Efficacy of a probiotic bacterium to treat bats affected by the disease white-nose syndrome

Tina L. Cheng1*, Heather Mayberry2,3, Liam P. McGuire2,4, Joseph R. Hoyt1, Kate E. Langwig1,5, Hung Nguyen1, Katy L. Parise6, Jeffrey T. Foster6, Craig K. R. Willis2, Auston Marm Kilpatrick1 and Winifred F. Frick1,7

1Department of Ecology and Evolutionary Biology, University of California, 1156 High Street, Santa Cruz, CA 95064, USA; 2University of Winnipeg, Winnipeg, MB R3B 2E9, Canada; 3University of Toronto, 3359 Mississauga Road, Mississauga, ON L5L 1C6, Canada; 4Texas Tech University, Lubbock, TX 79409, USA; 5Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA; 6University of New Hampshire, Durham, NH 03824, USA; and 7Bat Conservation International, PO Box 162603, Austin, TX 78716, USA

Summary

1. The management of infectious diseases is an important conservation concern for a growing number of wildlife species. However, effective disease control in wildlife is challenging because feasible management options are often lacking. White-nose syndrome (WNS) is an infectious disease of hibernating bats that currently threatens several North American species with extinction. Currently, no effective treatments exist for WNS.

2. We conducted a laboratory experiment to test the efficacy of probiotic treatment with Pseudomonas fluorescens, a bacterium that naturally occurs on bats, to reduce disease severity and improve survival of little brown bats Myotis lucifugus exposed to Pseudogymnoascus destructans, the fungal pathogen that causes WNS.

3. We found that application of the probiotic bacteria at the time of fungal infection reduced several measures of disease severity and increased survival, whereas bacterial treatment prior to pathogen exposure had no effect on survival and worsened disease severity.

4. Synthesis and applications. Our results suggest that probiotic treatment with Ps. fluorescens has potential for white-nose syndrome disease management, but the timing of application is critical and should coincide with natural exposure of bats to P. destructans. These results add to the growing knowledge of how natural host microbiota can be implemented as a biocontrol treatment to influence disease outcomes.

Key-words: bats, conservation, disease management, Geomyces destructans, microbial biocontrol, Myotis lucifugus, probiotics, Pseudogymnoascus destructans, Pseudomonas fluorescens, white-nose syndrome

Introduction

Emerging infectious diseases can cause dramatic population declines and extinctions (De Castro & Bolker 2004). Effective tools for controlling wildlife diseases are often lacking and disease management can be challenging to implement (Langwig et al. 2015a). However, development of viable pathogen control tools allows for targeted treatment of endangered populations to prevent disease-induced extinction (Haydon et al. 2006; Chang et al. 2007).

White-nose syndrome (WNS) is a recently emerged multihost disease that has caused severe declines in hibernating bat populations throughout eastern and midwestern North America and threatens several bat species with extinction (Frick et al. 2010, 2015; Langwig et al. 2012, 2015b). WNS is characterized by epidermal invasion by the fungal pathogen, Pseudogymnoascus destructans (Pd) (Meteyer et al. 2009; Lorch et al. 2011; Warnecke et al. 2012), which causes mortality by disrupting physiological processes (electrolyte imbalance, hypotonic dehydration and increased arousals from torpor) and depleting energy reserves during hibernation (Reeder et al. 2012; Warnecke et al. 2012; Verant et al. 2014). Pd infection and host mortality occur primarily during hibernation when bat
hosts are torpid for extended periods (Langwig et al. 2015c). Impacts are highly variable, with some species experiencing severe population declines and/or site level extirpations (e.g. *Myotis lucifugus* and *Myotis septentrionalis*), while other species are far less impacted (e.g. *Eptesicus fuscus, Myotis leibii* (Frick et al. 2010, 2015; Langwig et al. 2012). Currently, no effective treatments for WNS have been demonstrated. Given the rapid declines caused by the disease, a treatment or control strategy for WNS is urgently needed.

The use of naturally occurring microbiota to treat disease is widespread in agriculture (Weller 1988) and has increased recently for both human (Borody & Khoruts 2012) and wildlife disease (Bletz et al. 2013). For example, several species of bacteria have recently been examined as possible treatment agents for the amphibian pathogen, *Batrachochytrium dendrobatidis* (*Bd*), including the bacterium, *Janthinobacterium lividum*, which was isolated from a *Bd*-resistant salamander, *Plethodon cinereus* (Brucker et al. 2008). Application of these bacteria in a laboratory study prevented fungal infection and the disease, chytridiomycosis, and eliminated the severe mortality (85%) normally caused by *Bd* in a highly susceptible frog species, *Rana muscosa* (Harris et al. 2009). More broadly, the skin microbiome has the potential to provide an important protective community for hosts against pathogens (Belden & Harris 2007), and this barrier could be particularly important for bats exhibiting downregulated immune function during hibernation (Meteyer et al. 2009; Moore et al. 2013).

Bacteria from the *Pseudomonas fluorescens* species complex isolated from bat skin have demonstrated growth inhibition of *Pd in vitro*, suggesting that these bacteria could be useful for WNS management (Hoyt et al. 2015). These bacteria are also widely used as probiotics in agriculture (Cook & Baker 1983; Weller & Cook 1983; Keel et al. 1992) and aquaculture (Gram et al. 1999) and have been investigated as a tool for protecting wild amphibian populations against chytridiomycosis (Lauer et al. 2007; Myers et al. 2012). A related species, *Pseudomonas veronii*-like PAZ1, was recently isolated in Europe and has exhibited strong anti-*Pd* properties in *vitro* (Fritz, Pham & Zaspel 2012). *Pseudomonas* bacteria interact with fungi via multiple pathways including the production of antimicrobial metabolites (e.g. 2-4 DAPG) and competition for iron via siderophore production (Ligon et al. 2013; Mascuch et al. 2015).

We conducted *in vitro* treatment experiments with captive bats to test the effectiveness of a strain of *Ps. fluorescens* (designated Pf1) isolated from a big brown bat *Eptesicus fuscus* (Hoyt et al. 2015) to reduce WNS disease severity and mortality in the highly impacted species, the little brown bat *M. lucifugus*. We examined efficacy of two application periods relative to when bats acquire *Pd* infection to provide greater flexibility for field application. First, we examined effectiveness of applying Pf1 simultaneously with *Pd* inoculation to simulate treatment of bats in early winter when most bats first acquire infection (Langwig et al. 2015c). We also examined applying Pf1 3 weeks prior to *Pd* inoculation to emulate treatment in fall before the onset of hibernation and *Pd* infection (Langwig et al. 2015c). We hypothesized that treatment both prior to and simultaneous with *Pd* exposure would reduce disease severity and mortality.

**Materials and methods**

**Experimental design**

We randomly assigned 85 wild-collected, male little brown bats into five groups of 17 individuals each (Simultaneous Pf1 application, Pre-exposure Pf1 application, *Pd* Control, Pf1 Control and Sham Control) (see Table S1, Supporting Information). Each treatment group was housed separately in a nylon mesh cage, and all six cages were placed in a single hibernaculum chamber (CARON, Model 6040-1, Marietta, OH, USA) that was maintained at a constant temperature of 7 °C and 98% relative humidity. Little brown bats naturally cluster during hibernation so we housed bats in each treatment group together in a cage to reduce stress. To accommodate clustering as well as constraints on space within the chamber and the number of bats we were permitted to collect from the wild, our design resulted in a single replicate cage per treatment. Previous laboratory infection experiments of bats and WNS have followed a similar design (Lorch et al. 2011; Warnecke et al. 2012; Johnson et al. 2014). In our experiment, co-housing may have influenced survival and arousal patterns if behaviour of individuals within a group influenced cohabitating bats (Turner et al. 2015), but was unlikely to confound disease severity metrics measured on individual bats. Replicating cages within treatments would have required many more bats, which was not possible given the severe declines experienced by this species due to WNS.

**Animal collection and handling**

We collected bats on 29 November 2013 from a hibernaculum in central Manitoba, Canada, where WNS has not been detected. At the time of collection, we swabbed bats for *Pd* by rubbing sterile polyester swabs (Fisherbrand synthetic tipped applicators 23-400-116) five times on the forearm and five times on the muzzle (Langwig et al. 2015c) and stored swab samples in RNAlater at −20 °C until testing. We extracted swabs and tested for *Pd* using quantitative polymerase chain reaction (qPCR) (Muller et al. 2013) to confirm that collected individuals were negative for *Pd*. Prior to the first treatment, we measured mass (±0.1 g) and forearm length (±0.01 mm) to calculate body condition index (mass/forearm length). We banded each bat with a lipped forearm band (Porzana Ltd., East Sussex, UK) and attached an iButton (Thermochron DS1922L-F5#) to record skin temperature (*T*<sub>s</sub>) every 10 min. We modified iButtons following Reeder et al. (2012) to reduce their mass and marked each iButton with a unique pattern for individual identification. Bats in each cage were provided with a single water dish (Petri plate filled with water), which was refilled twice each week by a tube extending outside the chamber to prevent disturbance. All bats were handled for the same amount of time and received application of *Ps. fluorescens, Pd* or sham inoculum according to their treatment group (Table S1).
PREPARATION AND APPLICATION OF SHAM INOCULUM

We prepared 1X PBS (autoclave sterilized at 121 °C for 30 min) to use as the sham inoculum and diluent in *Ps. fluorescens* and *Pd* inocula.

PREPARATION AND APPLICATION OF *PS. FLUORESCENS* (PF1) TREATMENT INOCULUM

We used a strain of PF1 that was cultured from a wild-caught big brown bat *E. fuscus* in Virginia in March 2012. This strain exhibited the strongest inhibitory effects against *Pd* growth in vitro of the five bacterial strains collected from bats in a prior study (Hoyt et al. 2015). To prepare the PF1 treatment inoculum, we prepared 1:10 dilutions of PF1 stock culture (stored at −80 °C in glycerol and defrosted on ice to room temperature) on Luria agar plates. Colony-forming units were counted to determine concentration, and initial stock was diluted in 1X PBS to make a working inoculum at 1.8 × 10⁸ CFU mL⁻¹. Bats were sprayed with PF1 inoculum on both wings and the tail membrane using a 100-mL generic plastic cosmetic perfume atomizer (ASIN: B018TTEQUS; Amazon.com, Inc., Seattle, WA, USA). Bats received two sprays on each side of each wing and one spray on each side of the tail membrane. Each spray delivered approximately 170 μL of probiotic inoculum, for a total of 1.2 mL total for each bat (2.2 × 10⁸ CFUs). We used a plastic sheet to cover the torso of the bat to prevent dampening the fur while exposing the wings to the spray. PF1 inoculum was sourced from the same stock (stored at −80 °C in 30% glycerol) and prepared identically according to the procedures detailed above for Pre-exposure and Simultaneous PF1 treatments.

PREPARATION AND APPLICATION OF *P. DESTRUCTANS* INOCULUM

We obtained a wild strain of *Pd* from an infected *Perimyotis subflavus* in New Brunswick, Canada, isolated on 4 April 2013 (Karen Vanderwolke, Canadian Wildlife Federation). We cultured *Pd* on Sabouraud Dextrose Agar with chloramphenicol and gentamicin at 7 °C for 30–60 days until cultures produced characteristic curved conidia. *Pd* cultures were harvested and diluted with 1X PBS to make an inoculum of concentration 2.5 × 10⁶ conidia mL⁻¹ (Lorch et al. 2011). We inoculated each bat with 20 μL of *Pd* inoculum containing 5 × 10⁵ conidia. Inoculum was applied and spread onto the dorsal side of both wings with a pipette. We selected this dose and method of application because it produced skin lesions, increased arousal frequencies and behavioural changes characteristic of WNS in previous experiments (Lorch et al. 2011; Warnecke et al. 2012).

EXPERIMENTAL PROTOCOL

We examined five metrics of WNS severity that characterize different stages of disease (McGuire et al. 2016). These include *Pd* load based on qPCR of swabs ( Muller et al. 2013), surface area of the wing covered in *Pd* by histological examination, the percent of visible ultraviolet (UV) florescence from *Pd* invasion on the wing (Turner et al. 2014), number of invasive tissue lesions by histological examination (Meteyer et al. 2009; Reeder et al. 2012) and changes in hibernation torpor patterns (Reeder et al. 2012; Warnecke et al. 2012).

We continuously monitored bats using motion-sensitive infrared cameras mounted inside the hibernation chamber. Individuals were identified based on symbols on each iButton datalogger. We removed and examined individuals within 24 h of observing the following behaviours: (i) arousal on three consecutive days, (ii) shaky and/or lethargic movements during arousal, (iii) roosting solitarily at the bottom of the cage or on the floor or (iv) roosting with wings spread out (Warnecke et al. 2012). We weighed bats and assessed health status based on active or lethargic response to handling. If individuals were not responsive during handling, bats were humanely euthanized by decapitation under isoflurane anesthesia.

At the termination of the experiment or when bats were removed and euthanized, individuals were swabbed using the protocol described above. We photographed the left ventral wing using UV transillumination (368-nm wavelength and 2-s exposure) to measure florescence. UV photographs were analysed using the colour-matching application in Photoshop (Appendix S1). For histology, we removed the wing section from the fifth digit to the body, rolled the tissue around dental wax dowels and stored it in neutral-buffered formalin. Histological sections were prepared at the Wisconsin Veterinary Diagnostic Laboratory. Rolled wing sections were trimmed in six cross sections per bat and stained with periodic acid–Schiff. Prepared sections were examined under a light microscope at 400–600×. We identified and counted WNS lesions, defined as cup-like skin erosions filled with *Pd* conidia (Meteyer et al. 2009) for each tissue section of each bat (Fig. S1). We also approximated, to the nearest 5%, the fraction of the epidermal surface covered with *Pd* on each tissue section per bat (Fig. S1). We scored all histology slides blind to the treatment group. We quantified torpor patterns based on data recorded by iButtons attached to each bat. We defined torpor bout duration (TBD) as the time in days that a bat was torpid between arousals. Arousals, in which *Tₚsk* was within 10 °C of the maximum *Tₚsk* recorded for each individual, were used to distinguish long bouts of steady-state torpor (Reeder et al. 2012).

Bats in this region enter hibernation between early September and late October (Norquay & Willis 2014). Therefore, we monitored bats for ≤ a 210-day hibernation period beginning on 1 October 2014 halfway through this period. Morbidity increased significantly in our Sham Control group after 185 days (≥ 6 months) into hibernation, indicating that survival was compromised after 185 days in captivity regardless of WNS status (all Sham Control bats tested negative for *Pd*). Therefore, we examined differences in survival among groups up to day 185 to exclude mortality unrelated to WNS. We defined mortality as the proportion of bats in each treatment group that had died or been euthanized by day 185.

STATISTICAL ANALYSES

All statistical analyses were performed using R v. 3.0.2 (R Core Team 2016). We considered individual bat as the experimental unit for all analyses. For each metric of WNS severity, we included initial body mass index, treatment group, number of days in hibernation and an interaction between treatment group and number of days in hibernation as fixed effects. There were a total of ten candidate models in each model set (Table S2), and we selected the best-supported model by the Akaike Information Criterion (AIC) (Burnham & Anderson 2002). We used a
Results

DISEASE SEVERITY AND SURVIVAL

Disease severity increased over time, differed among treatments and was not correlated with initial body mass index (Figs 1 and 2; Table S3). Severity in Simultaneous Pf1 bats was significantly reduced compared to Pd Control bats in four out of five disease severity metrics (number of lesions, surface fungi, UV fluorescence and TBD) (Figs 1 and 2; Table S4; Fig. S2). In contrast, severity in Pre-exposure Pf1 bats was significantly greater than Pd Control bats for all five metrics (number of lesions, surface fungi, UV fluorescence, Pd load and TBD) (Figs 1 and 2; Table S4; Figure S2), and thus, the data did not support our a priori predictions about the impact of this treatment. The zero-inflated model indicated that Simultaneous Pf1 treated bats with lesions had marginally fewer lesions than Pd Control bats (P = 0.06; Table S4), but were not significantly different in the probability of having at least one lesion compared to Pd Control bats (P = 0.25; Table S4). In contrast, Pre-exposure Pf1 bats had a marginally greater probability of acquiring a lesion compared to Pd Control (P = 0.06; Table S4), whereas those that had lesions did not differ significantly in the number of lesions compared to Pd Control bats (P = 0.65; Table S4).

Survival varied between treatments (Fig. 1; Table S2). The Pf1 Control group had the highest survival, and bats treated with Pf1 at the time of exposure to Pd had significantly higher survival compared to untreated Pd-infected bats and equal or higher survival than the Sham Control group. Most other comparisons were not significant, including the comparison of Pre-exposure Pf1 bats and untreated infected bats (Pd Control group). Although Simultaneous Pf1 bats had higher survival than Pd and Sham Control bats for much of the experiment, substantial mortality in the final 10 days eliminated these differences.

DISEASE PREVALENCE

There were no differences between Pf1-treated and Pd-infected groups in the fraction of bats detected with Pd based on qPCR or those with superficial surface fungi based on histology (Fig. S3; Table S3). In contrast, fewer Simultaneous Pf1 bats had detectable sign of WNS by UV florescence, disrupted torpor patterns, or mortality compared to Pd Control bats, whereas more Pre-exposure Pf1 bats had detectable WNS by UV florescence and disrupted torpor patterns compared to Pd Control bats (Fig. S3; Table S4).

Fig. 1. Comparison of white-nose syndrome (WNS) severity among treatment groups along a gradient in disease progression. WNS disease severity is shown along a progression of increasing disease manifestation (indicated by the right-facing arrow): exposure to Pd (Pd load) leads to superficial (Surface Fungi) and dermal invasion (UV/Lesions), which leads to increased arousals [torpor bout duration (TBD)], which results in fat depletion and death (Mortality). WNS disease severity for Sham Control (purple), Pf1 Control (grey), Pd Control (red), Simultaneous Pf1 (orange) and Pre-exposure Pf1 (blue-green) are shown for six disease metrics. Pd load, Surface Fungi, UV fluorescence and Lesions: Circles show the average (±1 SE) number of lesions, per cent surface fungi on each tissue section, per cent area with fluorescing Pd and log_{10}(Pd load) for each of 17 individual bats per treatment group. Lines show the best-fitting model (Table S4). TBD: Points indicate the duration of time since last arousal and segments connect torpor bouts of individual bats in each treatment group. Lines show the predicted TBD over time from the best-fit model (Table S4). The slope of the dashed lines was not significantly different from zero, and the slopes of solid lines formed three groups which were significantly different from each other: Simultaneous Pf1 = Sham Control = Pf1 Control < Pd Control < Pre-exposure Pf1 (Table S4). Survival curves: The fraction of each group of 17 bats remaining alive for the first 185 days of hibernation (125 days post-Pd inoculation) is shown. Shared letters (A, B) at the end of each line indicate treatment groups that do not differ significantly in pairwise log-rank survival tests (Table S2).
**Discussion**

Our results indicate that *Ps. fluorescens* application is non-detrimental to bats and that treatment with strain Pf1 at the same time bats are exposed to *Pd* could reduce WNS severity and mortality and, therefore, reduce impacts of WNS on bat populations. Bats treated with Pf1 and not infected with *Pd* had the highest survival and...
fat stores, suggesting that Pf1 treatment did not have any pathological effects in the absence of Pd. In addition, bats treated with Pf1 at the same time as Pd exposure had higher survival compared to untreated infected bats (Pd Control) and lower disease severity across most of the disease metrics we examined.

The disparity in disease outcomes between Pre-exposure and Simultaneous Pf1 bats suggests that the mechanism for successful Pf1 application is dependent on treatment timing. Neither Pre-exposure nor Simultaneous Pf1 treatment prevented Pd colonization since Pd was detected on all bats in these groups and fungal loads were similar on Simultaneous Pf1 and Pd Control bats. However, bats infected with Pd and treated with Pf1 simultaneously had significantly reduced Pd invasion (assessed by UV and histology) and showed no increase in arousal frequency, which has been proposed as a precursor to disease-induced mortality (Reeder et al. 2012; Warnecke et al. 2012, 2013; Verant et al. 2014). We found no evidence that our application of Pf1 persisted or amplified on bats during the experiment because bats in Pf1-treated groups did not differ in prevalence of Ps. fluorescens by the end of the experiment (Appendix S2; Table S6). Thus, interaction between Pf1 and Pd is likely restricted to short-term impacts following application and potentially functions by delaying fungal invasion via production of antifungals (Brucker et al. 2008), resource competition (e.g. iron sequestration; Ligon et al. 2013; Mascuch et al. 2015) or reducing colonization (Warnink et al. 2011). In contrast, pre-exposure application of Pf1 appears to compromise some degree of natural resistance, potentially by disrupting the natural skin microbiome, and resulted in higher severity of Pd invasion (although without a detectable increase in mortality). Given the importance of treatment timing, application of Pf1 should take place in early hibernation at sites where Pd has established and when Pd prevalence is near 100% (Langwig et al. 2015a).

Our study suffers from pseudoreplication (Hurlbert 1984), in that we had a single cage for each of our treatments. We grouped bats in a single cage for ethical reasons because little brown bats are highly social and cluster during hibernation. Replication of experimental groups (cages) was also limited by the number of hibernation chambers available, space within each chamber, and, most importantly, the number of bats we could collect from the wild. Cage effects are most likely to influence comparisons of torpor patterns, which could in turn affect mortality, if an arousal by one bat disturbed other individuals in that cage (Turner et al. 2015). However, differences among groups in other metrics of severity (Pd load by qPCR, UV fluorescence, lesions, surface fungi) are less likely to be caused by cage effects because all infected bats were inoculated with a single high dose of Pd. Results from all five WNS metrics were very similar; thus, it is more likely that differences in all disease metrics were due to treatments rather than cage effects, but cage effects cannot be ruled out.

Our results suggest that Ps. fluorescens could be a useful tool for disease management of wild bat populations affected by WNS. Pseudomonas bacteria are commonly found on hibernating bats throughout eastern North America (Hoyt et al. 2015) (Appendix S2; Table S5). Thus, treatment with this bacterium effectively represents bio-augmentation, which is beneficial in reducing impacts of non-target effects. Due to the rapid spread of WNS and the urgency for action, we recommend a small-scale field trial as the next step in development of Pseudomonas as a treatment for WNS. Such a trial should be conducted in a human-made habitat (e.g. a mine, to prevent potential non-target effects on cave-dwelling organisms) with treatment timed to coincide with Pd infection. These trials should furthermore be conducted within an adaptive management framework and in compliance with biological control protocols for other systems (e.g. chytridiomycosis; Woodhams et al. 2011). Future studies might also improve treatment efficacy by determining the mechanism of Ps. fluorescens-Pd inhibition and by applying different doses and strains of Ps. fluorescens. Given the uncertainty associated with the mechanism of action, treatment with Ps. fluorescens should be focused at sites where Pd has recently arrived and where bats are likely to decline precipitously, and to focus on the most susceptible species, rather than at sites where bats are persisting with Pd (Langwig et al. 2015a).

The importance of resident microbes in health and disease is becoming increasingly evident (Cho & Blaser 2012). Concurrently, recent advancements in microbial biotechnology and bioinformatics have expanded the potential of probiotics to be developed as tools in disease management (Rebollar et al. 2016). The recent emergence of several virulent, multihit and rapidly spreading infectious diseases (e.g. chytridiomycosis, white-nose syndrome, Batrachochytrium salamandrivorans, west nile virus) highlights the critical need for rapid development of

---

**Table 1.** Summary of the degree of white-nose syndrome (WNS) disease severity relative to Pd Control for Pre-exposure Pf1 and Simultaneous Pf1 treatment groups. Red upward facing arrows represent increased WNS disease severity; blue downward facing arrows represent decreased WNS disease severity. Associated P-values compare the treatment group to Pd Control (Table S4). Gradient in red and blue colour represents significance levels, with solid blue/red indicating significance at α < 0.05 and muted colours representing marginal significance at α < 0.10. Black bars represent no significant difference compared to Pd Control.

<table>
<thead>
<tr>
<th>WNS metric</th>
<th>Simultaneous Pf1</th>
<th>Pre-exposure Pf1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesions (Histology)</td>
<td>$p = 0.06$</td>
<td>$p = 0.05$</td>
</tr>
<tr>
<td>Surface Pd (Histology)</td>
<td>$p = 0.001$</td>
<td>$p = 0.001$</td>
</tr>
<tr>
<td>UV</td>
<td>$p = 0.001$</td>
<td>$p = 0.001$</td>
</tr>
<tr>
<td>Pd load (qPCR)</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td>Torpor bout length</td>
<td>$p &lt; 0.001$</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>

---

**Fig. 2.** Summary of the degree of white-nose syndrome (WNS) disease severity relative to Pd Control for Pre-exposure Pf1 and Simultaneous Pf1 treatment groups. Red upward facing arrows represent increased WNS disease severity; blue downward facing arrows represent decreased WNS disease severity. Associated P-values compare the treatment group to Pd Control (Table S4).

Gradient in red and blue colour represents significance levels, with solid blue/red indicating significance at α < 0.05 and muted colours representing marginal significance at α < 0.10. Black bars represent no significant difference compared to Pd Control.
disease management tools for conservation (Voyles et al. 2014; Langwig et al. 2015a). Probiotics represent a promising alternative to traditional antimicrobials. Our experiment provides an important step in the development of *Ps. fluorescens* as a probiotic agent that could be further developed as a disease management tool against WNS. Further progress in the protection of imperilled bat populations in North America will require an adaptive management approach that should include field trials for probiotics and other biological agents (Cornelison et al. 2014) and exploration of other interventions, such as habitat decontamination (Bosch et al. 2015) and microclimate manipulations (Boyles & Willis 2010; Langwig et al. 2012; Johnson et al. 2014; Grieneisen et al. 2015; Wilcox & Willis 2016). Rapidly leveraging results of these studies is important for directing long-term strategies to halt the rapid decline and extirpation of many bat populations.

**Acknowledgements**

Funding was provided by USFWS: F12AP01081; NSF: DEB-1115895 and DEB-1336290; and Bat Conservation International. We thank the many members of the Willis Lab (Ally Menzies, Alana Wilcox, Dylan Baloun, Mary-Anne Collis, Quinn Webber, Kristina Muise and Christina Davy) and the staff of the University of Winnipeg Amimal Facility (Dan Wasylow, Robyn Cole) for their assistance in collecting bats, monitoring and helping to conduct the experiment. We thank Melissa Behr and Kevin Keel for their assistance with histological preparation and training. We acknowledge the technical support from Benjamin Abrams, UCSC Life Sciences Microscopy Center, and ManinderPaul Sidhu for their assistance with microscope photography of histological slides. We thank Karen Van derwolf for providing *Pseudogymnoascus destructans* culture. We extend our sincere gratitude to Rain Ackerman and the Ackerman family for their support.

**Data accessibility**

Data are available from Dryad Digital Repository http://dx.doi.org/10.5061/dryad.p1bd1 (Cheng et al. 2016).

**References**


