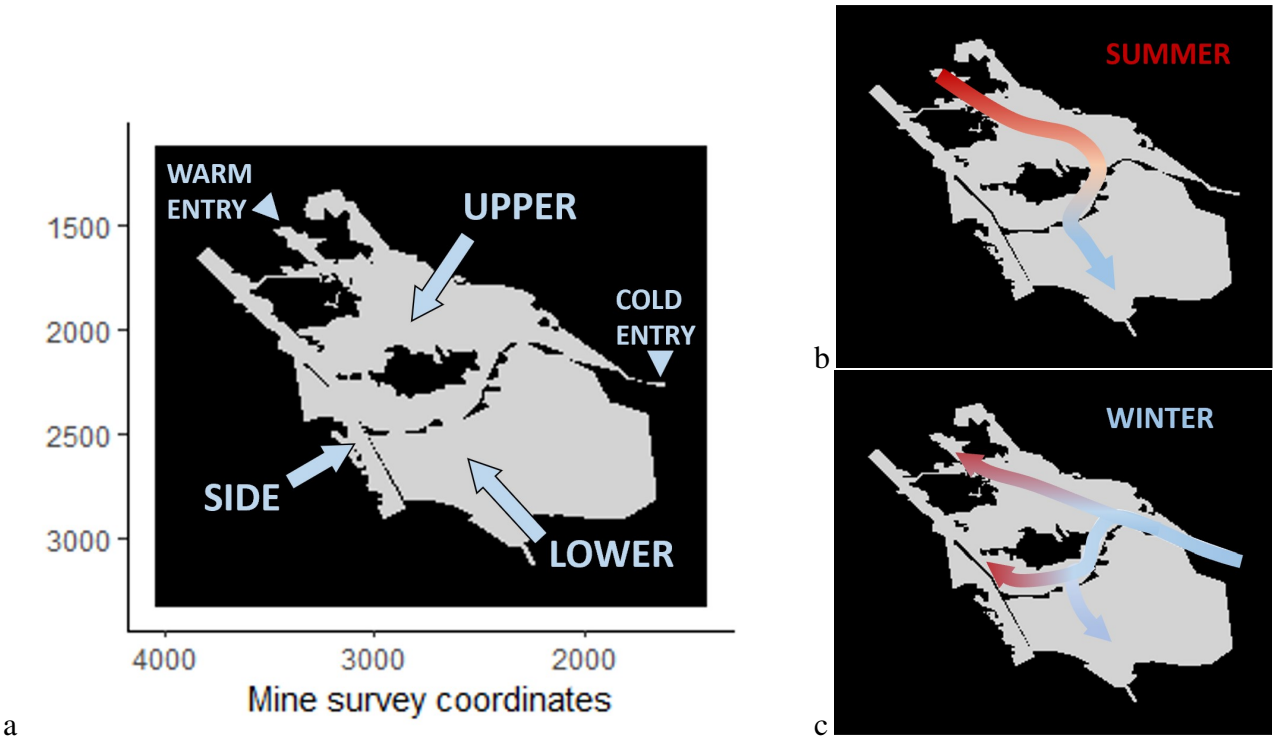


Figure 3.1: A schematic representation of this study's subterranean system. We explicitly model microclimates across three different chambers: Upper, Lower, and Side. Due to the system's shape and changes in elevation, airflow is expected to shift seasonally, with microsites near the warm entrance being most similar to surface conditions in the summer, and microsites near the cold entrance being most similar to outside conditions in the winter. As air flows through the system it is expected that cool air will sink and settle into the lowest elevation areas while warm air will rise to higher elevations. Simultaneously, contact with rock walls pulls air temperature closer to the mean annual surface temperature analog for the system, creating a heat source or sink depending on air temperature.

ing the outer casing, solidifying microchip lead connections to the battery using conductive silver epoxy, and painting the entire device save for the humidity sensor with waterproof epoxy sealant. Before modifying all data loggers, proposed modifications were performed on approximately ten loggers and tested under laboratory conditions for several months in a temperature-controlled refrigerated unit with built-in fan to allow for variable humidity, simulating underground conditions. Loggers were hung from cavern walls and ceilings using drywall screws placed in rock crevices, approximating areas where bats would be expected to roost when possible (Figure 3.2.a). Despite laboratory success with modified logger survival under extended cave-like refrigerated conditions with 100% relative humidity, 34 data loggers contained usable data when retrieved. These 34 loggers were relatively evenly distributed throughout the mine system, with a total of 12 loggers

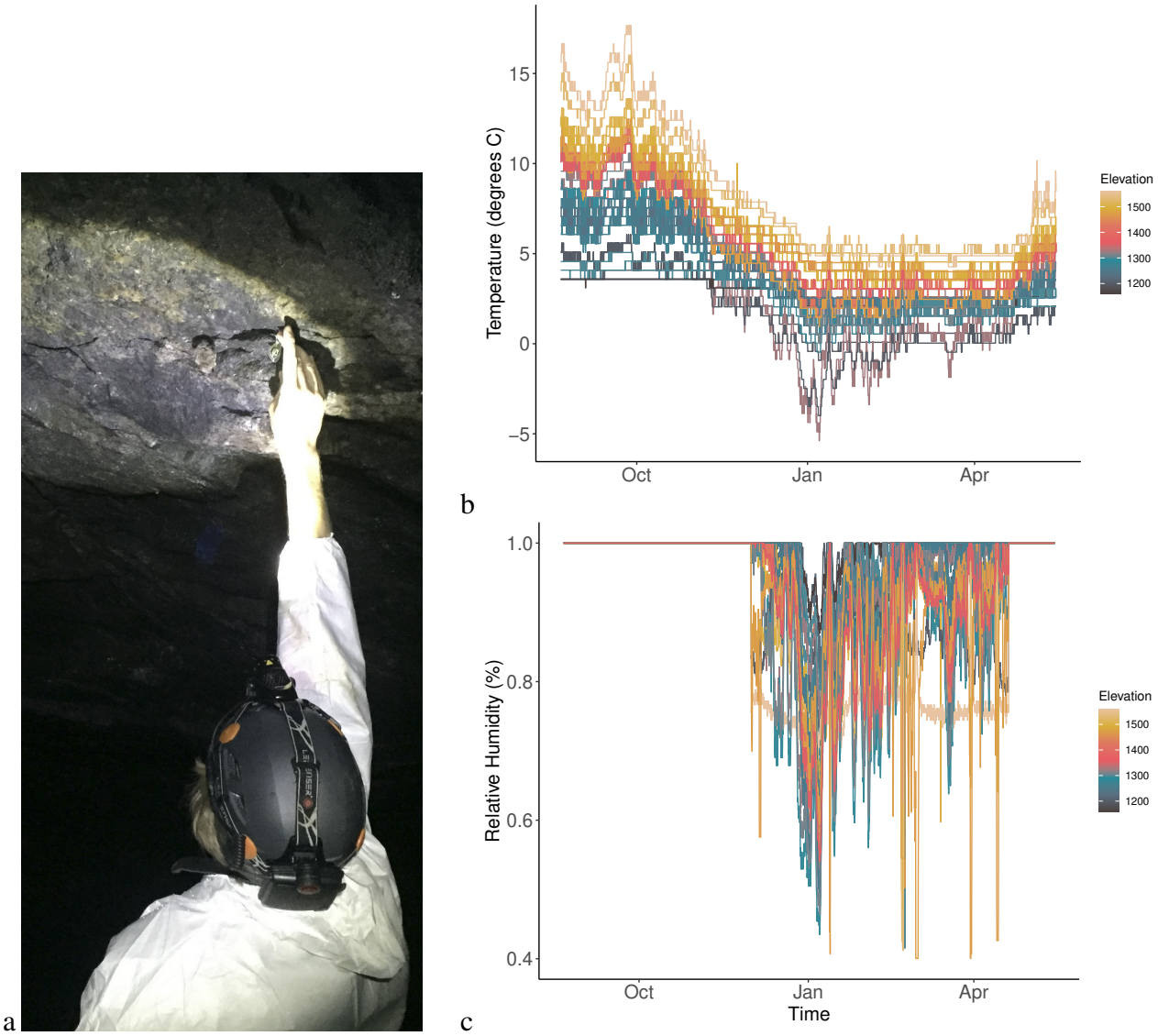


Figure 3.2: (a) Placement of a modified temperature/humidity data logger near a torpid bat within our subterranean system. Data loggers recorded (b) temperature and (c) relative humidity every two hours. Each line depicts the microclimate signature for a given data logger. For visualization purposes, we color data loggers by elevation, with high elevations being closest to surface level and low elevations being the deepest underground microsites of the system. There are clear spatial patterns within the data, with low elevation microsites having some of the lowest temperature and highest relative humidity signatures.

spanning the *M. lucifugus* hibernation roosting areas of the three chambers and 22 microsites where *M. lucifugus* are not generally found during mid-winter surveys (Figure 3.3 main panel).

Data loggers took temperature and relative humidity readings every two hours from August 15th, 2017, to April 30th, 2018 (Figure 3.2.b and c, respectively). Temperature and relative humidity traces contained clearly non-realistic reading errors (e.g. sporadic -40^o temperature readings, relative humidity traces greater than 100% and less than 0%). These were corrected by removing the errant values and replacing with the previous recording for temperature, and by assuming 100% relative humidity in warm months (see Appendix Figure B.1). These microclimate traces of the hibernation season were used to generate summary statistics for initial model selection. We used temperature to convert relative humidity readings into ambient water vapor pressure deficit using the same method as Haase *et al.* (2019a), which is a more useful statistic in the context of bat hydration and physiology (Kurta, 2014). Because we assume 100% relative humidity, and hence saturated vapor pressure and a water vapor pressure deficit of 0 kPa, during warm months when cool dry air is not flowing through the mine system, we only use the data set where this assumption does not apply to generate summary statistics.

To pair microclimates with spatial location in the mine, we used survey maps from when the mine was functioning to assign each data logger x and y coordinates and approximate elevation. Given that airflow within the mine is nonlinear, with walls, wide pillars, and curved passages directing airflow, we developed a logger placement network (see Appendix Figure B.2), such that data loggers without significant barriers between them are connected by an edge that is the Euclidean distance in three dimensional space between those two points. Using this, we measured the distances to warm and cold entrances as the shortest paths along this network, approximating distance of airflow from respective entrances to reach the microsite. We also measured the weighted "path to bats" as the sum of reciprocals of network path distance from the microsite to the centroids of recorded *M. lucifugus* roosting microsites within each chamber ($\Sigma(1/d_{\text{Upper centroid}} + 1/d_{\text{Lower centroid}} + 1/d_{\text{Side centroid}})$). Then, the degree of positive correlation of

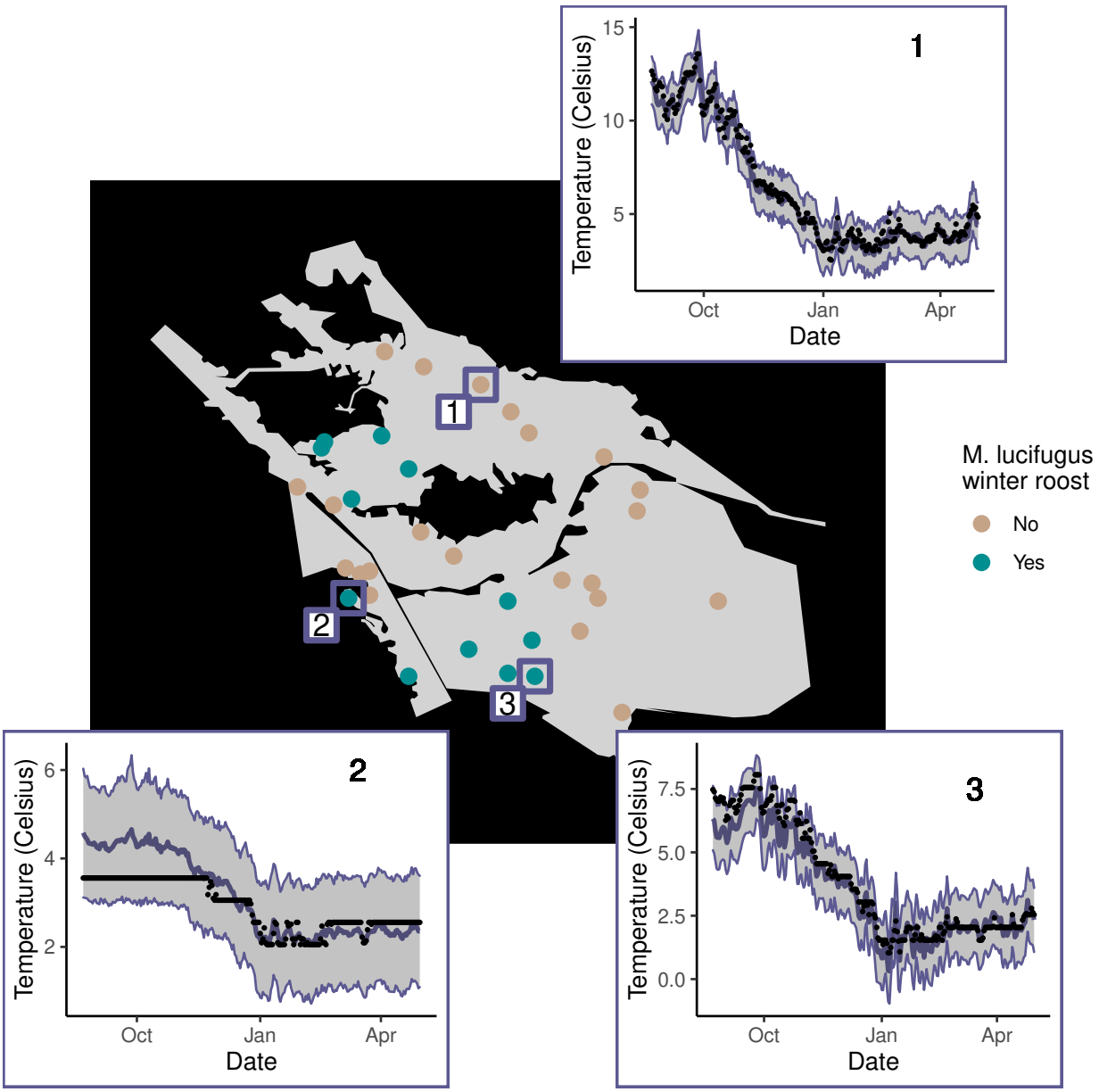


Figure 3.3: Data logger placement throughout the subterranean system. Data loggers are relatively widely distributed, covering both areas where bats are commonly found roosting during midwinter surveys (green dots) and areas where they are not (tan dots). The three insets depict temperature recordings and hierarchical model predictions of average daily temperature for representative samples of the [1] Upper, [2] Side, and [3] Lower chambers. Insets depict longitudinal data and predictions for given microsites, with black dots representing temperature recorded by data loggers and purple lines representing predicted average daily temperature with a 95% credible interval. Note that temperatures are highest and most variable in the Upper chamber, and most stable in the Side chamber. The Side chamber (inset 2) also depicts best how data loggers lose resolution in measurement accuracy by recording 0.5 degree Celsius temperature increments that may not capture minor daily variation in temperature predicted in the hierarchical model.

path to bats with roosting probability was interpreted as the relative strength of influence for roosting affinity, i.e. the degree to which bats roost in a site due to neighboring bat roosting activity.

Daily surface temperature data was gathered using the Coupled Model Intercomparison Project (CMIP5) (Taylor *et al.*, 2012). We used the surface temperature readings for the longitude and latitude that closest approximated the study hibernaculum's location. We used daily averages for the 2017-2018 and projected 2047-2048 (RCP 2.6 model) hibernation seasons, and used the previous year's worth of daily average temperature to calculate mean annual surface temperature each day.

3.2.2 Logistic roost occurrence model

To determine which factors were significantly correlated with bat hibernation roost occurrence, we performed a form of boosted logistic regression (Friedman *et al.*, 2000) implemented in Bayesian format to predict bat occupancy throughout the system. We aggregated a list of potential covariates based on each microsite's spatial location and microclimate output. Because researchers often assume bats will seek out the most stable subterranean conditions for hibernation, we included spatial covariates that reflect the reduced influence of above ground forces and increased influence of the more stable MAST, i.e. distances from the warm and cold entrances and elevation. Summary statistics (i.e. mean, standard deviation, maximum, minimum, and median) were included for microsite temperature and water vapor pressure deficit to represent microclimate influence. We included interaction terms of spatial covariates to reflect the three-dimensional cavern space, as well as interaction terms of mean temperature and water vapor pressure to reflect the potential for bats to favor optimal peaks in temperature or water vapor pressure deficit, or for important interaction between the two closely related variables (Kurta, 2014). Because bats transition from swarming activity to hibernation in the fall, we included mean and standard deviation in temperature for September, October, and November, to test if particular periods of early hibernation might be significant for hibernation roost establishment. Finally, to test the effects of sociality on roost locations, we included the path to bats covariate. See Table B.1 in the Appendix for a list of all covariates considered.

When all covariates were gathered, we calculated Spearman's rank correlation coefficient using the PerformanceAnalytics packages in R (Peterson *et al.*, 2018) to identify variables that were closely related enough to present identifiability issues in the model. When two variables had a greater than 0.9 correlation coefficient, one was rejected, with the more data-inclusive variable favored where applicable (e.g. mean hibernation temperature favored over mean November temperature). The remaining covariates were then centered and scaled ($cov = (obs - \mu_{obs})/\sigma_{obs}$) for comparison of coefficient strength and fed into a Bayesian logistic regression model, custom coded and implemented in R (R Core Team, 2021) using the rjags package (Plummer, 2021). For each known microsite occupancy there is a probability of roost occurrence (ϕ) that is a logit function of the spatial and environmental covariates and their respective coefficients. Coefficients were initiated with a Normal distribution and noninformative priors. Starting with the full model containing all covariates that were not rejected for correlation, we performed backward model selection guided by change in DIC to select a final reduced logistic model to use in our hierarchical model.

In-sample validation was performed by training the model on covariate data for all microsites and comparing values of ϕ, the probability of roost occurrence, to observed occurrence. We also performed out-of-sample prediction using k-fold cross validation, randomly removing three microsites from the data set and predicting ϕ for omitted microsites using covariates, repeated over 500 iterations. Logistic model Bayesian analyses included two chains with an adaptation period of 10,000 iterations, followed by model prediction over 40,000 iterations with a 20,000 iteration burn in period.

3.2.3 Microclimate prediction model

In order to predict the factors that influence hibernation roost establishment, it is important that we understand how microsite microclimates develop and change with the outside world. To this end, we developed a model to predict underground temperature throughout our study environment based on surface-level conditions. This model's structure was informed by expert knowledge regarding this particular subterranean system and our microclimate observations throughout hiber-

nation. As such, it was specific to our system but the general structure may apply to predicting microclimates in other subterranean systems. We developed our model's form by considering basic physical principles: warm air rises, and heat will transfer from warmer to colder objects. Then, cavern walls that correlate consistently with MAST (Perry, 2013) acted as a heat source or sink depending on whether air was cold or hot, respectively. Because we based our model on established principles of natural systems, we were able to make inference to inform on expected microclimates under predicted above-ground changes, such as climate change. Although the relationships of airflow and temperature throughout the system may appear complex, we used a relatively straightforward parsimonious model to describe the spatially-changing influences of above-ground airflow and underground MAST temperature on within-cavern air temperature. We did not focus on prediction of water vapor pressure because our covariate selection process to predict roosting sites revealed that water vapor pressure was not an important predictor.

Our temperature prediction model built predictions along a time series, with each daily prediction of mean average temperature in a given microsite being a function of the previous day's temperature plus some change in temperature dependent on microsite location and surface conditions. We predicted microclimates on the initial day as a function of mine structure and system-specific expectations. In our case, this meant delineating microsites into three general location groupings: the Upper chamber, Lower chamber, and Side chamber (Figure 3.1). We expected in our system that throughout the summer, warm air will settle into the mine, simultaneously warming any super-cooled air from the previous winter and being cooled down to the local MAST-related analog (henceforth simply "MAST analog"). We used the second half of August as our start point, as the deepest microsites should have been sufficiently rewarmed to MAST analog conditions by this time. We chose a peak in mean daily surface temperature as our start point so there is no potential for microsites to be warmer than surface temperature as a result of lag effects from previous days. Thus, microsite temperature was greater than or equal to MAST analog temperature and below surface temperature.

Average daily temperature for the ith microsite on the first day ($\mu_{Temp,1,i}$) in each of the three chambers was phenomenologically modeled as a function of decrease in temperature from the primary entrance in the chamber down the elevation gradient, starting with surface temperature at highest elevation down to the MAST analog at lowest elevation (See Appendix Equation B.1). With average daily temperature at time $t = 1$ established for all microsites, subsequent average daily temperature ($\mu_{Temp,t,i}$) was modeled as a combination of temperature change due to airflow from the surface and the pull of temperature toward the MAST analog, with the percent contribution (ν) of each mediated by estimated airflow (Equation 3.1, see Appendix B.1). We assigned microsites as having high, medium, or low airflow, based on system observations. High airflow indicated microsites located in large open channels that connect directly to outside entrances. Medium airflow indicated microsites in relatively open but highly columnar space, so the air stream is easily broken. Low airflow microsites were located in small nooks that are offset from larger chambers. In equation 3.1, $f(\alpha_i, T_{out,t-3:t})$ indicates a regression on change in outside temperature over the past three days mediated by the spatially-derived parameter α, and $g(T_{MAST,t})$ refers to the change of within-chamber air temperature toward the daily MAST analog ($T_{MAST,t}$) due to contact with cavern walls. We modeled three days of change in outside temperatures because preliminary cross-correlation analysis between outside temperature and microsite temperature revealed that this was the longest period over which lag effects of outside temperature affected any site underground (see Appendix Figure B.3). Because airflow changed significantly in our system when surface temperature was lower than the MAST analog, we predicted model parameters separately for warm airflow days ($T_{out,t} > T_{MAST,t}$) and cold airflow days ($T_{out,t} < T_{MAST,t}$), designated with subscript w or c, respectively. All estimated parameters were initiated with noninformative priors. See Appendix B.1 for further details.

$$
\begin{aligned}
\mu_{Temp,t,i} &= \begin{cases} \nu_{i,w} f(\alpha_{i,w}, T_{out,t-3:t}) + (1-\nu_{i,w}) g_w(T_{MAST,t}) & T_{out,t} \geq T_{MAST,t} \\ \nu_{i,c} f(\alpha_{i,c}, T_{out,t-3:t}) + (1-\nu_{i,c}) g_c(T_{MAST,t}) & T_{out,t} < T_{MAST,t} \end{cases} \\
T_{t,i} &\sim \text{Normal}(\mu_{Temp,t,i}, \sigma_T^2)
\end{aligned} \tag{3.1}
$$

We performed in-sample validation of the microclimate model output by comparing predicted daily mean temperature to data logger observations for each microsite, and ensuring that daily variance in temperature predictions did not significantly exceed data logger measurement variance. The microclimate model is computationally slow due to a large amount of informing microclimate data, so our Bayesian analysis included two chains with an adaptation period of 1,000 iterations, followed by model prediction over 3,000 iterations with a 2,000 iteration burn in period.

3.2.4 Hierarchical model

In order to elevate our logistic roost occurrence model's predictive capacity, we used the microclimate model to predict relevant environmental covariates that feed hierarchically into the logistic roost occurrence model. The resulting hierarchical model is similarly computationally slow as the microclimate model, so we used the same Bayesian analysis protocol for adaptation, burn in, and prediction. We primarily used in-sample validation in the hierarchical model because the model's one week run-time makes repeated sampling intractable. In-sample validation included comparing model predictions of relevant microclimate covariates and probability of roost occurrence (ϕ) to system observations. We did perform an informal out-of-sample validation by predicting probability of roost occurrence in microsites where we did not have microclimate data to inform predictions, but knew that bats were or were not present during midwinter surveys.

We also used our model to predict underground microclimates as a function of above-ground conditions under climate change. To do so, we used estimated surface temperatures under an RCP 2.6 model projected 30 years in the future (2047-48 hibernation season) (Taylor *et al.*, 2012). We used our hierarchical model with estimated parameter values to calculate expected microclimate

covariates and roosting occurrence probability under these projected environmental conditions. To estimate model predictions of microclimate covariates and probability of roost occurrence throughout the entire subterranean system, we use the akima package in R (Akima & Gebhardt, 2021) to interpolate model expectations between and beyond data logger locations. This was performed for both the 2017-18 predictions informed by data logger measurements and the 2047-48 predictions under assumed climate change.

3.3 Results

Table 3.1 details the final results of our logistic roost occurrence model selection. We identified

Table 3.1: Selection of logistic roost occurrence model informed by measured microclimates. Models with a difference in DIC greater than 100% of the full model's penalized deviance were considered significantly less descriptive, and were rejected.

Model	Penalized deviance	ΔDIC
Full model	3.19	0
Selected model: Intercept + distance to warm + September standard deviation in temperature + mean hibernation temperature + path to bats	5.39	2.20
Selected $-$distance to warm	17.53	14.34
Selected $-$September standard deviation in temperature	21.83	18.64
Selected $-$mean hibernation temperature	22.60	19.41
Selected $-$path to bats	38.09	34.9

distance from warm entrance, mean hibernation temperature, standard deviation of temperature in September, and path to bats as significant covariates in determining roost occurrence. Distance to warm entrance, mean temperature, and path to bats were positively correlated with roost occurrence, while September standard deviation in temperature was negatively correlated with roost occurrence. Path to bats registered as most significant in both change in DIC and coefficient values, followed by mean hibernation temperature and September standard deviation in temperature. Distance from warm entrance and path to bats are spatial covariates that do not change over time,

but we were able to predict hibernation mean and September standard deviation in temperature through our hierarchical microclimate model. Figure 3.3 depicts representative microclimate prediction output from each of the three chambers. The predicted standard deviation of daily temperature (σ_T) was 0.64, which is comparable to the advertised +/- 0.5 degree Celsius resolution inherent in data logger measurements.

For our microclimate predictions to hierarchically inform the logistic model, we calculated mean hibernation temperature as the mean of predicted daily temperature ($\mu_{Temp,t,i}$) over the modeled time period and September standard deviation in temperature as the standard deviation of predicted daily temperature throughout September (Figure 3.4, see Appendix B.4). Our model captured mean hibernation temperature with impressive accuracy (observed vs. predicted Adjusted $R^2 = 0.93$, $p < 0.0001$) (Figure 3.4.a,b). September standard deviation demonstrated a clear relationship between observations and predictions, though goodness of fit was not as precise (observed vs. predicted Adjusted $R^2 = 0.23$, $p = 0.002$). This may be expected given that the lack of resolution in data logger measurements is unable to capture minor temperature variation in stable conditions (e.g. Figure 3.3 inset 2). However, importantly, our predictions did capture a decrease in standard deviation in areas where midwinter roosts are found relative to areas where they are not found (Figure 3.4.d). This was consistent with the logistic model, which predicted a negative correlation between roost occurrence and September standard deviation in temperature.

The predicted values in Figure 3.4.b and d are covariates for inference in the logistic layer of the hierarchical model. The result is an estimation of microsite roosting probability that is even more precise than the already reasonably successful logistic model making direct inference with data-based covariates (Figure 3.5.a,b). In fact, we can see that any microsites for which there was a large uncertainty in roosting probability for either the out-of-sample or in-sample data-informed logistic model, the uncertainty is greatly reduced by using the hierarchical model. As a result, the hierarchical model is capable of accurately predicting all measured microsites as having ($\phi > 50\%$) or not having roosts ($\phi < 50\%$) (Figure 3.5.a,b), with one exception that is particularly close in proximity to a roost-present microsite. Interestingly, areas of high roosting probability

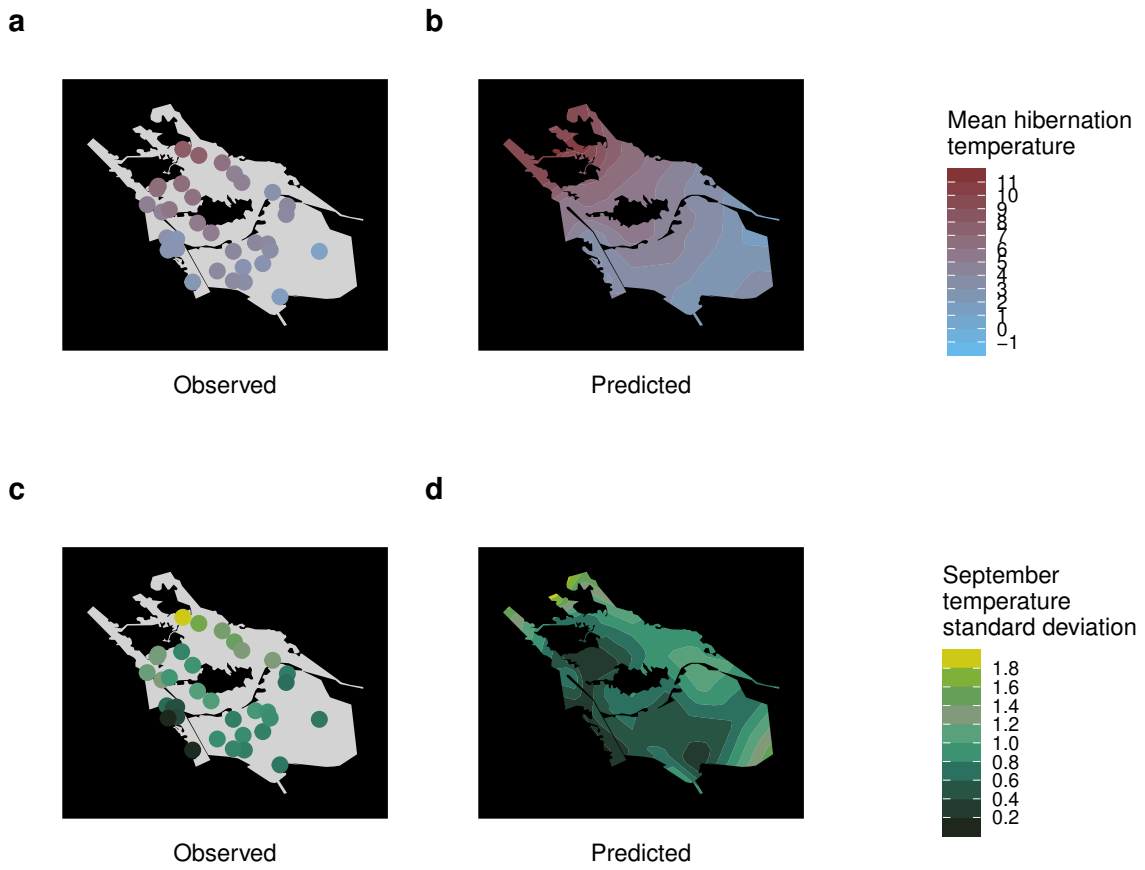


Figure 3.4: Measured and predicted microclimate-related variables relevant to the selected logistic model. (a) Observed and (c) predicted mean hibernation temperature show similar trends, with lower average temperatures at lower elevations farther from the high elevation warm entrance. (b) Observed September standard deviation in temperature is lowest in the side chamber and highest closest to the warm entrance. While there is some evidence that areas where bats commonly roost have lower standard deviation in temperature, these differences are more pronounced in (d) model predictions. Observations (a,b) are represented as individual points for each data logger, while space between model predictions (c,d) was estimated using interpolation (Akima & Gebhardt, 2021).

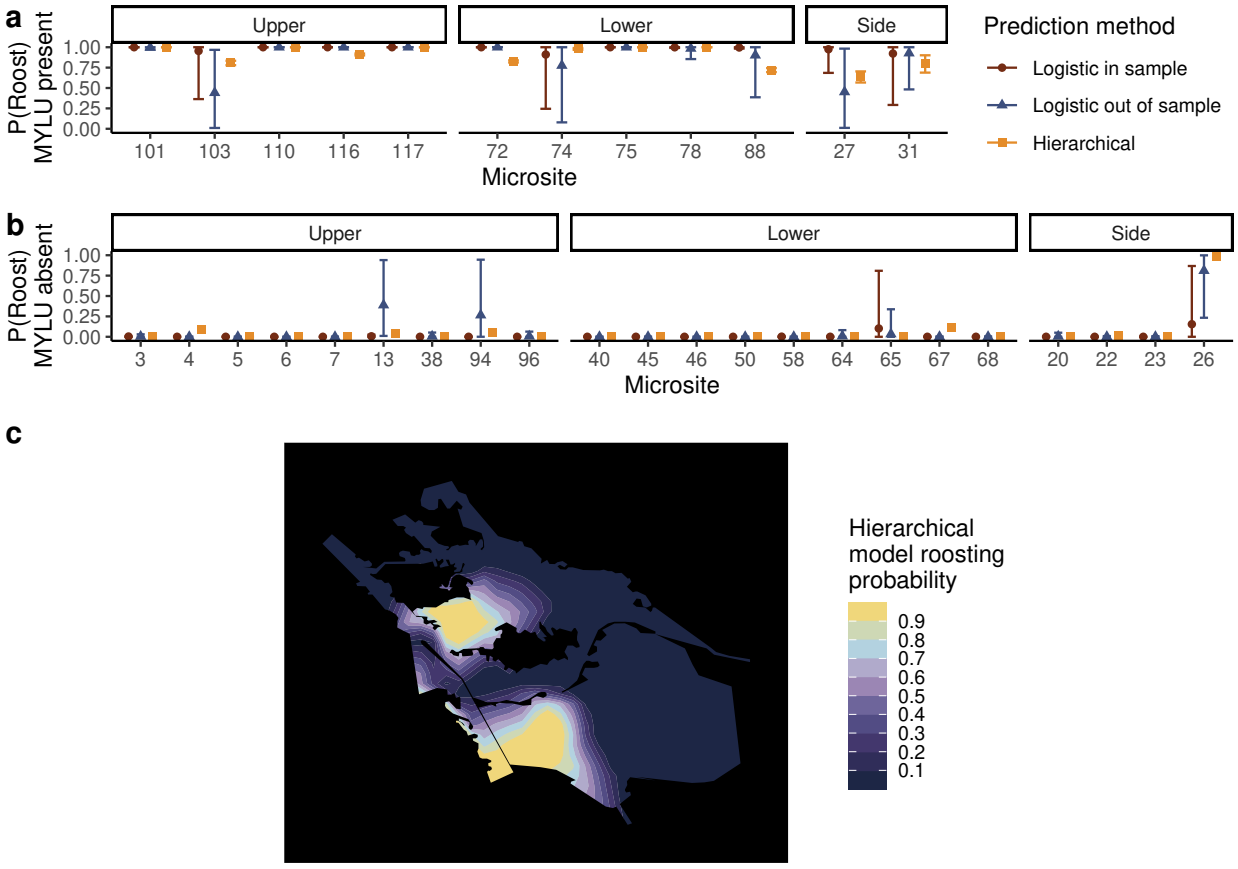


Figure 3.5: Mean and 95% credible intervals of model predictions for probability of bat roosting for microsites (a) with and (b) without regularly observed *M. lucifugus* roosts. Hierarchical model predictions (orange) have significantly reduced uncertainty in probability compared to either in-sample or out-of-sample predictions using the logistic model informed by data logger measurements. (c) Hierarchical modeling of microclimate prediction informing logistic roosting probability throughout the subterranean system. Areas of high bat roosting probability are present in all three chambers, including pockets of the Side chamber where *M. lucifugus* are regularly observed roosting but data logger measurements are not available.

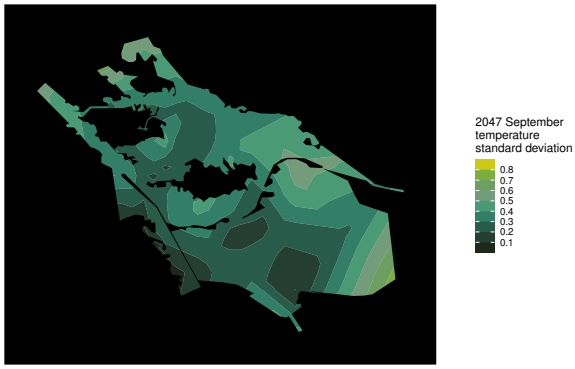
between the Lower and Side chambers appear contiguous, which may be possible due to small connecting chambers not depicted in our rough visual approximation of tunnel boundaries or logger network (see Appendix Figure B.2). In pockets of the Side chamber where several data loggers malfunctioned there are areas of high roosting probability (Figure 3.5.c), which is consistent with mid-winter survey observations of bats roosting. Additionally, we predicted that areas on the right side of the Lower chamber (Figure 3.5.c) where data were largely not collected were unsuitable for bat roosting, which is also consistent with midwinter survey observations. These microsites act as an additional form of out-of-sample validation where microclimate data collection was not possible.

To examine the potential effects of climate change on hibernaculum roosting potential, we used projected global surface data from 2047-48 to inform our hierarchical model (Figure 3.6). We find that under the RCP 2.6 climate change prediction model, mean hibernation temperature throughout the hibernaculum is increased by approximately two degrees Celsius (Figure 3.6.a), and while in general September standard deviation in temperature holds similar patterns of being greater closer to mine entrances, the overall standard deviation is significantly lower throughout the hibernaculum (Figure 3.6.b). This suggests that hibernaculum microsites overall could be both warmer and more stable than current conditions. As a result, there is a significant increase in predicted within-hibernaculum range of suitable roosting microsites (Figure 3.6.c).

3.4 Discussion

Subterranean environments provide opportunity to examine ecological niche space in relatively undisturbed and stable natural systems (Mammola *et al.*, 2019b). It is important that we define the realized niche used by bat populations to survive hibernation so we can manage expectations when anthropogenic disturbances occur. While bats are often regarded as seeking “optimal" microclimates and stable conditions for hibernation (Elliott & Clawson, 2001; Tuttle & Kennedy, 2011), attempts to characterize roost suitability in subterranean systems with variable microclimates show that these assumptions may be an oversimplification (Boyles *et al.*, 2017). The breadth of the *M.*

a



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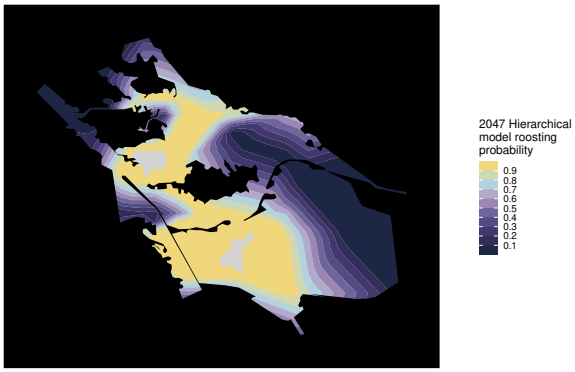

c

Figure 3.6: Predicted (a) mean hibernation temperature, (b) September standard deviation in temperature, and (c) bat roosting probability using daily temperature predicted using the GFDL-CM3 (NOAA, USA) RCP 2.6 model for the 2047-48 hibernation season. Compared to predicted 2017-18 values in Figure 3.4, mean temperature is in general increased, and September standard deviation decreased, resulting in an expansion of suitable roosting habitat in all hibernaculum chambers.

lucifugus hibernation niche appears greater than expected, which may contribute to observed survival when *Pd* is introduced to the system. To our knowledge, our efforts are among the most thorough and complete description of spatiotemporally variable microsites available for bat roosting within a single hibernaculum. By assembling this complex data set, we were able to parameterize a novel hierarchical modeling framework that accurately predicts both spatiotemporal changes in microclimate as a function of above-ground conditions, and probability of bat roost occurrence throughout the hibernaculum. In doing so, we describe the realized niche space for a population of free-ranging *M. lucifugus* (Figure 3.5), as well as how that space changes with external influences (Figure 3.6). The flexibility of our approach paves the way for forecasting microclimate availability under different scenarios to evaluate risk associated with disturbances to the available niche space.

To evaluate niche response to environmental disturbances, we first have to define the niche boundaries of preferred hibernation microsites. In general, these *M. lucifugus* roost in deeper areas of the subterranean system, which is generally where the most stable conditions occur (Perry, 2013). This is in keeping with general assumptions that bats prefer more stable conditions (Elliott & Clawson, 2001; Tuttle & Kennedy, 2011), but observed significant differences in variation (Figure 3.3 insets 2,3) suggest that this population has a relatively wide realized niche where temperature variation is concerned (Boyles *et al.*, 2017). In this hibernaculum, there was a positive correlation with overall hibernation temperature, suggesting that bats may be choosing roosting microsites to avoid excessively cold temperatures. However, extremes like temperature minimum and maximum were not significant in our logistic model. This may be because bats have ways of mitigating the effects of brief energetically harmful shifts in microclimate, such as clustering behavior, using neighbors as insulation to prevent energetic and evaporative water loss (Clawson *et al.*, 1980; Roverud & Chappell, 1991; Canals *et al.*, 1997; Boratyński *et al.*, 2015). The potential impact of clustering or similar behavior is further supported by the importance of the path to bats covariate in the logistic model, which suggests that there may be a strong social component to the realized niche space. In our system, *M. lucifugus* are not found roosting in microsites with colder

winter temperatures during mid-winter surveys, but it would be be worth examining longitudinal microclimate data in conjunction with mid-hibernation bat movement to evaluate how changes in microclimate over time may affect bat roost location preference (Ryan *et al.*, 2019; Golas *et al.*, in review). Overall, we find evidence that hibernation roost occurrence is influenced by a combination of microclimate profiles, microsite stability, and sociality.

In addition to these three factors, by connecting underground microclimates to above-ground conditions, we demonstrate an important temporal component to the realized niche of hibernation roost establishment. We suspect that roosts are established early in the hibernation season, potentially by the first individuals to settle underground, given a strong negative correlation between roosting preference and standard deviation in temperature in September specifically, as opposed to later months (Table 3.1). This may explain why distance to the warm entrance, which is the primary source of airflow and microclimate variability in warm Fall months, is significantly correlated with roost establishment, as opposed to the cold entrance. However, microclimates can change seasonally, such that an optimal Fall microsite might be detrimental in Winter or Spring. As such, it may be beneficial for a bat to have a wide range of roosting preferences rather than optimizing for a singular temperature value (Boyles *et al.*, 2017). By developing tolerance for a variety of conditions, bats can wait out less ideal microclimates rather than arouse from torpor to move locations (Ryan *et al.*, 2019). The majority of hibernation energy expenditure comes from arousal events rather than torpor itself (Thomas *et al.*, 1990). Then, tolerance of variance in temperature could be a life-saving benefit that prevents unnecessary arousal when conditions change, even if those new conditions are not as physiologically ideal as the previous.

Non-ideal microclimate tolerance may have significant impact when we consider the impacts of water vapor pressure and *Pd* on the bat hibernation niche. We do not yet incorporate water vapor pressure into our microclimate predictions based on our logistic model selection results, but evaporative water loss due to disrupted skin membranes is an important driver of WNS pathology (Cryan *et al.*, 2010; Willis *et al.*, 2011; Cryan *et al.*, 2013; McGuire *et al.*, 2021a). We suspect that water vapor pressure was not significantly correlated with roost occurrence in this hibernac-

ulum because the entire subterranean system is essentially saturated (100% possible water vapor pressure for a given ambient temperature) in the Fall months when roosts are being established. However, water vapor pressure may be important for *M. lucifugus* survival in this system. Unlike other hibernacula, where longitudinal measurements of roosting microclimates often reveal constant saturation (Haase *et al.*, 2019a), we find significant drops in relative humidity due to airflow through the cold entrance (Figure 3.4.c). *Pd* generally requires high humidity environments, and sudden drops in temperature and humidity could disrupt fungal growth (Verant *et al.*, 2012). On the other hand, low humidity environments can result in excessive evaporative water loss, making drier microclimates outside the fundamental niche range for hibernating bats (Haase *et al.*, 2019a). The persistence of bats through drier periods suggests tolerance of variation in water vapor pressure similar to temperature variation tolerance.

Importantly, our prediction modeling framework's flexibility and parsimony makes it an excellent framework for further evaluating bat hibernation and survival under changing conditions. While others have had impressive success reproducing subterranean microclimates in natural systems using complex model approaches (Jernigan & Swift, 2001), we provide a more universal approach that requires only minor customization based on the system's spatial characteristics. Because our model uses widely available surface-level data to predict underground microclimate availability as it affects bat roost preference, we can easily make predictions, such as expected suitable hibernation roost niches under climate change (Figure 3.6). Our predictions under this climate change forecast suggest that warmer temperatures and reduced variance in September microclimates may drastically increase the spatial niche for *M. lucifugus* in this hibernaculum. However, given more stable conditions overall, this increase in warmer temperatures may be a reflection of reduced cold dry airflow coming through the cold entrance in winter, resulting in consistently high humidity. This could result in increased *Pd* growth, and subsequently increased WNS mortality and reduced realized niche space (Hayman *et al.*, 2016; Haase *et al.*, 2019a). However, if climatic change is gradual compared to bat reproductive life cycles, bats may be capable of evolving tolerance for *Pd* presence before microclimate-induced *Pd* overgrowth becomes overwhelming

(DiRenzo *et al.*, 2018; Gignoux-Wolfsohn *et al.*, 2021; Golas *et al.*, 2021). Thus, when considering prediction of ecophysiological host-pathogen dynamics under climate change scenarios, it would be worth implementing within an evolutionary framework to account for potential adaptive changes in niche space (Golas *et al.*, 2021).

Considering WNS as shared niche space between the fungus *Pd* and hibernating bats, we require hibernation microclimate data to evaluate WNS risk, but these are difficult to obtain and sometimes wholly unavailable. For example, the actual hibernation locations of western bats are largely unknown (McClure *et al.*, 2020), and in the east there are efforts to alter microclimates to increase airflow and make them more suitable for bat survival (Turner *et al.*, 2021). While initial results have been promising (Turner *et al.*, 2021), there is concern that such manipulations could alter available microclimates in unexpected ways, potentially reducing niche space and survival potential for some bat populations. Novel technological and modeling advancements can help to address this data deficiency (Golas *et al.*, in review). We can use our approach to consider the potential results of theoretical new hibernaculum entrances. By estimating changes in temperature variability in the early Fall and average hibernation temperature as a result of alterations, we can predict whether bats would be attracted to roosting microsites prior to making irreversible changes to sensitive hibernacula. A predictive approach to subterranean microclimate availability opens the door for many new possibilities for evaluating bat roost potential and subsequent survival with consequences for conservation and management action.

Chapter 4

Behavioral and physiological adaptations interact to allow white nose syndrome survival in hibernating bats

4.1 Introduction

Niche space is a combination of physiological requirements for survival (fundamental niche) and biotic interactions (realized niche) that define the space occupied by a population (Hutchinson, 1957). It can be difficult to identify drivers of niche in natural systems due to complexity and stochasticity, though pairing behavioral observations with detailed environmental data can begin to define realized niche space (Golas *et al.*, in prep). We can further define a population's fundamental niche using ecophysiological models that describe hard limits to the environmental conditions in which an individual can survive (Hayman *et al.*, 2016; Haase *et al.*, 2019a). By combining ecophysiological models alongside experienced environmental conditions largely determining survival, we can estimate population and individual traits that allow organisms to inhabit their niche (Golas *et al.*, in review). However, a population's niche is capable of expanding or contracting with changes in community interactions (Bruno *et al.*, 2003). We often consider biotic interactions changing niche space via direct interspecific interactions, such as predation or competetive inhibition (Connell, 1961; Williams & Martinez, 2000), but less frequently do we consider the indirect effects that can occur in niche partitioning and evolution (Holt, 1996; Jachowski *et al.*, 2014). For example, in the case of white-nose syndrome (WNS) in bats, the keratinolytic fungus *Pseudogymnoascus destructans* (*Pd*) disrupts susceptible bats' skin membranes, changing how they interact with their environments and restricting the physiological limits of their fundamental niches (Cryan *et al.*, 2010; Willis *et al.*, 2011; Cryan *et al.*, 2013; Hayman *et al.*, 2016). As a result, environmental conditions that used to be 'survival space' capable of maintaining healthy populations have

become ecological traps (Schlaepfer *et al.*, 2002; Leach *et al.*, 2016; Golas *et al.*, in review), resulting in the deaths of millions of bats (Blehert *et al.*, 2009; Frick *et al.*, 2010; O'Shea *et al.*, 2016). Behavioral adaptations may be capable of mitigating disease processes (Clawson *et al.*, 1980; Brownlee-Bouboulis & Reeder, 2013; Boratyński *et al.*, 2015), but these have not yet been examined alongside ecophysiological models that relate WNS mortality to experienced environmental conditions. To assess population viability of hibernating bats infected with *Pd*, we need to consider a broader range of behavioral and physiological interactions between hosts, pathogen, and environment that define the direct and indirect effects on fundamental and realized niche ranges.

Multiple hypotheses for behavioral and physiological survival mechanisms have arisen to describe inter- and intra-specific variation in host response to *Pd*. Mortality seems to be closely linked to localized environmental conditions (microclimates) experienced during hibernation (Cryan *et al.*, 2010; Langwig *et al.*, 2012; Cryan *et al.*, 2013). Bats that succumb to WNS arouse more frequently, leading to increased energetic loss and eventual starvation (Reeder *et al.*, 2012). Studies suggest that this arousal is a function of microclimate temperature and humidity, with *Pd* loads increasing bat energy loss and water loss, triggering the lowered metabolism of torpor to rise back to euthermic levels (Hayman *et al.*, 2016; Haase *et al.*, 2019a). *Pd* growth is also a function of microclimate (Verant *et al.*, 2012), meaning that to survive infection hibernating bats must balance conditions that satisfy their own physiological needs while preventing excessive *Pd* growth (Hayman *et al.*, 2016). Depending on metabolic parameters (e.g. metabolic rate, evaporative water loss rate), the niche of microclimates in which an individual can survive hibernation may be very wide or narrow, and may overlap significantly with the *Pd* fundamental niche. While this theory of torpor arousal has been validated in both laboratory and field settings (Haase *et al.*, 2019a; Golas *et al.*, in review), anomalies of unexpectedly high population survival rates in an otherwise high mortality species (Cheng *et al.*, 2019; Golas *et al.*, in prep) suggest that we require a better understanding of variation in and interactions between bat physiology, environmental conditions, and behaviors influencing host metabolism and *Pd* presence.

To best assess how host interactions with their environment impact WNS outcomes, we need to combine detailed longitudinal data to characterize experienced microclimates with host metabolic traits and processes (Golas *et al.*, in review). Microclimates available for hibernation change with time; they are often highly dependent on mean annual surface temperature, but depending on hibernaculum structure there can be significant microclimate variation associated with changes in daily above ground conditions (Perry, 2013; McClure *et al.*, 2020; Golas *et al.*, in prep). Despite common assertions that bats preferentially hibernate in stable microclimates (Elliott & Clawson, 2001; Tuttle & Kennedy, 2011), there can be significant variation in temperature and humidity at hibernation microsites (Boyles *et al.*, 2017; Golas *et al.*, in prep). Variability in humidity may be especially important to capture, as there is a growing body of evidence suggesting that hibernating bat arousal patterns and WNS mortality are closely related to environmental water vapor pressure (Cryan *et al.*, 2010; Willis *et al.*, 2011; Cryan *et al.*, 2013; McGuire *et al.*, 2021a). Microclimate extremes can be untolerable for *Pd growth* and more energetically taxing for hibernating bats (Verant *et al.*, 2012; Hayman *et al.*, 2016). However, local exaptation for metabolic traits determining bat response to microclimate may widen the hibernation niche range beyond *Pd* limits, contributing to survival. Bat populations can exhibit significant intraspecific variability in these traits (Burnett, 1983; Cryan *et al.*, 2010). For example, big brown bats living in dry areas may be adapted for reduced evaporative water loss (Klüg-Baerwald & Brigham, 2017), and along a latitudinal gradient, bat populations experience differing temperature-dependent torpid metabolic rates (Dunbar & Brigham, 2010). Prominent mechanistic models of hibernation survival with and without *Pd* rely on metabolic parameters to determine arousal frequency (Hayman *et al.*, 2016; Haase *et al.*, 2019a). However, these models have been parameterized using literature values or experimental data from laboratory settings, sampling populations that might differ from the population of interest. A recent study accounts for natural intraspecific and individual variation in bat ecophysiological parameters by estimating them in a model fitting the relationship of measured torpor bout lengths to microclimates (Golas *et al.*, in review). By pairing ecophysiological parameter estimation with longitudinal microclimate data and spatial hibernation observations, we can simul-

taneously estimate the metabolic trait profiles required to explain observed bat survival in a local population and determine whether bats preferentially use energetically favorable microclimates that preclude survival.

While microclimate use should have a strong effect on WNS survival (Hayman *et al.*, 2016), there are multiple other behavioral strategies that could help mitigate disease impact that are not included in current ecophysiological models. Clustering of bats spatially has the potential to reduce energetic loss, as is seen in other animals that rest in groups, reducing heat loss to the environment by insulating exposed areas with other bodies (Clawson *et al.*, 1980; Roverud & Chappell, 1991; Canals *et al.*, 1997). Clustering also has the potential to reduce evaporative water loss by covering exposed skin membranes (Boratyński *et al.*, 2015), which similarly has the potential to increase torpor length and reduce arousal frequency (Haase *et al.*, 2019a). However, clustering behavior could also prove detrimental when disturbances such as disease of arousing neighbors might lead to an increase in energetically costly arousal events (Thomas *et al.*, 1990; Langwig *et al.*, 2012; Reeder *et al.*, 2012). Another potential behavioral adaptation for survival is grooming to clear fungus. Bats need to arouse from torpor periodically, and may exhibit active behaviors, such as flying, drinking, and grooming, during these periods of euthermia. While some studies show that experimentally infected bats reduce grooming behavior (Wilcox *et al.*, 2014; Bohn *et al.*, 2016), others have shown a significant increase in grooming during arousal compared to uninfected bats (Brownlee-Bouboulis & Reeder, 2013). Attempts to visualize clustering and grooming behaviors in natural free-ranging populations can be difficult given the logistical constraints of recording video underground in the dark without ready power supply (Hayman *et al.*, 2017), and experimental studies to estimate the potential benefits of these strategies require elaborate equipment and removal of wild animals from natural environments that might impact outcomes (Boratyński *et al.*, 2015; Golas *et al.*, in review). It is worth testing the potential for these behaviors to impact survival outcomes, which we can do by amending ecophysiological models designed to explore microclimate use hypotheses (Hayman *et al.*, 2016; Haase *et al.*, 2019b) with mathematical representations of other survival-related behaviors. Thus, a robust evaluation of how bats interact with

their environments to survive WNS will incorporate population variation in physiological traits, longitudinal hibernaculum microclimate data, and a variety of behavioral mechanisms.

We aim to test the relative importance of four different factors affecting WNS survival in natural settings: physiological adaptation, microclimate use, clustering behavior, and grooming behavior. We will evaluate an important hibernaculum wherein the *Myotis lucifugus* population has thrived with no indications of significant die-offs despite regional mass mortality (Cheng *et al.*, 2019; Golas *et al.*, in prep). To compare the impact of potential WNS survival factors, we plan to use a combination of ecophysiological modeling and detailed hibernaculum microclimate data to estimate survival as a function of energetic use, similar to Golas *et al.* (in review). This approach relies on bat-specific torpor bout length measurements and conditions experienced by the individual, but to evaluate microclimate choice as a survival-driving behavior, we need to be able to consider microclimates not used for hibernation in addition to those the bats experience. Thus, to evaluate the suitability for hibernation survival of any given location within the hibernaculum (microsite), we will have to adapt these methods to encompass longitudinal environmental data without explicit data on bat arousal activity. We convert ecophysiological models to estimate hourly energy expenditure and arousal frequency, allowing us to test models under different metabolic parameterizations and accept or reject them based on biologically realistic survival and torpor bout length outcomes. By amending ecophysiological models with mathematical terms representing clustering and grooming behaviors, we develop a suite of models that predict WNS outcomes based on metabolic traits, microclimate use, and exhibited combinations of behaviors. Starting with literature-based, biologically relevant priors, we use approximate Bayesian computation to simultaneously estimate metabolic parameters and identify behavioral models capable of describing biologically realistic survival across this system. Then, we can examine parameter estimates for deviance from literature-based values suggesting local physiological adaptation, and we can make inference on important survival behaviors based on model feasibility. As a result, we improve our understanding of how a population from a species in decline can use metabolic and behavioral traits in variable environmental conditions to thrive in the face of a devastating disease.

4.2 Materials and Methods

4.2.1 System description

For our study, we chose to investigate *M. lucifugus* because there are measured literature-based estimates for parameters of interest in the metabolic equations (Hayman *et al.*, 2016; Haase *et al.*, 2019a), and this once widely abundant species has experienced significant die offs since *Pd* introduction (Cheng *et al.*, 2019). Despite severe mortality events across eastern North America, our study hibernaculum did not experience any significantly increased mortality in *M. lucifugus* following *Pd* introduction Cheng *et al.* (2019). The hibernaculum is composed of three main chambers (Upper, Lower, and Side), each with microsites where *M. lucifugus* have been observed hibernating during midwinter surveys and areas where they have not been observed (Golas *et al.*, in prep). We used modified iButton data loggers to measure temperature and relative humidity every two hours at hibernation and non-hibernation microsites throughout the hibernaculum (Golas *et al.*, in prep) (see Appendix). We used microclimate data collected from October 1st, 2017, to April 15, 2018, for a total 197 days of hibernation, which was informed by regional surveys of Fall swarming and Spring emergence. Microclimate data were used in this study as inputs for metabolic equations to determine arousal frequency and energy use throughout hibernation. In order to estimate hourly energy expenditure, we repeated the previous hour's measurement for hours where measurements were not taken. Erroneous measurements were adjusted as described by Golas *et al.* (in prep) and relative humidity converted to water vapor pressure using the same methods as (Haase *et al.*, 2019a). Further details regarding the study hibernaculum and microclimate data collection can be found in Golas *et al.* (in prep).

4.2.2 Model descriptions

To estimate energetic expenditure as a function of microclimate, we used the metabolic equations presented by Haase *et al.* (2019a). While there is evidence that actual torpor bout duration may on average be shorter than model expectations due to stochasticity in natural systems (Golas *et al.*, in review), we considered the equations as presented because they have been validated

using *M. lucifugus* torpor bouts directly, and we did not have microclimate-dependent torpor bout lengths in natural systems available for this species. We implemented the metabolic equations that describe torpor bouts sequentially, starting with a cooling phase (Haase *et al.*, 2019b), then torpor, arousal, euthermia, and back to cooling. We continued this cycle until hibernation was over, and finished with a final arousal if the bat was not already euthermic. We used an average literature-based length for euthermia, but the lengths of the three other phases were microclimate-dependent (see Appendix). The length of cooling and arousal periods were calculated using the microclimate when torpor began because these periods generally last less than 2 hours, and our microclimate measurements were taken every two hours. Torpor bout length was calculated using a rolling estimate. Each hour of torpor, we first calculated energy and water loss from the metabolic equations based on the current hour's microclimate. We then recalculated expected torpor bout length based on the cumulative microclimates experienced from the torpor's start to the current hour (Figure 4.1). Once the number of hours spent in torpor exceeded the microclimate-based torpor length es-

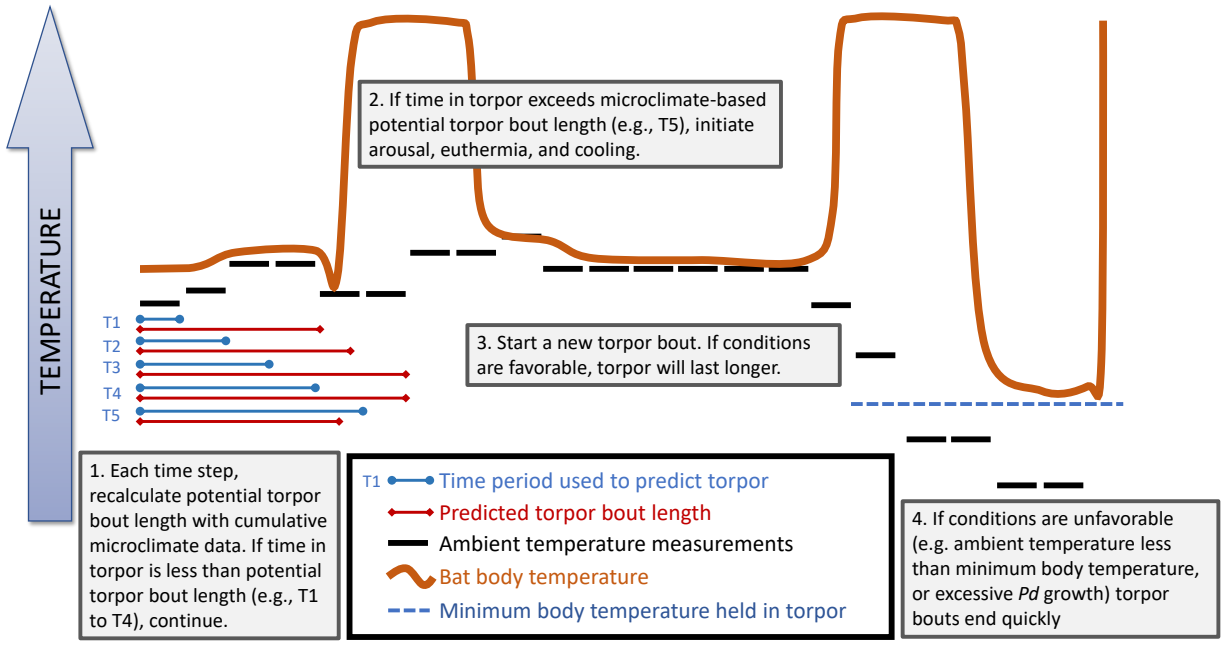

Figure 4.1: A schematic representing the decision-making process of how simulations determine torpor bout length and arousal frequency based on longitudinal microclimate data.

timate, arousal was initiated. In simulations with *Pd*, fungal growth accumulated every hour based on ambient water vapor pressure and bat skin temperature (Hayman *et al.*, 2016). Torpor bout duration decreases with increased *Pd* growth due to increased energy expenditure and evaporative water loss (Hayman *et al.*, 2016; Haase *et al.*, 2019a).

We incorporated other behavioral adaptations for WNS survival as mathematical terms in the metabolic equations. Clustering was estimated as the percent of energy used in torpor while clustered within a large group of other individuals as compared to energy used while hibernating individually (Canals *et al.*, 1997). We multiplied this cluster factor times the denominators of torpor length equations to extend maximum possible torpor length through reduced energy consumption and evaporative water loss (see Appendix). We applied the cluster factor only to the torpid period, not arousal or cooling, because to our knowledge *M. lucifugus* arouse individually, rather than in groups (Hayman *et al.*, 2017), so insulatory benefits of warming the self while surrounded by cold individuals may be minimal. Similar to how fungal growth was estimated as square centimeters accumulated per hour, we estimated grooming rate as square centimeters of fungus removed per hour. Per previous studies, we allowed bats 22% of the euthermic period to remove fungus via grooming (Brownlee-Bouboulis & Reeder, 2013). The maximum possible grooming rate estimate (Table 4.1) suggests that *M. lucifugus* could groom fungus from the entire wing surface during a single arousal, roughly 20 cm^2 over 15 minutes (approximately 22% of 1.1 hours) (Haase *et al.*, 2019a). This was probably an over-estimation of bat capabilities, but it set a biologically-relevant limit to guide our investigation.

Given these metabolic equations and additional mechanisms, we simulated six different scenarios for a bat hibernating in a given microclimate: solitary hibernation without *Pd*, solitary hibernation with *Pd*, clustered hibernation without *Pd*, clustered hibernation with *Pd*, solitary hibernation with *Pd* and grooming, and clustered hibernation with *Pd* and grooming. Using these scenarios, we established four separate models representing different combinations of behavioral mechanisms. Each model contains a hibernation scenario without *Pd* (solitary or clustered) and a hibernation scenario with *Pd* (solitary, clustered, solitary grooming, or clustered grooming).

Table 4.1: Parameters estimated via approximate Bayesian computation. Literature-based values were used to develop distributions for latin hypercube sampling.

Parameter	Definition and units	Value	Distribution	Reference
$T_{tor,min}$	Minimum body temperature maintained in torpor (°C)	2	Uniform(0.5, 5)	(Hock, 1951; Hanus, 1959; Speakman *et al.*, 1991; Haase *et al.*, 2019a)
TMR_{min}	Torpid metabolic rate (ml O_2 g^{-1} $hour^{-1}$)	0.03	Uniform(0.003, 0.15)	(Haase *et al.*, 2019a)
C_t	Torpid conductance (ml O_2 g^{-1} $°C^{-1}$)	0.2	Uniform(0.02, 1)	(McNab, 1980; Haase *et al.*, 2019a)
$t_{tor,max}$	Maximum torpor duration (hours)	1300	Uniform(650, 1950)	(Brack Jr & Twente, 1985)
$rEWL_{body}$	Rate of cutaneous evaporative water loss from the body (mg hr^{-1} ΔWVP^{-1} cm^{-2})	0.1	Uniform(0.01, 0.5)	(Haase *et al.*, 2019a)
M_{body}	Body mass (grams)	-	Uniform(7.46, 10.51)	(Cheng *et al.*, 2019)
p_{fat}	Pre-hibernation fat stores as percent of body mass	-	Uniform(0.152, 0.335)	(Cheng *et al.*, 2019)
$clusterfac$	Reduction in energy/water loss due to clustering as a percent of loss while solitary (%)	-	Uniform(50, 100)	(Canals *et al.*, 1997)
$groom$	Rate of *Pd* removal by grooming (cm^2/hour)	-	Uniform(0, 100)	This study

Because microclimate use was an inherent component of all simulations, we refer to the four models as: Microclimate, Microclimate + Clustering, Microclimate + Grooming, and Microclimate + Clustering + Grooming, depending on which additional behavioral hypotheses were being tested in the model. For each model, we tracked the cumulative energy consumption throughout hibernation with and without *Pd* and compared to the individual's fat stores. Individuals that did not consume as much fat as they had stored were marked as surviving, while those that did not were marked as dead. We also tracked the number of arousals through the hibernation period, and used this to estimate average torpor bout length as hibernation length divided by total number of arousals.

4.3 Parameter estimation

To screen for potential interactions between behavioral and physiologically adaptive hypotheses, we needed to identify which of the four models are relevant to our system and estimate parameters relevant to metabolic traits, but the complications of applying multiple complex models to longitudinal microclimate data and simulating latent variables like irregularly-timed arousals made likelihood evaluation infeasible. To bypass the need for likelihoods, we used approximate Bayesian computation (ABC) parameter estimation (Toni *et al.*, 2009; Beaumont, 2010) to test for potential local physiological adaptation in metabolic traits that might differ from values measured in other populations. We were then able to gain insight into behavioral adaptations that describe WNS survival by excluding models for which we could not identify parameters sets that resulted in biologically realistic outcomes.

We performed ABC parameter estimation in three steps: 1. Randomly draw a parameter set from literature-based, biologically relevant prior distributions. 2. Simulate hibernation for each of the six scenarios comprising the four models, for all thirty-four microsites with measured microclimates. 3. Evaluate output for each model through a rejection algorithm, rejecting the parameter set for the given model if the output does not meet biologically realistic criteria. Then, the parameter sets passing the rejection algorithm are a form of posterior distribution for parameter estimation. Parameters to estimate were chosen based on previous work by Golas *et al.* (in review) using

partial rank correlation coefficient parameter sensitivity analysis (Marino *et al.*, 2008) to identify parameters to which torpor bout length is sensitive. We generated 200,000 unique, random parameters sets for simulation using a Latin hypercube sampling (LHS) matrix (McKay *et al.*, 2000). The parameters sampled in the LHS matrix were torpid thermal conductance, torpid minimum metabolic rate, evaporative water loss, minimum body temperature defended while in torpor, maximum length of torpor, clustering factor, grooming rate, body size, and percent of body weight that is fat. For the first three parameters, we defined the prior distributions by starting with literature-based values measured from *M. lucifugus* populations (Haase *et al.*, 2019a) and multiplying by 0.1 and 5 to define the minimum and maximum limits for Uniform distributions, respectively. Similarly the Uniform distribution range of maximum length of torpor was determine by multiplying a literature-derived estimate by 0.5 and 1.5. For minimum torpid body temperature, we used the same biologically relevant range that Golas *et al.* (in review) used for parameter estimation. Clustering factor was estimated in a range from 50% to 100% and grooming fungal removal rate from 0 to 100 cm^2 per hour. Due to the lack of detailed investigation for these mechanisms in hibernating bats, we used ranges representing values with no impact to extremes that push assumptions of biological realism. Thus, all parameter prior distributions were based in biological realism but allowed for significant variation that might result from local adaptation. The ranges for body mass and percent fat were estimated using field data presented in previous studies from bats captured at this hibernaculum (Cheng *et al.*, 2019). Parameter prior distributions are detailed in Table 4.1.

We established our ABC rejection algorithm to realistically represent *M. lucifugus* hibernation in our study hibernaculum. The algorithm stated that, for a parameter set under a given model to be accepted, then for all microsites where *M. lucifugus* have been regularly observed hibernating: 1. Bats must survive hibernation. 2. The bat's average torpor length without fungus must fall within 9 to 31 days (Reeder *et al.*, 2012). 3. The bat's average torpor length with *Pd* must fall within 6.8 and 21.6 days (Reeder *et al.*, 2012). Following parameter estimation, we evaluated parameter posterior distributions for correlation using Spearman's rank correlation coefficient calculated using the 'PerformanceAnalytics' package in R (Peterson & Carl, 2020). We tested the microclimate use

hypothesis that bats preferentially hibernate in physiologically favorable conditions by separately averaging torpor bout lengths calculated using non-rejected parameter sets across microsites that do and do not have hibernating *M. lucifugus* in each of the three hibernaculum chambers. We used t-tests to compare average torpor bout length estimates between chambers and paired t-tests to compare expected torpor bout lengths in microsites where *M. lucifugus* are regularly found versus microsites where they are not.

All code was custom-written in R (R Core Team, 2021). The LHS matrix was generated using the 'lhs' package, and simulations were run in parallel using the 'doParallel' package (Carnell, 2021; Corporation & Weston, 2020). Figures were generated using the 'tidyverse' and 'ggplot2' packages (Wickham *et al.*, 2019; Wickham, 2016).

4.4 Results

Figure 4.2 depicts the estimated cumulative fat consumption through hibernation for the six different simulations using the literature values (Table 4.1) at a microsite where bats commonly hibernate. Without fungus, the solitary bat survived hibernation with remaining fat stores to spare. However, when *Pd* was introduced, arousal frequency, and subsequently energy expenditure, increased to the point where the bat exhausted all energy stores midwinter. In fact, despite no observed mass mortality, literature-based parameters of the original microclimate model suggested that, without additional behavioral mechanisms, bats die of WNS across all microsites where microclimate measurements were taken (see Appendix Figure C.2). We see in this example that clustering reduced energy consumption both with and without *Pd*, but did not prevent *Pd*-induced mortality. Grooming, however, eliminated enough fungal growth that even though fat consumed was greater than without fungus, the individual still had ample stores come hibernation's end. While the literature parameterization provided a representative example of expected model performance, torpor bout length outcomes were not biologically relevant for any of the four models (see Appendix Figure C.2. The Microclimate and Microclimate + Clustering models are excluded because bats are not predicted to survive *Pd* infection in a microsite where they are observed

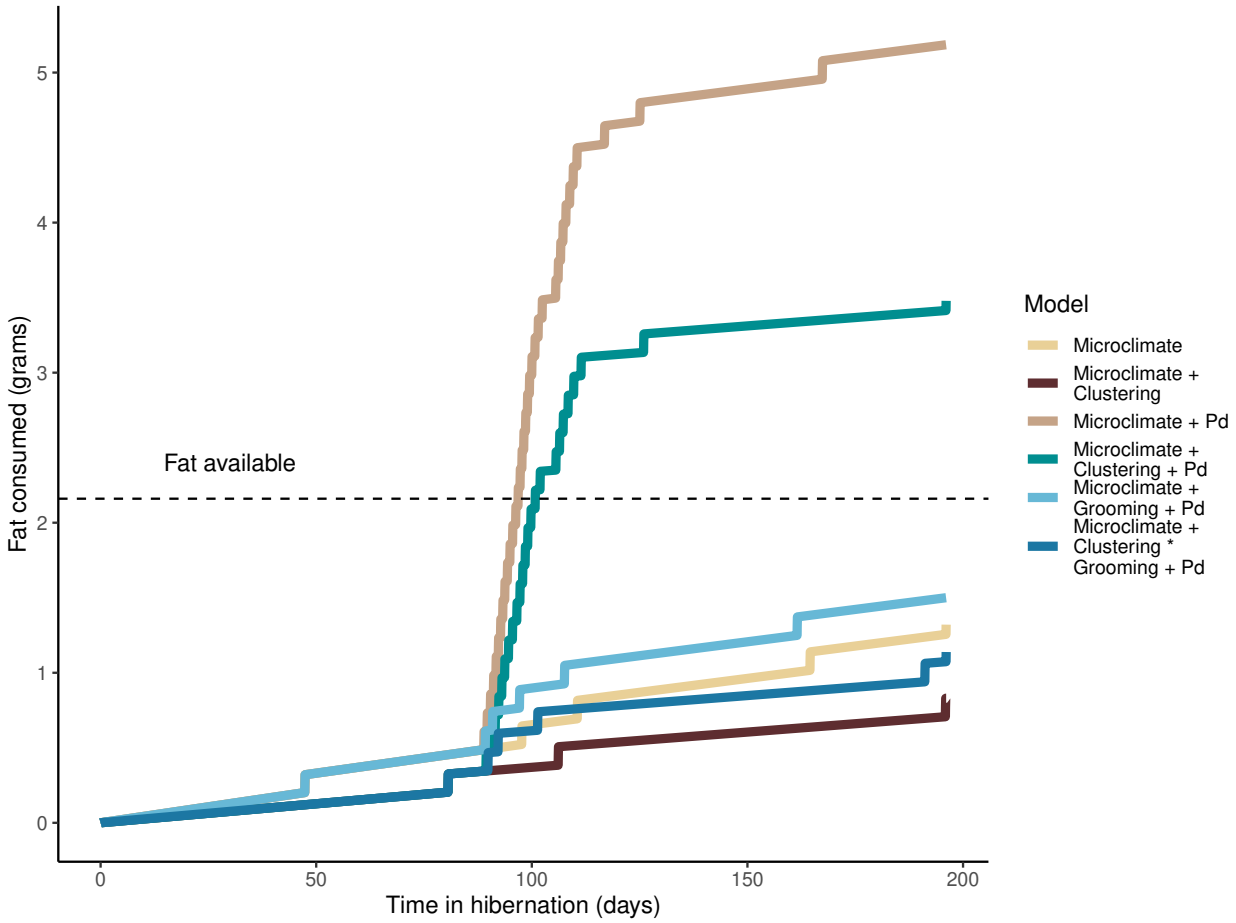

Figure 4.2: Example output of each of the six simulations using the same literature-based parameter set. Body mass = 9g, percent fat = 24%, clustering factor = 60%, and grooming rate = 20cm^2/hr, and all other parameters are listed in Table 1. For this parameterization, the bat will survive hibernation with *Pd* if grooming is an included mechanism, but without grooming, *Pd* infection causes bats to swiftly lose energy stores mid-winter when hibernaculum humidity levels decline.

hibernating without mortality. The Microcliamte + Grooming and Microclimate + Clustering + Grooming models passed the ABC rejection algorithm for this particularly energetically favorable microsite, but were rejected by the algorithm at less energetically favorable microsites where bats are still observed surviving hibernation. Thus, we needed to estimate parameters in order to determine if the incorporation of local adaptation in metabolic traits allows for survival across all hibernation microsites.

Across the 200,000 parameter sets evaluated through ABC, no parameter sets fit passed the rejection algorithm for the Microclimate or Microclimate + Clustering models (Figure 4.3). No

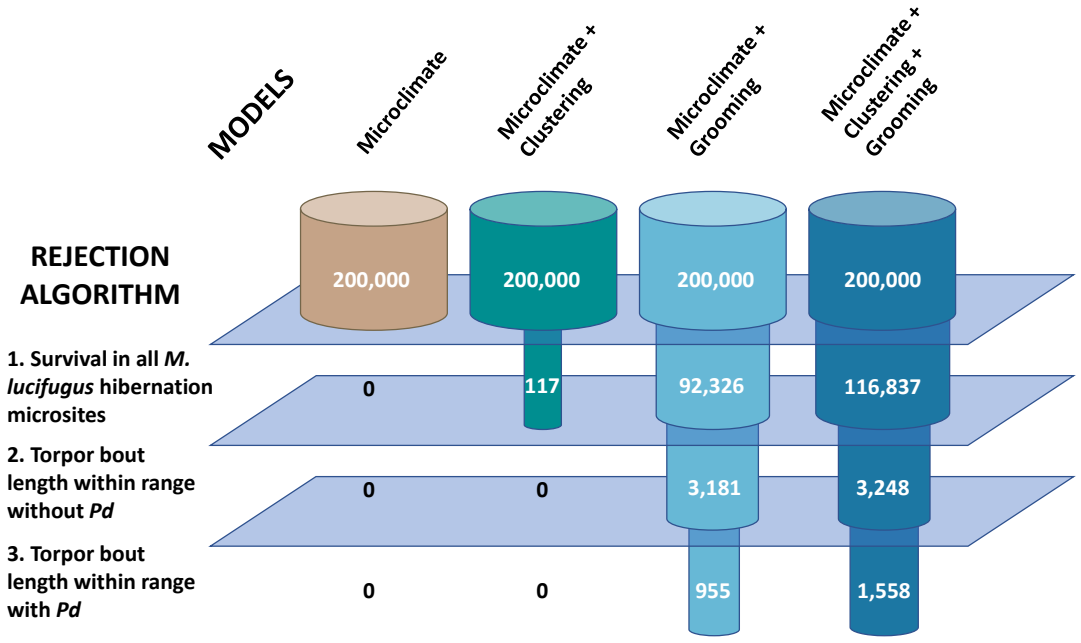

Figure 4.3: Schematic representation of approximate Bayesian computation parameter estimation under four different models. In the ABC rejection algorithm, we reject parameter sets if they do not meet three biologically relevant criteria. Note that each model contains two simulation scenarios, one with and one without *Pd*. Only the Microclimate + Grooming and Microclimate + Clustering + Grooming models exhibit parameter sets that passed all three criteria of the rejection algorithm, and are therefore biologically feasible. Thus, the Microclimate and Microclimate + Clustering models are rejected.

matter how energetically favorable a given parameter set was, none were capable of generating survival with *Pd* across all *M. lucifugus* hibernation microsites without additional behavioral adaptation beyond microclimate use. While clustering did allow for survival across all microsites used for hibernation in 117 parameter sets, application of the torpor length criterion excluded all parameter sets (Figure 4.3). Parameter sets where average torpor bout lengths were within literature-based range for microsites with high energetic requirements (i.e. 'non-optimal' microclimates) would result in bats unrealistically over-performing with average torpor bout lengths that are too long to be realistic in hibernation microsites with low energetic requirements (i.e. 'optimal' microclimates) (see Appendix Figure C.2). In addition, these parameter sets were very heavily weighted toward individuals that are particularly large with relatively high percentage of body fat, suggesting that only more energetically prepared individuals could survive, which was contradictory to observa-

tions in this hibernaculum (Cheng *et al.*, 2019). In contrast, the Microclimate + Grooming model resulted in 955 successful parameter sets, while the Microclimate + Clustering + Grooming model resulted in 1,558 parameter sets (Figure 4.3). This suggests that removal of *Pd* or mitigation of *Pd* growth were highly necessary adaptations for survival in the context of this hibernaculum. Thus, we reject the Microclimate and Microclimate + Clustering models and accept the Microclimate + Grooming and Microclimate + Clustering + Grooming models as being biologically feasible.

Several estimated posterior densities for metabolic traits were significantly off-set from literature-based values (Figure 4.4). In particular, the minimum body temperature that bats hold in torpor was significantly higher than literature estimates for the Microclimate + Clustering + Grooming model, peaking around 3^o Celsius (Figure 4.4.a). The evaporative water loss rate of the body was significantly lower for the Microclimate + Grooming model but not the Microclimate + Clustering + Grooming model (Figure 4.4.b). However, in the Microclimate + Clustering + Grooming model, there is strong negative correlation between evaporative water loss rate and the clustering factor percentage (see Appendix Figure C.3). When we estimated effective evaporative water loss rate by multiplying evaporative water loss rate by clustering factor, as in our metabolic equations, the effective evaporative water loss rate was significantly lower for the Microclimate + Clustering + Grooming model, with a strikingly similar distribution to the Microclimate + Grooming model (Figure 4.4.c). While there was not a significant offset of conductance or minimum metabolic rate in torpor posterior densities (Figure 4.4.d,e), the latter was heavily weighted toward higher values. These parameters existed primarily as a ratio to each other in the metabolic equations, so unsurprisingly there was a reasonable amount of correlation between the two in both Microclimate + Grooming and Microclimate + Clustering + Grooming models (see Appendix Figure C.3). When we observed the posterior density for conductance divided by torpid metabolic rate (Figure 4.4.f), we found that the parameter ratio credible interval was heavily weighted to be lower than the literature-based ratio of prior parameters, though literature-based values were still within credible intervals. Credible intervals included literature-based values in other parameters (see Appendix Figure C.4). *Pd* removal rate via grooming was most significant at rates greater than 20 cm^2/hr

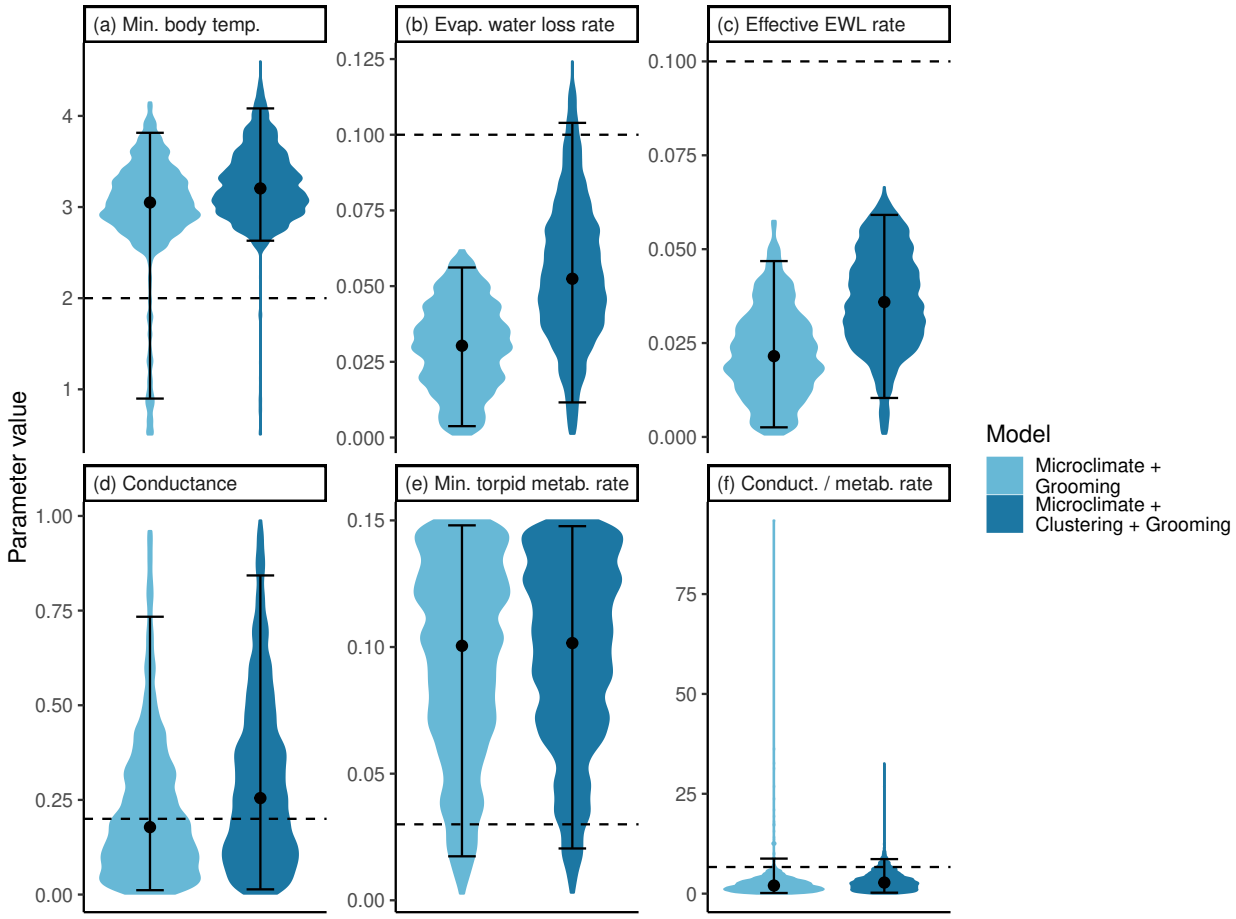

Figure 4.4: Posterior densities for parameters estimated via approximate Bayesian computation. Dashed horizontal lines represent literature-based parameterizations (see Table 1 for parameter descriptions and units). Colored violin plots represent posterior densities for their respective models. Circles represent the estimated median, and error bars represent the 95% credible interval. (a) Minimum body temperature held in torpor is similarly high for both models and significantly greater than literature values for the Microclimate + Clustering + Grooming model. (b) Evaporative water loss rate of the body is significantly lower than literature values in the Microclimate + Grooming model, but not the Microclimate + Clustering + Grooming model. However, multiplying evaporative water loss rate by the clustering factor (c) to estimate effective evaporative water loss rate results in both models being significantly lower than the literature value. (d) Torpor conductance and (e) minimum metabolic rate while torpid are not estimated as significantly different from literature values. (f) The posterior density of the ratio of conductance divided by metabolic rate, as present in metabolic equations, seems to converge on a value that is less than that expected by literature-based parameters. However, despite being heavily weighted toward lower values, the literature-based ratio is still within the credible intervals.

(see Appendix). Importantly, the posterior distribution of body mass or hibernation fat percentage was such that individuals of all observed body sizes could have survived *Pd* infection in any hibernation microsites (see Appendix Figure C.4).

Although microclimate use alone did not describe survival in all microsites, there was evidence of bats choosing energetically favorable microclimates within the system. In each chamber, paired t-tests indicated that for a given parameter set the estimated average torpor bout length at hibernation microsites was greater than estimated average torpor bout length if *M. lucifugus* were to hibernate at microsites where they are not found hibernating midwinter ($p < 0.001$ for each of three chambers, Figure 4.5). However, the expected average torpor bout length was also differ-

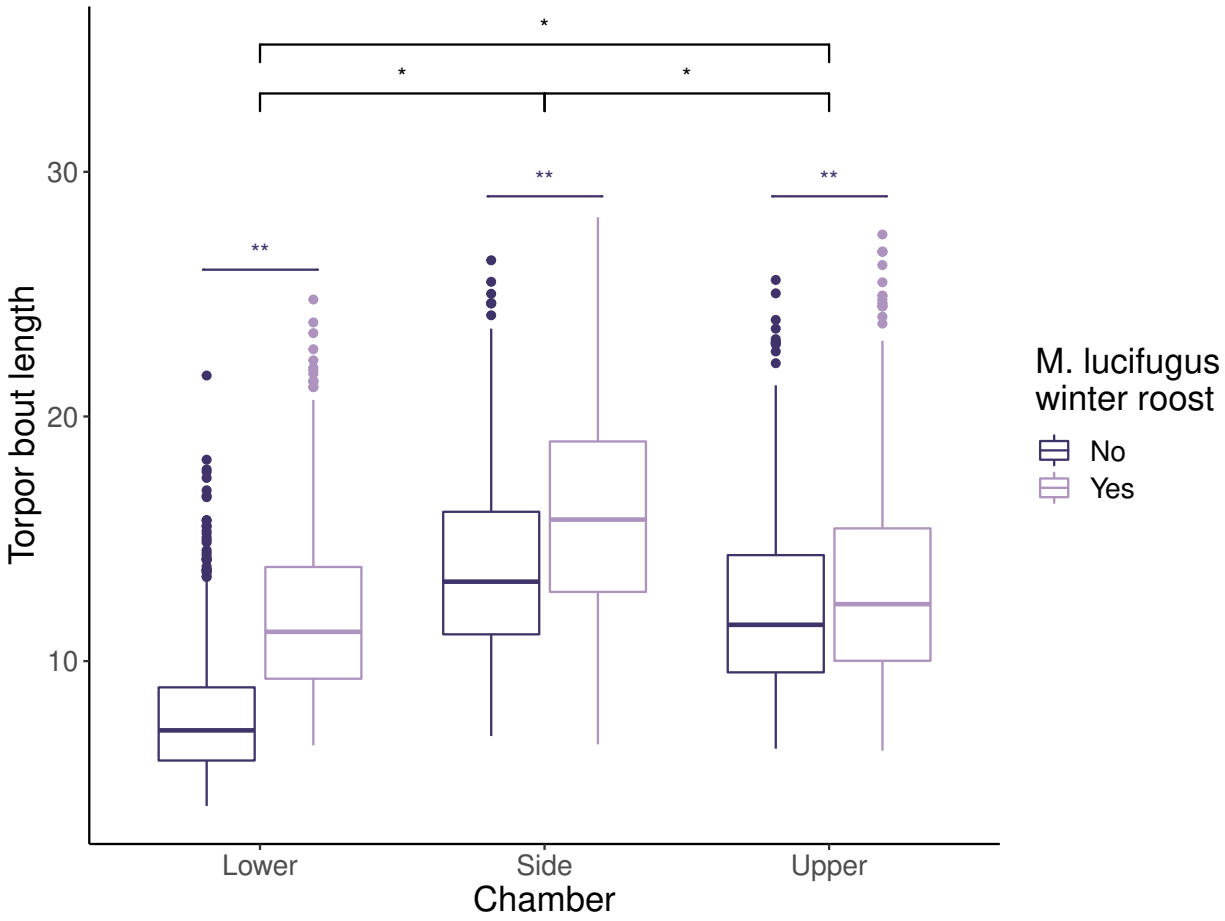

Figure 4.5: Box plots of the average torpor bout length of each parameterization in three different hibernaculum chambers for microsites with (light) and without (dark) hibernating *M. lucifugus* populations. * = significant difference determined by t-test, $p < 0.001$. ** = significant difference determined by paired t-test, $p < 0.001$.

ent across chambers, with the Side chamber being most energetically favorable (supports longer average torpor bouts) and the Lower chamber being least energetically favorable (Figure 4.5).

4.5 Discussion

Local adaptation (Gignoux-Wolfsohn *et al.*, 2021; Golas *et al.*, in review), microclimate use (Hayman *et al.*, 2016; Haase *et al.*, 2019a), and clustering (Langwig *et al.*, 2012; Hayman *et al.*, 2017) and grooming (Brownlee-Bouboulis & Reeder, 2013) behaviors have all been implicated in WNS outcomes individually, but this is the first study to test multiple hypotheses simultaneously. By comparing behavioral and physiological mechanisms using a unique approach of pairing fine-scale spatial and temporal data with mechanistic modeling, we can evaluate the relative importance of these factors in describing WNS survival in a natural system. Our models create biologically realistic predictions of torpor arousal and energetic expenditure when we examine the output at individual microsites (Figure 4.2). Periods of torpor result in a slow and steady fat consumption, while periods of arousal result in sudden rapid consumption. Per our expectations given a sudden reduction in water vapor pressure throughout the hibernaculum around day 100 (see Appendix Figure C.1), there is an increase in arousal frequency as microclimate conditions are less ideal for prolonged torpor. Accordingly, the steady build of *Pd* load in early hibernation results in many sequential short-lived torpor events during this relatively dry period when *Pd* is present without grooming. This seems to mimic the daily arousals experienced by bats succumbing to WNS as observed by Reeder *et al.* (2012). Ultimately, we find evidence that grooming behavior and local adaptation of metabolic traits are essential to describe observed patterns of survival, though the most biologically realistic model incorporates all four hypotheses, with evidence of interactions between them.

Microclimate use in hibernation has been repeatedly established as influential to hibernation and WNS survival (Cryan *et al.*, 2010; Willis *et al.*, 2011; Langwig *et al.*, 2012; Cryan *et al.*, 2013; Hayman *et al.*, 2016). Despite long-held beliefs that bats hibernate in stable, physiologically optimal conditions (Elliott & Clawson, 2001; Tuttle & Kennedy, 2011), we know now that the niche

range of utilized hibernation microclimates is wider than previously theorized (Boyles *et al.*, 2017; Golas *et al.*, in prep). By testing ecophysiological models against variable, longitudinal microclimate data, we now have evidence that the microclimates used to survive WNS in this hibernaculum are not all physiologically optimal (Figure 4.5), but they are physiologically survivable. The fact that the range of physiologically survivable microclimates is wider than expected may help explain why bats are not seeking out stable conditions as previously assumed. That said, while *M. lucifugus* do not choose to hibernate in a single optimal microsite of the hibernaculum, they are choosing the optimal locations within a given section of the hibernaculum (Figure 4.5). This is despite the fact that, in terms of metabolic expectations, there exist microsites that are superior for energy conservation, particularly in the Side chamber (Figure 4.5). Thus, *M. lucifugus* in this hibernaculum overwinter in physiologically beneficial microclimates, if not physiologically optimal. The flexibility observed in *M. lucifugus* microclimate use may prove favorable in coming years as climate change alters underground microclimate availability (McClure *et al.*, 2020; Golas *et al.*, in prep). This could lead to necessary range shifts or extirpation events if bats cannot tolerate the changing conditions (Humphries *et al.*, 2002). Bat responses to projected changes will likely depend heavily on the level of local physiological adaptation for consistently used hibernation microclimates.

The importance of physiological adaptation in this system becomes evident when we examine the results of parameter estimation. For example, minimum metabolic rate in torpor is estimated as high compared to literature-based values (Figure 4.4.a), which is consistent with *M. lucifugus* in this hibernaculum preferring warmer microsites for hibernation (Golas *et al.*, in prep). In particular, evaporative water loss is hypothesized as a primary driver of increased arousal leading to mortality in bats with WNS (Cryan *et al.*, 2010; Willis *et al.*, 2011; Cryan *et al.*, 2013) and has been explored and validated experimentally (Ben-Hamo *et al.*, 2013; McGuire *et al.*, 2017) and through modeling efforts (Haase *et al.*, 2019a). Per our parameter estimation, the evaporative water loss rate of the body is lower than the 0.1 $\frac{mgH_2O}{hr*\Delta WVP*cm^2}$ measured in Montana bats (Haase *et al.*, 2019a), with a mean of 0.055 for the Microclimate + Clustering + Grooming model, and 0.03 for the Microclimate + Grooming model (Figure 4.4.b). The fact that this *M. lucifugus* population ex-

hibits a lower estimated evaporative water loss rate than those measured in the Montana hibernaculum is in keeping with expected population variation across humidity gradients (Klüg-Baerwald & Brigham, 2017). We expect bats that hibernate consistently in drier microclimates to be locally adapted to prevent evaporative water loss (Klüg-Baerwald & Brigham, 2017), and in winter this hibernaculum exhibits significant drops in water vapor pressure across all microsites (Golas *et al.*, in prep), compared to the microclimates in the Montana hibernaculum that were stable throughout hibernation with no significant water vapor pressure deficit noted (Haase *et al.*, 2019a). Thus, the reason this hibernaculum's *M. lucifugus* population did not exhibit significant declines in WNS may be related not just to the fact that the microclimates in this hibernaculum are drier, but also physiological exaptations of bat populations consistently using this hibernaculum. This has potential consequences for attempts to mitigate WNS impact by altering hibernaculum microclimates (Turner *et al.*, 2021). Bat populations may not be adapted to handle drastic changes in available microclimates. We require more studies pairing metabolic traits with experienced microclimates (Golas *et al.*, in review) to better predict how populations might respond to such environmental changes before they are employed widespread as a mitigation strategy. Such studies should also carefully consider bat clustering behavior as well, as this appears to alter effective physiological parameters.

At face value, clustering does not appear to be necessary for WNS survival in this system (Figure 4.3). However, when we consider the effective metabolic rates experienced by clustered bats, it appears that clustering emerges as a latent mechanism in both models, as evidenced by reduced effective evaporative water loss rate and conductance relative to metabolic rate (Figure 4.4.c,f). As a result of this, the Microclimate + Grooming and Microclimate + Clustering + Grooming models are functionally equivalent, and the differences in number of parameter sets fit could be a function of the Microclimate + Clustering + Grooming model being more complex and therefore more flexible in parameterization. Then, clustering could still be an essential part of how *M. lucifugus* survive WNS in this hibernaculum. Literature-based parameters were generally measured in individuals rather than clusters to make estimation of metabolic parameters more precise. To

determine the full impact of clustering on hibernating bat physiology and realized niche range and further validate our findings, it would be worthwhile to measure metabolic processes in groups to better estimate the physiological benefits (Canals *et al.*, 1997; Roverud & Chappell, 1991; Boratyński *et al.*, 2015). Despite the apparent effects of clustering behavior, microclimate use, and physiological adaptation on WNS survival, none of our models were able to recreate biologically realistic WNS survival throughout the hibernaculum without the addition of grooming behavior as well.

In our models, grooming to reduce *Pd* load is a necessary mechanism to allow WNS survival throughout the hibernaculum. More specifically, our metabolic equations model the rate of *Pd* removal, and we refer to it as grooming based on observed behaviors in *Pd*-infected bats (Brownlee-Bouboulis & Reeder, 2013). Given that increased grooming is not a universal response to *Pd* infection (Bohn *et al.*, 2016), we might consider that other potential mechanisms for mitgation or reduction of *Pd* growth exist, such as immune system activation (Field *et al.*, 2015). There is evidence for selection of immune function in WNS survivors in the northeast US region (Gignoux-Wolfsohn *et al.*, 2021), but the lack of mortality and absence of fat-related selection common to high-mortality hibernacula (Cheng *et al.*, 2019) suggests that the selective forces of WNS in this hibernaculum are weak, and that mortality was prevent by a combination of environmental conditions and exaptations. This is further supported by the fact that any bats with body mass and fat percentage in ranges sampled in this hibernaculum before *Pd* introduction (Cheng *et al.*, 2019) could survive winter in any hibernation microsite (see Appendix Figure C.4). Our functional form for modeled grooming behavior suggests that bats will need to clean at least approximately 20 cm^2 per hour to effectively control *Pd* growth enough to allow survival at all hibernation microsites (Figure C.4). Given a little under a quarter of the arousal period spent grooming (Brownlee-Bouboulis & Reeder, 2013) and a little over an hour of time spent in arousal (Haase *et al.*, 2019a), this equates to approximately an area of 5 cm^2 effectively groomed per arousal period. We suspect that this is biologically feasible; further experimental studies such as those performed by Brownlee-Bouboulis & Reeder (2013) may be able to further validate these grooming rates by measuring conidial growth

pre- and post-arousal in experimentally infected bats. For now it remains that behavioral fungal mitigation could be an important, oft-overlooked function of WNS survival.

Anthropogenic stressors like climate change and introduced disease increase our need to better understand bat hibernation capabilities so that we can best predict at-risk populations and optimize management strategies. In this study we have developed a novel method of estimating important metabolic traits in natural settings with minimal invasion into the hibernaculum. This is increasingly important in preventing unnecessary arousal and energy loss as more and more bat populations are threatened by WNS (Speakman *et al.*, 1991; Cheng *et al.*, 2021). When we consider the niche of hibernating bats as the microclimates in which they can safely hibernate, there is an indirect contraction of the fundamental niche when the parasitic *Pd* is introduced due to changes in how hosts respond physiologically to hibernation microclimates (Hayman *et al.*, 2016; Haase *et al.*, 2019a). Overlap in niche range with *Pd* results in microclimates that once served as survival space becoming ecological traps (Golas *et al.*, in review). However, survival spaces may be maintained despite *Pd* presence if other factors can mitigate disease impact. Here, we find evidence of niche maintenance indirectly through hygienic behavior removing *Pd* affecting host physiology and directly through conspecific facilitation (Bruno *et al.*, 2003) of clustering behavior reducing the impact of metabolic processes. These strategies are successful in the context of a population that appears to have local metabolic adaptations to using physiologically beneficial microclimates, resulting in exaptations for WNS survival. Overall, this perspective captures relevant detail of a very complex system and allows us to isolate the impact of multiple mechanisms within their interactions in order to separate out their relative importance. We believe that an approach similar to that described here could prove useful in learning important natural history while providing important information for population management with the benefit of not requiring intensive handling and experimentation. Our study makes strong inference based on detailed hibernaculum measurements and an understanding of system biology. Using field-gathered data to inform and enhance modeling efforts (Restif *et al.*, 2012), we demonstrate essential mechanisms that describe observed survival in a natural system where previous methods were inadequate. In doing so, we are able to

define processes that directly and indirectly affect *M. lucifugus* niche range. Through innovative technology to collect vital field information combined with advanced modeling, we can further enhance our efforts to conserve bat biodiversity.

Chapter 5

Conclusions

In this dissertation, I have explored the environmental, physiological, and behavioral factors that define the realized hibernation niche of hibernating bats affected by *Pseudogymnoascus destructans* (*Pd*) causing white nose syndrome (WNS). My goal was to further define how hosts interact with their environments to survive infection with a deadly pathogen. In doing so, I aimed to bridge the laboratory-field gap by using field data to parameterize mechanistic models that describe driving processes in complex natural systems. As a result, I am able to gain insight into physiological traits that influence WNS outcomes without extensive handling of bats or potentially harmful midwinter intrusion of hibernacula. Each chapter builds on these themes by advancing our use of ecophysiological models and detailed microclimate data to describe bat behavioral observations.

In Chapter 2, I used unique microclimate and torpor bout length data from free-ranging *Eptesicus fuscus* to identify the drivers of energetically costly arousal. I found that torpor bout length in *E. fuscus* is physiologically limited by mostly temperature-related cues, but torpor bouts are often cut short, with bats arousing before physiological limits are reached due to natural system stochasticity. In doing so, I estimated important population variation in physiological traits, identifying differences in evaporative water loss rate, conductance, and metabolic rate compared to literature-based parameters. As a result, I was able to make population-specific predictions of expected restrictions in niche range with *Pd* introduction.

In Chapter 3, I defined the niche axes that determine hibernation location for *Myotis lucifugus* within a hibernaculum exhibiting spatiotemporally variable microclimates. I developed a hierarchical model that had two layers. In the first, I developed a predictive microclimate model, fit to a detailed longitudinal data set of temperature and humidity measured at microsites with and without hibernating bats, that used above-ground temperature to estimate the underground spatiotemporal distribution of temperatures throughout the hibernaculum. In the second layer, these temperature

predictions were used to estimate environmental covariates that fed into a logistic model predicting the probability of hibernation roost occurrence throughout the hibernaculum. Through this, I identified that hibernation microsite choice is dependent on a combination of sociality, warmer average temperatures, and microsite stability, though variability across hibernation microsites suggests that *M. lucifugus* are capable of occupying a larger niche space than previously recognized. Given its flexible and parsimonious nature, my predictive model is also capable of evaluating microsite favorability under changing hibernaculum conditions, demonstrating a potential spatial niche expansion under climate change. However, this expansion is contingent upon potential further range contraction that might occur as a result of improved conditions for *Pd* growth.

Thus, in Chapter 4, I expanded our understanding of how physiological adaptations interact with available microclimates and other behavioral mechanisms to impact WNS survival in the same hibernaculum as Chapter 3. I tested four different hypotheses for how *M. lucifugus* survive *Pd* infection in this hibernaculum by using ecophysiological models informed by longitudinal microclimate data to estimate metabolic parameters and select behavioral models that are biologically realistic. I found that survival is contingent on physiological exaptations to relatively dry microclimates and grooming behavior to reduce *Pd* burden. In addition, bats appear to select hibernation microsites that are physiologically favorable, and while the modeled clustering mechanism was not essential to survival, there were latent effects of clustering permeating throughout selected models. Thus, because *M. lucifugus* and Pd niche ranges overlap so heavily, in order to survive, bats reduce *Pd* load and subsequent influence, and they further expand niche range through relaxation of physiological constraints by clustering.

Importantly, I find that there is an important oft overlooked temporal component to the hibernation niche. While bats establish roosting microsites very early in the hibernation season (Chapter 3), the microclimates experienced at these sites remain physiologically survivable throughout hibernation, despite *Pd* presence and energetically unfavorable extremes (Chapter 4). In fact, temporary extreme changes in temperature or humidity that are outside the niche range of *Pd* may help bat survival of WNS, even if those changes are energetically unfavorable for bat hibernation.

Studies that assume bats are using stable microclimates for hibernation, or average values over the hibernation season, may be overlooking important system variation. That bats can use these conditions successfully for hibernation is likely at least in part a result of behavioral adaptations to mitigate metabolic losses (Chapter 4). This suggests that there should be further experimental investigation into the physiological benefits and potential consequences of behaviors like clustering and grooming.

Future efforts to characterize WNS outcomes in the field should further integrate and expand the technological and modeling innovation pioneered in these studies. Detailed descriptions of temperature microclimate and humidity, paired with behavioral observations, can allow strong inference into bat physiological properties (Chapter 2). But this inference is thus far based on bat survival of WNS. To push these advances forward, we should consider performing similar work in populations of known mortality outcomes, pairing ecophysiological models with mark-recapture studies. This could improve evaluations of population risk assessment, while also allowing investigation of selection on traits that improve survival outcomes (Gignoux-Wolfsohn *et al.*, 2021). In addition, we should find ways to pair hibernation microclimate measurements and within-hibernaculum bat movement (Ryan *et al.*, 2019) to better evaluate how microclimate availability and changes impact microclimate use and survival outcomes.

Ultimately, it appears as though the niche range of hibernating bats is wider than has previously been assumed. *Pd* indirectly reduces the bat's fundamental niche through physiological alterations, leading to mass mortality in bats unable to escape niche overlap with *Pd* growth. However, some bats appear capable of surviving WNS by mitigating niche restriction via *Pd* removal, increasing niche range through clustering as conspecific facilitation, and utilizing niche space that does not overlap with the *Pd* niche, even if it is temporarily unfavorable for hibernation. This suggests that, at least in this hibernaculum, bats are surviving as a function of exaptations, rather than current adaptation due to selection by *Pd*. My investigation reveals a series of important interactions between behavior and physiology that can change under different environmental conditions.

This is important to consider when developing WNS management strategies. Considering that that WNS survival is heavily dependent on experienced microclimates (Hayman *et al.*, 2016; Haase *et al.*, 2019a), there is a natural step from wanting to prevent WNS mortality to changing hibernaculum microclimates to better support survival (Turner *et al.*, 2021). However, a suitable microclimate is not necessarily enough to prevent mass mortality (Chapter 4). Our parameter estimation suggests that bats have physiological exaptations for survival microclimates that contribute to successful hibernation despite *Pd* presence. Then, if hibernaculum alterations push microclimates outside the niche range of resident populations, it could make the hibernaculum inhabitable or lead to further mortality. Here I provide methods to evaluate important physiological traits such that we can estimate hibernation success under a variety of hypothetical hibernaculum alteration scenarios. These tools can be used to investigate the potential impacts of anthropogenic alterations prior to implementation.

Perhaps more so than many diseases, WNS demonstrates the complexity inherent in natural systems, with individual outcomes heavily dependent on environmental factors, physiological responses, host-pathogen interactions, and population-level activities. With so many driving forces affecting individual and population outcomes of disease, it is important that we find ways to embrace this complexity. Laboratory-based experiments might be capable of measuring individual factors with precision, but they do so at the expense of removing other interactive variables. Thus, a robust approach to predicting WNS outcomes will integrate and test multiple hypotheses simultaneously, incorporating laboratory-based data while allowing flexibility for population variation. I have adapted a suite of tools to help this type of investigation, including ensemble modeling of multiple mechanistic models representing hypotheses, hierarchical models with complex natural system representations underlying regressive covariate selection, and approximate Bayesian computation allowing simultaneous parameter estimation and comparison of complex ecophysiological models. To make inference on specific systems, I gathered necessary data through fieldwork, which was itself informed by previous generations of models, representing a model-guided fieldwork approach (Restif *et al.*, 2012). Throughout my dissertation I have made inference to help

guide management efforts and suggested further lines of investigation, highlighting the most relevant paths to further understand WNS survival. I hope that this work represents the next iterative step to conservation of the wide and magnificent biodiversity of bats.

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Appendix A

Chapter 2 supplemental materials

A.1 Data logger details

We contracted Phase IV Engineering (Boulder, CO) to develop miniature data loggers that record ambient temperature and relative humidity each hour (Figure 1.a). Data loggers were waterproofed by spraying water-proof epoxy over all surfaces except the humidity sensor, and sealed in a water-permeable pouch to allow water vapor to reach the humidity sensor while preventing condensation from forming over the sensor. In total, the materials sutured to bats did not exceed 0.5 grams. We only attached data loggers to study subjects that weighed at least 20 grams to avoid adding mass that exceeded 5% of body mass. Loggers were tested in laboratory conditions prior to deployment on bats. Further details regarding data loggers are available in an *in prep* manuscript that can be made available upon request (*pers. comm.* Paul Cryan).

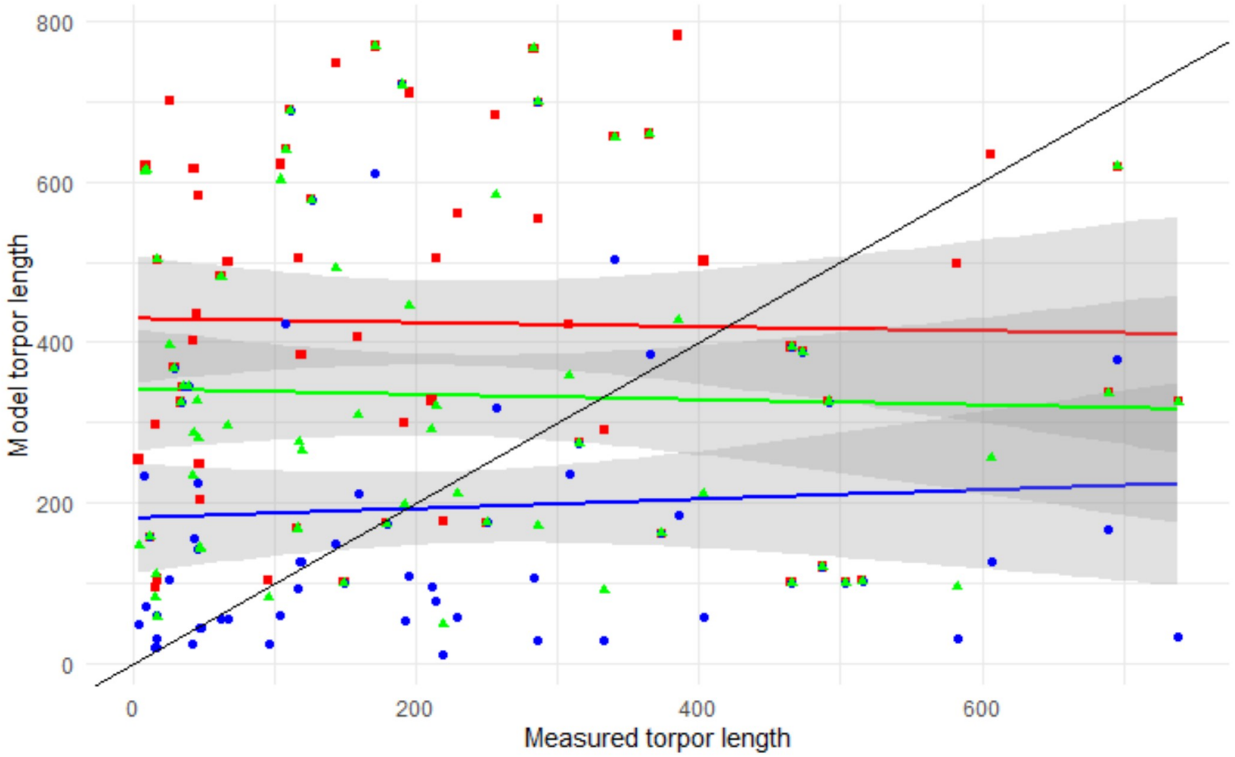


Figure A.1: Applying the energetics (red), hydration (blue), or scaled hydration model (green, see Appendix A.2) with literature-based parameters does not result in any correlation between observed torpor bout durations (in hours) and model expectations.

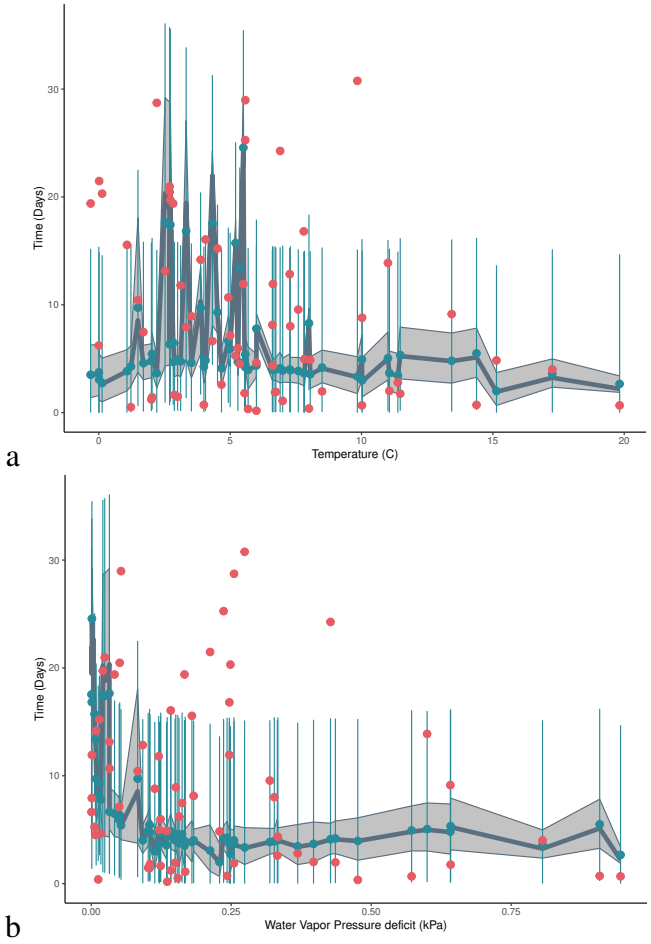


Figure A.2: Fitting the energetics and hydration models (teal) to torpor bout data (red points) directly rather than as ecophysiological boundaries to potential behavior. Doing so results in poor fits that ignore longer torpor bout durations in ranges of (a) temperature and (b) water vapor pressure deficit.

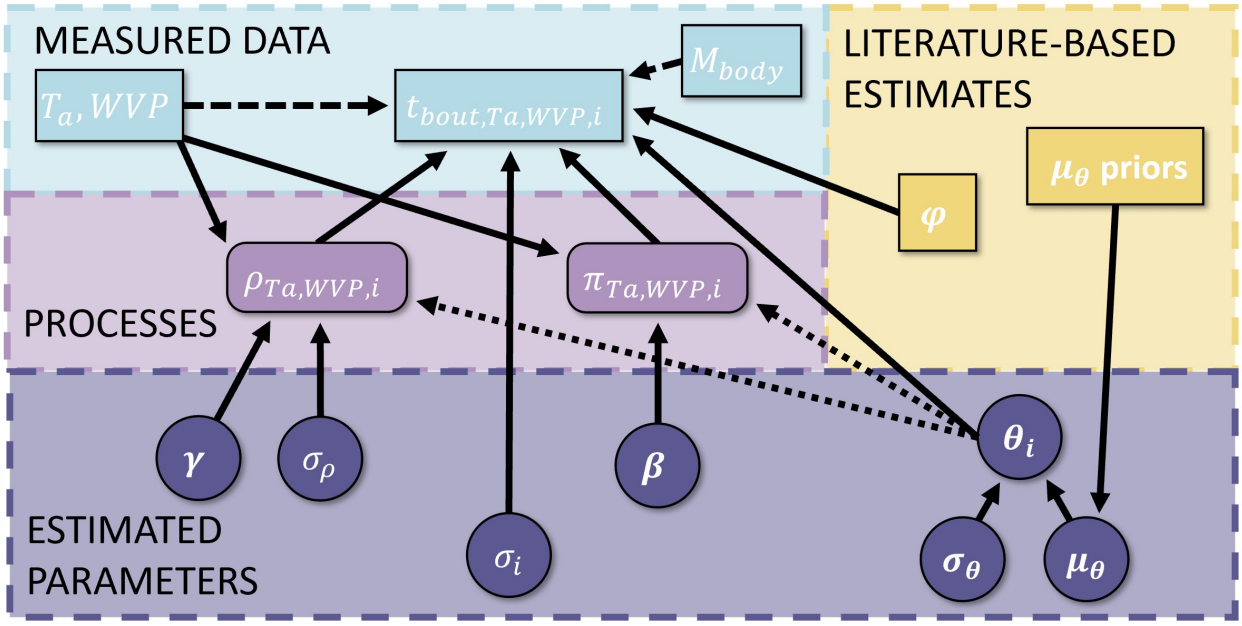


Figure A.3: Directed Acyclic Graph of our Bayesian hierarchical ensemble model that inputs measured values of ambient temperature (T_a), water vapor pressure (WVP), and bat pre-hibernation mass (M_{body}) to predict torpor bout duration. There are two processes incorporated: 1. The ensemble process (π) is a percentage dictating the weighted average of the energetics and hydration models. 2. In treating ecophysiological model predictions as a biological limit to behavior, we predict that actual torpor bout duration will be a percentage (ρ) of energetics and hydration model outputs. For one subset of parameters (ϕ), literature-based estimates were applied directly as model inputs. For the others, literature-based estimate were used as priors (μ_θ **priors**) to develop distributions for estimating population-level parameters (μ_θ), which were then used to estimate individual-level parameters (θ_i). Dotted lines indicate that one estimated parameter ($T_{tor,min}$) was used to calculate the water vapor pressure deficit, and therefore has a direct influence on the estimation of ρ and π. Dashed lines indicate independent variables affecting the dependent variable.

A.2 Estimating *Eptesicus fuscus* cutaneous evaporative water loss rate

Because we do not have a measure of partioned evaporative water loss (i.e., rates of loss for body, wings, and respiration) for *E. fuscus* as we do for *M. lucifugus*, we used published total evaporative water loss rates to approximate a scaled measure for *E. fuscus*. Klüg-Baerwald & Brigham (2017) report evaporative water loss rates under given environmental conditions. We use the reported body mass, temperature, and relative humidity to estimate water vapor pressure deficit and respiratory water vapor loss per Haase *et al.* (2019a). Then, we determine the evaporative water loss that is cutaneous (total minus respiratory) and multiply cutaneous loss by a scalar parameter (δ) to estimate *E. fuscus* cutaneous evaporative loss as a percentage of measured *M. lucifugus* cutaneous evaporative water loss from Haase *et al.* (2019a). We find that based on these two studies, the expected cutaneous evaporative water loss of *E. fuscus* is approximately 27% of *M. lucifugus*. As expected per our parameter estimation (Figure 2.3.a), this rescaling suggests that *E. fuscus* experience significantly lower rates of evaporative water loss than the population of *M. lucifugus* measured by Haase *et al.* (2019a).

Appendix B

Chapter 3 supplemental materials

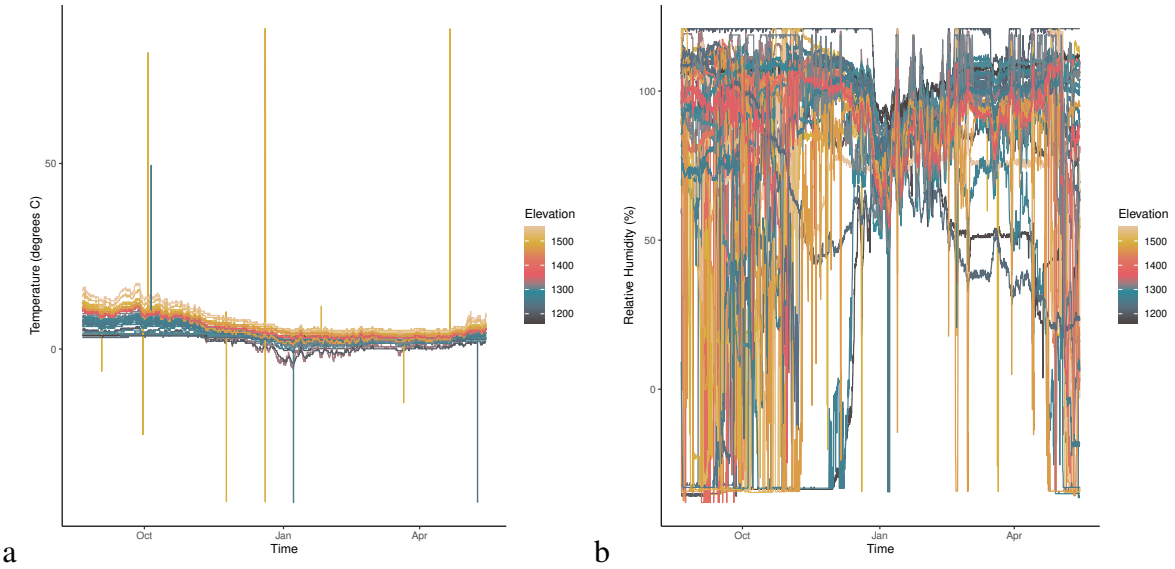


Figure B.1: Raw temperature and relative humidity readings prior to cleaning from data loggers. (a) Errant temperature values are particularly easy to pick out as large sudden spikes. (b) There are periods of high variability in readings during warm months that are likely erroneous given a large number of unrealistic values. However, in cold months, all loggers converge on a similar pattern that we interpret as correct readings.

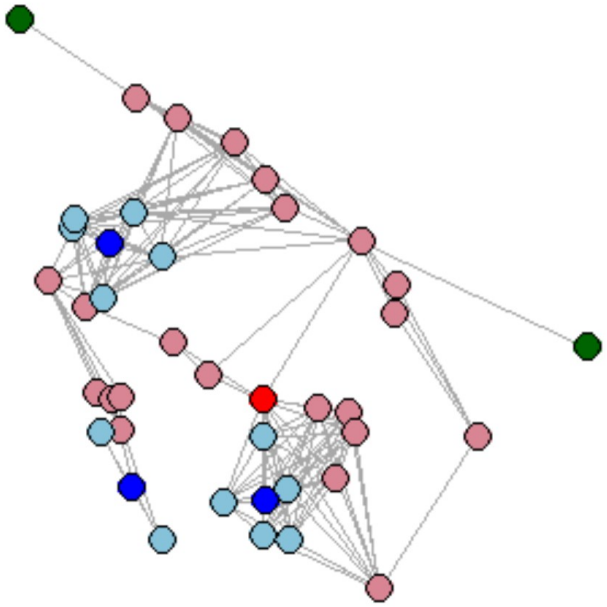


Figure B.2: Representation of logger connectivity network used to generate spatial covariates used in the logistic model. Nodes represent individual data loggers (light red are microsites without roosting *M. lucifugus*, light blue are microsites with roosting *M. lucifugus*), warm and cold entrances (green), roosting centroid for each chamber (dark blue), and estimated midpoint between the Upper and Lower chambers (bright red).

Table B.1: List of all spatial and environmental covariates considered for logistic regression of roost occurrence.

Covariate	Eliminated by correlation coefficient?	Eliminated by ΔDIC?	Included in selected model
SPATIAL			
Distance to warm entrance	No	No	Yes
Distance to cold entrance	No	Yes	-
Elevation	No	Yes	-
Path to bats	No	No	Yes
TEMPERATURE			
Hibernation mean	No	No	Yes
Hibernation standard deviation	No	Yes	
Hibernation maximum	Yes	-	-
Hibernation minimum	No	Yes	-
Hibernation median	Yes	-	-
September mean	Yes	-	-
September standard deviation	No	No	Yes
October mean	Yes	-	-
October standard deviation	Yes	-	-
November mean	Yes	-	-
November standard deviation	Yes	-	-
WATER VAPOR PRESSURE			
Mean	No	Yes	-
Standard deviation	No	Yes	-
Maximum	No	Yes	-
Minimum	No	Yes	-
Median	Yes	-	-
INTERACTION TERMS			
Distance to warm * Elevation	No	Yes	-
Distance to cold * Elevation	No	Yes	-
Distance to warm * Distance to cold	No	Yes	-
Distance to warm * Distance to cold * Elevation	No	Yes	-
Mean hibernation temperature squared	No	Yes	-
Water vapor pressure squared	No	Yes	-
Mean hibernation temperature * Water vapor pressure	No	Yes	-

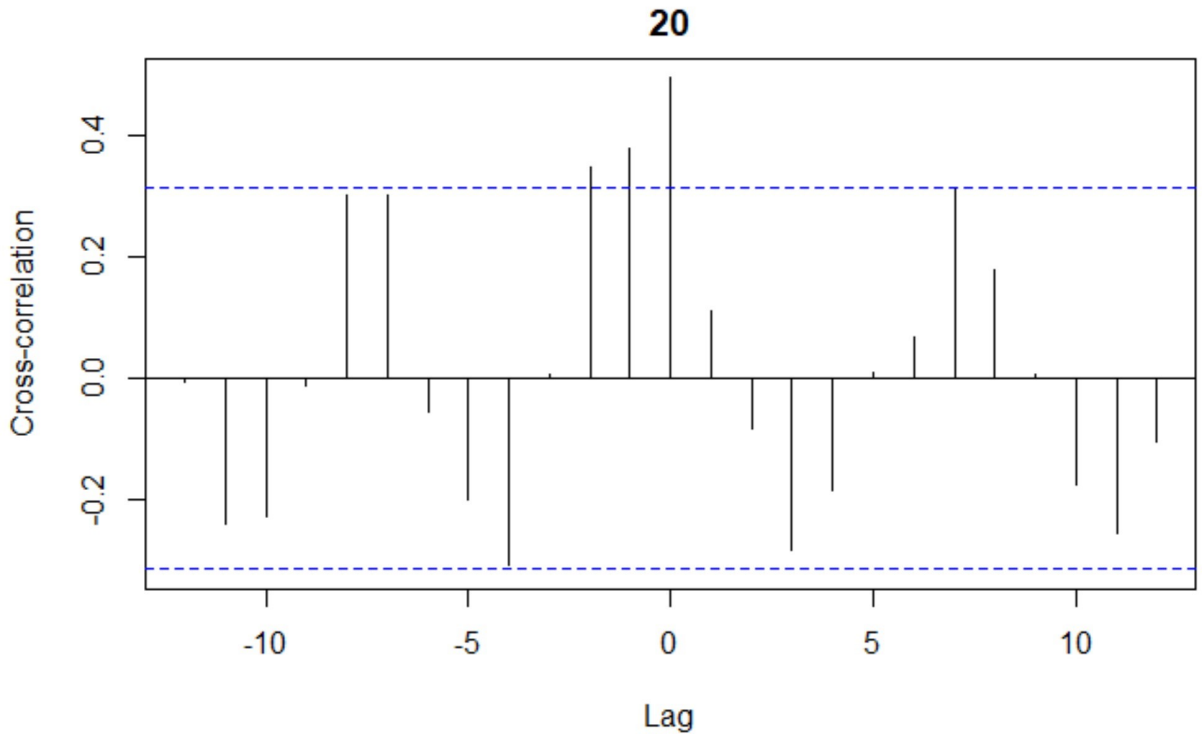


Figure B.3: Evidence of cross-correlational lag between above-ground temperature and a given microsite. The maximum significant lag period across measured microsites was three days.

B.1 Equations for microsite temperature prediction

Equation B.1 describes prediction of underground microclimates on the first day of the predicted time period. U, L, and S are equal to 1 if microsite i is in the Upper, Lower, or Side chamber, respectively, and they are equal to 0 otherwise. Microsites in the Upper chamber follow an exponential decay from mean daily temperature outside ($T_{out,t}$) through the warm entrance down to the system midpoint, wherein large un-mined walls create an airflow bottleneck. Then the Lower chamber microsites are a more linear diffusion of temperature from the midpoint ($T_{mid,t}$) down to the MAST analog ($T_{MAST,t}$). The Side chamber is accessed by a different passage in the Upper chamber located at similar elevation as the Upper/Lower midpoint, so it is modeled as an exponential decay in temperature from $T_{mid,1}$ down to the MAST analog. Differences in airflow and openness between chambers result in different slopes in temperature decrease along the elevation gradient. Microsite elevation is scaled between 0 and 1 ($\text{Elev}_i = (\text{Elevation above sea floor} - 1100)/1600$) to facilitate parameter estimation. The cooling rates (α) for each chamber use non-informative Gamma priors, and variance (σ_T^2) uses non-informative Normal priors.

$$
\begin{aligned}
\mu_{T,1,i} = \quad & (U_i)(1-L_i)(1-S_i)[(T_{out,1} - T_{mid,1})e^{-\alpha_U(\text{Elev}_{out} - \text{Elev}_i)} + T_{mid}] + \\
& (1-U_i)(L_i)(1-S_i)[T_{mid} + \alpha_L(\text{Elev}_i - \text{Elev}_{mid})] + \\
& (1-U_i)(1-L_i)(S_i)[(T_{mid,1} - T_{MAST,1})e^{-\alpha_S(\text{Elev}_{S.entry} - \text{Elev}_i)} + T_{MAST,1}] \\
T_{1,i} \sim \quad & \text{Normal}(\mu_{T,1,i}, \sigma_T^2)
\end{aligned}
\tag{B.1}
$$

Following day one, subsequent average daily temperatures are calculated as the combination of a regression function of the change in outside daily temperature over the past three days caused by airflow from above ground and exponential decay toward MAST analog caused by contact with cavern walls (Equation B.2). We expect that effects of outside temperature will vary across spatial location within the system, with deeper microsites with less airflow having more minimal and

longer lag response compared to microsites closer to surface-level entrances (Boyles *et al.*, 2017). Thus, the site-specific coefficients for daily change in temperature (δ_i) are themselves functions of distances to the warm entrance ($d_{warm,i}$) and cold entrance ($d_{cold,i}$) and elevation, described by (Equation B.4). In addition to δ_k-mediated regression of outside change in temperature, we include the main effects of each covariate with effect size γ. Opposing the impact of airflow from the surface, subterranean air temperature is pulled toward cavern wall temperature (similar to MAST analog) with a rate of $\lambda_{wall,w/c}$. The percent contributions of airflow regression versus cavern wall contact are mediated by the factor ν that indicates low, medium, or high airflow at a given microsite. ν distributions use noninformative Beta priors, λ_{wall} distributions use noninformative Gamma priors, and δ and γ distributions use noninformative Normal priors.

$$\mu_{T,t,i} = \begin{cases} (\nu_{1,w}\text{Airflow}_{high} + \nu_{2,w}\text{Airflow}_{med} + \nu_{3,w}\text{Airflow}_{low})* & \\ (\mu_{T,t-1,i} + \delta_{1w,i}(T_{out,t} - T_{out,t-1}) + \delta_{2w,i}(T_{out,t-1} - T_{out,t-2}) + & \\ \delta_{3w,i}(T_{out,t-2} - T_{out,t-3}) + \gamma_{1w}d_{warm,i} + \gamma_{2w}d_{cold,i} + & T_{out,t} \geq T_{MAST,t} \\ \gamma_{3w}\text{Elev}_i + \gamma_{4w}T_{out,t} + \gamma_{5w}T_{out,t-1} + \gamma_{6w}T_{out,t-2} + \gamma_{7w}T_{out,t-3}) + & \\ (1 - \nu_{1,w}\text{Airflow}_{high} - \nu_{2,w}\text{Airflow}_{med} - \nu_{3,w}\text{Airflow}_{low})* & \\ ((\mu_{T,t-1,i} - MAST_t)e^{-\lambda_{wall,w}} + MAST_t) & \\ & \\ (\nu_{1,c}\text{Airflow}_{high} + \nu_{2,c}\text{Airflow}_{med} + \nu_{3,c}\text{Airflow}_{low})* & \\ (\mu_{T,t-1,i} + \delta_{1c,i}(T_{out,t} - T_{out,t-1}) + \delta_{2c,i}(T_{out,t-1} - T_{out,t-2}) + & \\ \delta_{3c,i}(T_{out,t-2} - T_{out,t-3}) + \gamma_{1c}d_{warm,i} + \gamma_{2c}d_{cold,i} + & T_{out,t} < T_{MAST,t} \\ \gamma_{3c}\text{Elev}_i + \gamma_{4c}T_{out,t} + \gamma_{5c}T_{out,t-1} + \gamma_{6c}T_{out,t-2} + \gamma_{7c}T_{out,t-3}) + & \\ (1 - \nu_{1,c}\text{Airflow}_{high} - \nu_{2,c}\text{Airflow}_{med} - \nu_{3,c}\text{Airflow}_{low})* & \\ ((\mu_{T,t-1,i} - MAST_t)e^{-\lambda_{wall,c}} + MAST_t) & \end{cases} \tag{B.2}$$

$$T_{t,i} \sim \text{Normal}(\mu_{T,t,i}, \sigma_T^2) \tag{B.3}$$

$$\begin{aligned}
\delta_{k,w/c,i} = \quad & \lambda_{0,k,w/c} + \lambda_{1,k,w/c} d_{warm,i} + \lambda_{2,k,w/c} d_{cold,i} + \\
& \lambda_{3,k,w/c} \text{Elev}_i + \lambda_{4,k,w/c} d_{warm,i} d_{cold,i} + \lambda_{5,k,w/c} d_{warm,i} \text{Elev}_i + \\
& \lambda_{6,k,w/c} d_{cold,i} \text{Elev}_i + \lambda_{7,k,w/c} d_{warm,i} d_{cold,i} \text{Elev}_i + \\
& \lambda_{8,k,w/c} U_i + \lambda_{9,k,w/c} L_i + \lambda_{10,k,w/c} S_i
\end{aligned} \tag{B.4}$$

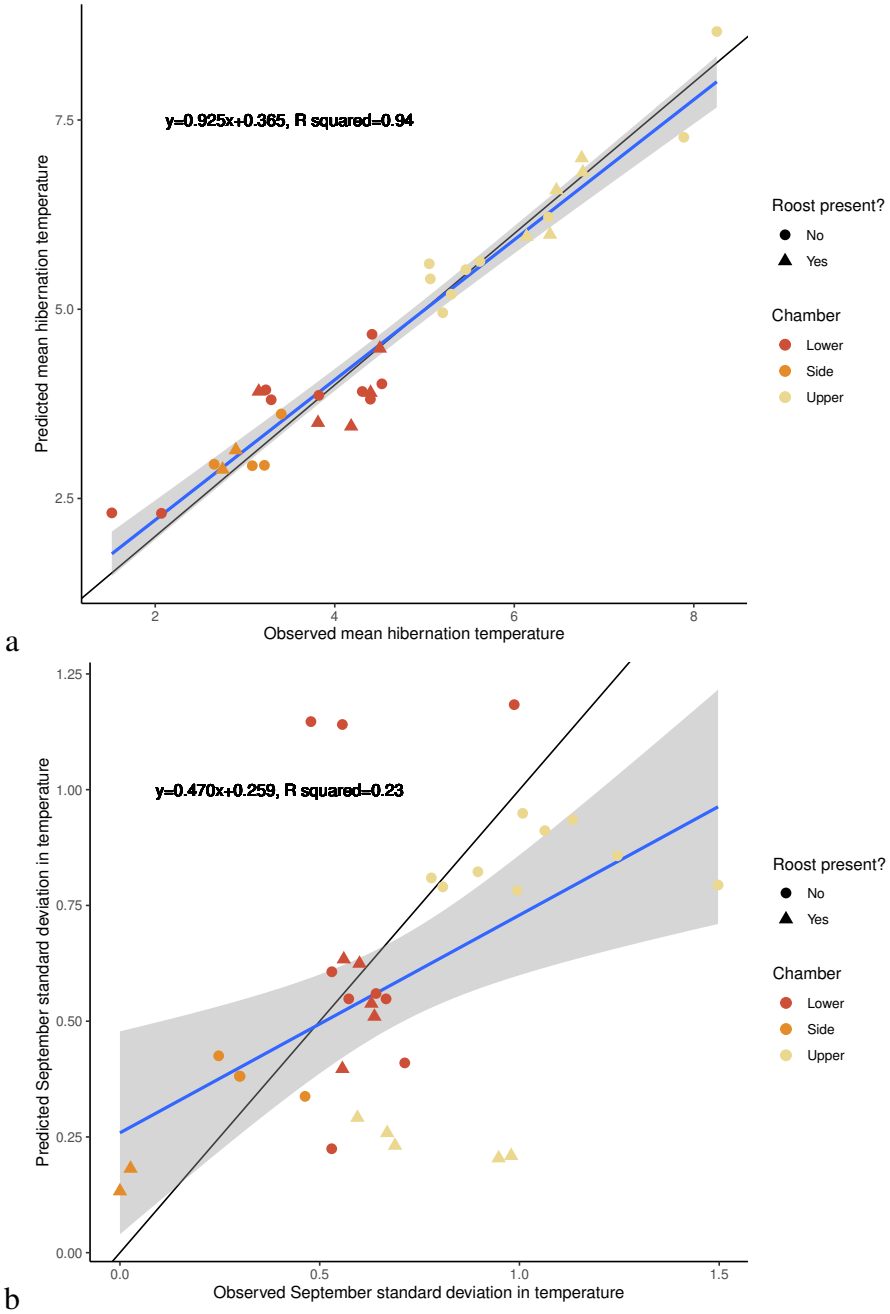


Figure B.4: Regression between observed and predicted (a) mean hibernation temperature and (b) September standard deviation in temperature. The black line has a slope of 1 and intercept of 0. The blue line and shaded area represent linear regression with a 95% confidence interval, the equation of which is inset in the figure. Note that while there is significant deviation between observed and predicted September standard deviation in temperture at some microsites, in general within-chamber relationships are maintained wherein bat roosts have lower standard deviation than non-roost microsites.

Appendix C

Chapter 4 supplemental materials

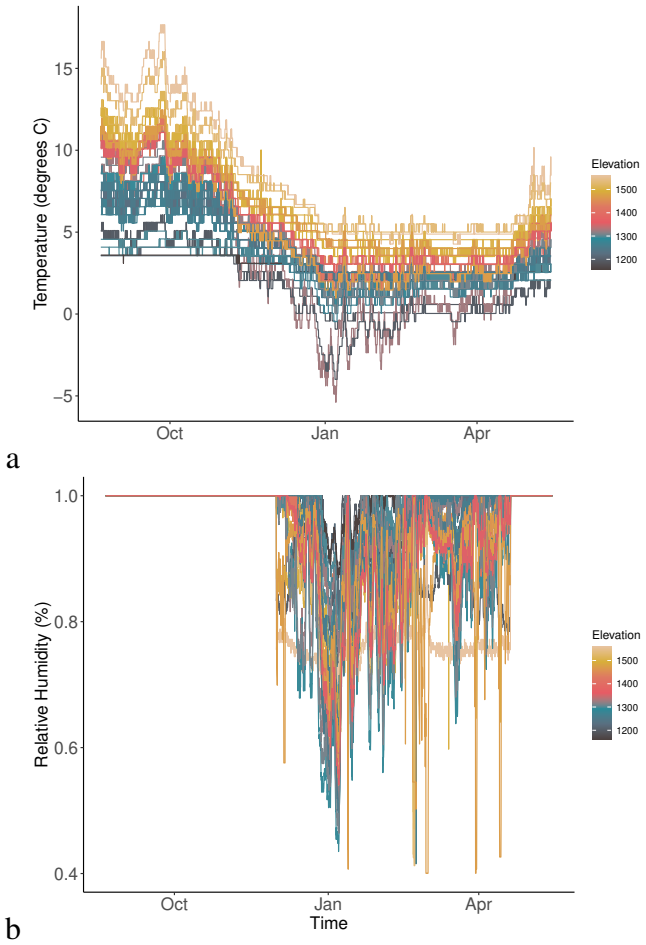


Figure C.1: (a) Temperature and (b) relative humidity data used in ecophysiolgoical models to generate torpor bout duration. Reprinted with permission from Golas *et al.* (in prep). Refer to Golas *et al.* (in prep) for further details.

C.1 Ecophysiological equation modifications

Our model uses the same equations to estimate time in torpor and energy consumed as Haase *et al.* (2019a), with the addition of time in cooling period taken from Haase *et al.* (2019b). We alter the equations to incorporate clustering by multiplying the denominator determining time in torpor and the cutaneous evaporative water loss by a percentage representing the ratio of energy lost clustered compared to energy lost as an individual (Canals *et al.*, 1997).

$$t_{tor,energetics} = \begin{cases} t_{tor,max}/Q_{10}^{\left(\frac{T_a - T_{tor,min}}{10}\right)} * clusterfac & T_a > T_{tor,min} \\ \frac{t_{tor,max}}{1+(T_{tor,min}-T_a)*\left(\frac{C_t}{TMR_{min}}\right)*clusterfac} & T_a \leq T_{tor,min} \end{cases} \tag{C.1}$$

$$CEWL = (SA_{body} * rEWL_{body} + SA_{wing} * rEWL_{wing})\Delta WVP * clusterfac \tag{C.2}$$

For grooming, we simply subtract from the *Pd* reservoir on the bat based on time spent clearing fungus at rate $groom$ during the arousal period. Then, following arousal, that amount of *Pd* will be as in Equation C.3. Parameter definitions and values are as described in Hayman *et al.* (2016).

$$Pd_{t+1} = Pd_t + \beta_1 * (T_{eu} - T_{min}) * (1 - e^{(\beta_2 * (T_{eu} - T_{max}))}) * \frac{\mu_1 * RH_t}{1 + \mu_2 * RH_t} * t_{eu} - groom * t_{eu} * 0.22 \tag{C.3}$$

For further details regarding model structures, code used to generate simulations is available at: https://github.com/bengolas/WNSSurvivalAdaptations

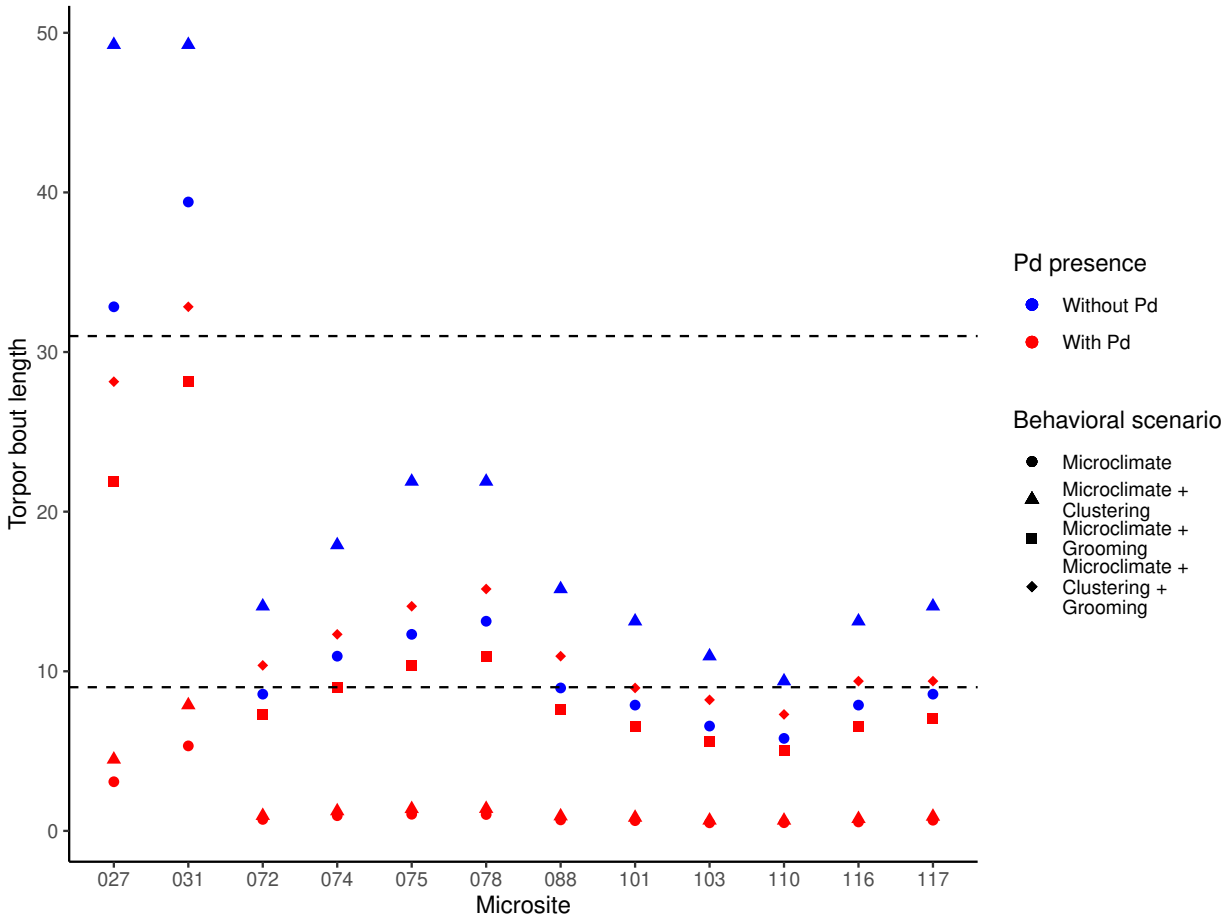
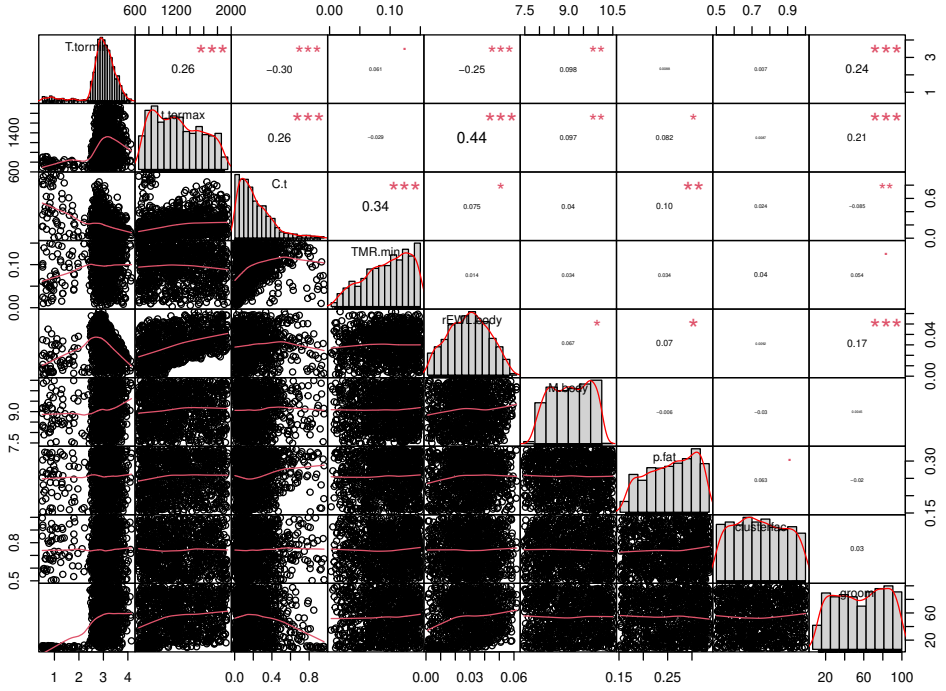
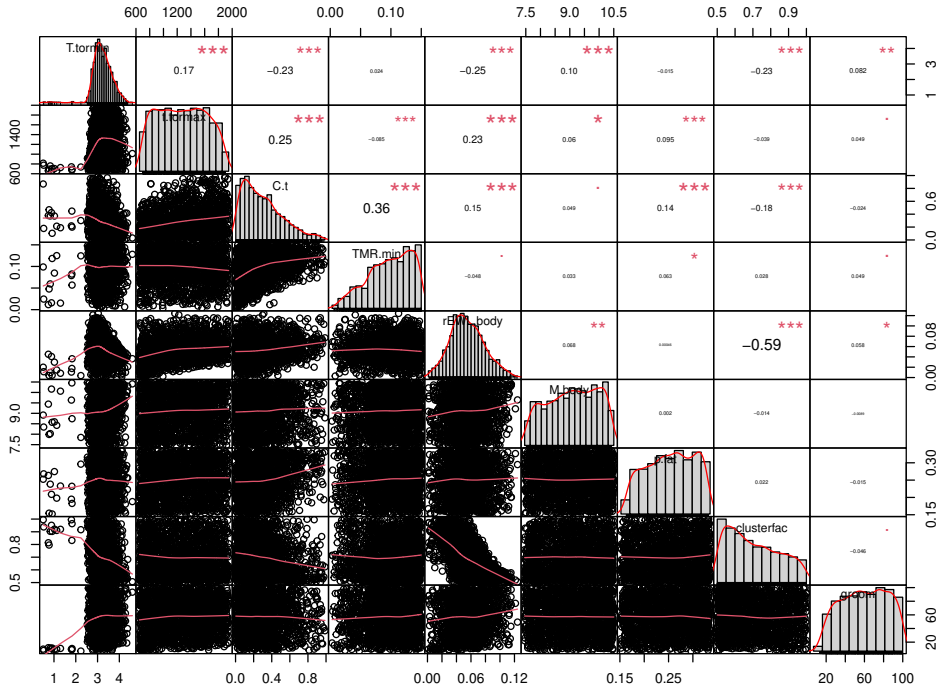


Figure C.2: Example equation output of torpor bout length for each of the six scenarios across microsites where *M. lucifugus* are found hibernating midwinter. Dotted lines indicate the approximate Bayesian computation rejection algorithm criteria for boundaries of average torpor bout length throughout hibernation without *Pd*. This literature-based parameter set would be excluded in the ABC process because in all four models (see text), there are microsites where expected average torpor bout length is greater than rejection algorithm criteria allow (microsites 27 and 31). The Microclimate and Microclimate + Clustering models would be excluded due to predicted death (average torpor bout length of near 0) in many microsites when *Pd* is introduced.



a



b

Figure C.3: Correlation between parameters in posteriors from approximate Bayesian computation for the (a) Microclimate + Grooming and (b) Microclimate + Clustering + Grooming models. Note the higher correlation between conductance (C.t) and torpid metabolic rate (TMR.min) in both models, and between evaporative water loss and clustering factor in the Microclimate + Clustering + Grooming model.

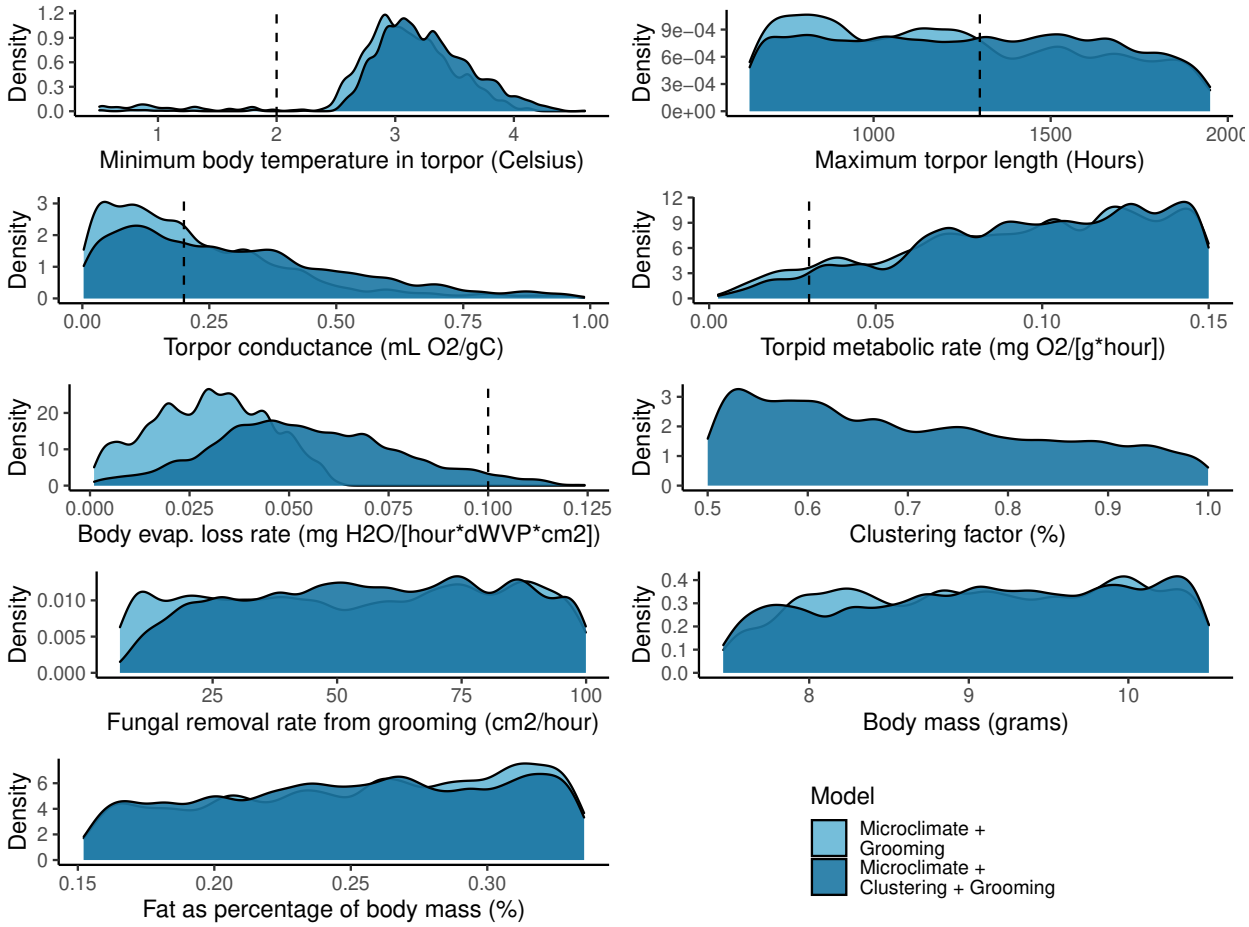


Figure C.4: Posterior densities for all estimated parameters from approximate Bayesian computation. Vertical dotted lines represent literature-based point estimates used to develop prior distributions.