Introduction

Invasive fungal diseases are exerting a growing threat on wildlife populations, causing profound ecological disruptions and posing significant challenges to conservation efforts. Fungal diseases such as chytridiomycosis in amphibians and sudden oak death in trees have highlighted the vulnerability of various species to invasive fungi. These pathogens exhibit a complex relationship with temperature, with variations influencing both their growth and the susceptibility of the host species. Some hosts are more susceptible to fungal pathogens at certain temperatures, while others may experience heightened resistance. This is particularly true for white-nose syndrome (WNS) in bats. First discovered in 2006, WNS has decimated bat populations across North America. Caused by the cold loving fungus *Pseudogymnoascus destructans* (Pd), it affects bats during hibernation in winter, significantly decreasing their survival rates. However, some bat populations have shown signs of recovery in regions where WNS has been present for decades. This recovery is not uniform and appears to be temperature dependent. This variance is likely due to the fact that Pd grows more slowly at temperatures nearing freezing, leading to less severe infections because of reduced exposure to the fungus.

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 experimentall 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

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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.

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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.

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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.

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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

# 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.