# Host abundance and heterogeneity in infectiousness determine extent of the environmental reservoir

Nichole A. Laggan[1*], Katy L. Parise[2], J. Paul White[3], Heather M. Kaarakka[3], Jennifer A. Redell[3], John E. DePue[4], William H. Scullon[5], Joseph Kath[6], Jeffrey T. Foster[2], A. Marm Kilpatrick[7], Kate E. Langwig[1], Joseph R. Hoyt[1]

[1]Department of Biological Sciences, Virginia Polytechnic Institute, Blacksburg, VA, 24060 USA

[2]Pathogen and Microbiome Institute, Northern Arizona University, Flagstaff, AZ, 86011, USA

[3]Wisconsin Department of Natural Resources, Madison, WI, 53707, USA

[4]Michigan Department of Natural Resources, Baraga, MI 49870, USA

[5]Michigan Department of Natural Resources, Norway, MI 49908, USA

[6]Illinois Department of Natural Resources, Springfield, IL, 62702, USA

[7]Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, 95064, USA

***Correspondence to**: Nichole A. Laggan: nicholelaggan@gmail.com

**Key words**: emerging infectious disease, environmental pathogen reservoir, pathogen shedding, host infectiousness, environmental transmission, white-nose syndrome

**Open Research statement**: Data will be made available through the Dryad Digital Repository before publication or upon reviewer request.

## Abstract

Environmental pathogen reservoirs exist for many globally important diseases and can fuel disease outbreaks, affect pathogen evolution, and increase the threat of host extinction. Differences in pathogen shedding among hosts can create mosaics of infection risk across landscapes by increasing pathogen contamination in high use areas. However, how the environmental reservoir establishes in multi-host communities and the importance of factors like host-specific infection and abundance in environmental contamination and transmission remain important outstanding questions. Here we examine how *Pseudogymnoascus destructans,* the fungal pathogen that causes white-nose syndrome in bats, invades and establishes in the environment. We quantified dynamic changes in pathogen shedding, infection intensities, host abundance, and the subsequent propagule pressure imposed by each species within the community. We find that the initial establishment of the pathogen reservoir is driven by different species within the community than those that are responsible for maintaining the reservoir over time. Our results also show that highly shedding species do not always contribute the most to pathogen reservoirs. More broadly, we demonstrate how individual host shedding rates scale to influence landscape-level pathogen contamination.

## Introduction

Emerging infectious diseases threaten efforts to conserve global biodiversity (Daszak et al. 2000, Taylor et al. 2001, Jones et al. 2008, Fisher et al. 2012). In some disease systems, pathogens may survive for long periods of time in the environment in the absence of a living host (Turner et al. 2016, Plummer et al. 2018, Islam et al. 2020). Pathogen persistence in the environment allows for transmission independent of infected hosts, can exacerbate disease impacts, and increase the risk of host extinction (de Castro and Bolker 2005, Mitchell et al. 2008, Almberg et al. 2011, Hoyt et al. 2020). However, pathogen contamination in the environment is not homogenous, rather, variation in the extent of the environmental reservoir is likely driven by a complex process of pathogen shedding from hosts within the community that lead to subsequent transmission events.

Infected hosts can vary in the amount of pathogen they shed into the environment with some hosts producing disproportionately high amounts of pathogen, independent of host contacts (Sheth et al. 2006, Chase-Topping et al. 2008, Lawley et al. 2008, Direnzo et al. 2014). Research has shown that inherent variation in host shedding can be driven by differences in behavior (Godfrey 2013, Rushmore et al. 2013, VanderWaal and Ezenwa 2016), innate susceptibility (Searle et al. 2011, Gervasi et al. 2013), space use (Brooks-Pollock et al. 2014), and infection severity (Lloyd-Smith et al. 2005, Munywoki et al. 2015). In multi-host disease systems, the variation produced through community composition and species abundance play key roles that determine transmission dynamics (Kilpatrick et al. 2006, Paull et al. 2012).

Host abundance within a community can interact with host shedding to moderate transmission from a particular species (Lloyd-Smith et al. 2005, Paull et al. 2012, Kilonzo et al. 2013). Species that have low rates of shedding, but are highly abundant, may contribute more to

transmission than might be expected based on the amount of pathogen they shed (Peterson and McKenzie 2014, Scheele et al. 2017). Conversely, a species that has high rates of shedding or is highly infectious, but at low abundance, may contribute less to transmission than other species (Lloyd-Smith et al. 2005, Kilpatrick et al. 2006). In addition, for some wildlife infectious diseases, infectiousness, shedding, and impacts are positively correlated (Langwig et al. 2016, Brannelly et al. 2020) such that hosts that are initially important in transmission suffer from high impacts and become less important contributors to the environmental reservoir over time. Heterogeneity in pathogen shedding and how it influences disease dynamics is important for many disease systems (Sheth et al. 2006, Chase-Topping et al. 2008, Henaux and Samuel 2011, Brooks-Pollock et al. 2014, Direnzo et al. 2014, Slater et al. 2016), but how differences in host shedding scale to a community-level and influence the environmental reservoir is rarely linked together.

White-nose syndrome (WNS) is an emerging infectious disease caused by the fungal pathogen *Pseudogymnoascus destructans* (Lorch et al. 2011, Warnecke et al. 2012*),* that has had devastating effects on bat populations (Langwig et al. 2012, Frick et al. 2015, Langwig et al. 2016). White-nose syndrome exhibits seasonal infection dynamics that are driven by the environmental reservoir and host-pathogen ecology (Langwig et al. 2015a, Hoyt et al. 2021, Langwig et al. 2021). *Pseudogymnoascus destructans* can persist for long periods of time in the environment, which results in widespread infection when hosts return to hibernacula (subterranean sites where bats hibernate in the winter) in the fall (Lorch et al. 2013, Hoyt et al. 2015, Langwig et al. 2015a, Campbell et al. 2019, Hoyt et al. 2020, Hicks et al. 2021). During this time, susceptible bats become infected or reinfected by *P. destructans* when they come into contact with the environmental reservoir upon entering hibernacula (Langwig et al. 2015a). Over

the winter hibernation period, *P. destructans* grows into the skin tissue, causing deleterious physiological changes, including increased arousals from hibernation, weight loss, dehydration, and ultimately death (Warnecke et al. 2013, Verant et al. 2014, McGuire et al. 2017, Hoyt et al. 2021). During hibernation, susceptible bat species vary greatly in their infection intensities and three species have suffered declines that exceed 95% (Langwig et al. 2012, Langwig et al. 2016, Hoyt et al. 2020, Hoyt et al. 2021). While species vary in infection intensities, species abundance also varies greatly within bat communities (Langwig et al. 2012, Frick et al. 2015), which may also influence pathogen contamination in the environment.

Contamination of *P. destructans* in the environment increases after the first year of invasion (Hoyt et al. 2020) and the extent of the environmental reservoir has been linked to increased pathogen prevalence and increased pathogen loads for bats across the globe (Hoyt et al. 2020, Hoyt et al. 2021). As a result, bat mortality also increases with higher levels of environmental contamination (Hoyt et al. 2018, Hoyt et al. 2020, Hicks et al. 2021). However, how the environmental pathogen reservoir becomes established in these multi-host communities has not been investigated.

Environmental transmission is an important driver of infectious disease dynamics and understanding the factors that lead to pathogen establishment in the environment is crucial for disease control and prevention. Here we use a unique dataset that encompasses the stages of *P. destructans* invasion and establishment to capture pathogen accumulation in the environmental reservoir across 21 sites in the Midwestern, United States. Using these data, we explored potential differences in pathogen shedding among species. We also assessed the relationship between bat infections and the amount of pathogen shed into the environment by each species present in the community. Because we hypothesized that bat species abundance would play a key

104 role in site-level contamination and pathogen establishment of the environmental reservoir, we
105 also explored how differential pathogen shedding among species and their abundance influences
106 the pathogen pressure within the community.
107
108 **Methods**
109 *Sample collection and quantification*
110 We quantified *P. destructans* fungal loads on bats and from hibernacula substrate
111 throughout bat hibernation sites in the Midwestern, United States. Samples were collected from
112 21 sites in Wisconsin, Illinois, and Michigan over seven years from pathogen invasion to
113 establishment. Hibernacula were visited twice yearly, once during early hibernation (November
114 to December) and once during late hibernation (March to April) to capture differences in
115 infection and environmental contamination at the beginning and end of hibernation. During each
116 visit, we counted the total number of bats within each site by species (*Eptesicus fuscus, Myotis*
117 *lucifugus, Myotis septentrionalis* and *Perimyotis subflavus*). We collected epidermal swab
118 samples from bats within sites to quantify bat infections (fungal loads) and determine infection
119 prevalence. Samples were collected using previously established protocols that consisted of
120 rubbing a polyester swab dipped in sterile water over the muzzle and forearm of the bat five
121 times (Langwig et al. 2015a, Hoyt et al. 2016).
122 We also collected environmental substrate swabs from hibernacula walls and ceilings
123 directly under hibernating bats to measure the amount of *P. destructans* shed into the
124 environment. To capture site-level environmental contamination, *P. destructans* was collected
125 from the environment in locations more than two meters away from any roosting bats, but in
126 areas that bats might roost. These environmental substrate samples were taken by swabbing an

127 area of substrate equal to the length of a bat's forearm (36-40 mm) five times back and forth, as
128 described previously (Langwig et al. 2015b). We preserved *P. destructans* DNA samples by
129 storing all swabs in salt preservation buffer (RNAlater; Thermo Fisher Scientific) directly after
130 collection. DNA was extracted from all samples with a modified Qiagen DNeasy Blood &
131 Tissue Kit (Langwig et al. 2015b). The presence and quantity of *P. destructans* was determined
132 by quantitative Polymerase Chain Reaction (qPCR) (Muller et al. 2013).
133 To verify that fungal loads measured using qPCR accurately reflected viable fungal
134 spores in the environment, that would be capable of infecting a host, we collected additional
135 substrate swab samples from a subset of locations that were paired with swabs used for qPCR.
136 These samples were cultured by streaking the substrate swab across a plate containing Sabouraud
137 Dextrose Agar treated with chloramphenicol and gentamicin. The plates were stored at 4 °C and
138 colony forming units (CFU's) of *P. destructans* were quantified within six weeks of initial
139 inoculation. We paired substrate samples analyzed using qPCR to determine $\log_{10}$ *P. destructans*
140 loads for comparison with colony forming units obtained from culture samples to validate
141 viability. There was a significant relationship between quantity of DNA measured through qPCR
142 and the number of *P. destructans* CFU's (Figure S1). This suggests that qPCR was a valid
143 method to estimate the extent of *P. destructans* in the environment and supports that qPCR
144 results are reflective of the number of infectious propagules in the environment and not relic
145 DNA (Figure S1).
146 All research was approved through Institutional Animal Care and Use Committee
147 protocols: Virginia Polytechnic Institute: 17-180; University of California, Santa Cruz:
148 Kilpm1705; Wisconsin Endangered/Threatened Species Permit 882 & 886; Michigan
149 Department of Natural Resources permit SC-1651; Illinois Endangered/ Threatened Species

Permit 5015, 2582 and Scientific Collections permit NH20.5888; US Fish and Wildlife Service Threatened & Endangered Species Permit TE64081B-1.

*Data analysis*

We separated invasion stage into two distinct categories: "invasion" which included the first year the pathogen arrived, as described previously (Langwig et al. 2015b, Hoyt et al. 2020), and "establishment" which included the second and third years of *P. destructans* presence in a site when species declines begin to occur, which corresponds to the epidemic phase as has been previously noted (Langwig et al. 2015b, Hoyt et al. 2020). We used these stages to capture pathogen shed into the environment before and after pathogen accumulation in the environment occurred (invasion and establishment, respectively) and to examine the dynamic changes between stages of pathogen invasion and establishment.

We first examined the presence and quantity of *P. destructans* on each species and the amount each species shed into the environment. We used mixed effect models with $\log_{10}$ environmental fungal load collected under each bat as our response variable, bat species interacting with pathogen invasion stage as our predictor and site as a random effect. We similarly compared infection intensity among species using the same model as described above, but with $\log_{10}$ fungal loads on bats as the response variable. We examined differences in infection prevalence by species using a generalized linear mixed effects model with a binomial distribution and a logit link with species as our predictor and bat infection prevalence (0|1) as our response and site as a random effect.

To examine how differences in infection on each bat contributed to contamination of the environment under each individual, we used a linear mixed effects model to explore the relationship between infection on each individual bat and pathogen shed into the environment

under each individual. In this analysis, we used paired $\log_{10}$ environmental *P. destructans* loads under a bat as our response variable with $\log_{10}$ bat fungal loads interacting with species as our predictor and site as a random effect. We combined the invasion and established stages since the amount of pathogen shed into the environment was hypothesized to be a product of how infected the host was, and therefore, comparable across years.

We investigated the role of species abundance in environmental contamination of *P. destructans*. We tested for differences among species abundance within each site by using a linear mixed effects model with species as our predictor and $\log_{10}$ average abundance during early hibernation (before over-winter declines occur) as our response and included site as a random effect for both invasion stages. To explore how species abundance influenced the degree of pathogen contamination within sites, we used a linear mixed effect model with $\log_{10}$ environmental fungal loads collected > 2m from any bat during late hibernation as our response variable and $\log_{10}$ average abundance interacting with species identity as our predictors with site as a random effect.

Finally, we calculated differences in propagule pressure among species by multiplying infection prevalence by the species abundance within each site to get the number of infected individuals. We then multiplied the number of infected individuals by the average amount of fungal spores (conidia) shed into the environment by each species in each site. We used a generalized linear mixed effect model with a negative binomial distribution to account for dispersed counts with an interaction between invasion stage and species as our predictor and propagule pressure as our response. We performed this analysis for both invasion and establishment during late hibernation to investigate how pathogen pressure may differ across stages of pathogen invasion when bats are heavily shedding into the environment. To assess if

196 propagule pressure was a suitable metric for pathogen invasion of the environmental reservoir,
197 we examined the relationship between site-level environmental contamination, as described
198 above, and sum propagule pressure for each site using a linear mixed effects model with site
199 included as a random effect. All analyses were conducted in R v.4.2.1 (R Core Team 2022).
200 Mixed-effects models were run using the package "lme4" (Bates et al. 2015) except for analyses
201 using a negative binomial distribution which were ran using "glmmTMB" (Brooks et al. 2017)
202
203 ***Results***
204 We found that pathogen shedding (the amount of pathogen under bats) varied among the
205 four species (Figure 1). During initial pathogen invasion into bat communities, we found that on
206 average individual *M. septentrionalis* (Figure 1A; Table S1; intercept =-3.79 ± 0.23) contributed
207 more pathogen into the environment than *E. fuscus* (coeff = -0.77 ± 0.37, P = 0.04) and *M.*
208 *lucifugus* (coeff= -0.58 ± 0.27, P = 0.03), but did not differ statistically from individual *P.*
209 *subflavus* (coeff =-0.21 ± 0.38, P = 0.58). In the established stage (Figure 1B), we found support
210 for higher shedding in *M. lucifugus* (intercept = -3.38 ± 0.11) than *E. fuscus* (coeff = -0.80 ±
211 0.14, P < 0.0001) and *P. subflavus* (coeff = -0.62 ± 0.13, P < 0.0001), but similar shedding to *M.*
212 *septentrionalis* (coeff = -0.38 ± 0.24, P = 0.11).
213 Our results showed consistent support that host infection intensity predicted the amount
214 of pathogen shed into the environment for all species (Figure 2C; Table S4; host infection and
215 shedding relationship: *M. lucifugus* slope = 0.38 ± 0.05, P < 0.001; *E. fuscus* slope = 0.33 ± 0.07,
216 P < 0.00001; *M. septentrionalis* slope = 0.41 ± 0.10, P = 0.00003; *P. subflavus* slope = 0.33 ±
217 0.08, P = 0.00006). Importantly, we found no support for differences among species in the slope
218 between environmental pathogen shedding and host infection level (Figure 2C; Table S4; all P >

0.05), suggesting that differences in shedding were primarily driven by the extent of infection on individual bats. *Eptesicus fuscus* (intercept = -3.37 ± 0.16) had significantly lower infection levels than all other species within the community (Figure 2B; Table S3; *M. lucifugus* coeff = 1.38 ± 0.15, P < 0.0001; *M. septentrionalis* coeff = 1.08 ± 0.21 , P < 0.0001; *P. subflavus* coeff = 1.30 ± 0.17, P < 0.0001) In addition, we found that *M. lucifugus* (intercept = 2.19 ± 0.27) had the highest infection prevalence, with 87% of hosts infected on average across the invasion and established stages (Figure 2A; Table S2; *E. fuscus* coeff = -0.79 ± 0.31, P < 0.01; *M. septentrionalis* coeff = -0.83 ± 0.35, P < 0.05; *P. subflavus* coeff = -1.10 ± 0.24, P < 0.0001).

In addition to differences in infection intensity, species also varied in abundance, with *M. lucifugus* (intercept = 1.63 ± 0.19) being the most abundant species across sites, with an average population size of 39.81 ± 7.76 before declines from WNS (invasion) (Figure 3; Table S5; *E. fuscus* coeff = -0.65 ± 0.24, P < 0.01; *M. septentrionalis* coeff = -0.60 ± 0.21, P < 0.01; *P. subflavus* coeff = -0.41 ± 0.23, P < 0.08). During the established stage, while population declines were occurring, *M. lucifugus* (intercept = 1.28 ± 0.13) remained the most abundant species within the community with an average population size of 18.20 ± 7.41 (Figure 3; Table S6; *E. fuscus* coeff = -0.54 ± 0.16, P < 0.001; *M. septentrionalis* coeff = -0.76 ± 0.14, P < 0.0001; *P. subflavus* coeff = -0.38 ± 0.15, P < 0.01). *M. septentrionalis* had the lowest abundance in the community with an average colony size of 11.22 ± 5.50 individuals before disease impacts and declined to an average of 3.52 ± 4.12 individuals in a site during the established stage (Figure 3; Table S5-7). Furthermore, host abundance was important in influencing the degree of site-level environmental pathogen contamination (Figure S2; Table S7; *M. lucifugus* slope = 0.40 ± 0.14, P = 0.03).

241 Finally, when we combined infection prevalence, species abundance, and pathogen
242 shedding into the metric of propagule pressure, we found that during the first year of invasion,
243 *M. septentrionalis* (Figure4A; Table S8; intercept = 7.85 ± 0.66) had higher propagule pressure
244 than *P. subflavus* (coeff = -2.81 ± 1.01, P < 0.01) and *E. fuscus* (-4.28 ± 0.85, P < 0.0001), but
245 not *M. lucifugus* (coeff = 0.43 ± 0.73, P = 0.55). In years following invasion, once *P. destructans*
246 is established, *M. lucifugus* (Figure 4B; intercept = 11.08 ± 0.56) had consistently higher
247 propagule pressure and contributed more pathogen to the environmental reservoir than all other
248 species (Figure 4B; *M. septentrionalis* coeff = -4.14 ± 0.96, P < 0.0001; *E. fuscus* coeff = -4.18 ±
249 0.68, P < 0.0001; *P. subflavus* coeff = -2.53 ± 0.68, P = 0.002). *Myotis septentrionalis* (Figure
250 4B; intercept = 6.94 ± 0.95) no longer contributed more than *P. subflavus* (coeff = 1.62 ± 1.03, P
251 = 0.11) and *E. fuscus* (coeff = -0.04 ± 1.02, P = 0.97) due to their rarity following disease-
252 induced declines (Figure 4). Mean environmental contamination in areas > 2m from bats
253 increased with total propagule pressure at a site (summed propagule pressure among species)
254 (Figure 4C; relationship between environmental contamination and propagule pressure intercept
255 = -5.48 ± 0.14, slope = 0.12, P = 0.002).
256
257 ***Discussion***
258 Host heterogeneity in pathogen shedding and subsequent environmental transmission can
259 have dramatic impacts on community-level disease outcomes. Our findings demonstrate how
260 host heterogeneity in infection intensity can lead to increased pathogen shedding, which is
261 consistent with research in other systems (Lloyd-Smith et al. 2005, Matthews et al. 2006, Chase-
262 Topping et al. 2008, Direnzo et al. 2014, Munywoki et al. 2015, Maguire et al. 2016,
263 VanderWaal and Ezenwa 2016). In addition, our study demonstrates that host heterogeneities in

abundance and infection interact to influence the extent of the environmental reservoir and the contribution of species, which varied over time. We identified a shift in the species responsible for the greatest environmental reservoir contamination between disease stages, suggesting that reservoir contamination is a dynamic process that varies over time.

Our findings suggest that the observed heterogeneities in pathogen shedding are primarily due to variation in infection intensities among species within the community. The relationship between individual host shedding into the environment and host infection intensity did not differ among species, suggesting that individuals of different species with the same infection intensity are equally efficient at depositing pathogen into the environment. Instead, the severity of infection, which varied among species, determined how much pathogen is shed per individual. Some species such as *E. fuscus* had low level infections, while the other species such as *M. septentrionalis* and *M. lucifugus* were much more infected and contributed more pathogen to the environmental reservoir (Figure 2B).

Disease-caused declines in several species (Figure 3) resulted in large changes in species abundance and community composition. We found evidence that species abundance predicted site-level contamination (Figure S2), and this supported the need to account for both species' infection and abundance when determining the contribution to the environmental reservoir. To combine these factors, we used propagule pressure or "introduction effort" which is a fundamental ecological metric that determines whether a biological invasion will be successful in reaching establishment (Lockwood et al. 2005), and is especially applicable to emerging infectious diseases. Our results indicated that during the initial invasion of *P. destructans*, both *M. lucifugus* and *M. septentrionalis* had higher propagule pressure than the other two species (Figure 4A), which was not apparent through examination of only pathogen shedding. The high

287 infection levels of *M. septentrionalis* resulted in a large contribution to the initial establishment
288 of the environmental reservoir despite their low relative abundance (Figure 2B; Figure 3).
289 However, in subsequent years, as this species declined precipitously (Figure 3), their
290 contribution was greatly reduced and equivalent to less infected species (e.g. *E. fuscus* and *P.*
291 *subflavus*, Figure 4B).

292 Our results suggest that host abundance, shedding, and infection prevalence are
293 collectively required to describe pathogen contamination in the environment. We found that
294 environmental contamination in the sampled locations that were not associated with bats
295 increased as propagule pressure increased (Figure 4C), suggesting that propagule pressure is an
296 accurate metric for measuring species contributions to the environmental reservoir. For example,
297 while *E. fuscus*, *M. septentrionalis*, and *P. subflavus* eventually contributed similar propagule
298 pressures during disease establishment, this was likely driven by different factors that need to be
299 considered when evaluating their influence on the reservoir. *Eptesicus fuscus* had low infection
300 intensity but had moderate prevalence (Figure 2A-B) and moderate abundance (Figure 3) which
301 elevated its contribution. *Perimyotis subflavus* had high infection levels, but reduced prevalence
302 (Figure 2A-B) compared to the other highly infected species and was only present in moderate
303 abundances (Figure 3). Finally, *M. septentrionalis* was highly infected (Figure2B), but at low
304 abundance across communities (Figure 3), which reduced its overall importance in years
305 following initial pathogen invasion. *Myotis lucifugus* maintained high propagule pressure
306 through the invasion and establishment of *P. destructans* (Figure 4A-B), suggesting that the
307 presence of *M. lucifugus* within a community will result in rapid establishment of *P. destructans*
308 in the environment and maintenance of environmental contamination within a site over time. Our
309 results highlight how both species infection intensity and abundance must be considered to

evaluate the influence of pathogen shedding on environmental reservoir establishment, subsequent environmental transmission and how this can change dynamically across disease stages.

Understanding environmental reservoir dynamics is crucial for many globally important disease systems. Discerning how variation across species contributes to maintenance of the environmental reservoir is important in determining the extent of epidemics, predicting long-term impacts on host communities, and developing control strategies. We demonstrate that the establishment and maintenance of the environmental reservoir is strongly influenced by both heterogeneity in pathogen infection and species abundance. Evaluating these effects together through the metric of propagule pressure allowed us to capture which species within the community contributed to pathogen invasion success and ultimately the maintenance of indirect transmission which is an important driver of infection and mortality (Hoyt et al. 2018). Broadly, our results demonstrate that multiple factors of species heterogeneity can scale to influence environmental reservoir dynamics within communities.

**Acknowledgements:**

The research was funded by the joint NSF-NIH-NIFA Ecology and Evolution of Infectious Disease award DEB-1911853, NSF DEB-1115895, USFWS (F17AP00591), and by the NSF GRFP. We would like to acknowledge landowners for access to sites, and M.J. Kailing for assisting culture collection and B. Heeringa and D. Kirk for field assistance.

333 **Author Contributions:**

334 NAL and JRH wrote the original draft of the manuscript. JRH, KEL, and AMK designed
335 methodology; NAL, JRH, KEL, AMK, JPW, HMK, JAR, JED, WHS, and JK collected the data;
336 NAL conducted the laboratory experiment; JTF and KLP supervised and performed sample
337 testing; NAL analyzed the data with assistance from KEL and JRH; All authors contributed
338 critically to draft revision.

339

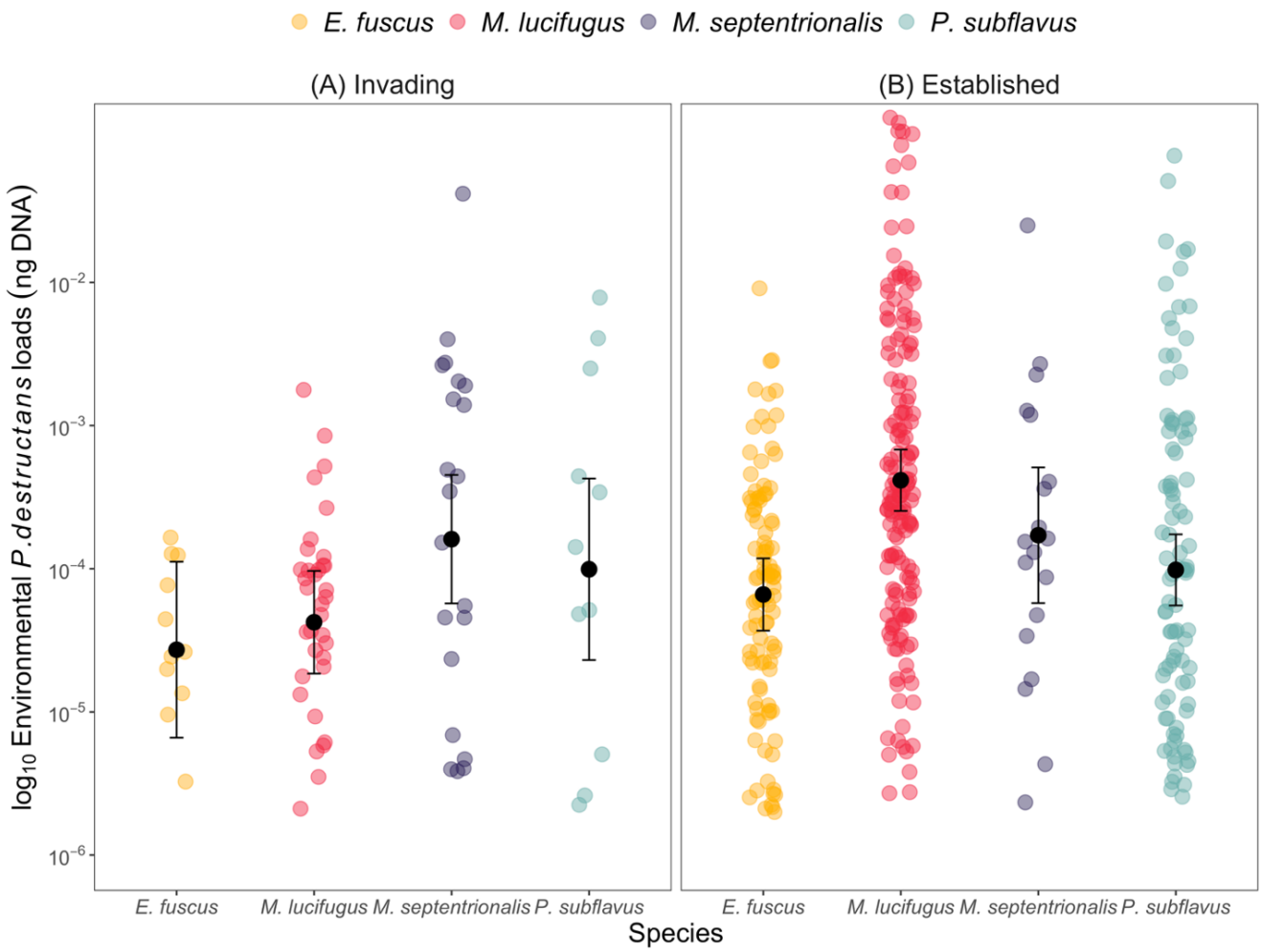
340 **Conflict of Interest:** The authors declare no conflicts of interest

341 **Figure captions:**

342 **Figure 1**: **Differences in pathogen shedding among species during reservoir invading and**
343 **established stages.** $\log_{10}$ *P. destructans* environmental loads (ng DNA) among species during
344 pathogen invasion (A) and establishment (B) in late hibernation. Each point represents the $\log_{10}$
345 environmental *P. destructans* loads from under an individual bat. Black points represent the
346 estimated mean and bars indicate ± standard error for each species.
347
348 **Figure 2: Bat infection prevalence, intensity, and the relationship between host infection**
349 **and pathogen shedding.** For all panels, bat species are displayed by color. Black points indicate
350 mean bat infections by species and bars represent ± standard error. (A) Bat infection prevalence
351 for each species during pathogen invasion in late hibernation. (B) Bat $\log_{10}$ *P. destructans* loads
352 (ng DNA) for each species during pathogen invasion in late hibernation. (C) The relationship
353 between $\log_{10}$ *P. destructans* loads (ng DNA) on an individual bat and the amount of $\log_{10}$ *P.*
354 *destructans* (ng DNA) in the environment directly underneath each individual during pathogen
355 invasion and establishment in late hibernation. The gray solid line shows the 1:1 line, and points
356 along this line would indicate that the amount of *P. destructans* on bats was equivalent to the
357 amount shed into the environment. Solid colored lines indicate statistical support for a positive
358 relationship. Each individual bat is represented by a point.
359
360 **Figure 3. Changes in host abundance between invading and established years.** Species
361 within the community are differentiated by color and points represent a species population at an
362 individual site. Species abundance in the invasion (light gray box) and established years (dark
363 gray box) in late hibernation within each site. Circular points indicate the mean abundance for
364 each year and bars denote ± standard error.
365
366 **Figure 4**. **Differences in propagule pressure among species during invading and established**
367 **stages and the relationship between propagule pressure and environmental contamination.**
368 (A-B) Propagule pressure (# of pathogen particles) by species during late hibernation in (A)
369 invading and (B) established stages. Colored points represent populations of species within a
370 site, black points represent the mean and bars indicate ± standard error for each species. Point
371 size is weighted by species population abundance. (C) Relationship between $\log_{10}$ propagule

pressure and $\log_{10}$ mean site-level contamination. Site-level contamination was assessed in samples > 2m away from roosting bats within sites during late hibernation in the established stage. Points indicate the sum propagule pressure at a site for each year and shape denotes invasion stage. The total environmental contamination increased as propagule pressure increased within a site (Environmental contamination: intercept = -5.48 ± 0.14, slope = 0.12, relationship between environmental contamination and propagule pressure P = 0.002).

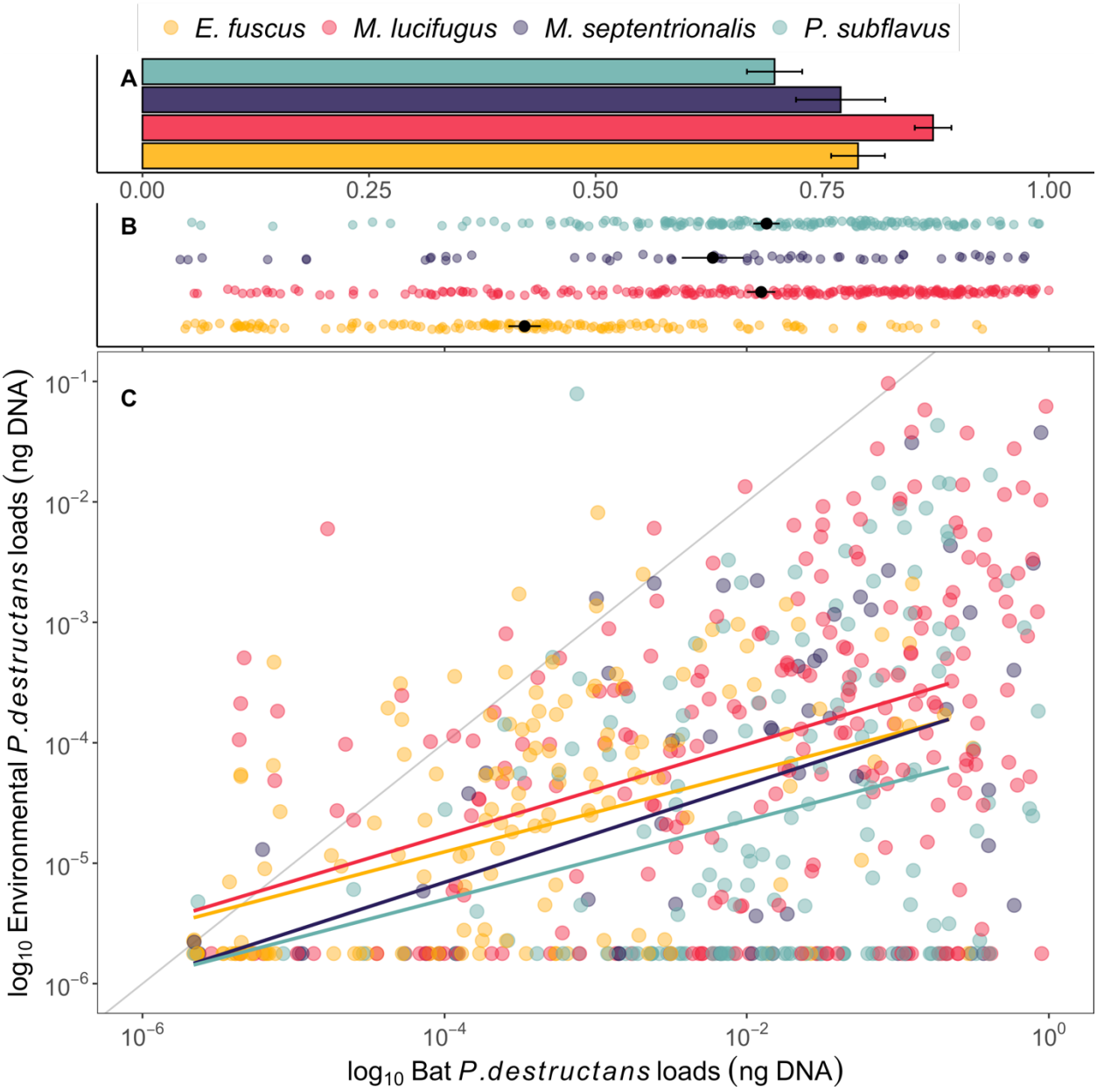
378 **Figure 1**




379

380 **Figure 2**

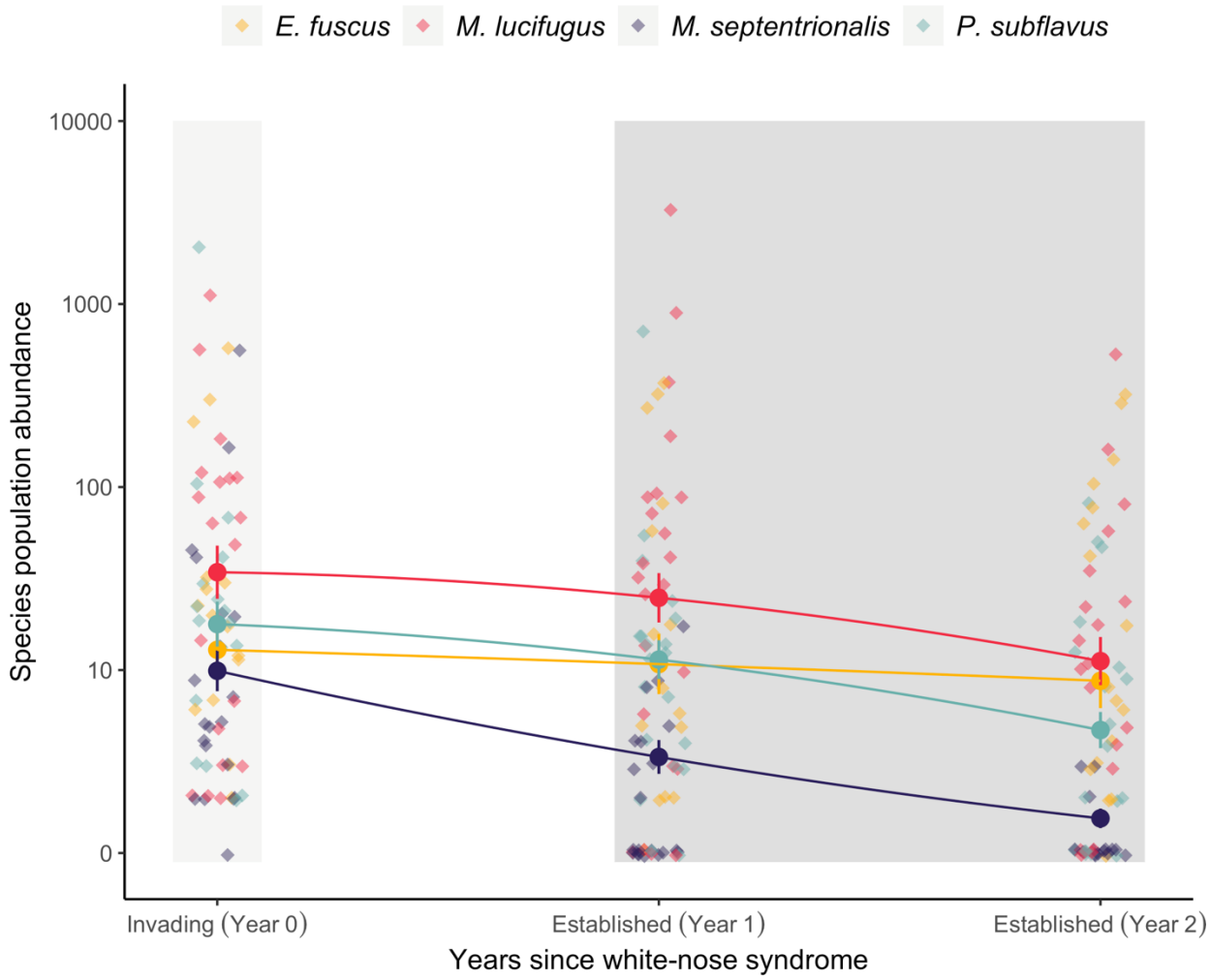
381




382

383

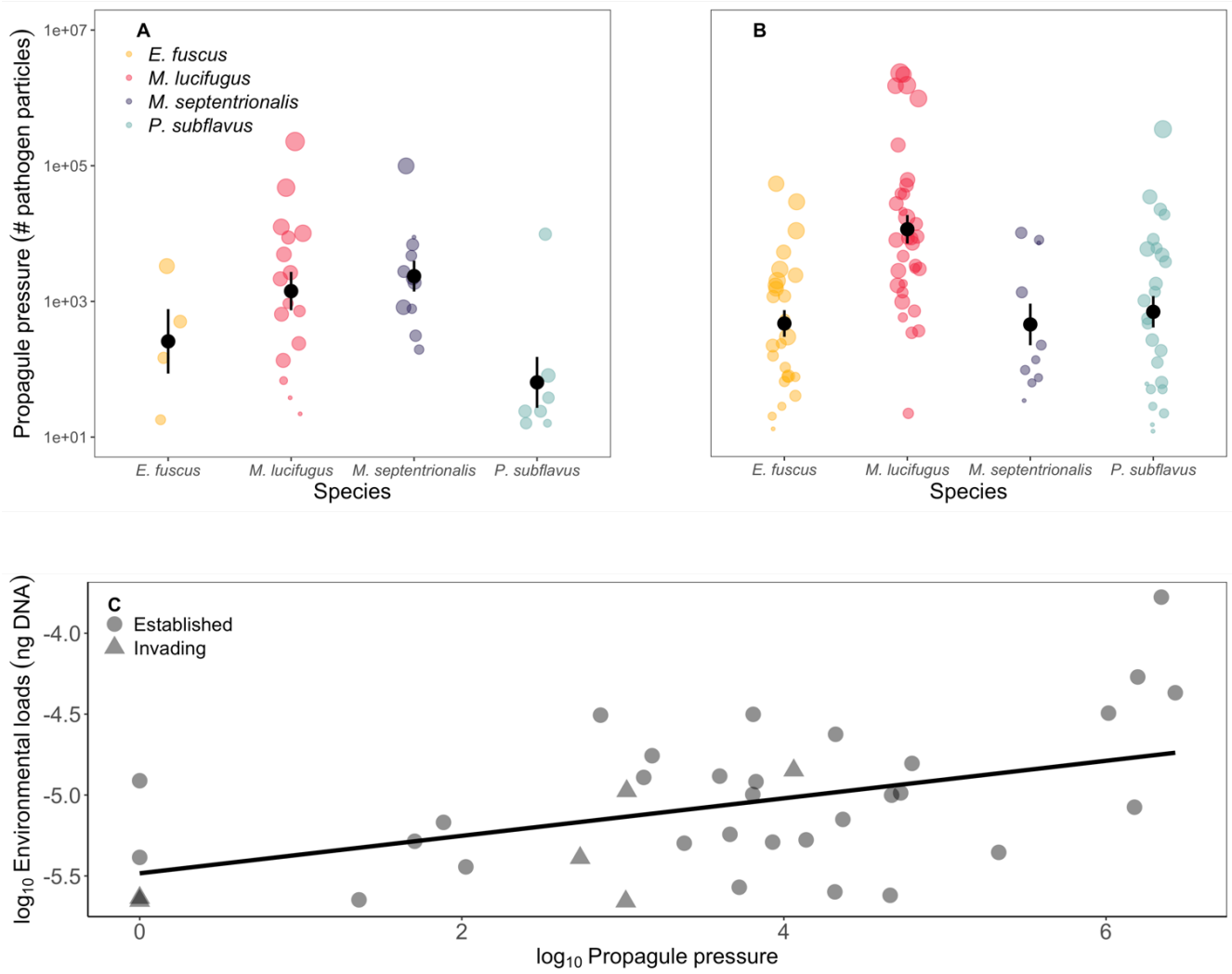
384 **Figure 3**




385

386

387 **Figure 4**




388

389

# References


Almberg, E. S., P. C. Cross, C. J. Johnson, D. M. Heisey, and B. J. Richards. 2011. Modeling routes of chronic wasting disease transmission: environmental prion persistence promotes deer population decline and extinction. PLoS One **6**:e19896.

Bates, D., M. Mächler, B. M. Bolker, and S. C. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**:1-48.

Brannelly, L. A., H. I. McCallum, L. F. Grogan, C. J. Briggs, M. P. Ribas, M. Hollanders, T. Sasso, M. Familiar López, D. A. Newell, and A. M. Kilpatrick. 2020. Mechanisms underlying host persistence following amphibian disease emergence determine appropriate management strategies. Ecology Letters.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielson, H. J. Skaug, M. Maechler, and B. M. Bolker. 2017. glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. The R Journal **9**:378-400.

Brooks-Pollock, E., G. O. Roberts, and M. J. Keeling. 2014. A dynamic model of bovine tuberculosis spread and control in Great Britain. Nature **511**:228-231.

Campbell, L. J., D. P. Walsh, D. S. Blehert, and J. M. Lorch. 2019. Long-term survival of Pseudogymnoascus destructans at elevated temperatures. Journal of Wildlife Diseases.

Chase-Topping, M., D. Gally, C. Low, L. Matthews, and M. Woolhouse. 2008. Super-shedding and the link between human infection and livestock carriage of Escherichia coli O157. Nat Rev Microbiol **6**:904-912.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science **287**:443.

de Castro, F., and B. Bolker. 2005. Mechanisms of disease-induced extinction. Ecology Letters **8**:117-126.

Direnzo, G. V., P. F. Langhammer, K. R. Zamudio, and K. R. Lips. 2014. Fungal infection intensity and zoospore output of Atelopus zeteki, a potential acute chytrid supershedder. PLoS One **9**:e93356.

Fisher, M. C., D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, and S. J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature **484**:186-194.

Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B. A. Nickel, K. E. Langwig, J. T. Foster, K. E. Barlow, T. Bartonicka, D. Feller, A. J. Haarsma, C. Herzog, I. Horacek, J. van der Kooij, B. Mulkens, B. Petrov, R. Reynolds, L. Rodrigues, C. W. Stihler, G. G. Turner, and A. M. Kilpatrick. 2015. Disease alters macroecological patterns of North American bats. Global Ecology and Biogeography **24**:741-749.

Gervasi, S. S., J. Urbina, J. Hua, T. Chestnut, R. A. Relyea, and A. R. Blaustein. 2013. Experimental evidence for American bullfrog (Lithobates catesbeianus) susceptibility to chytrid fungus (Batrachochytrium dendrobatidis). Ecohealth **10**:166-171.

Godfrey, S. S. 2013. Networks and the ecology of parasite transmission: A framework for wildlife parasitology. Int J Parasitol Parasites Wildl **2**:235-245.

Henaux, V., and M. D. Samuel. 2011. Avian influenza shedding patterns in waterfowl: implications for surveillance, environmental transmission, and disease spread. J Wildl Dis **47**:566-578.

Hicks, A. C., S. Darling, J. Flewelling, R. von Linden, C. U. Meteyer, D. Redell, J. P. White, J. Redell, R. Smith, D. Blehert, N. Rayman, J. R. Hoyt, J. C. Okoniewski, and K. E. Langwig. 2021. Environmental transmission of Pseudogymnoascus destructans to hibernating little brown bats. biorxiv.

Hoyt, J. R., A. M. Kilpatrick, and K. E. Langwig. 2021. Ecology and impacts of white-nose syndrome on bats. Nature Reviews Microbiology:1-15.

Hoyt, J. R., K. E. Langwig, J. Okoniewski, W. F. Frick, W. B. Stone, and A. M. Kilpatrick. 2015. Long-term persistence of Pseudogymnoascus destructans, the causative agent of white-nose syndrome, in the absence of bats. Ecohealth **12**:330-333.

437 Hoyt, J. R., K. E. Langwig, K. Sun, G. Lu, K. L. Parise, T. Jiang, W. F. Frick, J. T. Foster, J. Feng, and A. M.
438 Kilpatrick. 2016. Host persistence or extinction from emerging infectious disease: insights from
439 white-nose syndrome in endemic and invading regions. Proceedings of the Royal Society B:
440 Biological Sciences **283**.
441 Hoyt, J. R., K. E. Langwig, K. Sun, K. L. Parise, A. Li, Y. Wang, X. Huang, L. Worledge, H. Miller, J. P. White,
442 H. M. Kaarakka, J. A. Redell, T. Görföl, S. A. Boldogh, D. Fukui, M. Sakuyama, S. Yachimori, A.
443 Sato, M. Dalannast, A. Jargalsaikhan, N. Batbayar, Y. Yovel, E. Amichai, I. Natradze, W. F. Frick, J.
444 T. Foster, J. Feng, and A. M. Kilpatrick. 2020. Environmental reservoir dynamics predict global
445 infection patterns and population impacts for the fungal disease white-nose syndrome.
446 Proceedings of the National Academy of Sciences:201914794.
447 Hoyt, J. R., K. E. Langwig, J. P. White, H. M. Kaarakka, J. A. Redell, A. Kurta, J. E. DePue, W. H. Scullon, K.
448 L. Parise, and J. T. Foster. 2018. Cryptic connections illuminate pathogen transmission within
449 community networks. Nature:1.
450 Islam, M. S., M. H. Zaman, M. S. Islam, N. Ahmed, and J. D. Clemens. 2020. Environmental reservoirs of
451 Vibrio cholerae. Vaccine **38 Suppl 1**:A52-A62.
452 Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global
453 trends in emerging infectious diseases. Nature **451**:990-U994.
454 Kilonzo, C., X. Li, E. J. Vivas, M. T. Jay-Russell, K. L. Fernandez, and E. R. Atwill. 2013. Fecal shedding of
455 zoonotic food-borne pathogens by wild rodents in a major agricultural region of the central
456 California coast. Appl Environ Microbiol **79**:6337-6344.
457 Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra, and L. D. Kramer. 2006. Host heterogeneity
458 dominates West Nile virus transmission. Proceedings of the Royal Society B-Biological Sciences
459 **273**:2327-2333.
460 Langwig, K. E., W. F. Frick, J. T. Bried, A. C. Hicks, T. H. Kunz, and A. M. Kilpatrick. 2012. Sociality, density-
461 dependence and microclimates determine the persistence of populations suffering from a novel
462 fungal disease, white-nose syndrome. Ecol Lett **15**.
463 Langwig, K. E., W. F. Frick, J. R. Hoyt, K. L. Parise, K. P. Drees, T. H. Kunz, J. T. Foster, and A. M. Kilpatrick.
464 2016. Drivers of variation in species impacts for a multi-host fungal disease of bats. Philosophical
465 Transactions of the Royal Society B: Biological Sciences **10.1098/rstb.2015.0456**.
466 Langwig, K. E., W. F. Frick, R. Reynolds, K. L. Parise, K. P. Drees, J. R. Hoyt, T. L. Cheng, T. H. Kunz, J. T.
467 Foster, and A. M. Kilpatrick. 2015a. Host and pathogen ecology drive the seasonal dynamics of a
468 fungal disease, white-nose syndrome. Proceedings of the Royal Society B: Biological Sciences
469 **282**:20142335.
470 Langwig, K. E., J. R. Hoyt, K. L. Parise, J. Kath, D. Kirk, W. F. Frick, J. T. Foster, and A. M. Kilpatrick. 2015b.
471 Invasion Dynamics of White-Nose Syndrome Fungus, Midwestern United States, 2012-2014.
472 Emerging Infectious Disease **21**:1023-1026.
473 Langwig, K. E., J. P. White, K. L. Parise, H. M. Kaarakka, J. A. Redell, J. E. DePue, W. H. Scullon, J. T. Foster,
474 A. M. Kilpatrick, and J. R. Hoyt. 2021. Mobility and infectiousness in the spatial spread of an
475 emerging fungal pathogen. J Anim Ecol **90**:1134-1141.
476 Lawley, T. D., D. M. Bouley, Y. E. Hoy, C. Gerke, D. A. Relman, and D. M. Monack. 2008. Host
477 transmission of Salmonella enterica serovar Typhimurium is controlled by virulence factors and
478 indigenous intestinal microbiota. Infect Immun **76**:403-416.
479 Lloyd-Smith, J. O., S. J. Schreiber, P. E. Kopp, and W. M. Getz. 2005. Superspreading and the effect of
480 individual variation on disease emergence. Nature **438**:355-359.
481 Lockwood, J. L., P. Cassey, and T. Blackburn. 2005. The role of propagule pressure in explaining species
482 invasions. Trends Ecol Evol **20**:223-228.

Lorch, J. M., C. U. Meteyer, M. J. Behr, J. G. Boyles, P. M. Cryan, A. C. Hicks, A. E. Ballmann, J. T. H. Coleman, D. N. Redell, D. M. Reeder, and D. S. Blehert. 2011. Experimental infection of bats with Geomyces destructans causes white-nose syndrome. Nature **480**:376-378.

Lorch, J. M., L. K. Muller, R. E. Russell, M. O'Connor, D. L. Lindner, and D. S. Blehert. 2013. Distribution and environmental persistence of the causative agent of white-nose syndrome, Geomyces destructans, in bat hibernacula of the eastern United States. Applied and Environmental Microbiology **79**:1293-1301.

Maguire, C., G. V. DiRenzo, T. S. Tunstall, C. R. Muletz-Wolz, K. R. Zamudio, and K. R. Lips. 2016. Dead or alive? Viability of chytrid zoospores shed from live amphibian hosts. Diseases of Aquatic Organisms **199**:179-187.

Matthews, L., J. C. Low, D. L. Gally, M. C. Pearce, D. J. Mellor, J. A. Heesterbeek, M. Chase-Topping, S. W. Naylor, D. J. Shaw, S. W. Reid, G. J. Gunn, and M. E. Woolhouse. 2006. Heterogeneous shedding of Escherichia coli O157 in cattle and its implications for control. Proc Natl Acad Sci U S A **103**:547-552.

McGuire, L. P., H. W. Mayberry, and C. K. Willis. 2017. White-nose syndrome increases torpid metabolic rate and evaporative water loss in hibernating bats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology **313**:R680-R686.

Mitchell, K. M., T. S. Churcher, T. W. Garner, and M. C. Fisher. 2008. Persistence of the emerging pathogen Batrachochytrium dendrobatidis outside the amphibian host greatly increases the probability of host extinction. Proc Biol Sci **275**:329-334.

Muller, L. K., J. M. Lorch, D. L. Lindner, M. O'Connor, A. Gargas, and D. S. Blehert. 2013. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of Geomyces destructans. Mycologia **105**:253-259.

Munywoki, P. K., D. C. Koech, C. N. Agoti, N. Kibirige, J. Kipkoech, P. A. Cane, G. F. Medley, and D. J. Nokes. 2015. Influence of age, severity of infection, and co-infection on the duration of respiratory syncytial virus (RSV) shedding. Epidemiol Infect **143**:804-812.

Paull, S. H., S. Song, K. M. McClure, L. C. Sackett, A. M. Kilpatrick, and P. T. J. Johnson. 2012. From superspreaders to disease hotspots: linking transmission across hosts and space. Frontiers in Ecology and the Environment **10**:75-82.

Peterson, A. C., and V. J. McKenzie. 2014. Investigating differences across host species and scales to explain the distribution of the amphibian pathogen Batrachochytrium dendrobatidis. PLoS One **9**:e107441.

Plummer, I. H., C. J. Johnson, A. R. Chesney, J. A. Pedersen, and M. D. Samuel. 2018. Mineral licks as environmental reservoirs of chronic wasting disease prions. PLoS One **13**:e0196745.

Rushmore, J., D. Caillaud, L. Matamba, R. M. Stumpf, S. P. Borgatti, and S. Altizer. 2013. Social network analysis of wild chimpanzees provides insights for predicting infectious disease risk. J Anim Ecol **82**:976-986.

Scheele, B. C., D. A. Hunter, L. A. Brannelly, L. F. Skerratt, and D. A. Driscoll. 2017. Reservoir-host amplification of disease impact in an endangered amphibian. Conserv Biol **31**:592-600.

Searle, C. L., S. S. Gervasi, J. Hua, J. I. Hammond, R. A. Relyea, D. H. Olson, and A. R. Blaustein. 2011. Differential Host Susceptibility to Batrachochytrium dendrobatidis, an Emerging Amphibian Pathogen. Conservation Biology **25**:965-974.

Sheth, P. M., A. Danesh, A. Sheung, A. Rebbapragada, K. Shahabi, C. Kovacs, R. Halpenny, D. Tilley, T. Mazzulli, K. MacDonald, D. Kelvin, and R. Kaul. 2006. Disproportionately high semen shedding of HIV is associated with compartmentalized cytomegalovirus reactivation. J Infect Dis **193**:45-48.

Slater, N., R. M. Mitchell, R. H. Whitlock, T. Fyock, A. K. Pradhan, E. Knupfer, Y. H. Schukken, and Y. Louzoun. 2016. Impact of the shedding level on transmission of persistent infections in Mycobacterium avium subspecies paratuberculosis (MAP). Vet Res **47**:38.

Taylor, L. H., S. M. Latham, and M. E. Woolhouse. 2001. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci **356**:983-989.

Turner, W. C., K. L. Kausrud, W. Beyer, W. R. Easterday, Z. R. Barandongo, E. Blaschke, C. C. Cloete, J. Lazak, M. N. Van Ert, and H. H. Ganz. 2016. Lethal exposure: An integrated approach to pathogen transmission via environmental reservoirs. Scientific reports **6**:27311.

VanderWaal, K. L., and V. O. Ezenwa. 2016. Heterogeneity in pathogen transmission: mechanisms and methodology. Funct. Ecol. **30**:1606-1622.

Verant, M. L., M. U. Carol, J. R. Speakman, P. M. Cryan, J. M. Lorch, and D. S. Blehert. 2014. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. BMC physiology **14**:10.

Warnecke, L., J. M. Turner, T. K. Bollinger, J. M. Lorch, V. Misra, P. M. Cryan, G. Wibbelt, D. S. Blehert, and C. K. R. Willis. 2012. Inoculation of bats with European Geomyces destructans supports the novel pathogen hypothesis for the origin of white-nose syndrome. Proceedings of the National Academy of Sciences of the United States of America **109**:6999-7003.

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