





CONTRIBUTED PAPER

Cooling of bat hibernacula to mitigate white-nose syndrome

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Article Impact Statement: Cooling historically unimportant hibernacula provides bats with critical habitat and is an important tool for combating white-nose syndrome.

Abstract

White-nose syndrome (WNS) is a fungal disease that has caused precipitous declines in several North American bat species, creating an urgent need for conservation. We examined how microclimates and other characteristics of hibernacula have affected bat populations following WNS-associated declines and evaluated whether cooling of warm, little-used hibernacula could benefit bats. During the period following mass mortality (2013–2020), we conducted 191 winter surveys of 25 unmanipulated hibernacula and 6 manipulated hibernacula across Pennsylvania (USA). We joined these data with additional datasets on historical (pre-WNS) bat counts and on the spatial distribution of underground sites. We used generalized linear mixed models and model selection to identify factors affecting bat populations. Winter counts of *Myotis lucifugus* were higher and increased over time in colder hibernacula (those with midwinter temperatures of 3–6 °C) compared with warmer (7–11 °C) hibernacula. Counts of *Eptesicus fuscus*, *Myotis leibii*, and *Myotis septentrionalis* were likewise higher in colder hibernacula (temperature effects = −0.73 [SE 0.15], −0.51 [0.18], and −0.97 [0.28], respectively). Populations of *M. lucifugus* and *M. septentrionalis* increased most over time in hibernacula surrounded by more nearby sites, whereas *Eptesicus fuscus* counts remained high where they had been high before WNS onset (pre-WNS high count effect = 0.59 [0.22]). Winter counts of *M. leibii* were higher in hibernacula with high vapor pressure deficits (VPDs) (particularly over 0.1 kPa) compared with sites with lower VPDs (VPD effect = 15.3 [4.6]). Counts of *M. lucifugus* and *E. fuscus* also appeared higher where VPD was higher. In contrast, *Perimyotis subflavus* counts increased over time in relatively warm hibernacula and were unaffected by VPD. Where we manipulated hibernacula, we achieved cooling of on average 2.1 °C. At manipulated hibernacula, counts of *M. lucifugus* and *P. subflavus* increased over time (years since manipulation effect = 0.70 [0.28] and 0.51 [0.15], respectively). Further, there were more *E. fuscus* where cooling was greatest (temperature difference effect = −0.46 [SE 0.11]), and there was some evidence there were more *P. subflavus* in hibernacula sections that remained warm after manipulation. These data show bats are responding effectively to WNS through habitat selection. In *M. lucifugus*, *M. septentrionalis*, and possibly *P. subflavus*, this response is ongoing, with bats increasingly aggregating at suitable hibernacula, whereas *E. fuscus* remain in previously favored sites. Our results suggest that cooling warm sites receiving little use by bats is a viable strategy for combating WNS.

KEYWORDS

disease ecology, *Eptesicus fuscus*, hibernacula microclimate, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis*, *Perimyotis subflavus*, *Pseudogymnoascus destructans*, ecología de enfermedades, microclima de hibernáculos, *Eptesicus fuscus*, *Myotis leibii*, *Myotis lucifugus*, *Myotis septentrionalis*, *Perimyotis subflavus*, *Pseudogymnoascus destructans*

Resumen

El síndrome de nariz blanca (SNB) es una enfermedad fúngica que ha causado declinaciones precipitadas en varias especies de murciélagos norteamericanos, creando una necesidad urgente por conservarlas. Analizamos cómo los microclimas y otras características de

los hibernáculos han afectado a las poblaciones de murciélagos después de declinaciones asociadas al SNB y evaluamos si el enfriamiento de hibernáculos cálidos con poco uso podría beneficiar a los murciélagos. Durante el periodo posterior a una mortalidad masiva (2013 - 2020), realizamos 191 censos invernales en 25 hibernáculos sin manipulación y en seis hibernáculos manipulados localizados en Pensilvania (EUA). Juntamos estos datos con conjuntos adicionales de datos de los conteos históricos (previos WNS) de murciélagos y de la distribución espacial de sitios subterráneos. Usamos modelos mixtos lineales generalizados y selección de modelos para identificar los factores que afectan a las poblaciones de murciélagos. Los conteos invernales de *Myotis lucifugus* fueron más altos e incrementaron con el tiempo en los hibernáculos fríos (aquellos con temperaturas de 3 - 6° C registradas a mitad del invierno) en comparación con los hibernáculos cálidos (7 - 11° C). Los conteos *Eptesicus fuscus*, *M. leibii*, y *M. septentrionalis* fueron igualmente más altos en los hibernáculos fríos (efectos de la temperatura = -0.73 [ES 0.15], -0.51 [0.18], y -0.97 [0.28], respectivamente). Las poblaciones de *M. lucifugus* y *M. septentrionalis* fueron las que más incrementaron con el tiempo en los hibernáculos rodeados por más sitios cercanos, mientras que los conteos de *E. fuscus* permanecieron altos en donde ya habían sido altos antes del comienzo del SNB (el efecto del conteo alto previo al SNB = 0.59 [0.22]). Los conteos invernales de *M. leibii* fueron más altos en los hibernáculos con altos déficits de presión de vapor (DPV) (particularmente por encima de los 0.1 kPa) en comparación con los sitios con un DPV menor (efecto del VPD = 15.3 [4.6]). Los conteos de *M. lucifugus* y *E. fuscus* también fueron más altos en donde el DPV era alto. Al contrario, los conteos de *Perimyotis subflavus* incrementaron con el tiempo en hibernáculos relativamente cálidos y no se vieron afectados por el DPV. En donde alcanzamos un promedio de enfriamiento de 2.1° C de los hibernáculos, los conteos de *M. lucifugus* y *P. subflavus* incrementaron con el tiempo (años desde el efecto de manipulación = 0.70 [0.28] y 0.51 [0.15], respectivamente). Además, encontramos más *E. fuscus* en donde el enfriamiento fue mayor (efecto de la diferencia en temperatura = -0.46 [ES 0.11]), y hubo algunas evidencias de que había mayor cantidad de *P. subflavus* en las secciones del hibernáculo que permanecieron cálidas después de la manipulación. Estos datos muestran que los murciélagos están respondiendo efectivamente al SNB mediante la selección de hábitat. En el caso de *M. lucifugus*, *M. septentrionalis* y posiblemente *P. subflavus*, esta respuesta es persistente, con los murciélagos agrupándose cada vez más en hibernáculos adecuados, mientras que *E. fuscus* permanece en sitios favorecidos previamente. Nuestros resultados sugieren que el enfriamiento de los sitios cálidos que reciben poco uso por parte de los murciélagos es una estrategia viable para combatir al SNB.

Enfriamiento de los Hibernáculos de Murciélagos para Mitigar el Síndrome de Nariz Blanca

关键词: 大棕蝠(*Eptesicus fuscus*), 冬眠洞微气候, 美东小足蝠(*Myotis leibii*), 小棕蝠(*Myotis lucifugus*), 北长耳蝠(*Myotis septentrionalis*), 三色蝠(*Perimyotis subflavus*), 疾病生态学, 假裸囊菌属锈腐病菌(*Pseudogymnoascus destructans*)

【摘要】

白鼻综合征 (white-nose syndrome, WNS) 是一种真菌性疾病, 现已导致北美多个蝙蝠物种数量急剧减少, 迫切需要进行保护。本研究调查了微气候和冬眠洞的其它特征如何影响因WNS发生衰退的蝙蝠种群, 并评估了对温暖且较少使用的冬眠洞降温能否帮助蝙蝠获益。我们在蝙蝠大规模死亡后的一段时间内 (2013–2020年), 对美国宾夕法尼亚州25个未经人为操控的冬眠洞和6个人为操控的冬眠洞进行了191次冬季调查, 并将这些数据与额外的数据集相结合, 包括蝙蝠历史数量统计 (2013年之前) 和地下位点的空间分布数据。接下来, 我们用广义线性混合模型和模型选择的方法确定了影响蝙蝠种群的因素。与温度较高 (7–11°C) 的冬眠洞相比, 较冷的冬眠洞 (冬中温度为3–6°C) 中小棕蝠 (*Myotis lucifugus*) 冬季的数量更多, 且随时间推移而增加。大棕蝠 (*Eptesicus fuscus*)、美东小足蝠 (*M. leibii*) 和北长耳蝠 (*M. septentrionalis*) 也在较冷的冬眠洞中数量更多 (温度影响分别为-0.73 [标准误为0.15], -0.51 [0.18] 和 -0.97 [0.28])。随着时间推移, 小棕蝠和北长耳蝠在周围位点较多的冬眠洞中种群增长最多, 而大棕蝠在经历WNS之前就存在较高的种

群数量,之后也维持着较高数量(WNS前种群数量多的影响为0.59 [0.22])。与蒸气压差(vapor pressure deficits, VPD)较低的位点相比,高VPD(特别是超过0.1 kPa)的冬眠洞中美东小棕蝠冬季数量更多(VPD的影响为15.3 [4.6])。小棕蝠和大棕蝠也在高VPD的位点数量更多。相反,三色蝠(*Perimyotis subflavus*)则在较温暖的冬眠洞中数量随时间推移而增加,且不受VPD的影响。当我们对冬眠洞平均降温2.1°C时,小棕蝠和三色蝠的数量随时间推移而增加(自操控以来年数的影响分别为0.70[0.28]和0.51[0.15])。此外,在降温幅度最大的冬眠洞中出现了更多大棕蝠(温差影响为-0.46[0.11]),并且有证据表明,在人为操控后保持温暖的冬眠洞中出现了更多三色蝠。以上数据表明,蝙蝠正在通过栖息地选择有效地响应WNS的影响。在小棕蝠、北长耳蝠,可能还有三色蝠中,这种响应是持续发生的,表现为蝙蝠越来越多地聚集在合适的冬眠洞中越冬,而大棕蝠则倾向于留在以前选择的冬眠洞中。我们的结果表明,对蝙蝠很少使用的温暖位点进行降温是防治WNS的可行策略。【翻译:胡怡思;审校:聂永刚】

INTRODUCTION

Disease is increasingly recognized as a threat to biodiversity (De Castro & Bolker, 2005). Environmentally persistent fungi are particularly concerning disease agents because their capacity to infect hosts is decoupled from host density, allowing hosts to be extirpated (Fisher et al., 2012; Kuris et al., 2014). Managers charged with conserving species declining from environmentally persistent fungi therefore face complex problems that often lack clear solutions. For example, chytridiomycosis has been a threat to amphibians for more than 20 years, and conservationists still lack proven tools for mitigating the disease in nature (Garner et al., 2016; Peters et al., 2019; Skerratt et al., 2016). As a result, calls for translating knowledge of disease ecology into management strategies have increased (Canessa et al., 2018; Peters et al., 2019).

Another wildlife disease caused by an environmentally persistent fungus, and for which management strategies remain elusive, is white-nose syndrome (WNS) (Bernard et al., 2020). WNS is caused by the fungus *Pseudogymnoascus destructans* (Pd), which was first detected in North America in 2006 (Blehert et al., 2009). Since its introduction, Pd has spread across much of North America, where it currently causes WNS in 12 bat species (White-nose Syndrome Response Team, 2021). Overlap between the current geographic extent of WNS and ranges of hibernating bat species varies from 32% in *Eptesicus fuscus* to 93% in *Myotis sodalis*. Declines in winter counts in affected areas ranges from 28% in *M. sodalis* to nearly 100% in *M. septentrionalis* (Cheng et al., 2021), although there is substantial variability among sites (Turner et al., 2011). The scope and extent of declines suggest WNS poses an extreme threat to *M. septentrionalis*, *M. lucifugus*, and *Perimyotis subflavus* and a serious threat to *E. fuscus* (Cheng et al., 2021). The disease would further pose a serious risk to bat fauna in the southern hemisphere, should Pd be introduced there (Turbill & Welbergen, 2020).

Concerns for the future of North American bat populations have led government agencies and private organizations to invest heavily in researching tools for wildlife managers (Bernard et al., 2020). Examples include fungal biocontrol agents (Hoyt et al., 2019) and vaccines (Rocke et al., 2019), but translating any tool into an effective management strategy

is challenging. For example, handling or otherwise disturbing bats during hibernation to apply treatment will cause bats to arouse from torpor and use energy reserves (Thomas et al., 1990). Furthermore, applying treatments at the scale required to effect bat populations (Fletcher et al., 2020) will be difficult given the cryptic nature of bats. Finally, treatment efforts may be counterproductive if they limit hosts' adaptive responses to Pd (Maslo et al., 2017).

Host adaptation to pathogens has been observed in response to rabbit hemorrhagic disease (Schwensow et al., 2020), devil facial tumor disease (Jones et al., 2008), and WNS (Cheng et al., 2019). Such adaptations may represent opportunities to align management efforts with selective pressures instead of fighting against them. For example, Pd grows optimally from 12 to 15.8 °C and grows more slowly at colder temperatures (Verant et al., 2012). Many caves and mines that North American bats have historically used for hibernation are therefore suitable for Pd. While hibernating, bats are vulnerable to cutaneous infection with Pd, which can cause a cascade of physiological effects, including dehydration, increased frequency of arousals from hibernation, depleted fat stores, and, ultimately, death (Verant et al., 2014). However, both field and laboratory studies show higher survival among bats hibernating in cold microclimates, where fungal growth rates are reduced (Johnson et al., 2014; Langwig et al., 2012; Lilley et al., 2016), and remnant populations of several susceptible species have been found in colder roosting sites than was observed prior to the arrival of WNS (Johnson et al., 2016). Such habitat shifts may be critical for some bat species or populations because no large-scale evolutionary response to infection by Pd has yet been observed (Lilley et al., 2020).

The knowledge that cold temperatures are important for surviving WNS may be leveraged to develop management strategies. Specifically, prioritizing relatively colder hibernacula for conservation or creating suitable conditions for hibernation by manipulating unsuitable sites or low-quality habitat may benefit bats affected by WNS (Sewall et al., 2016). Cooling underground temperatures by altering airflow has long been used to improve low-quality habitat and to restore imperiled habitat (Richter et al., 1993; Tuttle, 2017, 2019; Tuttle & Kennedy, 2002). Metabolic rates of hibernators decline as ambient

temperature approaches a minimal threshold, although cold temperatures can impose other physiological costs (Boyles et al., 2020). Thus, colder hibernacula that remain above the minimal threshold could provide a thermal refuge from high fungal growth rates while in some cases providing additional metabolic benefits if free-ranging bats can discover and thrive in such modified habitats. In addition to temperature, bats are also affected by hibernacula humidity. Although less studied than temperature, humidity influences evaporative water loss and indirectly the rate of costly arousals from torpor during hibernation (Ben-Hamo et al., 2013). Humidity also influences growth of Pd (Marroquin et al., 2017) and therefore may also drive variation in WNS severity within and across hibernacula.

We sought to determine how microclimates and other characteristics of hibernacula have affected populations of overwintering bats following WNS-associated declines and to evaluate potential management strategies. Specifically, our objectives were to evaluate how hibernacula microclimates (temperature, humidity) and other hibernacula variables (pre-WNS use of hibernacula, availability of nearby sites) have influenced populations of hibernating bats (across time in the endemic phase) and to assess whether manipulative cooling of warm, little-used hibernacula could benefit bats.

METHODS

Study system

We examined survey and microclimate data from 31 hibernacula, including underground caves, mines, and tunnels widely distributed across Pennsylvania, in the mid-Atlantic region of the United States (Appendix S1). We focused on 5 bat species for which we had sufficient data: *E. fuscus*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, and *P. subflavus*. The pathogen Pd was first detected in Pennsylvania hibernacula in the winter of 2008–2009 (hereafter, winters are denoted by the second calendar year, here 2009) and was widespread at known hibernacula by 2012 (Hefernan & Turner, 2016). Severe population declines of several hibernating bat species were observed throughout 2009–2012 in Pennsylvania (Ingersoll et al., 2016; Turner et al., 2011); less marked changes occurred thereafter. We therefore focused on bat population changes since 2012.

Data collection

Bat counts were compiled from a long-term program of bat monitoring conducted by the Pennsylvania Game Commission (PGC). The PGC has surveyed hibernacula during winters since the mid-1980s with standardized methods. Surveys were conducted at nearly all sites at least once every 5 years, and typically once every 2 years. Surveys undertaken outside a 3-month period from December 21 to March 23 were excluded from analyses. During each survey, biologists unobtrusively identified bats visually to the species level and recorded the number of each species present. Bats that could not be confidently identified were recorded as unknown (<1%) and excluded from analyses. Starting in the 1990s, the section (areas with similar

environmental conditions) of the hibernaculum in which bats were observed was also noted, and hibernacula temperatures were collected with a laser-guided infrared thermometer adjacent to hibernating bats in each section. Relative humidity data loggers were deployed at a subset of sites beginning in the 2000s (Appendix S2).

Hibernacula manipulations

To assess whether cooling of warm, little-used sites would result in increased use by bats, we manipulated 6 sites, including 1 limestone mine, 2 limestone caves, 2 sandstone mines, and 1 abandoned railroad tunnel to cool interior temperatures. The median section temperature for these sites before manipulation was 10 °C. The modification of each site was accomplished from 2015 to 2018 by one-time engineering of entrances to hibernacula, taking into consideration the aboveground topography, entrance characteristics, and the underground structure of the sites. Specifically, 2 of the limestone sites were manipulated by creating additional entrances that accepted cold air while venting warm air through other entryways, whereas the third site had a steel door removed and a bat-friendly gate installed in its place. Conversely, we manipulated the 2 sandstone mines by sealing the entrances that allowed cold air to flow downslope and leave these sites, leaving the upslope entrances accessible to bats. Finally, the railroad tunnel had an earthen mound created directly in front of, but not restricting access to, its entrance. The height was made taller than the entrance, with the goal of keeping cool air from escaping, while still venting warmer air. All the manipulations occurred outside the hibernation period to avoid bat disturbance. Cooling began as soon as external temperatures dropped below internal temperatures. Surveys were conducted as described above before and after site manipulations.

Data analyses

We completed distinct analyses for each species and for manipulated and unmanipulated hibernacula. For unmanipulated hibernacula, we evaluated recent changes in bat populations with 2 sets of analyses. Both were conducted using generalized linear mixed models (GLMMs) and a response of bat count by hibernacula section. In the first set, we related recent changes in bat count to temperature (hereafter temperature analysis) in a data set of 167 surveys over 8 years (2013–2020) in 60 sections within 25 hibernacula. For each species, the global model included the predictor variables: year (years since 2012), temperature (average midwinter temperature of a section in degrees Celsius), natural logarithm of the number of other known underground sites within 10 km of the hibernaculum entrance (LnSiteNum) (value assumed to be correlated with the number of other known and unknown local hibernacula), natural logarithm of the highest known count of the species at the site in the 10 years prior to arrival of WNS in the state (LnHighCount), sequential day of winter, beginning 1 December (survey day),

and all interactions among year, temperature, and LnSiteNum. All models also included random effects of section nested in site (a hibernaculum). For each species, we first evaluated model type, comparing model fit among Poisson and negative binomial distributions with and without a constant zero-inflation parameter. The best fit to the data was obtained with a zero-inflated Poisson (ZIP) model in *M. septentrionalis* and negative binomial (NB) models in the other species. We used a model-selection approach with the small-sample-size-corrected Akaike's information criterion (AICc) to identify the model that best fit the data and alternative models with some support.

In the second set of analyses of unmanipulated hibernacula, we related recent changes in bat count to temperature and water vapor pressure deficit (VPD), where air is fully saturated with water vapor at $VPD = 0$. We assumed higher VPD correlates with higher evaporative water loss in bats and poorer conditions for Pd growth. This analysis (hereafter temperature-and-VPD analysis) was conducted in the subset of hibernacula for which humidity data were recorded: 51 surveys over 7 years (2014–2020) in 16 sections in 8 unmanipulated hibernacula. The global model included year, temperature, VPD, and all interactions. All models also included random effects of section nested in site. We chose the model type and selected the best model as in the temperature analysis. Here, the best fit to the data was obtained with negative binomial models in *M. lucifugus* and *E. fuscus*, Poisson models in *P. subflavus*, and ZIP models in *M. leibii*. We had insufficient data to model *M. septentrionalis* in this analysis.

For the analysis of the 6 manipulated hibernacula, we first used a paired *t*-test to compare site averages of midwinter temperature before and after manipulation. We then investigated changes in bat counts following manipulation relative to average values from 2012 to manipulation with data from 73 surveys (including 24 post manipulation) of 12 surveyed sections of the 6 manipulated hibernacula. We used linear mixed-effects models (LMMs) of the log response ratio of bat count. Bat count models were analyzed for *M. lucifugus*, *E. fuscus*, and *P. subflavus*, for which we had sufficient data. Predictors were years since manipulation, either temperature or average temperature difference of a section in degrees Celsius from before to after manipulation (TempDiff), and the interaction. All models included random effects of section nested in site. We then used model selection with AICc. Additional details on data analyses are included in Appendix S2.

RESULTS

Bat population change with temperature in unmanipulated hibernacula

A model with the 3-way interaction of year \times temperature \times LnSiteNum best predicted *M. lucifugus* counts at unmanipulated sites in the temperature analysis (Table 1, Appendix S3). Specifically, *M. lucifugus* counts increased substantially since 2012 only in colder hibernacula, and these increases were most pronounced when the colder hibernacula were located near a greater number of nearby underground sites (NB GLMM:

TABLE 1 Best models for each set of analyses, and significant effects

Analysis species	Best model (with significant effects underlined) ^a
Unmanipulated hibernacula, full dataset, temperature	
<i>M. lucifugus</i>	<u>Year*Temp*LnSiteNum</u>
<i>E. fuscus</i>	<u>Year + Temp + LnHighCount</u>
<i>P. subflavus</i>	<u>Year*Temp + Temp*LnSiteNum</u>
<i>M. leibii</i>	Year + <u>Temp</u> + LnHighCount
<i>M. septentrionalis</i>	<u>Year*LnSiteNum</u> + <u>Temp</u> + SurveyDay
Unmanipulated hibernacula, subset of data, temperature and VPD	
<i>M. lucifugus</i>	<u>Year*Temp*VPD</u>
<i>E. fuscus</i>	<u>Year*Temp*VPD</u>
<i>P. subflavus</i>	<u>Year*Temp</u>
<i>M. leibii</i>	<u>VPD</u>
Manipulated hibernacula	
<i>M. lucifugus</i>	<u>Years-since-manip</u>
<i>E. fuscus</i>	<u>TempDiff</u>
<i>P. subflavus</i>	<u>Years-since-manip</u>

^aVariable names are as in main text and Supplemental Methods of Supporting Information; significance is at alpha level of 0.05.

Asterisk represents interaction and all subsidiary effects; all models also contain the random effects of section nested in site.

3-way interaction = -0.071 [SE 0.029], $p = 0.01$; model conditional pseudo- $R^2 = 0.36$) (Figure 1). The best model for *M. lucifugus* from the temperature-and-VPD analysis had low support (Appendices S4 & S5), but this model suggested that such population increases in *M. lucifugus* in cold sites may be further reinforced in hibernacula with higher VPD (Table 1, Appendix S6).

In the temperature analysis for *E. fuscus*, a model with the main effects of year, temperature, and LnHighCount (and no interactions) best predicted *E. fuscus* count (NB GLMM: year = 0.098 [SE 0.044], $p = 0.03$; temperature = -0.73 [0.15], $p < 0.001$; LnHighCount = 0.59 [0.22], $p = 0.007$; model conditional pseudo- $R^2 = 0.45$) (Table 1, Appendix S7). Specifically, *E. fuscus* populations increased since 2012; more bats were found in colder hibernacula and where there had been higher *E. fuscus* counts prior to the onset of WNS (Figure 2). The best model for *E. fuscus* from the temperature-and-VPD analysis had low support (Appendices S3 & S8). This model suggested that bat counts in *E. fuscus* may be higher in hibernacula with both colder temperatures and higher VPD (Table 1, Appendix S9).

In the temperature analysis for *P. subflavus*, a model with the 2-way interactions of year \times temperature and temperature \times LnSiteNum best predicted *P. subflavus* count (Table 1, Appendix S10). This model was not well supported, but the second part suggests that *P. subflavus* counts were higher in warm, isolated hibernacula and in cold hibernacula with more nearby sites (Appendix S11). The first part of the temperature analysis was also consistent with results from the better-supported temperature-and-VPD analysis. In that temperature-and-VPD analysis, a model with the 2-way interaction of year \times temperature best predicted *P. subflavus* count (Poisson GLMM:

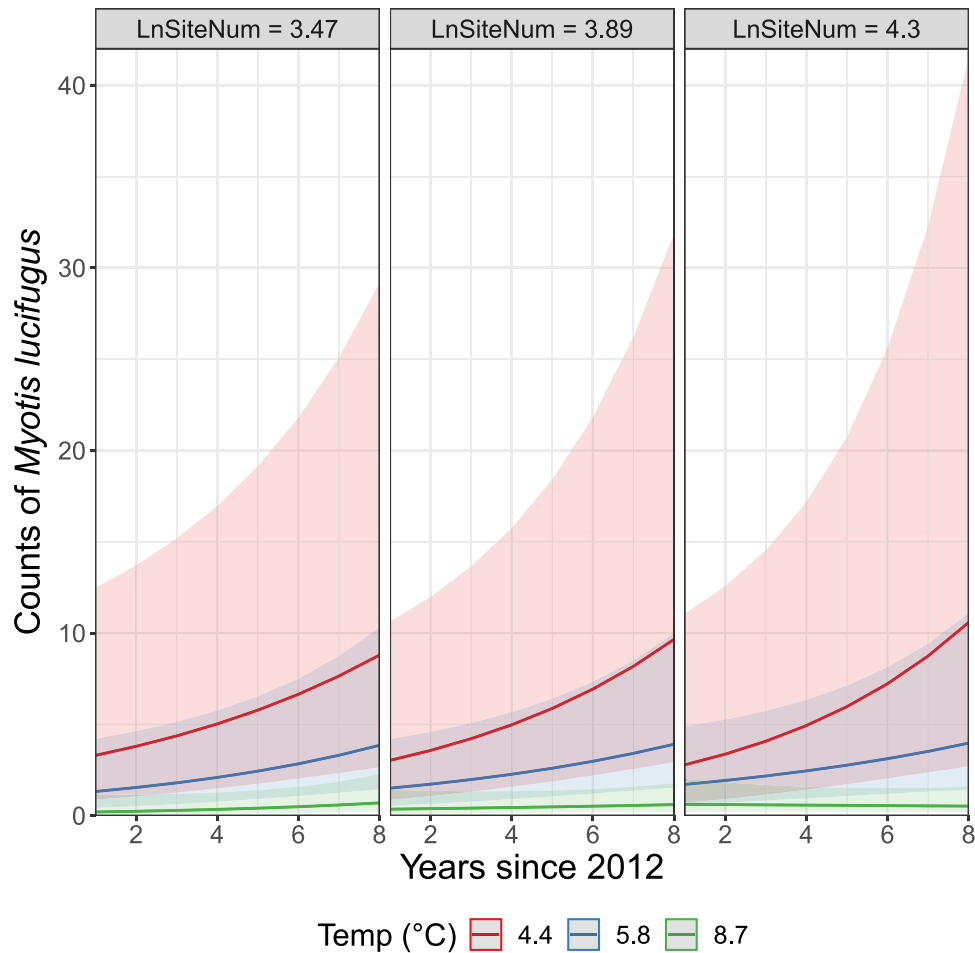


FIGURE 1 In unmanipulated hibernacula, effects of temperature, year, and number of nearby alternative hibernacula on counts of *Myotis lucifugus* (lines, temperature quartiles; panels, Ln number of sites [LnSiteNum] quartiles)

year \times temperature interaction = 0.12 [SE 0.03], $p = 0.0006$; model conditional pseudo- $R^2 = 0.79$) (Table 1 & Appendix S12). Specifically, *P. subflavus* populations decreased after 2012 in colder sites and increased slightly in warmer sites (Figure 3).

In the temperature analysis for *M. leibii*, several models had some support ($\Delta\text{AICc} < 2$; Appendix S13), but in all of them, temperature was the sole significant variable. In the best model, the main effects of year, temperature, and LnHighCount (and no interactions) predicted *M. leibii* count (NB GLMM: year = 0.14 [SE 0.08], $p = 0.11$; temperature = -0.51 [0.18], $p = 0.004$; LnHighCount = 0.87 [0.48], $p = 0.07$; model conditional pseudo- $R^2 = 0.68$) (Table 1 & Appendix S13); more *M. leibii* were observed in colder sites (Figure 4a). In the temperature-and-VPD analysis for *M. leibii*, 3 models had some support ($\Delta\text{AICc} < 2$) (Appendix S14), but in all of them, VPD was the sole significant variable. In the best model, VPD predicted *M. leibii* count (ZIP GLMM: VPD = 15.3 [4.6], $p = 0.0009$; R^2 value unavailable for a zero-inflated model) (Table 1, Appendix S14); more *M. leibii* were observed where the VPD was higher (Figure 4b).

In the temperature analysis for *M. septentrionalis*, a model with the year \times LnSiteNum interaction and the main effects of temperature and survey day best predicted count (ZIP GLMM: year \times LnSiteNum interaction = 0.57 [SE 0.17], $p = 0.001$; temperature = -0.97 [0.28], $p = 0.0007$; survey day = -0.0029 [0.0136], $p = 0.83$; R^2 value unavailable for a zero-inflated model) (Table 1, Appendix S15). Specifically, *M. septentrionalis* populations were larger in cold hibernacula. Populations of *M. septentrionalis* also increased since 2012 in hibernacula with many nearby sites, whereas they decreased elsewhere (Figure 5). We did not have sufficient data to complete a temperature-and-VPD analysis for *M. septentrionalis*.

Influence of hibernacula manipulation on bat populations

Manipulation decreased temperatures in hibernacula by 2.1 °C on average (paired t -test, manipulation = -2.10 [SE 0.55], $p = 0.004$). Temperatures did not change equally in all measured

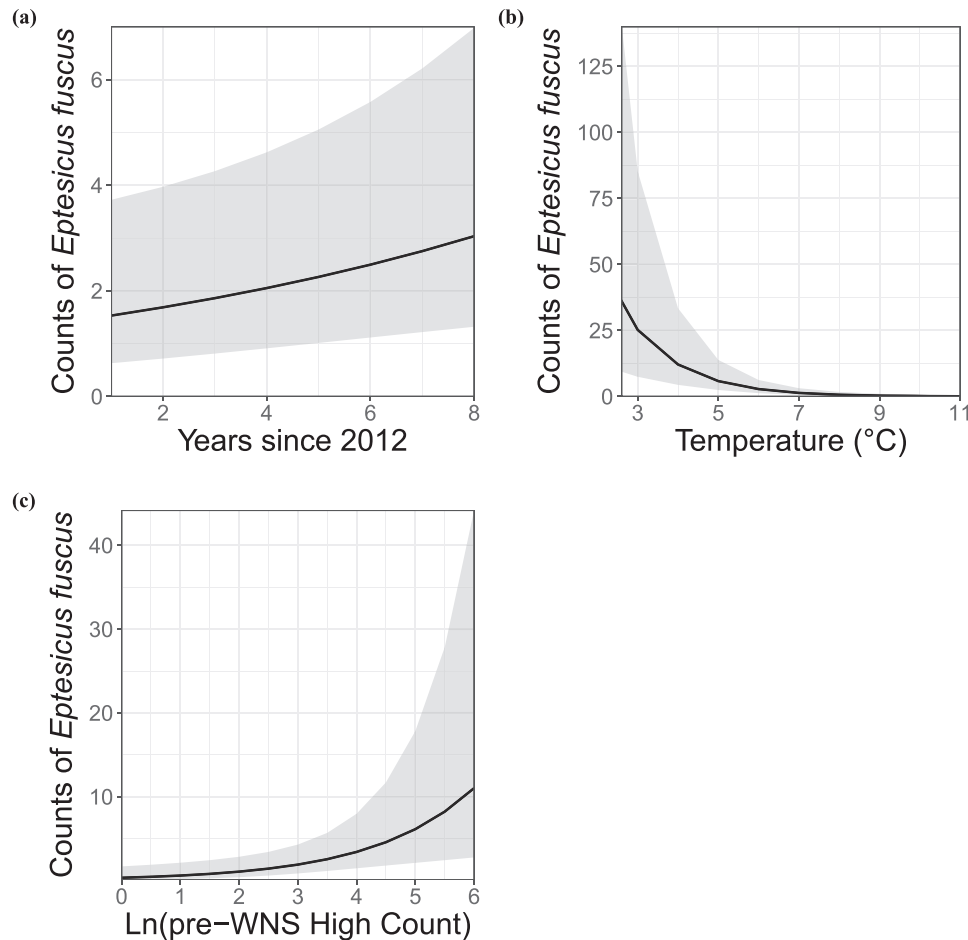


FIGURE 2 In unmanipulated hibernacula, counts of *Eptesicus fuscus* (a) since 2012, (b) relative to temperature, and (c) winter populations before the onset of white-nose syndrome (WNS) (shading, 95% confidence intervals)

sections of hibernacula, and different patterns were observed by section even in the same hibernaculum (Figure 6a). The best models for *M. lucifugus* had the single fixed effect of years since manipulation (Table 1, Appendix S16), indicating counts increased with time since manipulation relative to the premanipulation period (LMM: years since manipulation = 0.70 [0.28], $p = 0.03$; model $R^2 = 0.65$) (Figure 6b). The best model for *E. fuscus* had the single fixed effect of TempDiff (Table 1, Appendix S17), indicating counts were higher relative to the premanipulation period in sections of manipulated sites that had been cooled to a greater extent by manipulation (LMM: TempDiff = -0.46 [0.11], $p = 0.01$; model $R^2 = 0.91$) (Figure 6c). The best count model for *P. subflavus* had the single fixed effect of years since manipulation (Table 1, Appendix S18), indicating counts increased with time since manipulation relative to the premanipulation period (LMM, years since manipulation = 0.51 [0.15], $p = 0.005$; model $R^2 = 0.90$) (Figure 6d). An alternate model with some support ($\Delta\text{AICc} = 1.1$) (years since manipulation) indicated the same effect of time since manipulation and that *P. subflavus* counts tended to be higher in warmer sections of hibernacula following manipulation (Figure 6e & Appendix S18 & S19).

DISCUSSION

Several bat species responded to WNS by selecting hibernacula with microclimate conditions less favorable to Pd growth. This knowledge can be used by wildlife managers to enhance recovery of imperiled species. Specifically, we found that in the years following mass mortality from WNS, *M. lucifugus*, *E. fuscus*, *M. leibii*, and *M. septentrionalis* increased or had higher counts in colder hibernacula, particularly where average mid-winter temperatures were 3–6 °C. We also found evidence that *M. lucifugus*, *E. fuscus*, and *M. leibii* increased or had higher counts in hibernacula with higher VPD, particularly where VPD was over 0.1 kPa. Our finding that relatively cold hibernacula are important to several bat species is consistent with our earlier observations of a shift to colder hibernacula (Johnson et al., 2016) and are intuitive given that Pd grows more slowly (Verant et al., 2012) and bats conserve more energy (to an extent) (Boyles et al., 2020) in colder conditions. The role of VPD is less studied, but like temperature, lower humidity is associated with reduced fungal growth at 13 °C (Marroquin et al., 2017). Use of colder hibernacula with higher VPD should therefore benefit bats affected by WNS, although it may not be uniformly so.

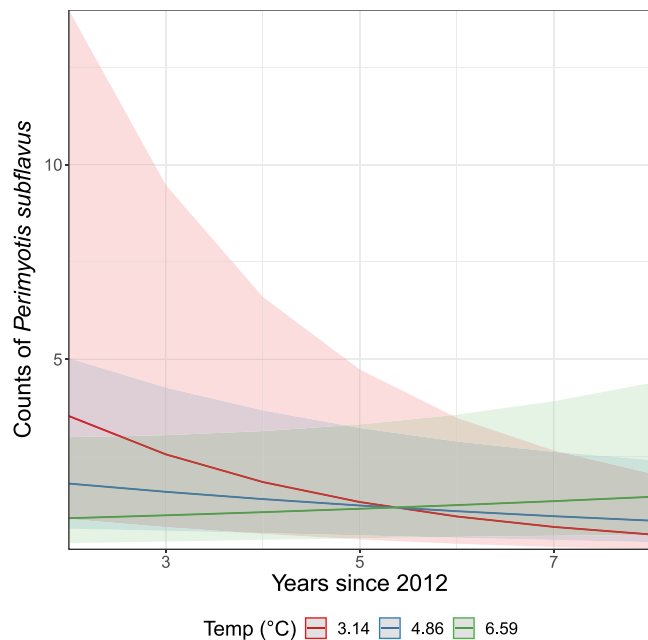


FIGURE 3 In unmanipulated hibernacula, effects of temperature on counts of *Perimyotis subflavus* since 2012 (lines, temperature quartiles; shading, 95% confidence intervals)

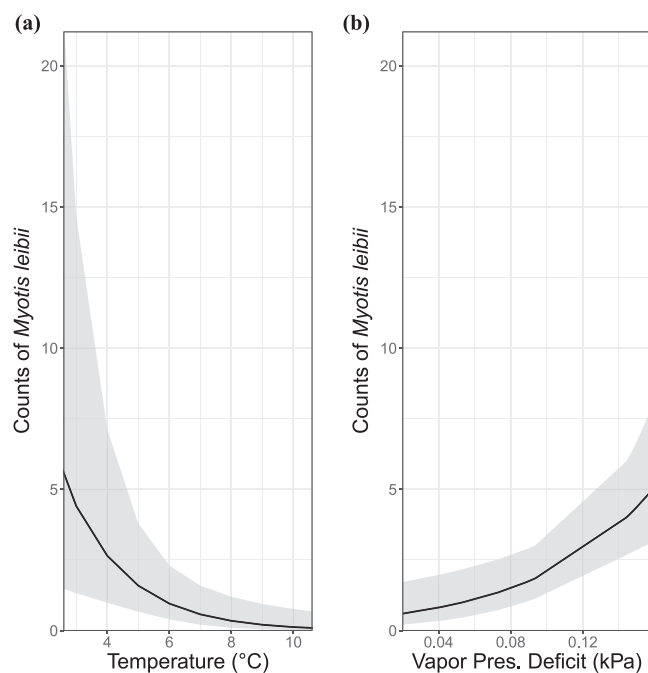


FIGURE 4 In unmanipulated hibernacula, (a) temperature and (b) vapor pressure deficit relative to counts of *Myotis leibii* (shading, 95% confidence intervals)

Hibernation in sites with temperatures below minimum thresholds may impose physiological costs (Boyles et al., 2020), whereas hibernation in locations with higher VPD may lead to more frequent arousals from hibernation (Ben-Hamo et al., 2013) and more rapid depletion of winter energy reserves. Thus, our results suggesting several bat species increase or are found

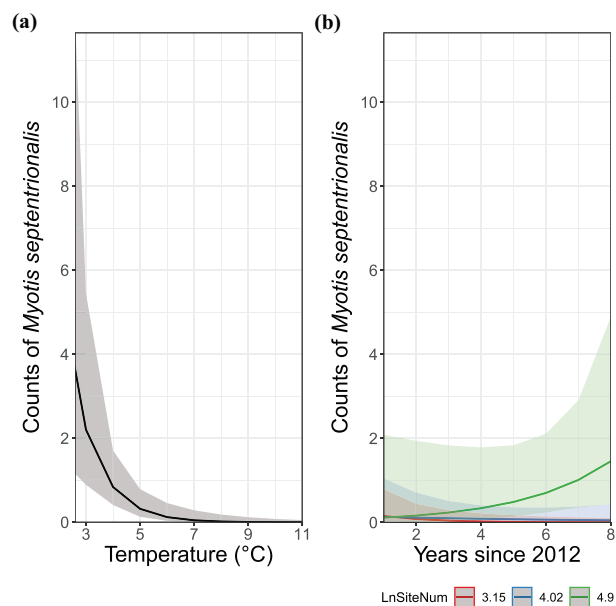


FIGURE 5 In unmanipulated hibernacula, counts of *Myotis septentrionalis* relative to (a) temperature and (b) time since 2012 (lines, quartiles of Ln number of sites [LnSiteNum]; shading, 95% confidence intervals)

at higher numbers in hibernacula that are both colder and have higher VPD are interesting because they suggest the combined benefits of Pd avoidance and metabolic savings in these environments outweigh potential costs.

Our results from manipulated sites were also consistent with this conclusion. Specifically, *M. lucifugus* increased after cooling warm (approximately 10 °C) hibernacula by an average of 2.1 °C, and *E. fuscus* counts increased more in areas with greater cooling. These results suggest that manipulating hibernacula temperatures can provide habitats that bats can discover. More broadly, these data provide an example of how studying host responses to pathogens can be used to devise management strategies to promote recovery from wildlife disease.

One exception to these patterns was in *P. subflavus*, in which counts decreased over time in unmanipulated hibernacula colder than 6 °C. This decline agrees with recent surveys of hibernacula in New Brunswick, Canada, where temperatures average <5 °C during winter and *P. subflavus* has been extirpated due to WNS (Vanderwolf & McAlpine, 2021). Prior to WNS, *P. subflavus* often inhabited relatively warm hibernacula and was uncommon during winter at the northern edge of its range, where hibernacula are often colder (Kurta & Smith, 2014; Vanderwolf & McAlpine, 2021). This may indicate that *P. subflavus* is less capable of hibernating in cold habitats in the presence of Pd than other species. However, we found some evidence for more *P. subflavus* in cold hibernacula surrounded by many alternative sites, similar to our previous observations (Johnson et al., 2016). Cooling hibernacula also resulted in increasing numbers of *P. subflavus* over time, apparently because even after manipulation, more complex underground sites continued to provide some warm areas where these bats tended to be found. Spatial variability in available temperatures throughout a hibernaculum

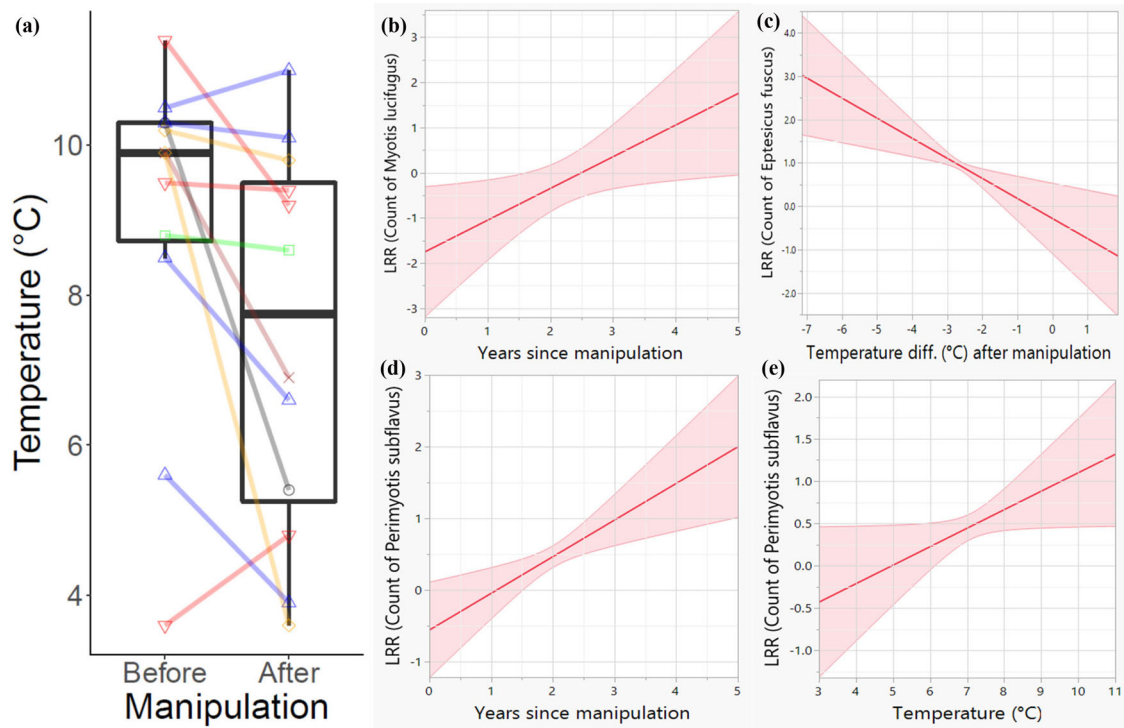


FIGURE 6 (a) Midwinter temperatures in hibernacula before and after manipulation (i.e., cooling) (boxes, interquartile range; whiskers, most distant points within 1.5 times the interquartile range; matching color and symbols on lines, sections in the same hibernaculum). In manipulated hibernacula, (b) counts of *Myotis lucifugus* and (d) *Perimyotis subflavus* relative to time since manipulation; (c) counts of *Eptesicus fuscus* relative to the amount of hibernacula cooling; and (e) counts of *P. subflavus* relative to temperature in a competing model that included time since manipulation and hibernacula temperature and predictor variables

may be beneficial to individual bats, allowing selection of microclimates based on their condition (Boyles et al., 2007). Similarly, management for multiple bat species may be best achieved by tailoring prioritization plans or manipulation to favor a variety of microclimates within and among hibernacula (Sewall et al., 2016).

Increased use of cold hibernacula differs from historical patterns of habitat selection for *M. lucifugus* in the region (Johnson et al., 2016). Lilley et al. (2018) suggest that hibernacula once favorable for North American cave-hibernating species could now serve as ecological traps because of their suitability for Pd. More recently, Hopkins et al. (2021) found that 52% of *M. lucifugus* hibernating in Michigan and Wisconsin continue to overwinter in sites warmer than 8 °C and exhibit a marginal preference for warmer sites despite lower recapture rates, and presumably survival, in these environments. In contrast, we found *M. lucifugus* increasing most in cold hibernacula surrounded by a greater density of underground sites, indicating that bats are avoiding low-quality habitats when higher-quality habitats are available. We also found no evidence that counts of *M. lucifugus*, *M. septentrionalis*, or *P. subflavus* were greatest in hibernacula with large populations prior to WNS, further indicating a shift in habitat use and avoidance of ecological traps. We found some evidence that *P. subflavus* used warmer sections of manipulated hibernacula and that counts in those manipulated sites and at warmer unmanipulated sites increased over time. We found no evidence of a shift in habitat selection

in *E. fuscus*, a cold-roosting species, which was found in greater numbers in sites where counts were high before arrival of WNS. Thus, our results do not suggest that warm hibernacula act as temperature-based ecological traps.

Our finding that counts of *M. lucifugus* and *M. septentrionalis* in cold hibernacula were correlated with the density of nearby underground habitats suggests bats are immigrating to suitable sites. Banding studies of *M. lucifugus* indicate that although philopatry to winter sites is high, movement does occur, even during winter (Humphrey & Cope, 1976; Norquay et al., 2013). Further, the number of hibernating *M. sodalis* increased faster than possible via annual reproduction following restoration of air flow in Wyandotte Cave, Indiana (Richter et al., 1993), and colonized Magazine Mine, Illinois, shortly after the end of mining operations (Kath, 2002). Similarly, at 1 manipulated site in our study, the count of *M. lucifugus* was just 2 individuals in the year after manipulation, but had reached 43 individuals at the same site 3 years later. These examples suggest bat species can respond to changes to habitat quality on a landscape scale through dispersal. Manipulating hibernacula microclimates is therefore a powerful tool for managing WNS because it capitalizes on bats' response to Pd and established ability to discover suitable environments. With the infrequency of sites with midwinter temperatures <6 °C across the mid-Atlantic region of North America, aggregation of survivors into a few sites may place vulnerable populations at risk to natural disasters such as flooding


or anthropogenic ones like vandalism or persecution. Hibernacula manipulation provides managers with a technique that could reduce the risk of catastrophic loss of 1 or a few critical sites.

Changes in disease outcomes can arise from changes to the host, pathogen, or environment. Because environmentally persistent fungi are not likely to be extirpated as host density declines (Kuris et al., 2014), environmental modification, leveraging host genetic or behavioral response to the disease, may promote long-term bat persistence. Although cooling hibernacula may not be appropriate in all climates (Johnson et al., 2021), this strategy can provide important thermal refugia, especially where sites colder than 6 °C are rare and where warmer sites are relatively abundant and underutilized by bats. We therefore recommend a landscape-scale approach to selecting sites where management efforts can have the greatest impact. For example, cooling hibernacula surrounded by a high density of alternative, but warm, sites may result in a greater increase in bats than managing a hibernaculum where several cold habitats are already available nearby. A somewhat different approach may be needed in regions where available hibernacula have temperatures that are too variable or cold. There, modifications to achieve microclimate stability or increased temperature could be more important than cooling. Finally, we urge the continued preservation of hibernacula with temperatures historically preferred by bats because these sites may once again become priority hibernacula if bats adapt to WNS in a way that allows them to return to these sites (Sewall et al., 2016).

ACKNOWLEDGMENTS

Funding for this research came from RCN and Indiana Bat Conservation Funding and PGC grant 4000023784 to BJS. The authors thank C. Butchkoski, C. Eichelberger, J. Sinclair, L. DeWolski, and C. Hauser for field assistance. The authors thank J. Kennedy for assisting with gating several sites, the United States Fish and Wildlife Service (USFWS) Partners for Fish and Wildlife program and Armstrong Drilling Inc. for excavation and gating, and J. Arway (USFWS), L. Zimmerman (Pennsylvania Fish and Boat Commission), and the Western Pennsylvania Conservancy for site acquisition. Finally, the authors thank the Pennsylvania Department of Conservation of Natural Resources, J. P. Espy, G. Czmor, R. Burd, R. Kromer, and many others for access to the sites included in this study. Financial support for this study was acquired by GT, BS, and DB. This study was conceptualized by GT, BS, and JJ. The methods were designed by GT, BS, JJ, and DB. Project supervision and administration was conducted by GT, MS, and DB. Data were collected by GT and MS, curated by MS and JJ, and analyzed by BS. Visualizations were produced by BS and TL. The original manuscript was drafted by GT, BS, TL, and JJ. The final manuscript was edited and reviewed by GT, BS, MS, TL, DB, and JJ.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Turner, G. G., Sewall, B. J., Scafani, M. R., Lilley, T. M., Bitz, Danieland, & Johnson, J. S. (2022). Cooling of bat hibernacula to mitigate white-nose syndrome. *Conservation Biology*. *36*:e13803. <https://doi.org/10.1111/cobi.13803>