Changing Relationship Between Temperature and Pathogen Growth on Bats with White-nose Syndrome

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ACADEMIC ABSTRACT

Emerging infectious diseases (EID) pose significant threats to biodiversity. Human influence over the environment has increased opportunities for the introduction of novel pathogens to naïve hosts, potentially leading to host extinction. Understanding mechanisms of host persistence is critical for effectively conserving species affected by EIDs. Our study investigated the disease dynamics of white-nose syndrome (WNS), caused by the fungal pathogen *Pseudogymnoascus destructans* (*Pd*), in little brown bats (*Myotis lucifugus*) across a spatiotemporal gradient. We explored the relationship between bat roosting temperatures and *Pd* growth rates across three phases of pathogen invasion comprising years since WNS has been present at sites: invasion (0-3), established (4-8), and endemic (9+ years). Data used by this study comes from a combination of field-based data collection in New York where WNS has been present the longest and data from a long-running project which includes from other locations in the Northeast and Midwest regions of the United States. Our results reveal a weakening interaction between temperature and fungal growth rates time with WNS progresses. We additionally observed a decrease in early hibernation fungal loads and variation in infection prevalence over time, suggesting the onset of a coevolutionary relationship between bats and *Pd*. This study highlights the importance of investigating changing disease dynamics when understanding the reasonings for host persistence.

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PUBLIC ABSTRACT

Emerging infectious diseases threaten species with the risk of extinction. Human activities have altered habitats which has increased the spread of new pathogens to vulnerable host populations. This research focuses on white-nose syndrome (WNS), an emerging disease caused by the fungal pathogen *Pseudogymnoascus destructans* (*Pd*). The arrival of *Pd* to North America resulted in widespread declines in little brown bat (*Myotis lucifugus*) populations, however, some populations persist at stable or growing rates. This study aims to investigate how the relationship between the growth rate of *Pd* and bat hibernation temperature may have changed over time. We used a combination of contemporary data collected in New York and a long-running dataset that documents the invasion and establishment of *Pd* across the Northeast and Midwestern regions of the United States to investigate fungal growth rates during different phases of *Pd* invasion: invasion, established, and endemic phases. Our results indicate the relationship between temperature and pathogen growth rate has weakened over time, suggesting potential changes in the host-pathogen relationship. Additionally, we found changes in fungal loads and infection prevalence throughout hibernation, suggesting the foundation of a coevolutionary relationship between bats and *Pd*. This research highlights the importance of understanding changes in disease dynamics to help understand how other species at risk of emerging infectious diseases may be able to persist.

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Chapter 1 – Introduction

Increases in the emergence of novel infectious diseases are a key threat to global biodiversity (Daszak et al. 2000). Over the past century, humans have radically changed the environment through development, natural resource extraction, and agriculture (Jones et al. 2008). Environmental changes, including habitat encroachment and fragmentation, have repercussions for habitats crucial for community health. Habitat encroachment increases the chances of human-wildlife interactions, which may also provide opportunities for the introduction of new pathogens to both susceptible and naïve hosts (Bradley and Altizer 2007). Further, habitat fragmentation may temporarily increase wildlife interactions which provides increased opportunities for interspecific and intraspecific pathogen transmission (Hochachka and Dhondt 2000, Patz et al. 2004).

When coupled with other ecological stressors, the introduction of novel pathogens may heighten the risk of extinction for some hosts (de Castro and Bolker 2005). Generally, factors that increase the probability of extinction include transmission modes that continue to facilitate pathogen transmission even after host populations have declined to low densities (de Castro and Bolker 2005). Prevailing theory suggests that as host populations decline due to disease, pathogen transmission should decrease as hosts become rare and experience fewer interactions, thus lowering transmission (Anderson and May 1991, McCallum et al. 2001, Lloyd-Smith et al. 2005). As the population of susceptible hosts diminishes, R_0, or the intrinsic rate of increase for a pathogen, also decreases, thereby allowing host populations to potentially avoid extinction by disease (Hochachka and Dhondt 2000). However, susceptible host population sizes are less critical if pathogen transmission is frequency-dependent. Under this scenario, pathogens continue to infect new hosts even as host populations become smaller due to interactions such as mating

or social behaviors (Anderson and May 1991, Augustine 1998, Altizer et al. 2003, de Castro and Bolker 2005, McCallum et al. 2009). Additionally, environmental pathogen persistence that is not host-dependent can increase the probability of host extinction as interactions between hosts and the environment sustain transmission as populations decline (Johnson and Speare 2003, de Castro and Bolker 2005, Hoyt et al. 2015). Additionally, for pathogens that infect multiple hosts that vary in mortality, less impacted hosts may continue to facilitate transmission to hosts that are more impacted (de Castro and Bolker 2005, Lips et al. 2006, Frick et al. 2010). However, in some host-pathogen systems, despite the presence of multiple attributes that may promote extinction, some hosts are able to persist with pathogens for unknown reasons (Woodworth et al. 2005, Epstein et al. 2016, Langwig et al. 2017). Ultimately, changes in the host, pathogen, or environment could facilitate host persistence with disease. Therefore, effectively conserving species threatened by diseases requires an understanding of the mechanisms that enable species persistence (Langwig et al. 2015c).

Hosts may use a variety of mechanisms to avoid extinction. Selective pressure exerted by a pathogen may select for resistant or tolerant traits within host populations (e.g. evolutionary rescue) (Roy and Kirchner 2000, Gomulkiewicz and Shaw 2013, DiRenzo et al. 2018). Resistance refers to the physiological ability of a host to decrease pathogen growth rate, in turn reducing disease severity (Roy and Kirchner 2000, Raberg et al. 2007). In contrast, tolerance refers to host ability to reduce pathogenic damage whereby enduring high pathogen loads (Roy and Kirchner 2000, Raberg et al. 2007). The selection for these traits is not mutually exclusive and may be present in varying degrees within populations. However, resistance and tolerance are negatively correlated with one another which suggests trade-offs between the two traits (Raberg et al. 2007).

Pathogens may also undergo selective pressure that may also result in host persistence. Pathogens that cause severe disease may reduce populations quickly, and in turn, reduce transmission and pathogen fitness (known as the transmission-virulence trade-off) (Anderson and May 1982). In this scenario, if hosts develop immunity after infection or if the susceptible population is not replenished quickly enough for sustained transmission, pathogen fitness (the number of new hosts infected) is reduced. Therefore, the trade-off hypothesis predicts that transmission and pathogen fitness are optimized at intermediate levels of virulence to balance the costs of replication and damage done to hosts (Anderson and May 1982, Alizon et al. 2009). Evidence supporting the trade-off hypothesis was observed during the introduction of a highly virulent pathogen to cull invasive European rabbits (*Oryctolagus cuniculus*) in Australia (Fenner and Woodroofe 1965). The viral pathogen initially reduced rabbit populations by >95%, but the virus gradually evolved less virulent traits (Fenner and Woodroofe 1965). Additionally, rabbits also evolved resistance, establishing a long-term coevolutionary dynamic between the host and pathogen (Alves et al. 2019).

Environmental conditions also may contribute to host persistence. Hosts may find refuge from pathogens by selecting environments that reduce the severity of disease or reduce transmission. Examples of this type of refuge involve habitats that restrict the replication of a pathogen, such as in extreme temperatures or altitudes (Andre et al. 2008, Mosher et al. 2018, Hopkins et al. 2021). Hawaiian honeycreepers find refuge from the protozoan parasite *Plasmodium relictum,* which is responsible for avian malaria, by selecting higher altitudes (van Riper et. al 1986, Woodworth et. al 2005). The mosquito vector's thermal tolerance is not compatible with the cooler temperatures found at higher altitudes, allowing honeycreepers to avoid transmission and persist in this refuge (van Riper et. al 1986, Woodworth et. al 2005).

Additionally, certain environments may provide refuge through interactions that increase host survival. First, the environment may interact with hosts to enhance host survival by allowing for more effective immune responses against pathogens (Lorch et. al 2016). One example of this interaction can be found in snakes with snake fungal disease (*Ophidiomyces ophiodiicola*) where hosts have been documented to spend more time basking in the sun, which likely interacts with their physiology to help mount a more effective immune response (Puschendorf et. al 2011). Second, the environment may also directly interact with pathogens to inhibit replication, which may aid in host survival (Langwig et. al 2012). As an example, some amphibian populations show higher survival with chytridiomycosis (*Batrachonchytrium dendrobatitis*) in warmer and drier environments, which inhibits pathogen replication (Rollins-Smith et al. 2011).

White-nose syndrome (WNS), a disease of hibernating bats (Blehert et al. 2009, Gargas et al. 2009), was predicted to cause host extinctions (Frick et al. 2010), but some host populations continue to survive with disease for unclear reasons. White-nose syndrome is caused by a fungal pathogen, *Pseudogymnoascus destructans* (*Pd*), (Lorch et al. 2011, Warnecke et al. 2012) and was likely introduced to New York in the early 2000s through human activity (Drees et al. 2017). This pathogen originated in Eurasia where it likely coevolved with bats for thousands of years and where transmission is reduced compared to North America (Drees et al. 2017, Palmer et al. 2018, Hoyt et al. 2020). Upon the detection of *Pd* in North America during the 2006-2007 winter, mass mortalities of bat populations led to widespread declines and changes in community composition (Frick et al. 2010, Ford et al. 2011, Langwig et al. 2012, O'Keefe et al. 2019). As of 2024, *Pd* has now spread across 43 states in the U.S. and 9 Canadian territories (White-nose Syndrome Response Team 2024).

Bats first become infected with *Pd* during late fall when they return to the caves and mines that they use for mating and hibernation (Langwig et al. 2015a). *Pseudogymnoascus destructans* is a psychrophilic fungus and can survive in the cool, stable environment of caves and mines (Gargas et al. 2009, Hoyt et al. 2015). As *Pd* replicates on bats, conidia are shed into the environment where it persists on substrates used by bats during hibernation, which has led to widespread contamination of hibernacula (Lorch et. al 2013, Hoyt et. al 2015). Consequentially, transmission is primarily driven by the environmental reservoir (Hoyt et al. 2015, Langwig et al. 2015a, Hoyt et al. 2020) although further transmission could also occur through direct bat-to-bat contact during mating (Thomas et al. 1979, Hoyt et al. 2020) or clustering behavior during hibernation (Hoyt et al. 2018).

During hibernation, bats lower their body temperature to ambient conditions within hibernacula which allows *Pd* to replicate and invade their epidermis and connective tissue (Meteyer et al. 2009, Langwig et al. 2015a, Langwig et al. 2016). Infections cause evaporative water loss and electrolyte imbalances that results in bats arousing more frequently from hibernation (Warnecke et al. 2012, Warnecke et al. 2013, Verant et al. 2014). Each arousal requires bats to utilize their limited energy reserves to increase body temperature, thereby depleting stored fat (Cryan et al. 2010, McGuire et al. 2017). Ultimately, the lack of resources to mitigate water loss and starvation frequently leads to mortality (Warnecke et al. 2012, Warnecke et al. 2013, Verant et al. 2014). If a bat survives hibernation to migrate to summer and spring habitats, it may be able to clear the infection in several weeks upon returning to regular euthermic conditions (Meteyer et al. 2011, Langwig et al. 2015a, Fuller et al. 2020). However, clearing the infection is energetically costly and may negatively impact reproductive success during the summer (Francl et. al 2012, Pettit and O'Keefe 2017).

Before *Pd* arrived in North America, little brown bats were one of the most abundant bat species on the continent (Langwig et al. 2012). Following the introduction of *Pd*, little brown bats declined by over 90% and many local to sub-regional populations have been extirpated (Frick et al. 2010). Because transmission appears to be density-independent (Langwig et al. 2017) and is driven through the environmental reservoir (Hoyt et al. 2015), bats still become infected each hibernation season despite the dramatic declines in population sizes (Langwig et al. 2015a, Hoyt et al. 2020). However, despite continued transmission, some populations have avoided extirpation and are persisting at stable or growing population growth rates (Langwig et al. 2017). These observations are notably present in New York, where *Pd* was originally introduced and sites have experienced WNS the longest (Hoyt et al. 2021).

The temperature of bat roosts inside hibernacula appears to be a key factor in allowing bats to persist with WNS. *Pseudogymnoascus destructans* grows optimally ~13 C ° (Verant et al. 2012), with slower growth at cooler temperatures. Consequentially, bats roosting at cooler temperatures have experienced less fungal growth, had higher individual survival, and experienced lower population declines (Langwig et al. 2012, Langwig et al. 2016, Hopkins et al. 2021). As a result, persisting bat populations now roost at temperatures that are ~1 C ° cooler than before disease arrival which likely reduces fungal growth (Hopkins et al. 2021).

Changes in roosting temperature alone do not fully explain bat persistence with WNS. Using epidemiological models to fit data collected from persisting and epidemic populations, bats in persisting populations experienced lower fungal growth rates than bats in epidemic, declining populations, consistent with the evolution of resistance (Langwig et al. 2017). In addition, bats from populations where WNS has been present for at least 10 years had higher

survival at warm temperatures compared to WNS naïve bat populations, providing further support for host population adaptations (Grimaudo et al. 2022).

Lastly, changes in the pathogen could contribute to differences in disease dynamics over time. North American *Pd* is clonal and isolates causing severe disease and population declines in some areas are genetically indistinguishable from isolates found on surviving persisting bats (Drees et al. 2017). However, phenotypic differences can occur in the absence of genetic differences, and preliminary observations suggests that *Pd*'s growth rate has changed at some temperatures since the arrival of WNS (Laggan et al. in prep). Ultimately, survival of little brown populations may not stem from a single independent change in the pathogen, host, or environment. Instead, these mechanisms might be interactive and collectively lead to host persistence. This study aims to explore host persistence by examining how some of these persistence mechanisms may have changed over time. Specifically, the goal of this research is to identify changes in the temperature-dependent pathogen growth rates on hosts by comparing across a spatio-temporal invasion gradient. The WNS system provides a unique lens to investigate these questions as the results could shed light on how disease dynamics may change over time in other emerging infectious disease systems.

Chapter 2 – Changing Relationship Between Temperature and Pathogen Growth on Bats with White-nose Syndrome

Introduction

Increases in emerging infectious diseases of wildlife pose a significant threat to the conservation of biodiversity (Daszak et al. 2000). Environmental modifications by humans have led to habitat alterations which provide increased opportunities for novel pathogen introductions to wildlife communities and have increased opportunities for interspecific and intraspecific pathogen transmission (Hochachka and Dhondt 2000, Patz et al. 2004, Bradley and Altizer 2007, Jones et al. 2008). Transmission modes that facilitate pathogen transmission at low host population densities may elevate the risk of host extinction (de Castro and Bolker 2005). If pathogen transmission is frequency-dependent, susceptible hosts continue to be infected as populations decline due to interactions such as mating or social behaviors (Anderson and May 1991, Augustine 1998, Altizer et al. 2003, McCallum et al. 2009). Additionally, environmental pathogen persistence may increase the probability of host extinction as hosts continue to interact with contaminated environments and become infected (Johnson and Speare 2003, Hoyt et al. 2015). Lastly, if pathogens can transmit to multiple hosts at varying degrees of mortality, less impacted hosts may serve as pathogen reservoirs and facilitate transmission to hosts that have experienced more mortality (Fenton and Pedersen 2005, Lips et al. 2006). Even though these mechanisms may increase extinction risk, some hosts predicted to face extinction now persist despite ongoing transmission from highly virulent novel pathogens (Fenner and Woodroofe 1965, Woodworth et al. 2005, Epstein et al. 2016, Langwig et al. 2017).

Host persistence can be attributed to changing conditions in hosts, pathogens, or environments, which may also act together to facilitate survival (Grimaudo et al. 2022). Host populations may experience evolutionary rescue through the selection of resistant traits, that reduce pathogen loads, or increase tolerant host traits, which in turn reduce physiological damage caused by the pathogen (Roy and Kirchner 2000, Raberg et al. 2007, Gomulkiewicz and Shaw 2013, DiRenzo et al. 2018). Additionally, hosts may alter their behavior through pathogen avoidance, isolation, and changes in social networks to ensure persistence (Loehle 1995). Highly virulent pathogens may also experience selective pressure if host populations are rapidly reduced through trade-offs between transmission and virulence, resulting in a reduction in virulence that enables survival of host populations and continued transmission (Anderson and May 1982, Alizon et al. 2009).

The environment is often a key factor in persistence in host-pathogen systems. First, hosts may utilize environments that enable persistence through mechanisms that indirectly increase host survival. For example, some amphibians susceptible to infection by a fungal pathogen, *Batrachochytrium dendrobatidis (Bd),* are documented to have reduced immune responses at cooler temperatures (Rollins-Smith et al. 2011). This physiological interaction with the environment allows *Bd* to replicate on hosts at cooler temperatures but encourages persistence in warm stable environments through appropriate immune responses (Rollins-Smith et al. 2011). Second, hosts may select environmental extremes to reduce pathogen virulence or transmission. For example, Hawaiian birds avoid the protozoan parasite (*Plasmodium relictum*), responsible for avian malaria, by selecting high altitude environments and thus temperatures lower than the mosquito vector's thermal tolerance (van Riper et al. 1986, Woodworth et al. 2005).

White-nose syndrome (WNS) was predicted to cause extinction in some hibernating bat species (Frick et al. 2010), but some host populations have persisted with disease for uncertain reasons (Blehert et al. 2009, Gargas et al. 2009). The disease is caused by the fungal pathogen *Pseudogymnoascus destructans* (*Pd*) which was likely transported from Eurasia to New York in the early 2000s through human activity (Lorch et al. 2011, Warnecke et al. 2012, Drees et al. 2017). Despite being responsible for mass mortalities in bat populations and dramatic changes to community composition (Frick et al. 2010, Ford et al. 2011), *Pd* transmission continues in surviving bat populations. Continued transmission is likely due to conidia shed from previous winter infections that persist on abiotic environmental substrates, resulting in a heavily contaminated environmental reservoir inside caves and mines that bats use for mating and hibernation (Hoyt et al. 2015, Langwig et al. 2015a, Hoyt et al. 2020). During hibernation, bats reduce their body temperature to ambient conditions which unfortunately allows the psychrophilic fungus to replicate and invade their epidermis and connective tissues (Gargas et al. 2009, Meteyer et al. 2009, Langwig et al. 2015a, Langwig et al. 2016). The increase in fungal loads over hibernation causes physiological imbalances, resulting in bats arousing more frequently (Warnecke et al. 2012, Warnecke et al. 2013, Verant et al. 2014). Bats must use their limited energy reserves to increase their body temperature with each arousal, eventually leading to the depletion of stored fat (Cryan et al. 2010, McGuire et al. 2017). Without resources to compensate for these losses, mortality often occurs (Warnecke et al. 2012, Warnecke et al. 2013, Verant et al. 2014). Additionally, WNS may cause bats to suffer exposure-related mortality if they emerge from hibernacula prematurely in search of resources (Blehert et. al 2009, Gargas et. al 2009).

The introduction of *Pd* to North America reduced one of the most continentally abundant bat species, little brown bats (*Myotis lucifugus*), by over 90% and extirpated many populations (Frick et al. 2010, Langwig et al. 2012). However, some populations have avoided extirpation and now survive at stable or increasing population growth rates despite ongoing infection (Langwig et al. 2017). These population trends are notably present in New York where *Pd* was originally introduced and populations have experienced WNS the longest (Hoyt et al. 2021). The roosting temperature of bats appears to be a key factor in persistence as populations have shifted their preferred roosting temperature by ~1 C° cooler (Hopkins et al. 2021). This shift in temperature reflects movement away from *Pd*'s thermal optimum of ~13 C°, which likely reduces fungal growth and increases survival (Langwig et al. 2012, Verant et al. 2012, Langwig et al. 2016, Hopkins et al. 2021). Given the established relationship between bat roosting temperature and pathogen growth rate, this study seeks to further explore persistence in little brown bats. The goal of this research is to identify changes in the temperature-dependent pathogen growth rate on hosts across a spatio-temporal gradient. The WNS system provides unique opportunities to answer these questions as results could shed light on how disease dynamics may change over time in other emerging infectious disease systems.

Methods

Existing Dataset

To investigate how contemporary fungal growth differed from historical fungal growth patterns, we used an existing dataset that spans a large temporal and spatial scale (Langwig et al. 2015b, Langwig et al. 2015c, Hoyt et al. 2016, Hoyt et al. 2018, Hoyt et al. 2020, Hopkins et al. 2021, Laggan et al. 2022, Hoyt et al. 2023, Kailing et al. 2023, Langwig et al. 2023). This dataset includes fungal loads collected from bats over the course of twelve years and across midwestern (MI, IL, WI) and eastern (NY and VA) states (Table 1).

Table 1: Overview of the data from the data used for this project. The invasion phase represents years when WNS was present from 0-3 years, the established phase represents years when WNS was present from 4-8 years, and the endemic phase represents 9+ years with WNS. Statewide total samples represent the sample size of fungal load swabs collected from each state.

State	Invasion Phase	Established Phase	Endemic Phase	Statewide Total Samples
NY	130	117	317	564
VA	154	43	11	208
MI	612	1135	0	1,747
IL	330	321	207	858
WI	1188	1807	0	2,995

Contemporary New York Data

In addition to the existing dataset, we also visited 6 sites in New York during the winter hibernation period of 2022-202. These hibernacula were selected based on the availability of historical New York data during the initial invasion of *Pd* (Langwig et al. 2015c) and based on

their proximity to the origin of the introduction of *Pd* to North America. White-nose syndrome was detected in these sites between 2006-2009, therefore at least 14 years elapsed by the start of this project (Frank et. al 2019, Szymanski et. al 2009). From pre-WNS, little brown bat populations in these sites declined dramatically (90%-99%) with the arrival of WNS but have since stabilized (Langwig et. al 2017). Three sites were abandoned deep cement mines (Williams Hotel Mine, Williams Lake Mine, Williams Preserve Mine), one was an abandoned iron mine (Fahnestock Mine), and the remaining two were natural Karst cave systems (Schoharie Cave, Hailes Cave). Each site was visited twice to collect on-bat fungal loads and record bat roosting temperatures: once during early hibernation (October-November 2022) and once during late hibernation (March 2023).

To obtain on-bat fungal loads, we sought to sample ~20 little brown bats opportunistically at each site. Bats were chosen randomly throughout the site to capture a range of hibernation temperatures. Each bat was swabbed with a moist sterile swab on their muzzles and wings (Langwig et al. 2015a). The swabs were preserved in RNALater and frozen at -80 C for later DNA extraction and quantification by qPCR (Muller et al. 2013). The temperature of the substrate adjacent to each bat was recorded to reflect the bat's roosting temperature. All bats were banded with a unique 2.9 mm Porzana aluminum band during the first visit in early hibernation for reidentification on our second visit. Additionally, we glued small pieces of reflective material on their heads to assist with finding the originally sampled bats in late hibernation. Data was collected under New York State Department of Environmental Conservation Scientific Collections permit #1808.

Statistical Analysis

All statistical analyses were conducted in RStudio version 2023.06.0 (R Core Team 2022) using the tidyverse, lme4, and glmmTMB packages (Bates et. al 2015, Brooks et. al 2017, Wickham et. al 2019). To examine on-bat fungal growth rates, I first calculated the change in fungal quantities on bats (e.g. fungal growth rate) on a population level according to each site and hibernation season. This was done by first grouping fungal load data by site, the winter season sampled, and the timing of our site visits (early or late hibernation) to calculate mean population-level fungal loads for early and late hibernation at each site and winter season. I then paired the early and late mean hibernation loads to calculate the days between sampling events. The mean fungal loads were then used to calculate weekly fungal growth rates on a $\log_{10}$ scale to ensure a normal distribution (Eq. 1).

$$Weekly\ Growth\ Rate = \log_{10}\left\{\left(\frac{Late\ Hibernation\ Loads}{Early\ Hibernation\ Loads}\right)^{\left[\frac{1}{\left(\frac{DaysBetweenSampling}{7}\right)}\right]}\right\} \quad \textbf{(Eq. 1)}$$

Additionally, I calculated individual bat's weekly fungal load changes using paired early and late fungal loads from marked recaptured individuals. The time between sampling events was also calculated for each individual. To account for instances when a bat was not infected during early hibernation but was infected during late hibernation, I added 0.0001 to all fungal loads in our data to ensure a rate would be able to be calculated (Zuur et. al 2009). Individual-level weekly fungal growth rates were then calculated using Equation 1.

I classified sites in accordance with their time since WNS arrival and examined this temporal scale on a factored level, a continuous series, or categorized within phases of invasion. I found that categorizing the number of years since WNS was present at each site into the following phases maximized the number of data points within each bin allowing for the best

balance between phases: invasion (WNS present for 0-3 years), established (WNS present for 4-8 years), and endemic (WNS present 9+ years), although patterns were qualitatively the same if I used data at a continuous level.

I used interactive linear models (R Core Team 2022) in our population-scale analysis with average weekly fungal growth rate as our response variable and average early hibernation temperature interacting with invasion phase as our predictor variables. I additionally used interactive linear mixed models (Bates et. al 2015) in our individual-scale analysis with early hibernation temperature of recaptured bats interacting with invasion phase as the predictor variables and weekly fungal growth rates as the response variable with the addition of site as a random effect to account for multiple individuals sampled within each site. Final models were selected based on AIC values, with lower values indicating higher model support (Burnham and Anderson 2002).

To explore possible explanations for changes in the temperature-fungal growth rate analysis, we also investigated how early hibernation on-bat *Pd* loads may have changed over time as changes in the total amount of pathogen on bats would also change growth rates. This analysis was completed on a population scale using a linear model with the phase of invasion as the predictor variable and the site-averaged early hibernation *Pd* load as the response variable.

Lastly, using data from NY where WNS has been present the longest, I also examined trends in fungal loads and infection prevalence over time on bats. To examine fungal loads, I used an interactive linear mixed model with fungal loads of individual bats as the response variable and hibernation date interacting with winter year as our predictor variables with a random effect for site. To examine infection prevalence, I used an interactive generalized linear

mixed model (Brooks et. al 2017) with a binomial distribution and a logit link with infection status (1|0) of individual bats as our response and hibernation date interacting with winter year as our predictor variables with a random effect for site.

Results

In early winter 2022-2023, we swabbed and banded 101 little brown bats in six New York sites. On our return visit in late hibernation, we recaptured 13 of the original 101 bats and swabbed an additional 76 bats. The existing dataset included a total of 6,271 unique swabs (Table 1) and 337 paired swabs during early and late hibernation from recaptured individuals.

Temperature – Pathogen Growth Rate Interaction

When examined on the population-level scale (Table 2), the on-bat growth rate of *P. destructans* exhibited a temperature-dependent relationship during the invasion phase with lower growth rates at cooler temperatures and higher growth rates at warmer temperatures (invasion β=0.00846 +/- SE: 0.0021, p-value<0.001, Figure 1). However, this relationship significantly weakened during the established phase (established versus invasion interactive coef =0.066714 +/- SE: 0.0261, p-value= 0.0119). Likewise, the weekly growth rate of *Pd* did not show a clear temperature-dependent relationship during the endemic phase (endemic slope β= 0.00481+/- SE 0.0039, p-value= 0.219 and did not differ from either the invasion phase (invasion versus established coef = -0.04529 +- SE 0.0336, p-value=0.181) or established (established versus endemic coef = 0.021427 +- SE 0.0339, p-value=0.529) phases.

Table 2: Statistical results of the temperature-dependent growth rate of *Pd* at the population-scale.

Table 2

Response variable: Weekly fungal growth rate – Population Scaled

Interactive linear model:

(population weekly growth rate) ~ (average early hibernation temperature) * phase

Model reference level: invasion phase

Term	*Estimate*	*Standard Error*	*P value*
Intercept	0.266494	0.018178	< 0.001
Invasion Phase (reference)	0.008455	0.002063	< 0.001
Established Phase	0.066714	0.026074	0.0119
Endemic Phase	0.045288	0.033608	0.1807

Model reference level: endemic phase

Term	*Estimate*	*Standard Error*	*P value*
Intercept	0.311781	0.028268	< 0.001
Endemic Phase (reference)	0.004810	0.003894	0.219
Invasion Phase	-0.045288	0.033608	0.181
Established Phase	0.021427	0.033889	0.529

When examined on an individual-level scale using recaptured bats, the weekly growth rate of *Pd* also has a significant temperature-dependent relationship during the invasion phase (invasion β=0.007338 +/- SE: 0.003, t-value=2.264, Figure 2). However, the relationship significantly weakened in the latter phases (established versus invasion coef=0.059754+/- SE: 0.0258, t-value= 2.312; endemic versus invasion coef= 0.082107+/- SE 0.0311, t-value= 2.639). However, unlike the population-level model, the temperature-dependent relationship in the individual-level model's endemic phase significantly weakened compared to the invasion phase

(invasion versus endemic coef = -0.082107 +/- SE 0.0311, t-value= -2.639) and marginally from the established phase (established versus endemic coef = -0.022352 +/- SE 0.02, t-value= -1.106) (Table 3).

Table 3: Statistical results of the temperature-dependent growth rate of *Pd* at the individual-scale.

Table 3

Response variable: Weekly fungal growth rate – Individual Scaled

Interactive linear mixed model:

(Individual weekly growth rate) ~ (average early hibernation temperature) * phase + (1|site)

Model reference level: invasion phase

Term	*Estimate*	*Standard Error*	*T value*
Intercept	0.277393	0.02698	10.282
Invasion Phase (reference)	0.007338	0.00324	2.264
Established Phase	0.059754	0.025843	2.312
Endemic Phase	0.082107	0.031108	2.639

Model reference level: endemic phase

Term	*Estimate*	*Standard Error*	*T value*
Intercept	0.359499	0.017863	20.126
Endemic Phase (reference)	-0.002208	0.002896	-0.762
Invasion Phase	-0.082107	0.031108	-2.639
Established Phase	-0.022352	0.020206	-1.106

Early Hibernation Loads

To standardize the timing of early hibernation sampling across all phases, we analyzed early hibernation loads sampled from bats in November. When compared to the initial invasion

phase (invasion intercept coef = -3.424 +/- SE 0.1159, p-value <0.001, Figure 3), early hibernation loads appeared to be similar during the established phase (established versus invasion coef = -0.281, +/- SE: 0.1675, p-value = 0.096) and endemic phase (endemic versus invasion coef = -0.3492 +/- SE 0.2834, p-value = 0.373) (Table 4).

Table 4: Results of site average on-bat fungal loads across phases of invasion during early hibernation.

Table 4

Response variable: Fungal loads

Linear model:

(fungal loads) ~ (phase)

Model reference level: Invasion phase

Term	*Estimate*	*Standard Error*	*P value*
Intercept	-3.424	0.1159	< 0.001
Established Phase	-0.281	0.1675	0.096
Endemic Phase	-0.3492	0.3903	0.373

New York Fungal Loads and Infection Prevalence Trends

There were six winter seasons available for this analysis, two seasons were dropped due to low sample sizes and a lack of sampling over the winter season. As a result, data from New York was available for analysis during the invasion and endemic phase. Bats began hibernation with higher fungal loads during the invasion phase (endemic versus invasion coef = -1.15636 +/- SE: 0.0539, t-value = -3.732) (Fig. 4). Increases in fungal loads occurred during each phase and more increases were seen during the endemic phase (Table 5), likely due to survival bias as bats die after reaching an infection threshold (Langwig et. al 2016). Infection prevalence did not

appear to differ between the invasion and endemic phase (endemic versus invasion coef: -0.8141 +/- SE 0.9583, p-value = 0.3956) (Fig. 5, Table 6). However, there was some support for decreasing prevalence during the winter of 2022-2023 compared to other years (Supp. Figure 2 Supp. Table 2).

Table 5: Results from changes in fungal loads at New York sites across phases of invasion.

Table 5

Response variable: Fungal load

Interactive linear mixed model

(fungal load) ~ (hibernation date* (phase) + (1|site)

Model reference level: invasion

Term	*Estimate*	*Standard Error*	*T value*
Intercept (Invasion early hibernation loads)	-3.49018	0.31173	-11.196
Invasion overwinter Pd load change	0.1196	0.05394	2.217
Endemic early hibernation loads	-1.15636	0.05394	-3.732
Endemic overwinter Pd load change	0.30893	0.06058	5.1

Table 6: Results from changes in infection prevalence at New York sites across phases of invasion.

Table 6

Response variable: Infection status (0 or 1)

Interactive generalized linear mixed model with a binomial distribution and a logit link:

(infection status) ~ (hibernation date) * (phase) + (1|site), family binomial

Model reference level: Invasion

Term	*Estimate*	*Standard Error*	*P value*
Intercept (Invasion early hibernation prevalence)	1.7623	0.9917	0.0756
Invasion overwinter prevalence change	0.1982	0.1855	0.2852
Endemic early hibernation prevalence	-0.8141	0.9584	0.3956
Endemic overwinter prevalence change	-0.1247	0.1924	0.5171

Figures

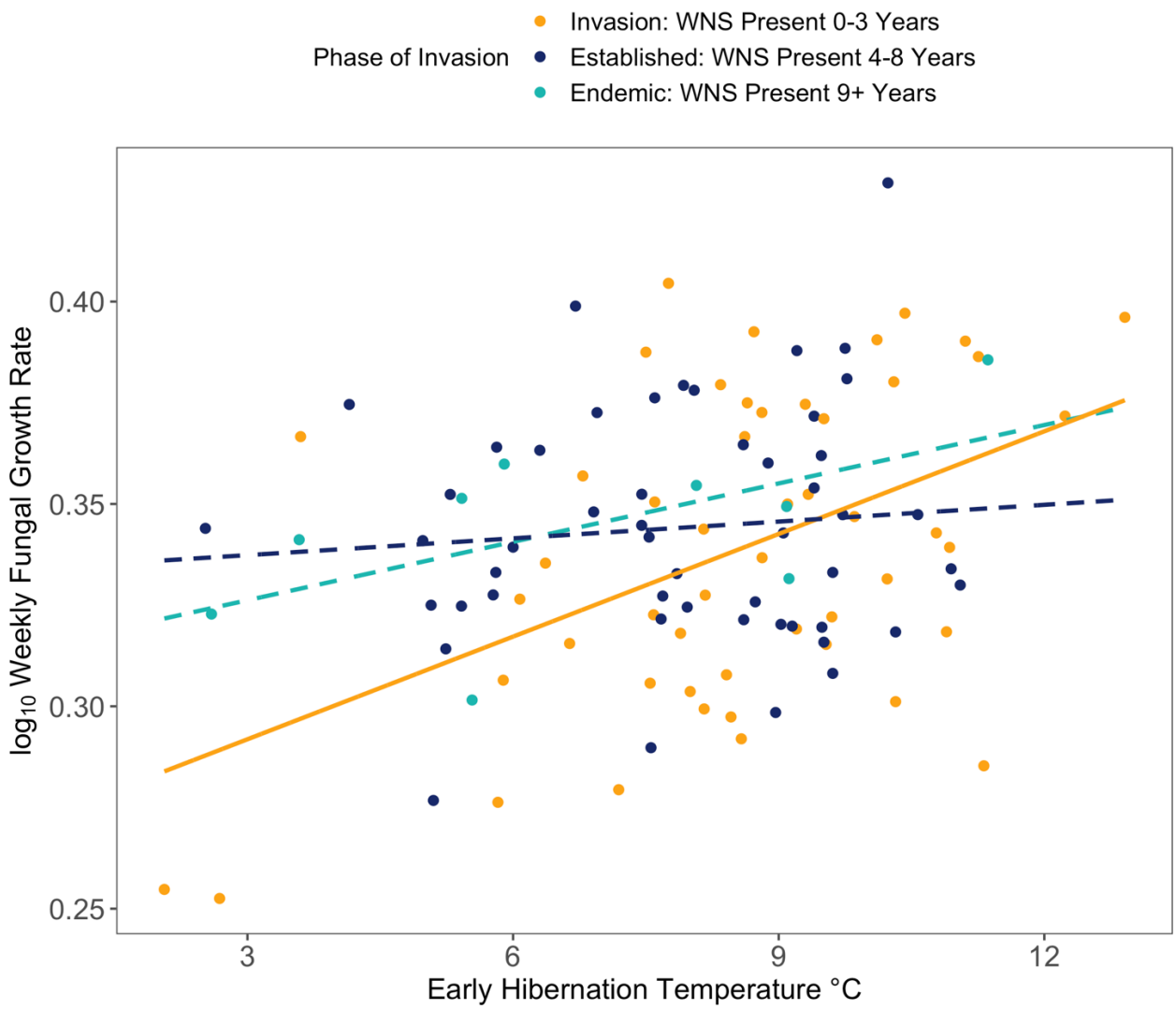


Figure 1. Weekly on-bat growth rate of *Pd* examined on a population-level scale. Solid line represents a significant temperature-dependent relationship while the dashed lines represent a non-significant temperature-dependent relationship. The growth rate is highly temperature-dependent during the invasion phase. As sites reach the established phase, the relationship between temperature and growth rate significantly weakens. The endemic phase appears to show an intermediate, but non-significant relationship between temperature and growth rate and is not statistically differentiated from the other two phases.

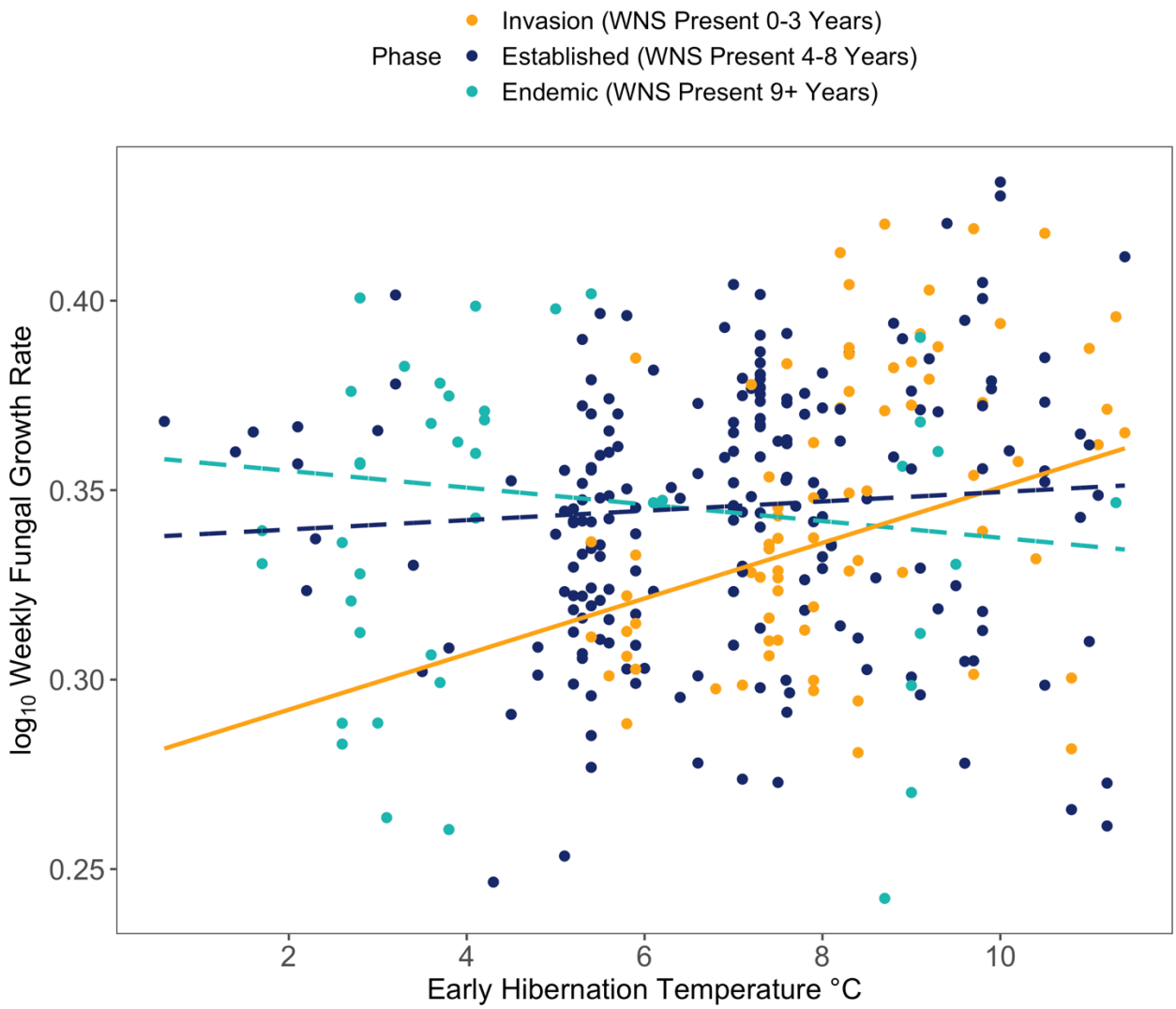


Figure 2. The weekly on-bat growth rate of *Pd* examined on an individual-level scale using marked and recaptured bats. Solid line represents a significant temperature-dependent relationship while the dashed lines represent a non-significant temperature-dependent relationship. The growth rate is highly temperature-dependent during the invasion phase. The established and endemic phases also show a significant weakening of the interaction between temperature and growth. Contrasting the population-scale, both the established and endemic phase are statistically different from the invasion phase. Note the trends in early hibernation temperature as each phase advances, indicating changes in preferred roosting temperatures.

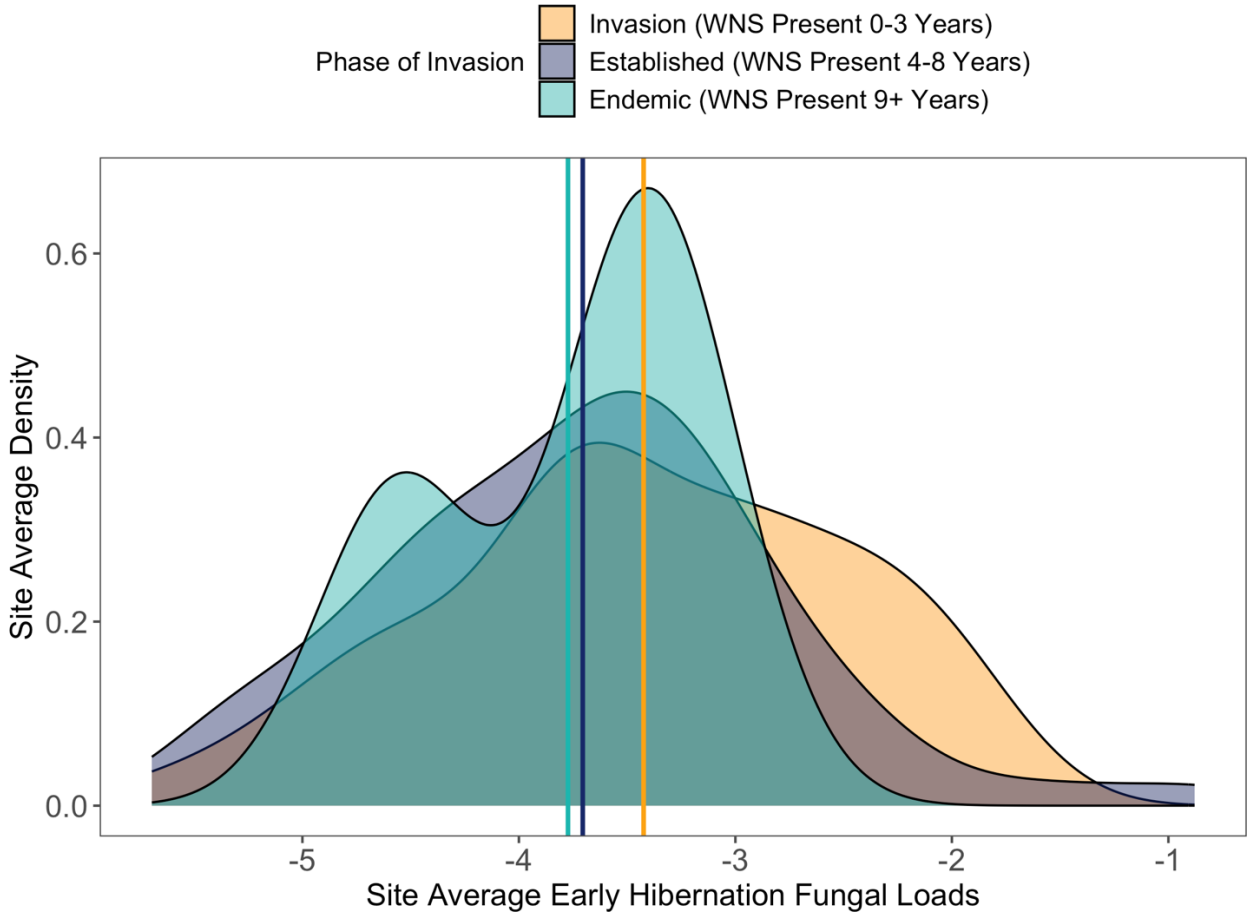


Figure 3. Early hibernation on-bat *Pd* loads examined on a population scale shown by density and by the average load by phase (vertical lines). Curved lines represent kernel density estimates as a smoothed histogram). Bats experience similar levels of *Pd* loads during all phases, suggesting no change in transmission from the environmental reservoir.

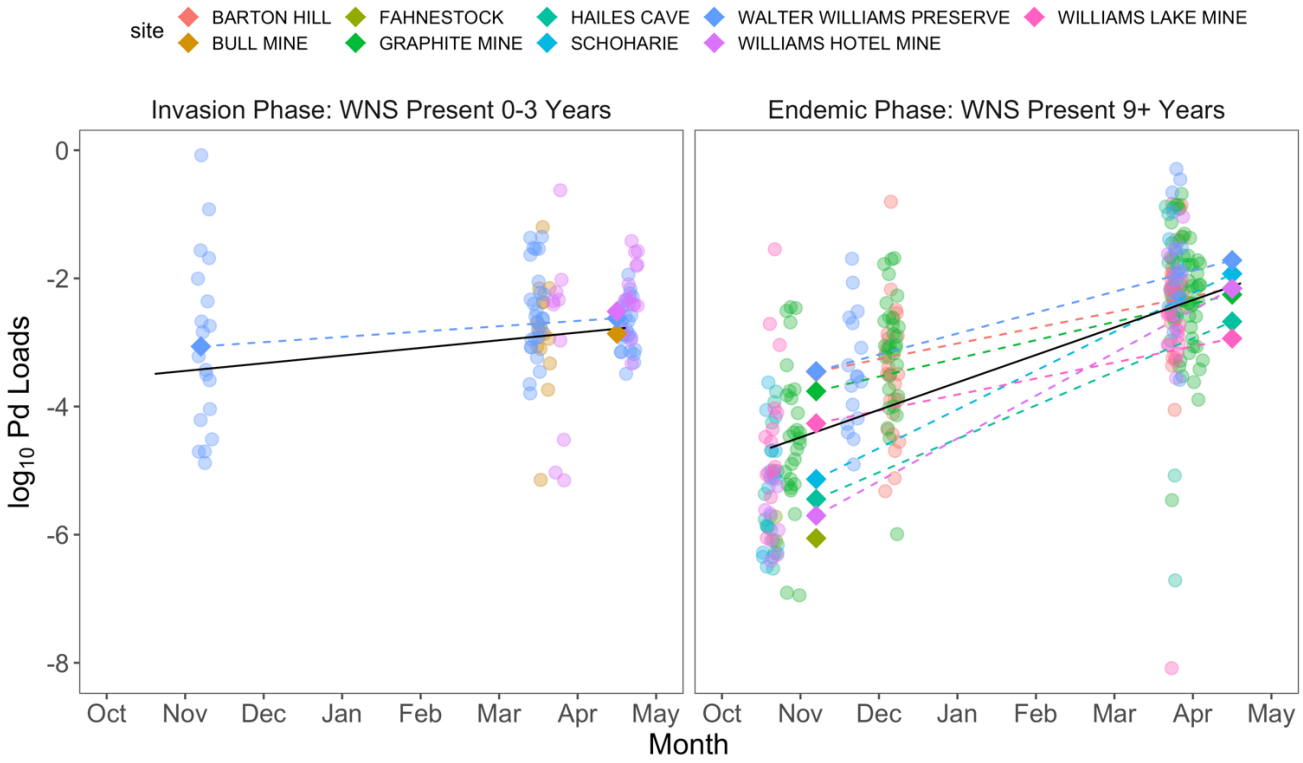


Figure 4. On-bat fungal loads in New York sites. Panels represent phases of invasion. Circles represent individual sampled bats while the diamonds represent site average loads during early and late hibernation. Solid lines are model-predicted fungal loads as hibernation progresses and dashed lines connect site average loads.

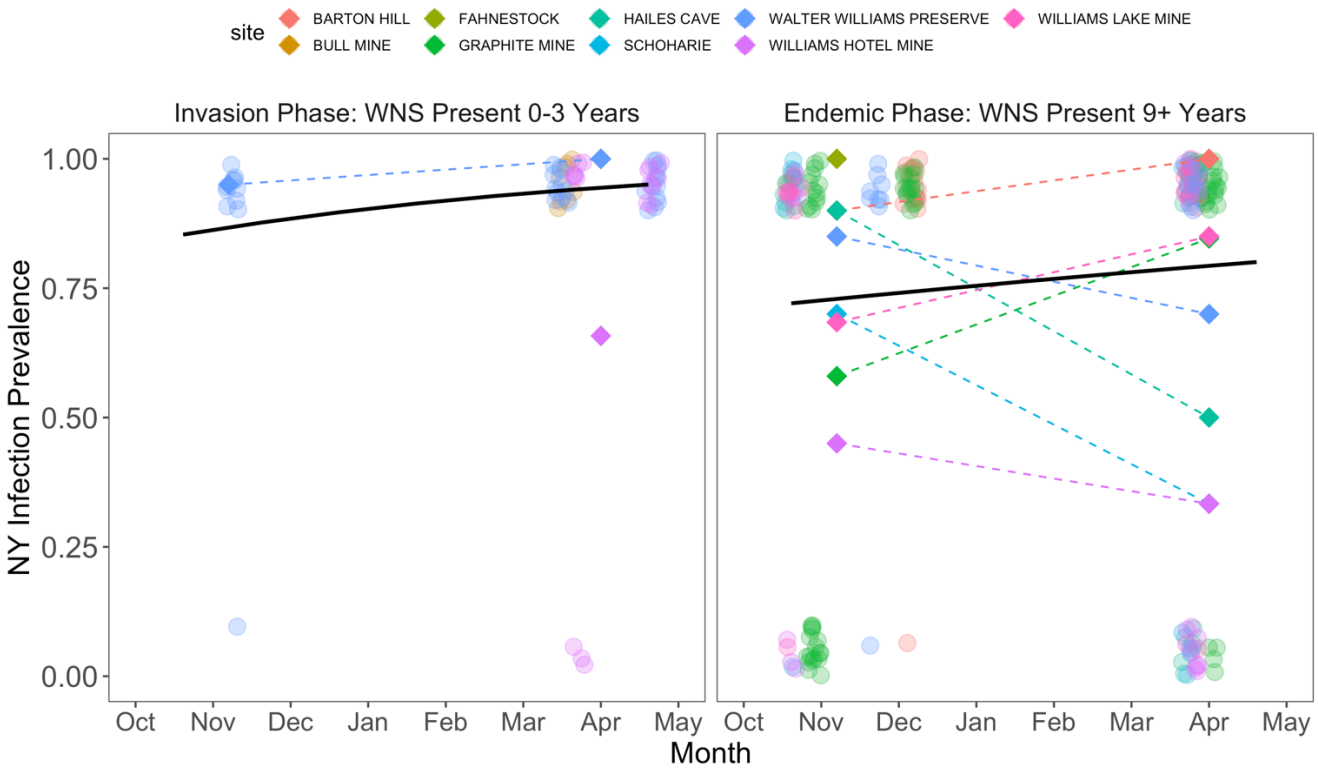


Figure 5. Temporal changes in infection prevalence from New York sites throughout phases of invasion as shown by the panels. Individual bats are denoted by the points and the lines represent the model's infection prevalence as hibernation progresses. Diamonds represent average site infection prevalence during early or late hibernation. Solid lines represent changes in infection prevalence throughout hibernation and dashed lines connect site averaged infection prevalence during early and late hibernation.

Discussion

Our results show that the relationship between early hibernation roosting temperature and the growth rate of *Pd* has changed over time. During the initial years when *Pd* first arrived, we found lower fungal rates at cooler temperatures and higher fungal growth rates at warmer temperatures on both population and individual scales. As *Pd* became established within sites, we found that the temperature-fungal growth rate interaction weakened in both populations and individuals. Once *Pd* became endemic within the community, we continued to see a weaker relationship between temperature and fungal growth rate, however, these results are less clear. Notably, when this relationship is examined during the endemic phase in individuals, the interaction appears to begin showing elevated growth rates at cooler temperatures, however, this is likely to be attributed to an overall decrease in preferred roosting temperature, poor sample size at warmer temperatures, and general removal of bats from warmer temperatures by earlier phases of *Pd*-related mortalities.

Changes in *Pd* growth rates cannot be explained alone by greater exposure to the environmental reservoir when bats return to hibernacula because the early hibernation on-bat fungal loads remain consistent between phases (Figure 3). However, we did find support for a decrease in early fungal loads during the winters of 2019, 2022, and 2023 when compared to 2011 (Supplemental Figure 2). This could be partially attributed to early sampling timing during later years (~15 days earlier), resulting in less time for *Pd* to grow on bats. It is therefore possible that infection may be changing as *Pd* becomes endemic in sites, although additional research is needed to understand the consistency of this result.

Changes in the temperature fungal growth rate relationship may be due to changes in the hosts, pathogen, or both. Prior work shows that persisting bat populations have lower loads and increased survival by the end of hibernation (Langwig et. al 2017, Grimaudo et. al 2022). This pattern is consistent with the evolution of resistant traits in little brown bat populations and may also explain how pathogen growth rates have decreased at warmer hibernation temperatures. However, increases in bat resistance do not fully explain higher fungal growth rates at colder temperatures in the latter two phases compared to the invasion phase, which could be due to changes in the pathogen itself. The trade-off hypothesis predicts that pathogens will evolve to an intermediate form of virulence by balancing pathogen fitness (transmission and replication) with virulence (Anderson and May 1982, Alizon et al. 2009). The increased fungal growth rate at cooler temperatures may be the pathogen's response to bats shifting their average roosting temperature to cooler microclimates, further from *Pd*'s thermal optimum. To continue to infect hosts, the pathogen may need to replicate faster at cooler temperatures, potentially sacrificing the high virulence at warm temperatures seen during the invasion phase. However, host and pathogen evolution does not occur in a vacuum and likely both are responding to each other. The Red Queen hypothesis suggests that a beneficial adaptation by the host will be countered by an adaptation by the pathogen, leading to coevolution between the two (Decaestecker et. al 2007). Thus, the weakening interaction between temperature and fungal growth rate is likely due to evolution in both the host and the pathogen occurring simultaneously.

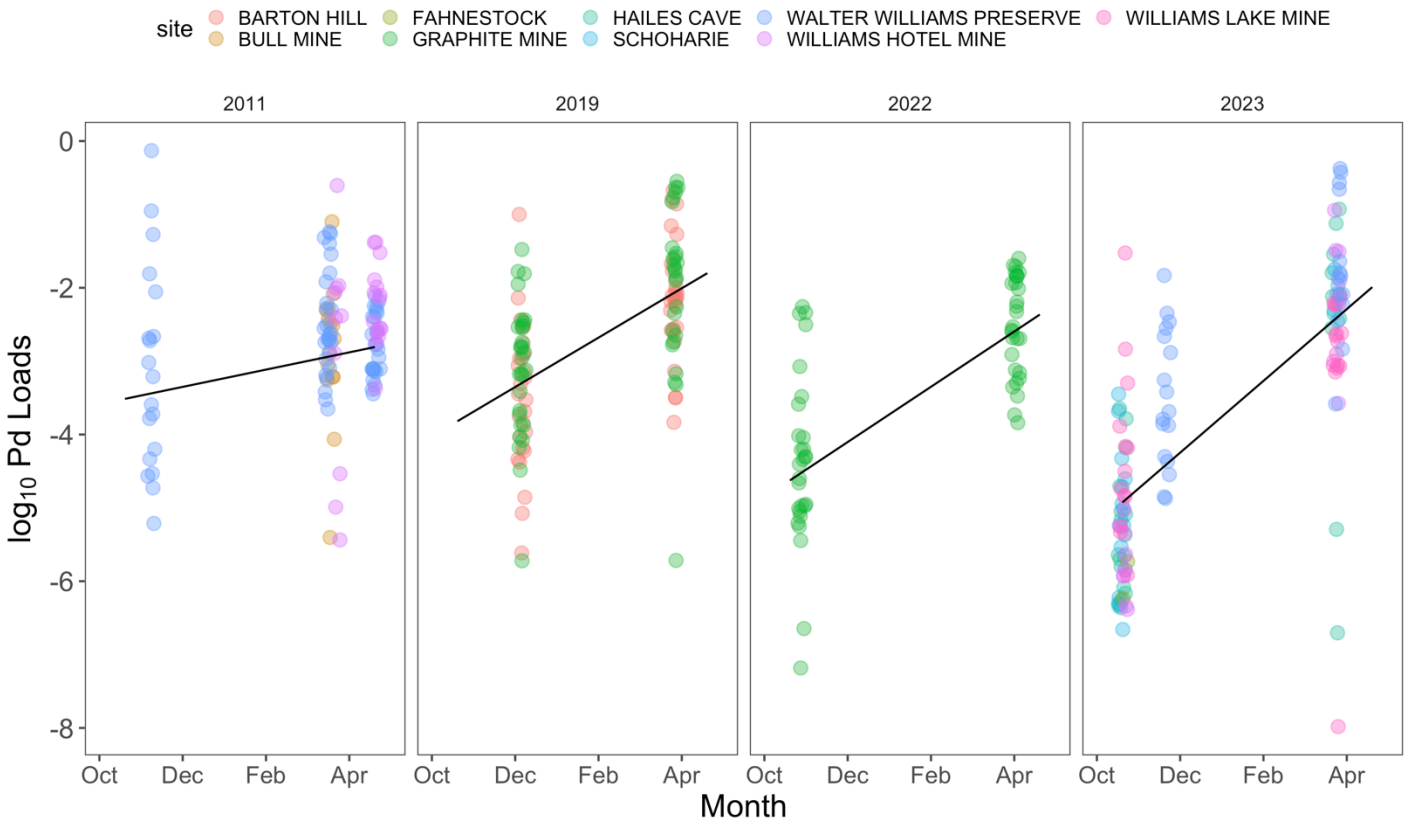
Possible further evidence for this rapid coevolution between little brown bats and *Pd* can be detected due to changes in pathogen prevalence throughout hibernation. During the early years of *Pd* introduction to sites, all bats were infected with *Pd,* and this was the case during most of the years following pathogen introduction. However, during the endemic phase of

invasion from sampling at New York sites, there is considerably more variation in infection prevalence among individuals in both early and late hibernation (Fig. 5, Supp. Fig. 2). Given that *Pd* has been in these sites for at least 15 years, it is possible a change in infection dynamics between the bats and *Pd* has arisen. However future research should be completed to examine trends across a larger spatial and temporal scale and to investigate the mechanisms explaining changes in overwinter infection status.

Changes in roosting temperatures of bats may have influenced support for some of the temperature and *Pd* growth rate relationships we observed (Hopkins et al. 2021). First, as previously discussed, bats that preferred to hibernate at warmer temperatures have been disproportionately removed from populations (Hopkins et al. 2021), therefore the number of individuals that can be sampled at warmer temperatures is smaller. However, previous research has shown that bats who have persisted with *Pd* show greater survival and lower growth rates at warmer temperatures compared to *Pd* naïve bats hibernating at warm temperatures (Grimaudo et al. 2022) suggesting Pd exerted selective pressure on those populations to be better able to cope with infection and that survival bias of contemporary bats at warm temperatures may not substantially impact our results. Second, the quantified *Pd* loads obtained during late hibernation may not be a full representation of the population during early hibernation. If a bat experiences a rapid fungal growth rate, mortality may occur before we can sample the population, thus resulting in a survivorship bias (Langwig et. al 2016). However, prior research has also shown that bats in populations that have experienced *Pd* carefully budget their energy reserves and arouse less frequently compared to naïve populations therefore bat populations after *Pd* invasion are likely better equipped to handle fungal growth rate and their data may show less survivorship bias (Lilley et. al 2016, McGuire et. al 2021, Langwig et. al 2023).

The WNS system provides a unique opportunity to study emerging infectious disease dynamics as the pathogen is still invading sites across North America. Rather than witnessing predicted extinctions, we have observed the development of persisting populations. This system provides the opportunity for researchers to understand how invading pathogens may become endemic and could provide insight into how to prepare for and respond to future emerging infectious diseases. Notably, this system provides the opportunity to study the development of coevolution between hosts and novel pathogens. Future research should focus on disentangling the mechanisms of persistence including the development of host resistance and changing pathogen virulence. Additionally, efforts should be made to understand differences in host persistence with WNS across the bat community affected by WNS. Though persisting little brown bat populations show stability, other affected species such as *Myotis septentrionalis* and *Myotis sodalis* continue to decline or have unstable population growth rates (Hoyt et. al 2021). Investigating the different disease dynamics within species and identifying attributes responsible for variation in host-specific outcomes with WNS may further enable conservation measures.

Supplement



Supplemental Figure 1. On-bat fungal loads in New York sites. Panels show a winter season (e.g. panel 2011 represents the 2010-2011 winter). Points represent individual sampled bats while the line denotes model-predicted fungal loads.

Supplemental Table 1: Results from changes in fungal loads at New York sites across the winter seasons of 2011, 2019, 2022, and 2023.

Supplemental Table 1

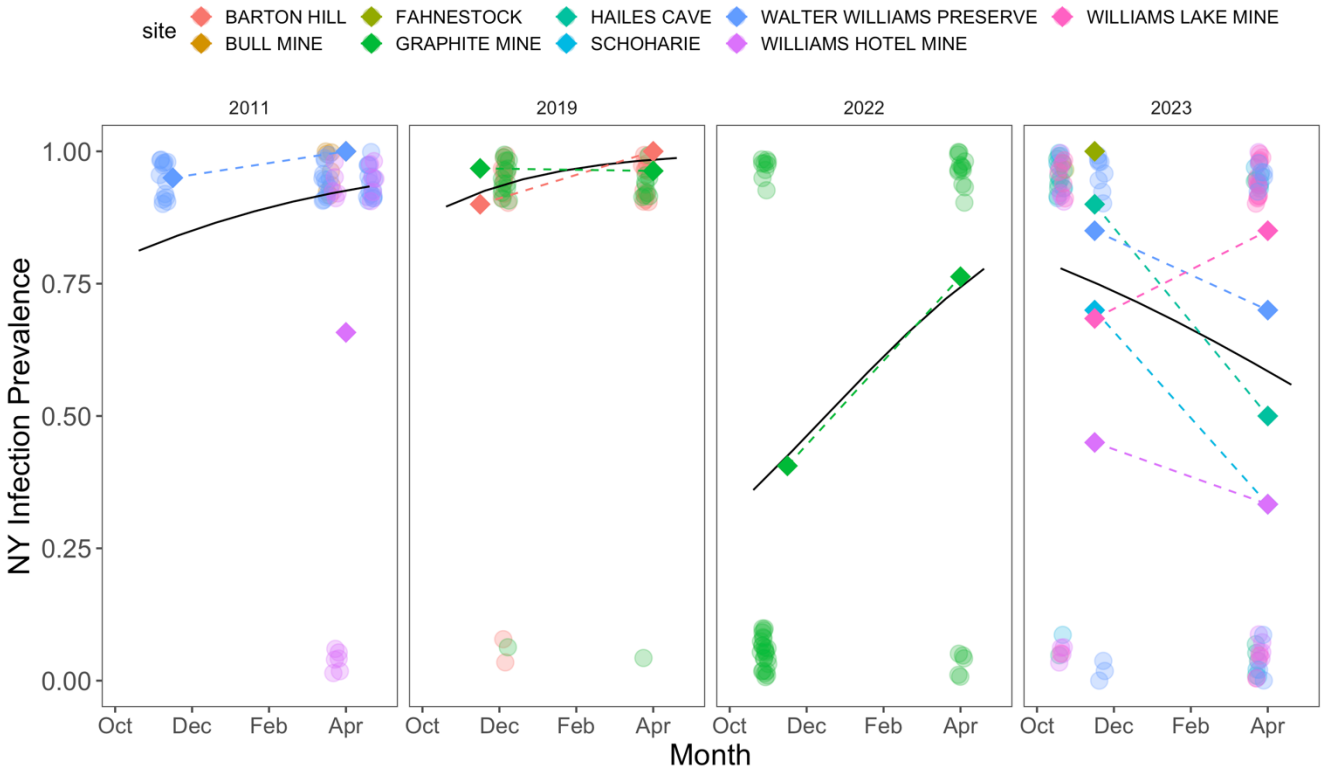
Response variable: Fungal load

Interactive linear mixed model

(fungal load) ~ (hibernation date* (winter season) + (1|site)

Model reference level: 2011

Term	*Estimate*	*Standard Error*	*T value*
Intercept (2011 early hibernation loads)	-3.51391	0.29847	-11.773
2011 overwinter Pd load change	0.11767	0.05275	2.231
2019 early hibernation loads	-0.30508	0.40311	-0.757
2022 early hibernation loads	-1.11133	0.41316	-2.690
2023 early hibernation loads	-1.41179	0.30943	-4.563
2019 overwinter Pd load change	0.21873	0.07451	2.936
2022 overwinter Pd load change	0.25903	0.07417	3.492
2023 overwinter Pd load change	0.37153	0.06559	5.664



Supplemental Figure 2: Temporal changes in infection prevalence from New York sites throughout winter seasons. Individual bats are denoted by the points and the lines represent the model's infection prevalence as hibernation progresses. Diamonds represent average site infection prevalence during early or late hibernation. Solid lines represent changes in infection prevalence throughout hibernation and dashed lines connect site averaged infection prevalence during early and late hibernation.

Supplemental Table 2: Results from changes in infection prevalence at New York sites across the winter seasons of 2011, 2019, 2022, and 2023.

Supplemental Table 2

Response variable: Infection status (0 or 1)

Interactive generalized linear mixed model with a binomial distribution and a logit link:

(infection status) ~ (hibernation date) * (winter season) + (1|site), family binomial

Model reference level: 2023

Term	*Estimate*	*Standard Error*	*P value*
Intercept (2023 early hibernation prevalence)	1.25995	0.40879	0.00206
2023 overwinter prevalence change	-0.17038	-2.502	0.01233
2011 early hibernation prevalence	0.20512	0.98008	0.83422
2019 early hibernation prevalence	0.88822	1.08248	0.41191
2022 early hibernation prevalence	-1.83479	0.79429	0.02089
2011 overwinter prevalence change	0.36721	0.20377	0.07154
2019 overwinter prevalence change	0.53941	0.31924	0.09109
2022 overwinter prevalence change	0.47542	0.11192	< 0.001

Chapter 3 – Conclusion

The goal of this research was to examine how changing disease dynamics relating to temperature and pathogen growth rate may contribute to the persistence of a host with an emerging infectious disease. By using the white-nose syndrome system to examine this question, we found several signs of a changing relationship between little brown bats and *P. destructans.* First, our results showed that the relationship between little brown bat's early hibernation temperature and the fungal growth rate weakened throughout the advancement of *Pd* invasion phases. Second, we observed similar early hibernation loads across the invasion and established phases, indicating the changes in fungal growth rate cannot be explained by solely by greater exposure to the environmental reservoir. Third, we found evidence of decreased fungal loads and decreased infection prevalence as the phase of invasion advanced throughout New York sites, however more research is needed to explore and validate these results.

Broadly, the results from this research demonstrate that little brown bats and *P. destructans* may be beginning to coexist within sites with long-established persisting bat populations. The development of a coevolutionary dynamic between little brown bats and *Pd* would allow for the long-term persistence of bats and hopefully, extinction avoidance. However, questions relating to the persistence of these populations remain. More research is needed to investigate the evolution of resistant and tolerant traits within bats. Additionally, more work is needed to identify evolutionary changes related to virulence that *Pd* may have experienced from the new environment or hosts since its introduction to North America. Ideally, these questions could be explored in a controlled experiment to disentangle the contributions of the host and pathogen that enable persistence by inoculating naïve and persisting bats with contemporary and historical *Pd* strains to identify different disease outcomes. Identifying and quantifying the

changes in both bats and *Pd* will enable researchers and managers to better identify mechanisms of persistence and enhance conservation strategies. In addition, answering these questions will enrich the field of disease ecology by providing an example of host persistence due to evolutionary changes in a vertebrate system which may help understand other host-pathogen dynamics.

As we anticipate the continued emergence of infectious diseases in wildlife, results from this research offer additional resources for disease ecologists to predict how hosts in other systems may persist with disease. Factors of hosts, pathogens, and the environment may interact differently in other systems, but all three must interact under the correct conditions for hosts to experience disease. Identifying the changes in these factors will enable the conservation of species at risk of extinction by disease. This research underlines the importance of monitoring these systems over a larger spatio-temporal scale to identify important interactions and how they change as hosts and pathogens coevolve.

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