Introduction Notes

1. Turner, G. G., Sewall, B. J., Scafini, M. R., Lilley, T. M., Bitz, D., & Johnson, J. S. (2022). Cooling of bat hibernacula to mitigate white‐nose syndrome. *Conservation Biology*, *36*(2), e13803.

Several bat species responded to WNS by selecting hibernacula with microclimate conditions less favorable to Pd growth. Specifically, found that in the years following mass mortality from WNS, LBB, BBB, M. leibii, and NLEB increased or had higher counts in colder hibernacula, particularly where average mid-winter temperatures were 3-6 C.

1. Moore, M. S., Field, K. A., Behr, M. J., Turner, G. G., Furze, M. E., Stern, D. W., ... & Reeder, D. M. (2018). Energy conserving thermoregulatory patterns and lower disease severity in a bat resistant to the impacts of white-nose syndrome. *Journal of Comparative Physiology B*, *188*, 163-176.

Little brown bats are more susceptible to WNS than big brown bats. Significantly more Pd-exposed little brown myotis developed Pd lesions than did Pd-exposed big brown bats. This finding is in line with field observations of differences in Pd prevalence and of mortality (Turner et al. 2011; Langwig et al. 2012, 2015; Frick et al. 2015).

1. Langwig, K. E., Frick, W. F., Bried, J. T., Hicks, A. C., Kunz, T. H., & Marm Kilpatrick, A. (2012). Sociality, density‐dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white‐nose syndrome. *Ecology letters*, *15*(9), 1050-1057.

In the past three decades a number of pathogens have invaded new regions and caused declines across entire communities of hosts (Riper et al. 1986; Lips et al. 2006; LaDeau et al. 2007). Clustering behavior facilitated high transmission regardless of colony size, with infected individuals having approximately the same number of contacts in small colonies as they did in larger populations. This pattern is consistent with transmission being frequency-dependent. Found that declines in Indiana bats were greater under more humid conditions, which suggest that growth of the fungus, and either intensity or prevalence of infections may be higher in more humid conditions. Also found that for little brown bats declines were higher in hibernacula with higher temperatures. This suggests that increased pathogen growth observed in the lab across the range of temperatures measured in hibernacula 3-15 C (Gargas et al. 2009; Chaturvedi et al. 2010) is important. It is possible that the lower declines observed in Indiana bats compared to LBB may be partly due to the cooler temperatures where Indiana bats hibernate.

1. Lilley, T. M., Johnson, J. S., Ruokolainen, L., Rogers, E. J., Wilson, C. A., Schell, S. M., ... & Reeder, D. M. (2016). White-nose syndrome survivors do not exhibit frequent arousals associated with Pseudogymnoascus destructans infection. *Frontiers in Zoology*, *13*, 1-8.

Despite infection with Pd the remnant population displayed less frequent arousals from torpor and lower torpid body temperatures than bats that died from WNS during the peak of mortality. The hibernation patterns of the remnant population resembled pre-WNS patterns with some modifications. Suriviing LBB do not experience the increase in periodic arousals from hibernation typified by bats dying from WNS, despite the presence of Pd on their skin. These patterns may reflect the use of colder hibernacula microclimates by WNS survivors, and/or may reflect differences in how these bats respond to the disease. Found that surviving bats had significantly lower mean torpor skin temperature compared to pre-WNS or peak-WNS bats. Bats can arouse less during hibernation to save fat and hibernate at lower temperatures to increase energy conservation and slow Pd growth. The microclimate a bat hibernates in is critical to the successful completion of a hibernation period (Kokurewicz 2004), because of their thermoconforming nature (but see Willis et al. 2005). Both Johnson et al. 2014 and Grieneisen et al. 2015 under controlled conditions, and Langwig et al. 2012 under field conditions, demonstrated a significant enhanced survival at lower temperatures in Pd affected bats. We found that post-WNS bats demonstrated the lowest torpor skin temperature (1.9 +- 1.4 C). But whether individual bats have altered their behavior to seek out colder microclimates or what we are seeing is the result of selection cannot be distinguished with the data available. One can speculate that a portion of the historic population has preferred colder microclimates in hibernacula, or used entire hibernacula that are not suitable for Pd growth and that influence torpor behavior. A colder microclimate at a certain point allows for longer hibernation bouts and lower energy expenditure but comes at the expense of expending more energy to arouse from torpor.

1. Lindauer, A. L., Maier, P. A., & Voyles, J. (2020). Daily fluctuating temperatures decrease growth and reproduction rate of a lethal amphibian fungal pathogen in culture. *BMC ecology*, *20*, 1-9.

Emerging infectious diseases are contributing to species die-offs worldwide. Pathogens are exposed to variable temperatures across daily, seasonal, and annual scales. Exposure to temperature fluctuations may reduce pathogen growth and reproduction, which could affect pathogen virulence, transmission, and environmental persistence with implications for disease. Observed decreased Bd growth and reproduction in a diurnally fluctuating thermal environment as compared to cultures grown at constant temperatures within the optimal Bd thermal range. Also found that Bd exhibits temperature-induced trade-offs under constant low and constant high temperature conditions.

1. Herczeg, D., Holly, D., Kásler, A., Bókony, V., Papp, T., Takács‐Vágó, H., ... & Hettyey, A. (2023). Amphibian larvae benefit from a warm environment under simultaneous threat from chytridiomycosis and ranavirosis. *Oikos*, *2023*(11), e09953.

Study showed increased higher survival of agile frogs and common toads when they were in temperature conditions beyond what is ideal for both Bd and ranavirosis (Rv), suggesting that amphibians can find refuge and survive in conditions with these temperature conditions. In this case 30 C

1. Turner, A., Wassens, S., Heard, G., & Peters, A. (2021). Temperature as a driver of the pathogenicity and virulence of amphibian chytrid fungus Batrachochytrium dendrobatidis: A systematic review. *The Journal of Wildlife Diseases*, *57*(3), 477-494.

Temperature is one of the most important environmental factors influencing the outcome of interactions between Bd and amphibian hosts. Bd displays optimal growth between 17 and 25 C, and fails to grow at 28 C (Johnson et al. 2003; Piotrowski et al. 2004; Kriger & Hero 2008). However, Bd is able to survive at 4 C and can, therefore, overwinter in its hosts (Piotrowski et al. 2004; Bosch et al. 2007).

1. Hayman, D. T., Pulliam, J. R., Marshall, J. C., Cryan, P. M., & Webb, C. T. (2016). Environment, host, and fungal traits predict continental-scale white-nose syndrome in bats. *Science advances*, *2*(1), e1500831.

We model the growth dynamics of Pd and energetic requirements of WNS-affected hibernating bats under a range of environmental conditions. Populations of little brown bat in the northeastern United States and Canada have been more affected by WNS than any other species (Frick et al. 2010). Model Pd growth as a function of body temperature and RH and incorporate this into an energetic model across a range of ambient temperatures. The model predicts that hibernating little browns infected with Pd can make energy reserves last 6 months at both ambient temperatures between 1 and 6 C and <98% RH. M. lucifugus experimentally infected with European and North American isolates of Pd died within 70 to 120 days at 7C and 97% RH (Warnecke et al. 2012). Model suggests that behavioral and/or physiological traits may have evolved or been preadapted in the European species to increase survival with Pd infection, whereas in Little and Big brown bats, such traits have not evolved. Findings suggest that environmental conditions and basic host traits alone may explain much of the variability in disease outcomes among species of bats infected by Pd in North America and Europe. The importance of RH to Pd growth is plausible, because conidial fungi such as Pd are more likely to germinate and degrade nutrient substrates in the presence of high moisture levels in their environments (Hajek et al. 1990). Bats that use microhabitats with lower RH will be less susceptible to WNS. In most caves, RH reaches 100% far from entrances but can vary throughout, and RH is affected by ambient temperatures, airflow, and atmospheric pressure (Perry 2013). Typically, bats use hibernation sites with 90 to 100% RH (Thomas & Cloutier 1992). The three most affected species M. lucifugus, M. septentrionalis, and P. subflavus, consistently roost in the most humid locations within hibernacula and are regularly observed with condensation of their fur (Cryan et al. 2010; Langwig et al. 2012), while three less severely affected species M. sodalist, M. leibii, and E. fuscus tend to select drier areas within hibernacula. Increasing humidity in the presence of Pd generally decreases bat survival (Langwig et al 2012). M. lucifugus is in a positive water balance at 2 and 4 C only at >= 99% RH (Thomas and Cloutier 1992). Understanding the interactions between water vapor pressure and EWL (Ben-Hamo et al. 2012, 2013). Survival was most sensitive to changes in body mass, with increasing body mass decreasing mortality (possibly due to increased fat available and decreased thermal conductance), followed by lower temperature at and above which bats are thermally neutral and minimal resting metabolic rate occurs. Strong selection pressure for traits that lead to larger body sizes, and hibernation in colder and drier sites. Increased metabolic rates may occur through alternative mechanisms in the absence of increased arousal frequency (Verant et al. 2014). Other mechanisms that may lead to decreased survival of WNS-affected bats include altered physiological processes during winter, such as fungal damage to wing membranes potentially disrupting blood circulation, water and electrolyte balance, or immune function (Cryan et al. 2010, 2013; Warnecke et al. 2013; Bouma et al. 2013; Meteyer et al. 2012). Because Pd growth is what influences arousal (Warnecke et al. 2012; Reeder et al. 2012). One arousal bout of M. lucifugus hibernating at 5C consumes the same amount of fat energy as 67 days spent in torpor (Thomas et al. 1990). Temporal dynamics of WNS as it spreads (Maher et al. 2012).

CITED

W. F. Frick, J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, T. H. Kunz, An emerging disease causes regional population collapse of a common North American bat species. *Science* **329**, 679–682 (2010).

L. Warnecke, J. M. Turner, T. K. Bollinger, J. M. Lorch, V. Misra, P. M. Cryan, G. Wibbelt, D. S. Blehert, C. K. R. Willis, Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 6999–7003 (2012).

A. E. Hajek, R. I. Carruthers, R. S. Soper, Temperature and moisture relations of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymantriidae). *Environ. Entomol.* **19**, 85–90 (1990).

R. W. Perry, A review of factors affecting cave climates for hibernating bats in temperate North America. *Environ. Rev.* **21**, 28–39 (2013).

D. W. Thomas, D. Cloutier, Evaporative water loss by hibernating little brown bats, Myotis lucifugus. *Physiol. Zool.* **65**, 443–456 (1992).

P. M. Cryan, C. U. Meteyer, J. G. Boyles, D. S. Blehert, Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biol.* **8**, 135 (2010).

K. E. Langwig, W. F. Frick, J. T. Bried, A. C. Hicks, T. H. Kunz, A. M. Kilpatrick, Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecol. Lett.* **15**, 1050–1057 (2012).

M. Ben-Hamo, A. Muñoz-Garcia, C. Korine, B. Pinshow, Hydration state of bats may explain frequency of arousals from torpor. *FASEB J.* **26**, 886.11 (2012).

M. Ben-Hamo, A. Muñoz-Garcia, J. B. Williams, C. Korine, B. Pinshow, Waking to drink: Rates of evaporative water loss determine arousal frequency in hibernating bats. *J. Exp. Biol.* **216**, 573–577 (2013).

M. L. Verant, C. U. Meteyer, J. R. Speakman, P. M. Cryan, J. M. Lorch, D. S. Blehert, White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. *BMC Physiol.* **14**, 10 (2014).

P. M. Cryan, C. U. Meteyer, D. S. Blehert, J. M. Lorch, D. M. Reeder, G. G. Turner, J. Webb, M. Behr, M. Verant, R. E. Russell, K. T. Castle, Electrolyte depletion in white-nose syndrome bats. *J. Wildl. Dis.* **49**, 398–402 (2013)

L. Warnecke, J. M. Turner, T. K. Bollinger, V. Misra, P. M. Cryan, D. S. Blehert, G. Wibbelt, C. K. R. Willis, Pathophysiology of white-nose syndrome in bats: A mechanistic model linking wing damage to mortality. *Biol. Lett.* **9**, 20130177 (2013).

H. R. Bouma, R. H. Henning, F. G. M. Kroese, H. V. Carey, Hibernation is associated with depression of T-cell independent humoral immune responses in the 13-lined ground squirrel. *Dev. Comp. Immunol.* **39**, 154–160 (2013).

C. U. Meteyer, D. Barber, J. N. Mandl, Pathology in euthermic bats with white nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. *Virulence* **3**, 583–588 (2012).

D. M. Reeder, C. L. Frank, G. G. Turner, C. U. Meteyer, A. Kurta, E. R. Britzke, M. E. Vodzak, S. R. Darling, C. W. Stihler, A. C. Hicks, R. Jacob, L. E. Grieneisen, S. A. Brownlee, L. K. Muller, D. S. Blehert, Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLOS One* **7**, e38920 (2012).

D. W. Thomas, M. Dorais, J.-M. Bergeron, Winter energy budgets and cost of arousals for hibernating little brown bats, *Myotis lucifugus*. *J. Mammal.* **71**, 475–479 (1990).

S. P. Maher, A. M. Kramer, J. T. Pulliam, M. A. Zokan, S. E. Bowden, H. D. Barton, K. Magori, J. M. Drake, Spread of white-nose syndrome on a network regulated by geography and climate. *Nat. Commun.* **3**, 1306 (2012).

1. M. Ben-Hamo, A. Muñoz-Garcia, J. B. Williams, C. Korine, B. Pinshow, Waking to drink: Rates of evaporative water loss determine arousal frequency in hibernating bats. *J. Exp. Biol.* **216**, 573–577 (2013).

Hibernation is not a constant state of reduced body temperature and metabolic rate; rather it comprises bouts of torpor interspersed with periods of arousal, when the animal returns to its normothermic body temperature and metabolic rate (French 1985). Periods of arousals can last 350 h but rarely exceed 24 h (Geiser & Ruf, 1995). Although bats arouse for only 5-10% of the time they are hibernating, arousals can account for over 85% of a hibernating bat’s energy expenditure (Wang 1978; Thomas et al. 1990; Geiser & Ruf 1995; Dunbar & Thomasi 2006; Jonasson & Willis 2012). Because arousals involve such high energetic costs, one might assume their occurrence to be obligatory and adaptive. Presumably, critical processes or functions that must be periodically restored at normothermic body temperature for the organism’s survival necessitate these arousals. Number of arousals was positively correlated with total evaporative water loss of bats in dry air but not humid air.

CITED

French, A. R. (1985). Allometries of the durations of torpid and euthermic intervals during mammalian hibernation: a test of the theory of metabolic control of the timing of changes in body temperature. *Journal of comparative Physiology B*, *156*, 13-19.

Geiser, F., & Ruf, T. (1995). Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. *Physiological zoology*, *68*(6), 935-966.

Korine, C., & Pinshow, B. (2004). Guild structure, foraging space use, and distribution in a community of insectivorous bats in the Negev Desert. *Journal of Zoology*, *262*(2), 187-196.

Dunbar, M. B., & Tomasi, T. E. (2006). Arousal patterns, metabolic rate, and an energy budget of eastern red bats (Lasiurus borealis) in winter. *Journal of Mammalogy*, *87*(6), 1096-1102.

Thomas, D. W., Dorais, M., & Bergeron, J. M. (1990). Winter energy budgets and cost of arousals for hibernating little brown bats, Myotis lucifugus. *Journal of mammalogy*, *71*(3), 475-479.

Wang, L. CH 1978. Energetic and field aspects of mammalian torpor: the Richardson's ground squirrel. *Strategies in the cold: natural torpidity and thermogenesis (L. CH Wang and J. W. Hudson, eds.). Academic Press, New York*, 109-145.

1. Zhang T, Chaturvedi V, Chaturvedi S (2015) Novel *Trichoderma polysporum* Strain for the Biocontrol of *Pseudogymnoascus destructans*, the Fungal Etiologic Agent of Bat White Nose Syndrome. PLoS ONE 10(10): e0141316. https://doi.org/10.1371/journal.pone.0141316

Control of Pd in affected sites is urgently needed to break the transmission cycle while minimizing any adverse impact on the native organisms. Isolated a novel strain of *Trichoderma polysporum* (Tp) from one of the caves at the epicenter of WNS zoonotic. Tp WPM 39143 restricted Pd colony growth in dual culture challenges. Results suggest Tp WPM 39143 is a promising candidate for further evaluation as a biocontrol agent of Pd in WNS affected sites.

1. Micalizzi, E. W., Mack, J. N., White, G. P., Avis, T. J., & Smith, M. L. (2017). Microbial inhibitors of the fungus Pseudogymnoascus destructans, the causal agent of white-nose syndrome in bats. *PLoS one*, *12*(6), e0179770.

After the winter, surviving bats can rid themselves of Pd (Langwig et al. 2015; Meteyer et al. 2011) and quickly heal their skin lesions (Fuller et al. 2011). However, because Pd persists in hibernacula by growing saprotrophically when bats are absent (Lorch et al. 2013; Reynolds & Barton 2014); it is possible that healthy bats could be infected when entering contaminated hibernacula (Langwig et al. 2015; Reynolds et al. 2015). Model predictions have suggested that under certain circumstances, reducing the growth of Pd in hibernacula may mitigate or prevent WNS-associated colony collapse (Reynolds et al. 2015; Meyer et al. 2016). We identified 145 microbes that inhibit the growth of Pd to some extent, and 53 that completely or nearly completely inhibited Pd (Micalizzi et al. 2017).

1. Gabriel, K. T., McDonald, A. G., Lutsch, K. E., Pattavina, P. E., Morris, K. M., Ferrall, E. A., ... & Cornelison, C. T. (2022). Development of a multi-year white-nose syndrome mitigation strategy using antifungal volatile organic compounds. *Plos one*, *17*(12), e0278603.

In an effort to mitigate precipitous declines in bat populations due to WNS, we have developed and implemented a multi-year mitigation strategy. Mitigation approach involved in situ treatment of bats at the colony level through aerosol distribution of antifungal volatile organic compounds (VOCs) that demonstrated an in vitro ability to inhibit Pd conidia germination and mycelial growth through contact-independent exposure. The VOCs evaluated have been identified from microbes inhabiting naturally-occuring fungistatic soils and endophytic fungi. These VOCs are of low toxicity to mammals and have been observed to elicit antagonism of Pd at low gaseous concentrations. Pd is a psychrophilic ascomycete that has been identified as the etiological agent responsible for the deadly EFD among North American bats known as WNS. While torpid, the body temperature of bats often fall within the growth range of Pd (0 to 20 C) (Verant et al. 2012).

A number of potential mitigation methods have been developed or experimentally tested against Pd or treating WNS, including chemical agents (Cornelison et al. 2014; Boire et al. 2016; Padhi et al. 2017; Gabriel et al. 2018; Micalizzi & Smith 2020; Rusman et al. 2020), microbial antagonists (Cornelison et al. 2014; Hoyt et al. 2015; Zhang et al. 2015; Cheng et al. 2017; Micalizzi et al. 2017; Singh et al. 2018; Hoyt et al. 2019), environmental modulation (Wilcox & Willis 2016; Marroquin et al. 2017), UV light exposure (Palmer et al. 2018; Hartman et al. 2020; Kwait et al. 2022), antibiotics (Court et al. 2017), vaccination (Rocke et al. 2019), and electrolyte supplementation (McGuire et al. 2019), among others. Of these the greatest interest has been in the use of chemical and microbial agents to inhibit the growth and pathogenicity of Pd. There is not enough evidence to make any conclusions as to whether the treatments were effective at increasing bat survivorship. The application of these methods in other hibernacula that have greater complexity, such as natural caves will introduce challenges that may hinder application and make distribution of treatment formulation through these structures more difficult.

Citations:

Cornelison CT, Gabriel KT, Barlament C, Crow SA. Inhibition of *Pseudogymnoascus destructans* Growth from Conidia and Mycelial Extension by Bacterially Produced Volatile Organic Compounds. Mycopathologia. 2014 Feb 1;177(1):1–10. pmid:24190516

Boire N, Zhang S, Khuvis J, Lee R, Rivers J, Crandall P, et al. Potent Inhibition of Pseudogymnoascus destructans, the Causative Agent of White-Nose Syndrome in Bats, by Cold-Pressed, Terpeneless, Valencia Orange Oil. PLOS ONE. 2016 Feb 5;11(2):e0148473. pmid:26849057

Padhi S, Dias I, Bennett JW. Two volatile-phase alcohols inhibit growth of *Pseudogymnoascus destructans*, causative agent of white-nose syndrome in bats. Mycology. 2017 Jan 2;8(1):11–6.

Gabriel KT, Kartforosh L, Crow SA, Cornelison CT. Antimicrobial Activity of Essential Oils Against the Fungal Pathogens *Ascosphaera apis* and *Pseudogymnoascus destructans*. Mycopathologia. 2018 Dec 1;183(6):921–34. pmid:30306397

Padhi S, Dias I, Korn VL, Bennett JW. *Pseudogymnoascus destructans*: Causative Agent of White-Nose Syndrome in Bats Is Inhibited by Safe Volatile Organic Compounds. Journal of Fungi. 2018 Jun;4(2):48. pmid:29642609

Micalizzi EW, Smith ML. Volatile organic compounds kill the white-nose syndrome fungus, *Pseudogymnoascus destructans*, in hibernaculum sediment. Can J Microbiol. 2020 Oct 1;66(10):593–9. pmid:32485113

Rusman Y, Wilson MB, Williams JM, Held BW, Blanchette RA, Anderson BN, et al. Antifungal Norditerpene Oidiolactones from the Fungus *Oidiodendron truncatum*, a Potential Biocontrol Agent for White-Nose Syndrome in Bats. J Nat Prod. 2020 Feb 28;83(2):344–53. pmid:31986046

Cornelison CT, Keel MK, Gabriel KT, Barlament CK, Tucker TA, Pierce GE, et al. A preliminary report on the contact-independent antagonism of *Pseudogymnoascus destructans* by *Rhodococcus rhodochrous* strain DAP96253. BMC Microbiology. 2014 Sep 26;14(1):246. pmid:25253442

Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF, Kilpatrick AM. Bacteria Isolated from Bats Inhibit the Growth of *Pseudogymnoascus destructans*, the Causative Agent of White-Nose Syndrome. PLOS ONE. 2015 Apr 8;10(4):e0121329. pmid:25853558

Zhang T, Chaturvedi V, Chaturvedi S. Novel *Trichoderma polysporum* Strain for the Biocontrol of *Pseudogymnoascus destructans*, the Fungal Etiologic Agent of Bat White Nose Syndrome. PLOS ONE. 2015 Oct 28;10(10):e0141316. pmid:26509269

Cheng TL, Mayberry H, McGuire LP, Hoyt JR, Langwig KE, Nguyen H, et al. Efficacy of a probiotic bacterium to treat bats affected by the disease white-nose syndrome. Journal of Applied Ecology. 2017;54(3):701–8.

Micalizzi EW, Mack JN, White GP, Avis TJ, Smith ML. Microbial inhibitors of the fungus *Pseudogymnoascus destructans*, the causal agent of white-nose syndrome in bats. PLOS ONE. 2017 Jun 20;12(6):e0179770. pmid:28632782

Singh A, Lasek-Nesselquist E, Chaturvedi V, Chaturvedi S. *Trichoderma polysporum* selectively inhibits white-nose syndrome fungal pathogen *Pseudogymnoascus destructans* amidst soil microbes. Microbiome. 2018 Aug 8;6(1):139. pmid:30089518

Hoyt JR, Langwig KE, White JP, Kaarakka HM, Redell JA, Parise KL, et al. Field trial of a probiotic bacteria to protect bats from white-nose syndrome. Sci Rep. 2019 Jun 24;9(1):9158. pmid:31235813

Wilcox A, Willis CKR. Energetic benefits of enhanced summer roosting habitat for little brown bats (*Myotis lucifugus*) recovering from white-nose syndrome. Conservation Physiology. 2016 Jan 1;4(1). pmid:27293749

Marroquin CM, Lavine JO, Windstam ST. Effect of Humidity on Development of *Pseudogymnoascus destructans*, the Causal Agent of Bat White-Nose Syndrome. nena. 2017 Mar;24(1):54–64.

Palmer JM, Drees KP, Foster JT, Lindner DL. Extreme sensitivity to ultraviolet light in the fungal pathogen causing white-nose syndrome of bats. Nat Commun. 2018 Jan 2;9(1):35. pmid:29295979

Hartman CJ, Mester JC, Hare PM, Cohen AI. Novel inactivation of the causative fungal pathogen of white-nose syndrome with methoxsalen plus ultraviolet A or B radiation. PLOS ONE. 2020 Sep 11;15(9):e0239001. pmid:32915896

Kwait R, Kerwin K, Herzog C, Bennett J, Padhi S, Zoccolo I, et al. 2022. Whole-room ultraviolet sanitization as a method for the site-level treatment of Pseudogymnoascus destructans. Conservation Science and Practice. 2022;4:e623.

Court MH, Robbins AH, Whitford AM, Beck EV, Tseng FS, Reeder DM. Pharmacokinetics of terbinafine in little brown myotis (*Myotis lucifugus*) infected with *Pseudogymnoascus destructans*. American Journal of Veterinary Research. 2017 Jan 1;78(1):90–9. pmid:28029293

Rocke TE, Kingstad-Bakke B, Wüthrich M, Stading B, Abbott RC, Isidoro-Ayza M, et al. Virally-vectored vaccine candidates against white-nose syndrome induce anti-fungal immune response in little brown bats (*Myotis lucifugus*). Sci Rep. 2019 May 1;9(1):6788. pmid:31043669

McGuire LP, Mayberry HW, Fletcher QE, Willis CKR. An experimental test of energy and electrolyte supplementation as a mitigation strategy for white-nose syndrome. Conservation Physiology. 2019 Jan 1;7(1). pmid:30805191

1. Hoyt JR, Langwig KE, White JP, Kaarakka HM, Redell JA, Parise KL, et al. Field trial of a probiotic bacteria to protect bats from white-nose syndrome. Sci Rep. 2019 Jun 24;9(1):9158. pmid:31235813

In free-flying experiment, treatment with the probiotic, *P. fluorescens*, increased apparent overwinter survival more than five-fold by extending the last date of detection by a month into early spring. Although over half of *P. fluorescens*-treated bats still likely died from WNS over the winter.

1. Kwait R, Kerwin K, Herzog C, Bennett J, Padhi S, Zoccolo I, et al. 2022. Whole-room ultraviolet sanitization as a method for the site-level treatment of Pseudogymnoascus destructans. Conservation Science and Practice. 2022;4:e623.

Surviving bats clear infection each summer but are re-infected upon return to the hibernaculum. Therefore, addressing environmental reservoirs is critical for managing WNS. Ultraviolet light (UV) is known to kill Pd in the lab.

1. McGuire LP, Mayberry HW, Fletcher QE, Willis CKR. An experimental test of energy and electrolyte supplementation as a mitigation strategy for white-nose syndrome. Conservation Physiology. 2019 Jan 1;7(1). pmid:30805191

Infected bats in the Pedialyte-supplemented group generally avoided the Pedialyte and preferentially drank plain water. We did not observe any differences in survival, arousal frequency or blood chemistry, but bats in the Pedialyte-supplemental group and higher fungal load and more UV fluorescence than the control group. This approach to supplement electrolytes was shown to be ineffective.

1. Sewall, B. J., Turner, G. G., Scafini, M. R., Gagnon, M. F., Johnson, J. S., Keel, M. K., ... & Overton, B. E. (2023). Environmental control reduces white‐nose syndrome infection in hibernating bats. *Animal Conservation*, *26*(5), 642-653.

We used a small captive environmental control strategy to manage Pd within its environmental reservoir where the pathogen is endemic. The strategy centers on the application of Polyethylene Glycol 8000 (PEG) to roost substrates in summer, prior to bat hibernation, as a means to disrupt environmental transmission to bats in early winter. In the field trial, Pd load and infection extent both declined substantially in free-ranging M. lucifugus after treatment relative to controls, with declines exceeding effects of inter-site and inter-annual variation. Pathogen prevalence and load also declined.

1. Meierhofer, M. B., Johnson, J. S., Perez‐Jimenez, J., Ito, F., Webela, P. W., Wiantoro, S., ... & Mammola, S. (2024). Effective conservation of subterranean‐roosting bats. *Conservation Biology*, *38*(1), e14157.

Bats are a keystone species in some subterranean ecosystems as the guano they deposit is one of the main sources of energy that moves through the subterranean food web (Pilo et al. 2023; Sakoui et al. 2020). In addition, they may serve as an umbrella species in the protection of subterranean species, including a wealth of invertebrates and microorganisms (Frick et al. 2020; Phelps et al. 2016; Tanalgo et al. 2018). Climate change is altering microclimatic conditions in subterranean environments (Mammola, Piano et al. 2019; Sanchez-Fernandez et al. 2021), whereas broad-scale habitat change at the surface, including urbanization and deforestation, affects the quality of habitats underneath (Couton et al. 2023; Whitten 2009). Factors such as diseases (Hoyt et al. 2021), overexploitation (e.g., tourism, guano harvesting, hunting), and direct habitat destruction and alteration (mining) (Chaber et al. 2021; Ferreira et al., 2022; Frick et al. 2020; Furey & Racey 2016; Piano et al., 2020) all can result in local population declines of subterranean biota, including roosting bats. Although bats are reservoirs for several viruses (Letko et al. 2020; Van Brussel & Holmes 2022; Wu et al. 2016), including harboring related coronaviruses (Li et al. 2005; Murakami et al. 2020), the idea that bats are the origin of the coronavirus pandemic is based on unproven assumptions and oversimplifies the process of disease spillover (Plowright et al. 2017).

1. Johnson, J. S., Scafini, M. R., Sewall, B. J., & Turner, G. G. (2016). Hibernating bat species in Pennsylvania use colder winter habitats following the arrival of white-nose syndrome. *Conservation and ecology of Pennsylvania’s bats*, 181-199.

Found that after WNS the majority of Little Brown Bats, tri-colored bats, and big brown bats occupied sections of hibernacula that were colder than sections with the largest aggregations prior to the onset of WNS. Furthermore, found members of each species in sections with colder minimum temperatures than sections reported as occupied before WNS. At least 3 species of hibernating bats in Pennsylvania have changed their winter behavior in the years following dramatic population declines, providing management agencies with options for enhancing winter habitat. Although temperatures represent an energetic stress during normothermy, temperatures between 0 and 10 C (Heldmaier et al. 2004) are associated with the greatest reductions in metabolic rate, and, therefore, energy used during torpor. Temperatures below freezing, as well as temperatures > 10 C, are associated with higher torpid metabolic rates and higher rates of arousal from hibernation, resulting in morae energy spent during hibernation, and a decrease in the potential hibernating period (Thomas et al. 1990; Buck and Barnes 2000; Geiser 2004). Boyles et al. (2007) found that little brown myotis with lower body mass, and presumably lower fat reserves, hibernated in colder areas (promoting longer torpor bouts and more energy savings) than individuals with greater body mass within the same hibernaculum (CHECK THIS TO SEE HOW THEY DID THIS, BECAUSE BATS MOVE). Thus, hibernacula temperatures have a profound impact on bats during their winter hibernation. Hibernacula with high species diversity commonly provide a range of temperatures that meet the hibernation preferences of various species throughout the hibernation season.

1. Loeb, S. C., & Winters, E. A. (2022). Changes in hibernating tricolored bat (Perimyotis subflavus) roosting behavior in response to white‐nose syndrome. *Ecology and Evolution*, *12*(7), e9045.

Prior to WNS, 95% of tri-colored bats roosted in the back portion of the tunnel that was the warmest, after WNS a significant increase in the proportion of bats using the front, colder portions of the tunnel. Greater use of the colder sections of the tunnel by tricolored bats could have led to increased survival due to slower growth rates of the fungus that caused WNS in colder temperatures or decreased energetic costs associated with colder hibernation temperatures. While some species have gone extinct in the face of disease, others manage to persist, albeit at lower abundance. Persistence to pathogens can be attained through resistance or tolerance, where resistance is the ability to limit infection and tolerance is the ability to limit the effects of the pathogen (Brannelly et al. 2021; Raberg et al. 2007; Roy & Kirchner 2000).

1. Crowley, L. N. (2024). *Predicting Fungal Growth at the Edge of the Range: Impacts of Microclimate on Probability of White-Nose Syndrome in Southeastern Hibernacula* (Master's thesis, Austin Peay State University).

Findings suggest that atypical hibernacula (culverts) exhibited lower humidity resulting in reduced fungal growth and an atypical hibernating behavior (shallow and short torpor bouts), which may contribute to disease evasion.

1. Cheng, T. L., Bennett, A. B., Teague O'Mara, M., Auteri, G. G., & Frick, W. F. (2024). Persist or Perish: Can Bats Threatened with Extinction Persist and Recover from White-nose Syndrome?. *Integrative and Comparative Biology*, *64*(3), 807-815.

Found evidence that bats surviving WNS are exhibiting mechanisms of avoidance (by selecting microclimates within roosts) and tolerance (by increasing winter fat reserves), which may help avoid costs of immunopathology incurred by a maladaptive host resistance response.

1. Frick, W. F., Johnson, E., Cheng, T. L., Lankton, J. S., Warne, R., Dallas, J., ... & McGuire, L. P. (2022). Experimental inoculation trial to determine the effects of temperature and humidity on White-nose Syndrome in hibernating bats. *Scientific reports*, *12*(1), 971.

Only 37% of bats experimentally inoculated with Pd at the start of the experiment showed any infection response or disease symptoms after 83 days of captive hibernation. There was no evidence that temperature or humidity influenced infection response. Temperature had a strong effect on fungal growth on media plates, but the influence of humidity was more variable and uncertain.

1. Boyles, J. G., Brack Jr, V., & McGuire, L. P. (2023). Balancing costs and benefits of managing hibernacula of cavernicolous bats. *Mammal Review*, *53*(3), 133-142.

Managers suggest modifying hibernacula to meet conditions historically thought to minimize energy expenditure during hibernation. Hibernaculum manipulations carry high risk because cave systems used by bats have all the hallmarks of systems prone to falling into ecological traps. The author’s model shows an oversimplification of physiological and environmental conditions are very risky when realistic levels of variation in ambient conditions are included. Realistic natural conditions in many or most hibernacula mean that modifications to the microclimate may produce modest energy savings for hibernating bats while potentially exposing them to substantial long-term fitness declines. Due to the risks of creating ecological traps and negative energetic consequences, we generally urge caution when modifying subterranean sites for bat use, and specifically suggest that if hibernacula are modified, the primary goal should be to maximize spatial gradients and minimize temporal variability in ambient conditions (temperature and humidity), as opposed to aiming to achieve a specific midwinter temperature. Coarse environmental manipulations are feasible by altering airflow. Because of the relative tractability of altering microclimate, cave manipulations have long been suggested as a management strategy for hibernating bats (Richter et al. 1993; Tuttle & Kennedy 2002; Mitchell-Jones 2004). There is recognition that hibernation as a life history strategy includes a host of costs and benefits (Frech 2000; Humphries et al. 2003; Ruf et al. 2012; Nowack et al. 2019). It is increasingly accepted that hibernating mammals rarely choose to maximize the expression of hibernation unless they are nearing starvation (Boyles et al. 2020). Effective strategies to slow down the spread of Pd or limit effects on bats remain elusive (Bernard et al. 2020), and WNS continues to wreak havoc on cavernicolous bat populations in North America. As starvation is thought to be the ultimate cause of death in most WNS-affected bats, management strategies designed to increase pre-hibernation energy stores (e.g., increased foraging opportunities; Bernard et al. 2021) or decrease the rate of energy expenditure (e.g., hibernaculum manipulations) have predominated. Potential harm to other inhabitants of subterranean ecosystems is often noted (Meierhofer et al. 2022), so many proponents of manipulating subterranean sites suggest manipulation of unused artificial mines as the safest option (Sewall et al. 2016). Bats have an amazing ability to find new hibernacula, either through exploration or information transfer among individuals (Richter et al. 1993). Cues available during pre-hibernation investigations are all bats have available to decide where to hibernate. At artificially manipulated sites those immediate cues may be unreliable if they relate differently to midwinter conditions than do cues in unmanipulated sites. Hibernating bats also have limited opportunities to ‘escape’ a trap, because they spend most of the season in torpor and attempts to move to alternative sites might be fatal. Given the highly mobile nature of bats and the concerns associated with disturbing bats during hibernation, we cannot currently gather survival and reproductive data in the detail necessary to estimate fitness and evaluate success of site manipulations. While manipulations generally lower overall temperatures in a site, nuances of microclimate manipulation are completely unknown. Occasionally, reference is made to maintaining spatial variability in microclimate (Sewall et al. 2016; Turner et al. 2022), but we know of no attempts to control temporal variability of microclimates, which is as important or more important than maintaining a spatial gradient. Thus, it became common to use abstractions like mean midwinter temperatures as proxies for temperatures within a hibernaculum, though they poorly describe conditions used by bats within, throughout, or across seasons of hibernation (Boyles et al. 2017). This represents a case of the ‘tyranny of the golden mean’ (Bennett 1987) and is problematic because physiology and energetics of hibernating bats are not governed by mean temperatures across hibernation, but rather vary with changes in environmental conditions in real time. Over the course of the winter, variation in temperature can result in a bat spending as much energy hibernating in a location with an average midwinter temperatures of 3 C (sometimes considered ideal for bat hibernation) as one hibernating at 9 C (often considered warmer than ideal for bat hibernation; (Boyles & McKechnie 2010). This is because metabolic rate greatly increases below 0 C and gradually increases above 10 C. So it is riskier to hibernate at cooler temperatures if ambient temperatures fluctuate within a site. For example, it is riskier for bats if ambient temperatures go below 0 C than it is if temperatures go above 10 C. A common argument is that cooling a subterranean site brings it closer to the mean midwinter temperature that minimizes torpid metabolic rate and thus affords bats the best opportunity to survive winter with limited fat stores. An emphasis should be placed on creating hibernacula with the largest possible spatial gradients and smallest possible temporal gradients in temperature and air moisture. Such sites would allow WNS-affected bats to choose cooler temperatures if necessary (sensu Hopkins et al. 2021) while allowing healthier bats and species that need warmer temperatures to access those microclimates as well. Targeted manipulations that decrease of increase temperature and air moisture in limited parts of hibernacula have potential to achieve outcomes desired by some management agencies while minimizing unrecognized risks.

1. Lewis, M. A., Turner, G. G., Scafini, M. R., & Johnson, J. S. (2022). Seasonal roost selection and activity of a remnant population of northern myotis in Pennsylvania. *PLoS One*, *17*(7), e0270478.

Roost selection during spring, summer, and fall.

1. Brown, R. L., Loeb, S. C., Bridges, W. C., & Rodriguez, S. L. (2024). Tricolored Bat (Perimyotis subflavus) microsite use throughout hibernation. *Journal of Mammalogy*, gyae109.

Most tricolored bats roosted in the back part of a tunnel where temperatures were warmer and disturbance was reduced. About 20% of bats roosted in the front of the tunnel where roost temperatures were significantly colder and VPD was higher but more variable, and human disturbance was also higher. The proportion of bats in each section did not differ throughout the winter. Results suggest that no optimum temperature exists but VPD and disturbance are also likely important factors driving microsite use.

1. Brownlee-Bouboulis, S. A., & Reeder, D. M. (2013). White-nose syndrome-affected little brown myotis (Myotis lucifugus) increase grooming and other active behaviors during arousals from hibernation. *Journal of Wildlife Diseases*, *49*(4), 850-859.

WNS-affected bats exhibited significantly higher rates of grooming, relative to unaffected bats, at the expense of time that would otherwise be spent inactive. In captivity.

1. Grieneisen, L. (2011). Hibernacula microclimate and white-nose syndrome susceptibility in the little brown myotis (Myotis lucifugus).

Male bats with WNS has increased survivability over WNS-affected female bats and WNS bats below the ideal growth range of the fungus had increased survival over those housed at warmer temperatures.

1. Vanderwolf, K. J., & McAlpine, D. F. (2021). Hibernacula microclimate and declines in overwintering bats during an outbreak of white‐nose syndrome near the northern range limit of infection in North America. *Ecology and Evolution*, *11*(5), 2273-2288.

A 99% decrease in overwintering bat population in New Brunswick was observed between 2011 and 2015. No P. subflavus were observed between 2011-2015 and the species appears to be extirpated from these sites. Bats did not appear to choose hibernacula based on winter temperatures, but dark zone (zone where no light penetrates) winter temperatures did not differ among study sites. Winter dark zone temperatures were warmer and less variable than entrance or above ground temperatures. Pd growth was observed on hibernating bats in New Brunswick during early winter surveys (November) even though hibernacula temperatures were colder than optimum for in vitro Pd growth. This suggests that cold hibernacula temperatures encountered near the apparent northern range limit for Pd do not sufficiently slow fungal growth to prevent the onset of WNS and associated bat mortality over the winter.

1. Boyles, J. G., & Willis, C. K. (2010). Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white‐nose syndrome?. *Frontiers in Ecology and the Environment*, *8*(2), 92-98.

Use a model to present evidence that artificial warming of localized areas within hibernacula could increase survival of WNS-affected bats during winter by lessening the energetic costs of periodic arousals. The model suggests that localized thermal refugia of 28 C could improve survival by up to 75%, depending on how WNS acts to disrupt energy balance.

1. Dzal, Y., McGuire, L. P., Veselka, N., & Fenton, M. B. (2011). Going, going, gone: the impact of white-nose syndrome on the summer activity of the little brown bat (Myotis lucifugus). *Biology letters*, *7*(3), 392-394.

Documented a 78% decline in summer activity of M. lucifugus coinciding with the arrival and spread of WNS. We suggest that mortality of M. lucifugus in winter hibernacula if reflected by reduced levels of activity in the summer and that WNS affects the entire bat population of an area, and not only individual hibernacula.

1. Perea, S., Ferrall, E. A., Morris, K. M., Pattavina, P. E., Sharp, N., & Castleberry, S. B. (2024). A decade of hibernating bat communities along the periphery of a region of white‐nose syndrome. *The Journal of Wildlife Management*, *88*(1), e22506.

Examined trends in hibernating bat populations at 11 hibernacula in Northern Georgia and Alabama, USA from 2013-2022. Tricolored bats and northern long-eared bats declined by >90% in the first 5 years after WNS detection in the region. No northern long-eared bats were reported since 2019, but tricolored bat populations stabilized following initial declines.

# Discussion:

These results potentially could muster more extreme management options such as sealing hibernacula that are population sinks for Little Brown Bats and promoting those hibernacula that have better inside microclimate conditions that reduce the growth of Pd resulting in lower mortality.