

Determinants of *Pseudogymnoascus destructans* within bat hibernacula: Implications for surveillance and management of white-nose syndrome

Michelle L. Verant^{1,2}  | Elizabeth A. Bohuski² | Katherine L. D. Richgels² |
Kevin J. Olival³ | Jonathan H. Epstein³ | David S. Blehert²

¹School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

²U.S. Geological Survey – National Wildlife Health Center, Madison, WI, USA

³EcoHealth Alliance, New York, NY, USA

Correspondence

Michelle L. Verant
Email: michelle_verant@nps.gov
and

David S. Blehert
Email: dblehert@usgs.gov

Present address

Michelle L. Verant, Biological Resources Division, Wildlife Health Branch, National Park Service, Fort Collins, CO, USA

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Abstract

1. Fungal diseases are an emerging global problem affecting human health, food security and biodiversity. Ability of many fungal pathogens to persist within environmental reservoirs can increase extinction risks for host species and presents challenges for disease control. Understanding factors that regulate pathogen spread and persistence in these reservoirs is critical for effective disease management.
2. White-nose syndrome (WNS) is a disease of hibernating bats caused by *Pseudogymnoascus destructans* (*Pd*), a fungus that establishes persistent environmental reservoirs within bat hibernacula, which contribute to seasonal disease transmission dynamics in bats. However, host and environmental factors influencing distribution of *Pd* within these reservoirs are unknown.
3. We used model selection on longitudinally collected field data to test multiple hypotheses describing presence-absence and abundance of *Pd* in environmental substrates and on bats within hibernacula at different stages of WNS.
4. First detection of *Pd* in the environment lagged up to 1 year after first detection on bats within that hibernaculum. Once detected, the probability of detecting *Pd* within environmental samples from a hibernaculum increased over time and was higher in sediment compared to wall surfaces. Temperature had marginal effects on the distribution of *Pd*. For bats, prevalence and abundance of *Pd* were highest on *Myotis lucifugus* and on bats with visible signs of WNS.
5. *Synthesis and applications.* Our results indicate that distribution of *Pseudogymnoascus destructans* (*Pd*) within a hibernaculum is driven primarily by bats with delayed establishment of environmental reservoirs. Thus, collection of samples from *Myotis lucifugus*, or from sediment if bats cannot be sampled, should be prioritized to improve detection probabilities for *Pd* surveillance. Long-term persistence of *Pd* in sediment suggests that disease management for white-nose syndrome should address risks of sustained transmission from environmental reservoirs.

KEYWORDS

bat hibernacula, disease management, environmental reservoirs, epidemiology, fungal pathogen, *Pseudogymnoascus destructans*, surveillance, temperature, white-nose syndrome

1 | INTRODUCTION

Fungal diseases are increasing globally and threaten human health, food security and biodiversity (Fisher et al., 2012; Skerratt et al., 2007). For many infectious diseases, density of available hosts regulates pathogen transmission and mortality rates (De Castro & Bolker, 2005). However, pathogens with alternate reservoirs can escape host density dependence and have the potential to drive host species to extinction (McCallum & Dobson, 1995). Thus, ability to survive and remain infectious in environmental reservoirs complicates efforts to control fungal pathogens. Understanding factors that regulate pathogen spread and persistence in environmental reservoirs, and how these reservoirs relate to infections in hosts, is critical for effective disease surveillance and control (Haydon, Cleaveland, Taylor, & Laurenson, 2002).

White-nose syndrome (WNS) is a fungal disease of North American hibernating bats caused by *Pseudogymnoascus destructans* (*Pd*) (Blehert et al., 2009). Following presumed introduction of *Pd* from Eurasia (Hoyt et al., 2016; Wibbelt et al., 2010), WNS has caused rapid population declines among several species of North American bats (Frick et al., 2015). The fungus invades the skin surface of bats' wings during hibernation (Lorch et al., 2011; Meteyer et al., 2009), resulting in altered behaviours (Reeder et al., 2012; Warnecke et al., 2012; Wilcox et al., 2014), physiological disruptions, and death of susceptible species (Cryan, Meteyer, Boyles, & Blehert, 2010; Cryan et al., 2013; Verant et al., 2014; Warnecke et al., 2013). Since identified in New York in 2007, WNS has continued to spread across the continent changing the composition of bat communities and threatening some species with extinction (Frick et al., 2010, 2015; Thogmartin et al., 2013).

Pseudogymnoascus destructans is a psychrophilic fungus (Gargas, Trest, Christensen, Volk, & Blehert, 2009; Minnis & Lindner, 2013) that can grow on a variety of substrates (Raudabaugh & Miller, 2013; Reynolds & Barton, 2014) and has been detected in environments of hibernacula (caves and mines) that harbour bats affected by WNS (Lindner et al., 2011; Vanderwolf, Malloch, & McAlpine, 2016). Additionally, *Pd* can persist in these environments through summer, when bats are absent, and following extirpation of a bat population

by WNS (Lorch, Muller, et al., 2013). These findings indicate that environmental reservoirs of *Pd* within hibernacula are potential sources of infection for bats (Langwig, Frick, et al., 2015) and could increase extinction risks for bat populations by reducing dependencies on host density for *Pd* transmission. Environmental reservoirs also offer alternative media for *Pd* surveillance when collecting samples directly from bats is not possible. However, interpretation of results from environmental sampling is hindered by lack of knowledge about spatial and temporal relationships between distributions of *Pd* in the environment and on bats within a hibernaculum.

In this study, we compared relative effects of spatial, temporal, climatic and host factors on distribution of *Pd* within a hibernaculum. For environmental substrates, we hypothesized that presence-absence and abundance of *Pd* would be correlated with location in a hibernaculum (Vanderwolf, Malloch, McAlpine, & Forbes, 2013), time since first detection of WNS at the site (Langwig, Hoyt, et al., 2015), time of year (Langwig, Frick, et al., 2015) and temperature at the sampling location (Verant, Boyles, Waldrep, Wibbelt, & Blehert, 2012). Additionally, we hypothesized that *Pd* on bats would correlate with species and temperature (Langwig et al., 2016), sex (Grieneisen, Brownlee-Bouboulis, Johnson, & Reeder, 2015), body condition and visual evidence of WNS (Janicki et al., 2015; Turner et al., 2014). We tested these hypotheses using a multi-step modelling approach applied to six hibernacula at different stages of WNS, from first introduction of *Pd* to establishment of WNS within a bat population undergoing severe declines.

2 | MATERIALS AND METHODS

2.1 | Data collection

We selected six hibernacula in the eastern U.S. that harboured populations of hibernating bats at different stages of WNS (Table 1, Figure S1). Three hibernacula with epidemic populations had either recent detections or no evidence of WNS with minimal to no observed population declines at the start of the study. Three hibernacula with endemic populations had been affected by WNS for 4 years or more

TABLE 1 Attributes of study hibernacula

Site ID	County, state	Structure	Year WNS Confirmed, status	Daily mean temperature, °C (SD) ^a
NY1	Albany, NY	Cave, one entrance with streams	2008, Endemic	5.48 (2.04)
NY2	Sullivan, NY	Cave, multi-entrance and elevations with stream	2009, Endemic	9.32 (0.96)
NY3	Ulster, NY	Limestone mine, multi-entrances, downslope to deeper areas	2008, Endemic	4.9 (1.91)
KY	Trigg, KY	Cave, multi-entrances with stream	2011, Epidemic	12.44 (3.34)
TN	Fentress, TN	Cave, multi-entrances and elevations with stream	2013, Epidemic	9.15 (3.22)
WI	Grant, WI	Lead mine, single-adit	2014, Epidemic	9.11 (1.53)

SD, Standard deviation; WNS, White-nose syndrome.

^aTemperature was recorded using iButton data loggers at multiple locations within all hibernacula from 2012 to 2014, and within WI, TN and KY in 2015.

with severe population declines. Additionally, each hibernaculum represented a range of characteristics suitable for hibernating bats (Swezey & Garrity, 2011) and historically harboured over 1,000 *Myotis lucifugus*.

We measured temperature at 75 locations within each hibernaculum using data loggers (iButton, model numbers DS 1923-F5# and DS 1922 L-F5#; Maxim Integrated, San Jose, CA). Data loggers were separated equally into three sampling regions that varied by distance from the site entrance (14–336 m across all sites) and that encompassed historic or observed bat roosting locations. Data loggers were installed on walls or ceilings and were assigned coordinates based on distance from the entrance of the hibernaculum (X) and height from the floor (Z). Temperature was recorded every 6 hr from August 2012 to August 2014 at all sites and for an additional year (August 2014 to August 2015) at three of the sites (WI, TN and KY). Data were downloaded every August, and any missing or malfunctioning data loggers were replaced. Daily temperature parameters were summarized for each sampling location, including mean (T_{dmean}), minimum (T_{dmin}), maximum (T_{dmax}), SD (T_{dstd}) and range (T_{drange}).

Environmental substrates (sediment or wall surfaces) were sampled for *Pd* at each data-logger location within each hibernaculum three times per year in summer (August), early hibernation (November/December) and late hibernation (March) to coincide with the hibernation phenology of bats and seasonal dynamics of WNS (Janicki et al., 2015; Langwig, Frick, et al., 2015; Norquay & Willis, 2014). March sampling trips were delayed at NY1 to May 2013 and at NY2 to June 2013 and 2014 because of ice obstructing site entrances. Similarly, we were unable to enter NY2 in December 2013. Wall surfaces were sampled using a sterile polyester swab (Puritan, Guilford, ME) pre-moistened with sterile water and rolled across a c. 5-cm diameter surface within 30 cm of each data logger ($n = 69$ per site). Sediment samples ($n = 15$ per site) were collected from a transect along the length of each region within each hibernaculum using a sterile tongue depressor (Puritan) to scrape surface sediment into a sterile sample bag (Fisher Scientific, Madison, WI).

Bats hibernating within sampling regions of each site were hand-captured during late hibernation in 2013 and 2014 (with the exception of NY2 as only a few bats were observed during the study); at the WI site, bats were also sampled in 2015. Sampled species included *M. lucifugus*, tri-coloured bats *Perimyotis subflavus* and Indiana bats *Myotis sodalis* because they were present within the hibernacula and are susceptible to WNS. A total of 15–72 bats roosting within 2 m of a data logger were sampled per site each year. Bats were sampled for *Pd* using a sterile pre-moistened nylon-flocked swab (Puritan Purflock® Micro Ultrafine Tip) gently rolled along the right forearm and 1-cm strip of adjacent wing tissue three times each on the dorsal and ventral surfaces. The dorsal surface of the extended right wing was then examined under a hand-held UV light (385 nm) for fluorescence characteristic of WNS (Turner et al., 2014). A UV score (a qualitative estimate for the extent of WNS pathology) was determined for each bat based on the estimated proportion of wing surface exhibiting fluorescence (0 [0%], 1 [<25%], 2 [<50%], 3 [<75%], 4 [>75%]). A similar scoring system was applied to the amount of visible fungus on the extended wing.

Sex and species were recorded for each bat, and weight and forearm length were measured to calculate body mass index (BMI; mass [g]/forearm length [mm]; Chappell & Titman, 1983).

All samples were stored at c. 4°C during transport and then frozen at –80°C. Samples were analysed for *Pd* using the intergenic spacer-based quantitative PCR assay (Muller et al., 2013) following optimized protocols (Verant, Bohuski, Lorch, & Bleher, 2016). Duplicate standard curves of genomic DNA of *Pd* were included on each reaction plate to quantify *Pd* in each sample.

TABLE 2 Candidate models used to evaluate hypotheses describing presence–absence or abundance of *Pseudogymnoascus destructans* within hibernacula (sites)

Model	Covariate	Description
<i>Environment</i>		
Spatial	X	Distance from the entrance
	Z	Height from the floor
	Type	Sediment or wall-swab
Temporal	WNStime	Time since first confirmation of WNS in bats at the site. Each unit coincides with a sampling interval (approximately every 4 months)
	Time of year	Time of year sample was collected; early hibernation, late hibernation, summer
Climate	T_{dmean}	Daily mean temperature
	T_{drange}	Daily temperature range
<i>Bats</i>		
Spatial	Region ^a	Sampling region within the site (near entrance, middle, deep)
	Z ^b	Height from the floor
Temporal	WNSWinters ^c	Winters since first confirmation of WNS in bats at a site
Climate	T_{dmean}	Daily mean temperature within 2 m of roost location
	T_{drange}	Daily temperature range within 2 m of roost location
Host	Species	<i>Myotis lucifugus</i> , <i>Perimyotis subflavus</i> or <i>Myotis sodalis</i>
	Sex	Male, female
	BMI	Body mass index (mass [g]/forearm [mm])
Clinical signs	Fungus	Extent of visible fungus on the wing
	UV	Extent of UV fluorescence on the wing

^aIncluded in models using full dataset.

^bIncluded in models using subset dataset with paired temperature information because we included temperature at roost location as a covariate. X was not included due to collinearity with other covariates in models.

^cOmitted in models using subset dataset due to a singularity in response by site, which was included as a random effect. WNS, white-nose syndrome.

TABLE 3 Comparisons of candidate models for *Pseudogymnoascus destructans* in environmental substrates from bat hibernacula

Models ^a	k	AIC _c	ΔAIC _c	w
<i>Presence-absence</i>				
Spatial + Temporal + Climate	10	1,109.54	0	0.92
Spatial + Temporal	8	1,114.44	4.9	0.08
Climate	4	1,358.75	249.22	0
Intercept	2	1,378.93	269.39	0
<i>Abundance</i>				
Spatial + Temporal + Climate	13	1,062.74	0	0.77
Spatial + Temporal	11	1,065.16	2.42	0.23
Climate	7	1,075.14	12.4	0
Intercept	5	1,075.26	12.52	0

^aModels are ranked based on corrected Akaike's information criterion (AIC_c) and relative fits are shown with Akaike weights (w) which sum to 1. k is number of parameters. All presence-absence models included a random intercept of time since first detection of white-nose syndrome (WNS) in bats at the site. All abundance models included a random slope by sample type and a random intercept by site. See Table 2 for model descriptions.

TABLE 4 Comparisons of candidate models for *Pseudogymnoascus destructans* on hibernating bats

Model ^a	k	AIC _c	ΔAIC _c	w
<i>Presence-absence</i>				
Location + Host + Clinical signs	12	285.5	0	0.73
Host + Clinical signs	11	287.66	2.16	0.25
Location + Clinical signs	8	292.68	7.18	0.02
Clinical signs	5	300.82	15.32	0
Location + Host	10	306.28	20.78	0
Host	7	309.3	23.8	0
Location	6	314.84	29.33	0
Intercept	3	333.89	48.40	0
<i>Abundance</i>				
Location + Host + Clinical signs	12	647.05	0	0.98
Host + Clinical signs	9	655.27	8.22	0.02
Location + Clinical signs	8	661.88	14.84	0
Clinical signs	5	680.07	33.02	0
Location + Host	10	706.29	59.24	0
Host	7	714.92	67.87	0
Location	6	737.93	90.88	0
Intercept	3	756.46	109.41	0

^aModels are ranked based on corrected Akaike's information criterion (AIC_c) and relative fits are shown with Akaike weights (w) which sum to 1. k is number of parameters. Random intercepts for all presence-absence models were by year and site, and for all abundance models were by site. Temperature parameters did not improve model fit (Table S2), so model results are shown from the full dataset without temperature. See Table 2 for model descriptions.

Bats were handled in accordance with protocols approved by the Institutional Animal Care and Use Committee of the U.S. Geological Survey National Wildlife Health Center and permits from collaborating management agencies. The National White-Nose Syndrome Decontamination protocol (U.S. Fish and Wildlife Service, 2012) was followed for each site visit.

2.2 | Model selection

We used a candidate model selection approach (Bolker et al., 2009; Zuur, Ieno, Walker, Saveliev, & Smith, 2009) to evaluate multiple *a priori* hypotheses describing the distribution of *Pd* within hibernacula (Table 2). This modelling framework reduces the number of competing models and the likelihood of type-1 error. With this approach, the best-fit models highlight important correlates of *Pd* distribution within

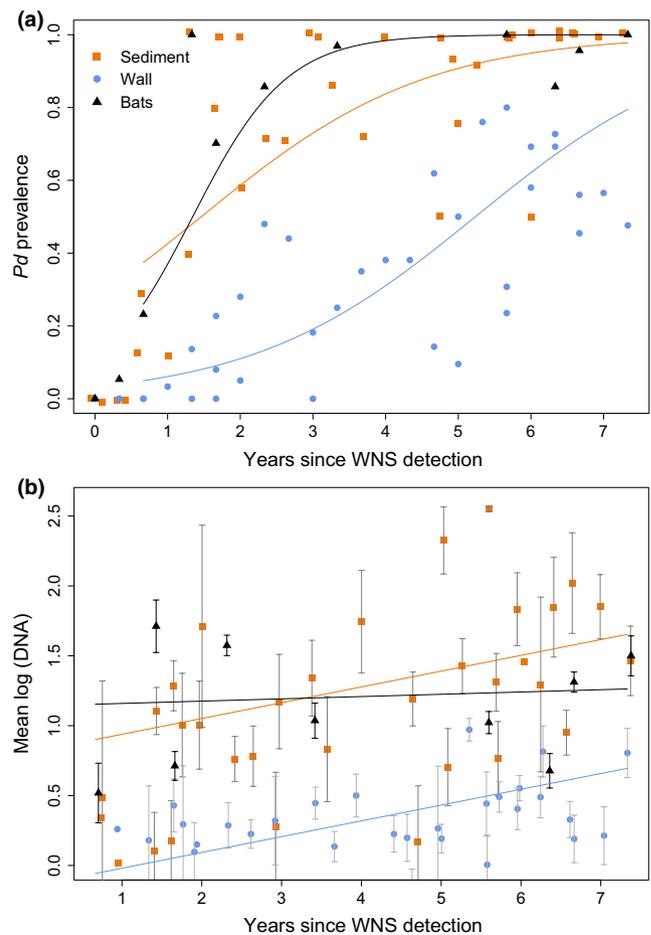


FIGURE 1 Comparison of bat wing swabs (triangles), sediment samples (squares) and wall-surface swabs (circles) collected within study hibernacula for prevalence (a) and abundance (b) of *Pseudogymnoascus destructans* (*Pd*). Points represent data summarized by sample type, site and time. Abundance includes *Pd*-positive samples only with standard errors. Solid lines are generalized linear model fits for prevalence or abundance vs. WNS time and sample type. These model fits are provided for illustrative purposes to show the general trend for each sample type over time in years since first confirmation of white-nose syndrome (WNS) at a site

a hibernaculum and are not intended to optimize predictive power. Due to loss or malfunction of data loggers, paired temperature measurements were not available for all samples. Thus, we used a two-stage model selection approach where we compared all but climate models using the full dataset, and then we compared the best-fit model to alternative models using a subset dataset, which included temperature data for the sample location. We only included mean and range of daily temperatures in models for the day the sample was collected due to collinearity with other temperature parameters. For environmental samples, we compared four models with the full dataset (Table S1) and four models using the subset dataset (Table 3). For bat samples, we compared eight models with the full dataset (Table 4) and eight models with the subset dataset (Table S2).

We fit generalized linear mixed models of presence-absence of *Pd* with a Bernoulli distribution and logit link using function *glmer* in the *lme4* package (Bates, Maechler, Bolker, & Walker, 2015) in R v3.1.2 (R Development Core Team, 2008). For abundance models, we fit linear mixed models with a *Gaussian* distribution using function *lmer* on log₁₀ transformed DNA values with only *Pd*-positive samples included in the response (all zeros were omitted). All continuous covariates were scaled and centred to allow for direct comparisons of parameter estimates in final models (Gelman, 2008; Schielzeth, 2010). Covariance among explanatory variables was assessed using Pearson’s correlation coefficients, and any potential for collinearity was further evaluated using variance inflation factors.

Best-fit models were determined by $\Delta AIC_c < 2$ (Burnham & Anderson, 2002). Correct identification of models and random effect structures were assessed using plots of residuals vs. fitted values and residuals vs. covariates (Bolker et al., 2009). Autocorrelation was assessed by plotting the autocorrelation function of the residuals in the final models, and goodness-of-fit was calculated as *pseudo-r*² values (Johnson, 2014) using the *r.squaredGLMM* function in the *MuMIn* package (Bartoń, 2015).

3 | RESULTS

Study hibernacula represented a range of attributes and temperatures characteristic of caves and mines selected by bats for hibernation (Table 1, Figure S2). We confirmed WNS in bats by histopathology for the first time in January 2013 and March 2014 at two study hibernacula (TN and WI, respectively) that had no detection of *Pd* in the environment or on bats at the start of our study.

Prevalence estimates of *Pd* on bats at the time WNS was first detected in a population were low but increased to near 100% prevalence within hibernacula affected by WNS for 3 years or more (Figure 1a). We did not detect *Pd* in environmental samples at the time WNS was first detected in bats at the TN and WI sites. First detection of *Pd* in sediment samples from both sites occurred two sampling intervals (c. 8 months) after WNS was first detected in bats. Upon

TABLE 5 Summary of best-fit models for *Pseudogymnoascus destructans* in environmental substrates of bat hibernacula

Model term	Covariates	Estimate	SE	95% CI	
<i>Presence-absence</i>					
Intercept		0.09	0.68	-1.24 to 1.42	
Spatial	X	-0.25	0.14	-0.52 to 0.01	
	Z	0.63	0.11	0.42-0.83	
	Type (wall-swab)	-3.91	0.31	-4.53 to -3.3	
Temporal	WNStime	0.21	0.05	0.11-0.30	
	Time of year	Late hibernation	0.30	0.32	-0.33 to 0.93
		Summer	-0.32	0.33	-0.97 to 0.34
Climate	<i>T</i> _{dmean}	-0.22	0.16	-0.54 to 0.09	
	<i>T</i> _{drange}	-0.29	0.11	-0.5 to -0.07	
No. of observations: 1,316; random intercept: WNStime					
<i>Abundance</i>					
Intercept		1.62	0.26	1.1-2.14	
Spatial	X	-0.14	0.06	-0.26 to -0.02	
	Z	0.05	0.05	-0.05 to 0.14	
	Type (wall-swab)	-1	0.15	-1.29 to -0.71	
Temporal	WNStime	-0.02	0.01	-0.05 to 0	
	Time of year	Late hibernation	-0.13	0.08	-0.29 to 0.02
		Summer	-0.1	0.09	-0.27 to 0.07
Climate	<i>T</i> _{dmean}	-0.08	0.06	-0.21 to 0.04	
	<i>T</i> _{drange}	-0.1	0.04	-0.18 to -0.01	
No. of observations: 505; random slope: type; random intercept: site					

Reference is a sediment sample collected during early hibernation.

Model term	Covariates		Estimate	SE	95% CI
<i>Presence-absence</i>					
Intercept			-0.72	2.78	-6.18 to 4.74
Location	WNSWinters		0.95	0.38	0.2-1.7
	Region	Middle	-0.12	0.47	-1.03 to 0.8
		Deep	-0.62	0.46	-1.52 to 0.28
Host	Species	MYSO	-2.5	0.91	-4.27 to -0.71
		PESU	0.31	0.62	-0.9 to 1.52
	BMI	0.33	0.25	-0.16 to 0.82	
	Sex (M)	0.52	0.39	-0.23 to 1.28	
Clinical signs	Fungus		-0.06	0.41	-0.87 to 0.75
	UV		0.97	0.23	0.52-1.42
No. of observations: 567; random intercepts: year and site					
<i>Abundance</i>					
Intercept			0.51	0.33	-0.14 to 1.16
Location	WNSWinters		0.10	0.06	-0.02 to 0.23
	Region	Middle	0.19	0.09	0.02-0.37
		Deep	-0.07	0.08	-0.24 to 0.09
Host	Species	MYSO	-0.81	0.19	-1.19 to -0.43
		PESU	-0.25	0.14	-0.53 to 0.02
	BMI	-0.09	0.04	-0.17 to -0.02	
	Sex (M)	-0.08	0.07	-0.22 to 0.06	
Clinical signs	Fungus		0.2	0.05	0.11-0.29
	UV		0.16	0.03	0.1-0.22
No. of observations: 340; random intercept: site					

MYSO, *Myotis sodalis* Indiana bat; PESU, *Perimyotis subflavus* tri-coloured bat.

Reference is a female *Myotis lucifugus* little brown bat without visible fungus roosting near the entrance of a hibernaculum.

initial detection, prevalence of *Pd* in sediment was low but increased to c. 80% prevalence after 3 or more years. First detection of *Pd* in wall-surface samples lagged about 1 year after first detection of WNS in bats. Prevalence and abundance of *Pd* in wall-surface samples increased at a slower rate and remained lower than in sediment and on bats within the site (Figure 1b).

Presence-absence and abundance of *Pd* in environmental samples from hibernacula was best described by spatial, temporal and climate covariates (presence-absence model $pseudo-r^2 = .51$ [fixed effects], $.69$ [fixed and random effects]; abundance model $pseudo-r^2 = .26$ [fixed effects], $.39$ [fixed and random effects]; Table 3). Spatial covariates had the largest effect (based on parameter coefficients farthest from zero) in the best-fit models (Table 5). Detection probability and abundance of *Pd* were significantly higher in sediment compared to wall-surface samples. Additionally, probability of detecting *Pd* increased with height from the floor for wall-sample locations, and abundance of *Pd* was higher when sampled closer to the entrance. The mean range of daily temperatures at sample locations had a small yet significant effect, with higher detection probabilities and abundance of *Pd* in locations with more stable temperatures. Time also had a small

TABLE 6 Summary of best-fit models for *Pseudogymnoascus destructans* on hibernating bats

effect on detection of *Pd*, but not abundance of *Pd*, increasing significantly since first detection of WNS in bats at a site. Non-significant parameters included in both best-fit models were time of year and mean daily temperature at sample location.

Presence-absence and abundance of *Pd* on hibernating bats were best described by a combination of spatial, temporal and host covariates (presence-absence $pseudo-r^2 = .38$ [fixed effects], $.89$ [fixed and random effects]; abundance $pseudo-r^2 = .31$ [fixed effects], $.58$ [fixed and random effects]; Table 4). For bats, temperature covariates did not improve model fits (Table S2). Species had the largest effect in the best-fit models (Table 6), with significantly lower detection probabilities and abundance of *Pd* on *M. sodalis* compared to *M. lucifugus*. Across all species, probability of detecting *Pd* increased with time since first confirmation of WNS in the population, and abundance was higher on bats with lower BMI and on those roosting in the middle of a hibernaculum compared to other regions of the site at the time of sampling. Abundance of *Pd* was also higher on bats with clinical signs of WNS (UV fluorescence and visible fungus), but detection of *Pd* was only positively correlated with fluorescence of wing skin under UV light. Sex was included in both of the best-fit models but was non-significant.

4 | DISCUSSION

Understanding determinants of pathogen distribution in hosts and environmental reservoirs is necessary for optimal disease surveillance and control strategies (Haydon et al., 2002). In the context of WNS, *Pd* is known to colonize bats and substrates of underground hibernacula (Lorch, Muller, et al., 2013); however, factors influencing distribution and persistence of the fungal pathogen had not been elucidated. In this study, we define presence-absence and abundance of *Pd* in environmental substrates and on bats within a hibernaculum from first introduction of the fungus to an established state. These analyses can be used to inform surveillance and management of WNS.

Results indicate that bats are the primary means by which *Pd* is introduced into a hibernaculum. Thus, if collection of samples from hibernating bats is feasible and acceptable, this method is most likely to facilitate early detection of *Pd* within that population. Similar to other

studies (Janicki et al., 2015; Langwig et al., 2016), higher probability of detecting *Pd* on *M. lucifugus* compared to other co-habitant species (Figure 2) indicates that when possible, *M. lucifugus* should be prioritized for sampling. Results also demonstrate that use of longwave UV light (385 nm) to identify hibernating bats with fluorescence characteristic of WNS (Turner et al., 2014), and targeting those animals for sampling, will further increase probability of detecting *Pd* in a wild population.

When bats cannot be sampled, testing environmental samples from a hibernaculum for *Pd* provides an alternative option for pathogen surveillance. Further, time of year did not correlate with detection probability in environmental samples, demonstrating the utility of this method for identifying *Pd* in sites that are inaccessible during winter. Higher probability of detecting *Pd* in floor sediment compared to wall-surface samples (Figure 1) indicates that sediment is the preferred sample type when conducting environmental surveillance for site-level detection of *Pd* based on an appropriate sampling design (Lorch, Muller, et al., 2013).

Similar to previous studies, we found that detecting *Pd* in environmental samples from a hibernaculum reliably indicates that *Pd* is present in the resident bat population (Langwig, Hoyt, et al., 2015; Lindner et al., 2011). We further demonstrated that detection of *Pd* in the environment of a hibernaculum, even with a robust sampling design, can lag up to 1 year following first detection of infected bats within the site. Thus, failure to detect *Pd* in environmental samples from a hibernaculum does not confirm absence of the pathogen in the bat population.

Accumulation of *Pd* in the hibernaculum environment may reflect deposition of fungal material shed by bats or growth of the fungus. Molecular methods used in this study cannot confirm viability, but *Pd* has been isolated from sediments of hibernacula using live-culture methods (Lorch, Lindner, et al., 2013). Relatively high abundance of *Pd* within sediment compared to on wall surfaces (Figure 1b) also suggests growth of the fungus in sediment. Nonetheless, biases associated with sample volume or extraction efficiency between sample types should be considered (Verant et al., 2016).

Higher prevalence and abundance of *Pd* in floor sediment are consistent with distributional patterns reported for other fungi in caves and likely reflect availability of organic matter (Dickson & Kirk, 1976; Vanderwolf et al., 2013). In contrast, vertical surfaces appear to be less uniform reservoirs of *Pd*, with distributions related more to the presence of hibernating bats. Consistent with preferences of hibernating bats to roost near cave ceilings, we demonstrated a higher probability of detecting *Pd* in wall-surface samples collected farther from hibernaculum floors. Moreover, in accordance with observations of hibernating bats affected by WNS shifting roost locations (Cryan et al., 2010), we detected greater abundance of *Pd* at environmental sampling locations closer to hibernaculum entrances. Although we did not explicitly link detection of *Pd* on roosting bats with specific environmental sampling locations, prevalence of *Pd* in environmental samples has been shown to be higher when collected near (within 20 cm of) hibernating bats (Langwig, Hoyt, et al., 2015).

Reducing environmental reservoirs of a pathogen or blocking transmission to susceptible hosts from the environment can

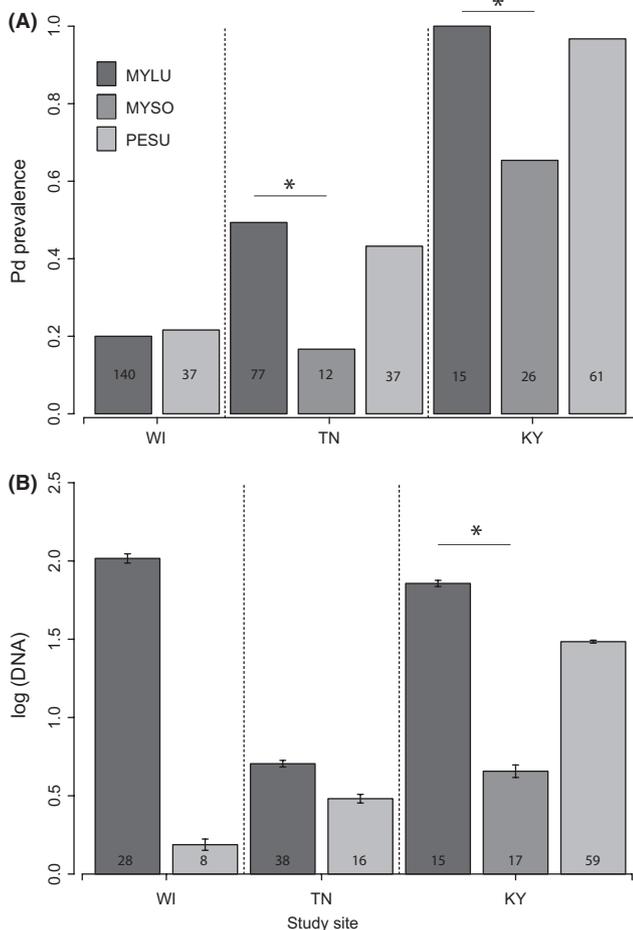


FIGURE 2 Comparison of prevalence (a) and abundance (b) of *Pseudogymnoascus destructans* in wing-swab samples from bats hibernating within study hibernacula. Only sites containing multiple species, *Myotis sodalis* (MYSO), *Myotis lucifugus* (MYLU) or *Perimyotis subflavus* (PESU), are included. MYSO were excluded from (b) for TN because of a low sample size ($n = 2$). Numbers within bars are sample sizes. Error bars denote $\pm SE$. Asterisks denote species that had significant differences in the generalized linear mixed model results

be accomplished through strategic environmental modifications (Campbell-Lendrum et al., 2005). This is standard practice for mitigating diseases in humans and livestock (Barger, 1999; Singh & Tham, 1988) and is useful for free-ranging wildlife when treatment of individual animals is not feasible. The role of environmental reservoirs of *Pd* as sources of infection for bats (Frick et al., 2017; Langwig, Frick, et al., 2015) suggests that reducing abundance of *Pd* in a hibernaculum during summer, when bats are largely absent, may reduce incidence and intensity of infection in bats that return for hibernation. Our results, however, suggest that decontamination of a hibernaculum immediately following first detection of *Pd* in bats at that site may not be productive given the scarcity of *Pd* in environmental reservoirs at this time. However, reducing the amount of *Pd* in the environment of a hibernaculum in years following first detection of the pathogen may help moderate increased rates of transmission and mortality in bats in subsequent years (Frick et al., 2017; Langwig, Hoyt, et al., 2015).

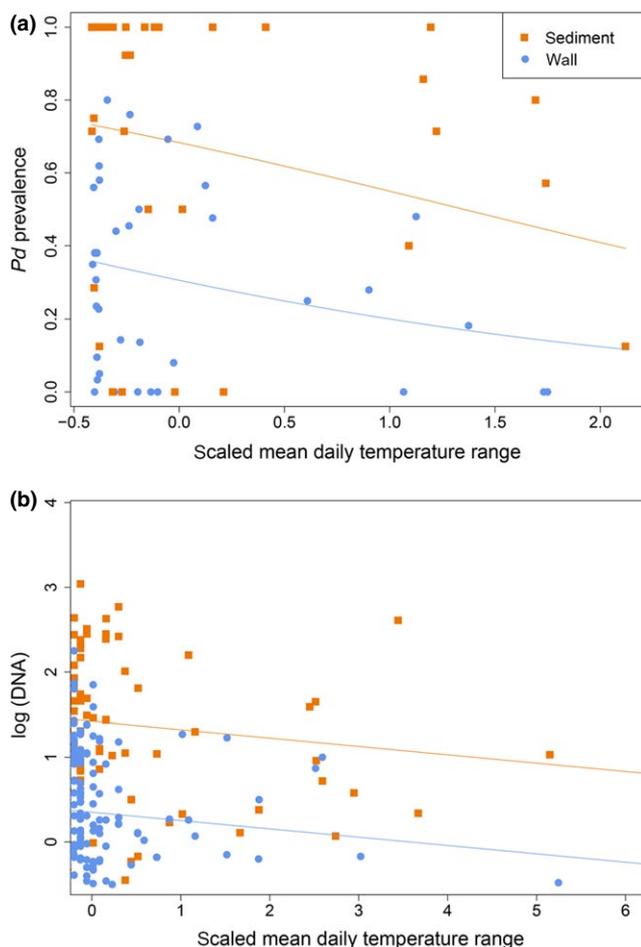


FIGURE 3 Relationships between mean daily temperature range and prevalence (a) or abundance (b) of *Pseudogymnoascus destructans* in sediment (squares) and wall-surface swabs (circles) at sampled locations within study hibernacula. Solid lines for abundance show model fits from the best-fit model by sample type. Solid lines for prevalence represent a simplified generalized linear mixed model presented for illustration purposes. This model includes mean daily temperature range (scaled and centred) and type as covariates in a binomial distribution of prevalence weighted by sample size

Further research on transmission of *Pd* between environmental substrates and bats is needed to elucidate the importance of environmental reservoirs in WNS disease dynamics.

Lowering the temperature within a hibernaculum has been proposed as a strategy for managing WNS based on observations of lower mortality in bats hibernating under colder conditions (Grieneisen et al., 2015; Johnson et al., 2014; Langwig et al., 2012). This may be related to lower growth rates of *Pd* at reduced temperature (Verant et al., 2012) resulting in lower abundance of *Pd* on bats (Langwig et al., 2016) or other physiologic effects of temperature on hibernating bats (Boyles, Dunbar, Storm, & Brack, 2007; Humphries, Thomas, & Speakman, 2002; Thomas & Cloutier, 1992). Although we detected a small effect of temperature variability on distribution and abundance of *Pd* in the environment (Figure 3), results of this study do not, within the range of temperatures suitable for bat hibernation, indicate a clear role for colder temperature in reducing growth rates and abundance of *Pd* in environmental reservoirs or on hibernating bats.

Results of this study expand our understanding of the epidemiology of WNS within bat hibernacula by identifying determinants of *Pd* within environmental substrates and on hibernating bats, from first introduction of the fungus to establishment of WNS in the bat population. This study was designed to capture fine-scale dynamics of *Pd* within hibernacula in the eastern and mid-western U.S. Additionally, conclusions provide a framework to inform WNS surveillance and management in other geographical areas in response to this unprecedented epidemic.

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AUTHORS' CONTRIBUTIONS

M.L.V., D.S.B., K.J.O. and J.H.E. conceived the ideas and designed the study; M.L.V. and E.A.B. collected the data; M.L.V., E.A.B. and K.L.D.R. analysed the data; M.L.V., K.L.D.R. and D.S.B. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the U.S. Geological Survey ScienceBase Catalog <https://doi.org/10.5066/f77d2sp5> (Verant et al., 2017).

ORCID

Michelle L. Verant  <http://orcid.org/0000-0001-6994-6257>

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