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



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Environmental control reduces white-nose syndrome infection in hibernating bats

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Keywords

bat hibernacula; environmental reservoir; *Eptesicus fuscus*; fungal pathogen; *Myotis lucifugus*; *Pseudogymnoascus destructans*; sapronotic disease agent; white-nose syndrome..

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Abstract

Infectious diseases caused by invasive, environmentally persistent fungal pathogens have increasingly endangered global biodiversity, yet disease management remains a major conservation challenge. A prominent example is white-nose syndrome (WNS), a disease caused by the invasive fungal pathogen *Pseudogymnoascus destructans* (*Pd*) that has devastated populations of multiple North American bat species, but for which few effective management tools exist. Here, we propose that strategies to delay environmental transmission of *Pd* during early winter could limit WNS disease effects across winter, benefitting bats. We used a small captive experiment and a multi-year field trial on wild, free-ranging bats to assess an environmental control strategy to manage *Pd* within its environmental reservoir in Pennsylvania, USA, where the pathogen has become 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 captive experiment, environmental transmission of *Pd* to immunologically naïve little brown myotis (*Myotis lucifugus*) occurred from roost substrates inoculated with *Pd*, but the application of PEG to these substrates effectively blocked this transmission. 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 substantially after PEG treatment in big brown bats (*Eptesicus fuscus*). No negative effects of PEG treatment were observed in body condition or colony counts of bats or in the microbial community. Together, these results are consistent with effective environmental control of *Pd* and reduced WNS disease effects in bats within contaminated hibernacula. The results also highlight the potential of carefully designed environmental control strategies for managing environmentally persistent pathogens.

Introduction

Infectious disease has increasingly endangered global biodiversity (Daszak, Cunningham, & Hyatt, 2000; Joseph *et al.*, 2013), with disease caused by invasive, environmentally persistent fungal pathogens presenting an especially dire emerging threat to wild plants and animals (Fisher *et al.*, 2012). Such diseases can have rapid, severe, and

widespread impacts on hosts (Scheele *et al.*, 2019), and can drive long-term changes in host populations and ecological communities across large geographic scales (Elliott & Swank, 2008). Effective management of such infectious diseases of wildlife remains a major challenge for conservation (Martin *et al.*, 2019), and progress will require a more holistic understanding of complex host–pathogen–environment interactions.

One important example is white-nose syndrome (WNS), an infectious disease of hibernating bats. Since its initial detection in New York, USA, in 2006 (Bleher *et al.*, 2009; Rubin, 2020), *Pseudogymnoascus destructans* (*Pd*), the invasive fungal pathogen that causes WNS (Lorch *et al.*, 2011; Warnecke *et al.*, 2012), has caused repeated epizootic outbreaks in hibernacula (Frick *et al.*, 2010), with disease impacts and long-term population responses varying by bat species, hibernacula characteristics, and local pathogen abundance (Hoyt *et al.*, 2020; Haase *et al.*, 2021; Turner *et al.*, 2022). The disease has now been confirmed on 12 hibernating North American bat species in 38 of the United States and 7 Canadian provinces (WNS Response Team, 2022), leading to a serious or pervasive threat to at least 5 of these species (Cheng *et al.*, 2021). Further, there is the potential for serious risk to additional hibernating bats should *Pd* spread beyond its current range (Lilley *et al.*, 2020; Turbill & Welbergen, 2020).

Managers have few tools to protect susceptible bats from WNS, however. Widely used management methods (e.g. restrictions on hibernacula access or decontamination protocols; USFS, 2012; USFWS, 2020) may effectively slow human-mediated contamination or limit additive impacts on bats. Yet, such methods are unlikely to stop bat-mediated or environmental spread of *Pd* or limit WNS impacts on bats once a site is contaminated. Other proposed management methods are likely ineffective or counterproductive (e.g. culling infected bats; Hallam & McCracken, 2011). Many proposed treatments (summarized by Hoyt *et al.*, 2019) have yet to demonstrate effectiveness in benefitting bats under field conditions; cannot be easily scaled up to benefit colonies (e.g. because they require capture and individualized treatment of many bats); cause disruption of bats during their critical hibernation period or may have negative or unknown side effects on bats, groundwater and troglobitic or troglomorphic organisms (Rocke *et al.*, 2019; Sewall *et al.*, 2022).

The existence of multiple modes of disease transmission can also hinder the development of effective management approaches (Burns *et al.*, 2021). For instance, *Pd* is readily transmitted from bat to bat (Lorch *et al.*, 2011), but is also capable of exhibiting dormancy as spores as well as active organismal growth and replication in cave or mine environments (Raudabaugh & Miller, 2013; Reynolds & Barton, 2014; Urbina *et al.*, 2021), and can persist environmentally for months or years in the absence of bat hosts (Lorch *et al.*, 2013; Hoyt *et al.*, 2015; Urbina *et al.*, 2021). Further, infections occur repeatedly in the same hibernacula in winters subsequent to the initial establishment of *Pd* (Dobony *et al.*, 2011), and high *Pd* prevalence and load on bats may persist in hibernacula even years after extreme declines in the local bat colony (Frick *et al.*, 2017). Together, these patterns are consistent with contaminated hibernacula serving as extensive environmental reservoirs for *Pd* during bats' active period from late spring to early fall (Frick *et al.*, 2017). Further, although it has yet to be formally demonstrated, these findings and recent modeling efforts (Meierhofer *et al.*, 2021) suggest environmental transmission – the substrate-to-host movement of a pathogen – in

winter may initiate and drive seasonal cycles of WNS infection.

Environmental transmission remains poorly studied in wildlife diseases generally (Joseph *et al.*, 2013), but in diseases with environmental transmission, diminishing the environmental reservoir of the pathogen or reducing transmission from this reservoir to the host is essential to limiting pathogen prevalence and disease impacts (Kuris, Lafferty, & Sokolow, 2014). While control of environmental transmission may be particularly challenging where complete local eradication of the disease agent is necessary to prevent host extirpation (e.g. Martin *et al.*, 2019), disease management may often be effective without pathogen extirpation when interventions target critical stages of disease dynamics, such as during periods of heightened host susceptibility or when host encounters with infective stages in the environment are elevated (Altizer *et al.*, 2006). In this context, the strong seasonality of WNS provides an opening that could be exploited to benefit bats. Specifically, WNS disease effects are mostly restricted to winter hibernation, and the prevalence and load of *Pd* on hibernating bats increase progressively across the winter (Langwig *et al.*, 2015; Frick *et al.*, 2017). Harm to bats is alleviated at spring emergence from hibernation, however: euthermy skin temperatures of active bats rise above ranges associated with optimal growth of *Pd* (Verant *et al.*, 2012), bat immune responses re-engage rapidly upon attaining euthermy (Field *et al.*, 2018), damage to bats from WNS heals within weeks (Meteyer *et al.*, 2011; Fuller *et al.*, 2020) and the prevalence and load of *Pd* on bats drop off rapidly, reaching near zero by late summer and fall (Langwig *et al.*, 2015).

Together, these results suggest that delaying environmental transmission of *Pd* to bats in early winter could reduce disease effects attained by late winter (Hoyt *et al.*, 2020), and thereby facilitate bat survival to spring emergence, with potential for subsequent recovery. We, therefore, developed an environmental control strategy for WNS with the goal of reducing environmental transmission in early winter. This strategy had three key elements. First, our group has identified polyethylene glycol, a compound widely used in agriculture and medicine, as a promising agent that could reduce or delay environmental transmission of *Pd*. High-molecular-weight configurations, such as Polyethylene Glycol 8000 (hereafter, PEG), have low toxicity in mammals (INCHEM, 2020). Importantly, this compound is not biocidal, as such substances have sometimes had inadvertent and undesirable effects in underground environments (e.g. Bastian *et al.*, 2010). Rather, in the laboratory, PEG increases matric-induced water stress and inhibits mycelial growth and conidial germination in a limited set of fungi that includes *Pd* (Raudabaugh *et al.*, 2011; Raudabaugh & Miller, 2013). Second, we developed delivery methods (portable backpack sprayers to apply PEG in solution to hibernacula substrates) that are suitable for application of PEG to remote hibernacula and difficult-to-access areas within hibernacula. The application was with off-the-shelf, portable, commercial backpack sprayers (Husqvarna Four-Gallon Professional Sprayer) and gas-powered backpack foggers (Stihl SR450)

(Appendix S2, Supporting Information). Finally, we targeted interventions to the critical period of WNS disease dynamics, with the goal of delaying *Pd* transmission when bats return to hibernacula in fall. This timing had the additional benefit of avoiding harmful disturbance to bats during hibernation.

We sought to evaluate the effectiveness of this environmental control strategy but proceeded in a cautious stepwise manner out of concern for declining bat host species. The first study we reported here was a small experiment on captive bats in a semi-natural setting (hereafter, the ‘captive experiment’). Objectives were to clarify mechanisms of disease transmission, to evaluate whether PEG application could reduce disease effects and to determine the most effective technique for PEG application (direct application to bat wings or roost treatment). We hypothesized that environmental transmission would occur; that PEG would reduce WNS infection extent while limiting WNS-induced shortening of torpor bout lengths and that both application techniques would be beneficial for bat hosts relative to controls. The second study was a small field trial of free-ranging bats (hereafter, the ‘field trial’), with the objective of evaluating the effectiveness of PEG in benefitting wild individuals of two bat species. We hypothesized that pathogen prevalence, pathogen load and infection extent would be reduced following roost treatment and that these reductions would be maintained over multiple treatment years. Finally, colony counts and winter body condition were hypothesized to be unaffected by treatment (since these variables are affected by female reproductive activity and foraging occurring in the active seasons, whereas we studied male-dominated colonies during winter), but these variables were monitored to detect potential negative secondary effects of treatment.

Materials and methods

Set-up of the captive experiment

In the captive experiment, we studied 21 adult male *Myotis lucifugus* (little brown myotis), a species highly affected by WNS (Cheng *et al.*, 2021). Bats were presumably immunologically naïve to WNS, as they were collected during hibernation in fall of 2015 from an area outside the WNS-affected zone. They were brought to the experimental site, a small, abandoned limestone mine in Blair County, Pennsylvania, USA, that had been previously contaminated with *Pd*.

In advance of the experiment, we installed three cages at the experimental site at locations determined by environmental swabbing and qPCR (see Methods below) to be negative for *Pd*. We also harvested *Pd* from a separate location within the same experimental site, and 9 days before the start, we inoculated three aliquots of 10 g of sterilized sediment with 1500 spores and applied the mixture to the wall and cage substrates within each cage. This procedure enabled a standardized environmental exposure to *Pd* within cages while avoiding the possibility of introduction of any different strains of *Pd* or other microorganisms to the experimental site.

To evaluate the effect of PEG on environmental transmission, one cage was used for the positive control (no PEG

application), one for the bat application treatment (PEG applied to the left wing of each bat to attain -3 MPa of matric stress, but not applied to right wings or roost substrates) and one for the roost treatment (PEG not applied to wings, but PEG was applied to the roost substrate to attain -6 MPa of matric stress). Bats were fitted with skin temperature data loggers (Reeder *et al.*, 2012) and placed in cages on 6 November 2015. Body condition did not differ by treatment group and we detected no *Pd* or WNS infection on any bat from swab samples or ultraviolet imagery (see Methods below). The bats were then allowed to hibernate within cages. The study was continued until a predation event occurred between January 4 and 11. This event shortened the planned duration of the study (the achieved duration was 59–65 days) and limited data collection to wing data from 12 bats and logger data from 6 bats. Additional methods for the captive experiment are in Appendix S1.

Set-up of the field trial

In the field trial, we studied free-ranging bats from wild populations of both *M. lucifugus* and the less affected *Eptesicus fuscus* (big brown bat) (Frank *et al.*, 2014) in central Pennsylvania, USA. To reduce potential impacts of the study, we used anthropogenic or highly modified hibernacula that were used primarily by male bats. Sites were an abandoned railroad tunnel in Clearfield County (hereafter, the PEG treatment site) with concrete and brick surfaces, a commercial limestone cave in Centre County (sham control site) that had been substantially modified for tourist access and another (different from the captive experiment) abandoned limestone mine in Blair County (no-spray control site). All hibernacula are geographically close (<90 km apart), have been contaminated with WNS since at least 2014 and have similar microclimates (with midwinter temperatures averaging -4°C and high humidity).

During the summers of 2018 and 2019, we sprayed ceiling, wall and floor substrates of the PEG treatment site in the absence of bats with a thin film of PEG/distilled water solution to control *Pd* environmental growth, replication and/or spore germination and we sprayed substrates of the sham control site with a similar thin film of an equal volume of distilled water only. The no-spray control site was not sprayed. We applied a concentration of PEG (0.264 g of PEG/g of water) that, in laboratory studies (Raudabaugh & Miller, 2013), was sufficient to produce environmental matric stress of -10 MPa and completely halt *Pd* growth and germination.

To evaluate the effects of treatment, we captured bats from all sites during March 14–23 one year before (2018) the treatment and sham treatment were first applied and during each of the subsequent 2 years (2019 and 2020) that followed treatments. In each year, we captured 20–25 *M. lucifugus* at each site and 20–25 *E. fuscus* at the treatment site only (this species was rare at the sham and control sites). Additional methods for the field trial are in Appendix S2.

Data collection methods for bats

To confirm the pathogen was absent from bats at the beginning of the captive experiment, and to assess the effect of

treatment on pathogen prevalence and load in the field trial, we used genetic analysis of swab samples. We swiped a sterile swab with five unique passes on the dorsal side of each bat's right wing. Genetic analysis of *Pd* on swab samples was completed by the University of California, Davis School of Veterinary Medicine in the captive experiment and in the field trial in 2018–2019 and by the University of Pennsylvania School of Veterinary Medicine in the field trial in 2020. Genetic analysis used quantitative polymerase chain reaction (qPCR) of *Pd* (Muller *et al.*, 2013). Samples were run in duplicates and repeated for high (>35) cycle threshold (C_t) values. Bats were considered negative when mean *Pd* load was lower than the last standard ($C_t > 35$; or $\sim 0.03 \times 10^{-4}$ ng/ μ L) and positive otherwise.

In both studies, bat wings were examined by ultraviolet imagery to determine infection extent. At the beginning and end of the captive experiment, and each winter of the field trial, we took images of each wing of each bat while trans-illuminating it with ultraviolet (UV) light (12-volt lamp with 368 nm bulbs, Way Too Cool ultraviolet light table). We then used Image Pro Premier version 9.3 (Media Cybernetics) software on these images to quantify the percentage of the area of both wings with fluorescence characteristic of WNS lesions (Turner *et al.*, 2014).

In the captive experiment, bat wings were also examined by histology at the end of the study to confirm infection. The presence of cup-shaped intra-epidermal colonies of fungi with short, slightly curved arthroconidia characteristic of infection by *Pd* was considered positive evidence of WNS during histology (Meteyer *et al.*, 2009).

In addition, in the captive experiment, skin temperature data loggers were examined to measure torpor bout lengths and shorter torpor bouts represented a negative impact of WNS on bat hosts (Reeder *et al.*, 2012). For the field study, we used winter bat counts provided by the Pennsylvania Game Commission's hibernacula monitoring program (described in Turner *et al.*, 2022) to evaluate changes in bat colonies over time. During both studies, we measured body mass index (mass divided by right forearm length), a size-corrected measure of body condition. Additional descriptions of these methods are in Appendices S1 and S2.

Data analysis

In the captive experiment, pairs of groups were compared with t-tests, with alpha level adjusted for small sample sizes (de Winter, 2013). In the comparison of extent of infection by treatment, we used a Wilcoxon rank-sums test. Further analysis details are in Appendix S1.

In the field trial, for *M. lucifugus*, we used ANOVAs and post hoc Tukey honestly significant difference (HSD) tests to examine the effects of year, treatment and their interaction on pathogen load or infection extent; the interaction indicated how treatment affected bats differently at the PEG treatment and control sites after the onset of treatment and sham treatment. We also evaluated the overall effect of treatment using planned contrasts for before–after–control–impact (BACI) designs for multi-year, multi-site studies

(Schwarz, 2019). These models included treatment type (treatment or control), period (before or after treatment) and their interaction, plus the random effects of site and year. A significant contrast represented a consistently greater overall change at the treatment site than at control sites following the treatment event, after accounting for the effects of inter-site and inter-annual variation.

For *E. fuscus* at the PEG treatment site, we used Fisher's exact tests to evaluate *Pd* prevalence by year. We used Kruskal–Wallis rank-sums tests and post-hoc Steel–Dwass all-pairs tests to evaluate how year affected pathogen load and infection extent. All analyses were completed using JMP Pro 14.2.0 statistical software (SAS Institute Inc., Cary, North Carolina, USA, 1989–2019). Further analysis details are in Appendix S2.

Results

Captive experiment – Effect of PEG treatment

Within the bat application group, infection extent did not differ between treated and untreated wings (Appendix S3). Infection extent was greater in the bat application group than in the roost treatment group (bat application mean = 0.47%, roost treatment mean = 0.000086% and Wilcoxon rank-sum test, $X^2 = 4.43$, $P = 0.035$; Fig. 1). Similarly, among bats in the bat application treatment analyzed by histology ($n = 5$), all but one had mild-to-moderate WNS infections with cup-shaped intra-epidermal erosions containing colonies of *Pd* (Fig. 2a). In contrast, histology revealed that none of the roost treatment bats ($n = 7$) had cupping erosions or penetrating hyphae of *Pd* (Fig. 2b). There were no differences in torpor–bout length or date of death between the positive control and bat application groups (Appendix S3).

Field trial – Effect of PEG treatment in *M. lucifugus*

Although *Pd* prevalence did not change with treatment in *M. lucifugus* (*Pd* prevalence was 100% in all treatments and years), treatment was strongly associated with decreased *Pd* load. Specifically, *Pd* load decreased substantially at the PEG treatment site but less at control sites (change of +33% at no-spray control, –47% at sham control and –90% at the PEG treatment site in untransformed data from 2018 to the 2019–2020 average). In the model, the interaction between treatment and year predicted *M. lucifugus* pathogen load (ANOVA; $n = 206$, model $F_{8, 197} = 36.3$, $P < 0.0001$, $R^2 = 0.60$; treatment \times year interaction, $F_{4, 197} = 12.6$, $P < 0.0001$), with pathogen load consistently lower after treatment at the PEG treatment site but not consistently lower at the control sites (Tukey HSD test, Fig. 3a). Overall, *M. lucifugus* pathogen load decreased substantially more following treatment at the PEG treatment site than at control sites (BACI planned contrasts, model $F_{1, 200} = 42.5$, estimate = 2.38 [1.33, 3.43], $P < 0.0001$). This result implies a decrease in *Pd* load in the PEG treatment site that was 10.8

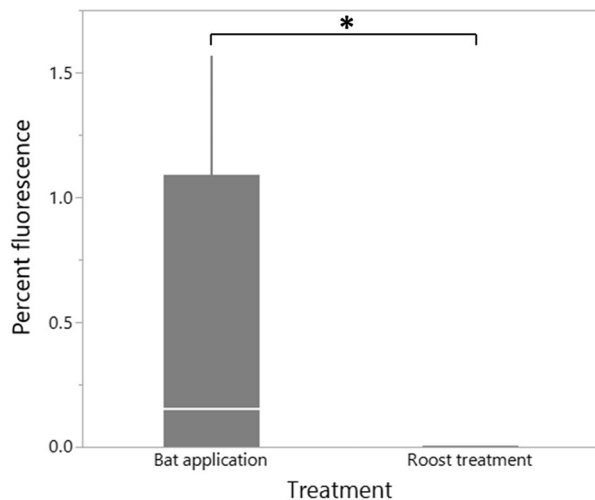


Figure 1 *Myotis lucifugus* infection extent by treatment in the captive experiment. In box-and-whisker plots in all figures, the solid box represents the 1st to 3rd quartile, the median is the white line, whiskers extend to the outermost data point within 1.5 times the interquartile range and points represent outliers. Asterisks represent the pairwise difference ($P < 0.05$).

times larger than in the control sites, after accounting for inter-site and inter-annual variation.

Treatment with PEG was also associated with decreased infection extent in *M. lucifugus*. Infection extent decreased more at the PEG treatment site than at control sites (change of -34% at no-spray control, -30% at sham control and -66% at PEG treatment site in untransformed data from 2018 to the 2019–2020 average). In the model, *M. lucifugus* infection extent varied with both treatment and year but not with the interaction between treatment and year (ANOVA; $n = 198$, model $F_{8, 189} = 8.97$, $P < 0.0001$, $R^2 = 0.28$; treatment, $F_{2, 189} = 24.6$, $p < 0.0001$; year, $F_{2, 189} = 6.86$, $P = 0.0013$; treatment \times year interaction, $F_{4, 189} = 1.81$, $P = 0.13$), with the additive effect of treatment and year resulting in lower infection extent after treatment at the PEG treatment site than before treatment or at the control sites (Tukey HSD test, Fig. 3b–d). Overall, *M. lucifugus* infection extent decreased more following treatment at the PEG treatment site than at control sites (BACI planned contrasts, estimate = 0.79 [0.013, 1.57], $P = 0.048$). This implies a decrease in infection extent in the PEG treatment site that was 2.2 times larger than in the control sites, after accounting for inter-site and inter-annual variation.

We did not find evidence that PEG treatment affected the body condition or colony counts of *M. lucifugus* (Supporting Information, Appendix S3).

Field trial – Effect of PEG treatment in *E. fuscus*

In *E. fuscus* at the PEG treatment site, prevalence of *Pd* decreased progressively across years (Fisher's exact test; $P < 0.0001$), with a decline of 68% in the proportion of

positive samples from 2018 (before treatment) to the 2019–2020 average (after treatment). Similarly, *Pd* load decreased by 49% in untransformed data from 2018 to the 2019–2020 average. In the model, year predicted *Pd* load (Kruskal–Wallis; $n = 65$, d.f. = 2, $\chi^2 = 14.57$, $P = 0.0007$), with *Pd* load being lower in 2020 than in 2018 (Steel–Dwass; Fig. 4a). Infection extent varied by year (Kruskal–Wallis; $n = 65$, d.f. = 2, $\chi^2 = 42.2$, $P < 0.0001$), however, and was lowest in 2019, highest in 2020 and intermediate in 2018 (Steel–Dwass, Fig. 4b). Both *Pd* load and infection extent were extremely low in *E. fuscus* relative to *M. lucifugus*: untransformed values in *E. fuscus* were 4 and 1 orders of magnitude smaller for *Pd* load and infection extent respectively. Finally, we did not find evidence that PEG treatment affected the body condition or colony counts of *E. fuscus* (Supporting Information, Appendix S3).

Discussion

Managers are in urgent need of effective tools to protect bats from WNS, especially after hibernacula become contaminated with *Pd*. We present here direct evidence that environmental transmission can initiate WNS infection in hibernating bats in early winter, confirming expectations from previous studies (e.g. Frick *et al.*, 2017; Meierhofer *et al.*, 2021). In the captive experiment, bats without previous exposure to *Pd* developed confirmed cases of WNS after exposure to contaminated surfaces. Direct application of PEG to bat wings did not stop WNS infection, perhaps because of a lack of adhesion of PEG to wings, grooming of PEG by bats or an ineffectiveness of PEG in stopping *Pd* growth, sporulation or germination on exposed bat skin. However, application of PEG to contaminated roost substrates prior to hibernation largely blocked environmental transmission to bats in early winter. Further, results from the field trial were consistent with PEG treatment reducing WNS effects in wild, free-ranging bats. In *M. lucifugus*, both *Pd* load and infection extent declined after treatment relative to controls, with declines exceeding effects of inter-site and inter-annual variation. In *E. fuscus*, *Pd* prevalence and load declined substantially after treatment, while changes in infection extent were inconsistent (but exceptionally low relative to *M. lucifugus*). Finally, no negative effects of treatment were observed in body condition or bat counts in either species. Together, these results suggest PEG treatment of roost substrates can safely reduce some WNS disease effects within contaminated hibernacula, likely by delaying environmental transmission in early winter and thereby reducing time available for disease effects to ramp up to high levels later in the season. These results imply that environmental control with PEG has potential to benefit bats susceptible to WNS within contaminated hibernacula.

Our findings were particularly intriguing because we addressed only one transmission mode (environmental transmission) in the field trial, while leaving another (host-to-host) transmission mode unaddressed. Pathogens with multimodal transmission can complicate management by providing alternate opportunities for pathogens to infect susceptible

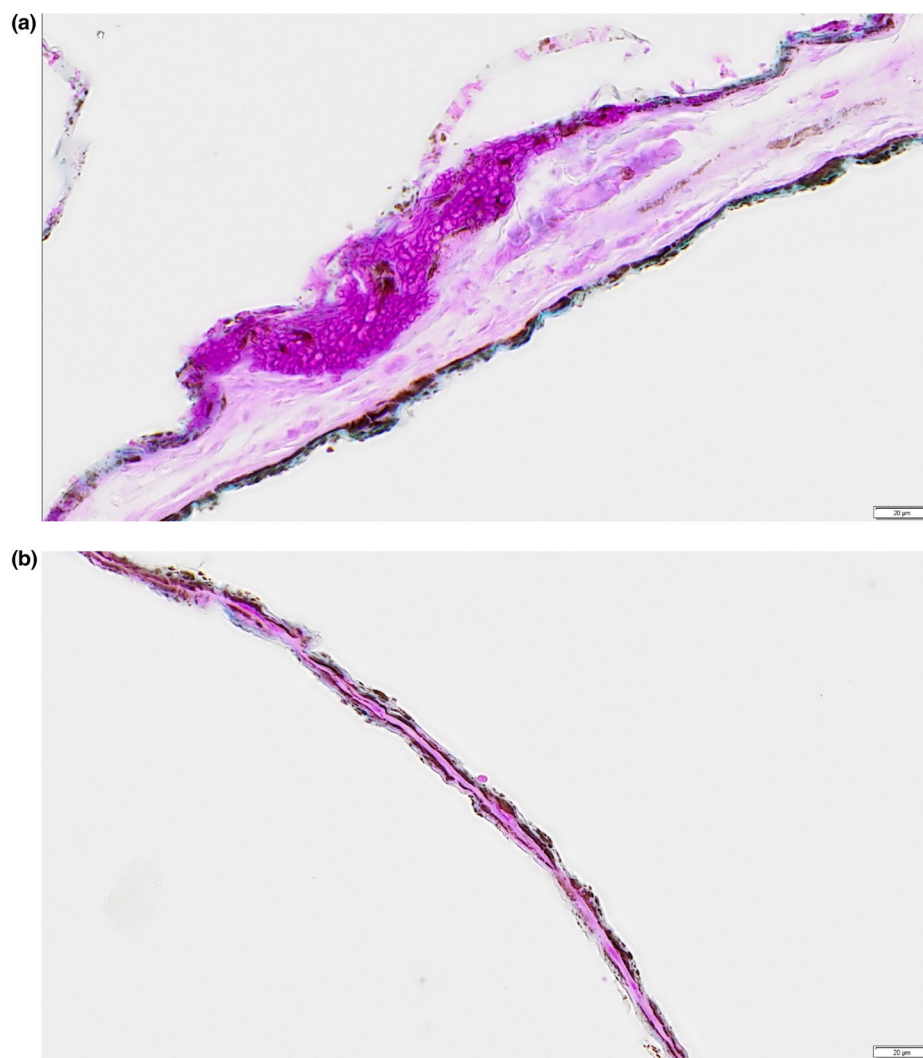


Figure 2 Example of histological cross sections of *M. lucifugus* wing tissues in (a) the bat application group and (b) the roost treatment group, in the captive experiment. Image in panel (a) shows cup-shaped intra-epidermal colonies and production of short, slightly curved arthroconidia characteristic of infection with *Pd*. Scale bar indicating 20 µm is shown in bottom right corner.

hosts (Burns *et al.*, 2021). For instance, bat movement among hibernacula is not uncommon during winter (Boyles, Dunbar, & Whitaker, 2006), and such movement could in theory circumvent local controls on environmental transmission if bats relocating from other contaminated hibernacula midwinter bring high levels of *Pd* load and infection with them and if they then transmit *Pd* to other bats. Environmental control strategies would ideally be paired with approaches that reduce bat-to-bat transmission (e.g. Rocke *et al.*, 2019) and those that limit effects on bats after they acquire the pathogen (e.g. Cornelison *et al.*, 2014; Court *et al.*, 2017; Ghosh *et al.*, 2018; Hoyt *et al.*, 2019) although such approaches are still in development. PEG, as a common excipient, may be well suited for coupling with other proposed treatments (e.g. Raudabaugh & Miller, 2015), which could provide synergistic effects that increase effectiveness (Charloq *et al.*, 2013).

The effectiveness of our environmental control strategy in limiting WNS effects despite the ongoing, unaddressed capacity of bats for host-to-host transmission provides evidence to support previous hypotheses that hibernacula reservoirs for *Pd* are a primary factor triggering seasonal WNS infection (Frick *et al.*, 2017; Meierhofer *et al.*, 2021). Both theory and empirical studies suggest the presence of biotic or abiotic reservoirs provides pathogens with heightened potential to impact host species (Rosa *et al.*, 2003; de Castro & Bolker, 2005; Fisher *et al.*, 2012). Similarly, reservoir dynamics appear to be particularly important in WNS; higher pathogen abundance in the environment is correlated with more rapid seasonal exposure to *Pd*, higher *Pd* load at the end of winter and more severe population impacts (Hoyt *et al.*, 2020). In this context, environmental control strategies targeting the pathogen in its environmental reservoir, such as with PEG (or via other means, e.g. Boire *et al.*, 2016;

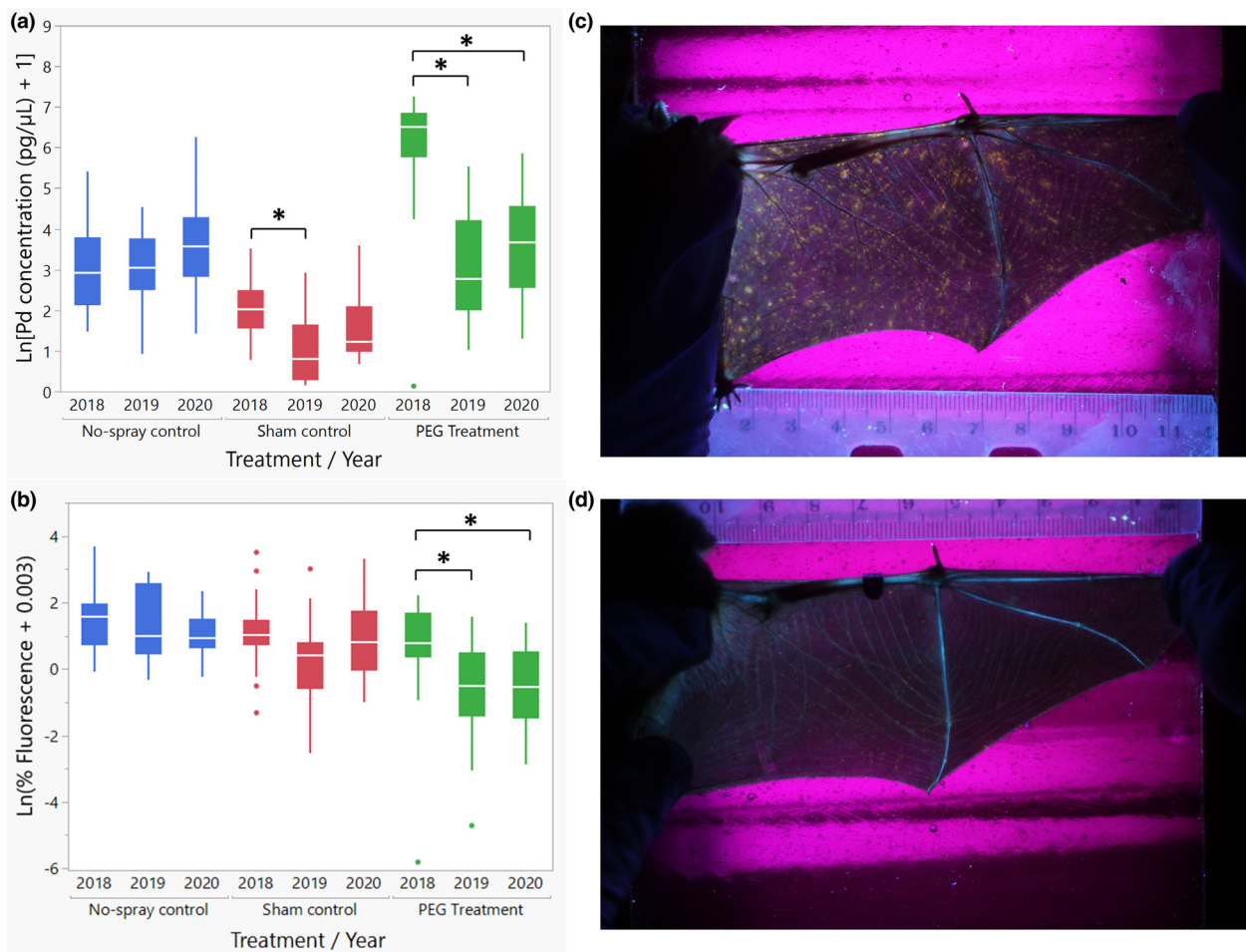


Figure 3 *Myotis lucifugus* (a) pathogen load and (b) infection extent in the no-spray control, sham control and PEG treatment hibernacula in the field trial; plus images of typical bat wings at the (c) no-spray control site and (d) PEG treatment site in 2019. PEG and sham applications occurred in the summer between the winters of 2018 and 2019 and were repeated in the summer between the winters of 2019 and 2020. Box-and-whisker plots are as described in Fig. 1. Asterisks represent pairwise differences ($P < 0.05$) among years within a treatment type. Images show bat wings trans-illuminated with ultraviolet light; the yellow-orange fluorescent patches represent WNS infection.

Palmer *et al.*, 2018), could benefit hosts by reducing pathogen abundance, inhibiting the pathogen's environmental growth or sporulation, reducing or delaying spore germination, disrupting or delaying environmental transmission, or altering the pathogen's ability to cause disease. Furthermore, while *E. fuscus* was previously known to have lower susceptibility to WNS than *M. lucifugus* (Frank *et al.*, 2014), our finding that *Pd* prevalence and load in *E. fuscus* decreased after treatment could also benefit *M. lucifugus*, by reducing any role the more common *E. fuscus* may have as a biotic reservoir in initiating seasonal infection during hibernation.

Our finding in the captive experiment that PEG application to contaminated roost substrates blocked environmental transmission for at least a 2-month period suggests that a primary mechanism of the environmental control strategy in the field trial was by delaying the onset of WNS infection. Even short delays in initial infection of bats may be critical

to reducing WNS effects and even mortality since WNS infection proceeds progressively across winter (Sewall, Turner, Gagnon, Field and Reeder, unpublished). In this context, delays in infection initiation on this time scale likely mean the disease does not have enough time to progress to cause the most severe disease effects by the end of the season (Hoyt *et al.*, 2020). Delays in infection might particularly benefit juveniles and adult female bats, which often enter hibernacula prior to adult males (Norquay & Willis, 2014), and thus, have longer potential periods of seasonal exposure to *Pd*. If so, reduced disease impacts in these groups may have outsized benefits for population-level reproduction and recruitment.

We have sought to apply a series of rigorous tests to the environmental control strategy, proceeding stepwise from the laboratory to controlled studies to small-scale trials at a limited number of less sensitive sites to larger-scale field trials.

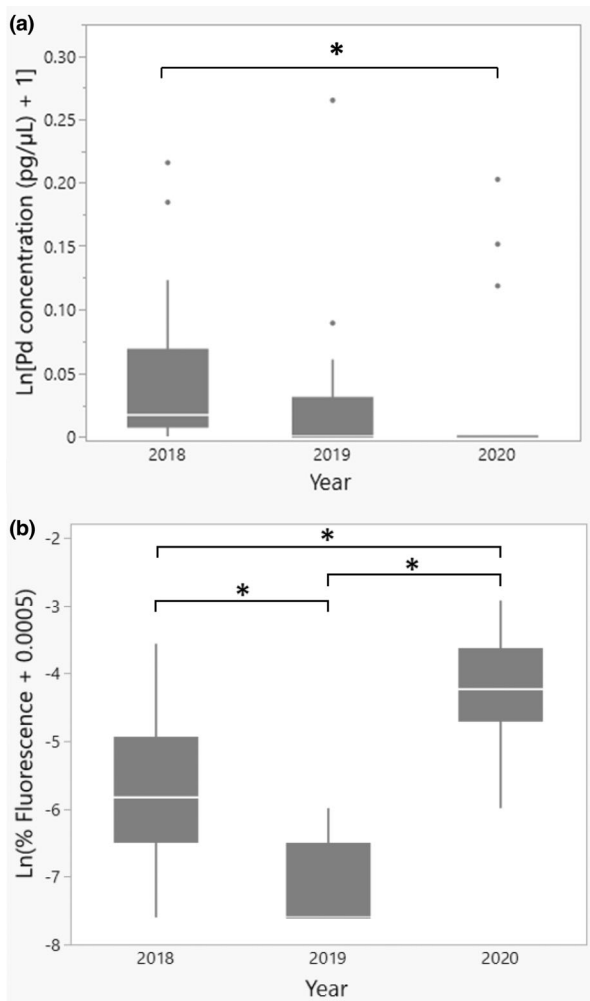


Figure 4 *Eptesicus fuscus* (a) pathogen load and (b) infection extent in the PEG treatment hibernaculum in the field trial. Treatment occurred in the summer between the winters of 2018 and 2019 and was repeated in the summer between the winters of 2019 and 2020. Box-and-whisker plots are as described in Fig. 1. Asterisks represent pairwise differences ($P < 0.05$) among years.

Our initial results from the laboratory (Raudabaugh *et al.*, 2011) and the results from both the captive study and small-scale field trial described here have each been consistent with expectations for successful environmental control. Evaluation of non-target impacts is another important step in evaluating a treatment approach, and in a companion study, we found negligible impacts of treatment on subterranean fungal and bacterial communities (Overton, Turner, Scafini, Johnson and Sewall, unpublished). One drawback of proceeding in a stepwise manner, however, is that out of appropriate caution, definitive larger-scale tests are delayed until after initial controlled and smaller-scale tests have been completed. For instance, due to the limited number of field sites in this study, we cannot yet definitively conclude that PEG treatment – and not variation due to microclimate (Turner

et al., 2022), inter-annual changes in the abundance of *Pd* within the reservoir (Hoyt *et al.*, 2020) that were not associated with PEG treatment or another unknown factor – caused the observed decrease in WNS disease effects (although the geographic proximity and microclimatic similarity of the sites in the field trial likely limited site-level differences to some extent). Further, beneficial responses were not consistently observed for infection extent in *E. fuscus*, although infection extent was extremely low in this species. Overall, our findings of improved indicators of WNS after treatment, combined with the lack of obvious negative effects on bats or microbial communities, together indicate studies of larger scope and duration are now warranted. Until such studies are completed, we recommend a precautionary approach, focusing initially on anthropogenic or heavily impacted sites and involving comprehensive surveys in advance of treatment to avoid sites with sensitive or rare target and non-target species or subterranean communities.

Our study demonstrated effectiveness in diminishing WNS effects on the time scale of 1–2 years, but such effectiveness could continue or even improve over time because PEG may indirectly suppress sporulation (Thapa *et al.*, 2016) and because *Pd* dormancy induced by PEG could limit its ability to compete with other microbes in the environment (Reynolds & Barton, 2014), gradually reducing pathogen density in the environmental reservoir. Furthermore, continued but more limited host exposure to the pathogen could help avert host population extirpation while facilitating the evolution of resistance or tolerance among bats (Kilpatrick, 2006), as likely occurred in evolutionary history among Eurasian bats (Lilley *et al.*, 2019). Environmental control of *Pd* transmission using PEG could also have other potential benefits if used proactively at early stages of *Pd* invasion into new areas, such as by slowing the rate of *Pd* spread among hibernacula or reducing the severity of mass mortality events at critical hibernation sites. Our approach to environmental control with PEG also has several other attractive features, including that high-molecular-weight PEG has low toxicity to mammals (INCHEM, 2020), is inexpensive and widely available and can be applied in the field even under difficult field conditions or rough terrain (Appendix S2). A particularly useful aspect is that it can be applied prior to bat return to hibernacula in the fall, thus providing a non-invasive approach that avoids disturbing bats during hibernation. While multifaceted approaches are likely needed to mitigate the effects of emerging infectious diseases of wildlife across species and habitats and over the long term (Scheele *et al.*, 2014), environmental control may nonetheless provide a much needed management tool to limit WNS effects or help depleted bat populations recover. More broadly, as invasive zoonotic or environmentally persistent pathogens increasingly threaten host species of conservation concern (Fisher *et al.*, 2012; Scheele *et al.*, 2019), our results, combined with those of others (Kuris, Lafferty, & Sokolow, 2014; Martin *et al.*, 2019), suggest environmental control provides an opportunity for limiting environmental transmission and managing emerging zoonotic and environmentally persistent diseases of wildlife.

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Author contributions

BJS, GGT, MRS, JSJ and BEO conceived the ideas, designed methodology, obtained funding, obtained approvals and led logistics; BJS analyzed the data and led the writing of the manuscript; and all authors collected the data, contributed critically to the drafts and gave final approval for publication.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Additional methods – captive experiment.

Appendix S2. Additional methods – field trial.

Appendix S3. Additional results.