

ARTICLE

Host infection and disease-induced mortality modify species contributions to the environmental reservoir

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Abstract

Environmental pathogen reservoirs exist for many globally important diseases and can fuel epidemics, influence pathogen evolution, and increase the threat of host extinction. Species composition can be an important factor that shapes reservoir dynamics and ultimately determines the outcome of a disease outbreak. However, disease-induced mortality can change species communities, indicating that species responsible for environmental reservoir maintenance may change over time. Here we examine the reservoir dynamics of *Pseudogymnoascus destructans*, the fungal pathogen that causes white-nose syndrome in bats. We quantified changes in pathogen shedding, infection prevalence and intensity, host abundance, and the subsequent propagule pressure imposed by each species over time. We find that highly shedding species are important during pathogen invasion, but contribute less over time to environmental contamination as they also suffer the greatest declines. Less infected species remain more abundant, resulting in equivalent or higher propagule pressure. More broadly, we demonstrate that high infection intensity and subsequent mortality during disease progression can reduce the contributions of high-shedding species to long-term pathogen maintenance.

KEYWORDS

emerging infectious disease, environmental pathogen reservoir, environmental transmission, host infectiousness, pathogen shedding, white-nose syndrome

INTRODUCTION

Emerging infectious diseases threaten efforts to conserve global biodiversity (Daszak et al., 2000; Fisher et al., 2012; Jones et al., 2008; Taylor et al., 2001). In some disease systems, pathogens may survive for long periods of time in the environment in the absence of a living host (Islam et al., 2020; Plummer et al., 2018; Turner et al., 2016). Pathogen persistence in the environment allows for transmission independent of infected hosts, can exacerbate disease impacts, and increase the risk of host extinction (Almberg et al., 2011; de Castro & Bolker, 2005; Hoyt et al., 2020; Mitchell et al., 2008). However, pathogen contamination in the environment is not homogeneous; rather, variation in the amount of pathogen in the environmental reservoir is likely to be driven by a complex process of pathogen shedding from hosts within the community leading 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 direct host contacts (Chase-Topping et al., 2008; Drenzo et al., 2014; Lawley et al., 2008; Sheth et al., 2006). Research has shown that variation in host shedding can be driven by differences in behavior (Godfrey, 2013; Rushmore et al., 2013; VanderWaal & Ezenwa, 2016), innate susceptibility (Gervasi et al., 2013; Searle et al., 2011), space use (Brooks-Pollock et al., 2014), and infection severity (Lloyd-Smith et al., 2005; Munywoki et al., 2015). In multi-host disease systems, variation in pathogen shedding, produced through community composition and species abundance, can play a key role in transmission dynamics (Esteban et al., 2009; 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 (Kilonzo et al., 2013; Lloyd-Smith et al., 2005; Paull et al., 2012). Species that have low rates of shedding, but are highly abundant, may contribute more to transmission than might be expected based on the per capita amount of pathogen they shed (Peterson & 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 (Kilpatrick et al., 2006; Lloyd-Smith et al., 2005). In addition, for some wildlife infectious diseases, infectiousness, shedding, and impacts are positively correlated (Brannelly et al., 2020; Langwig et al., 2016), such that hosts initially important for disease transmission, suffer from high disease-related mortality and become less important contributors to pathogen maintenance over time (Brannelly et al., 2018). Variation in pathogen shedding and how it influences disease dynamics is important for many disease systems

(Brooks-Pollock et al., 2014; Chase-Topping et al., 2008; Drenzo et al., 2014; Henaux & Samuel, 2011; Sheth et al., 2006; Slater et al., 2016), but how differences in host shedding scale to a community-level and influence the environmental reservoir are 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), which has had devastating effects on bat populations (Frick et al., 2015; Langwig et al., 2012, 2016). WNS exhibits seasonal infection dynamics that are driven by the environmental reservoir and host-pathogen ecology (Hoyt et al., 2021; Kailing et al., 2023; Langwig et al., 2021; Langwig, Frick, et al., 2015). *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 (Campbell et al., 2019; Hicks et al., 2021; Hoyt et al., 2015, 2020; Langwig, Frick, et al., 2015; Lorch et al., 2013). 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, Frick, et al., 2015). 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 often death (cumulative 95%–99% declines) (Hoyt et al., 2021; McGuire et al., 2017; Verant et al., 2014; Warnecke et al., 2013). During hibernation, susceptible bat species vary greatly in their infection intensities and three species have suffered declines that have exceeded 95% (Hoyt et al., 2020, 2021; Langwig et al., 2012, 2016). Species abundance also varies greatly within bat communities and during the epizootic (Frick et al., 2015; Langwig et al., 2012). Together, differences in pathogen shedding and species abundance may influence pathogen contamination in the environment.

The amount of *P. destructans* in the environment has been shown to increase after the first year of invasion (Hoyt et al., 2020) and contamination of the environmental reservoir has been linked to increased pathogen prevalence and loads for bats (Hoyt et al., 2020, 2021). As a result, bat mortality also increases with higher levels of environmental contamination (Hicks et al., 2021; Hoyt et al., 2018, 2020, 2023). However, the establishment of the environmental pathogen reservoir in these multi-host communities remains an important knowledge gap.

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 19 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 infection intensity and the amount of pathogen shed into the environment by each species present in the community. We hypothesized that bat species abundance would also play a key role in site-level contamination and environmental reservoir establishment, so we also explored how differential pathogen shedding among species and their abundance influences the propagule pressure within communities.

METHODS

Sample collection and quantification

We quantified *P. destructans* fungal loads on bats and from hibernacula substrate throughout bat hibernation sites in the midwestern United States. Samples were collected from 19 sites in Wisconsin, Illinois, and Michigan and each site included 3 years of pathogen data from invasion to establishment, which was collected over a 7-year period (Appendix S1: Table S1). Hibernacula were visited twice yearly, once during early hibernation (November to December) and once during late hibernation (March to April) to capture differences in infection dynamics and environmental contamination at the beginning and end of hibernation. During each visit, we counted the total number of bats within each site by species (*Eptesicus fuscus* [Big brown bat], *Myotis lucifugus* [Little brown bat], *Myotis septentrionalis* [Northern long-eared bat] and *Perimyotis subflavus* [Tricolored bat]; Appendix S1: Figure S1). We collected epidermal swab samples from bats within sites to quantify bat infection intensity (quantities of fungal DNA) and determine infection prevalence (Appendix S1: Table S2). Samples were collected using previously established protocols that consisted of rubbing a polyester swab dipped in sterile water over the muzzle and forearm of the bat five times (Hoyt et al., 2016; Langwig, Frick, et al., 2015).

To measure the amount of *P. destructans* shed into the environment we also collected environmental substrate swabs from beneath or directly adjacent to each hibernating bat (on hibernacula walls and ceilings) where they are in direct contact with the substrate. Samples collected in close proximity to bats have been shown to be strongly tied to the infection intensity of the bat (Hoyt et al., 2020; Langwig, Hoyt, et al., 2015). To capture independent site-level *P. destructans* environmental contamination, we collected swab samples as described above, but collected from the environment in locations more than 2 m away from

roosting bats, in areas where bats might roost. These samples were used to estimate the reservoir contamination across the site without targeting substrate used by specific bat species. These environmental samples were taken by swabbing an area of substrate equal to the length of a bat's forearm (36–40 mm) five times back and forth, as described previously (Langwig, Hoyt, et al., 2015). We preserved *P. destructans* DNA samples by storing all swabs in salt preservation buffer (RNAlater; Thermo Fisher Scientific) directly after collection. DNA was extracted from all samples with a modified Qiagen DNeasy Blood & Tissue Kit (Frick et al., 2015; Langwig, Hoyt, et al., 2015). The presence and quantity of *P. destructans* were determined by quantitative polymerase chain reaction (qPCR) (Muller et al., 2013).

To verify that fungal loads measured using qPCR accurately reflected viable fungal spores in the environment, that are able to infect a host, we collected additional substrate swab samples from a subset of locations that were paired with substrate swabs used for qPCR. These samples were cultured by streaking the substrate swab across a plate containing Sabouraud Dextrose Agar treated with chloramphenicol and gentamicin. The plates were stored at 4°C and colony forming units (CFUs) of *P. destructans* were quantified within 6 weeks of initial inoculation. We paired substrate samples analyzed using qPCR to determine $\log_{10}$ *P. destructans* loads for comparison with CFUs obtained from culture samples to validate viability. There was a significant relationship between the quantity of *P. destructans* DNA measured through qPCR and the number of CFUs (Appendix S1: Figure S2). This suggests that qPCR was a valid method to estimate the amount of *P. destructans* in the environment and supports that qPCR results are reflective of the number of infectious propagules in the environment and not relic DNA (Appendix S1: Figure S2).

All research was approved through Institutional Animal Care and Use Committee protocols: Virginia Polytechnic Institute: 17-180; University of California, Santa Cruz: Kilpm1705; Wisconsin Endangered/Threatened Species Permit 882 and 886; Michigan 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 and Endangered Species Permit TE64081B-1.

Data analysis

We separated the invasion stage into two distinct categories: “invasion” which included the first year the pathogen arrived, as described previously (Hoyt et al., 2020; Langwig, Hoyt, et al., 2015), and “establishment” which included the second and third years of *P. destructans*

presence in a site when bat species declines begin to occur, which corresponds to the epidemic stage as has been previously noted (Hoyt et al., 2020; Langwig, Hoyt, et al., 2015). We used these stages to capture pathogen shedding 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 bat species and the amount each bat species shed into the environment. We used mixed effects models with $\log_{10}$ environmental fungal load collected under each bat as our response variable, bat species as our predictor, and site as a random effect for both invasion stages. Tables are reported for model output by including *M. septentrionalis* as the reference level for the invasion stage and *M. lucifugus* during the established stage. These were chosen because they had the highest levels of shedding in the respective stages and demonstrated contrasts with all other species. 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. For this analysis we reported the table for the model output with *E. fuscus* as the reference level because it has the lowest levels of infection intensity and demonstrates contrasts to the other species. We examined differences in infection prevalence by bat species using a generalized linear mixed effects model with a binomial distribution and a logit link with species as our predictor, bat infection status (0|1) as our response, and site as a random effect. To examine within-host variation in pathogen shedding, we calculated the coefficient of variation of pathogen shedding for each species in both invasion stages.

To examine how differences in bat infection intensity contributed to contamination of the environment under each individual, we used a linear mixed effects model to explore the relationship between the infection intensity of each bat and the amount of 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 because 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 report the output of our model using estimated marginal means of linear trends to highlight the support for the relationship between bat fungal loads and environmental fungal loads beneath bats for all species and to display multiple species slope contrasts. To examine within-host variation in infection intensity, we

calculated the coefficient of variation for infection intensity for each species in both invasion stages.

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

Finally, we calculated differences in propagule pressure among bat species by multiplying pathogen prevalence by bat 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 shed into the environment by each species in each site. We analyzed the propagule pressure calculations using a generalized linear mixed effects model with a negative binomial distribution to account for dispersed population abundance counts with an interaction between species as our predictor and propagule pressure as our response for each invasion stage. We performed this analysis for invasion (year 0) and establishment (years 1–2) during late hibernation to investigate how pathogen pressure may differ across stages of pathogen invasion when bats are heavily shedding into the environment. For the invasion year, we report the table for model output with *M. septentrionalis* as the reference level as it has the highest level of propagule pressure compared with the other species. For the established stage we report using estimated marginal means of our generalized linear mixed effects model to highlight contrasts between multiple species. To assess if propagule pressure was a suitable metric for pathogen invasion of the environmental reservoir, we examined the relationship between site-level environmental contamination, as described above, and sum propagule pressure for each site using a linear mixed effects model with site included as a random effect.

All analyses were conducted in R v.4.2.1 (R Core Team, 2022). Mixed effects models were run using the package lme4 (Bates et al., 2015) except for analyses using a negative binomial distribution which were run using the glmmTMB package (Brooks et al., 2017). The reported estimated marginal means and estimated marginal means of linear trends were generated using the emmeans package (Lenth, 2022; Searle et al., 1980).

RESULTS

We found that pathogen shedding (the amount of pathogen detected under bats) and infection varied both within and among the four species present in the community (Figure 1, Appendix S1: Figure S3). During initial pathogen invasion into bat communities, we found that, on average, individual *M. septentrionalis* (Figure 1a; Appendix S1: Table S4; intercept = -3.72 ± 0.33) contributed more pathogen into the environment than *E. fuscus* (coefficient = -0.88 ± 0.33 , $p = 0.009$), *M. lucifugus* (coefficient = -0.52 ± 0.22 , $p = 0.02$), and *P. subflavus* (coefficient = -0.86 ± 0.35 , $p = 0.02$), and also had the greatest within-species variability (Appendix S1: Figure S3A). In the established stage

(Figure 1b; Appendix S1: Table S5), we found support for shifts in species shedding patterns with higher shedding in *M. lucifugus* (intercept = -3.41 ± 0.12) than *E. fuscus* (coefficient = -0.75 ± 0.14 , $p < 0.0001$) and *P. subflavus* (coefficient = -0.65 ± 0.13 , $p < 0.0001$), but similar shedding to *M. septentrionalis* (coefficient = -0.33 ± 0.24 , $p = 0.17$).

Our results showed consistent support that host infection intensity predicted the amount of pathogen shed into the environment for all species (Figure 2c; Appendix S1: Table S8; host infection and shedding relationship: *M. lucifugus* slope = 0.38 ± 0.05 , $p < 0.0001$; *E. fuscus* slope = 0.33 ± 0.07 , $p < 0.0001$; *M. septentrionalis* slope = 0.41 ± 0.10 , $p < 0.0001$; *P. subflavus* slope = 0.33 ± 0.08 , $p = 0.0001$). Importantly, we found no support for

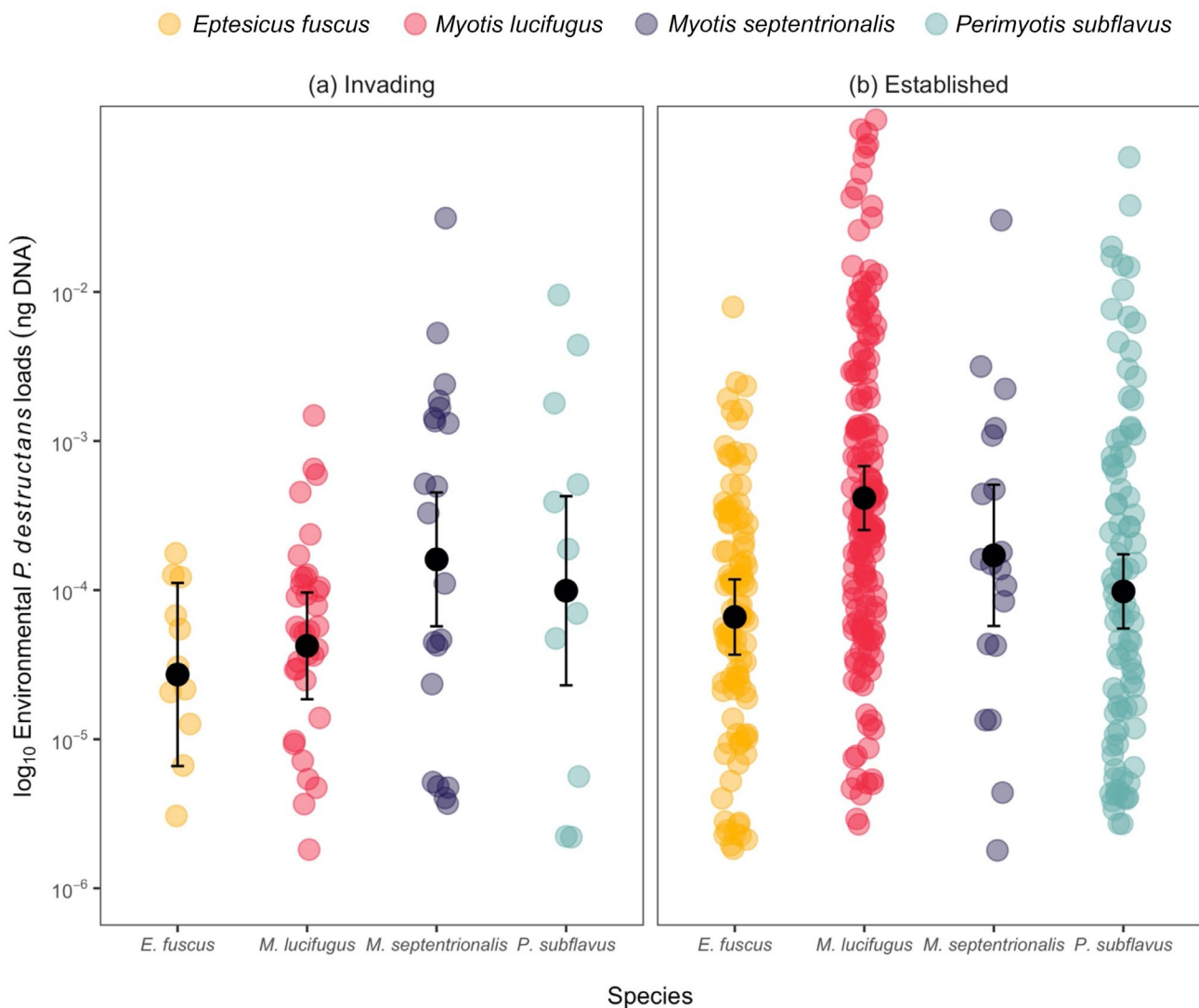


FIGURE 1 Differences in pathogen shedding into the environment among invading and established invasion stages. $\log_{10}$ *Pseudogymnoascus destructans* environmental loads (in nanograms DNA) among species during pathogen invasion (a) and establishment (b) in late hibernation. Each point represents the $\log_{10}$ environmental *P. destructans* loads from under an individual bat. Black points represent the estimated mean and bars indicate $\pm$ SE for each species. Samples collected that were beyond the limits of detection were set to $10^{-5.75}$ $\log_{10}$ *P. destructans* loads (in nanograms DNA).

differences among species in the slope between environmental pathogen shedding and host infection intensity (Figure 2c; Appendix S1: Table S8; all $p > 0.05$), suggesting that differences in shedding were primarily driven by the infection intensity of individual bats. *Eptesicus fuscus* (intercept = -3.37 ± 0.16) had significantly lower infection intensity than all other species within the community (Figure 2b; Appendix S1: Table S7; *M. lucifugus* coefficient = 1.38 ± 0.15 , $p < 0.0001$; *M. septentrionalis* coefficient = 1.08 ± 0.21 , $p < 0.0001$; *P. subflavus*

coefficient = 1.30 ± 0.17 , $p < 0.0001$), but had the greatest amount of variation for within-species infection intensity (Appendix S1: Figure S3B). 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; Appendix S1: Table S6; *E. fuscus* coefficient = -0.79 ± 0.31 , $p < 0.01$; *M. septentrionalis* coefficient = -0.83 ± 0.35 , $p = 0.02$; *P. subflavus* coefficient = -1.10 ± 0.24 , $p < 0.0001$).

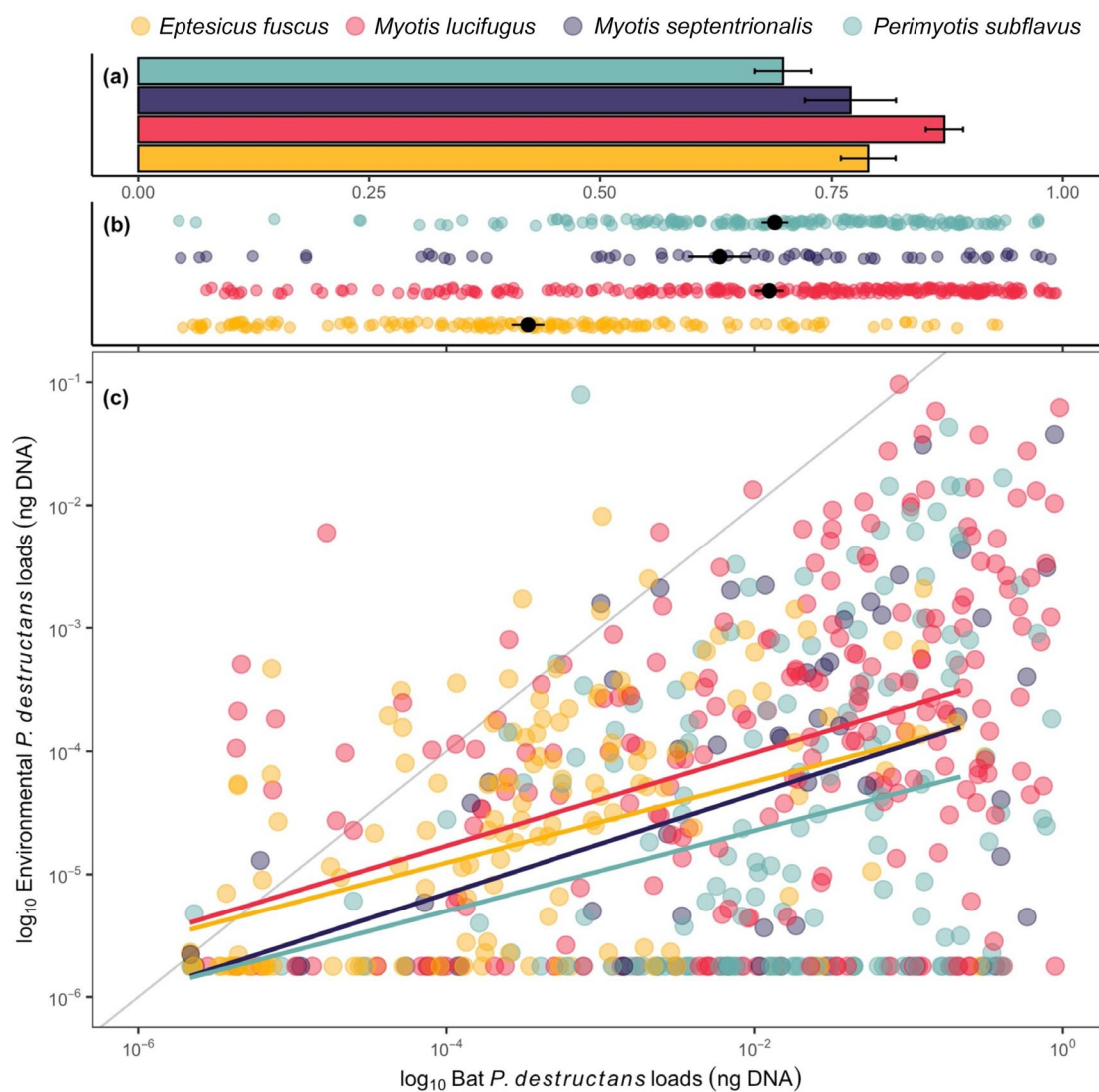


FIGURE 2 Bat infection prevalence, intensity, and the relationship between host infection and pathogen shedding. For all panels, bat species are displayed by color. Black points indicate mean bat fungal loads by species and bars represent $\pm$ SE. (a) Pathogen prevalence for each species during pathogen invasion in late hibernation. (b) Bat $\log_{10}$ *Pseudogymnoascus destructans* loads (in nanograms DNA) for each species during pathogen invasion in late hibernation. (c) The relationship between $\log_{10}$ *P. destructans* loads (in nanograms DNA) on an individual bat and the amount of $\log_{10}$ *P. destructans* (in nanograms DNA) in the environment directly underneath individuals during pathogen invasion and establishment in late hibernation. Colored lines show the relationship between $\log_{10}$ bat *P. destructans* loads and $\log_{10}$ environmental *P. destructans* loads fit with a linear mixed effects model. The gray solid line shows the 1:1 line, and points along this line would indicate that the amount of *P. destructans* on bats was equivalent to the amount shed into the environment. Each individual bat is represented by a point. Samples collected that were beyond the limits of detection were set to $10^{-5.75}$ $\log_{10}$ *P. destructans* loads (in nanograms DNA).

In addition to differences in infection intensity and prevalence, 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; Appendix S1: Table S9; *E. fuscus* coefficient = -0.65 ± 0.24 , $p < 0.01$; *M. septentrionalis* coefficient = -0.60 ± 0.21 , $p < 0.01$; *P. subflavus* coefficient = -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; Appendix S1: Table S10; *E. fuscus* coefficient = -0.54 ± 0.16 , $p < 0.001$; *M. septentrionalis* coefficient = -0.76 ± 0.14 , $p < 0.0001$; *P. subflavus* coefficient = -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; Appendix S1: Tables S9 and S10). Furthermore, host abundance was important in influencing the degree of site-level environmental pathogen contamination (Appendix S1: Figure S4 and Table S13; *M. lucifugus* slope = 0.25 ± 0.11 , $p = 0.03$).

Finally, when we combined infection prevalence, species abundance, and pathogen shedding into the metric of propagule pressure, we found that during the first year of invasion, *M. septentrionalis* (Figure 4a; Appendix S1: Table S11; intercept = 7.44 ± 1.05) had higher propagule pressure than *P. subflavus* (coefficient = -3.17 ± 1.25 , $p = 0.01$) and *E. fuscus* (-4.28 ± 1.21 , $p < 0.001$), but not *M. lucifugus* (coefficient = 0.87 ± 1.03 , $p = 0.39$). In the years following invasion, once *P. destructans* was established, *M. lucifugus* (Figure 4b; Appendix S1: Table S12; coefficient from estimated marginal mean = 10.75 ± 0.51) had consistently higher propagule pressure and contributed more pathogen to the environmental reservoir than all other species (Figure 4b; Appendix S1: Table S12; *M. lucifugus*–*M. septentrionalis* contrast = 4.24 ± 0.79 , $p < 0.001$; *M. lucifugus*–*E. fuscus*

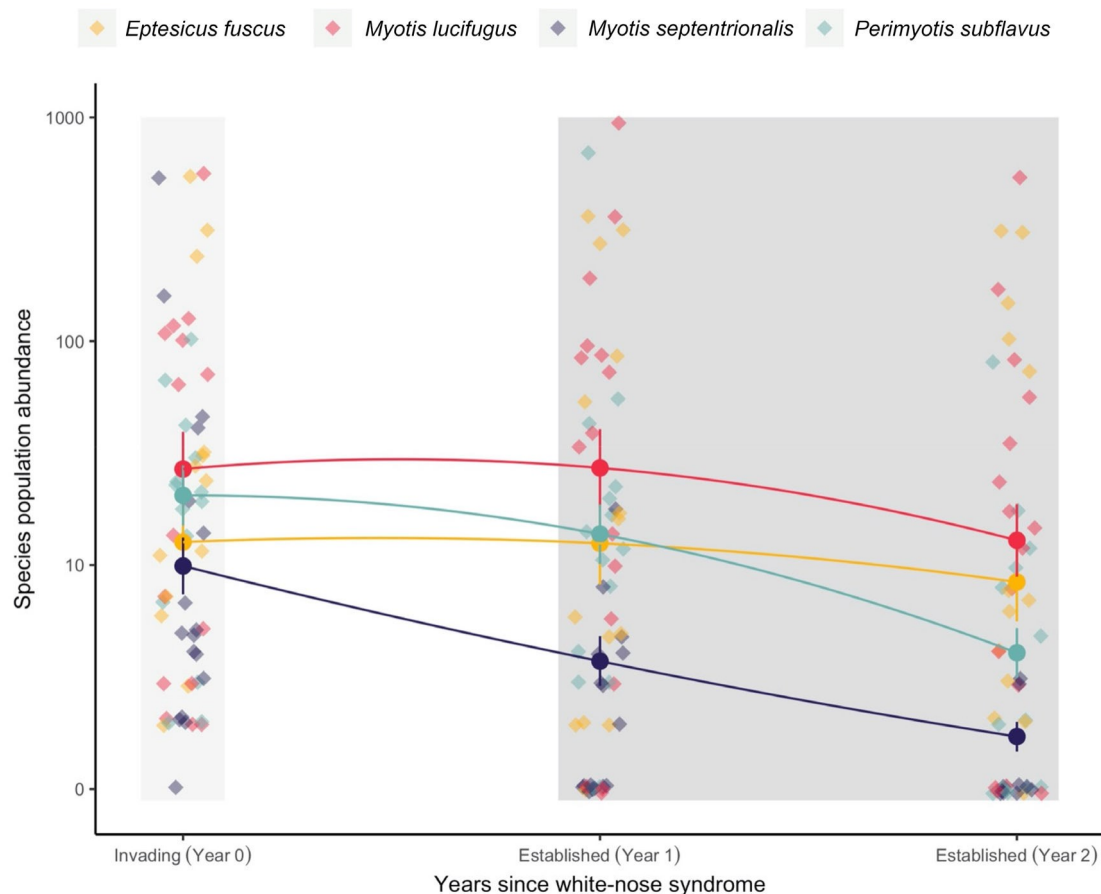


FIGURE 3 Changes in host abundance between invading and established years. Species within the community are differentiated by color and points represent a species population at an individual site. Species population abundance in the invasion (light gray box) and established years (dark gray box) in late hibernation within each site. Circular points indicate the mean abundance for each year and bars denote $\pm$ SE. For best data visualization, three data points have been excluded from the figure (1110 *M. lucifugus* and 1985 *P. subflavus* during the invading stage, and 3154 *M. lucifugus* during the established stage).

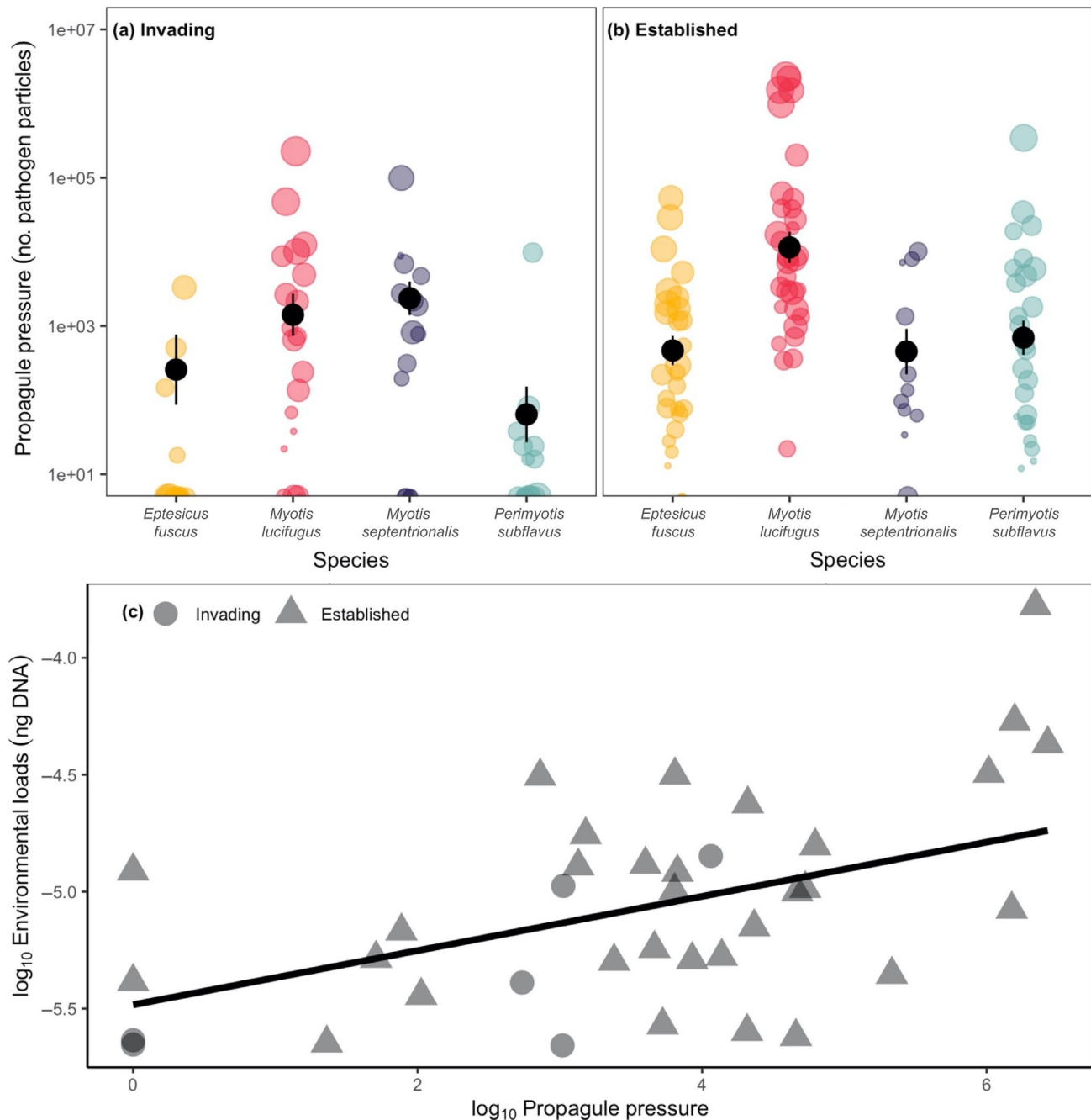


FIGURE 4 Differences in propagule pressure among species during invading and established stages and the relationship between propagule pressure and environmental contamination. (a, b) Propagule pressure (number of pathogen particles) by species during late hibernation in (a) invading and (b) established stages. Colored points represent populations of species within a site, black points represent the mean and bars indicate $\pm$ SE for each species. Point 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 >2 m 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 line shows the relationship between $\log_{10}$ propagule pressure and $\log_{10}$ environmental P. destructans loads fitted with a linear mixed effects model. 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 $p = 0.002$).

contrast = 3.84 ± 0.56 , $p < 0.0001$; *M. lucifugus*–*P. subflavus* contrast = 2.46 ± 0.54 , $p = 0.0001$). *M. septentrionalis* (Figure 4b; Appendix S1: Table S12; coefficient from estimated marginal mean = 6.52 ± 0.81) no longer

contributed more than *P. subflavus* (*M. septentrionalis*–*P. subflavus* contrast = -1.78 ± 0.85 , $p = 0.16$) or *E. fuscus* (*M. septentrionalis*–*E. fuscus* contrast = -0.39 ± 0.83 , $p = 0.97$) due to their rarity following disease-induced

declines (Figure 4). Mean environmental contamination in areas greater than 2 m from bats increased with total propagule pressure calculated at a site level (summed propagule pressure among species), suggesting the measure of propagule pressure accurately captures environmental contamination levels (Figure 4c; the relationship between environmental contamination and propagule pressure intercept = -5.48 ± 0.14 , slope = 0.12, $p = 0.002$).

DISCUSSION

Variations in pathogen shedding, and subsequent environmental transmission can have dramatic impacts on community-level disease outcomes. Our findings demonstrate how increases in host infection intensity can lead to increased pathogen shedding of *P. destructans* (Figure 2c), which is consistent with research in other systems (Chase-Topping et al., 2008; Drenzo et al., 2014; Lloyd-Smith et al., 2005; Maguire et al., 2016; Matthews et al., 2006; Munywoki et al., 2015; VanderWaal & Ezenwa, 2016). In addition, we demonstrated that changes in host abundance and differences in infection prevalence modified the species contributions to the environmental reservoir, which varied over time (Figures 2a, 3 and 4). Most importantly, while high infection intensity initially leads to high pathogen shedding for one species (Figure 2c), this also leads to elevated mortality (Langwig et al., 2016) and local extirpations (Appendix S1: Figure S1). In contrast, species with lower infection intensity and greater variation within species for infection intensity, such as *E. fuscus*, had reduced impacts (Figure 2b; Appendix S1: Figures S1 and S3B) (Langwig et al., 2016) and may be more important in pathogen maintenance than high shedding species that suffer severe mortality. Therefore, we identified a shift in the species responsible for the greatest environmental reservoir contamination between invasion stages, suggesting that contributions to the reservoir are a dynamic process that varies over time (Figure 4).

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 (Figure 2c; Appendix S1: Figure S5). Instead, the prevalence and intensity of infection, which did vary among species, influenced how much pathogen was shed on average per individual (Figure 2a–c). Some species such as *E. fuscus* had low-intensity infections despite moderate prevalence, while other species, *M. septentrionalis* and *M. lucifugus*, were much more heavily infected and shed more pathogen into the environment (Figure 2b,c). In addition, we saw shifts in host infection intensity between

invasion and establishment stages (Figure 2b) leading to shifts in shedding (Figure 1a,b). An increase in host infection prevalence between invasion stages has been previously described by Hoyt et al., 2020, where increases in pathogen contamination during the initial invasion stage, resulted in rapid reinfection and higher exposure doses in subsequent years. This influenced fungal burdens on bats and eventually caused population level declines (Hoyt et al., 2020; Langwig et al., 2021; Langwig, Hoyt, et al., 2015).

Disease-caused declines in several bat species (Figure 3), which have been shown to be strongly linked with the intensity of infection (Hopkins et al., 2021; Langwig et al., 2017), resulted in large changes in species abundance and community composition (Appendix S1: Figure S1). We found support that species abundance predicted site-level contamination (Appendix S1: Figure S4), and this reinforced the need to account for abundance when determining species-level contributions 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. We found that environmental contamination in the sampled locations that were not associated with an individual bat increased as our calculated propagule pressure metric increased (Figure 4c), suggesting that propagule pressure is an accurate way of measuring species contributions to the environmental reservoir.

During the initial invasion of *P. destructans*, both *M. lucifugus* and *M. septentrionalis* had higher propagule pressure than other species present (Figure 4a), which was not apparent through the examination of only pathogen shedding (Figure 1a). The high infection intensity of *M. septentrionalis* resulted in a large contribution to the initial establishment of the environmental reservoir despite their low relative abundance (Figures 2b and 3). However, in subsequent years, as this species declined precipitously (Figure 3; Appendix S1: Figure S1), their contribution was greatly reduced and equivalent to less infected species (e.g., *E. fuscus*, Figure 4b).

Our results suggest that host abundance, infection prevalence, and infection intensity (which influences shedding into the environment) are collectively required to describe species contributions to environmental pathogen contamination. For example, while *E. fuscus*, *M. septentrionalis*, and *P. subflavus* eventually contributed similar propagule pressures during the established invasion stage (Figure 4b), this was likely to have been driven by different factors that need to be considered when evaluating their influence on environmental contamination.

Eptesicus fuscus had low infection intensity and subsequently low declines, but had moderate pathogen prevalence (Figure 2a,b) and moderate abundance (Figure 3), which elevated its contribution. *Perimyotis subflavus* had high infection intensity, but reduced prevalence compared with the other heavily infected species (Figure 2a,b) and was only present in moderate abundances (Figure 3). Finally, *M. septentrionalis* was heavily infected (Figure 2b), but at low abundance across communities (Figure 3), which reduced its overall importance in the years following the initial pathogen invasion (Figure 4b). *Myotis lucifugus* maintained high propagule pressure through the invasion and establishment of *P. destructans* (Figure 4a,b), suggesting that the presence of *M. lucifugus* within a community will result in the rapid establishment of *P. destructans* in the environment, and maintenance of environmental contamination within a site over time (Appendix S1: Figure S4). We also found that within-species variation in infection and pathogen shedding changed across invasion stages by species (Appendix S1: Figure S3) which may be influenced by numerous factors (e.g., the timing of infection, microclimate use, etc.) and warrants further investigation. Our results highlighted numerous factors that must be considered to evaluate the influence of pathogen shedding on environmental reservoir maintenance, subsequent environmental transmission, and how this can change dynamically across invasion stages.

Understanding environmental reservoir dynamics is crucial for many globally important disease systems. Discerning how variation across species contributes to the 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 demonstrated that the establishment and maintenance of the environmental reservoir is strongly influenced by variation in pathogen prevalence, infection intensity, 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 demonstrated that multiple factors of species variability can scale to influence environmental reservoir dynamics within communities.

AUTHOR CONTRIBUTIONS

Nichole A. Laggan and Joseph R. Hoyt wrote the original draft of the manuscript. Joseph R. Hoyt, Kate E. Langwig, and A. Marm Kilpatrick designed the methodology. Nichole A. Laggan, Joseph R. Hoyt,

Kate E. Langwig, A. Marm Kilpatrick, J. Paul White, Heather M. Kaarakka, Jennifer A. Redell, John E. DePue, William H. Scullon, and Joseph Kath collected the data. Nichole A. Laggan conducted the laboratory experiment. Jeffrey T. Foster and Katy L. Parise supervised and performed sample testing. Nichole A. Laggan analyzed the data with assistance from Kate E. Langwig and Joseph R. Hoyt. All authors contributed critically to the draft revision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are available in Dryad from Laggan et al. (2023) at <https://doi.org/10.5061/dryad.ns1rn8pzz>.

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SUPPORTING INFORMATION

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

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