Title: Recovery of Myotis in Michigan’s Upper Peninsula

# **Introduction:**

The Anthropocene is characterized by profound, human-driven changes that are reshaping the planet’s ecosystems. Habitat degradation, climate change, the spread of invasive species, and the emergence of novel pathogens are defining characteristics of the Anthropocene, each contributing to the loss of biodiversity and ecosystem destabilization. As human activities accelerate environmental stressors, wildlife species face unprecedented challenges. Among these stressors, invasive fungal pathogens have caused some of the most severe die-offs ever recorded in wild species (Fisher et al. 2012). In the nineteenth century, *Phytophthora infestans*, the fungus responsible for late blight, caused widespread starvation and contributed to the downfall of the English government during the Irish potato famine (Fisher et al. 2012). More recently, *Cryphonectaria parasitica* (Chestnut blight) and *Ophiostoma novo-ulmi* (Dutch Elm disease) caused the near extinction of the American chestnut (*Castanea dentata*) and American Elm (*Ulmus americana*), leading to devastating ecological and economic impacts in eastern North America, forever changing the landscape (Fisher et al. 2012). Invasive fungal pathogens are particularly destructive because they can spread rapidly, are highly resistant and their ability to adapt quickly to new selection pressures (Fisher et al. 2020). Moreover, these threats are exacerbated by human-driven changes, including habitat degradation, the spread of invasive species, and shifting climate conditions. Understanding the environmental conditions that buffer wildlife communities from detrimental impacts has become a key research priority to guide conservation action.

Many fungal diseases exhibit temperature-dependent behavior, influencing their virulence, host susceptibility, and the severity of the disease they cause. Temperature affects pathogen growth rates and can influence the geographical distribution of these pathogens, making it an important factor in understanding their ecological impacts and developing effective management strategies. For example, temperature is an important factor influencing the outcomes of *Batrachochytrium dendrobatidis* (Bd), the fungus that causes chytridiomycosis in amphibians (Lindauer et al. 2020; Turner et al. 2021; Herczeg et al. 2023). Bd shows optimal growth between 17 and 25 °C but fails to grow or reproduce at 28 °C, which suggests that amphibians in warmer conditions may find refuge from the pathogen. Identifying these temperature buffers is essential for understanding and protecting the habitats species need to survive and potentially thrive despite fungal infections.

Bats present a compelling case study of the devastating effects of Anthropocene stressors. Habitat loss, climate change, energy production, and invasive pathogens all threaten bat survival (CITE). The largest threat is an invasive fungal pathogen known as white-nose syndrome (WNS), caused by the fungus *Pseudogymnoascus destructans* (Pd). First documented in New York during the winter of 2006–2007, WNS has since swept across North America, causing mortality rates as high as 99% in some bat populations (Meteyer et al., 2009; Kurta & Smith 2020; Hout et al., 2021; Vanderwolf & McAlpine, 2021; Perea et al. 2023). The disease disrupts hibernation by triggering frequent arousals, forcing bats to deplete their limited every reserves, ultimately leading to starvation (Frick et al. 2016). Temperature plays a critical role in shaping the development and spread of Pd. Like many fungal pathogens, Pd exhibits temperature-dependent behavior, thriving in cool, humid environments typical of bat hibernacula. Notably, higher fungal loads are associated with increased mortality, particularly in bats hibernating within temperature ranges conducive to fungal growth, with active growth beginning at 3°C and peak growth occurring between 12.5°C and 15.8°C (Verant et al., 2012; Langwig et al., 2012; Johnson et al., 2014, 2016; Hayman et al., 2016). Given that many cave-roosting bat populations are limited by the availability of suitable hibernacula (Davis & Hitchcock, 1965), the degradation of these habitats due to WNS represents a significant threat to population viability. The deleterious effects of WNS have led to local extirpation of species (Vanderwolf & McAlpine, 2021; Perea et al. 2024) and prompted the listing of several bat species under the Endangered Species Act, further intensifying regulatory and conservation efforts (Turner et al., 2011; Frick et al., 2015; Hoyt et al., 2021). Understanding how temperature influences pathogen growth and affects survival and behavior is important for developing strategies to mitigate the impacts of WNS.

The habitats and microclimates that bats select during winter are important to their survival. Bats face the challenge of balancing costs of prolonged torpor with the energy demands of arousals to euthermia. Prolonged torpor leads to physiological costs, including immune function suppression, dehydration and muscle atrophy, but euthermic arousals are energetically expensive, consuming 80-90% of their overwinter energy reserves. For example, a single arousal of *Myotis lucifugus* uses the equivalent fat reserves of 67 days spent in torpor (Thomas et al. 1990). WNS has fundamentally altered bat behavior, particularly with respect to their roosting site selection and hibernation strategies. Before the emergence of WNS, bats typically selected hibernacula with relatively warm and stable microclimates that allowed them to maintain periodic arousals, likely to avoid the physiological stressors of prolonged torpor (Thomas et al., 1990; Humphries et al., 2002; Hayman et al., 2016). However, microclimate diversity, or the presence of multiple microhabitats with varying temperatures within hibernacula, may offer important benefits for bats. Boyles et al. (2007) hypothesized that *Myotis lucifugus* would select warmer roost sites in good body condition but colder sites when in poor condition, indicating that the ability to select varying microclimates could help bats balance the trade-off between torpor and energy expenditure. In response to the pressures of WNS, many susceptible bats have shifted to colder roosting sites (Johnson et al. 2016; Turner et al. 2022; Loeb & Winters, 2022). This change in behavior appears to be a response to the increased mortality observed in warmer sites, where Pd grows more aggressively and forces more frequent arousals. In colder hibernacula, Pd’s growth is less pronounced, and the reduced frequency of arousals leads to lower energy expenditure and higher survival rates (Johnson et al., 2014, 2016; Langwig et al. 2012; Lilley et al. 2016). For example, remnant populations of *Myotis lucifugus* have been found hibernating in colder roosting sites post-WNS, compared to their pre-WNS preferences (Johnson et al. 2014, 2016; Langwig et al. 2012; Lilley et al. 2016; Turner et al. 2022; Loeb & Winters 2022), and bats are recovering from WNS-induced mortalities faster in sites with cooler microclimates (Turner et al. 2022). In New York, *Myotis lucifugus* had higher survival rates despite being pd-positive when their skin temperatures during torpor averaged 2℃ compared to peak-WNS mortality when skin temperature averaged 7℃ (Lilley et al., 2016) Similarly, in Pennsylvania, recovering populations of *Myotis lucifugus*, *Permyotis subflavus* (Tricolored bats*,* and *Eptesicus fuscus* (Big Brown Bats) changed their winter roosting behavior to colder sections of hibernacula following dramatic population declines caused by WNS (Johnson et al. 2016; Turner et al. 2022). The shift toward colder hibernacula after the onset of WNS highlights the importance of microclimate variability in bat survival and the potential for environmental manipulations to enhance survival rates in affected populations.

In the Upper Great Lakes region, suitable hibernacula are scarce. However, the western Upper Peninsula of Michigan is notable for its hundreds of mines excavated since the 1840s (Kurta & Smith 2014). Before WNS arrived, Kurta and Smith (2014) surveyed 119 subterranean sites, finding that *Myotis* species (Little Brown Bats and Northern Long-Eared Bats) comprised over 99% of the bat population. These bats were concentrated in a few large, complex mines, with temperature and the presence of warm air traps identified as key factors in sites selection. WNS reached Michigan’s Upper Peninsula in the winter of 2013-2014, resulting in a 90% decline in bat populations by 2020, from 138,068 to 13,988 in surveyed mines (Kurta & Smith 2020).

We investigated the environmental conditions within hibernacula that influenced the rate of population decline (“crash rate”) for each site. *Myotis* bats are recovering after the initial population crashes from WNS in some hibernacula. We hypothesized that the microclimate conditions within hibernacula that favor the growth and persistence of Pd (warm, high relative humidity) influenced both the rates of population decline and recovery. Specifically, we predicted that higher crash rates would correlate with slower recovery rates, while lower population crash rates would correlate with faster recovery rates. Additionally, we examined the rates of both population crashes and recovery in relation tomicroclimates factors within each hibernaculum to understand the microclimate conditions in Michigan that led to higher population crashes and subsequent recovery. Lastly, we hypothesized that evolutionary selective pressures or behavioral plasticity drove surviving bats to select hibernacula with cooler microclimates in response to WNS. We predicted that pre-WNS *Myotis* bats show a preference for relatively warmer hibernacula, as indicated by mean temperature, and post-WNS would demonstrate a shift toward cooler microclimates, reflecting a behavioral or evolutionary response to the disease.

## **Methods:**

## **Study area:**

Most hibernacula sites in Michigan are in the western third of the Upper Peninsula (UP) (46.79-47.43 N, 87.59-90.14 W). This region is sparsely populated by humans (US Census Bureau 2020) and is mostly covered by conifers and northern hardwoods. Elevation varies from 184 to 604 meters above sea level. The western UP has Lake Superior to the north and Wisconsin to the south and west.

Winters are snowy, long, and cold with the first minimum temperature of 0 ℃ occurring in early November and the last such temperature of the winter occurring in April (NOAA; Supplemental File). The cold climate results in overwintering populations of bats peaking by late October, and most individuals remain underground until late April (Kurta et al. 1997; Meyer et al. 2016; Stones & Fritz 1969; Wheeler 1982).

## **Data collection**

Bat counts were compiled from a long-term program of bat monitoring conducted by the Michigan Department of Natural Resources (MiDNR). The MiDNR has surveyed hibernacula since 1980 but regular and consistent surveys began in 1996.

This study utilized 598 surveys of 205 hibernacula across Michigan, the majority in the western Upper Peninsula (Kurta & Smith 2014). Mine characteristics, environmental conditions, and biological data were recorded at each site.

Mine Characteristics:

Data included the geological composition (ore type), the length of the main passage (passage length), and the number of levels and shafts accessible to humans.

Environmental Conditions:

Environmental conditions were recorded intermittently, depending on the equipment available at the time of the survey. The measurements recorded included whether the site had any standing water at the entrance or bottom of the shaft, external temperature, internal temperature, and relative humidity. Between 1980 and 2013, internal temperatures were recorded using mercury thermometers to measure air temperature. In 2014, laser infrared thermometers were introduced, measuring surface rock temperatures. Due to inconsistencies in temperature measurement methods and equipment, a mean temperature was calculated for each site and used as the primary temperature variable. Internal relative humidity was inconsistently recorded and therefore excluded from the analysis. Mine temperatures taken during a survey were often reported as a range of temperatures (e.g., 5-7 ℃), those ranges along with any additional temperature measurements reported were used to calculate the mean temperature of each site, the average number of temperatures that were used to calculate the mean temperature was 5.12 (2, 10).

Biological Data:

During each survey, the total number of bats were recorded and identified to species or genus. The focus was on *Myotis* species, which included Little Brown Bats and Northern Long-Eared Bats. *Eptesicus fuscus* (Big Brown Bats) and *Perimyotis subflavus* (Tri-colored Bats) were also noted but removed from the analysis because *Eptesicus fuscus* does not experience high mortality from WNS (CITE) and *Perimyotis subflavus* is historically rare in our study area. Due to the lack of differentiation between Myotis species in some surveys, total Myotis counts were used as the response variable.

To ensure that data reflected significant hibernacula, we excluded sites that were surveyed only once or had fewer than five bats recorded. This filtering resulted in 308 surveys across 42 sites, which were included in some analyses.

All surveys were conducted under approved wildlife protection regulations, and the protocol was designed to minimize any disturbance to the hibernating bats by limiting time spent within the mines.

## Data analysis

### Hypothesis 1:

To investigate our first hypothesis, we used data from 33 hibernacula sites that have adequate survey data pre- and post-WNS of which 23 of those sites have recovering populations of Myotis.

We calculated the population crash rate using the formula:

Population Crash Rate = 1 – (Minimum Survey Count / Mean Survey Count Before WNS).

This captures the overall population decline from a “steady state” before WNS to the minimum number of bats surveyed after the introduction of Pd.

We calculated the slope of recovery by first normalizing bat counts at each site to a range of [0, 1] using the formula:

Normalized Count = (count – minimum count) / (mean count before WNS / minimum count).

This ensures that the mean count before WNS “steady state” at each site is standardized to 1 and the minimum count to 0. We then fit a linear regression of normalized count against relative year (where year 0 is the year after WNS affected population crashes) to derive the slope of recovery for each site (either positive or negative).

**LM:** normalized count ~ relative year.

To investigate our first prediction that population crash rates caused by WNS are correlated with Myotis population recovery rates (N=33), we fit the following model:

**LM:** slope of recovery ~ population crash.

### Hypothesis 2:

After processing the data, we constructed two separate sets of models. One we used a Bayesian hierarchical regression model with a Beta family distribution for the likelihood to model crash rate as a function of mean temperature and log transformed passage length.

Second, we used the Student T-distribution for the likelihood to model the recovery rate as a function of mean temperature, the log transformed passage length, and an offset variable for the number of years of recovery to account for the difference in the duration of recovery between the sites.

We fit models using the `brm` function in R package (brms) (Bürkner 2017). We assumed weakly informative priors (Normal (0,1)) for the intercept and slope. We ran 4000 iterations each with 1000 warmup iterations and an adapt\_delta of 0.99. All models showed convergence with R-hat values close to 1.

We used leave-one-out cross-validation (LOO-CV) to compare model performance. Leave-one-out cross-validation provides a more robust estimate of predictive accuracy in Bayesian models. It is especially useful when handling small sample sizes or influential observations, as it avoids the biases inherent in other criteria such as AIC or WAIC.

### Hypothesis 3:

To investigate our third hypothesis that Myotis species selected different habitat post-WNS infection we used 3708 surveys from 42 sites, 168 surveys were conducted before WNS caused mortalities and 125 surveys were conducted after WNS caused mortalities.

We calculated the proportions of bats in each mine to the totals in each period (before and after). We then subtracted the proportions (period after – period before), therefore a positive number indicated that site held a higher relative proportion of bats post-WNS and a negative number indicated that site held a lower relative proportion of bats pre-WNS. We then fit the models:

**LM1:** proportions ~ mean\_temp

**LM2:** proportions ~ mean\_temp + mean\_temp2

These results indicated habitat selection compared to habitat availability. We removed unimportant sites (sites <100 bats) from our final analysis. The sites that were unimportant before WNS were still unimportant after WNS because of other habitat or microclimate conditions that the larger population of Myotis were not selecting.

## RESULTS:

### Hypothesis 1

We found support for our first hypothesis where sites with temperatures conducive to Pd growth exhibited a correlation between the rates of population decline and subsequent recovery. The model explained a significant negative relationship between the population crash rate and the population recovery rate (SE = 0.0316, F-statistic = 18.95, p-value = 0.0001436). The model explained 38.7% of the variance in the recovery slope (R2 = 0.3871, Adjusted R2 = 0.3667). The model (slope ~ crash) compared to the null model (slope ~ 1) indicated our model had a significantly better fit, with a lower AIC value than the null model, suggesting that the inclusion of population crash rate as a predictor improved model fit. An ANOVA comparison between the two models confirmed this finding, with a significant improvement in the model fit (p = 0.0001436).

Residuals are approximately normally distributed. The assumption of constant variance is not fully satisfied where residuals increasing in variance as the fitted values increase indicating heteroscedasticity. Additionally, the leverage plot identified a few outlier points that may be influencing the model results. However, despite some potential violations of the model assumptions, the linear model still provides valuable insights into the relationship between population crash rate and recovery rate.

We found support for our first hypothesis where sites with temperatures conducive to Pd growth exhibited a correlation between the rates of population decline and subsequent recovery. The sites with higher population declines had slower recovery rates of species because they are persistently dealing with Pd (adjusted R2 = 0.3667, p-value 0.0001436).

A graph of a recovery rate and the population crash

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***Figure 1.*** *Population crash rates (1 - (minimum count after WNS/mean pop count before WNS) compared to recovery rates of myotis bats in mines in Michigan’s upper peninsula.*

*Hypothesis 2*

We compared multiple models to assess the relationship between population crash rate using Leave-One-Out Cross-Validation (LOO-CV) (CITE) and assessed the Expected Log Pointwise Predictive Density (ELPD). The results indicate that mean temperature is the strongest predictor of population crash rate. The slight difference between the null model and crash ~ mean temp (ΔELPD = -0.391) suggests that mean temperature is a significant predictor but not dramatically different to the baseline model. Including more complex predictors (log(passage length), mean temp2) does not improve the model’s predictive accuracy, as evidenced by the lower ELPD values.

***Model:*** *(crash ~ mean temperature) was selected as the final model due to its higher predictive accuracy (as shown by LOO-CV) and simpler structure compared to model 2 and model 3, which included additional covariates without improving predictive performance.*

| **Model** | **ELPD** | **SE** |
| --- | --- | --- |
| Pop crash rate ~ mean temp | 21.188 | 5.4 |
| Pop crash rate ~ 1 | 20.797 | 5.4 |
| Pop crash rate ~ mean temp + mean temp2 | 20.414 | 5.4 |
| Pop crash rate ~ mean temp + log(passage length) | 19.638 | 5.0 |

This model included mean temperature as the sole predictor. The inclusion of extra covariates did not significantly improve predictive performance, as indicated by the small ELPD differences between the models and their standard errors. This suggests that mean temperature alone is a sufficient predictor of population crash rate for these sites.

The posterior mean estimate for the effect of mean temperature on crash rate was -0.23 (95% credible interval: 0, 45) suggesting that higher mean temperatures are associated with an increase in crash rate (Table 2). Each additional degree of temperature is expected to increase the crash rate by 23% (0, 45%).

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| A graph of a graph showing the temperature of a car  Description automatically generated with medium confidence | A graph of a curve  Description automatically generated |

***Figure 2 a & b.*** *The relationship between population crash rates (1 - (Minimum count / Maximum count)) and mean temperature. Colder sites had significantly smaller population crashes compared to warmer sites.* ***Note:*** *The green line represents the posterior mean estimate from the final Bayesian model (Crash rate ~ Mean Temperature). The size of the points represent the mean population size for each site before WNS.  The relationship between population recovery rates and mean temperature. Colder sites have faster recovery rates compared to warmer sites, however, not statistically significant.* ***Note****: The blue line represents the posterior mean estiamte from the final bayesian model (Slope ~ Mean Temperature).*

We compared multiple models to predict the relationship between the recovery rate (slope) of bat populations and microclimate variables within hibernacula, using Leave-One-Out Cross-Validation to assess model fit. The model **slope ~ mean temperature showed** the best predictive performance, with the highest ELPD value of 40.385, indicating that it is the most accurate model for predicting the recovery slope in this population (Table 2).

The next best model **slope ~ mean temperature + mean temperature2**, showed reasonably good fit, with an ELPD value of 39.285, further emphasizing the importance of hibernaculum temperature to bat recovery. The inclusion of the offset variable for recovery years (the number of years a site had to recover from its minimum population) did not improve the model’s accuracy. This result suggests that the number of recovery years provides little additional predictive value. One potential explanation is that the mean temperature likely has more direct impact on recovery than time. If the environmental conditions are unsuitable, recovery may not occur regardless of the amount of time that has passed since the population crash. Alternatively, it is possible that there has not been enough time since the initial population decline from WNS for recovery years to serve as a meaningful predictor in our models due to bats low fecundity rate (CITE). The posterior mean estimate for the effect of mean temperature on recovery rate was -0.25 (95% credible interval: -0.53 to 0.01), suggesting that higher mean temperatures are associated with a decrease in recovery rate, however, not statistically significant (credible interval overlaps with 0).

|  |  |  |
| --- | --- | --- |
| **Model** | **ELPD** | **SE** |
| Pop recovery rate (slope) ~ mean temp | 40.385 | 3.6 |
| Pop recovery rate (slope) ~ mean temp + mean temp2 | 39.285 | 3.6 |
| Pop recovery rate (slope) ~ mean temp + log(passage length) | 39.046 | 4.0 |
| Pop recovery rate (slope) ~ 1 | 38.808 | 3.7 |
| Pop recovery rate (slope) ~ 1 + offset(recovery years) | -42.5 | 2.2 |
| Pop recovery rate (slope) ~ mean temp + offset(recovery years) | -43.197 | 2.2 |
| Pop recovery rate (slope) ~ mean temp + log(passage length) + offset(recovery years) | -43.295 | 2.4 |
| Pop recovery rate (slope) ~ mean temp + mean temp2 + offset(recovery years) | -43.507 | 3.0 |

*Hypothesis 3*

We used a linear model to understand the habitats selected by Little Brown Bats (LBB) after the introduction of white-nose syndrome. Our analysis showed that sites with colder mean temperatures tended to hold a higher proportion of bats post-WNS infestation. The best-fitting model was proportion ~ mean temperature + mean temperature2, which included both mean temperature and its quadratic term to capture the non-linear relationship between temperature and habitat selection by LBBs.

We used AICc to compare model fit and select the best model (Table 3). The addition of the quadratic term significantly improved the model’s fit, as indicated by the F-test (F=9.788, p-value = 0.00366), suggesting that temperature has a non-linear effect on LBB habitat preference.

|  |  |  |
| --- | --- | --- |
| **Model** | **AIC** | **AICc** |
| Proportions ~ mean temperature + mean temperature2 | -110.60 | -109.31 |
| Proportions ~ mean temperature | -103.25 | -102.50 |
| Proportions ~ 1 | -98.75 | -98.39 |

A graph of a graph showing the temperature of a bat

Description automatically generated with medium confidence

***Figure 4****. The figure represents the proportion of Little Brown Bats (LBB) for each site from two periods (After WNS proportions – Before WNS proportions).* ***Note:*** *Blue dots indicate sites where the proportion of LBB increased post-WNS, while red dots represent sites with a decreased proportion of LBB post-WNS. The x-axis represents the mean temperature of each site, the black line is the best-fit quadratic regression model  (proportion of LBB ~ mean temperature + mean temperature2), with the shaded region showing the 95% confidence interval.*

*Adjusted r2 = 0.3171, p-value = 0.00106*

**Discussion**

Since the emergence of Pd, cave-dwelling bat populations have declined by as much as 90% (Kurta and Smith 2020). This devastating impact has prompted scientists and wildlife managers to develop strategies aimed at mitigating the disease’s effects and promoting the recovery of affected populations.

Various techniques have been explored, including fumigation, inoculation, vaccination, and UV treatment, each showing various degrees of success (Gabriel et al. 2018; Padhi et al 2018; Micalizzi & Smith 2020; Rusman et al. 2020; Hoyt et al. 2017, 2019; Palmer et al. 2018; Hartman et al. 2020; Kwait et al. 2022; Rocke et al. 2019; McGuire et al. 2019).

Our findings show that bats in cooler hibernacula tend to be more resilient to Pd infection. This is likely due to the temperature-dependent growth rate of the fungus, as temperatures around 4 ℃ inhibit its growth while still supporting bat hibernation. The relationship between cooler microclimate selection and increased survival has likely exerted strong evolutionary pressures, driving bats to hibernate in cooler conditions. Understanding these adaptive responses, while simultaneously developing methods to manipulate hibernacula temperatures to enhance overwintering survival, representing a pressing line of inquiry.

Our first hypothesis explored the relationship between the slope of recovery rate for each hibernacula site and the population crash rate. The results indicate that sites with a lower population crash rate also exhibited higher recovery rates. This recovery could partly be attributed to the presence of more bats in the sites following the crash. However, this does not fully explain why these sites had a less severe crash initially and then demonstrated a faster recovery. To address this, we used our second hypothesis to examine the microclimate conditions within these hibernacula that contributed to a less severe population crash. Our first hypothesis suggests that the same microclimate conditions that helped reduce the severity of the population crash after the introduction *Pseudogymnoascus destructans* (Pd) are also the factors that enable bats to recover. This finding becomes even more clear when we only include hibernacula that are recovering by removing those sites that have not recovered from their initial population crash. These findings are promising, as they suggest that certain hibernacula in Michigan provide the right microclimates that allow *Myotis lucifugus* to tolerate WNS, survive, and reproduce.

Our second hypothesis aimed to identify the microclimate conditions responsible for population crashes and subsequent recovery. We considered mean temperature and passage length (a proxy for site size) as key variables. It became clear that passage length was not an important variable for these response variables at this time, so we removed it from our model. The population crash rate model revealed considerable variation, as indicated by the loose fit of the regression line. This variability is likely due to the fact that many bats were susceptible to Pd when it was first introduced, and regardless of the microclimate conditions at many of these sites, those susceptible to the disease succumbed to it. We used mean temperature instead of other temperature variables because temperature measurements were taken during surveys, providing a snapshot of the site temperature at the time of each survey. By compiling the temperature measurements from all surveys and calculating a mean temperature for each site, we reduced the year-to-year variability that external temperature fluctuations might introduce.

Alternatively, if we had used minimum and maximum temperature, we would only have captured the extreme temperature at a single point within the site. We know the temperature can vary across a site, and not all measurements reflect the areas where the bats hibernate within a site. To minimize this variability and the potential for inaccuracies we used a mean temperature. However, the absence of consistent, accurate internal temperature measurements increased the variability in our models, which in turn reduced their fit. The variation in temperature that a hibernacula experiences throughout the year is likely important for fungal growth and bat recovery. Unfortunately, due to the lack of accurate year-round temperature data at each site, we could not incorporate this variation into our analysis.

Future steps will involve quantifying the seasonal temperature variation at each site and within each site used in our analysis and updating the model accordingly. The outliers and the strength of the models likely reflect some of the limitations of the current survey data. Nevertheless, the general trend is clear: sites that tend to be cooler, as indicated by the mean temperatures from the surveys, appear to be more beneficial for *Myotis lucifugus* in the presence of Pd. This is likely due to the temperature-dependent growth rate of Pd.

Our third hypothesis shows the limitations of our survey data but also indicates clues for management. Most sites did not gain or lose the proportion of bats that use those sites for hibernation, with some noticeable exceptions. Four hibernacula had a 10% decrease in the proportion of bats that use those sites after after WNS population crashes. From a management perspective this likely indicates sites with poor quality habitat that are being selected against in the presence of Pd. Three of these sites have high mean temperatures above 7 ℃, while one site has a cooler mean temperature just above 4 ℃. This outlier should be investigated further. Two hibernacula sites gained almost 10% proportionally more bats and one site gained more than 25% proportionally more bats post-WNS. These sites potentially indicate good quality habitat in the presence of Pd, and their microclimate conditions should be investigated further. Either their conditions are non-conducive to Pd growth or there are some other microbial conditions reducing Pd growth within the sites.

One important factor influencing Pd growth that we did not incorporate into our models is humidity. We excluded humidity measurements due to the lack of data at most sites. However, literature indicates that the most severely affected species (*M. lucifugus*, *Myotis septentrionalis*, and *Perimyotis subflavus*) tend to roost in the most humid locations within hibernacula (Cryan et al. 2010; Langwig et al. 2012; Hayman et al. 2016), often maintaining relative humidity levels around 90 to 100% (Thomas and Cloutier 1992).

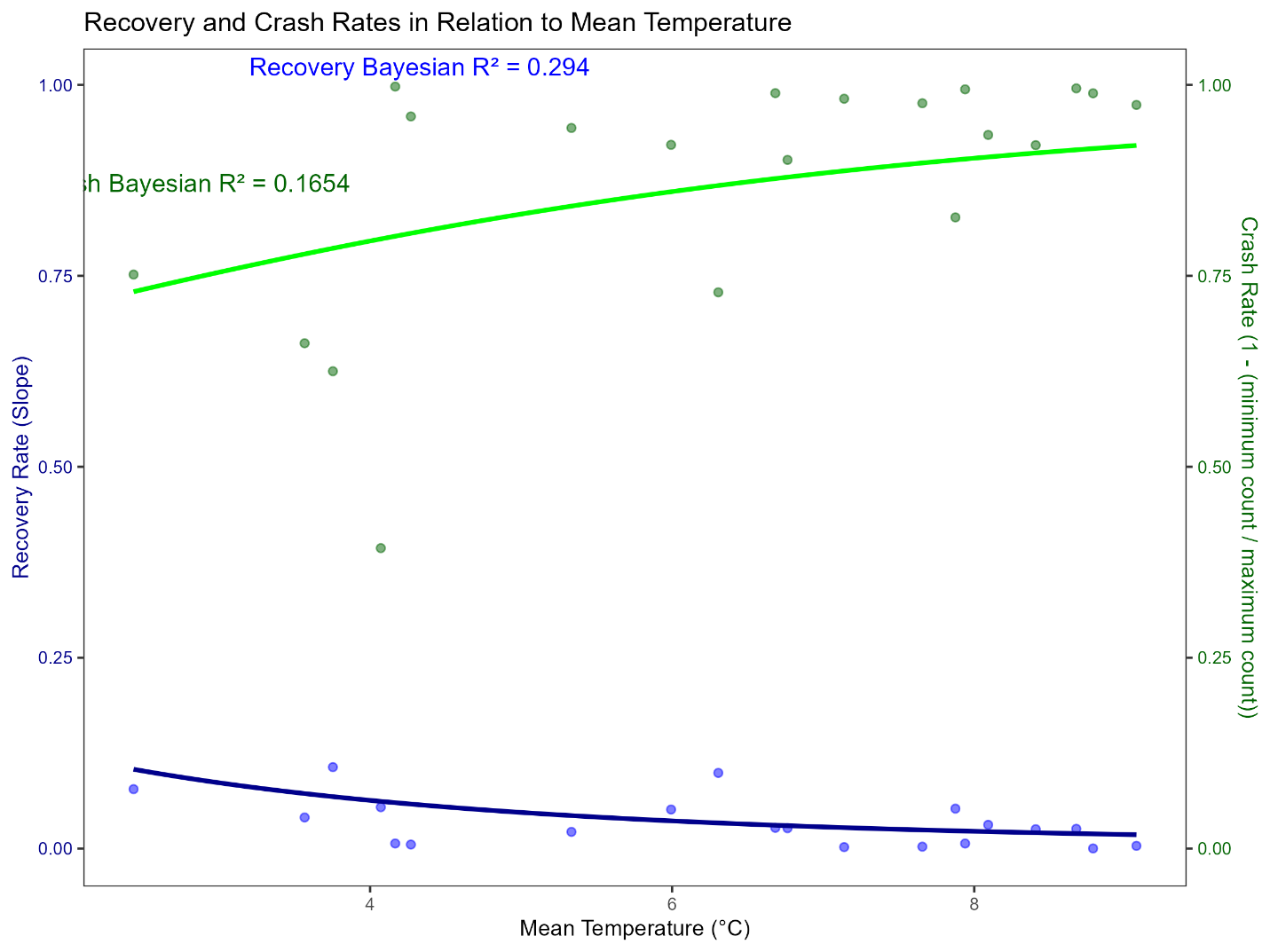
Research shows that increasing humidity in the presence of Pd generally correlates with decreased bat survival (Langwig et al. 2012), as Pd growth is positively associated with humidity (Hayman et al. 2016) and influences bat arousal patterns (Warnecke et al. 2012; Reeder et al. 2012; Nem-Hamo 2013). Notably, a single arousal bout of Myotis lucifugus hibernating at 5 ℃ consumes the equivalent fat energy of 67 days spent in torpor (Thomas et al. 1990).

Given this knowledge, it is reasonable to assume that the bats in these sites are experiencing high relative humidity levels. In many cases, relative humidity far from the entrance of a cave approaches 100% (Wigley 1969; Cigna, 2004). However, relative humidity, defined as the amount of water vapor present in the air relative to the maximum amount of water vapor the air can hold at saturation, is influenced by temperature. Warmer air can hold more water than colder air, meaning that the amount of water vapor in the air depends on the site temperature. Even though we did not directly account for relative humidity in our models, we understand that warmer sites will retain more moisture in the air at equivalent relative humidity levels, potentially promoting increased growth rates of Pd.

**Literature Cited**

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APPENDIX A:



A graph of a recovery rate and the population crash

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Figure . Population crash rates (1 - (minimum count after WNS/mean pop count before WNS) compared to recovery rates of myotis bats in mines in Michigan’s upper peninsula.

Hypothesis 2

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Model: (crash ~ mean temperature) was selected as the final model due to its higher predictive accuracy (as shown by LOO-CV) and simpler structure compared to model 2 and model 3, which included additional covariates without improving predictive performance.

|  |  |  |
| --- | --- | --- |
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The posterior mean estimate for the effect of mean temperature on crash rate was -0.23 (95% credible interval: 0, 45) suggesting that higher mean temperatures are associated with an increase in crash rate (Table 2). Each additional degree of temperature is expected to increase the crash rate by 23% (0, 45%).

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Figure . The relationship between population crash rates (1 - (Minimum count / Maximum count)) and mean temperature. Colder sites had significantly smaller population crashes compared to warmer sites. The green line represents the posterior mean estimate from the final Bayesian model (Crash rate ~ Mean Temperature). The size of the points represent the mean population size for each site before WNS. . The relationship between population recovery rates and mean temperature. Colder sites have faster recovery rates compared to warmer sites, however, not statistically significant. The blue line represents the posterior mean estiamte from the final bayesian model (Slope ~ Mean Temperature).

We compared multiple models to predict the relationship between the recovery rate (slope) of bat populations and microclimate variables within hibernacula, using Leave-One-Out Cross-Validation to assess model fit. The model **slope ~ mean temperature showed** the best predictive performance, with the highest ELPD value of 40.385, indicating that it is the most accurate model for predicting the recovery slope in this population (Table 2).

The next best model **slope ~ mean temperature + mean temperature2**, showed reasonably good fit, with an ELPD value of 39.285, further emphasizing the importance of hibernaculum temperature to bat recovery. The inclusion of the offset variable for recovery years (the number of years a site had to recover from its minimum population) did not improve the model’s accuracy. This result suggests that the number of recovery years provides little additional predictive value. One potential explanation is that the mean temperature likely has more direct impact on recovery than time. If the environmental conditions are unsuitable, recovery may not occur regardless of the amount of time that has passed since the population crash. Alternatively, it is possible that there has not been enough time since the initial population decline from WNS for recovery years to serve as a meaningful predictor in our models due to bats low fecundity rate (CITE). The posterior mean estimate for the effect of mean temperature on recovery rate was -0.25 (95% credible interval: -0.53 to 0.01), suggesting that higher mean temperatures are associated with a decrease in recovery rate, however, not statistically significant (credible interval overlaps with 0).

|  |  |  |
| --- | --- | --- |
| Model | ELPD | SE |
| Pop recovery rate (slope) ~ mean temp | 40.385 | 3.6 |
| Pop recovery rate (slope) ~ mean temp + mean temp2 | 39.285 | 3.6 |
| Pop recovery rate (slope) ~ mean temp + log(passage length) | 39.046 | 4.0 |
| Pop recovery rate (slope) ~ 1 | 38.808 | 3.7 |
| Pop recovery rate (slope) ~ 1 + offset(recovery years) | -42.5 | 2.2 |
| Pop recovery rate (slope) ~ mean temp + offset(recovery years) | -43.197 | 2.2 |
| Pop recovery rate (slope) ~ mean temp + log(passage length) + offset(recovery years) | -43.295 | 2.4 |
| Pop recovery rate (slope) ~ mean temp + mean temp2 + offset(recovery years) | -43.507 | 3.0 |

### Hypothesis 3.

We used a linear model to understand the habitats selected by Little Brown Bats (LBB) after the introduction of white-nose syndrome. Our analysis showed that sites with colder mean temperatures tended to hold a higher proportion of bats post-WNS infestation. The best-fitting model was proportion ~ mean temperature + mean temperature2, which included both mean temperature and its quadratic term to capture the non-linear relationship between temperature and habitat selection by LBBs.

We used AICc to compare model fit and select the best model (Table 3). The addition of the quadratic term significantly improved the model’s fit, as indicated by the F-test (F=9.788, p-value = 0.00366), suggesting that temperature has a non-linear effect on LBB habitat preference.

|  |  |  |
| --- | --- | --- |
| Model | AIC | AICc |
| Proportions ~ mean temperature + mean temperature2 | -110.60 | -109.31 |
| Proportions ~ mean temperature | -103.25 | -102.50 |
| Proportions ~ 1 | -98.75 | -98.39 |

A graph of a graph showing the temperature of a bat

Description automatically generated with medium confidence

Figure . The figure represents the proportion of Little Brown Bats (LBB) for each site from two periods (After WNS proportions – Before WNS proportions). Blue dots indicate sites where the proportion of LBB increased post-WNS, while red dots represent sites with a decreased proportion of LBB post-WNS. The x-axis represents the mean temperature of each site, the black line is the best-fit quadratic regression model (proportion of LBB ~ mean temperature + mean temperature2), with the shaded region showing the 95% confidence interval. Adjusted r2 = 0.3171, p-value = 0.00106.

Discussion

Since the emergence of Pd, cave-dwelling bat populations have declined by as much as 90% (Kurta and Smith 2020). This devastating impact has prompted scientists and wildlife managers to develop strategies aimed at mitigating the disease’s effects and promoting the recovery of affected populations.

Various techniques have been explored, including fumigation, inoculation, vaccination, and UV treatment, each showing various degrees of success (Gabriel et al. 2018; Padhi et al 2018; Micalizzi & Smith 2020; Rusman et al. 2020; Hoyt et al. 2017, 2019; Palmer et al. 2018; Hartman et al. 2020; Kwait et al. 2022; Rocke et al. 2019; McGuire et al. 2019).

Our findings show that bats in cooler hibernacula tend to be more resilient to Pd infection. This is likely due to the temperature-dependent growth rate of the fungus, as temperatures around 4 ℃ inhibit its growth while still supporting bat hibernation. The relationship between cooler microclimate selection and increased survival has likely exerted strong evolutionary pressures, driving bats to hibernate in cooler conditions. Understanding these adaptive responses, while simultaneously developing methods to manipulate hibernacula temperatures to enhance overwintering survival, representing a pressing line of inquiry.

Our first hypothesis explored the relationship between the slope of recovery rate for each hibernacula site and the population crash rate. The results indicate that sites with a lower population crash rate also exhibited higher recovery rates. This recovery could partly be attributed to the presence of more bats in the sites following the crash. However, this does not fully explain why these sites had a less severe crash initially and then demonstrated a faster recovery. To address this, we used our second hypothesis to examine the microclimate conditions within these hibernacula that contributed to a less severe population crash. Our first hypothesis suggests that the same microclimate conditions that helped reduce the severity of the population crash after the introduction *Pseudogymnoascus destructans* (Pd) are also the factors that enable bats to recover. This finding becomes even more clear when we only include hibernacula that are recovering by removing those sites that have not recovered from their initial population crash. These findings are promising, as they suggest that certain hibernacula in Michigan provide the right microclimates that allow *Myotis lucifugus* to tolerate WNS, survive, and reproduce.

Our second hypothesis aimed to identify the microclimate conditions responsible for population crashes and subsequent recovery. We considered mean temperature and passage length (a proxy for site size) as key variables. It became clear that passage length was not an important variable for these response variables at this time, so we removed it from our model. The population crash rate model revealed considerable variation, as indicated by the loose fit of the regression line. This variability is likely due to the fact that many bats were susceptible to Pd when it was first introduced, and regardless of the microclimate conditions at many of these sites, those susceptible to the disease succumbed to it. We used mean temperature instead of other temperature variables because temperature measurements were taken during surveys, providing a snapshot of the site temperature at the time of each survey. By compiling the temperature measurements from all surveys and calculating a mean temperature for each site, we reduced the year-to-year variability that external temperature fluctuations might introduce.

Alternatively, if we had used minimum and maximum temperature, we would only have captured the extreme temperature at a single point within the site. We know the temperature can vary across a site, and not all measurements reflect the areas where the bats hibernate within a site. To minimize this variability and the potential for inaccuracies we used a mean temperature. However, the absence of consistent, accurate internal temperature measurements increased the variability in our models, which in turn reduced their fit. The variation in temperature that a hibernacula experiences throughout the year is likely important for fungal growth and bat recovery. Unfortunately, due to the lack of accurate year-round temperature data at each site, we could not incorporate this variation into our analysis.

Future steps will involve quantifying the seasonal temperature variation at each site and within each site used in our analysis and updating the model accordingly. The outliers and the strength of the models likely reflect some of the limitations of the current survey data. Nevertheless, the general trend is clear: sites that tend to be cooler, as indicated by the mean temperatures from the surveys, appear to be more beneficial for *Myotis lucifugus* in the presence of Pd. This is likely due to the temperature-dependent growth rate of Pd.

Our third hypothesis shows the limitations of our survey data but also indicates clues for management. Most sites did not gain or lose the proportion of bats that use those sites for hibernation, with some noticeable exceptions. Four hibernacula had a 10% decrease in the proportion of bats that use those sites after after WNS population crashes. From a management perspective this likely indicates sites with poor quality habitat that are being selected against in the presence of Pd. Three of these sites have high mean temperatures above 7 ℃, while one site has a cooler mean temperature just above 4 ℃. This outlier should be investigated further. Two hibernacula sites gained almost 10% proportionally more bats and one site gained more than 25% proportionally more bats post-WNS. These sites potentially indicate good quality habitat in the presence of Pd, and their microclimate conditions should be investigated further. Either their conditions are non-conducive to Pd growth or there are some other microbial conditions reducing Pd growth within the sites.

One important factor influencing Pd growth that we did not incorporate into our models is humidity. We excluded humidity measurements due to the lack of data at most sites. However, literature indicates that the most severely affected species (*M. lucifugus*, *Myotis septentrionalis*, and *Perimyotis subflavus*) tend to roost in the most humid locations within hibernacula (Cryan et al. 2010; Langwig et al. 2012; Hayman et al. 2016), often maintaining relative humidity levels around 90 to 100% (Thomas and Cloutier 1992).

Research shows that increasing humidity in the presence of Pd generally correlates with decreased bat survival (Langwig et al. 2012), as Pd growth is positively associated with humidity (Hayman et al. 2016) and influences bat arousal patterns (Warnecke et al. 2012; Reeder et al. 2012; Nem-Hamo 2013). Notably, a single arousal bout of Myotis lucifugus hibernating at 5 ℃ consumes the equivalent fat energy of 67 days spent in torpor (Thomas et al. 1990).

Given this knowledge, it is reasonable to assume that the bats in these sites are experiencing high relative humidity levels. In many cases, relative humidity far from the entrance of a cave approaches 100% (Wigley 1969; Cigna, 2004). However, relative humidity, defined as the amount of water vapor present in the air relative to the maximum amount of water vapor the air can hold at saturation, is influenced by temperature. Warmer air can hold more water than colder air, meaning that the amount of water vapor in the air depends on the site temperature. Even though we did not directly account for relative humidity in our models, we understand that warmer sites will retain more moisture in the air at equivalent relative humidity levels, potentially promoting increased growth rates of Pd.

Literature Cited

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