

Exploring the drivers and consequences of emerging infectious disease of wildlife

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Exploring the drivers and consequences of variation in host response to emerging infectious
disease

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Academic Abstract

Emerging infectious diseases of wildlife have threatened host populations of diverse taxa in recent history, which is largely attributable to anthropogenic global change. In three data chapters, this dissertation examines the drivers of individual- to population-level variation in how host populations respond to novel and emerging pathogens. Each chapter explores these processes in bat populations of North America, predominantly the Northeast and Midwest regions of the United States, impacted by the emerging fungal pathogen that causes white-nose syndrome, *Pseudogymnoascus destructans*. In Chapter 2, I disentangle the effects of adaptive host traits and environmental influences in driving host population stabilization of the little brown bat (*Myotis lucifugus*), finding that host-pathogen coexistence in this system is the product of their complex interaction. In Chapter 3, I characterize the range-wide variation in white-nose syndrome impacts on a federally endangered and poorly studied species, the Indiana bat (*Myotis sodalis*), as well as environmental and demographic determinants of its declines over epidemic time. In Chapter 4, I explore the role of individual variation in roosting microclimate selection of little brown bats in driving their infection severity, yielding important insights into the pathophysiology and environmental dependence of white-nose syndrome. Ultimately, this dissertation characterizes complex drivers of variation in host responses to emerging and invading pathogens, yielding insights essential to the successful mitigation of their impacts.

Exploring the drivers and consequences of variation in host response to emerging infectious disease

Alexander Thomas Grimaudo

General Audience Abstract

In the same way that Covid-19 swept through our global human population in the year 2020, novel infectious diseases have threatened wildlife populations, sometimes to the point of extinction. Often, however, the processes driving the impacts of novel infectious diseases in wildlife are unknown, despite being important information to protect susceptible populations. In this dissertation, I explore how North American bat populations have been impacted by a recently emerged disease, white-nose syndrome, and what processes cause variation in how individual bats and bat colonies have responded to the disease. In Chapter 2, I explore how the little brown bat (*Myotis lucifugus*) has evolved to co-exist with its new pathogen and how this coexistence is affected by environmental conditions like temperature and humidity. In Chapter 3, I characterize variation in how populations of the Indiana bat (*Myotis sodalis*) have responded to white-nose syndrome and how environmental and demographic conditions have affected declines since the disease first emerged. In Chapter 4, I explore how the temperatures used by little brown bats during hibernation affect the severity of their infection, giving us important information on how bats survive with white-nose syndrome and the role of temperature. Altogether, the research in this dissertation describes complex interactions between hosts, pathogens, and their environment in driving the patterns we observe after the emergence of novel infectious diseases.

Dedication

This dissertation is dedicated to my friends and family.

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Chapter 1 – Introduction

Emerging infectious diseases of wildlife

Widespread global environmental change and threats to the conservation of biodiversity are defining characteristics of the Anthropocene (Young et al. 2016). Emerging infectious diseases (EIDs) of wildlife, characterized by an unprecedented increase in the prevalence and impact of infectious pathogens causing disease in host populations, are one such threat that has caused documented declines in host populations in recent history (Daszak et al. 2000, 2001). Notable examples that have pushed populations to the brink of extinction include chytridiomycosis in global amphibian communities (Scheele et al. 2019), avian malaria in birds of Hawaii (Paxton et al. 2016), facial tumor disease in Tasmanian devils (Hawkins et al. 2006), and white-nose syndrome of bats in North America (Langwig et al. 2016). During pathogen emergence and invasion, we often observe substantial variation among individual hosts and host populations in how they respond to the disease. The drivers of this variation and how it might be leveraged to mitigate the impact of EIDs is often unclear, particularly in the early stages of pathogen emergence, despite its importance for host conservation.

One common driver of variation in disease impacts is variation in the environmental context of host-pathogen interactions. Ambient environmental conditions such as temperature or humidity can have individual- to population-level impacts on infectious disease dynamics. For example, temperature is strongly associated with mosquito development and biting rates, each of which are important drivers of the epidemiology of vector-borne diseases such as malaria or Dengue (Beck-Johnson et al. 2013; Shapiro et al. 2017). Temperature can also influence the susceptibility of hosts to infection, as is the case in amphibians challenged by the emergence of chytridiomycosis globally (Cohen et al. 2017). Finally, following the establishment of infection,

temperature conditions can also be associated with the severity of infection or disease, as is the case in bats with white-nose syndrome (Hopkins et al. 2021; Langwig et al. 2016) or salmonid fish with proliferative kidney disease (Bailey et al. 2018). However, despite its importance, the environmental dependence of disease impacts following pathogen emergence is often difficult to characterize and unexplored in EID systems. My dissertation expands on our understanding of the environmental dependence of disease over epidemic time and host-pathogen coevolutionary history.

Host-pathogen coevolution following pathogen emergence can further generate spatial and temporal variation in disease impacts. Adaptive host traits, such as resistance or tolerance, can evolve in response to the emergence of a novel pathogen (Råberg et al. 2009; Restif & Koella 2004), itself able to respond evolutionarily through virulence or antigenic evolution (Marchi et al. 2021; de Roode et al. 2008). The directionality and rate of these coevolutionary processes will largely be driven by the underlying ecological and environmental context, resulting in a geographic mosaic of coevolution and host population impacts (Laine 2009; Parratt et al. 2016). In some cases, host-pathogen coevolution may result in “evolutionary rescue,” in which a declining host population stabilizes through the evolution of adaptive traits before extirpation occurs (Bell 2013; Bell & Gonzalez 2009; Carlson et al. 2014; Gonzalez et al. 2013). Host populations displaying adaptive traits may be prioritized for conservation action, as they represent a genetic pool to promote evolutionary rescue in other populations threatened by the pathogen. Often, however, it is difficult to identify host-pathogen coevolution when it has occurred, particularly if there are environmental covariates that could drive host population stabilization as well. Additionally, how host-pathogen coevolution interacts with environmental dependence of disease processes and how these associations may change over time is often

poorly understood. My dissertation expands on our understanding of host-pathogen coevolution and coexistence, their environmental limitations, and how they change over epidemic time.

Study system and context

The research presented in my dissertation explores the dynamics of white-nose syndrome (WNS), an emerging infectious disease of bats in the United States (Hoyt et al. 2021). WNS is caused by a fungal pathogen, *Pseudogymnoascus destructans* (Lorch et al. 2011; Warnecke et al. 2012), which has recently emerged in bat populations of North America, causing significant but variable population declines in hibernating bat populations (Blehert et al. 2009; Drees et al. 2017; Frick et al. 2015; Langwig et al. 2012, 2015b, 2016; Leopardi et al. 2015). The prevalence and impact of the disease is highly seasonal, occurring when bats return in fall to the caves and mines (“hibernacula”) in which they form colonies and hibernate over winter (Langwig et al. 2015a), environments that are contaminated by a reservoir of the pathogen (Hoyt et al. 2020; Laggan et al. 2023). The severity of infection and disease increases over winter, when bat body temperature and immune function drops during hibernation, until bats either die from the disease or emerge onto the landscape in spring and clear their infection (Langwig et al. 2015a).

There is substantial variation in the severity of population-level impacts of WNS both within and among host species (Cheng et al. 2021; Frick et al. 2015; Langwig et al. 2016; Thogmartin et al. 2012). While complete colony extirpations due to WNS have been observed, other colonies appear to stabilize and persist following initial declines, an observation initially made in little brown bat (*Myotis lucifugus*) populations in the northeast United States (Dobony et al. 2011; Langwig et al. 2012, 2017; Frick et al. 2015; Hoyt et al. 2021). Variation among hibernacula in temperature and humidity conditions is a potential source of population-level variation in WNS impacts (Langwig et al. 2012), as the growth of *P. destructans* is dependent on

temperature and humidity conditions (Marroquin et al. 2017; Verant et al. 2012). However, evidence also suggests that host-pathogen coevolutionary dynamics may produce variation among populations in WNS impacts as well, potentially through the evolution of host resistance (Langwig et al. 2017). The environmental dependence of pathogen dynamics and the wide variation in population-level impacts of WNS make it an excellent system in which to explore the dynamics of environmental disease dependence and host-pathogen coevolution.

Chapter Overview

My dissertation contains three data-driven chapters, each exploring dynamics of WNS at different scales and ecological contexts. In Chapter 2, I experimentally disentangle environmental effects and host adaptation in driving the stabilization and persistence of little brown bat (*Myotis lucifugus*) colonies impacted by WNS in New York (Grimaudo et al. 2022; Langwig et al. 2012). I found that neither environmental conditions nor the evolution of adaptive host traits independently drive host-pathogen coexistence in these colonies, but rather each interact to promote host population stabilization. Specifically, host traits adaptive for surviving WNS have evolved in these populations, a process that was likely driven by the particularly cold environmental conditions buffering the speed of population declines. However, these adaptive traits do not afford protection across all environmental conditions available, as severe disease and mortality is still observed in warm conditions and outside of intermediate levels of humidity. Additionally, the results of this chapter suggest that bat movement among various microclimates available within hibernacula over winter may be an important driver of WNS dynamics, a finding largely motivating the research presented in Chapter 4. In Chapter 3, I explore drivers of population-level impacts of WNS in the Indiana bat (*Myotis sodalis*), a federally endangered species, including the role of hibernaculum environmental conditions and host colony size

(Grimaudo et al. in review). I found that there was substantial variation in the impacts of WNS on Indiana bat colonies, ranging from complete extirpation to moderate declines and stabilization. The temperature of hibernacula was a strong predictor of the severity of declines and extinction risk, but this effect weakened as the epidemic progressed, potentially due to host-pathogen coevolution relaxing the environmental space of coexistence. Population simulations suggest that most extirpations that will occur already have, likely due to *P. destructans* finishing its invasion across the Indiana bat range. Additionally, I found that extirpation risk was particularly high for small colonies. These results illustrate the importance of both host population dynamics and environmental conditions in driving severity of declines and extinction risk from emerging pathogens. In Chapter 4, I explore how overwinter microclimate use by individual bats influences infection and pathophysiology dynamics of WNS. I successfully characterize an environment-pathophysiological pathway between microclimate temperature, infection severity, arousal frequency, and weight loss. Furthermore, I found a previously undescribed mechanism by which bats may mitigate the severity of their disease by behaviorally reducing the energetic cost of WNS-driven arousals from torpor. Finally, a model of on-host and environmentally dependent pathogen dynamics suggested that overwinter microclimate use is an important contributor to infection severity, improving predictability over conventional “snapshot” approaches that characterize environment-disease associations using single time point samples. Overall, these four chapters combine data across ecological scales to improve our understanding of emerging infectious disease dynamics, the environmental dependence of disease, and host-pathogen coevolution.

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Chapter 2 – Host traits and environment interact to determine persistence of bat populations impacted by white-nose syndrome

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Abstract

Emerging infectious diseases have resulted in severe population declines across diverse taxa. In some instances, despite attributes associated with high extinction risk, disease emergence and host declines are followed by host stabilization for unknown reasons. While host, pathogen, and the environment are recognized as important factors that interact to determine host-pathogen coexistence, they are often considered independently. Here, we use a translocation experiment to disentangle the role of host traits and environmental conditions in driving the persistence of remnant bat populations a decade after they declined 70-99% due to white-nose syndrome and subsequently stabilized. While survival was significantly higher than during the initial epidemic within all sites, protection from severe disease only existed within a narrow environmental space, suggesting host traits conducive to surviving disease are highly environmentally dependent. Ultimately, population persistence following pathogen invasion is the product of host-pathogen interactions that vary across a patchwork of environments.

Introduction

Emerging infectious diseases of wildlife have resulted in severe mortality events and regional to complete extinctions of host populations (Van Riper III et al. 1986; Antolin et al. 2002; LaDeau et al. 2007; Mccallum et al. 2009; Langwig et al. 2012; Scheele et al. 2019). In some instances, the presence of pathogen reservoirs, frequency-dependent transmission, and small pre-epidemic host population sizes suggest that host species will be driven to extinction (de Castro & Bolker 2005). Additionally, high initial host population declines leave remnant populations more vulnerable to stochastic and Allee effects that increase the likelihood of host extinction (Lande 1998; de Castro & Bolker 2005; Friedman & Yakubu 2012). However, following the initial epidemic and population declines, some host populations stabilize and persist for unknown reasons (Briggs et al. 2010; Langwig et al. 2012; Brannelly et al. 2021). For example, population persistence has been observed in several important disease systems including amphibians impacted by chytridiomycosis (Briggs et al. 2010; Scheele et al. 2015, 2017; Voyles et al. 2018; Brannelly et al. 2021), Tasmanian devils impacted by facial tumor disease (Epstein et al. 2016; Lazenby et al. 2018; Hohenlohe et al. 2019; Patton et al. 2020), birds impacted by avian malaria (Woodworth et al. 2005), and bats impacted by white-nose syndrome (Langwig et al. 2012; Reichard et al. 2014). While initial evidence suggested that these hosts would be extirpated by infectious disease, some populations have stabilized despite infection prevalence remaining high while others continue to decline or have gone extinct (Briggs et al. 2010; Langwig et al. 2012, 2016; Frick et al. 2015; Samuel et al. 2015; Scheele et al. 2019, 2017; Lazenby et al. 2018; Hoyt et al. 2020, 2021; Brannelly et al. 2021).

Potential drivers of host-pathogen coexistence include the evolution of host resistance, tolerance, and/or general vigor (Roy & Kirchner 2000; Restif & Koella 2004; Råberg et al. 2007,

2009; Best et al. 2008; Boots et al. 2009; Kutzer & Armitage 2016; Voyles et al. 2018; Wilber et al. 2021), environmental refugia from infection or severe disease (Tobler et al. 2007; Springer 2009; Schelkle et al. 2012; Zumbado-Ulate et al. 2014; Heard et al. 2015; Mosher et al. 2018), host demographic compensation (Arthur et al. 2004; Lachish et al. 2009; Scheele et al. 2015; McDonald et al. 2016; Spitzen-Van Der Sluijs et al. 2017), density-dependent transmission (Hochachka & Dhondt 2000; McCallum et al. 2001; Fenton et al. 2002; Lloyd-Smith et al. 2005), and attenuation of pathogen virulence (Levin & Pimentel 1981; Anderson & May 1982; Boots et al. 2004; Kerr et al. 2006; Wild et al. 2009; Cressler et al. 2016). However, studies investigating host coexistence with virulent pathogens frequently focus on a single aspect of the host-pathogen-environment interaction, despite evidence that such interactions may influence disease severity and scale up to population-level effects on the host (Laine 2007; Sadd 2011; Echaubard et al. 2014). For example, studies of amphibian chytridiomycosis have illustrated shifts in host responses following pathogen invasion that may be indicative of host resistance (Wilber et al. 2017; Voyles et al. 2018), but that host-pathogen coexistence is additionally modified by an interaction with environmental conditions (Forrest & Schlaepfer 2011; Scheele et al. 2015; Spitzen-Van Der Sluijs et al. 2017; Wilber et al. 2017). Given the potential for interactions between the host and environment to drive host population persistence, which may change as pathogen invasion progresses, understanding these interactions is essential for identifying the conditions necessary for host-pathogen coexistence following the invasion of a virulent pathogen.

White-nose syndrome (WNS) is an infectious disease of bats caused by the fungal pathogen *Pseudogymnoascus destructans* (Lorch et al. 2011; Warnecke et al. 2012; Minnis & Lindner 2013). In North America, the disease was first detected in New York state in 2006

(Blehert et al. 2009) and has since resulted in large mortality events and regional extinctions of once common bat species (Langwig et al. 2012, 2016; Frick et al. 2015). An environmental pathogen reservoir establishes following the introduction of *P. destructans* to hibernacula (Langwig et al. 2015b; Hoyt et al. 2020) and most populations decline greater than 90%, often resulting in complete local extirpations (Frick et al. 2010; Langwig et al. 2012). Following the establishment of the pathogen within hibernacula, bats become infected with *P. destructans* upon entering previously contaminated hibernacula in the fall (Langwig et al. 2015a), and both indirect and direct transmission result in widespread infection early in the seasonal epidemic (Lorch et al. 2011; Langwig et al. 2015a; Hoyt et al. 2018, 2020). However, bats that survive hibernation emerge onto the landscape in spring and clear infection (Meteyer et al. 2011; Langwig et al. 2015a; Fuller et al. 2020).

The growth of *P. destructans* is sensitive to environmental temperature (Verant et al. 2012) and humidity (Marroquin et al. 2017), resulting in environmental trends in population declines such that populations and species roosting in warmer and wetter environments have more severe declines (Langwig et al. 2012, 2016; Grieneisen et al. 2015; Lilley et al. 2018; Hopkins et al. 2021). However, several years following pathogen introduction, some colonies of little brown bats (*Myotis lucifugus*) in the northeast United States stabilized at 5-30% of their pre-epidemic population size following cumulative regional declines of 96% (Dobony et al. 2011; Langwig et al. 2012, 2017; Frick et al. 2015; Hoyt et al. 2021) despite infection prevalence remaining high (Langwig et al. 2017). The abiotic pathogen reservoir within hibernacula sustains a high prevalence of infection regardless of colony size, suggesting density-dependent transmission is not a driver of population persistence (Langwig et al. 2015a, b; Hoyt et al. 2016a, 2020). Compared to colonies undergoing epidemic conditions on the invasion front, bats in

persisting colonies display slower on-host pathogen growth rates, potentially a signature of host resistance (Langwig et al. 2017). However, persisting colonies also utilize colder hibernacula (Langwig et al. 2012; Hopkins et al. 2021), so lower pathogen growth rates may be a product of temperature operating independently of host characteristics. Several lines of evidence suggest that pathogen virulence attenuation is not a principal driver of host population persistence in this system. Few mutations have been identified in *P. destructans* since initial introduction to North America (Trivedi et al. 2017), and experimental inoculation of North American bats naïve to WNS shows similar virulence between North American and European isolates (Warnecke et al. 2012). Furthermore, *P. destructans* isolates in North America reproduce asexually (Palmer et al. 2014; Drees et al. 2017), and genetic analyses indicate shallow genetic diversity across the North American continent, with high connectivity and long-distance dispersal of the pathogen (Ren et al. 2012; Leopardi et al. 2015; Drees et al. 2017). Due to the high connectivity of the pathogen population in North America, isolates from stabilizing host populations and those undergoing severe declines are genetically indistinguishable (Ren et al. 2012; Leopardi et al. 2015; Drees et al. 2017), variation which should be detectable if pathogen virulence attenuation drives host-pathogen coexistence.

The relative role of host traits and environmental conditions in driving the persistence of little brown bat populations impacted by WNS is still unclear. Understanding the factors driving host persistence will provide empirical support for general theory on host coexistence with virulent pathogens and much needed information on this important and devastating wildlife disease. Ten years following the introduction of *P. destructans* and subsequent colony declines, we conducted a fully factorial translocation experiment to understand the mechanisms of population persistence. We leveraged the variable environmental conditions in hibernacula (Fig.

1, Supplemental Fig. 1, 2) and a previously conducted translocation experiment early in the epizootic to disentangle the relative roles of host traits and environmental conditions in driving disease severity and ultimately population persistence.

Materials and Methods

Translocation experiment

Two hibernacula of persisting little brown bat (*Myotis lucifugus*) colonies in New York and one hibernaculum previously extirpated of bats by white-nose syndrome (WNS) in Vermont were used in this study (Fig. 1). Prior to hibernation, we installed ten five-sided reptile cages (dimensions: 12”W, 18”L, 20”H) in known or historical roosting locations within each site (Supplemental Fig. 8). Each cage was mounted so that the open side was in contact with the hibernaculum surface, allowing bats to roost on the substrate. Cages were mounted high on site ceilings and were sealed to prevent predation of bats. Water was provided in poultry waterers in each cage.

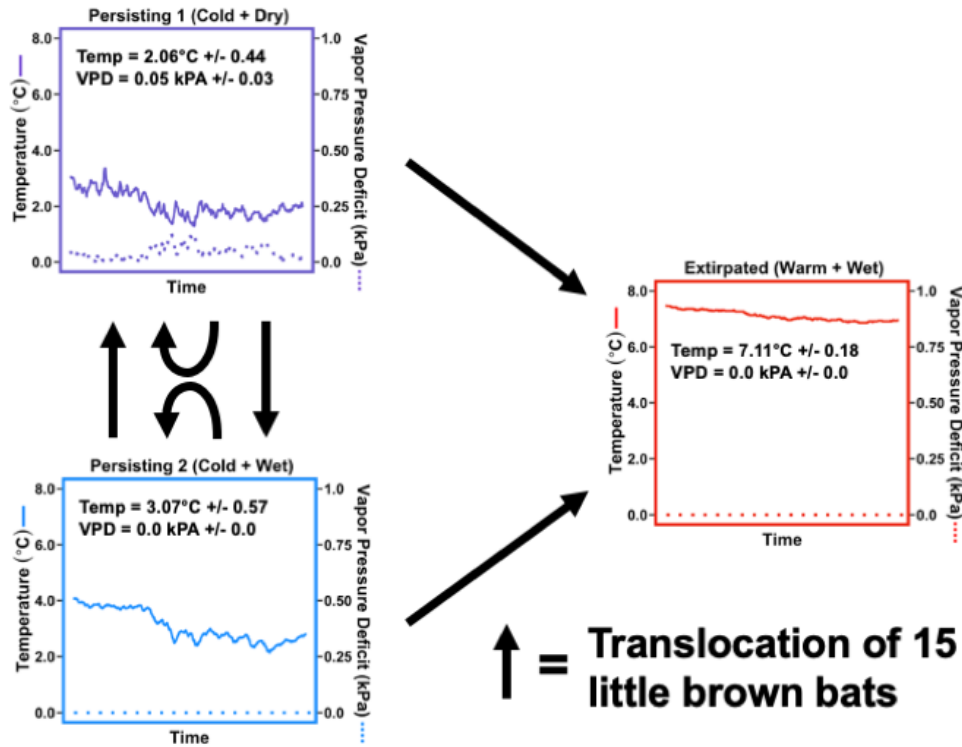


Figure 1: Schematic of the experimental design. Two sites in New York with known persisting colonies of little brown bats and one site that was previously extirpated of little brown bats were used in this study. The three sites are referred to as Persisting 1 (Cold + Dry), Persisting 2 (Cold + Wet), and Extirpated (Warm + Wet). Solid and dashed lines on plots correspond to temperature (Celsius) and vapor pressure deficit (kPa; higher values correspond to drier conditions), respectively, within each site over the course of this study. Mean +/- SD of temperature and vapor pressure deficit within each site is shown on each plot. Sites varied in their environmental conditions, with the persisting sites being colder than the extirpated site and Persisting 1 (Cold + Dry) being significantly drier than Persisting 2 (Cold + Wet). Persisting 2 (Cold + Wet) was similar in its humidity conditions to Extirpated (Warm + Wet). In early hibernation, 45 bats from each of the persisting sites ($n = 90$) were collected and randomly assigned a translocation site and cage (3 bats per cage). The translocation was fully factorial, with 15 bats from each site being caged within the same site, in the opposite persisting site, or the extirpated site. Bats remained caged within these sites until late hibernation, when survivors were returned to their site of origin.

In December 2018, in early hibernation, we returned to the two sites of persisting *M. lucifugus* colonies in New York. We collected 45 male *M. lucifugus* from each site and each received a unique forearm band. Individuals were randomly assigned to both a site and a cage

within that site with 3 individual bats from the same origin site sharing each cage. These assignments were in a full factorial design, such that of the 45 bats collected from each site, 15 were assigned to be caged within the same site, 15 within the opposite persisting site, and 15 within the previously extirpated site. We placed each bat individually in a cloth bag and transported them to their assigned site in a cooler so that they would remain in torpor and limit energy expenditure. We replicated the transportation disturbance across all treatment groups. Bats remained in their assigned cages for the duration of hibernation. All individuals were caged with at least one infected individual. In mid-hibernation, each site was visited once to record survival status through visual inspection (Persisting 1: 50 days; Persisting 2: 64 days; Extirpated: 71 days following initial translocation); no cages were opened or bats handled to minimize disturbance. In March 2019, in late hibernation, 110 days following the initial translocation, we returned to each of the three sites to collect data and terminate the experiment. We released all surviving individuals at their site of origin.

Disease severity metrics

Upon returning to each of the three sites, we recorded the survival status of each individual. To calculate the on-host pathogen growth rate of *Pseudogymnoascus destructans*, we collected swab samples from each individual bat in both December 2018 and March 2019 using a standardized swabbing protocol described in (Langwig et al. 2015a). We used real-time polymerase chain reaction (qPCR) to quantify fungal loads (described in Supplemental Information).

In December 2018 and March 2019, we weighed each bat using an electronic scale. We subtracted the late hibernation weight of each individual from their early hibernation weight to

calculate weight loss over the course of hibernation in grams. Additionally, in March 2019, we collected data on the severity of tissue invasion by transilluminating both wings of each individual using a 9-watt 368nm fluorescent light. Infected tissue fluoresces orange under ultraviolet light, verified by histology (Turner et al. 2014). We used the proportion of the plagiopatagium displaying orange fluorescence as the response variable in models of this disease severity metric (Supplemental Information).

Statistical analysis

We conducted all analyses using package “lme4” (Bates et al. 2015) in R v.3.6.0 (R Core Team 2019). We constructed separate models for each disease severity metric. To measure pathogen growth rate for each individual bat, we subtracted its early hibernation fungal load value from its late hibernation value to quantify the change in fungal loads. Bats that had no detectable fungus at the time of swabbing were assigned a value of $4.35e-03$ pg (equivalent to a Ct value of 40) for that swab sample (Langwig et al. 2016), or a single *P. destructans* conidia. We then added a constant of 10 and log₁₀-transformed the growth rate values. We used a linear regression with pathogen growth rate as the response variable and site (hibernaculum to which bats were translocated), origin site (hibernaculum from which bats originated), and their interaction as fixed effects to explore how pathogen growth varied across sites. Inclusion of cage ID as a random effect prevented model convergence and was dropped from the model, and model results were verified in a Bayesian framework using package “BRMS” with weakly-informed priors (normal distribution with mean of 0 and standard deviation of 5) (Bürkner 2017). To understand the relationship between roosting temperature and change in fungal loads, we constructed a linear mixed model with the average roosting temperature (data collected by

iButtons within each cage), origin site, their interaction, and the average vapor pressure deficit within a site as fixed effects and cage ID as a random effect. We used a generalized linear model with a gamma error distribution and log link to analyze differences in early hibernation pathogen loads between bats that originated in the two persisting sites. A generalized linear model was used because a linear model violated assumptions of normally distributed and homoscedastic error.

To test for differences in the severity of tissue invasion across the sites, we used a logistic regression with orange pixels indicating infection as successes and non-orange pixels as failures (generalized linear mixed model with binomial distribution and logit link), site and origin site as fixed effects, and cage ID as a random effect. Additionally, to test for differences in tissue invasion between caged bats and free-flying bats opportunistically sampled at the end of hibernation in each of the persisting sites, we used a logistic regression with the same response variable (generalized linear mixed model with binomial distribution and logit link) and site, caging status (caged vs. free-flying), and their interaction as fixed effects. To explore the differences in weight loss across the three sites, we used a generalized linear mixed model (gamma distribution, log link) with weight loss as the response variable, site and origin site as fixed effects, and cage ID as a random effect. A generalized linear mixed model was used because a linear model violated assumptions of normally distributed and homoscedastic error, and a gamma error distribution was used because weight loss is a continuous variable bounded by zero and infinity. To test for differences in late hibernation body mass between caged and free-flying bats in each persisting site, we used a linear regression with body mass as the response variable and an interaction term of site and caging status as the explanatory variable. We used a generalized linear mixed model (binomial distribution, logit link, cage ID as a random

effect) to explore the relationship between early hibernation body mass and survival. Finally, to investigate how survival varied across sites, we used a generalized linear mixed model (binomial distribution, logit link) with survival as the response variable, site and origin site as fixed effects, and cage ID as a random effect.

Results

Summary

Bats were translocated between two hibernacula with persisting colonies as well as to one hibernaculum previously extirpated of bats by WNS (Fig. 1). Bats were caged within each site, where they remained for the duration of winter, allowing us to quantify disease severity from the same individuals at both the beginning and end of hibernation. In all three sites, survival observed during the translocation experiment was higher than that observed within the same sites during the initial epidemic. This suggests little brown bats in persisting colonies may have unique host traits that promote surviving infection with *P. destructans*. However, disease severity and subsequently survival varied across the three hibernacula, suggesting environmental conditions potentially interact with circulating host traits to ultimately drive persistence.

Pathogen growth rate

At the time of collection in early hibernation, 88.89% (n=40) of individuals from Persisting 1 (Cold+Dry) had detectable *P. destructans* from swab samples, as did 97.78% (n=44) from Persisting 2 (Cold+Wet). All cages in each site had at least one infected bat at the beginning of the experiment. Additionally, at the time of collection, bats captured in Persisting 1 (Cold+Wet) had higher pathogen loads than bats that originated in Persisting 2 (Cold+Dry)

(generalized linear model with gamma error distribution and log link: $\beta = 0.282 \pm 0.094$ SE, $p = 0.003$; Supplemental Fig. 3).

Over the course of the experiment, the highest on-host pathogen growth rates were recorded in the previously extirpated site (Warm+Wet), which was 4.5° C warmer than either of the two persisting sites, on average. Pathogen growth rates in Persisting 2 (Cold+Wet) were slightly higher than in Persisting 1 (Cold+Dry), likely attributable to the 1° C warmer and more humid conditions in the former. We also detected an interactive effect of origin site (the site from which bats were collected at the beginning of the experiment) and translocation site on the pathogen growth rate, such that within the previously extirpated site (Warm+Wet), bats originating in Persisting 2 (Cold+Wet) had lower pathogen growth rates than bats that originated in Persisting 1 (Cold+Dry) (Fig. 2; Supplemental Fig. 4; Supplemental Table 1). Pathogen growth rate increased with average roosting temperature, consistent with the sensitivity of *P. destructans* to ambient thermal conditions (Verant *et al.* 2012; Langwig *et al.* 2016). However, due to the effect of origin site, the estimated relationship between average roosting temperature and pathogen growth rate was positive for bats that originated in Persisting 1 (Cold+Dry) but did not increase for bats that originated in Persisting 2 (Cold+Wet) (Fig. 2; Supplemental Table 2). Additionally, we found that pathogen growth on bat skin was generally lower in drier conditions. Interestingly, we observed a decrease in pathogen loads on five bats in Persisting 1 (Cold+Dry), four in Persisting 2 (Cold+Wet), and one in Extirpated (Warm+Wet).

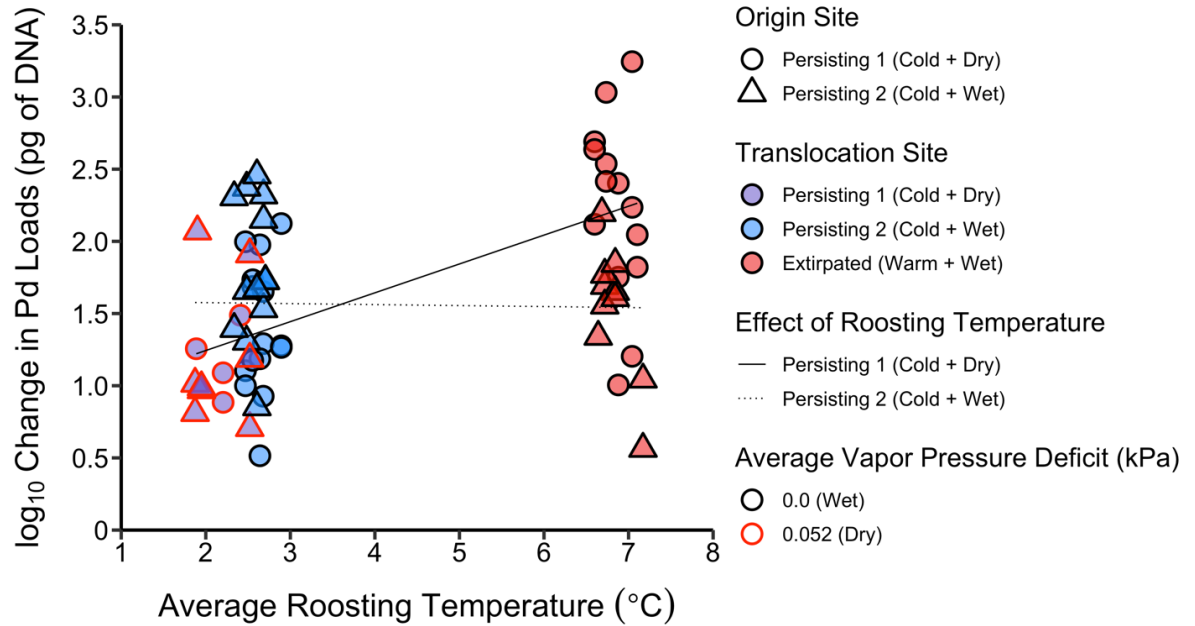


Figure 2: Relationship between average roosting temperature and pathogen growth rate on bats. The shape of data points denotes the persisting site from which individuals originated: Persisting 1 (Cold + Dry) = circle, Persisting 2 (Cold + Wet) = triangle. The color of data points represents the translocation sites: Persisting 1 (Cold + Dry) = purple, Persisting 2 (Cold + Wet) = blue, Extirpated (Warm + Wet) = red. The solid and dotted lines are model estimates for the effect of roosting temperature for bats that originated in the Persisting 1 (Cold + Dry) and Persisting 2 (Cold + Wet) sites, respectively. The outline of points represents average humidity conditions (vapor pressure deficit) within each site: 0.0 kPa (wet conditions) = black, 0.052 kPa (dry conditions) = red. As Extirpated (Warm + Wet) and Persisting 2 (Cold + Wet) were both at 100% relative humidity for the duration of the experiment, the humidity values are the same for these sites. A $\log_{10}$ change in Pd loads value of 1 is associated with no change in pathogen load from early to late hibernation, and data points above or below this value indicate an increase or decrease in pathogen load, respectively.

Infection severity and host body condition

The degree of tissue invasion and weight loss varied across the three sites (Fig. 3). Corresponding to the temperature-dependent pathogen growth rate, the severity of infection at Extirpated (Warm+Wet) was significantly higher than that observed at the similarly wet site, Persisting 2 (Cold+Wet) (Fig. 3A; Supplemental Table 3). However, contrary to the temperature-dependent pathogen growth rate observed, bats at Persisting 1 (Cold+Dry) displayed

significantly higher infection severity than that observed at Persisting 2 (Cold+Wet) and did not significantly differ from that observed in Extirpated (Warm+Wet). Across all sites, there was no clear effect of origin site on the degree of tissue invasion. Patterns of tissue invasion were mirrored by the degree of weight loss observed across the sites, as bats in Persisting 2 (Cold+Wet) had lower weight loss compared to either of the other sites (fig. 3B; Supplemental Table 4). The particularly dry conditions within Persisting 1 (Cold+Dry) may have resulted in the deviation from the temperature-pathogen growth relationship, exacerbating infection severity and weight loss despite a relatively low level of pathogen growth. Bats originating in Persisting 2 (Cold+Wet) lost slightly more weight than bats originating in Persisting 1 (Cold+Dry), but there was no support that weight loss was the product of an interaction between origin site and translocation site (interaction p-value>0.05).

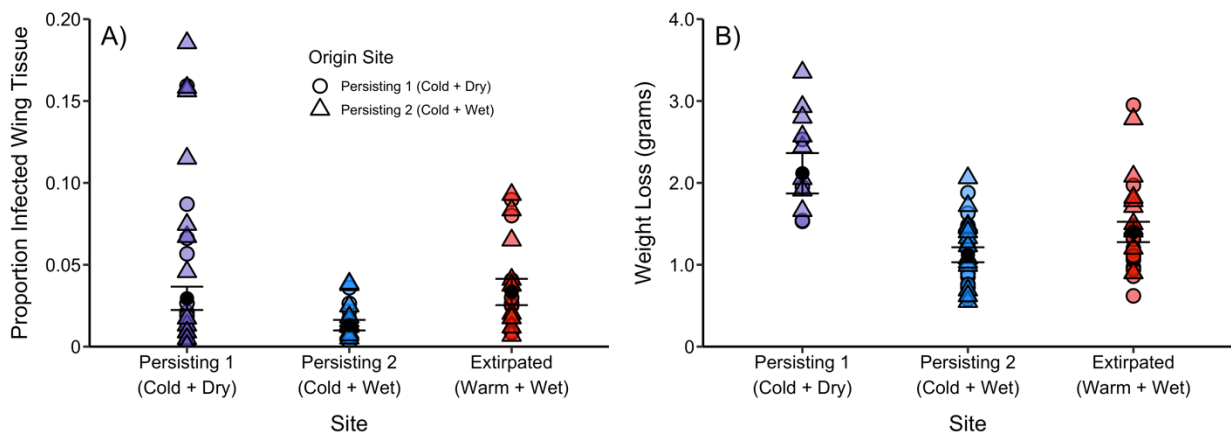


Figure 3: The **A)** proportion of wing tissue displaying signs of infection with *Pseudogymnoascus destructans* and **B)** amount of weight lost over hibernation in bats from each site. Black points and error bars represent model estimates $\pm$ standard error. Tissue invasion at Extirpated (Warm + Wet) was higher than that at the similarly humid Persisting 2 (Cold + Wet), suggesting that the higher fungal growth rate at the extirpated site resulted in greater tissue invasion and ultimately higher weight loss. The low humidity conditions at Persisting 1 (Cold + Dry) may have driven the observed increase in tissue invasion despite a lower pathogen growth rate. Low humidity conditions can exacerbate evaporative water loss from infected tissue, which may have increased arousal frequency and weight loss.

Survival

The highest survival was observed in Persisting 2 (Cold+Wet) (29 survivors of 30, 96.67% surviving), mirroring the pattern of infection severity and host body condition (Fig. 4A; Supplemental Table 5). The lowest observed survival occurred in Persisting 1 (Cold+Dry) (12 survivors of 30, 40% surviving) while Extirpated (Warm+Wet) displayed an intermediate level of survival (24 survivors of 30, 80% surviving). We found no effect of early hibernation body mass on survival (Supplemental Table 6). The higher degree of mortality at Extirpated (Warm+Wet) may have been related to the high pathogen growth rate within the site, while the high mortality at Persisting 1 (Cold+Dry) may be attributable to the particularly dry conditions, which appeared to exacerbate infection severity. Additionally, low humidity conditions increase evaporative water loss during infection with *P. destructans* (Mcguire *et al.* 2017), so the high degree of mortality within the cold and dry site may be attributable to the high infection severity driving higher rates of water loss (Cryan *et al.* 2010, 2013; Willis *et al.* 2011; Ehlman *et al.* 2013; Warnecke *et al.* 2013; Verant *et al.* 2014; Mcguire *et al.* 2017). However, in all sites, the level of survival observed was higher than that observed during the initial epidemic within the same sites, suggesting traits adaptive to surviving WNS may be promoting persistence in these populations (Fig. 4A).

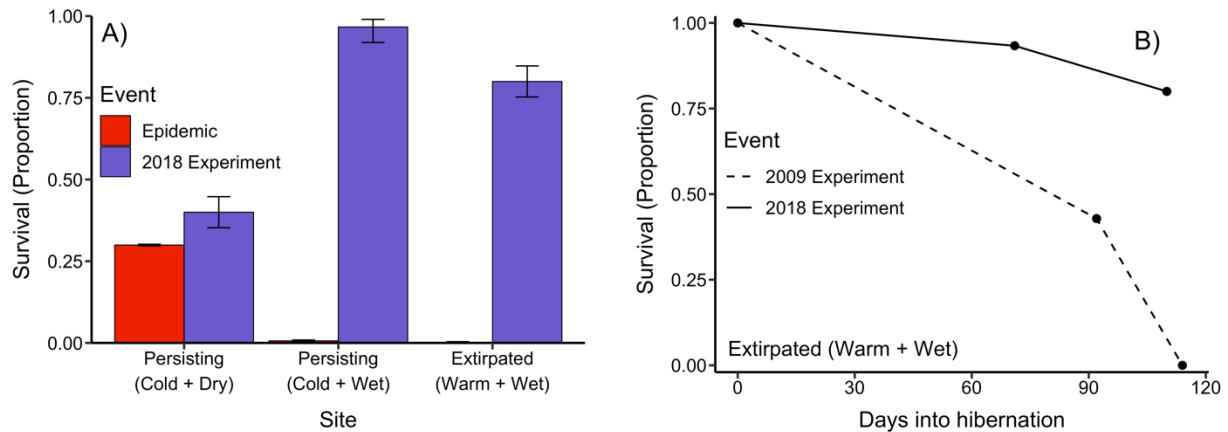


Figure 4: A) The proportion of bats surviving the initial epidemic (red bars) and 2018 experiment (blue bars) in each site. Error bars represent standard errors. Observed survival corresponded to the degree of tissue invasion and weight loss at each site. Despite the lowest pathogen growth rate, bats in Persisting 1 (Cold + Dry) had a high degree of infection severity, ultimately resulting in the lowest observed survival. In all three sites, we observed higher survival in this experiment than during the initial epidemic within the same sites, suggesting that WNS may have selected for host traits suitable to surviving the disease. **B)** Proportion of bats surviving translocation to the Extirpated (Warm + Wet) site in the 2018 experiment (solid line) and 2009 experiment (dashed line). Bats translocated to the extirpated site in 2009 were naïve to WNS, and consequently exhibited 100% mortality. Higher survival was observed in bats translocated to the site in 2018, suggesting that host traits beneficial to surviving WNS may not be circulating in these populations.

An additional line of evidence for adaptive traits promoting persistence in this system comes from comparison to a study conducted in 2009 in which bats naïve to WNS were translocated to the same extirpated site used in this study. In the historical experiment, all experimental bats died within 114 days (compared to 110 days, the length of this experiment; Fig. 4B) (Hicks *et al.* 2021). Effectively, this represents two replicates of extirpation within this site, in which all naïve bats succumbed to disease during the initial epidemic and subsequent re-introduction in 2009. However, in this translocation experiment, 24 of the 30 (80%) little brown bats we translocated from persisting colonies to the same extirpated site survived hibernation, suggesting traits adaptive to surviving infection may be circulating in these populations. Additionally, compared to the bats in the extirpated site during the historical translocation

experiment, bats in our study had low disease severity scores as indicated by UV fluorescence (Fig. 5; methods of comparison described in Supplemental Information). Among the two experiments, the faster speed of mortality in the historical experiment resulted in most of the histology scoring occurring in dead or moribund bats while in the contemporary experiment, wing invasion was predominately measured in live bats (N=64 alive, 17 dead; additional information on experimental design in Supplemental Information).

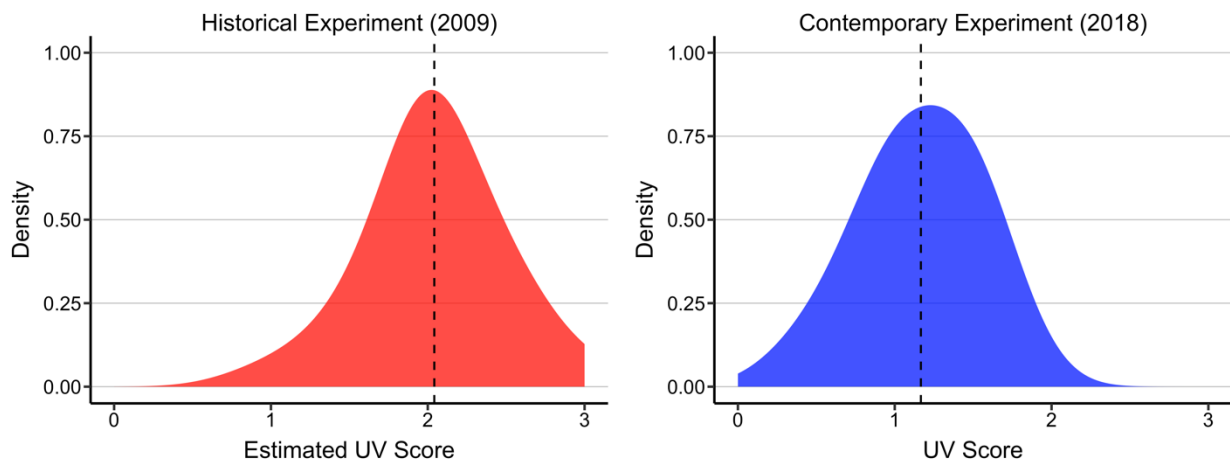


Figure 5: Density plots of infection severity as indicated by degree of orange fluorescence under ultraviolet light in bats from the historical and contemporary translocation experiments in Extirpated (Warm + Wet). A UV score of 0 = no orange fluorescence indicative of infection, 1 = 1-10% of wing area displays orange fluorescence, 2 = 10-50% of wing area displays fluorescence, and 3 = 50-100% of wing area displays fluorescence. Dashed lines correspond to average values. Bats translocated to the extirpated site in 2009 were naïve to WNS and displayed a higher degree of infection severity compared to the non-naïve bats translocated to the same site in 2018.

Comparisons with free-flying bats

At the end of the experiment in late hibernation, we sampled free-flying bats found roosting near the cages within each persisting site. At Persisting 1 (Cold+Dry), we found that free-flying bats had significantly lower tissue invasion compared to the bats within cages (Fig. 6; Supplemental Table 7). Conversely, free-flying bats at Persisting 2 (Cold+Wet) displayed a

similar degree of infection severity to caged bats within the same site. Free-flying bats had higher late hibernation body mass than caged bats in both persisting sites (Supplemental Fig. 5; Supplemental Table 8), and this difference was more pronounced in Persisting 1 (Cold+Dry).

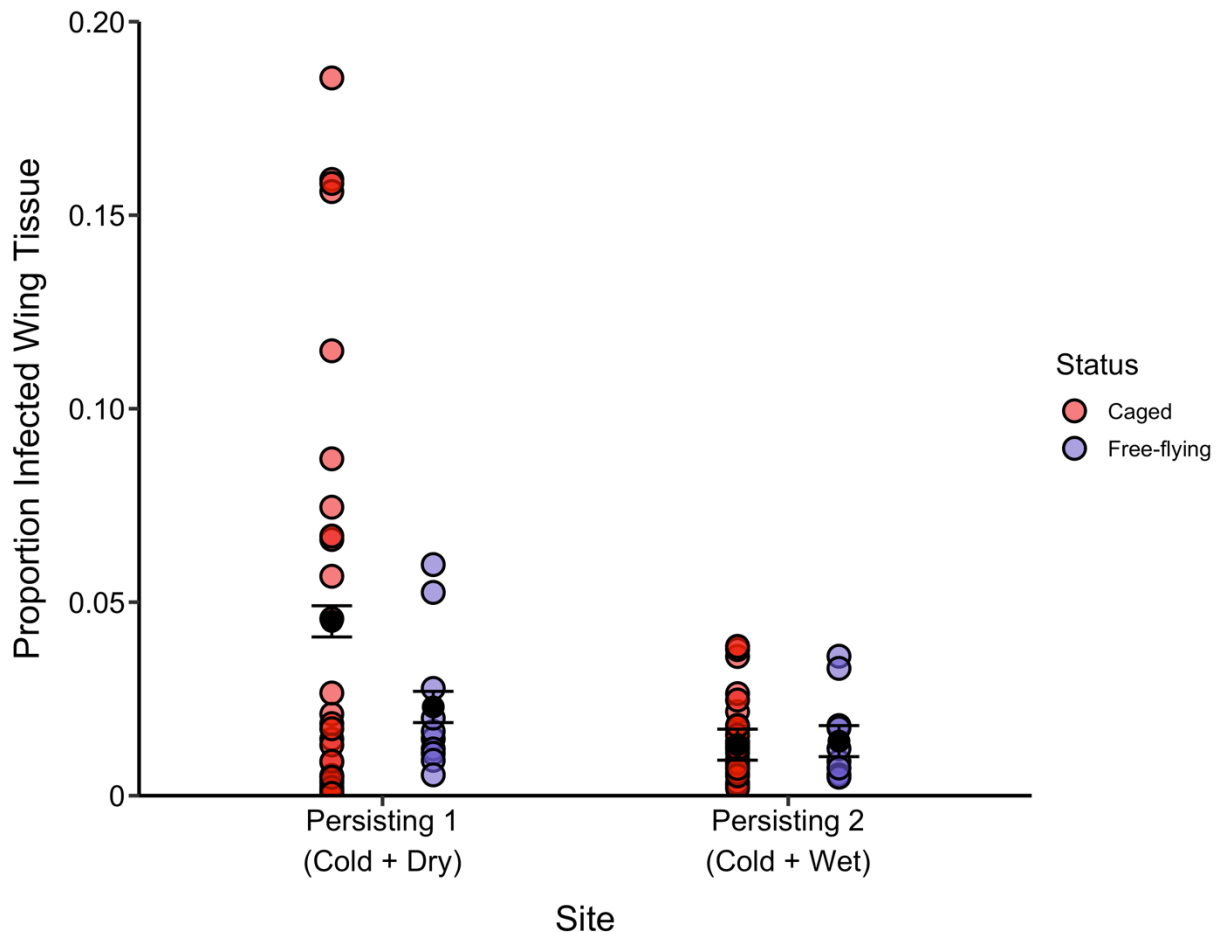


Figure 6: Proportion of infected wing tissue as indicated by orange fluorescence on caged and free-flying bats in each of the two persisting sites (Meteyer *et al.* 2009; Turner *et al.* 2014). Point colors represent caging stats: caged = red, free-flying = blue. Black points and error bars represent model estimates $\pm$ standard error. Free-flying bats were opportunistically captured at the end of hibernation at experiment termination. In Persisting 1 (Cold + Dry), where disease severity in caged bats was high, caged bats had significantly higher levels of tissue invasion compared to free-flying bats within the same site. However, this difference was not detected at Persisting 2 (Cold + Wet). This suggests that little brown bats in the cold and dry persisting site do not roost in dry microclimates for the entirety of hibernation, but rather move roosting locations periodically, as has been observed prior to the WNS epidemic. Moving amongst a variety of microclimates in this site may allow this persisting colony to mitigate disease severity.

Discussion

Our data suggest that environmental conditions interact with host traits to jointly drive persistence of host populations. Importantly, the survival we observed during this experiment was significantly higher than survival during the initial epidemic within the same sites in all cases, as well as during a similar experiment in 2009 within the extirpated site. We found evidence for increased on-host growth of *P. destructans* with increasing roosting temperature in bats that originated in Persisting 1 (Cold+Dry), but no relationship in bats that originated in Persisting 2 (Cold+Wet). Infection severity, host body condition, and survival also appeared to be influenced by site humidity, with higher disease severity and lower survival associated with over-winter exposure to the driest conditions in Persisting 1 (Cold+Dry). However, within the dry conditions, bats sampled from outside of cages in late hibernation displayed significantly lower infection severity compared to their caged counterparts, whereas no such difference was detected in Persisting 2 (Cold+Wet). This suggests that bats within Persisting 1 (Cold+Dry) may be utilizing a variety of microclimates and not remaining in these dry environments for the entire winter period, as the caged bats experienced. Furthermore, we observed declines in pathogen loads on 10 individuals, nine of which were in persisting sites. These data collectively suggest that persisting little brown bat colonies in the northeast United States may have evolved traits beneficial to surviving WNS (Auteri & Knowles 2020; Gignoux-Wolfsohn et al. 2021), but that these host traits interact with environmental conditions such that protection against severe disease and mortality depends to a strong degree on temperature and humidity.

We detected a positive relationship between average roosting temperature during hibernation and on-host pathogen growth rate. This is corroborated by data from the initial WNS epidemic, where the most severely impacted colonies were those hibernating in relatively warm

hibernacula (Langwig et al. 2012). Infection severity and over-winter weight loss showed a similar trend when humidity was high, with lower values occurring at Persisting 2 (Cold+Wet) compared to Extirpated (Warm+Wet). However, despite the coldest ambient conditions, infection severity and host weight loss were high under the dry conditions within Persisting 1 (Cold+Dry) and were comparable to the extirpated site. Under unfavorable ambient conditions, fungal pathogens may forgo reproduction and instead commit resources to within-host growth and the formation of spores that can survive stressful conditions. For example, *Metarhizium anisopliae* is a fungal pathogen of tick eggs that invades the egg tissue and undergoes growth (Valerio Garcia et al. 2005; Ment et al. 2010). Under humid, favorable conditions, the fungus will emerge from the egg to undergo asexual reproduction. However, under dry, unfavorable conditions, the fungus will instead remain within the egg host, continue to undergo growth, and produce environmentally resistant spores (Ment et al. 2010). We suggest that, similarly, exposure to dry conditions of Persisting 1 (Cold+Dry) over winter were unfavorable to the survival of *P. destructans* in superficial infections, and that the pathogen augmented tissue invasion to satisfy moisture requirements, resulting in a high degree of infection severity. Additionally, evaporative water loss from hibernating bats is highest in dry conditions (Thomas & Cloutier 1992; Ben-Hamo et al. 2013) and is exacerbated by infection with *P. destructans* (Mcguire et al. 2017), resulting in dehydration and increased arousal frequency to re-hydrate (Cryan et al. 2010, 2013; Willis et al. 2011; Ehlman et al. 2013; Warnecke et al. 2013; Verant et al. 2014; Mcguire et al. 2017). Increased frequency of arousal from torpor drives the premature depletion of fat reserves during hibernation, resulting in weight loss and starvation (Reeder et al. 2012; Warnecke et al. 2012). Therefore, the increased tissue invasion and evaporative water loss in Persisting 1

(Cold+Dry) may have operated synergistically to result in severe disease and ultimately the lowest observed survival.

Colonies of little brown bats in dry hibernacula may be persisting because of the availability of different microclimates. Microclimatic conditions are not uniform throughout an entire hibernation site, but vary with factors such as depth, air flow, and the height of the ceiling (Perry 2013). Some evidence suggests that bats may move to different roosting locations periodically during winter (Ryan et al. 2019), possibly in response to shifting costs associated with hibernation (Boyles et al. 2020), which could expose them to a variety of microclimates (Boyles et al. 2017). For example, some data suggest that bats may transition from roosting in relatively warm sections of hibernacula in early hibernation to the relatively cold sections by late winter (Ryan et al. 2019). In our study, bats were unable to select varying microclimates over the course of hibernation. However, we observed hundreds of little brown bats roosting in the area surrounding the cages during late winter in Persisting 1 (Cold+Dry), whereas less than a dozen individuals appeared to use that specific location in early hibernation, suggesting that bats do not roost in the same location for the entirety of hibernation in this site. Given that disease severity is highly dependent on environmental conditions within hibernacula, this movement behavior may have been pre-adaptive to surviving WNS if bats utilize microclimates that mitigate disease severity for at least part of hibernation. For example, movement within hibernacula may reduce the growth of *P. destructans* in late hibernation if bats move to the relatively cold conditions that slow pathogen growth, potentially affording them enough time to emerge from hibernation in spring and clear infection. Within Persisting 1 (Cold+Dry), free-flying bats sampled at the end of hibernation had significantly lower infection severity than caged bats, which is the expected pattern if movement within hibernacula is indeed beneficial to mitigating disease severity.

Furthermore, free-flying bats in both persisting sites had higher late hibernation body masses, and this was more pronounced in Persisting 1 (Cold+Dry). Behavioral responses that moderate the severity of disease have also been proposed for snake populations impacted by snake fungal disease (McKenzie et al. 2021), caused by the fungal pathogen *Ophidiomyces ophiodiicola* (Lorch et al. 2015). Snakes infected with *O. ophiodiicola* exhibit changes to their behavior that include increased surface activity and more time spent in exposed environments compared to their disease-free conspecifics (Lorch et al. 2015; McKenzie et al. 2021), potentially a sign of a behavioral fever response to infection (Burns et al. 1996). However, we make the important distinction here that because movement within hibernacula was observed in bats prior to the WNS epidemic, this behavior may have been pre-adaptive to surviving the disease rather than a direct response to the disease itself. Future research should investigate how the availability and utilization of varying environmental conditions can influence the dynamics of WNS, and how this may scale up to a population-level response.

During the initial epidemic, the cold conditions within hibernacula utilized by persisting colonies may have prevented total colony collapse, allowing standing genetic variation for favorable host traits to propagate (Langwig et al. 2012, 2016; Bell 2013; Grieneisen et al. 2015; Lilley et al. 2018). Previous research has also found genetic evidence from persisting little brown bat colonies indicative of a selective sweep following the invasion of *P. destructans* (Auteri & Knowles 2020; Gignoux-Wolfsohn et al. 2021). However, our data suggest that populations that appear to have evolved adaptive host traits are only afforded protection within a narrow environmental space. These processes have the potential to result in local adaptation, in which the evolutionary response of populations to WNS and the resulting dominant phenotype is specific to the local environmental conditions of hibernacula (Lilley et al. 2020). We detected an

effect of origin site on pathogen growth rate within Extirpated (Warm+Wet), potentially a signature of local adaptation to differing conditions in source hibernacula. However, for local adaptation to occur, the strength of selection must be high enough to combat the homogenizing effects of gene flow (Wright 1969; Felsenstein 1976; García-Ramos & Kirkpatrick 1997; Hendry et al. 2001), and current genetic evidence suggests a panmictic genetic landscape for bat populations (Burns et al. 2014; Johnson et al. 2015; Wilder et al. 2015; Talbot et al. 2016, 2017), but see (Miller-Butterworth et al. 2014; Davy et al. 2015).

Several host traits have been proposed as potential mechanisms of population persistence of little brown bats, including the evolution of host resistance or tolerance (Hoyt et al. 2016a; Langwig et al. 2016, 2017; Zukal et al. 2016), changes in fat deposition (Cheng et al. 2019), changes in hibernation physiology (Auteri & Knowles 2020; Gignoux-Wolfsohn et al. 2021), and reduced arousal frequency from hibernation (Lilley et al. 2016). While the objective of this study was not to identify the specific host trait potentially driving persistence, it is the first to connect host traits to individual-level survival rather than population-level trends, filling an essential gap in our understanding of WNS. However, potential mechanisms other than beneficial host traits are not necessarily absent and should be further explored. For example, while current genetic and phenotypic evidence suggests pathogen virulence attenuation is not a primary driver of host population persistence in this system (Ren et al. 2012; Warnecke et al. 2012; Palmer et al. 2014; Leopardi et al. 2015; Drees et al. 2017; Trivedi et al. 2017; Forsythe et al. 2018), it is possible that the signal of this mechanism has not yet been detected with the data on pathogen diversity currently available for North American isolates. Additionally, given that the data presented suggest an important role of environmental conditions in driving disease severity and potentially host persistence, shifting environmental conditions within hibernacula since the emergence of

WNS in North America could contribute to changing host population trajectories (Perry 2013; Mammola et al. 2019). However, there is no clear trend in changing underground conditions since pathogen invasion in our dataset (Supplemental Fig. 6), suggesting shifting thermal conditions are not likely to explain persistence in this system. However, because hibernacula temperatures are sensitive to aboveground conditions (Perry 2013), global climate change has the potential to alter the host-pathogen interaction in this system (Altizer et al. 2013; Mammola et al. 2019; Price et al. 2019). Finally, while current evidence does not suggest density-dependent transmission occurs in this system (Langwig et al. 2015a, b; Hoyt et al. 2016b, 2020), other density-dependent effects might be present if population density impacts infection severity rather than prevalence. For example, if conspecific behavior can exacerbate disease severity, then populations of lower density may be freed from detrimental conspecific effects. However, relatively small colonies of little brown bats exhibit population declines of the same magnitude of much larger colonies, a consistency which should not be observed if density-dependent effects drive population persistence (Langwig et al. 2012). Additionally, recent evidence suggests that hibernating bats continue to exhibit extensive social interactions following the arrival of WNS, suggesting a change in social behavior is not a principle contributor to population persistence (Hoyt et al. 2018). Finally, we found no support for a correlation between pathogen load and colony size, further suggesting density-dependent effects on disease severity are not a primary driver of persistence (Supplemental Fig. 7). Identifying the specific host traits contributing to the persistence of little brown bat colonies is essential to the successful management of the species and should be a primary focus of future research.

As *P. destructans* continues to spread throughout North America, bat population declines and regional extirpations will continue to occur. However, this study strongly suggests that prior

to the invasion of *P. destructans*, host traits conducive to surviving WNS circulated in little brown bat populations, which now offer some colonies imperfect protection from the disease. These host traits do not operate independently to promote population persistence with WNS, but rather interact strongly with environmental conditions, specifically temperature and humidity, to ultimately drive host-pathogen coexistence. Therefore, we should not expect to see all little brown bat populations across North America stabilize or rebound from declines, but rather the persistence of colonies with the correct combination of host traits and environmental conditions. Host population response to the invasion of a virulent pathogen will not be predictable by a single aspect of the host, environment, or pathogen. Rather, host-pathogen interactions and coexistence will be strongly mediated by environmental conditions, the result of which may be as variable as the environment itself (Parratt et al. 2016; Wilber et al. 2016). Underlying variation in host and pathogen populations will set the stage for subsequent coevolutionary processes and the likelihood of coexistence (Barrett & Schluter 2008; Bell 2013), but this interaction and the resulting host population response may be influenced by environmental conditions that vary over space and time (Wolinska & King 2009; Parratt et al. 2016), as illustrated by this study. Therefore, to achieve predictability in how emerging infectious diseases will impact host populations, it is essential to disentangle host-environment-pathogen interactions across a geographic and temporal mosaic of host-pathogen coevolution.

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Supplementary Information

Experimental methods: 2009 experiment and 2018 experiment

Bats were translocated to the previously extirpated site in both 2009 and 2018, in which they were caged and monitored over the course of hibernation. In the 2009 experiment, the entrance to the site was closed so that the bats could not exit the hibernaculum, effectively representing a large, site-wide cage. In the 2018 experiment, bats similarly could not leave the site, but were caged in groups of three in smaller cages mounted to the site's ceiling. In both experiments, bats had access to drinking water, either in the form of standing water within the site (2009 experiment) or chick waterers within cages (2018 experiment).

We compared the disease severity of little brown bats confined in the previously extirpated site during a similar translocation experiment in 2009 with that observed on the bats used in this study (Hicks *et al.* 2021). In the 2009 study, disease severity was measured on dead and moribund bats using a categorical score based on histology: 0 = no fungi suggestive of WNS, 1 = superficial and limited but suspicious of early WNS with hyphae in keratin and randomly into epidermis, but not yet forming distinctive cupping or dense packets, 2 = more extensive superficial infection with epidermal cupping packed with hyphae diagnostic of WNS, 3 = more severe fungal infection with tissue invasion including epidermal cupping packed with hyphae diagnostic of WNS, 4 = severe infection with tissue and wing damage worse than 3. In the present study, we used a similar categorical score of disease severity using the degree of tissue invasion indicated by fluorescence of tissue infected with *P. destructans*: 0 = no orange fluorescence indicative of infection, 1 = 1-10% of wing area displays orange fluorescence, 2 = 10-50% of wing area displays fluorescence, and 3 = 50-100% of wing area displays fluorescence. To estimate UV score from the historical dataset, we assigned histology scores of 0 and 1 a UV

score of 0, scores of 2 a UV score of 1, scores of 3 a UV score of 2, and scores of 4 a UV score of 3. Tissue does not display orange fluorescence until infection has been well established, and a histology score of 2 likely corresponds to when fluorescence will first be detected.

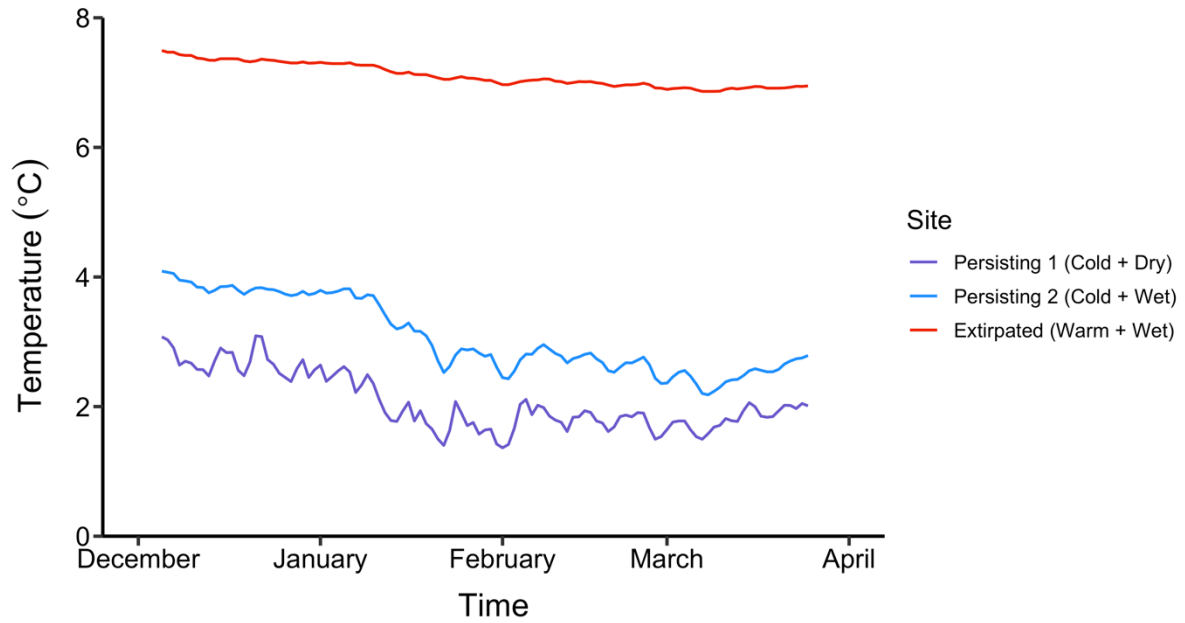
Real-time polymerase chain reaction (qPCR) methodology

Swab samples were preserved in RNAlater until extraction. We used real-time polymerase chain reaction (qPCR) targeting the multicopy intergenic spacer region of the rRNA gene complex to test swab samples for the presence of *P. destructans* DNA (Muller *et al.* 2013). We measured the quantity of *P. destructans* DNA in picograms from each swab sample based on the cycle threshold (C_t) value with a C_t cutoff of 40 cycles and a previously described standard quantification curve described in (Langwig *et al.* 2016).

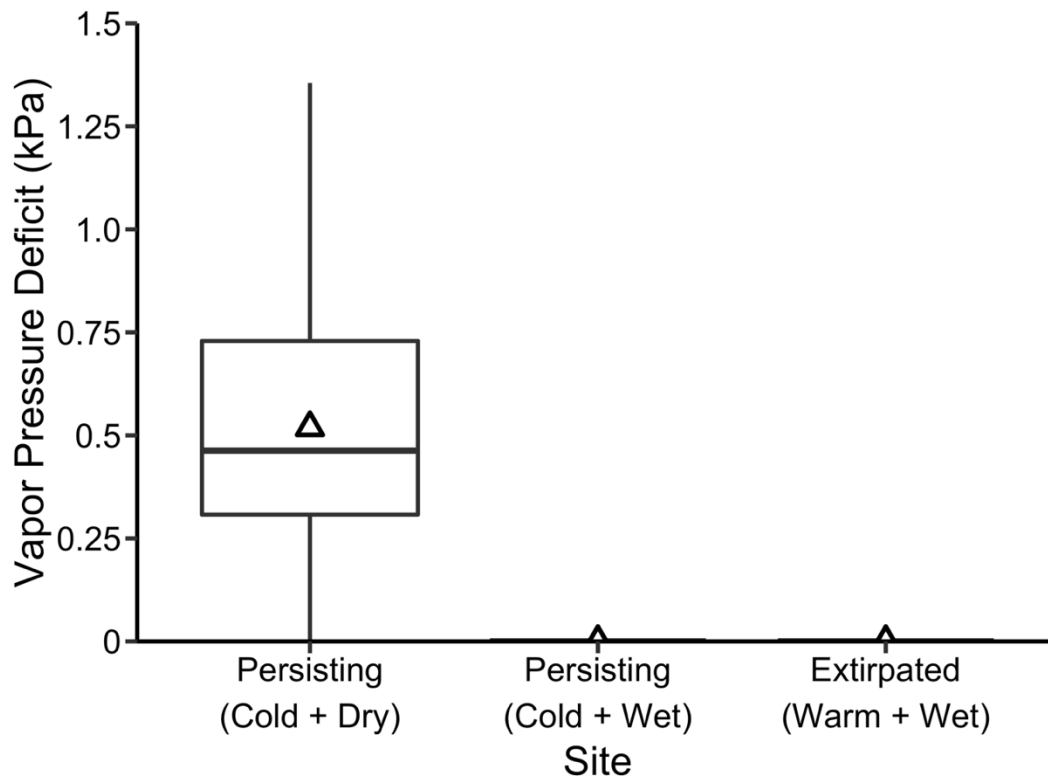
Ultraviolet transillumination

During ultraviolet transillumination, we photographed the wings of each bat using a Canon (Tokyo, Japan) EOS Rebel T6 (AF 15-55 mm lens, F-stop 22, ISO 1600, shutter speed 2.5 seconds, no filter). We used Adobe (San Jose, USA) Photoshop Elements Editor 15 to quantify the proportion of the plagiopatagium of each wing that displayed orange fluorescence indicative of infection with *P. destructans*. We first calculated the total number of pixels encompassed by the plagiopatagium, excluding bone area or area obstructed by fur. Then, we calculated the total number of orange pixels within the plagiopatagium. For each bat, pixel values from both wings were summed and proportions derived from these values. These image analyses were done by an individual blind to the origin of each bat and the hypotheses and predictions of the study.

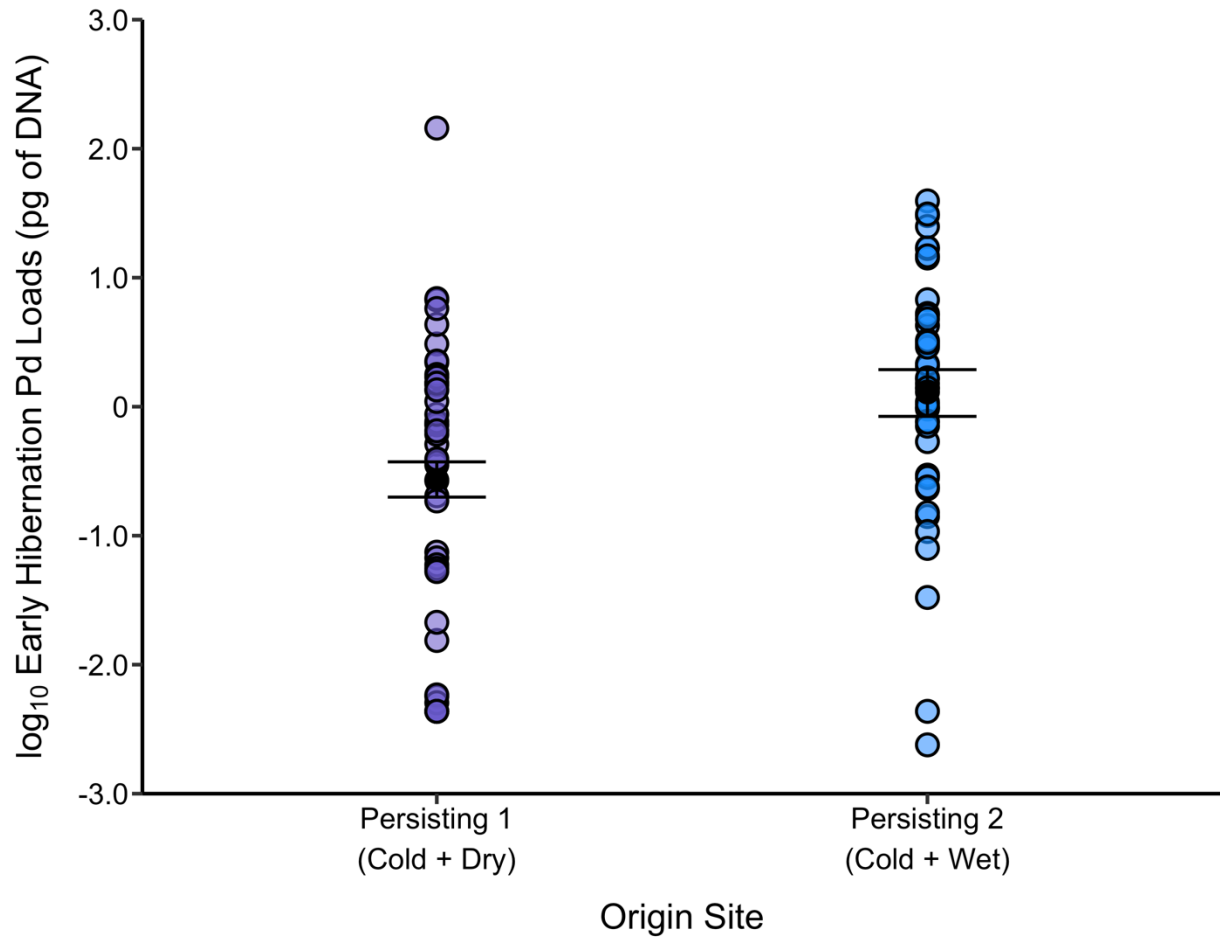
Supplemental figures



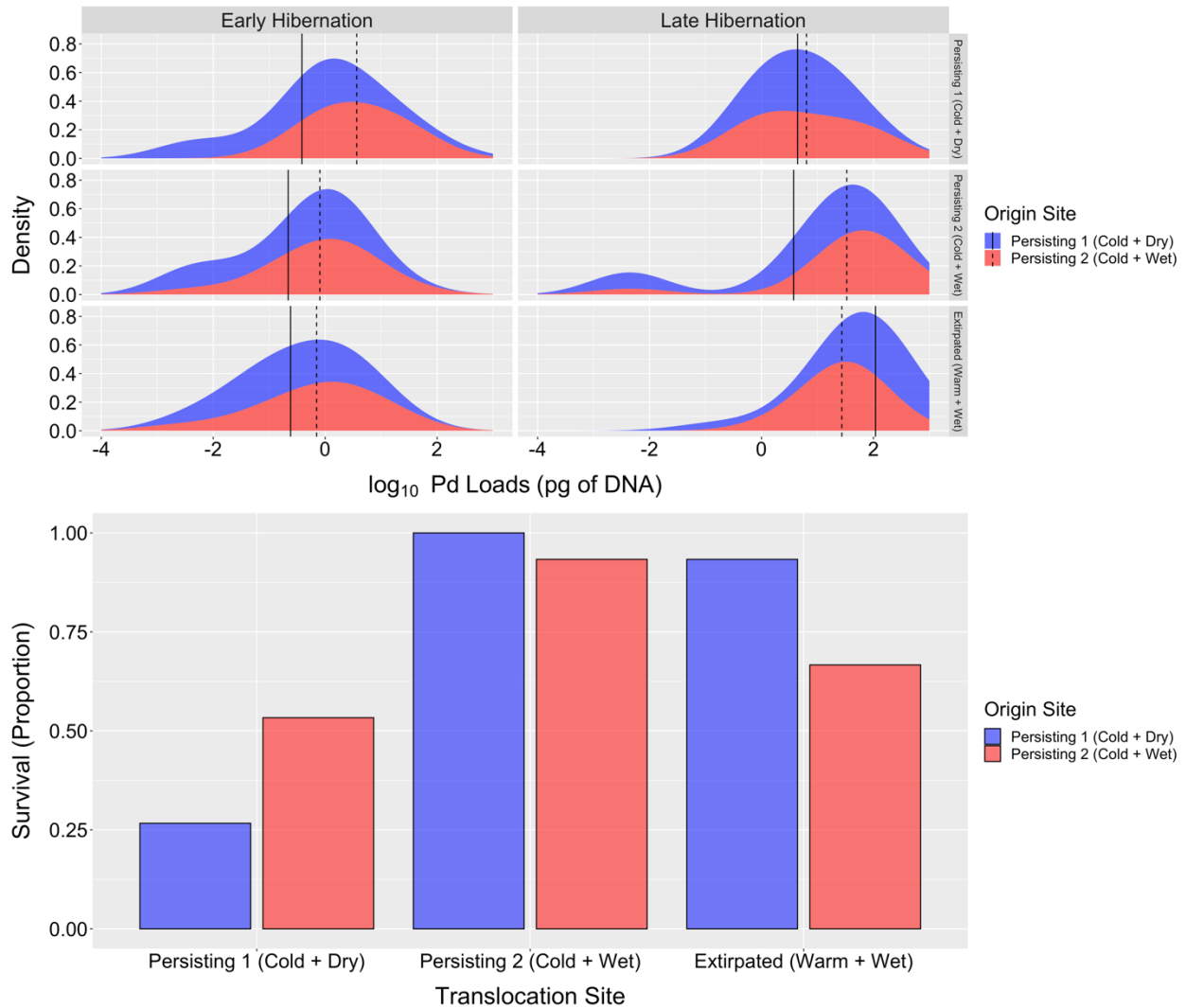
Supplemental Figure 1: Inter-site variation in temperature, plotted over time. The average temperature in Persisting 1 (Cold + Dry), Persisting 2 (Cold + Wet), and Extirpated (Warm + Wet) sites was $2.06^{\circ}\text{C} \pm 0.44 \text{ SD}$, $3.07^{\circ}\text{C} \pm 0.57 \text{ SD}$, and $7.11^{\circ}\text{C} \pm 0.18 \text{ SD}$, respectively.



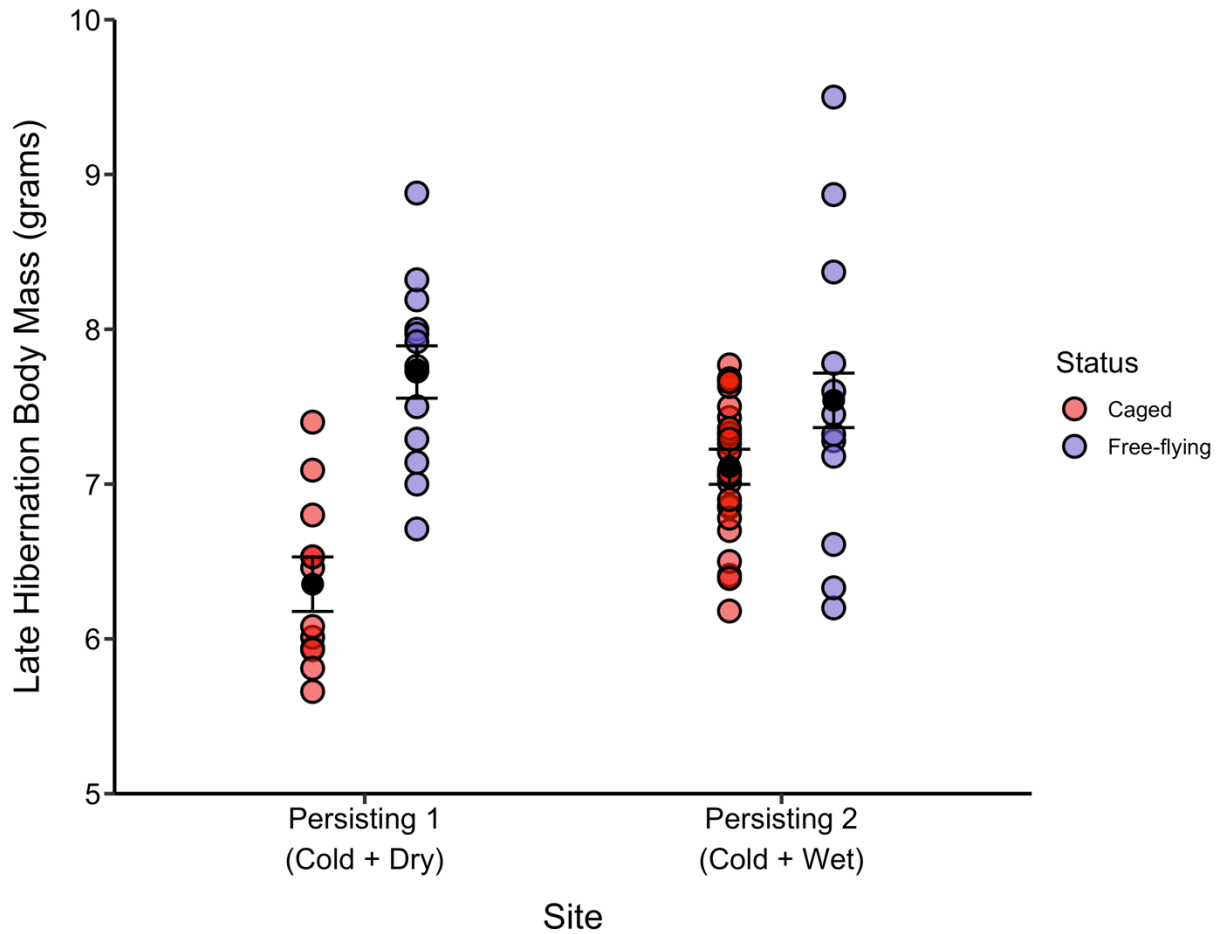
Supplemental Figure 2: Inter-site variation in vapor pressure deficit. White triangles indicate mean values. Vapor pressure deficit (VPD) is a measurement of the amount of moisture in the air and, given the temperature, its propensity to either condense or evaporate. Higher values of VPD correspond to environments in which moisture is more likely to evaporate, indicating a dry environment. The extremely low VPD values in the persisting cold and wet site and extirpated warm and wet site indicated that the air was nearly saturated and had a high propensity to condense on surfaces.



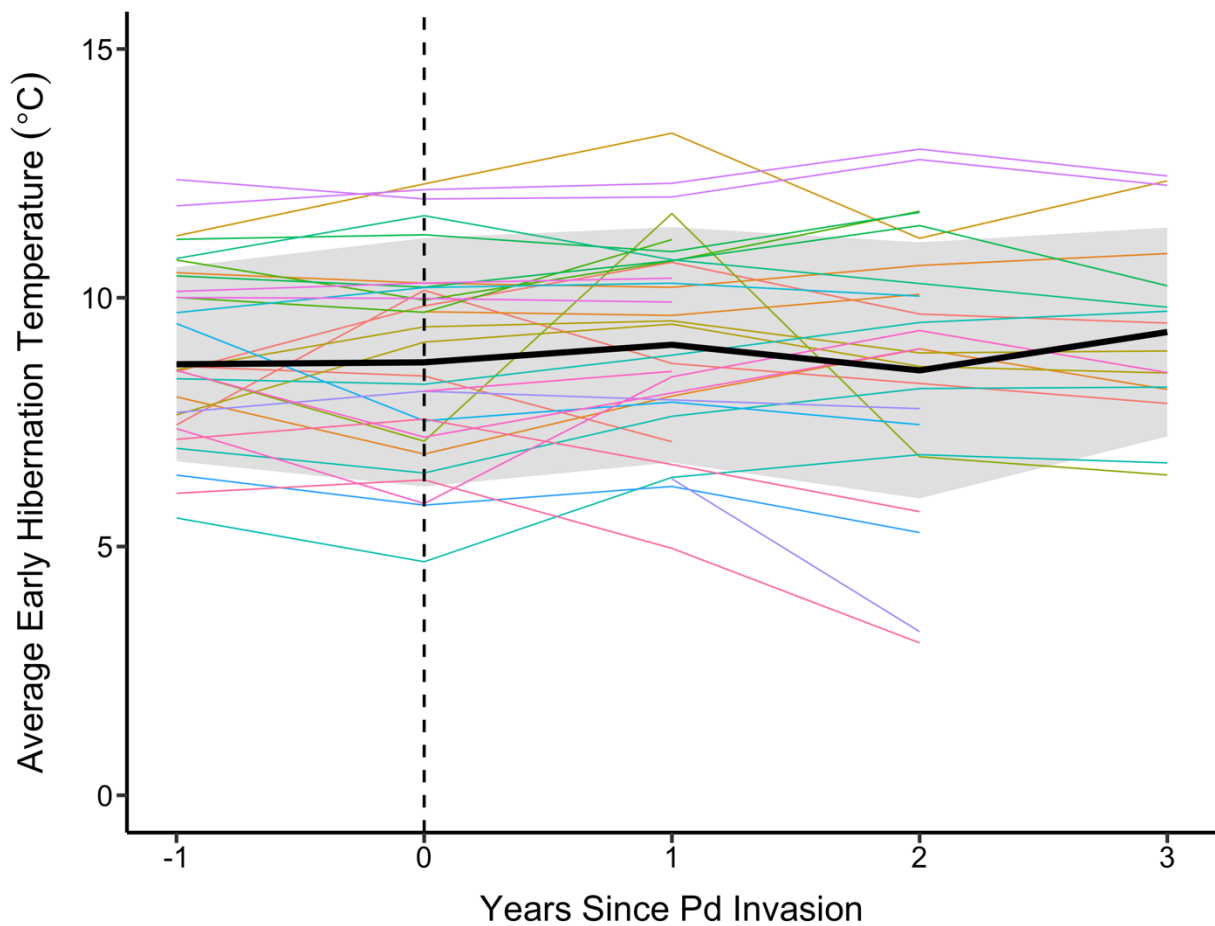
Supplemental Figure 3: Early hibernation pathogen loads (picograms of DNA). Black points and error bars are model estimates $\pm$ standard error. At the time of capture in December 2018, the pathogen load on bats that originated at the persisting cold and wet site were higher than those that originated in the persisting cold and dry site.



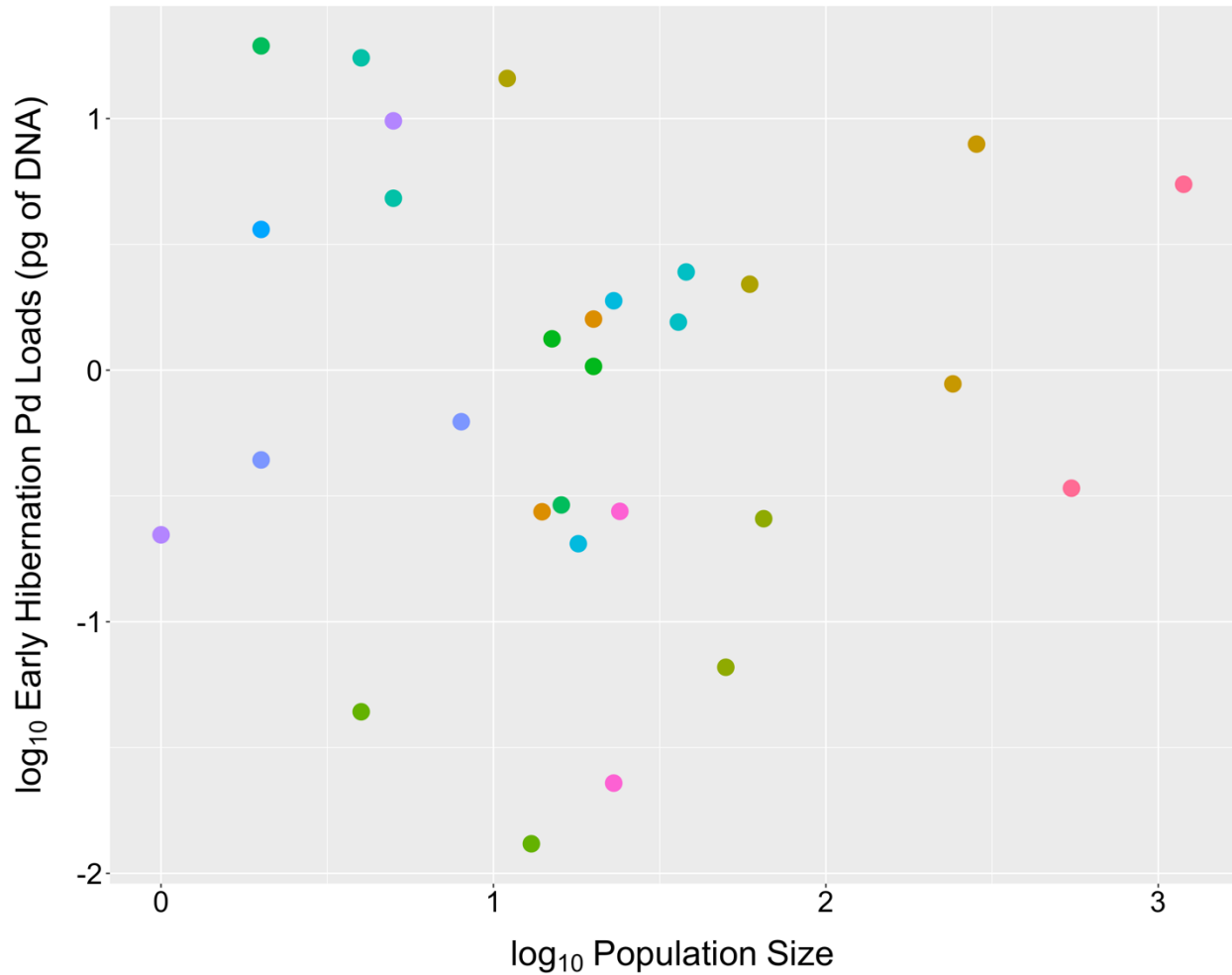
Supplemental Figure 4: The upper panel illustrates the changes in the frequency distribution of pathogen loads between early and late hibernation (columns) within each of the three sites (rows). The color of the density plots corresponds to bats from different origin sites: Persisting 1 (Cold + Dry) = blue, Persisting 2 (Cold + Wet) = red. Vertical lines represent the average pathogen load at each phase of hibernation for bats that originated in each persisting site: Persisting 1 (Cold + Dry) = solid line, Persisting 2 (Cold + Wet) = dashed line. We detected a significant effect of origin site on pathogen growth within Extirpated (Warm + Wet), in which bats that originated in Persisting 1 (Cold + Dry) had significantly higher pathogen growth than bats that originated in Persisting 2 (Cold + Wet). The lower panel illustrates differences in survival at each of the three translocation sites, with bar color representing different origin sites. While there were differences in survival across translocation sites in bats that originated in each persisting site, our model suggested no significant effect of origin site on survival.



Supplemental Figure 5: Late hibernation body mass in both caged and free-flying bats at both of the persisting colonies. Point colors represent caging stats: caged = red, free-flying = blue. Black points and error bars represent model estimates $\pm$ standard error. Free-flying bats were sampled from the area of the mine where the cages were installed at the end of the experiment. At the cold and dry persisting site, where disease severity was high, caged bats had significantly lower late hibernation body weights compared to free-flying bats.



Supplemental Figure 6: Average November temperatures across invasion years in 17 hibernacula with consecutive thermal data for at least four years overlapping the year of WNS detection within each site. There is not a clear signal of thermal conditions having shifted substantially since the invasion of *P. destructans* (linear mixed effects model with a site:section nested random effect: $\beta = 0.056 \pm 0.059$ SE, $p = 0.347$). Each line corresponds to an individual section within a hibernaculum, and sections within the same hibernaculum share a line color. The solid black line illustrates the average temperature across all sites and sections, and the gray polygon corresponds to $\pm$ one standard deviation.



Supplemental Figure 7: Plot of early hibernation pathogen loads against log₁₀ population sizes across 15 hibernacula following pathogen establishment. Point colors correspond to individual hibernacula. We find no clear relationship between pathogen load and colony size, suggesting disease severity is independent of population size (linear mixed effects model with a site random effect: $\beta = -0.051 \pm 0.247$ SE, $p = 0.839$).



Supplemental Figure 8: Cage installation in the hibernacula. Ten cages were installed in each site, and each contained three bats, all males and all having originated in the same persisting site. Each cage had a poultry waterer at the bottom of the cage to provide access to water as needed. Cages were predator-proofed by placing them high on ceilings and filling in gaps between cage and substrate with foam sealant.

Supplemental Tables

Supplemental Table 1: Model output of a multiple linear regression exploring the role of both origin site and translocation site on the change in fungal loads over the course of the experiment. Incorporating Cage ID as a random effect in the model resulted in a singularity issue, which is suggestive of an overly complex random effects structure. We re-ran this model in a Bayesian framework with the inclusion of the cage random effect and the model interpretation was the same. Therefore, for consistency, we have elected to report the frequentist model in the manuscript but have added reference to the identical Bayesian analysis in the caption text and included the data and associated code for both the frequentist and Bayesian model in a public GitHub repository: ELE-00803-2021.

Response variable: change in fungal loads

Multiple linear regression: (change in fungal loads) ~ (site)(origin site)*

Model reference level: persisting (cold + dry) translocation site, persisting (cold + dry) origin site

N = 65 (bats that were found dead at the end of the experiment were not used in this analysis)

<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	1.180	0.250	<0.001
Site(Persisting Cold + Wet)	0.214	0.281	0.449
Site(Extirpated Warm + Wet)	1.045	0.283	<0.001
Origin Site(Persisting Cold + Wet)	0.028	0.306	0.928
Site(Persisting Cold + Wet):Origin Site(Persisting Cold + Wet)	0.378	0.358	0.296
Site(Extirpated Warm + Wet):Origin Site(Persisting Cold + Wet)	-0.726	0.370	0.054

Supplemental Table 2: Model output of a linear mixed effects model exploring the role of, origin site, average roosting temperature, and average humidity conditions within a site on the change in fungal loads over the course of the experiment.

Response variable: change in fungal loads			
<i>Linear mixed effects model: (change in fungal loads) ~ (average roosting temperature)*(origin site)+(average vapor pressure deficit)+(1 Cage ID)</i>			
<i>Model reference level: persisting (cold + dry) origin site</i>			
<i>N = 65 (bats that were found dead at the end of the experiment were not used in this analysis)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	1.007	0.212	<0.001
Average roosting temperature	0.175	0.042	<0.001
Origin site(Persisting Cold + Wet)	0.855	0.278	0.003
Average vapor pressure deficit	-8.388	3.507	0.020
Average roosting temperature:Origin site(Persisting Cold + Wet)	-0.224	0.060	<0.001

Supplemental Table 3: Model output of a generalized linear mixed model (binomial error distribution, logit link) used to explore how infection severity differed between sites.

Response variable: infection severity			
<i>Generalized linear mixed model:</i>			
<i>(infection severity) ~ (site)+(origin site)+(1 Cage ID), family = binomial (link=logit)</i>			
<i>Model reference level: persisting (cold + dry) translocation site, persisting (cold + dry) origin site</i>			
<i>N = 81 (both alive and dead bats were used for this analysis. 8 dead bats were too decomposed to successfully investigate infection severity, and the photographs from 1 alive bat were of too low quality to use in these analyses)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	-3.530	0.286	<0.001
Site(Persisting Cold + Wet)	-0.828	0.351	0.018
Site(Extirpated Warm + Wet)	0.127	0.352	0.719
Origin Site(Persisting Cold + Wet)	0.143	0.286	0.617

Supplemental Table 4: Model output of a generalized linear mixed model (gamma error distribution, log link function) used to explore how host body condition differed between sites and origin site.

Response variable: weight loss
Generalized linear mixed model:
 (weight loss) ~ (site)+(origin site)+(1|Cage ID), family = Gamma (link = log)

Model reference level: persisting (cold + dry) translocation site, persisting (cold + dry) origin site

N = 65 (only data from bats alive at the end of the experiment was used in this analysis)

<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	0.632	0.135	<0.001
Site(Persisting Cold + Wet)	-0.635	0.142	<0.001
Site(Extirpated Warm + Wet)	-0.413	0.145	0.004
Origin site(Persisting Cold + Wet)	0.241	0.108	0.025

Supplemental Table 5: Model output of a generalized linear mixed model (binomial error distribution, logit link function) used to explore how host survival differed between sites and origin site.

Response variable: survival			
<i>Generalized linear mixed model:</i>			
<i>(survival) ~ (site)+(origin site)+(1 Cage ID), family=binomial (link=logit)</i>			
<i>Model reference level: persisting (cold + dry) translocation site, persisting (cold + dry) origin site</i>			
<i>N = 90 (all experimental bats were used in this analysis)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	-0.339	0.641	0.597
Site(Persisting Cold + Wet)	4.303	1.359	0.002
Site(Extirpated Warm + Wet)	2.192	0.909	0.016
Origin site(Persisting Cold + Wet)	-0.316	0.756	0.676

Supplemental Table 6: Model output of a generalized linear mixed model (binomial error distribution, logit link function) used to explore how the relationship between early hibernation body mass and host survival.

Response variable: survival			
<i>Generalized linear mixed model:</i>			
<i>(survival) ~ (early hibernation body mass)+(1 Cage ID), family=binomial (link=logit)</i>			
<i>N = 90 (all experimental bats were used in this analysis)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	1.864	4.314	0.666
Early hibernation body mass	-0.026	0.511	0.959

Supplemental Table 7: Model output of a generalized linear model (binomial error distribution, logit link) used to explore how infection severity differed between caged and free-flying bats in each of the two persisting sites.

Response variable: infection severity			
<i>Generalized linear model:</i>			
<i>(infection severity) ~ (site)*(caged), family=binomial (link=logit)</i>			
<i>Model reference level: persisting (cold + dry) translocation site, free-flying bats</i>			
<i>N = 78 (both alive and dead bats in cages were used for this analysis, excluding data from the extirpated site. Data from 58 caged bats from the two persisting sites as well as 20 free-flying bats were used in this analysis. Data from 10 free-flying bats was collected at each of the two persisting sites)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	-3.753	0.002	<0.001
Site(Persisting Cold + Wet)	-0.495	0.002	<0.001
Caged(Yes)	0.699	0.002	<0.001
Site(Persisting Cold + Wet):Caged(Yes)	-0.767	0.002	<0.001

Supplemental Table 8: Model output of a multiple linear regression used to explore how late hibernation body mass differed between caged and free-flying bats in each of the two persisting sites.

Response variable: late hibernation body mass			
<i>Multiple linear regression:</i>			
<i>(late hibernation body mass) ~ (site)*(caged)</i>			
<i>Model reference level: persisting (cold + dry) translocation site, free-flying bats</i>			
<i>N = 66 (Data from 41 alive, caged bats from the two persisting sites as well as 25 free-flying bats were used in this analysis. No data from the extirpated site was used. Late hibernation body mass data from 12 and 13 free-flying bats was collected at the cold/wet and cold/dry persisting sites, respectively)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	7.724	0.169	<0.001
Site(Persisting Cold + Wet)	-0.183	0.244	0.456
Caged(Yes)	-1.371	0.244	<0.001
Site(Persisting Cold + Wet):Caged(Yes)	0.941	0.322	0.005

Chapter 3 – Drivers of population dynamics of at-risk populations change with pathogen arrival

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Abstract

Successful wildlife conservation in an era of rapid global change requires understanding determinants of species population abundance and growth. However, when populations are faced with novel stressors, factors associated with healthy and growing populations can change, necessitating a shift in conservation strategies. For example, emerging infectious diseases can cause previously high-quality habitat to be associated with high pathogen transmission and population declines following pathogen arrival. Here, we paired a population dataset of 265 colonies of the federally endangered Indiana bat (*Myotis sodalis*) with 50.7 logger-years of environmental data to explore factors that affected colony response to white-nose syndrome (WNS), an emerging fungal disease. We found wide variation in colony responses to WNS, ranging from extirpation to stabilization and persistence. Simulating future population dynamics suggests that most extirpations have already occurred, as the pathogen has been present for several years in most colonies, and that small colonies were more susceptible to extirpation than large ones. Further, while temperature and humidity conditions of hibernacula appeared

unassociated with Indiana bat colony growth prior to WNS, extirpation risk following pathogen arrival was elevated in colonies that used colder and wetter hibernacula. Additionally, rates of decline were greater in colder hibernacula, opposite the association for a sympatric bat species. Overall, this study illustrates that emerging infectious diseases can change the factors associated with host population abundance and optimal growth, including through novel environmental associations, which can vary across host species. Consideration of these shifting associations and intrinsic differences between impacted host species will be essential to successful species conservation.

Introduction

Conserving global biodiversity in the Anthropocene requires a deep understanding of the factors that contribute to the resilience and growth of wildlife populations. The suite of biotic and abiotic conditions that can contribute to population growth, from ambient conditions like temperature (Woodworth et al., 2017), humidity (Lu and Wu, 2011), or precipitation (Masó et al., 2020), to community-level factors like predator density (Salo et al., 2010), competitor density (Gamelon et al., 2019), or pathogen prevalence (Hoyt et al., 2020), can further serve as targets for conservation and management. However, novel stressors such as climate change (Acevedo et al., 2020; Şekercioğlu et al., 2012; Van Dyck et al., 2015), invasive species introduction (Hagman et al., 2009), and habitat fragmentation and loss (Haddad et al., 2015; Mantyka-Pringle et al., 2015) can alter associations between biotic and abiotic conditions and population growth or stability. The form and magnitude of these altered associations is often ambiguous or difficult to characterize and can create the potential for a mismatch between conservation interventions and intended outcomes, possibly with negative effects on the targeted population (Sutherland et al., 2004). Therefore, understanding how novel stressors change the way wildlife populations

respond to biotic and abiotic conditions will be essential to their successful conservation in a rapidly changing world.

Emerging infectious diseases are one of many stressors that threaten wildlife populations and can result in host species declines or population extirpations (Altizer et al., 2013; Anderson et al., 2004; Daszak et al., 2001, 2000; Fisher et al., 2012; Rogalski et al., 2017), as evidenced by West Nile Virus and avian malaria in birds (Kilpatrick, 2011; Van Riper III et al., 1986), chytridiomycosis in amphibians (Skerratt et al., 2007), snake fungal disease (Lorch et al., 2016), sarcoptic mange in wombats (Martin et al., 2018), facial tumor disease in Tasmanian devils (Mccallum, 2008), and white-nose syndrome in bats (Hoyt et al., 2021). Early conservation measures to bolster the demographic resilience and adaptability of host populations might dampen the impact of disease following its arrival (Grant et al., 2017; Hodgson et al., 2015). However, it is often unclear whether those same conservation measures will promote the persistence of the host population during pathogen invasion when pathogen transmission dynamics (Huang et al., 2021; Leach et al., 2016) or environmental dependence of disease (Langwig et al., 2012; McNew et al., 2019; Samuel et al., 2015) can significantly shift the fitness landscape of the host. This information is particularly important for rare and endangered host species with limited habitat availability or population sizes before pathogen invasion, as their sensitivity to novel stressors and management interventions necessitates unequivocal conservation best practices.

The size of a host population can contribute to its response to pathogen invasion and ultimately its risk of extirpation. Greater standing genetic variation in large host populations may promote evolutionary adaptation to an invading pathogen before total population collapse occurs (Barrett and Schluter, 2008; Bell, 2013; Carlson et al., 2014; Gomulkiewicz and Holt, 1995;

Maslo and Fefferman, 2015). Large populations also have less risk of stochastic fadeout, which can be exacerbated in populations declining from disease (de Castro and Bolker, 2005; Frick et al., 2015; Langwig et al., 2012). However, if large populations also have high host densities, pathogen transmission may be elevated, leading to more rapid declines and greater extinction risk (Brunner et al., 2017; Cross et al., 2010; Cunningham et al., 2021; Rachowicz and Briggs, 2007; Smith et al., 2009; Storm et al., 2013). While there are clear theoretical mechanisms of how host population size can contribute to its response to an emerging infectious disease, this association is often left uncharacterized due to the difficulty of obtaining sufficient data, despite being essential to prioritizing populations for conservation action.

Environmental dependence of pathogen transmission or disease severity can create both hotspots of (Lambin et al., 2010; Ostfeld et al., 2005; Paull et al., 2012) and refugia from (Heard et al., 2015; Mosher et al., 2018; Puschendorf et al., 2011; Scheele et al., 2017a; Spitzen-Van Der Sluijs et al., 2017) infection and disease. Temperature, for example, can have complex effects on disease by influencing pathogen growth rates (Hopkins et al., 2021), pathogen transmission (Mordecai et al., 2019), host susceptibility to infection (Cohen et al., 2017), or disease severity following infection (Debes et al., 2017), processes that scale up to variation in population-level impacts on the host. These environmental effects can further exacerbate impacts to host populations if high-quality habitat prior to pathogen invasion becomes population sinks or ecological traps of high pathogen transmission and disease severity following disease emergence (Hopkins et al., 2021; Leach et al., 2016). Understanding environmental contributions to host population response offers a point of leverage for conservation efforts, as protection and enhancement of critical environmental conditions is fundamental to the conservation of threatened host species. Importantly, environmental associations need not take the same form

both pre- and post-pathogen arrival, and effective approaches to host species conservation must incorporate these dynamic processes.

White-nose syndrome (WNS) is an emerging infectious disease of hibernating bats caused by the fungal pathogen *Pseudogymnoascus destructans* (Lorch et al., 2011; Minnis and Lindner, 2013; Warnecke et al., 2012). The pathogen was introduced to North America in the early 2000s from its origin in Eurasia and has since spread widely through naïve hibernating bat populations (Blehert et al., 2009; Drees et al., 2017; Leopardi et al., 2015), causing severe population declines (Frick et al., 2015; Hoyt et al., 2021; Langwig et al., 2016, 2012).

Pseudogymnoascus destructans establishes an environmental pathogen reservoir within winter hibernacula (Hoyt et al., 2020, 2015; Laggan et al., 2023), the caves and mines where bats hibernate, and can persist for years in the absence of its host (Hoyt et al., 2015). This results in seasonal infection dynamics in which WNS prevalence increases as bats arrive in hibernacula in late fall but declines during spring and summer months when bats do not primarily use hibernacula for roosting and bats are often euthermic (K. E. Langwig et al., 2015). Infection severity increases over the winter hibernation period when body temperatures drop to approximately ambient conditions (K. E. Langwig et al., 2015; Langwig et al., 2017), allowing the cold-adapted fungus to invade the epidermal tissue (Lorch et al., 2011; Warnecke et al., 2013). Infection with *P. destructans* is accompanied by a suite of physiological changes that lead to dehydration (Cryan et al., 2013; McGuire et al., 2017; Verant et al., 2014), frequent arousals from torpor (Lilley et al., 2016; Reeder et al., 2012; Warnecke et al., 2012), emaciation, and ultimately mortality (Cryan et al., 2010; Warnecke et al., 2013).

The Indiana bat (*Myotis sodalis*) is a North American species that has experienced severe but variable population declines due to WNS (Langwig et al., 2012; Thogmartin et al., 2012).

Despite its status as endangered under the U.S. Endangered Species Act, little is known about drivers of variation in Indiana bat declines and persistence with disease, information important for its conservation. Environmental correlates of disease severity and population response have been previously reported for other species, particularly the little brown bat (*Myotis lucifugus*), where warmer hibernacula are associated with severe disease and declines (Grieneisen et al., 2015; Grimaudo et al., 2022; Hopkins et al., 2021; Langwig et al., 2016, 2012; Lilley et al., 2018), likely due to enhanced pathogen growth under those conditions (Marroquin et al., 2017; Verant et al., 2012). However, previous work has not found a clear association between temperature conditions of hibernacula and the response of Indiana bat colonies (Langwig et al., 2012). Evidence additionally suggests that for many species impacted by WNS, colony size may be related to the initial population response to the disease (Frick et al., 2015; Langwig et al., 2012). However, the particularly gregarious nature of Indiana bats (Clawson et al., 1980; Hardin and Hassell, 1970; Thomson, 1982) may result in constant population density across colony sizes and time if their propensity to form clusters remains unchanged, potentially keeping pathogen transmission and declines high regardless of colony size.

In this study, we pair an extensive bat population dataset curated by the U.S. Fish and Wildlife Service with a hibernacula environmental dataset to identify potential contributions of host population size and environmental conditions to Indiana bat population trends prior, during, and after pathogen arrival. Our data provide information on how population-level processes and variation in environmental conditions can generate heterogeneous host population responses to novel infectious diseases, lending insights into how to successfully manage their impacts.

Materials and Methods

Data collection

Population data

We analyzed data on population sizes of 265 colonies of hibernating Indiana bats from 15 U.S. states collected between 2003—2022 (Supplemental Table 1). Population data were collected by various federal and state agencies and were compiled by the U.S. Fish and Wildlife Service. For each colony, the year of WNS detection within their hibernaculum was recorded and used to assign ‘epidemic year,’ which is the year since WNS detection (e.g., epidemic year 0 corresponds to the year of WNS detection). We classified census data by epidemic phase, with the ‘Pre-Invasion’ phase being epidemic years less than or equal to 0, ‘Epidemic’ being epidemic years 1 through 5, and ‘Established’ being epidemic years 6 or greater. This classification reflects observed patterns in colony responses to WNS, in which major declines begin in the year following WNS detection (epidemic year 1) and continue for several years until population stability is achieved by epidemic year 6, on average (Hoyt et al., 2021). Overall, census data was available for 214 hibernacula during the ‘Pre-invasion’ phase, 251 hibernacula during the ‘Epidemic’ phase, and 106 hibernacula during the ‘Established’ phase.

We estimated the annual population growth rates, λ_i , for each Indiana bat colony for each winter with an available colony census. λ_i was derived by dividing the census value taken in a winter to the next most recent winter census value, separated by T years:

$$\lambda_i = \sqrt[T]{\left(\frac{N_i}{N_{i-T}}\right)} \quad (1)$$

Mean population growth rates, λ_m , for each colony in each epidemic phase were calculated by averaging all available annual lambda values for a colony within each epidemic phase. We omitted from the dataset any λ_i values that were calculated with N_{i-T} values less than 10, as calculations of λ_i are disproportionately sensitive at small colony sizes, producing misleadingly small or large λ_i values (Supplemental Figure 10). Values of T varied with a median value of 2 (Supplemental Figure 11).

We assigned a classification to each colony based on their overall response to WNS, referred to as ‘colony status.’ ‘Extirpated’ colonies were those with a most recent post-WNS arrival (epidemic year greater than or equal to 0) census value of 0, indicating total collapse of the colony. ‘Declining’ colonies were those that never exhibited a year of stable or positive annual colony growth (λ_i greater than or equal to 0) after epidemic year 1. Finally, ‘persisting’ colonies were those that had at least one year of stable or positive annual colony growth after epidemic year 1. These classifications were possible for 231 of the 265 colonies in the population dataset. The remaining 34 colonies did not have sufficient post-WNS detection census data to accurately assign a classification, as they did not have census data available following epidemic year 0.

Environmental data

Data on the temperature and humidity conditions of Indiana bat roosting locations within hibernacula were collected overwinter in a total of 48 hibernacula between 1994 and 2022, 53.9% of which was collected since 2015 and 10.8% of which was collected as a part of this study. In total, the environmental dataset we used in this study constitutes 50.7 logger-years of data on the environmental conditions within hibernacula (summarized in Supplemental Table 1).

Environmental data were recorded by a variety of data loggers, including Onset HOBO H8 Pro, Onset HOBO Pro V2, Onset HOBO Pendant, Onset HOBO XT, Madgetech TransiTempII-RH, Maxim Integrate iButton, and modified onset HOBO MX2303 data loggers (Supplemental Table 2). HOBO MX2303 units were modified into wet bulb psychrometers to estimate both temperature and humidity conditions within hibernacula (Supplemental Figure 9). Humidity was measured as vapor pressure deficit (VPD), which measures the difference between the amount of moisture in the air and how much moisture the air could hold at the same temperature when saturated. Higher VPD values correspond to drier conditions.

For each hibernaculum, we calculated the mean early hibernation (November and December) temperature and VPD across all available years of data. We quantified the environmental conditions present early in hibernation because they are most associated with initial infection severity and ultimately survival probability (Hopkins et al., 2021; Langwig et al., 2016). Mean VPD values were skewed towards zero with a long right tail, so we $\log_{10}$ -transformed the mean values before further analysis. To include untransformed VPD values of zero, a constant of 1×10^{-5} (same order of magnitude as the next-lowest non-zero VPD value) was added to all VPD values before transformation. Mean early hibernation temperature values were available for all 48 hibernacula and mean early hibernation VPD values were available for 34.

Statistical analyses

Effect of population size on declines

To descriptively illustrate how colony classification captured differences in λ_i across epidemic phases, colony status, and their interaction, we constructed a generalized linear mixed model with gamma error distribution, log link function, and colony ID as a random effect (Model

1; all models are described in Supplemental Table 10). We explored associations between λ_i (response variable) and $\log_{10}$ colony size (N_{i-T} in equation 1) interacting with epidemic phase (predictors) using a generalized linear mixed model with gamma error distribution and a log link with colony ID as a random effect (Model 2). Colony size was confounded with colony status and was therefore used in a different model exploring λ_i . We additionally assessed whether colony status was related to the size of the colony prior to the arrival of WNS by using a negative binomial model with log link function, the pre-WNS detection census value for each colony as the response variable, and colony status as the explanatory variable (Model 3).

Effects of environmental conditions on declines

To explore associations between environmental conditions within hibernacula and the response of Indiana bat colonies to WNS, we used a series of linear and generalized linear models. First, to test for the effect of mean early hibernation temperature, epidemic phase, and their interaction on the average annual colony growth value of a colony in an epidemic phase, λ_m , we used a generalized linear model with a gamma error distribution and log link function (Model 4). The same model structure was used for a separate model to determine whether mean early hibernation VPD, epidemic phase, and their interaction influenced colony growth, λ_m (Model 5). Because environmental conditions may have influenced colony size prior to WNS arrival, we also examined the relationship between environmental conditions of hibernacula and pre-WNS detection colony size by using two negative binomial generalized linear models with log link functions and mean early hibernation temperature (Model 6) or VPD (Model 7) as the predictor and the latest pre-WNS colony size for each colony as the response variable. All linear models satisfied assumptions of Gaussian error distributions and homoscedastic error. All analyses were

performed in R packages *stats* (R Core Team 2022), *glmmTMB* (Magnusson et al., 2017), and *MASS* (Ripley et al., 2013) in R v4.2.1.

Effect of little brown bat abundance on Indiana bat declines

Little brown bats (*Myotis lucifugus*) are a relatively abundant species in WNS-impacted communities and may contribute to community-wide disease dynamics (Laggan et al., 2023). However, because colony declines in little brown bats are also associated with the temperature and humidity of hibernacula, it is possible that any apparent associations between environmental conditions and Indiana bat declines could arise instead from the contribution of little brown bat abundance to Indiana bat survival and colony response. To identify a potential effect of little brown bat abundance on Indiana bat colony declines, we constructed a generalized linear mixed model with gamma error distribution and log link function with λ_i of Indiana bat colonies as the response variable, the $\log_{10}$ -transformed colony size of little brown bats (N_{i-T} of little brown bats) interacting with epidemic phase as the explanatory variables, and colony ID as a random effect (Model 8). Thirty Indiana bat hibernacula had sufficient little brown bat census data to be included in Model 8.

Colony extirpation simulation

We performed a simulation to predict the proportion of the 231 colonies with known population status that would be extirpated by year 2030. We first classified each colony as “small” (≤ 66 individuals) or “large” (>66 individuals) based on its most recent colony census value prior to the detection of WNS within its hibernaculum, using the median colony size of 66 as the cut-off. If a pre-WNS census value was not available due to survey gaps, then we used the

earliest census value post-WNS detection instead to classify the size of the colony. We constructed cumulative extirpation curves for each of these two datasets using colonies that were WNS-positive for at least seven years because the probability of becoming extirpated appeared to plateau following epidemic year 6. We fit logistic models to the two cumulative extirpation curves (Supplemental Figure 1, Supplemental Table 3) using the *stats* package in R (R Core Team 2022) and estimated the probability a colony was extirpated in epidemic year t if extant in epidemic year $t-1$ as the difference between the epidemic year model estimates for both small and large colonies. We then simulated the annual probability of extirpation by 2030 (binomial variable, 1 = extirpated 0 = extant) using the most recent known status of each colony as well as the epidemic year of its most recent census. To simulate colony extirpation, each small or large colony that was extant in year $t-1$ was assigned a new extirpation status in year t by drawing from a binomial probability distribution with the probability of extirpation corresponding to that calculated for each epidemic year using the cumulative extirpation curves for small and large colonies. The simulation was run for 1,000 iterations and summarized.

Results

General population trends

Population trends of Indiana bat colonies varied over time since pathogen arrival to their hibernacula (Figure 1, Supplemental Figure 2). Prior to the detection of WNS, colonies of Indiana bats were growing at an average rate of 0.9% annually. Following WNS detection within hibernacula, colonies declined significantly, with the largest declines in epidemic year two. Of the 231 colonies in our dataset, 56 (24.24%) were extirpated following the detection of WNS within their hibernacula and 53.57% of these extirpations occurred between epidemic years zero

and two (Supplemental Figure 3). Additionally, 72 of the 231 colonies (31.16%) continued to decline following WNS arrival and never achieved an annual lambda, λ_i , greater than or equal to 1.0 (stability) following WNS detection. The remaining 103 colonies (44.58%) were stable or growing for at least one year following the second year of the epidemic, and 47.57% of these had their first stable year by epidemic year three (Supplemental Figure 3). Population trends during the epidemic phase varied between extirpated, declining, and persisting colonies, with lower annual declines during the epidemic phase in persisting colonies compared to declining ($\beta = 0.218 \pm 0.043$ SE, $p < 0.001$) or extirpated ($\beta = 0.405 \pm 0.082$ SE, $p < 0.001$; Figure 1, Supplemental Table 4). Additionally, we found that extirpated colonies were, on average, significantly smaller than persisting ($\beta = -3.1678 \pm 0.356$ SE, $p < 0.001$) or declining ($\beta = -2.517 \pm 0.389$ SE, $p < 0.001$) colonies (Supplemental Figure 4, Supplemental Table 5). Pre-WNS arrival colony size in declining colonies was significantly lower than those in persisting colonies, but this effect was small ($\beta = -0.651 \pm 0.326$ SE, $p = 0.046$). We did not detect any statistically significant association between either counts of Indiana bats or little brown bats at the same site on annual Indiana bat colony growth, λ_i , regardless of epidemic phase (Supplemental Figure 5, 6, Supplemental Table 6, 7).

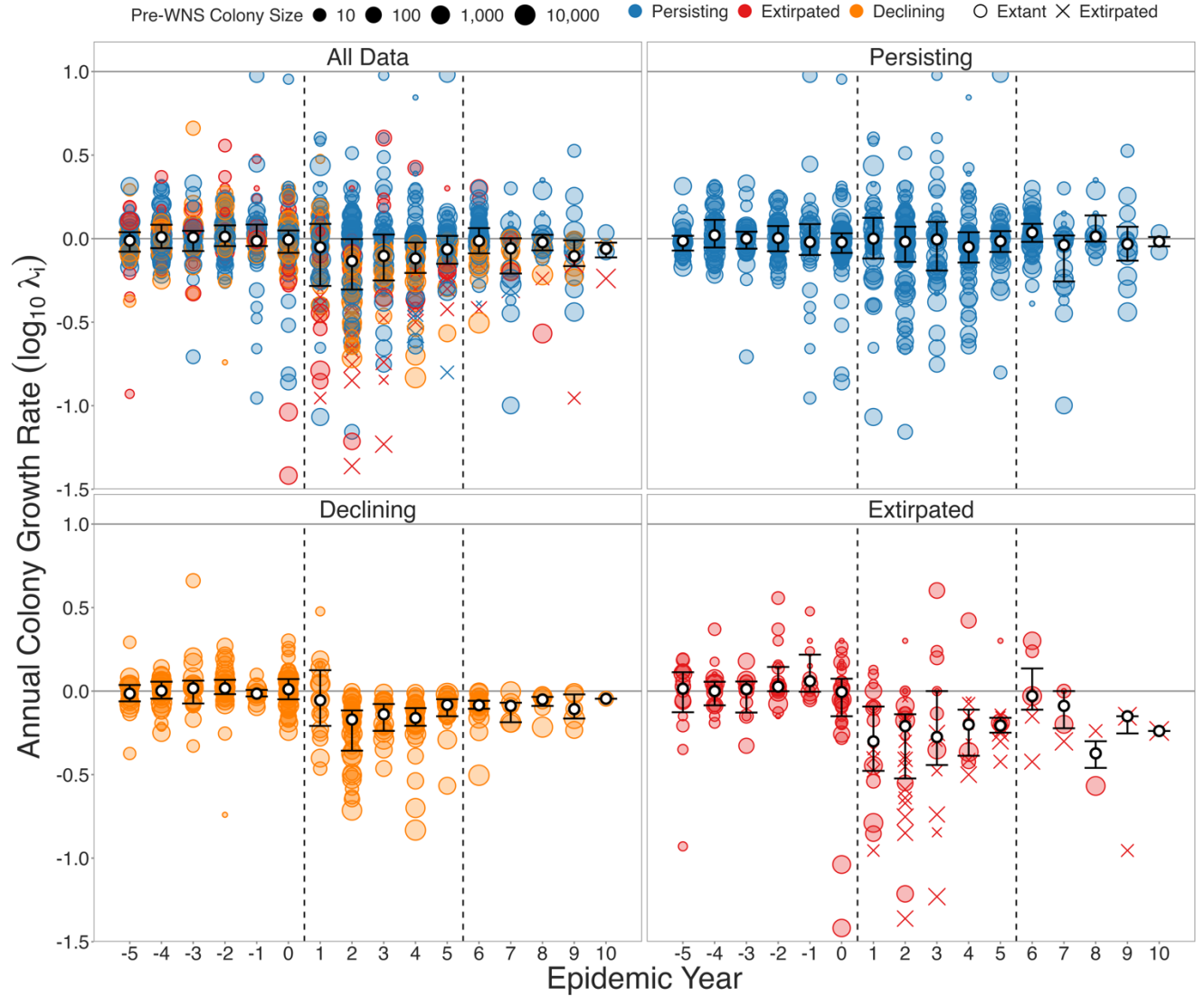


Figure 1: Population trends of Indiana bat colonies over time since detection of WNS.

Epidemic year 0 on the x-axis corresponds to the year in which WNS was detected within a hibernaculum. Vertical dashed lines separate epidemic phases (left to right: pre-invasion, epidemic, established). Annual population growth rates of 0 on the y-axis correspond to population stability, and values below or above 0 indicate declining or growing colonies, respectively. Points are colored by colony status, size corresponds to pre-WNS arrival colony size, and shape corresponds to the extinction status of the colony at each epidemic year (circle = extant, x = extirpated). White points and error bars show mean values and one standard error.

Colony extirpation simulation

Our simulation of future Indiana bat colony extirpations suggests that the rate of colony extirpation may slow in coming years (Figure 2), though the predicted asymptotes come with associated error and could be substantially different than what is ultimately observed. Most extirpations occur early in the epidemic and the probability of colony extirpation declines in later epidemic years (Supplemental Figure 1, 3). Therefore, our simulation predicts a decay in the rate of new extirpations, with 26.41% of total colonies (simulated range: 24.24 – 30.30%), or 61 of the 231 colonies in the dataset (simulated range: 56 – 70), extirpated by 2023. Because most extirpations occur early in the epidemic and most of the Indiana bat range has been affected by WNS for at least 5 years, this constitutes only an additional five extirpations from those recorded as of 2019. However, the number of predicted extirpations varied between small and large colonies. On average, 43.10% (range: 39.66 – 49.1%) of small colonies will be extirpated by 2030, corresponding to approximately 50 of the 116 small colonies in the dataset (range: 46 – 57), an increase of four extirpations from 2019. Conversely, only 9.57% (range: 8.70 – 12.17%) of large colonies will be extirpated, or approximately 11 of the 115 large colonies in the dataset (range: 10 – 14), an addition of a single extirpation from 2019.

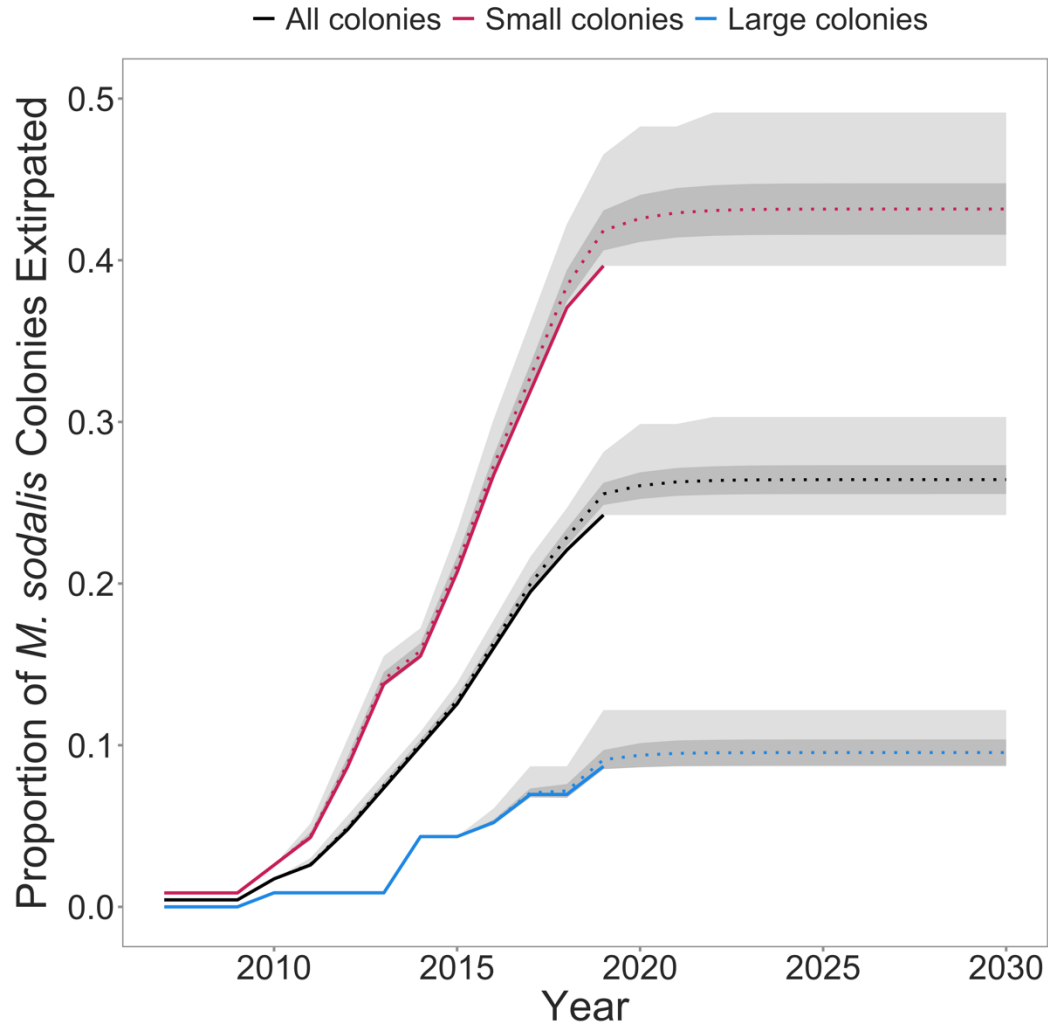


Figure 2: Simulated and empirical accumulation of extirpated Indiana bat colonies over time as a proportion of small, large, or all colonies. Solid lines show observed data and dotted lines show average simulated values for each year. The color of lines corresponds to dataset (black = all colonies, red = small colonies (≤ 66 individuals), blue = large colonies (> 66 individuals)). The dark gray shaded region corresponds to one standard deviation from the mean simulated value at each year. The light gray shaded region corresponds to the range of simulated values at each year.

Environmental associations

Associations between early winter temperature conditions within hibernacula and population growth rates varied with epidemic phase (Figure 3A, Supplemental Table 8). We did not detect a statistically clear association between average early hibernation temperature and

annual population growth of colonies prior to WNS arrival within hibernacula (temperature coefficient during Pre-invasion phase $\beta = -0.012 \pm 0.021$ SE, $p = 0.555$). However, following the detection of WNS (Epidemic phase), we found that colonies roosting at colder early hibernation temperatures experienced more severe declines (temperature coefficient during Epidemic phase: $\beta = 0.066 \pm 0.023$ SE, $p = 0.005$), a significantly different association than that observed in the Pre-invasion phase (interaction coefficient: $\beta = -0.078 \pm 0.031$ SE, $p = 0.013$). Correspondingly, the estimated mean early hibernation temperature of extirpated hibernacula was 5.338°C (± 0.708 SE, $p = <0.001$), colder than both persisting and declining colonies with estimates of 7.971°C (± 0.322 SE, $p = <0.001$) and 8.022°C (± 0.481 SE, $p = <0.001$), respectively (Figure 4A). Early winter temperature conditions of hibernacula used by persisting colonies did not significantly differ from those used by declining colonies ($\beta = -0.051 \pm 0.578$ SE, $p = 0.930$). Following the establishment of *P. destructans* within hibernacula, the positive association between average early hibernation temperature and population growth rates weakened towards the neutral association observed prior to the WNS epidemic (temperature coefficient during Established phase: $\beta = 0.037 \pm 0.025$ SE, $p = 0.138$), such that the association was not statistically different than pre-invasion ($\beta = -0.050 \pm 0.033$ SE, $p = 0.130$) or epidemic phases ($\beta = 0.028 \pm 0.034$ SE, $p = 0.403$). We found no statistically significant association between early hibernation temperature and pre-WNS Indiana bat colony sizes ($\beta = -0.082 \pm 0.054$ SE, $p = 0.126$; Supplemental Figure 7).

For vapor pressure deficit (VPD), we found no statistically clear relationships with colony growth, regardless of epidemic phase (Supplemental Figure 8, Supplemental Table 9). However, we found that VPD varied with site status, as extirpated colonies occurred in significantly more humid hibernacula than declining ($\beta = -2.227 \pm 0.764$ SE, $p = 0.007$) or

persisting colonies ($\beta = -2.169 \pm 0.685$ SE, $p = 0.003$; Figure 4B). We detected a slight negative association between early hibernation VPD and pre-WNS Indiana bat colony sizes indicating that, prior to the arrival of WNS, drier hibernacula were used by relatively smaller colonies on average compared to humid hibernacula ($\beta = -0.210 \pm 0.093$ SE, $p = 0.025$; Supplemental Figure 7).

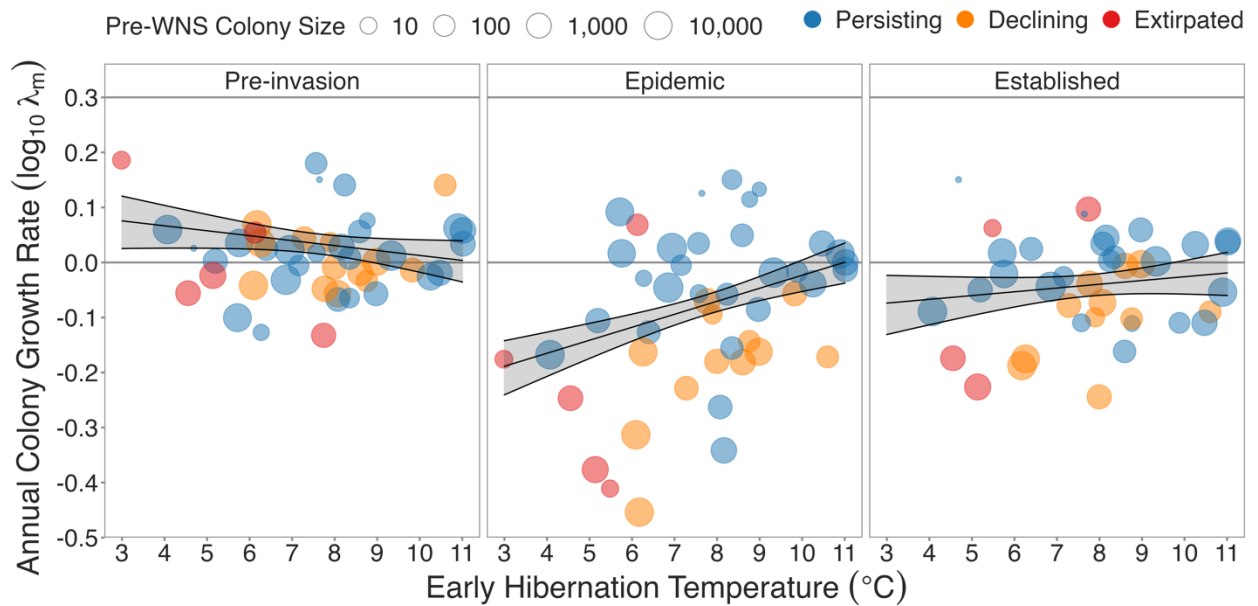


Figure 3: Annual colony growth rate over mean early hibernation temperature. Data are broken into separate panels by epidemic phase. A colony growth rate value of 0 corresponds to population stability, with values below or above 0 corresponding to colony decline or growth, respectively. Points are colored by colony status and point size corresponds to the most recent pre-WNS colony size. Solid black lines and shaded regions correspond to model estimates $\pm$ one standard error of the model predictions.

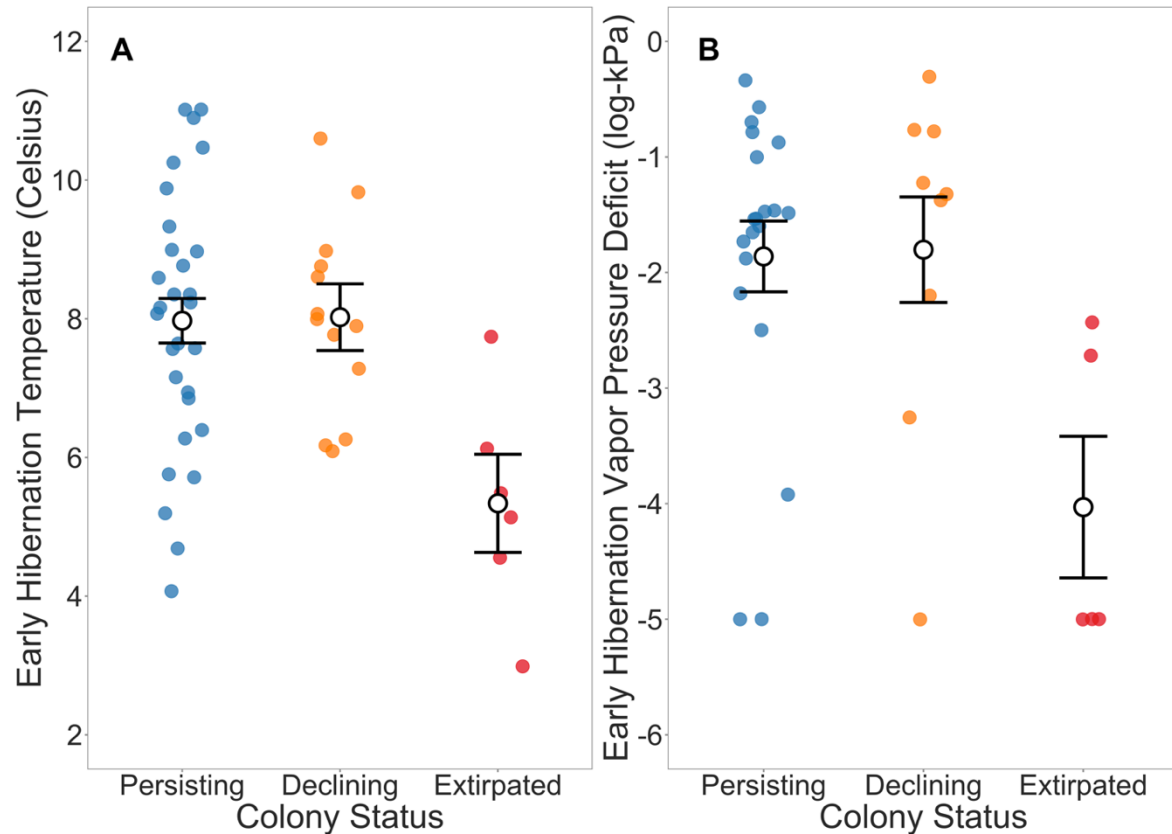


Figure 4: Effect of temperature and VPD on colony status. A) Mean early hibernation temperature and B) $\log_{10}$ vapor pressure deficit across persisting, declining, and extirpated colonies. Higher values of vapor pressure deficit correspond to drier conditions. Points are jittered and colored by colony status. White points and error bars correspond to model estimates $\pm$ one standard error of predicted mean.

Discussion

We found that the factors associated with Indiana bat population dynamics shifted over epidemic time. Prior to the arrival of WNS, we did not detect an effect of colony size, hibernaculum temperature, or humidity on colony growth. Following the invasion of *P. destructans*, however, responses of Indiana bat colonies varied from complete extirpation to persistence, with smaller colonies being more prone to extirpation. In addition, colonies in colder hibernacula experienced more severe declines than those in relatively warm hibernacula and were more likely to be extirpated, a novel association that arose during the WNS epidemic.

Colonies in relatively humid hibernacula were also at elevated risk of extirpation, potentially another association that arose following disease emergence. Once the pathogen became established in colonies, however, we found that the effects of environmental conditions weakened slightly, suggesting a possible return to the more neutral association observed prior to WNS arrival. Together, these data illustrate the potential of novel population stressors to shift the biotic and abiotic conditions optimal for population growth, an important consideration for the mitigation of their impacts.

Although population responses of Indiana bats were highly variable, many colonies in our dataset eventually stabilized in the presence of WNS. Colony persistence has been observed in other species of hibernating bats impacted by WNS in North America, such as the little brown bat (Dobony et al., 2011; Frick et al., 2015; Hoyt et al., 2021; Langwig et al., 2017, 2012), potentially the result of an interaction between evolutionary forces and favorable environmental conditions that allow for relatively high survival (Grimaudo et al., 2022; Hopkins et al., 2021; Langwig et al., 2017). Persisting Indiana bat colonies had less severe declines during the initial WNS epidemic than declining or extirpated colonies, potentially due to their larger colony sizes and warmer hibernaculum environments which buffered against unsustainable rates of mortality. However, stabilized colonies are not necessarily completely protected from extirpation, as they may be declining on average despite having a single year of colony stability or growth.

In accordance with prevailing theory, we found that extirpation risk was elevated in small colonies, potentially due to susceptibility to stochastic fadeout (Lande, 1993; Lande et al., 2003; Melbourne and Hastings, 2008) or demographic Allee effects (Gascoigne et al., 2009; Kramer et al., 2009; Stephens et al., 1999). Indiana bats are a highly gregarious species, historically having formed large clusters during hibernation (Clawson et al., 1980; Hardin and Hassell, 1970;

Thomson, 1982), a behavior potentially beneficial for limiting energy expenditure during arousals from torpor (Boyles et al., 2008). Sufficiently low colony sizes may prevent conspecifics from forming clusters, increasing per-arousal energy expenditure already exacerbated by infection with *P. destructans*, causing downstream effects on survival and ultimately leading to colony decline and extirpation. Further, the inability to form sufficiently large clusters may elevate emigration from reduced colonies, additionally contributing to their decline. If immigration to colonies occurs through individuals being led to hibernacula by conspecifics, then reduced colony sizes will further receive little immigration input, potentially slowing population growth in small colonies. Together, the consequences of reduced colony size may operate synergistically to elevate the risk of complete colony extirpation. More generally, our results suggest that pre-stressor population size, often a factor used to classify population risk, was positively associated with population resilience following pathogen arrival despite the potential for greater host density to amplify pathogen transmission.

Although small colonies were more likely to go extinct, we find that most colonies that will become extirpated have likely already done so due to the spread of WNS across the Indiana bat range. As the *P. destructans* invasion front advanced from its origin near Albany, New York, pathogen arrival was detected in Indiana bat colonies in every year between 2007 and 2017 in our dataset. However, all extant colonies are currently in later stages of the epidemic, when mortality is reduced and extirpation is less likely, resulting in a steep decline in the extirpation rate as the most recently infected colonies are either lost or stabilize. In the near future, declining colonies that have not yet been extirpated will either entirely collapse or settle into a stable population trajectory, ultimately determining the overall impact of WNS on Indiana bat colonies across their range.

We found that environmental conditions of hibernacula influenced Indiana bat colony response to WNS, an association that varied over epidemic time. In the years of high mortality and colony decline immediately following *P. destructans* arrival (epidemic phase), colonies using warmer hibernacula experienced lower declines than those in colder hibernacula. The role of temperature appeared to have arisen due to the emergence of WNS, as we did not detect the association prior to detection of the disease within hibernacula, illustrating the ability of novel infectious diseases to alter associations between environmental conditions and population growth. The effect of temperature extended to extirpation risk, as extirpated colonies used significantly colder hibernacula than extant colonies. Furthermore, while we did not detect an association between VPD within hibernacula and colony growth rates, we did find that extirpated colonies typically used more humid hibernacula than extant colonies, suggesting that humidity may additionally impact colony growth dynamics following WNS emergence. Importantly, we detected no association between pre-WNS colony size and temperature conditions within hibernacula, and a weak negative one for VPD, indicating that environmental conditions are unconfounded with colony size and operate independently to drive colony responses to WNS. Finally, while we did not investigate the role of temporal or spatial variation in temperature or humidity conditions within hibernacula on population response to WNS, it represents an additional potential driver of disease processes in this system. Future research is warranted to explore how variation in environmental conditions might influence disease processes from the individual- to population-level impacts on bat communities with WNS.

The positive association between hibernaculum temperature and colony response to WNS is opposite of that reported for the little brown bat, in which warmer hibernacula have higher disease severity and declines (Grieneisen et al., 2015; Hopkins et al., 2021; Langwig et al., 2016,

2012; Lilley et al., 2018), likely due to increased pathogen growth under those conditions (Grieneisen et al., 2015; Hopkins et al., 2021; Johnson et al., 2014; Langwig et al., 2016; Verant et al., 2012). We did not detect an association between little brown bat abundance within hibernacula and Indiana bat colony growth, suggesting that Indiana bat population declines at colder temperatures are not due to larger little brown bat colonies in cold hibernacula increasing environmental pathogen contamination (Laggan et al., 2023) or disturbance from torpor (Turner et al., 2014). Instead, this discrepancy might arise if the energy expended during arousals from torpor, which are more frequent in bats with WNS (Lilley et al., 2016; Reeder et al., 2012; Warnecke et al., 2012), is more important for surviving the disease for Indiana bats than little brown bats. The gregarious nature of Indiana bats and their propensity to form large clusters, which may further reduce cost of arousals from torpor (Boyles et al., 2008), lend possible credence to this explanation. Energy expended during arousals from torpor accounts for the majority of that spent during hibernation and is reduced under warmer conditions (Boyles and Willis, 2010; Thomas et al., 1990). The reduced cost of arousals in warm hibernacula potentially allowed Indiana bats to conserve enough energy to survive WNS over winter, buffering their overall declines. In cold hibernacula, however, the cost of additional arousals due to WNS may have been too great and resulted in premature energy depletion and mortality. For little brown bats, the disruption of water balance may instead be the physiological pathway that contributes most to mortality (Ben-Hamo et al., 2013; Cryan et al., 2010; Grimaudo et al., 2022; Thomas and Cloutier, 1992; Webb et al., 1995), which hypothetically scales positively with pathogen load. Given that the growth of *P. destructans* increases with hibernaculum temperature (Marroquin et al., 2017; Verant et al., 2012), little brown bat colonies in warm hibernacula could experience greater and unsustainable rates of water loss, resulting in their positive association between

hibernaculum temperature and colony decline whereas the opposite exists for Indiana bats.

Future research is warranted in identifying the physiological or ecological underpinnings of the opposite responses of Indiana and little brown bat colonies to hibernaculum temperatures when challenged by WNS.

The difference between Indiana and little brown bats in their colony response to WNS illustrates that in novel infectious disease systems with multiple host species, there may be species-specific differences in how environmental conditions influence population response to pathogen invasion. To mitigate the impact of invading pathogens on host populations, management strategies that consider inherent differences among host species are essential. For example, habitat manipulations meant to cool or warm hibernacula and benefit a target bat species could inadvertently harm another, so careful consideration of the bat communities present is essential (Boyles et al., 2023). Alternatively, the acquisition and conservation of diverse hibernacula critical to each species, such as those that offer protective microclimates or where colony populations are stabilizing, should be considered a high priority. Ultimately, the appropriate management strategy will be dependent on the community, environment, and disease conditions present, and management flexibility should reflect this potential for variation.

This study highlights how existing associations between biotic and abiotic conditions and population growth can be altered by novel stressors, including emerging infectious diseases. To protect vulnerable host populations from disease-driven extirpation, conservation measures should reflect these changing associations and consider differences among host species in shared habitat. Furthermore, it is crucial to conserve diverse habitats available to host species, as novel associations may arise and offer refugia from severe disease impacts. However, determining how to balance the conservation of habitat that is beneficial to one species while potentially

detrimental to another (i.e. conservation mismatch), such as the case with thermal conditions of Indiana and little brown bat hibernacula, deserves additional research. As novel host-pathogen interactions continue to arise, the successful mitigation of their impacts will be dependent on our ability to characterize and respond to how they interact with the unique ecological context in which they arise.

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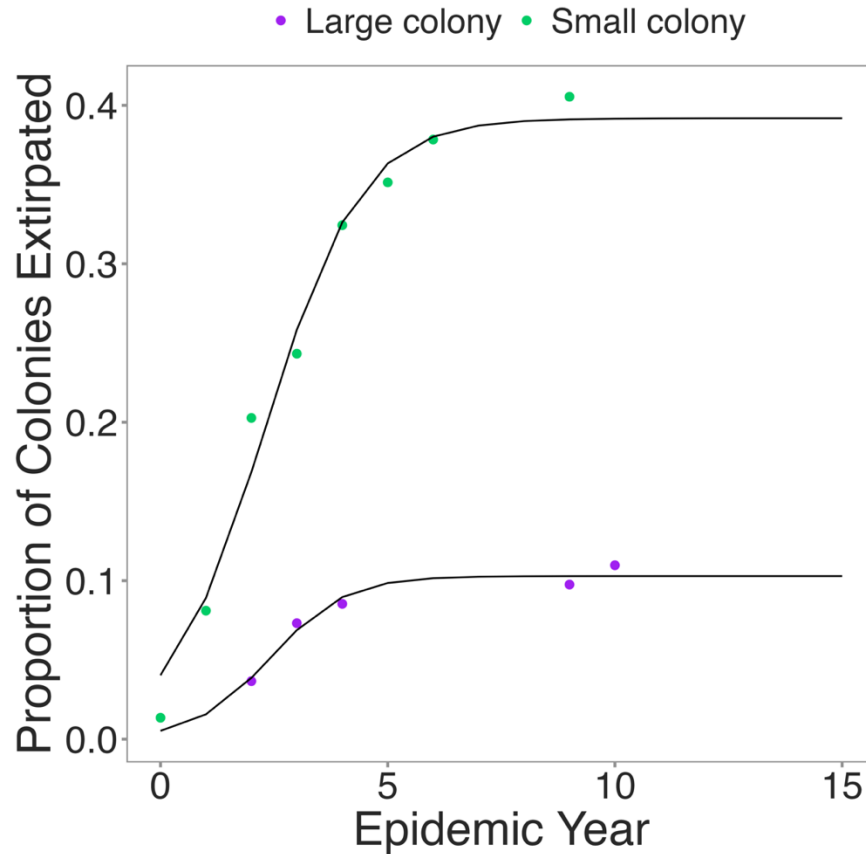
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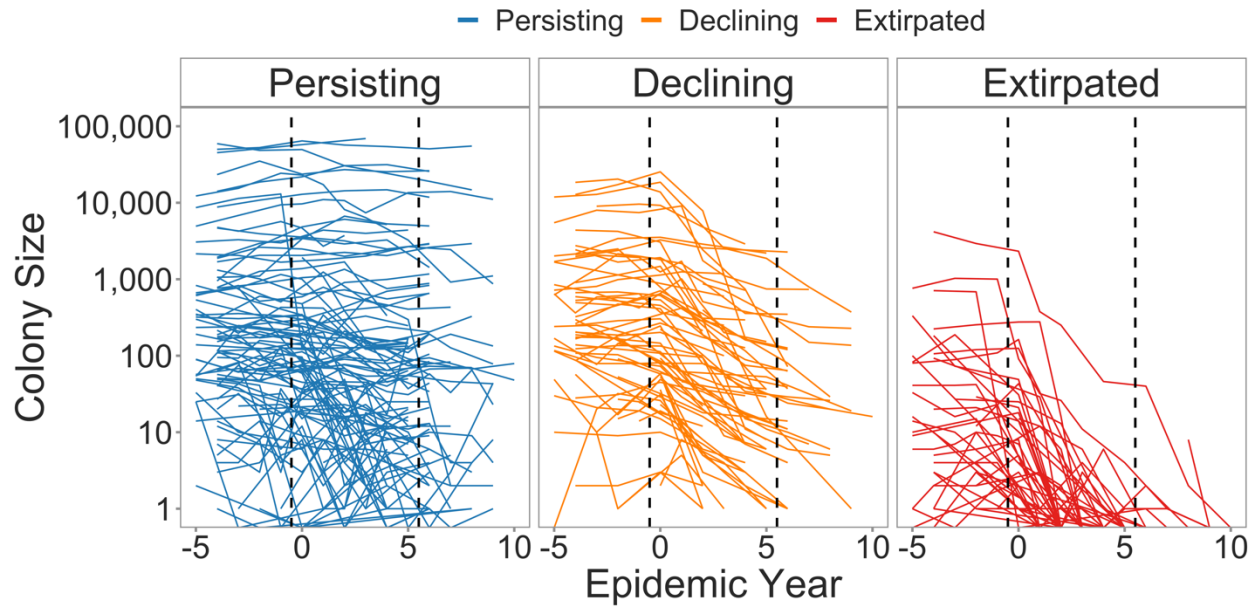
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Supplementary Information

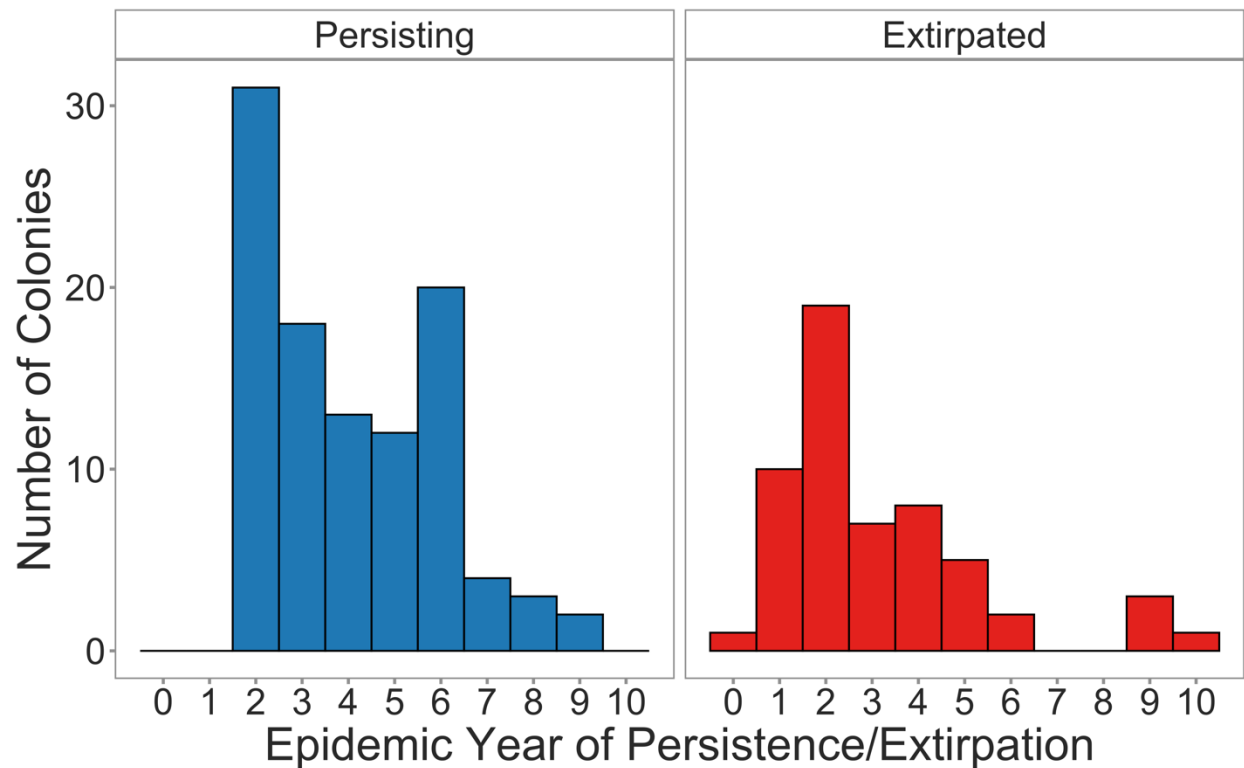
Supplemental Figures



Supplemental Figure 1: Plot of cumulative extirpations for both small (pre-WNS detection colony size of 66 or fewer individual *M. sodalis*) and large (pre-WNS detection colony size greater than 66 individual *M. sodalis*) colonies that have been WNS-positive for seven or more years ($n = 74$ small, 82 large colonies). Solid lines are estimates of logistic growth models fit to the cumulative extirpation data, from which the simulation extirpation probabilities were calculated.

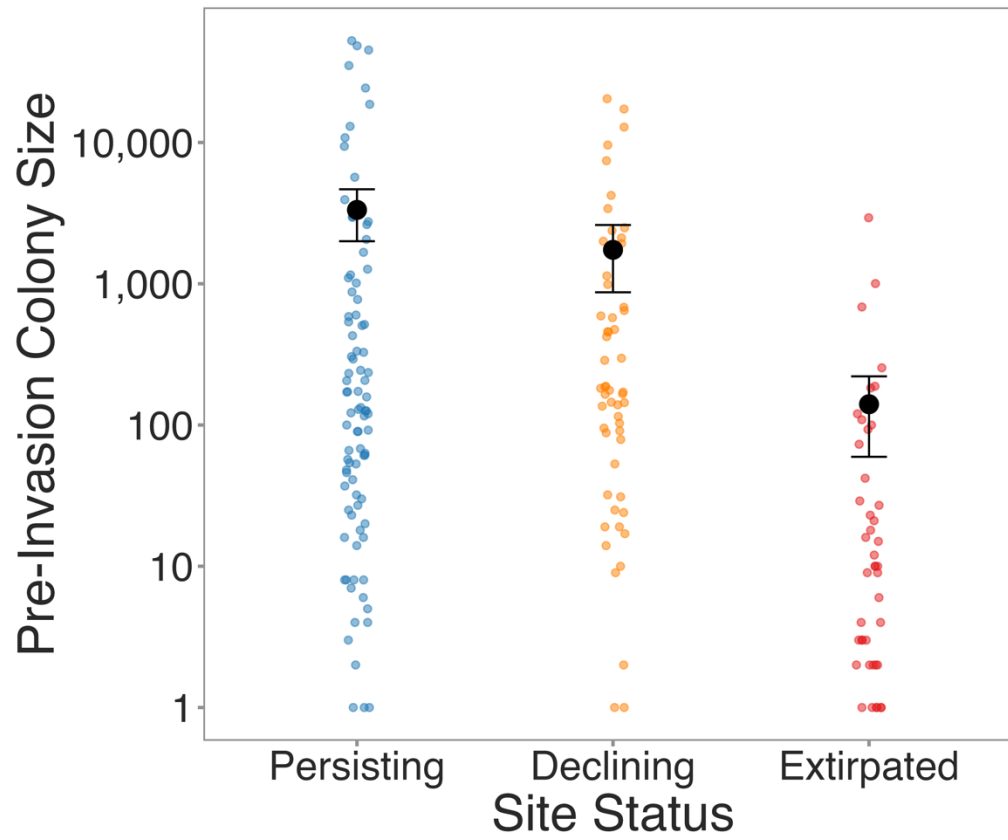


Supplemental Figure 2: Colony sizes of persisting, declining, and extirpated colonies over time since WNS detection. Epidemic year 0 on the x-axis corresponds to the year in which WNS was detected. Vertical dashed lines separate epidemic phases (pre-invasion, epidemic, established).

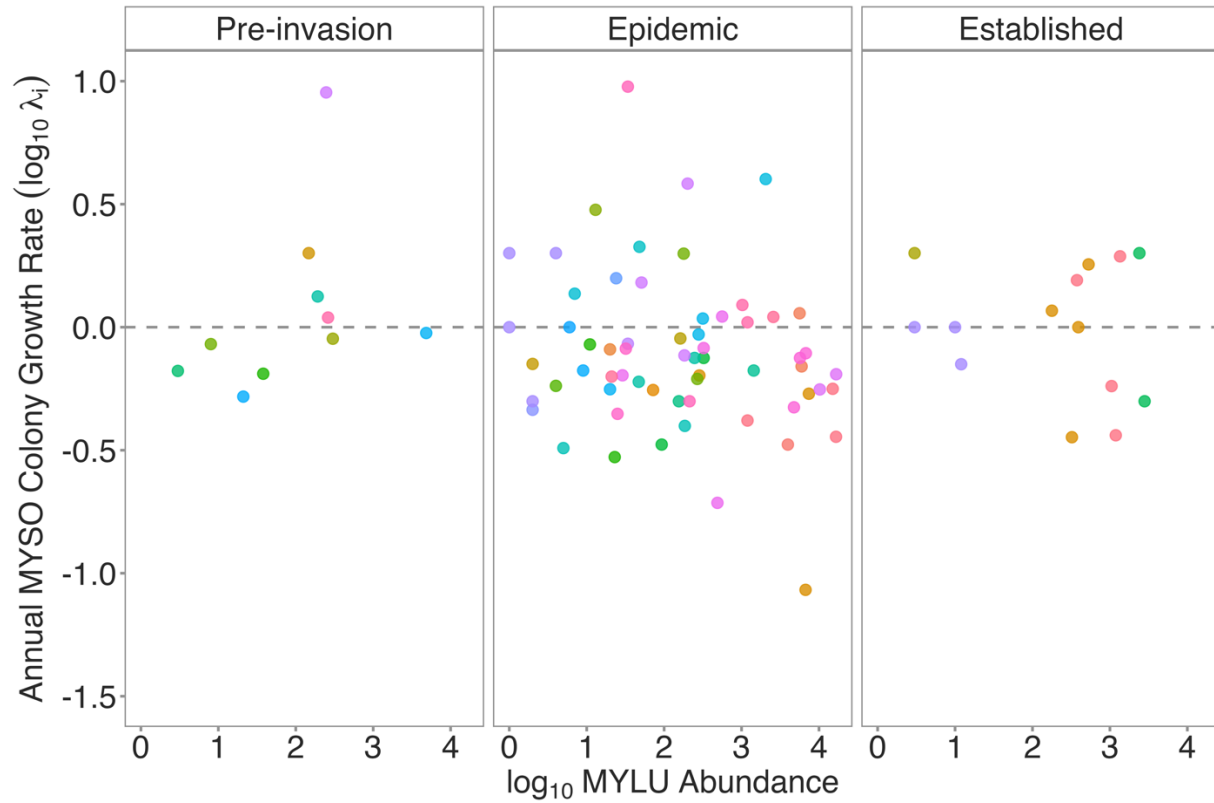


Supplemental Figure 3: Histogram of epidemic year in which stability or extirpation first occurs in persisting and extirpated colonies, respectively. Years of stability in epidemic year 0

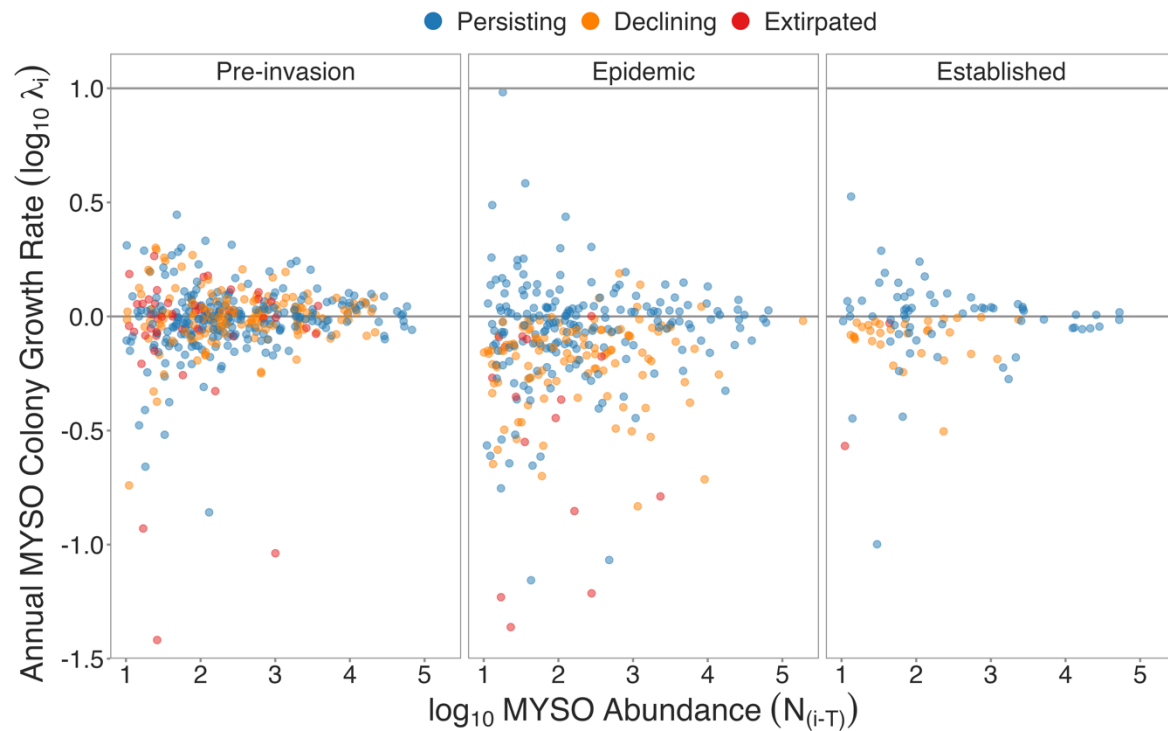
and 1 were not considered true stability events, as *P. destructans* is at sufficiently low prevalence at that point that the incidence of spurious population growth outside of the context of WNS is high.



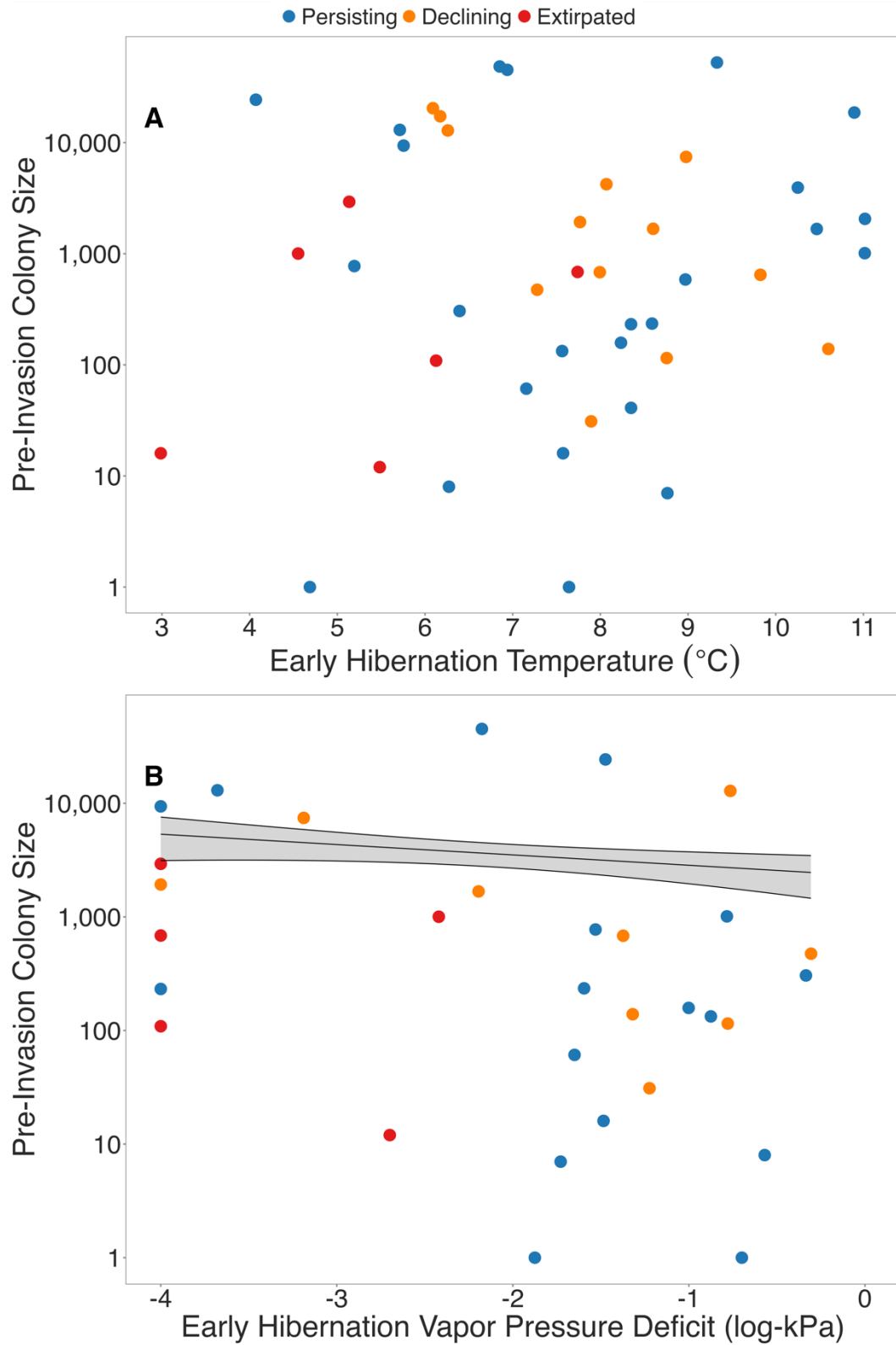
Supplemental Figure 4: Most recent colony sizes of persisting, declining, and extirpated colonies prior to the detection of WNS. Points are jittered and colored by site status.



Supplemental Figure 5: Annual Indiana bat (MYSO) colony growth rate by the $\log_{10}$ little brown bat (MYLU) abundance within the same hibernaculum in the previous winter. A colony growth value of 0 corresponds to colony stability, with values below or above 0 indicating colony decline and growth, respectively. Points are colored by unique hibernacula.

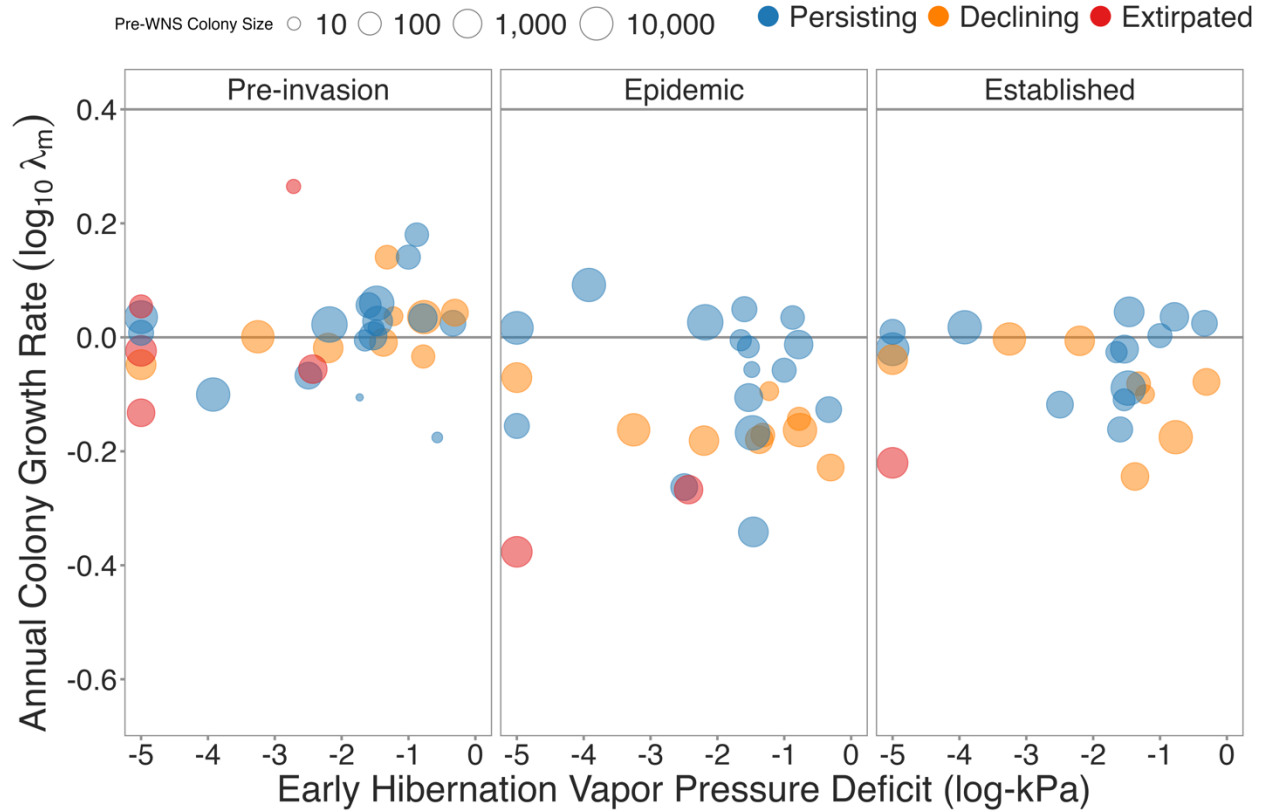


Supplemental Figure 6: Annual Indiana bat (MYSO) colony growth rate by the $\log_{10}$ Indiana bat abundance within the same hibernaculum in the previous winter. A colony growth value of 0 corresponds to colony stability, with values below or above 0 indicating colony decline and growth, respectively. Points are colored by colony status.

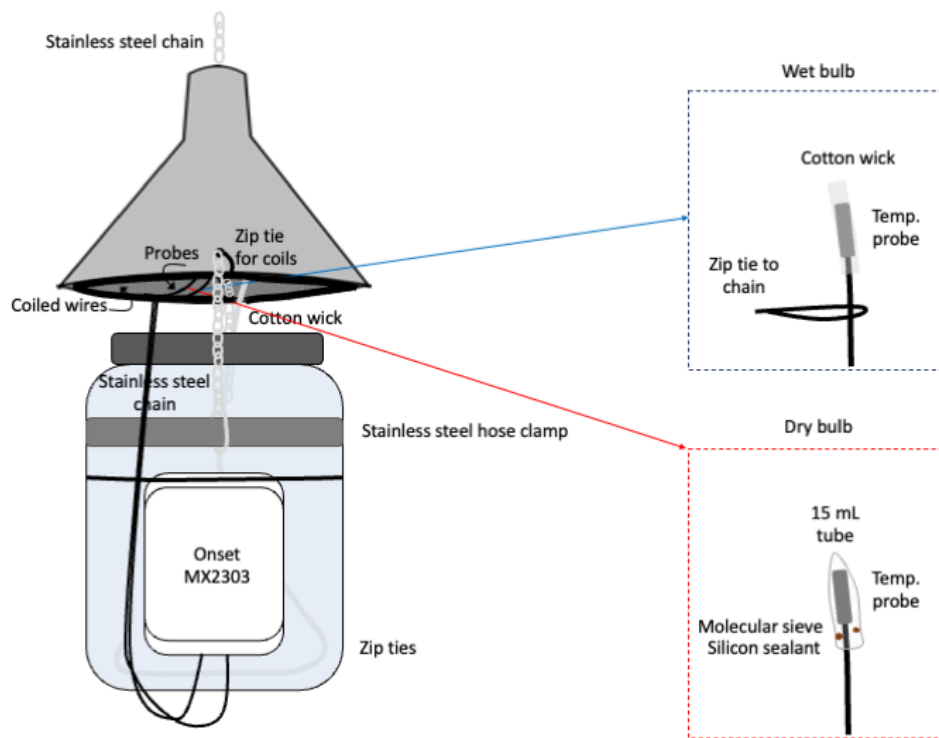


Supplemental Figure 7: Association between pre-WNS Indiana bat colony sizes and **A)** mean early hibernation temperature (Celsius) and **B)** $\log_{10}$ vapor pressure deficit. Points are colored by

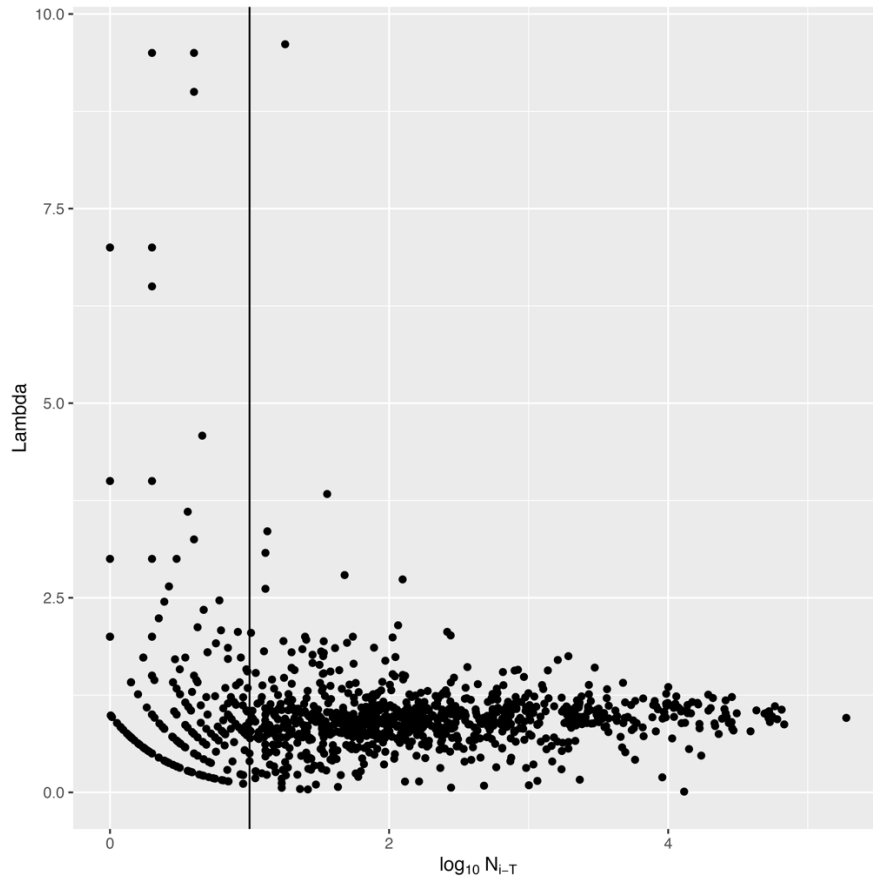
colony status. Solid line and shaded region correspond to model estimates $\pm$ one standard error of estimated mean.



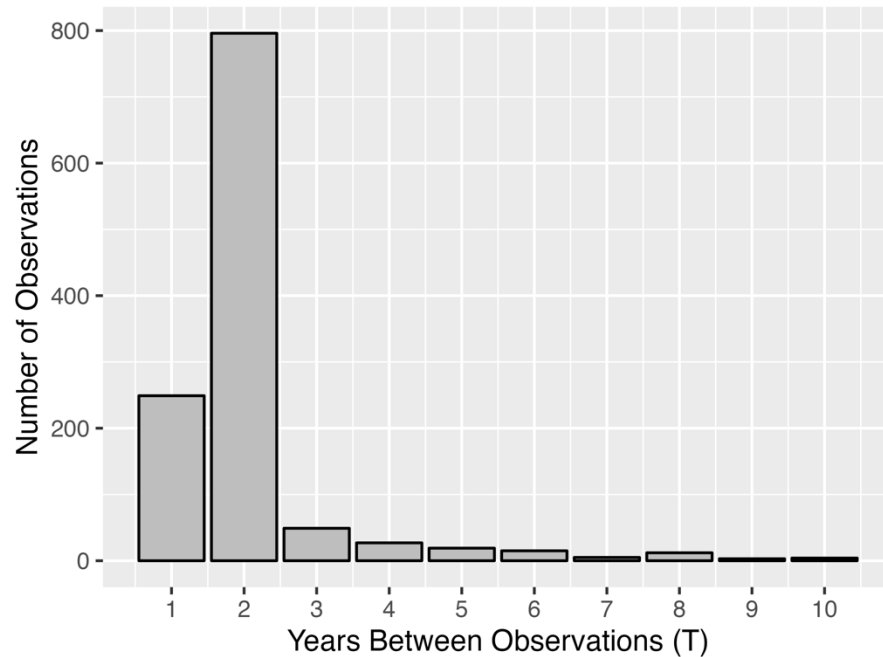
Supplemental Figure 8: Annual colony growth rate over mean early hibernation vapor pressure deficit. Lower VPD values correspond to more humid conditions. Data is broken into separate panels by epidemic phase. A colony growth rate value of 0 corresponds to population stability, with values below or above 0 corresponding to colony decline or growth, respectively. Points are colored by colony status and point size corresponds to the most recent pre-WNS colony size.



Supplemental Figure 9: Schematic of wet-bulb psychrometer used in this study. Wet-bulb psychrometers compare the temperature of wet bulbs and dry bulbs to estimate the vapor pressure deficit (VPD), or the difference between the measured vapor pressure and the vapor pressure at saturation at the given temperature. High values of VPD indicate low vapor pressure relative to what could be achieved at saturation, indicating dry environments. Low values of VPD indicate near- or at-saturation vapor pressure, corresponding to humid conditions.



Supplemental Figure 10: Scatterplot of annual colony growth rate, λ_i , by values of N_{i-T} used to calculate them. The black vertical line corresponds to the cutoff threshold of N_{i-T} , beneath which values of λ_i are excluded in our analyses due to being misleadingly sensitive to input from immigration.



Supplemental Figure 11: Histogram of the number of years between count observations, T , used to calculate annual colony growth rate values, λ_i .

Supplemental Tables

Supplemental Table 1: summary of origin of all Indiana bat count data we analyzed, including number of hibernacula per state, the range of years represented in their data, and the number of winter years of census data contributed to the dataset. The total winter years of count data contributed by each state was the number of census values from all hibernacula across all years of data. Also contains the number of hibernacula per state with both census and environmental logger data available, as well as the logger years of data from those hibernacula.

State	Number of hibernacula with census data	Range of years of census data	Total winter years of census data (% of all census data)	Number of hibernacula with both census and logger data (logger years)
Alabama	8	2009—2019	39 (3.14%)	0 (0)
Arkansas	8	2009—2019	39 (3.14%)	1 (0.17)
Illinois	9	2009—2022	48 (3.87%)	3 (13.99)
Indiana	19	2007—2019	113 (9.11%)	8 (10.43)
Kentucky	75	2006—2019	345 (27.8%)	4 (1.32)
Missouri	36	2005—2019	137 (11.04%)	3 (6.83)
North Carolina	2	2008—2014	6 (0.48%)	0 (0)
New Jersey	2	2004—2018	13 (1.05%)	0 (0)
New York	16	2003—2019	106 (8.54%)	8 (4.79)
Ohio	2	2007—2016	11 (0.89%)	0 (0)
Pennsylvania	15	2004—2019	52 (4.19%)	2 (0.33)
Tennessee	28	2007—2019	137 (11.04%)	4 (1.00)
Virginia	15	2005—2019	76 (6.12%)	4 (1.32)
Vermont	2	2003—2017	6 (0.48%)	1 (0.13)
West Virginia	28	2005—2019	113 (9.11%)	10 (10.42)

Supplemental Table 2: summary of the amount of environmental data collected by each logger model in this study.

Logger model	Total logger years of data (% of all environmental data)
Onset HOBO Pro	4.21 (8.26%)
Onset HOBO Pro V2	28.86 (56.70%)
Onset HOBO MX2303	5.52 (10.84%)
Onset HOBO XT	10.92 (21.45%)
Onset HOBO Pendant	0.17 (<1%)
Maxim Integrate iButton	0.37 (<1%)
Madgetech TransiTempII-RH	0.33 (<1%)
Unknown	0.51 (1%)

Supplemental Table 3: summary of the estimated parameters of a logistic regression fit to proportional cumulative colony extirpation data.

Cumulative Extirpation Curve -- Response variable: Proportion colonies extirpated				
<i>Logistic regression of form: $P = \frac{L}{1 + e^{-(a+bX)}}$ where:</i>				
<i>P = proportion of colonies extirpated; L = maximum value; a = intercept; b = growth rate</i>				
<i>X = epidemic year</i>				
<i>Colony size</i>	<i>Parameter</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Small	L	0.392	0.017	<0.001
	a	-2.165	0.322	0.001
	b	0.942	0.149	0.001
Large	L	0.103	0.005	0.003
	a	-2.028	0.908	0.084
	b	1.210	0.352	0.075

Supplemental Table 4: statistical output of a generalized linear mixed model exploring the association between epidemic phase, colony status, and their interaction with annual colony growth rate.

Model 1 -- Response variable: Annual colony growth rate (λ_i)			
<i>Generalized linear mixed model with gamma error distribution, log link function, random effect of colony ID (N = 857, groups = 182)</i>			
<i>Model reference level: Epidemic phase(Pre-invasion), Colony status(Persisting)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	0.028	0.032	0.386
Epidemic phase(Epidemic)	-0.039	0.041	0.353
Epidemic phase(Established)	0.005	0.061	0.929
Colony status(Extirpated)	-0.019	0.076	0.798
Colony status(Declining)	0.001	0.053	0.990
Epidemic phase(Epidemic):Colony status(Extirpated)	-0.385	0.112	<0.001
Epidemic phase(Established):Colony status(Extirpated)	-0.521	0.314	0.097
Epidemic phase(Epidemic):Colony status(Declining)	-0.219	0.068	0.001
Epidemic phase(Established):Colony status(Declining)	-0.233	0.106	0.028

Supplemental Table 5: statistical output of a linear model exploring the association between colony status and pre-WNS arrival colony size.

Model 3 -- Response variable: $\log_{10}$ Pre-WNS arrival colony size			
<i>Negative binomial generalized linear model with log link function (N = 189)</i>			
<i>Model reference level: Colony status(Persisting)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	8.112	0.204	<0.001
Colony status(Declining)	-0.651	0.326	0.046
Colony status(Extirpated)	-3.168	0.358	<0.001

Supplemental Table 6: statistical output of a generalized linear mixed model exploring the association between Indiana bat colony size in year $i-1$ with the annual colony growth to year i .

Model 2 -- Response variable: Annual colony growth rate (λ_i)			
<i>Generalized linear mixed model with gamma error distribution, log link function, random effect of colony ID (N = 866, groups = 191)</i>			
<i>Model reference level: Epidemic phase(Pre-invasion)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	-0.011	0.061	0.859
$\log_{10}$ census value (N_{i-1})	0.012	0.024	0.612
Epidemic phase(Epidemic)	-0.160	0.088	0.070
Epidemic phase(Established)	0.019	0.128	0.877
$\log_{10}$ census value (N_{i-1}):Epidemic phase(Epidemic)	-0.009	0.034	0.791
$\log_{10}$ census value (N_{i-1}):Epidemic phase(Established)	-0.035	0.052	0.493

Supplemental Table 7: statistical output of a generalized linear mixed model exploring the association between little brown bat colony size in year $i-1$ with the annual colony growth of Indiana bat colonies to year i .

Model 8 -- Response variable: Annual colony growth rate (λ_i)			
<i>Generalized linear mixed model with gamma error distribution, log link function, random effect of colony ID (N = 63, groups = 30)</i>			
<i>Model reference level: Epidemic phase(Pre-invasion)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	-0.243	0.421	0.564
Log ₁₀ MYLU census value (N_{i-1})	0.115	0.176	0.514
Epidemic phase(Epidemic)	0.055	0.467	0.906
Epidemic phase(Established)	0.798	1.750	0.648
Log ₁₀ MYLU census value (N_{i-1}): Epidemic phase(Epidemic)	-0.149	0.194	0.444
Log ₁₀ MYLU census value (N_{i-1}): Epidemic phase(Established)	-0.252	0.638	0.692

Supplemental Table 8: statistical output of a generalized linear model exploring the association between mean early hibernation temperature, epidemic phase, and their interaction and annual colony growth.

Model 4 -- Response variable: Mean annual colony growth rate (λ_m)			
<i>Generalized linear model with gamma error distribution and log link function (N = 115)</i>			
<i>Model reference level: Epidemic phase(Pre-invasion)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	0.146	0.165	0.376
Mean early hibernation temperature	-0.012	0.021	0.555
Epidemic phase(Epidemic)	-0.909	0.248	<0.001
Epidemic phase(Established)	-0.575	0.263	0.030
Mean early hibernation temperature: Epidemic phase(Epidemic)	0.078	0.031	0.013
Mean early hibernation temperature: Epidemic phase(Established)	0.050	0.033	0.130

Supplemental Table 9: statistical output of a generalized linear model exploring the association between mean early hibernation VPD, epidemic phase, and annual colony growth.

Model 5 -- Response variable: Mean annual colony growth rate (λ_m)			
<i>Generalized linear model with gamma error distribution and log link function (N = 81)</i>			
<i>Model reference level: Epidemic phase(Pre-invasion)</i>			
<i>Term</i>	<i>Estimate</i>	<i>Standard Error</i>	<i>P value</i>
Intercept	0.114	0.081	0.165
log ₁₀ mean early hibernation VPD	0.028	0.030	0.346
Epidemic phase(Epidemic)	-0.292	0.118	0.015
Epidemic phase(Established)	-0.251	0.125	0.048
Mean early hibernation VPD: Epidemic phase(Epidemic)	0.012	0.044	0.788
Mean early hibernation VPD: Epidemic phase(Established)	-0.034	0.046	0.469

Supplemental Table 10: names and descriptions of models reported in this study.

Model Name	Response Variable	Explanatory Variable(s)	Model type
Model 1	Annual colony growth (λ_i)	Epidemic phase + site status + epidemic phase * site status + (1 Site)	Generalized linear mixed model with gamma error distribution and log link function
Model 2	Annual colony growth (λ_i)	$\text{Log}_{10}(\text{colony size}, N_{i-1}) + \text{epidemic phase} + \text{Log}_{10}(\text{colony size}, N_{i-1}) * \text{epidemic phase} + (1 \text{site})$	Generalized linear mixed model with gamma error distribution and log link function
Model 3	Pre-WNS colony size	Site status	Negative binomial generalized linear model
Model 4	Average annual colony growth (λ_m)	Mean early hibernation temperature + epidemic phase + mean early hibernation temperature * epidemic phase	Generalized linear model with gamma error distribution and log link function
Model 5	Average annual colony growth (λ_m)	Mean early hibernation VPD + epidemic phase + mean early hibernation VPD * epidemic phase	Generalized linear model with gamma error distribution and log link function
Model 6	Pre-WNS colony size	Mean early hibernation temperature	Negative binomial generalized linear model
Model 7	Pre-WNS colony size	Mean early hibernation VPD	Negative binomial generalized linear model
Model 8	Annual colony growth (λ_i)	$\text{Log}_{10}(\text{colony size of MYLU}, N_{i-1}) + \text{epidemic phase} + \text{Log}_{10}(\text{colony size of MYLU}, N_{i-1}) * \text{epidemic phase} + (1 \text{site})$	Generalized linear mixed model with gamma error distribution and log link function

Chapter 4 – Variation in microclimate use shapes host infection and disease with a novel fungal pathogen.

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Abstract

Emerging infectious diseases can threaten host populations with severe declines and regional to complete extinction. However, we often observe individual-level variation in how hosts respond when challenged by the pathogen, which can scale up to population-level trends. The environmental dependence of disease is a common driver of such variation but is often characterized through traditional “snapshot” sampling approaches in which associations are drawn based on one-time sampling events that typically occur within a longer infection period. Therefore, failing to characterize environmental conditions experienced by hosts over the duration of their infection may hamper our understanding of environment-disease associations. Here, we pair hourly observations of microclimate conditions experienced by individual little brown bats (*Myotis lucifugus*) with white-nose syndrome (WNS) over the course of their months-long infection with disease severity data to explore the environmental dependence of its pathophysiology. We found that an association between arousal frequency and overwinter weight loss was moderated by a novel interaction with microclimate temperature, the result of lighter bats selecting colder microclimates than their heavier counterparts and losing less weight. Lighter bats moderated their weight loss by shortening the length of their arousals from torpor, potentially a physiological mechanism of disease mitigation in which they appear to reduce the energetic costs of infection-driven increases in arousal frequency. Finally, temperature-dependent models of pathogen growth parameterized with overwinter microclimate use data performed

better at predicting late hibernation pathogen loads than a model based on snapshot sampling, indicating overwinter microclimate use is an important driver of infection processes in this system. Ultimately, this work illustrates that longitudinal study of host environmental use over the course of infection can improve our understanding of environment-disease associations, information essential to the successful conservation of disease impacted hosts.

Introduction

The emergence of novel infectious diseases of wildlife has threatened diverse host populations in recent history. For example, in conjunction with other stressors characteristic of the Anthropocene, infectious diseases have contributed to the population declines and extinction threat in host species such as the northern long eared bat (*Myotis septentrionalis*; Cheng et al., 2021; Frick et al., 2015), Tasmanian devil (*Sarcophilus harrisii*; Cunningham et al., 2021; Hawkins et al., 2006), ‘Akikiki Hawaiian honeycreeper (*Oreomystis bairdi*; Paxton et al., 2016; Van Riper III et al., 1986), Panamanian golden frog (*Atelopus zeteki*; Lips et al., 2006; McCaffery et al., 2015), and the American chestnut tree (*Castanea dentata*; Anagnostakis, 1987; Van Drunen et al., 2017), among many others. However, we often observe variation in how host individuals and populations respond to disease, with some exhibiting severe disease and declines while others suffer few disease impacts (Briggs et al., 2005; Langwig et al., 2012; Scheele et al., 2017b). Identifying the drivers of such variation is a critical step in developing conservation strategies, as they offer a point of leverage to manipulate infectious disease systems to promote host survival. Successful conservation of host populations, therefore, will depend on the timely and effective identification of such drivers.

The environmental dependence of disease is one potential source of variation in disease impacts. For example, ambient environmental conditions such as temperature or humidity can influence important infectious disease processes such as transmission (Beck-Johnson et al., 2013; Shapiro et al., 2017), host susceptibility (Ben-Horin et al., 2013; Cohen et al., 2017), infection severity (Grimaudo et al., 2022; Hopkins et al., 2021; Langwig et al., 2016), and disease severity (Bailey et al., 2018; Paull and Johnson, 2011). Given that environmental conditions can vary over space and time, we often see corresponding variation in infectious disease impacts on the same scales (Parratt et al., 2016). However, disease-environment interactions are often characterized using “snapshot” approaches, in which the environmental conditions and infection/disease severity of the host at the time of sampling are used to draw conclusions on their association. Subsequently, our understanding of the environmental drivers of disease dynamics is often based solely on information gathered at a specific moment in the infection process of the host, which can be dynamic and span months or years (McLaughlin et al., 2000; Plowright et al., 2017; Stewart et al., 2003). What may be equally important, however, are the environmental conditions that a host experiences over the course of its infection, which are likely variable and information we fail to capture through snapshot approaches.

White-nose syndrome (WNS) is an emerging infectious disease of hibernating bats (Hoyt et al., 2021). The disease is caused by a fungal pathogen, *Pseudogymnoascus destructans* (Lorch et al., 2011; Minnis and Lindner, 2013; Warnecke et al., 2012), which was recently introduced to North America from its historical range in Eurasia and has since caused significant population declines in species of hibernating bats (Blehert et al., 2009; Drees et al., 2017; Frick et al., 2015; Hoyt et al., 2021; Langwig et al., 2016, 2015, 2012; Leopardi et al., 2015). The disease has strong seasonality in which bats become infected in autumn months when they return to their

winter hibernacula (Langwig et al., 2015), which are contaminated with an environmental reservoir of the pathogen (Hoyt et al., 2020, 2015; Laggan et al., 2023), and bats experience disease over hibernation until they die from infections or emerge in spring and can clear infection upon returning to euthermia. Infection with *P. destructans* initiates a physiological cascade that results in an increase in the frequency of arousal from hibernation (“arousal bouts”) (Lilley et al., 2016; Reeder et al., 2012; Warnecke et al., 2012), premature depletion of overwinter fat reserves, dehydration (Cryan et al., 2013; Mcguire et al., 2017; Verant et al., 2014), emaciation, and ultimately mortality (Figure 1; Cryan et al., 2010; Hoyt et al., 2021; Warnecke et al., 2013).

There is strong environmental dependence of infection and disease severity, with warmer hibernaculum temperatures typically being associated with greater infection intensity and colony declines (Grieneisen et al., 2015; Grimaudo et al., 2022; Hopkins et al., 2021; Langwig et al., 2016, 2012; Lilley et al., 2018; but see Grimaudo et al., 2023). However, our understanding of the role of temperature in driving WNS dynamics comes from classical snapshot approaches in which the microclimate roosting temperature of bats is recorded during discrete sampling events, which typically occur once in early and/or late hibernation, a months-long process. Therefore, standard sampling methodologies may fail to capture variation in the microclimate roosting temperature experienced by bats over the course of their infection, potentially limiting our understanding of the environmental dependence of WNS. Indeed, evidence suggests that, rather than remaining in the same roosting location for the entirety of hibernation, bats shift their location within a hibernaculum over the course of winter (Boyles et al., 2006; Daan and Wichers, 1968; Hardin and Hassell, 1970; Ryan et al., 2019). Hibernacula are spatially and temporally variable in ambient conditions such as temperature (Perry, 2013), so periodic movement behavior by hibernating bats could produce variation in the microclimate profiles they experience over

their infection with *P. destructans*. Lastly, because bats spend their winters hibernating in caves that are largely buffered from daily and seasonal climate extremes, WNS represents an ideal system by which to explore the effects of temperature variation on disease in the absence of extremes that can make isolating specific effects challenging.

In this study, we used individual data loggers to track the microclimate use of wild little brown bats (*Myotis lucifugus*) over the course of hibernation and their infection with *P. destructans*. By combining information on bat overwinter microclimate use with infection and disease severity metrics such as pathogen load, arousal frequency, and weight loss, we explored the association between microclimate conditions and disease dynamics otherwise unobservable through traditional snapshot approaches. Through this approach, we sought to further elucidate the role of temperature in influencing the pathophysiology of WNS (Figure 1; reprinted from Hoyt *et al.*, 2021), as well as how individual variation in environment use may produce variation in infection and disease outcomes. Finally, we developed within-host models of temperature-dependent pathogen growth to explore the importance of overwinter microclimate use in infection dynamics, hypothesizing that a model incorporating overwinter variation in temperature conditions will improve predictability of infection dynamics compared to a snapshot model. Ultimately, our data provide valuable information on the pathophysiology of WNS and the role of overwinter microclimate use in driving infection and disease dynamics in this system.

frequencies, DS1921G and DS1922L units logging every 120 and 60 minutes, respectively. In October and November of 2021 (early hibernation), we attached the data loggers to the backs of 287 individual adult little brown bats (200 males, 87 females) across seven hibernacula in Illinois (two hibernacula), Michigan (two hibernacula), Wisconsin (two hibernacula), and New York (one hibernacula) (Supplemental Figure 1; Supplemental Table 1). The data loggers recorded the skin temperature of each bat over the course of hibernation, providing information on microclimate use and arousal from torpor. In March and April of 2022 (late hibernation) we returned to each of the seven sites to retrieve the data loggers. Of the 287 data loggers deployed, 145 (50.52%) were found and retrieved. Of the 145 data loggers retrieved, 80 (55.17%) were still functional and were able to have their data off-loaded. Of the 80 functional data loggers we retrieved, 20 logged temperature data continuously throughout the entire winter period and 60 stopped logging data prior to their retrieval. The mean sampling period (number of days from logger deployment to the last day of logging) was 105.95 days (range: 92 – 155 days).

During hibernation, bat metabolism slows significantly, and their body temperature drops to near ambient temperature, a condition referred to as ‘torpor.’ Torpor is intermittently interrupted by bouts of ‘arousal’, which are euthermic periods during which bats are active and their body temperature increases. By recording the skin temperature of bats over hibernation, the data loggers captured bouts of both torpor and arousal, as well as the ambient temperature conditions present at roosting locations during bouts of torpor. Following the protocol of Reeder et al. 2012, we systematically classified the data collected by data loggers as either occurring during an ‘arousal’ or ‘torpor’ bout (Figure 2). First, we identified data points recorded during arousal bouts if they fell within 10°C of the maximum temperature recorded by the data logger. Often, however, this approach failed to capture data points that fell outside of the 10°C threshold

but were significantly warmer than the preceding or following bouts of torpor, indicating they were part of the arousal bout. To account for this, we allowed our classification algorithm to consider the two datapoints preceding or following an identified arousal bout using the 10°C threshold. If these ‘buffer’ data points were greater than 1°C higher than the closest non-arousal data point, they were classified as part of the arousal. All data points not classified as arousal bouts under this system were instead classified as occurring during torpor bouts. We removed data recorded on the days of sampling on which bats were handled to avoid including disturbance-related arousals in the data set.

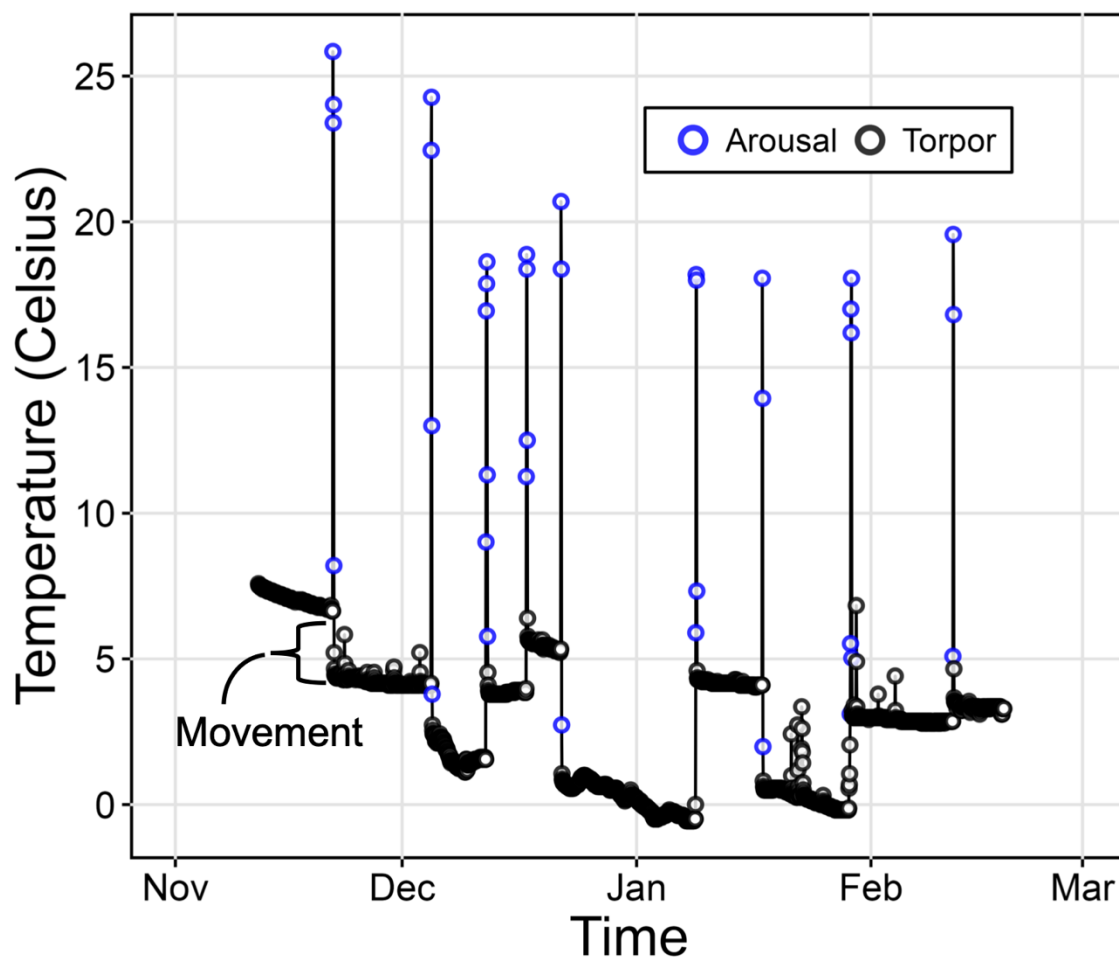


Figure 2: An example of the data collected by modified, temperature sensitive Thermocron iButton data loggers we deployed and retrieved from 80 little brown bats over the 2021-22 hibernation season. Point color corresponds to hibernation behavior (black = torpor bout, blue = arousal bout). Movement behavior is observable if the temperature preceding an arousal bout is different than that following an arousal bout, indicating the bat shifted to a new roosting location with a different microclimate.

We summarized the data collected by each data logger to characterize the overwinter microclimate use and arousal behavior of each individual. First, we calculated the ‘mean torpor bout temperature’ as the mean temperature recorded by the data logger during bouts of torpor. Second, to characterize the number of arousals from torpor by individual bats while controlling for differences in the length of the sampling period, we calculated ‘arousal frequency’ as the total number of arousal bouts divided by the sampling period length in days. Arousal frequency, therefore, corresponds to the number of arousals per day for each individual. Finally, we calculated the ‘mean arousal bout length’ of each individual bat as the average of the length of their arousal bouts in days.

Bat Sampling

In addition to deploying and retrieving data loggers during early and late hibernation site visits, respectively, we took swab samples on the forearm and muzzle of each bat following an established swabbing protocol (Langwig et al., 2015.) We stored swab samples in RNAlater until the pathogen load, or amount of *Pseudogymnoascus destructans* DNA on each swab sample (nanograms of DNA), could be quantified using real-time polymerase chain reaction (qPCR). Samples without detectable fungal DNA were assigned a pathogen load value corresponding to the 40ct qPCR lower detection limit. We measured roost site temperatures of each bat at each sampling event using a Fluke 62 Max IR laser thermometer. Additionally, we weighed each bat

to get an early and late hibernation body mass, which we used to calculate the rate of mass loss, a metric of weight loss that accounts for differences in the amount of time between sampling events ($((\text{early hibernation body mass} - \text{late hibernation body mass}) / \text{num. of days between sampling events})$). During the early hibernation sampling event, we fit an aluminum forearm band (2.9mm; Porzana Ltd., Icklesham, E. Sussex, U.K.) to each bat so that they could be individually identified.

Statistical Analysis

To explore how early hibernation roosting microclimate is associated with early hibernation infection severity, we constructed a generalized linear model with gamma error distribution, log link function, the early hibernation pathogen load as the response variable, and early hibernation roosting temperature (as measured by the Fluke laser thermometer at the time of sampling) as the explanatory variable. We assessed the role of early pathogen load in driving subsequent bouts of arousals with a generalized linear mixed model with gamma error distribution and log link function, random effect of site ID, arousal frequency as the response variable, and early hibernation pathogen load as the explanatory variable. We explored the association between arousal frequency, microclimate temperature, and mass loss using a generalized linear mixed model with gamma error distribution and log link function, daily mass loss as the response variable, and arousal frequency, mean torpor bout temperature, and their interaction as the explanatory variables. In turn, we tested the association between early hibernation body mass and early hibernation roosting temperature using a generalized linear model with gamma error distribution and log link function, early hibernation roosting temperature as the response variable, and early hibernation body mass as the explanatory

variable. We tested the association between mean arousal bout length and early hibernation body mass using a generalized linear mixed model with gamma error distribution and log link function, random effect of site ID, mean arousal bout length as the response variable, and early hibernation body mass as the explanatory variable. Finally, we explored the association between mass loss and arousal bout length with a generalized linear mixed model with gamma error distribution and log link function, site ID as a random effect, daily mass loss as the response variable, and mean arousal bout length as the explanatory variable. All analyses were performed using R package *stats* (R Core Team 2024), *glmmTMB* (Magnusson et al., 2017), and *lme4* (Bates et al., 2015) in R v4.2.3.

Pathogen growth model

To illustrate how incorporating data on microclimate use over the course of host infection may improve our understanding of infection dynamics, we constructed an individual-based model of temperature-dependent pathogen growth. The model combines data from the iButton data loggers with the qPCR-measured pathogen load values and a previously parameterized Logan-10 thermal performance curve of the intrinsic rate of growth of *P. destructans* across a range of ecologically realistic temperatures (Laggan et al., unpublished data, Logan et al., 1976; Supplemental Figure 2). The model takes the form of a hierarchical model of exponential population growth, with the intrinsic rate of growth, r , taking the form of a nested Logan-10 thermal performance curve (TPC):

$$\frac{dL}{dt} = a \left(\frac{1}{1 + ce^{-pT}} - e^{-\frac{T_{max}-T}{\Delta T}} \right)^{1/5} L_t \quad (1)$$

Where dL/dt is the instantaneous rate of growth of the pathogen population, a , c , and p are shape parameters of the TPC, T is the average torpor bout temperature on day t , T_{max} is the maximum threshold temperature of pathogen growth, ΔT is the width of the upper boundary between the maximum threshold temperature and the optimal temperature ($T_{max} - T_{optimal}$), and L_t is the pathogen population on day t (parameter values provided in Supplemental Table 2). The intrinsic rate of growth is scaled to its fifth root because the original TPC was fitted to pathogen growth data recorded on five-day intervals, thus we scale it here to its daily rate.

Starting with the qPCR-measured early hibernation pathogen load value, the model calculates the predicted load value of the following day ($t+1$) based on the load value and mean torpor bout temperature of the current day (t). The model iteratively updates the predicted fungal load value each day of hibernation for each bat, extending until the late hibernation sampling day. However, because some of the iButton data loggers did not record temperature data all the way until the late hibernation sampling day, we imputed the mean torpor bout temperature of missing days with the last recorded mean daily torpor bout temperature. This model is referred to as the “time-varying temperature” model of pathogen growth. We constructed a variant of the model that parameterized T instead with the early hibernation roost temperature recorded during the early hibernation sampling event and assumed that bats remained at that temperature for the duration of hibernation (i.e. T is not updated). We used this variant, “fixed temperature” model to resemble snapshot approaches to characterizing microclimate-disease associations and compared its predicted late hibernation pathogen load values (the model-predicted pathogen load value on the day of the late hibernation sampling event) to those of the time-varying temperature model, in which T was updated daily with the recorded iButton value. To test for differences between the two model types in the accuracy of their predicted late hibernation pathogen load values, we

calculated the deviance between their predicted values and the qPCR-measured load values, which was subsequently used as the response variable in a linear mixed model with ‘model type’ as the explanatory variable and a random effect of Site ID.

Results

Behavior and pathophysiology

Congruent with previously published data, we found that early hibernation pathogen load was significantly positively associated with early hibernation roosting temperature ($\beta = 1.039 \pm 0.166$ SE, $p < 0.001$; Supplemental Figure 3). In turn, early hibernation pathogen load was positively associated with arousal frequency, indicating that bats with more severe early infections aroused more frequently from torpor ($\beta = 0.056 \pm 0.023$ SE, $p = 0.016$; Supplemental Figure 4). We detected a significant interaction between the frequency of arousal from torpor and mean torpor bout temperature to drive observed rate of mass loss (Interaction term: $\beta = 1.435 \pm 0.728$ SE, $p = 0.049$; Figure 3). The interaction suggests that bats that roost at relatively warm temperatures over hibernation lose weight more rapidly if they arouse more frequently, whereas bats that roost in relatively cold microclimates have no such relationship. We found a significant positive relationship between early hibernation body mass and the temperature of roosting locations at the time of early hibernation sampling ($\beta = 0.055 \pm 0.014$ SE, $p < 0.001$; Supplemental Figure 5). Subsequently, mean arousal bout length was significantly positively related to early hibernation body mass, indicating that bats starting hibernation at relatively lower weights not only roosted in colder temperatures, but they also spent less time awake during bouts of arousal ($\beta = 0.092 \pm 0.030$ SE, $p = 0.002$; Figure 4A). Mean arousal bout length was positively related to the rate of mass loss, indicating that bats that took longer arousal bouts also

lost weight more rapidly ($\beta = 5.072 \pm 1.461$ SE, $p < 0.001$; Figure 4B). Therefore, our data indicate that bats roosting in colder roosting microclimates lost the same amount of weight regardless of arousal frequency (Figure 5).

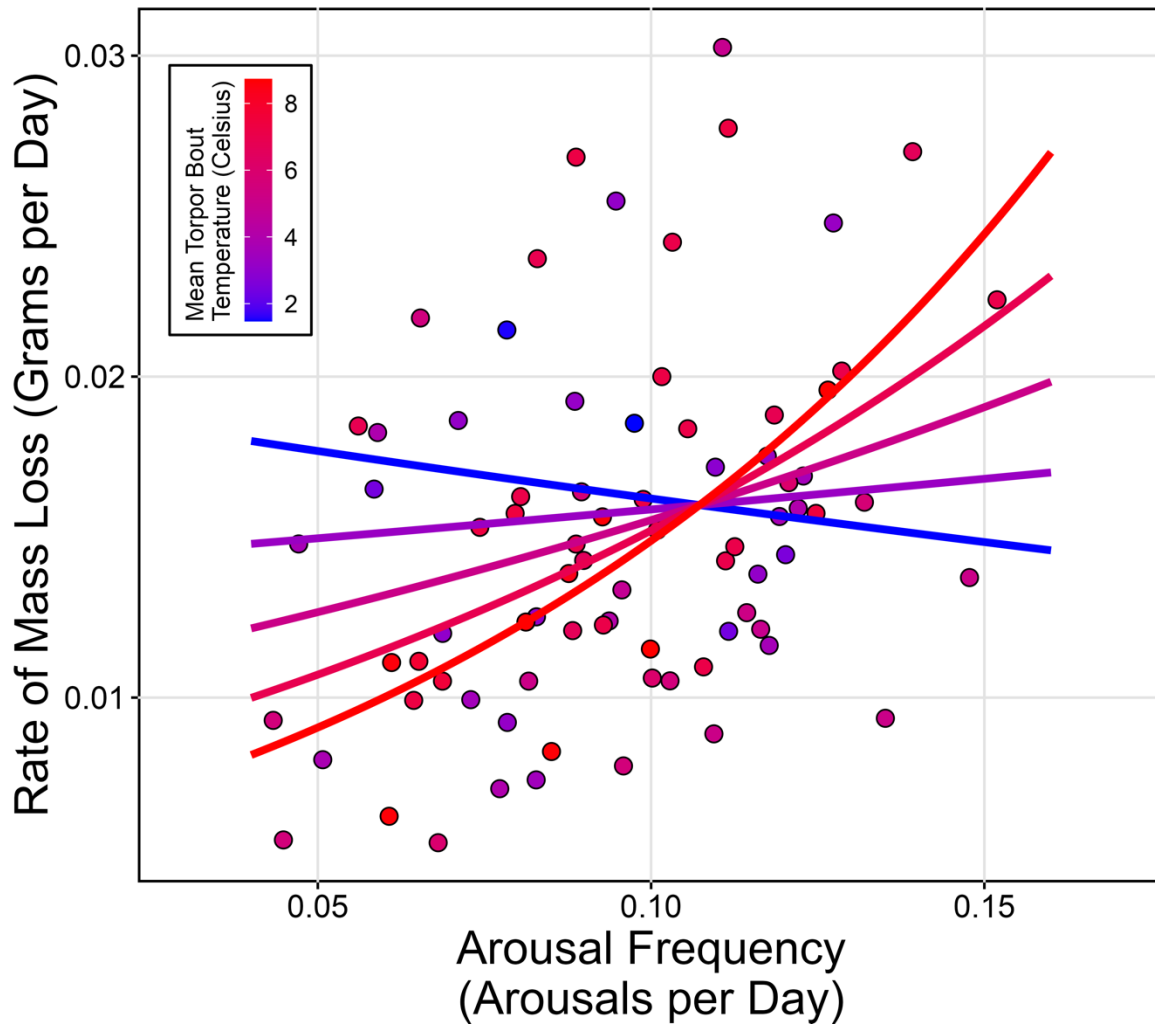


Figure 3: Interaction between arousal frequency and mean torpor bout temperature on daily mass loss. Point and line color correspond to mean torpor bout temperature. Each point corresponds to an individual bat and lines correspond to model estimates at various temperatures. Rate of mass loss is calculated as the total amount of mass (in grams) lost between early and late hibernation, divided by the number of days between sampling events. Similarly, arousal frequency is calculated as the total number of arousal bouts detected divided by the number of days between early and late hibernation sampling.

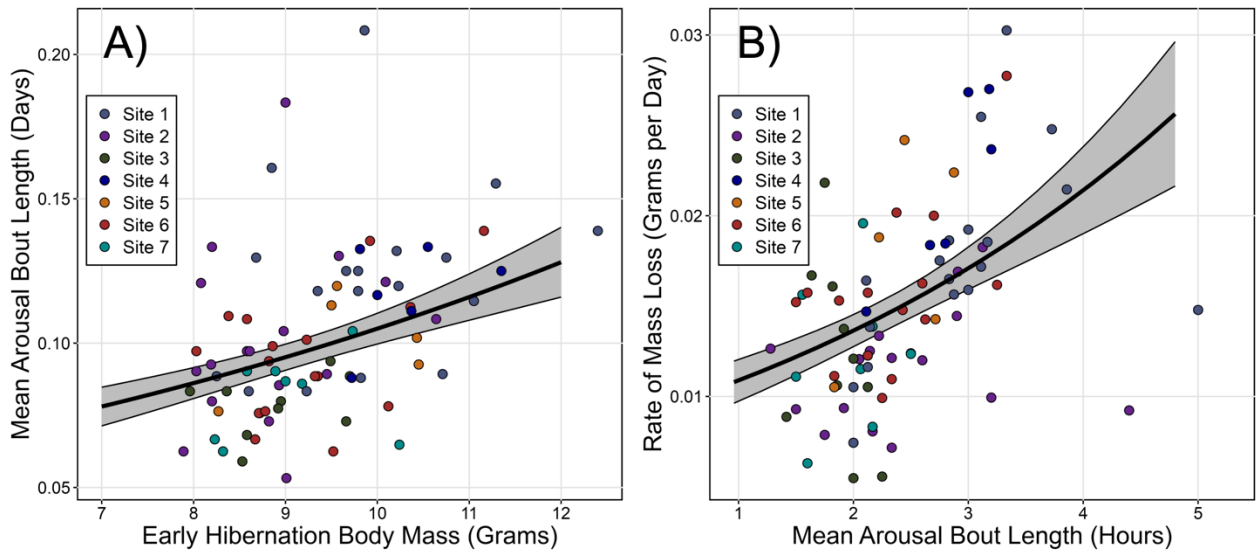


Figure 4: **A.** Association between early hibernation body mass and mean arousal bout length. Bats that started hibernation at heavier masses tended to stay awake longer during arousal bouts than lighter counterparts. **B.** Association between mean arousal bout length and daily mass loss. Individuals that had longer bouts of arousal also lost more weight. Each point corresponds to an individual bat and is colored by site. Solid black lines and gray shaded regions correspond to the model estimate $\pm$ one standard error.

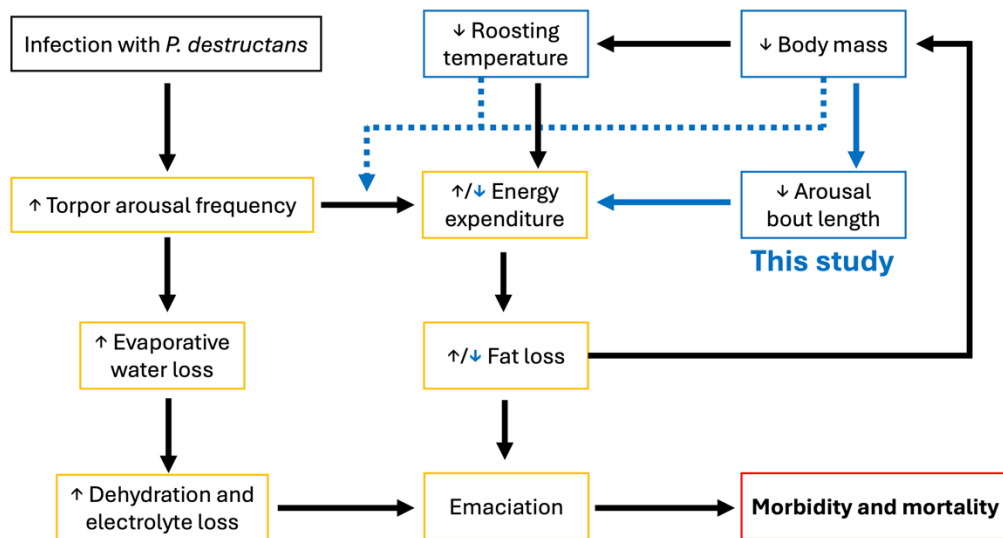


Figure 5: Conceptual diagram of the pathophysiological pathway of white-nose syndrome. Findings from this study are highlighted in blue. We found that body mass was positively associated with roosting temperature, driving an interaction between roosting temperature and arousal frequency on the rate of weight loss. Further, smaller bats shortened the length of their arousal bouts from torpor, lowering their energetic cost and further reducing the rate of mass loss.

Pathogen growth model

The average late hibernation pathogen load value, as measured by qPCR on swab samples taken in late hibernation, was -2.399 (± 0.991 SD) log-nanograms of fungal DNA. Relative to values measured by qPCR, both the time-varying and fixed temperature models overestimated late hibernation pathogen load (Figure 6A). However, the time-varying model of pathogen growth had significantly lower deviance from qPCR-measured values than the fixed temperature model, which was parameterized using only early winter roost microclimate data ($\beta = -4.516 \pm 0.862$ SE, $p < 0.001$; Figure 6B). Together, this suggests that incorporating information on host microclimate use over the course of infection can improve our understanding of the host-pathogen dynamics of white-nose syndrome, but that additional, potentially pathogen-reducing processes may also influence infection severity.

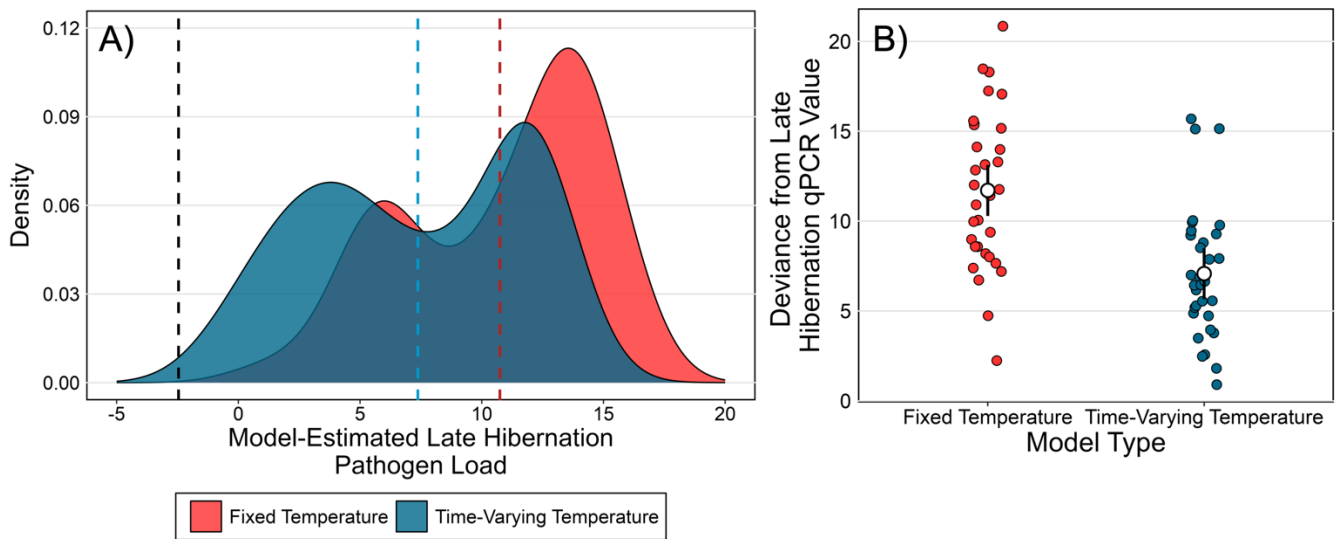


Figure 6: **A)** Density plot of model-estimated late hibernation pathogen loads. The blue density curve corresponds to the estimates produced by the time-varying temperature model of pathogen growth (blue dashed line corresponds to mean estimate). The red density curve corresponds to the estimates produced by the fixed temperature model of pathogen growth (red dashed line corresponds to the mean estimate). The black dashed line corresponds to the mean, qPCR-measured late hibernation pathogen load value on bats used in this study. **B)** Deviance of each model-estimated late hibernation pathogen load value from qPCR-measured values by model type. Red and blue points correspond to the deviance of the fixed temperature and time-varying temperature models, respectively. White points and error bars correspond to model-estimated mean deviance values and one standard error.

Discussion

Our data on the overwinter microclimate use and arousal behavior of bats with WNS revealed important and novel insights into bat-WNS pathophysiology. First, re-constructing the pathophysiological pipeline from early winter microclimate selection to infection severity, arousal frequency, and weight loss, revealed a previously undescribed environmental interaction and potential mechanism of host survival with disease. Specifically, we found an interaction between arousal frequency and microclimate temperature in driving overwinter weight loss. However, regardless of arousal frequency, bats with WNS may be able to modify the amount of weight they lost over winter to disease by altering the length of their arousal bouts from torpor.

Finally, comparing modeling approaches to pathogen growth dynamics suggested that overwinter microclimate use is an important contributor to host-pathogen interactions, but exploration of additional, potentially pathogen-reducing processes is warranted. Ultimately, by collecting host microclimate use data over the course of months-long infections, we discovered elements of environmental dependence of pathophysiology not otherwise detectable through snapshot approaches to characterizing environment-disease associations.

As has been described previously, we found that early hibernation roosting temperature was consequential on early hibernation pathogen load, with warmer temperatures resulting in more severe early infections (Grimaudo et al., 2022; Hopkins et al., 2021; Langwig et al., 2016). In turn, early hibernation pathogen load appeared to exacerbate arousal frequency, which has been previously associated with WNS (Lilley et al., 2016; Reeder et al., 2012; Warnecke et al., 2012). Arousal frequency interacted with mean torpor bout temperature, or the average roosting temperature of a bat over the course of its hibernation period, to drive overwinter mass loss, a potential indicator of WNS disease severity (Cheng et al., 2019; Lilley et al., 2016; McGuire et al., 2017; but see Langwig et al., 2023). Bats that tended to roost in relatively warm microclimates had a strong association between arousal frequency and mass loss, whereas those bats that roosted in relatively cold environments did not, indicating they lost similar amounts of weight regardless of how often they aroused from torpor. This novel signature of environmental dependence of disease may be the result of differing energy budgeting strategies of hibernation. First, we found that bats that started hibernation at a lighter body mass also tended to be those that roosted in colder microclimates, an important covariate of the previously described interaction. This could be because torpid metabolic rate is minimized under colder conditions (Geiser, 2004, 1988), and selecting such environments could be a strategy for lighter bats to

minimize additional costly energy expenditures (Boyles et al., 2007). In turn, those same bats spent less time awake during bouts of arousal from torpor than bats that tended to roost in relatively warmer microclimates. Therefore, because there was a positive association between arousal bout length and overwinter mass loss, it appears that lighter bats modified the amount of weight they lost over the course of their infection by shortening the length of their arousal bouts rather than making fewer arousals. This is further corroborated by previous findings illustrating that lighter bats with WNS lose more weight over hibernation (Langwig et al., 2023). Because an unsustainable rate of overwinter fat depletion and weight loss is believed to contribute to WNS mortality (Warnecke et al., 2013, 2012), this may represent a form of disease mitigation if bats are able to behaviorally reduce the energetic costliness of WNS-associated increases in arousal frequency (Råberg et al., 2009; Read et al., 2008). Importantly, this potential mechanism need not have evolved in response to WNS, but rather it could have been a mechanism of surviving the energetic demands of hibernation prior to the arrival of the disease (Czenze et al., 2017). Further, these data suggest that arousing from torpor offers a high enough fitness gain to justify burning available energy reserves. Heavy bats, which tended to roost in warmer locations, spent more time awake during arousals and subsequently burned through their fat reserves at a quicker rate. Additional research is warranted into the fitness advantage offered by extended arousal bouts, which occur even in the context of a novel infectious disease that strains the delicate balance of hibernation energetics.

Our pathogen growth modeling approach illustrated that overwinter microclimate use is an important factor in driving WNS infection dynamics. Compared to a model parameterized to reflect common snapshot approaches and which correspondingly assumed bats remained in the same microclimate from early to late hibernation, the time-varying pathogen growth model,

which was parameterized using microclimate use data from individual bats, more closely predicted late hibernation pathogen load. This indicates that overwinter microclimate use by bats is an important factor in subsequent infection dynamics. However, both models over-predicted late hibernation pathogen load, additionally indicating that other, potentially pathogen-limiting processes are also influential in this system. Our modeling approach did not allow for negative values of pathogen growth, sources of which could include host resistance or immune processes. Future research directions should include expanding such modeling frameworks to explore potential contributions of additional, pathogen-limiting processes in driving observed infection dynamics. Nevertheless, our modeling approach illustrates that snapshot classifications of environmental use by individual hosts will fail to capture important environmental influences in infection dynamics, information critical to the successful mitigation of emerging infectious diseases and conservation of the host species.

As emerging infectious diseases continue to threaten the conservation of diverse host taxa, it is essential to characterize infection dynamics quickly and effectively. Often, aspects of the dynamics of novel infectious diseases are environmentally dependent, information useful to mitigating their impacts (Bailey et al., 2018; Beck-Johnson et al., 2013; Ben-Horin et al., 2013; Cohen et al., 2017; Grimaudo et al., 2022; Hopkins et al., 2021; Langwig et al., 2016; Paull and Johnson, 2011; Shapiro et al., 2017). However, we illustrate here that incorporating data on environmental conditions present over the course of infection in individual hosts offers important insights into diseases processes not observable through common snapshot sampling approaches. Ultimately, the effectiveness of intervention strategies to mitigate the impacts of emerging infectious diseases of wildlife will depend on our understanding of their infection and disease

dynamics, which can be improved through longitudinal studies of infection processes in host species.

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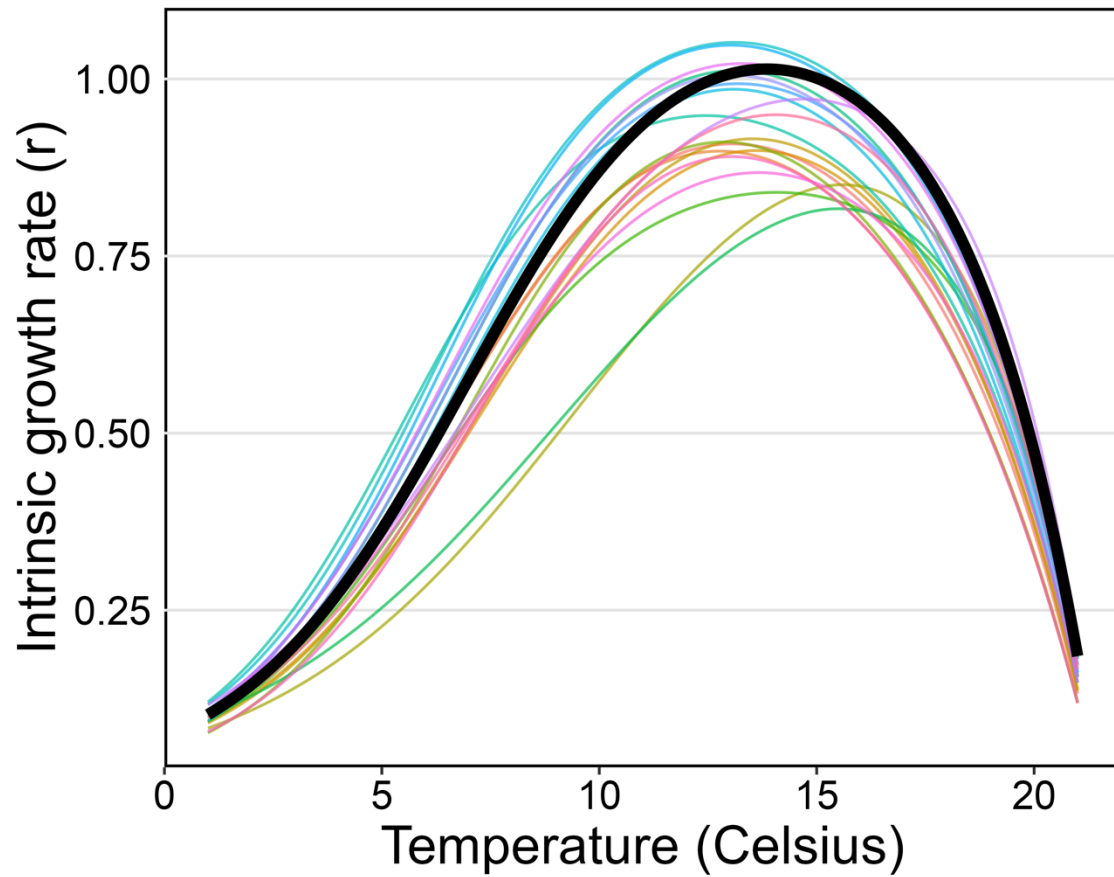
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Supplementary Information

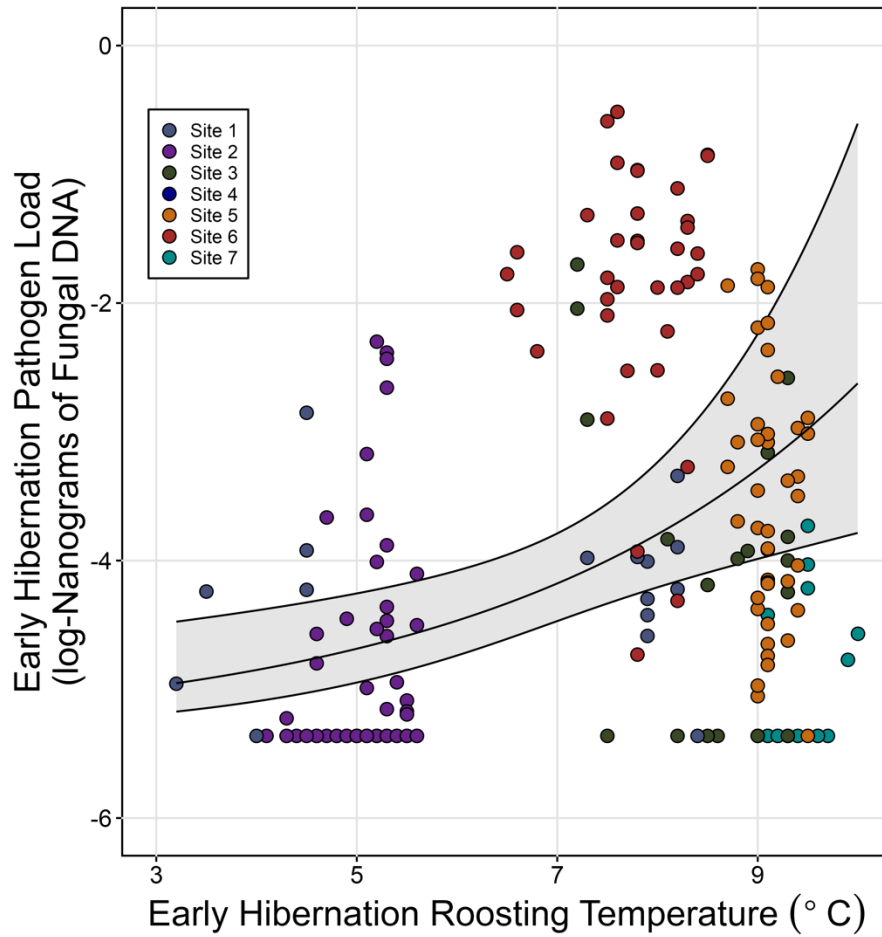
Supplemental Figures



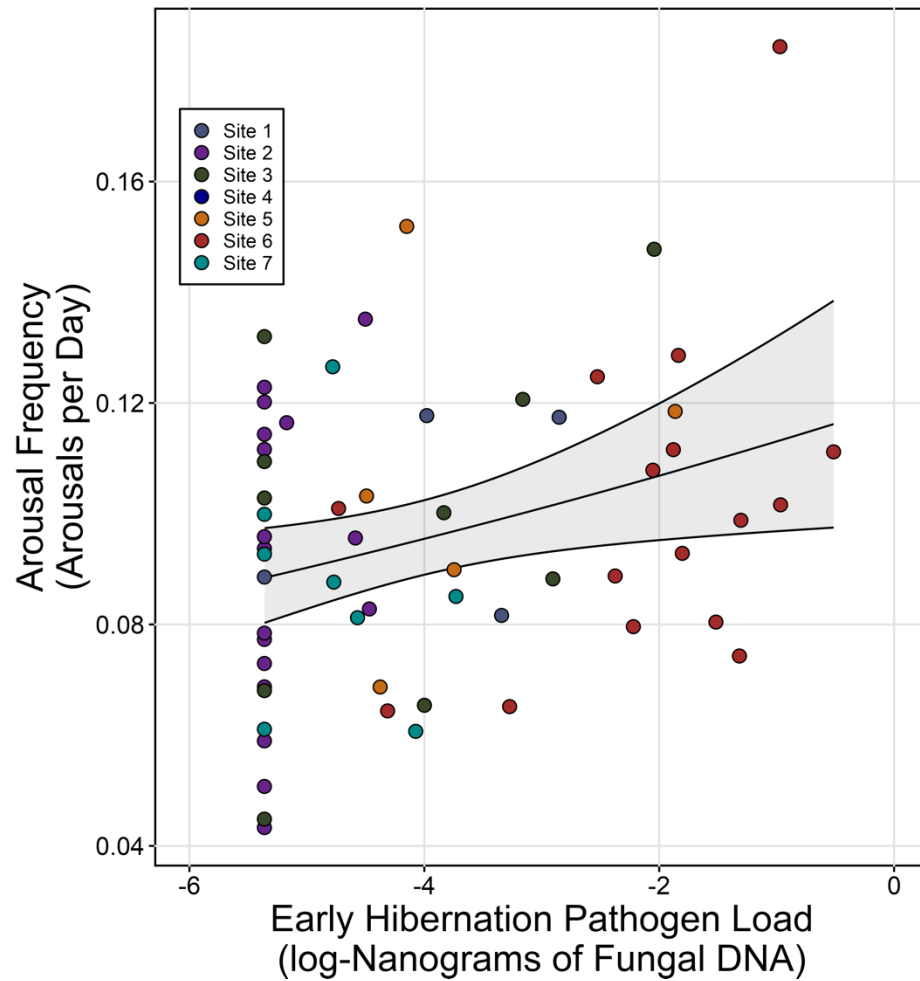
Supplemental Figure 1: Illustration of the temperature-sensitive data loggers we deployed on the backs of 287 individual hibernating little brown bats (*Myotis lucifugus*). The data loggers recorded the roosting microclimate temperature of the hibernating bats on regular intervals, capturing both torpor and arousal behavior.



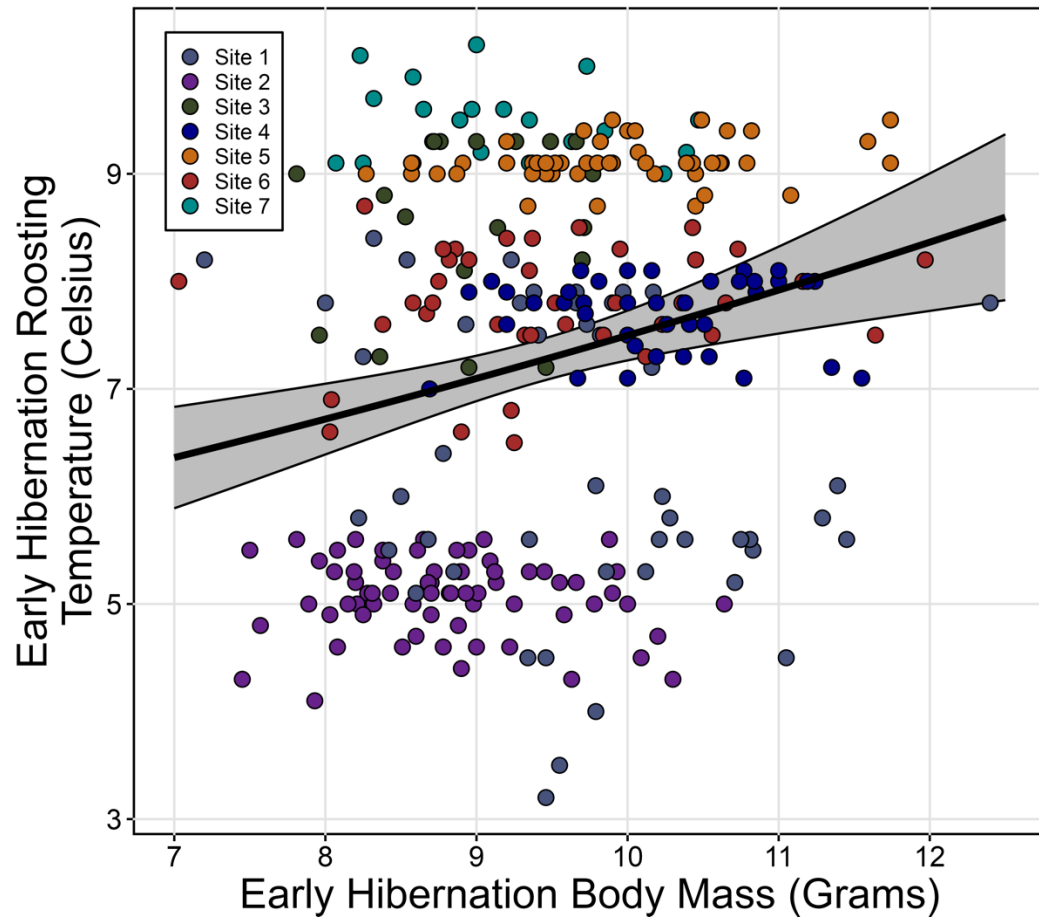
Supplemental Figure 2: Logan-10 thermal performance curve of the intrinsic rate of growth of *P. destructans*. Different lines correspond to the curves parameterized for different contemporary isolates of the pathogen, and the bold black line corresponds to a curve parameterized using the median parameter values of the fitted curves.



Supplemental Figure 3: Association between early hibernation roosting temperature and early hibernation pathogen load as measured by qPCR. Points are colored by site ID and the solid line and shaded ribbon indicate model estimate ± 1 standard error from the predicted mean.



Supplemental Figure 4: Association between early hibernation pathogen load and arousal frequency, or the number of arousals per day. Points are colored by site ID and the solid line and shaded ribbon indicate model estimate $\pm$ 1 standard error from the predicted mean.



Supplemental Figure 5: Association between early hibernation body mass and the temperature of roosting location during the early hibernation sampling event. Points are colored by site ID and the solid line and shaded ribbon indicate model estimate $\pm$ 1 standard error from the predicted mean.

Supplemental Tables

Supplemental Table 1: summary of the distribution of iButton data loggers by site, state, model, and sex of little brown bat.

Site	State	Num. Deployed DS1921G	Num. Deployed DS1922L	Num. Male MYLU	Num. Female MYLU
Site 1	Michigan	25	25	37	13
Site 2	New York	35	34	55	14
Site 3	Wisconsin	5	15	12	8
Site 4	Illinois	19	20	26	13
Site 5	Illinois	24	25	31	18
Site 6	Michigan	20	20	21	19
Site 7	Wisconsin	5	15	18	2

Supplemental Table 2: Parameter values of the Logan-10 thermal performance curve used in the pathogen growth model.

Parameter	Value
a	1.167
c	15.359
p	0.389
T_{max}	21.5
$T_{optimal}$	18.671

Chapter 5 – Conclusions

Summary and Synthesis

In this dissertation, I explore variation in how hosts respond to novel and emerging infectious diseases from the individual to population level. Utilizing field observational and experimental approaches as well as population modeling and simulation techniques, I disentangle wide variation and complex interactions in driving how bat populations of North America have responded to white-nose syndrome (WNS), an emerging infectious disease. Together, this research describes complex processes occurring at the scale of individual hosts to whole populations that ultimately drive infectious disease dynamics. The findings here offer information valuable to the successful conservation of host species threatened by emerging infectious diseases.

In Chapter 2, I examine the relative contribution of host-pathogen coevolution and environmental dependence of disease in driving the stabilization of little brown bat (*Myotis lucifugus*) populations following WNS emergence. I found that while host traits favorable to surviving WNS have evolved in these unique populations, they do not offer blanket protection across all environmental conditions. Rather, there exists a “Goldilocks” zone of cold temperature and intermediate humidity conditions within which adaptive host traits drive population stabilization, but outside of which high disease severity and mortality still occur. These findings offer important insight into the mechanistic underpinnings and limitations of the adaptive host trait that has evolved in this population but fall short of identifying its specific form. Interestingly, I observed high disease severity and mortality in dry conditions, under which I had predicted high survival due to the negative association between humidity and pathogen growth. Further, this result appeared to be driven by the inability of caged experimental bats to select

roosting microclimates, as free-flying bats in the same site did not display a similar degree of disease severity. Together, Chapter 2 contributed substantially to both our understanding of WNS and infection disease dynamics as well as the list of questions to motivate future research.

While I experimentally demonstrated evidence that adaptive host traits have evolved in persisting populations of little brown bats, the precise trait has yet to be identified and is not necessarily the only trait driving survival. However, some clues point to the potential mechanistic underpinnings of whatever that trait(s) may be. First, following initial infection in early hibernation, I observed a decline in the rate of pathogen growth, even leading to the *reduction* of infection intensity by late winter in some cases. As I articulated thoroughly in Chapter 2, this pattern is what would be expected if host resistance had evolved, a catch-all term that refers to mechanisms that actively reduce pathogen growth and infection intensity. Such mechanisms are diverse and can include immune responses, the facilitation of the growth of microbes inhibitory towards *P. destructans* in the host microbiome, and behavioral mechanisms of infection reduction such as grooming or thermoregulatory processes (e.g. behavioral fever). Whatever the mechanism may be, I have also shown that its efficacy is dependent on environmental conditions, another clue as to its specific form as processes such as immune function can be thermally dependent. Future research, potentially in the form of experimental infection trials, is warranted to identify the exact trait that has adaptively evolved in little brown bat populations, information that would be useful to their conservation.

The high disease severity and mortality observed in experimental bats in the dry persisting site in Chapter 2 was unexpected and presents another direction for future research. Pathogen growth experiments in-culture clearly indicate that *P. destructans* grows optimally under high humidity conditions. Further, at a population level, colonies of bats in relatively wet

hibernacula underwent more severe declines during the WNS epidemic than those in relatively dry hibernacula. Together, these observations would suggest dry conditions to be ideal for surviving WNS, which I predicted to be a primary result of Chapter 2. However, we observed the opposite, in which despite the low pathogen load in the dry persisting site, tissue invasion was exacerbated and resembled that observed in the warmest and wettest conditions. I hypothesize that this is the result of the moisture requirements of *P. destructans*. Given that there is little available water in dry ambient conditions, the only way for the pathogen to satisfy its moisture requirement could be to re-appropriate water from the tissue of the host. Under particularly dry conditions, then, we might expect tissue invasion to be exacerbated despite pathogen load being low as detected by swab sample, as the pathogen has transitioned from a superficial infection strategy to infecting deeper regions of the host epidermis. Additional research is needed in identifying the precise placement of humidity conditions in the pathophysiological pathway of WNS. Furthermore, free-flying, non-caged bats in the same site sampled at the same point in late hibernation had significantly lower tissue invasion than the caged experimental bats. This suggests that microclimate selection plays an important role in WNS dynamics, as the experimental bats were unable to select their roosting microclimate and thereby may have experienced more severe disease. In Chapter 4, I examined roosting microclimate data to explore this potential aspect of WNS dynamics, but the role of movement deserves further exploration in the future. Potentially, a similar experimental design to that in Chapter 2 could be used but incorporate the movement of bats between cages in different microclimates over the course of winter as the experimental premise. Regardless of study design, the movement behavior of bats during hibernation and subsequently their roost microclimate selection represents a deep pool of potential future research.

In Chapter 3, I describe variation in population-level impacts of WNS in the Indiana bat (*Myotis sodalis*), explore potential environmental and demographic drivers of population response, and simulate future extinction processes. I find substantial variation in colony-level responses to WNS in this species, with severe declines and high extirpation risk occurring in cold hibernacula with small initial colony sizes, although the effect of temperature wanes as epidemics progress. Interestingly, the association between hibernaculum temperature and colony response was opposite that of the sympatric little brown bat. Further, simulation of extirpation processes suggest that most WNS-driven Indiana bat colony extirpations that will occur already have, with most future extirpations primarily occurring in the smallest colonies.

Chapter 3 provided a plethora of new information on how Indiana bats have been impacted by WNS and how the disease dynamics vary in a multi-host community. The association between hibernaculum temperature and colony decline and extirpation risk was unexpected, as my results demonstrate that the coldest hibernacula were associated with the greatest WNS impacts, the opposite pattern observed in little brown bats. Why these species display opposite relationships with temperature conditions remains unclear, though I speculate in Chapter 3 that it could be driven by differences in their pathophysiological pathways. Specifically, this pattern could arise if the premature depletion of fat reserves due to increased arousals from torpor primarily drives mortality in the Indiana bat while water loss and dehydration drives mortality in the little brown bat. This hypothesis would be directly testable in an experimental infection setting in both species, paired with host respirometry and physiological data. Alternatively, this result may arise from more ecological mechanisms. While I did not detect an effect of little brown bat colony size on rates of colony growth in Indiana bats in their shared hibernacula, it is possible that the growth or decline of one population (or that of other

species present) could have corresponding effects in the other. Mechanisms such as competitive release could result in this pattern and might be explored in the future. Finally, while the results of my population simulation show an accumulation of Indiana bat colony extirpations, this may not reflect their long-term status. Once a colony is extirpated, we currently have no understanding of if the hibernaculum will remain devoid of bats or if it will be re-colonized. Further, what the rate of that re-colonization will be and its determinants are unknown, a question deserving future research and with conservation implications of high importance.

Finally, in Chapter 4, I explore how variation in microclimate use by individual little brown bats can influence the pathophysiology of WNS. I find that the temperature conditions in which bats roost during hibernation is consequential for the infection severity, but that individuals may mitigate their disease by behaviorally shortening WNS-driven arousal bouts, conserving critical overwinter fat stores and potentially improving their chances of survival. Furthermore, individual-based pathogen growth models parameterized using host microclimate use data suggest that microclimate selection is consequential for overwinter pathogen growth, improving predictability of on-host pathogen dynamics compared to common “snapshot” approaches to exploring environment-disease associations. Collectively, this work improves our understanding of the dynamics of white-nose syndrome as well as the drivers and consequences of variation in host population responses to emerging infectious diseases.

While the results of Chapter 4 provided important insight into the interaction between the temperature of bat roosting microclimates and the pathophysiology of WNS, I did not explore these processes with humidity. The results of the experiment in Chapter 2 suggested that humidity is an important component of infection and disease severity, but the limitations of microclimate logger technology and design prevented me from tracking the overwinter roost site

humidity of bats in the same way I tracked their thermal profiles. However, there is theoretical potential for humidity to similarly influence WNS dynamics. For example, if bats begin hibernation in the relatively warm and wet sections of hibernacula early in hibernation, these conditions will hypothetically exacerbate early pathogen growth. If they then transition to the cold and dry sections by late winter, which is what is typically observed, then the dry humidity conditions should reduce pathogen growth but potentially exacerbate tissue invasion in late winter. If this forces the pathogen to shift its strategy from superficial infection to deeper infection of the epidermis to satisfy moisture requirements, then there could be consequences on the disease severity and survival probability of the individual bat. Further, if individuals vary in how they select roosting microclimates, including for humidity, then there might be corresponding variation in these environment-pathophysiology interactions. Future research might prioritize exploring the role of humidity and its placement in the pathophysiological pathway of WNS.

Furthermore, while Chapter 4 identified potential new interactions in the WNS pathophysiological pathway, there is room for uncertainty around the directionality of the associations. As the design of this study was observational in nature, I relied on correlational data to link observed microclimate use, behavior, and WNS infection and disease severity, with the associated limitations in my ability to deduce the directionality of their relationships. To fully and confidently characterize the pathophysiological pathway of WNS, experimental infection studies paired with host physiology data are required, a potential direction for future research. This experimental design might be further paired with experimental variation of temperature and humidity conditions to explore questions around host microclimate variation and its interaction with WNS pathophysiology.

In writing this dissertation, I have attempted to contribute to our theoretical understanding of infectious disease processes and elucidate ecological mechanisms by which they produce variation in host populations. However, the primary objective and motivation of this work is to inform and advocate for conservation measures to mitigate the impacts of emerging infectious diseases to vulnerable populations. While emerging infectious diseases are among innumerable other threats to wildlife populations in the Anthropocene, human activity and globalization is causing them to emerge at an unprecedented frequency, threatening host populations across the globe and tree of life. I hope that the work contained within this dissertation might be instrumental in applying creative and effective techniques to protect not just bats from white-nose syndrome, but all wildlife populations threatened by novel pathogens. As being the species often responsible for the emergence of novel infectious diseases, we are also that responsible for their mitigation, and I hope the work contained herein will contribute to that end.