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Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome

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Seasonal patterns in pathogen transmission can influence the impact of disease on populations and the speed of spatial spread. Increases in host contact rates or births drive seasonal epidemics in some systems, but other factors may occasionally override these influences. White-nose syndrome, caused by the emerging fungal pathogen *Pseudogymnoascus destructans*, is spreading across North America and threatens several bat species with extinction. We examined patterns and drivers of seasonal transmission of *P. destructans* by measuring infection prevalence and pathogen loads in six bat species at 30 sites across the eastern United States. Bats became transiently infected in autumn, and transmission spiked in early winter when bats began hibernating. Nearly all bats in six species became infected by late winter when infection intensity peaked. In summer, despite high contact rates and a birth pulse, most bats cleared infections and prevalence dropped to zero. These data suggest the dominant driver of seasonal transmission dynamics was a change in host physiology, specifically hibernation. Our study is the first, to the best of our knowledge, to describe the seasonality of transmission in this emerging wildlife disease. The timing of infection and fungal growth resulted in maximal population impacts, but only moderate rates of spatial spread.

1. Introduction

Seasonality in pathogen dynamics influences the impact of disease on populations [1], and can enhance pathogen spread [2]. If the timing of peak infectiousness occurs when populations are highly mobile (e.g. during migration or dispersal), spatial spread will be maximized [3–6]. The timing of seasonal mortality can also influence disease impact: impacts will be additive and largest if there is seasonal density-dependent population regulation, and mortality from disease occurs after most density-dependent mortality [7–9]. For example, if birth pulses drive seasonal epidemics, then disease impacts may compensate for naturally occurring density-dependent mortality and dispersing infected young could lead to rapid spatial spread. Understanding the patterns and drivers of seasonality increases our understanding of disease impacts on populations and the rate of spread of invading pathogens.

Five mechanisms driving seasonality in transmission have been proposed for directly transmitted pathogens, and these may act independently or in concert with each other to drive transmission. First, sociality varies seasonally for many species and alters transmission by increasing or decreasing contact rates [5,10–12]. Mating frequently increases infectious contacts, whereas territoriality can decrease contact rates among hosts [2,5,13]. Second, seasonal birth pulses can increase

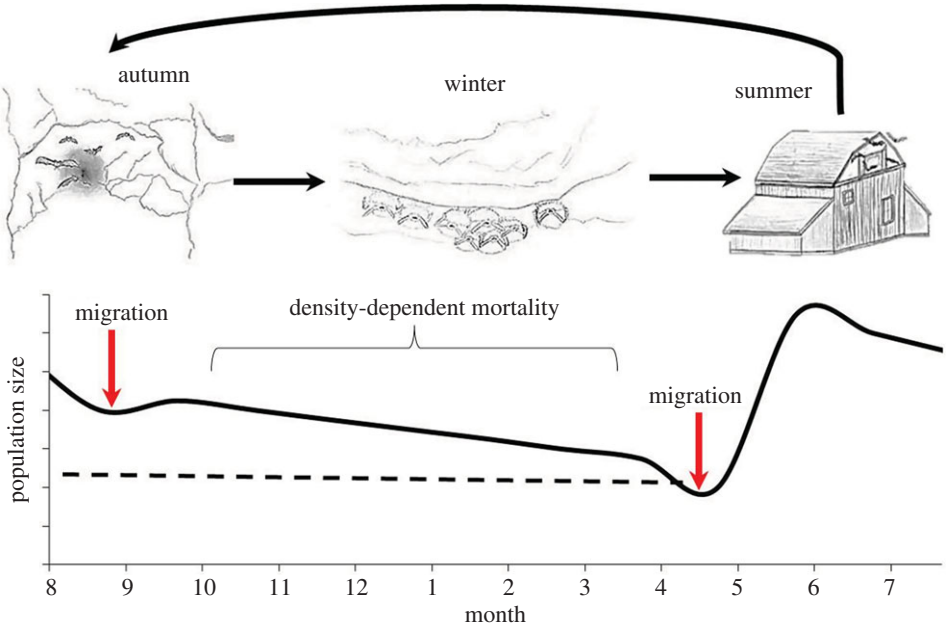

Figure 1. Seasonal life-history patterns of temperate hibernating bats and hypothesized trends in population size in the absence of disease. In the summer, female bats form single-species maternity colonies in human structures, trees or rock crevices, where they give birth to two (*Eptesicus fuscus*) or one (all other species) pup. During autumn, bats often mate at swarms in and around hibernation sites and use torpor intermittently. In winter, bats of multiple species enter prolonged periods of torpor (hibernation) inside hibernacula. Disease mortality that reduces populations down to the dashed black line may be compensatory mortality, whereas population decreases below the dashed black line will be additive with other sources and will lead to fewer reproducing individuals in summer. (Online version in colour.)

transmission by creating an influx of susceptible individuals into an otherwise mostly immune population [14–16]. Third, seasonal changes in host habitat use can influence the transmission and persistence of pathogens by altering contact with infective stages in the environment [17,18]. Fourth, climatic changes may influence the persistence of pathogens outside hosts, and can also change host behaviour, which may work in concert with other factors [19]. Finally, seasonal differences in host immune function also can alter growth of pathogens within hosts [20,21]. Hibernating bat species affected by the novel fungal disease, white-nose syndrome (WNS), exhibit seasonal differences in host physiology, habitat use and sociality, presenting a powerful opportunity to empirically test the influence of these factors on seasonal disease dynamics (figure 1).

WNS is caused by the fungus, *Pseudogymnoascus destructans* and emerged in North America in the winter of 2006 [22–24]. This disease currently threatens several hibernating bat species with extinction [25], has killed millions of individuals and has resulted in the collapse of little brown bat populations across eastern North America [25,26]. Morbidity and mortality appear to be linked to fungal invasion of tissues that disrupt bat physiology, and lead to dehydration and increased arousals [22] that deplete fat reserves [27]. *Pseudogymnoascus destructans* grows best at the cool temperatures at which many bats hibernate, with optimal fungal growth between 7°C and 16°C, and no growth above 20°C [28]. Hibernacula are known reservoirs for the fungus [29,30], and *P. destructans* can survive for long periods in the absence of bats [31].

We quantified seasonal patterns of *P. destructans* infection and pathogen loads (infection intensity) to examine the relative influences of colony size, birth pulses, habitat use and physiology on transmission (the change in prevalence over time) and pathogen amplification (the increase in infection intensity) on hosts. If colony size is the primary mechanism driving transmission, we predict that prevalence would increase faster in seasons when colonies are larger. A yearly birth pulse may be important in driving transmission if either colony size or acquired immunity is important, and would result in sharp increases in prevalence in mid-summer after females have given birth [32]. If contact with an environmental reservoir drives transmission, then prevalence would be predicted to increase significantly as bats contact infected environments. Hibernacula, where bats swarm and spend the winter are known reservoirs for the fungus [30], and it is unknown whether differences in seasonal exposure in different habitats may drive seasonal patterns of WNS. Finally, changes in host physiology, in particular, hibernation (when bats' lower their body temperatures for sustained periods to conditions where *P. destructans* can grow [32,33]) may drive seasonal transmission. Increases in infectiousness as the fungus grows on hibernating bats may drive winter transmission, with loads increasing throughout the winter. We examined the influence of these mechanisms on the seasonality of WNS by quantifying patterns of infection prevalence and pathogen loads on six bat species over the year at 30 hibernacula and maternity sites spanning much of the current distribution of the fungus.

2. Material and methods

(a) Study sites

We sampled bats at 30 hibernacula and maternity colonies in New York, Vermont, Massachusetts, Virginia, New Hampshire and Illinois where *P. destructans* had been present for at least 1 year. We sampled bats in one or more periods of their life cycle which roughly correspond with seasons, including early autumn swarm (late August to mid-September), late autumn swarm (late September to late October), early winter hibernation (November and December), late winter hibernation (March and early April), early summer maternity (May) and late summer maternity (late June to July; figure 2). Phenology varies by latitude; bats in southern sites have shorter hibernation seasons, swarm later in the autumn and return to maternity colonies earlier [34]. Winter colonies included one to six bat species: little brown myotis (*Myotis lucifugus*), northern long-eared myotis

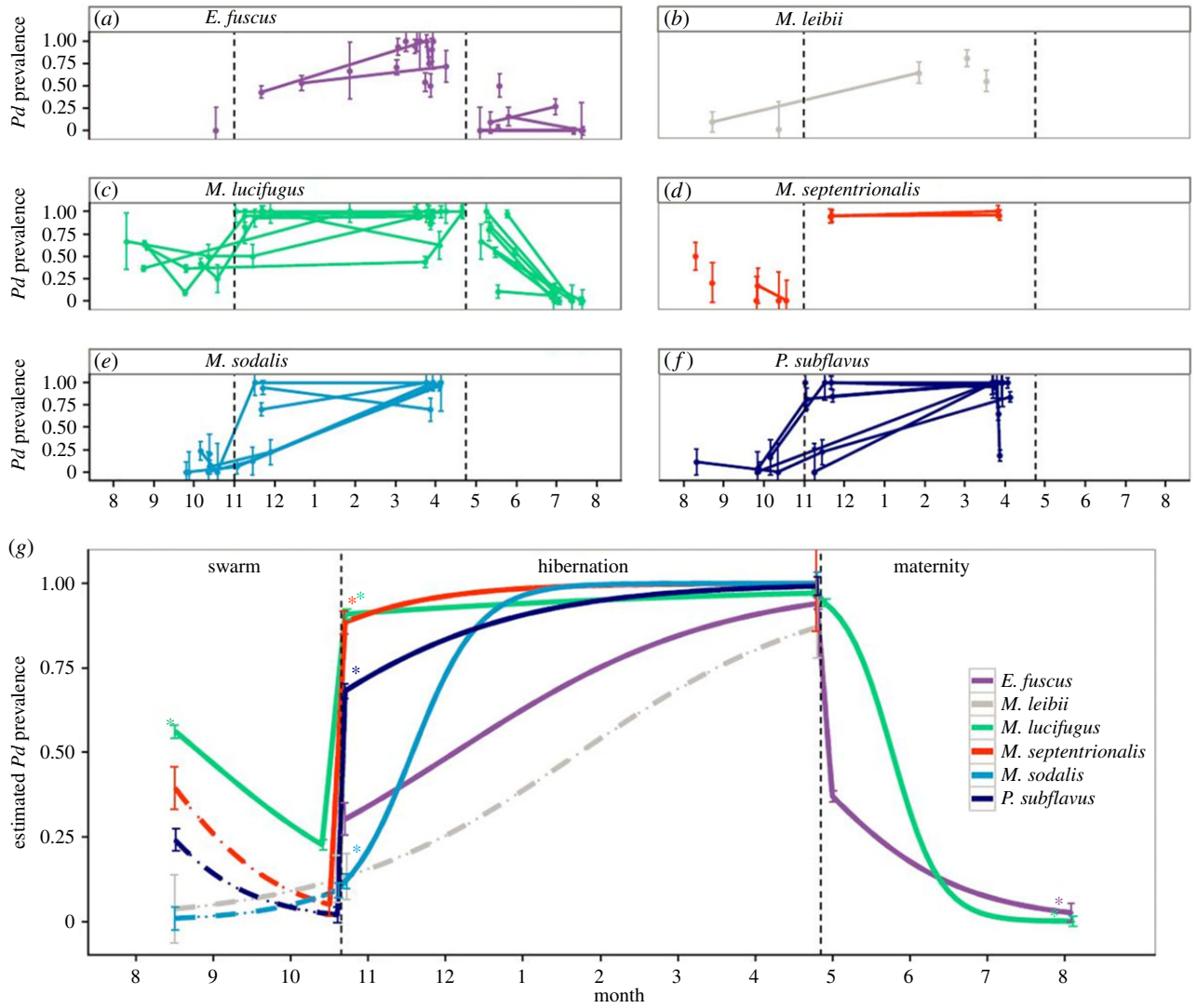

Figure 2. (*a–f*) Seasonal prevalence (±1 s.e.) of *Pseudogymnoascus destructans* for six species across all sites. Lines connect estimates from the same site. (*g*) Model predicted prevalence (± standard error of predicted mean) of *P. destructans* for six species from autumn to summer. Dashed lines indicate species that were poorly sampled across that season. Asterisks denote time points in which prevalence was significantly different ($p < 0.0001$ for all comparisons) than the previous season. Vertical dashed lines in all panels divide seasons (autumn swarm, winter hibernation, summer maternity.). (Online version in colour.)

(*Myotis septentrionalis*), eastern small-footed myotis (*Myotis leibii*), Indiana myotis (*Myotis sodalis*), tri-colored bats (*Perimyotis subflavus*) and big brown bats (*Eptesicus fuscus*). We sampled two species in the summer that roost in human dwellings (e.g. barns), little brown myotis and big brown bats. Other species' maternity sites are difficult to locate and therefore were not sampled. Hibernacula counts were conducted primarily during late winter visits. At maternity colonies, we conducted emergence counts twice to determine total colony size: once before, and once after the young of the year had become volant. We followed field hygiene protocols in accordance with United States Fish & Wildlife Service WNS Decontamination Guidelines, and individual state recommendations [35]. All research was conducted under protocol number 11-022 approved by the IACUC of Boston University.

(b) Sample collection and analysis

We sampled a mean of 12 individuals (±0.26, range: 5–80) of each species present at each site to determine infection prevalence and *P. destructans* infection intensity. We sampled bats by dipping a sterile polyester swab in sterile water to moisten it and then rubbing the swab five times across both the forearm and muzzle of a bat. Swabs were stored in RNAlater for preservation until extraction. Samples were tested for presence and quantity of *P. destructans* DNA using real-time PCR [36]. We quantified the amount of *P. destructans* based on the cycle threshold (C_t) value to estimate the fungal load on each bat, with a C_t cut-off of 40 cycles. The standard curve for quantification was generated using genomic DNA from *P. destructans* ATCC MYA-4855 quantified with the Quant-IT PicoGreen double-stranded DNA assay kit (Life Technologies, Carlsbad, CA) in conjunction with a DynaQuant 300 fluorometer (Harvard Bioscience, Inc., Holliston, MA). Serial dilutions of the DNA from 10 ng to 1000 fg were prepared and analysed with IGS qPCR, resulting in a significant curve from 17.33 to 30.74 C_t (ng of *P. destructans* $= -3.348{*}C_t + 22.049$, $r^2 = 0.986$).

(c) Statistical analysis

We used generalized linear-mixed models (function glmer in package lme4 [37] in R v. 3.02 [38]) to compare changes in *P. destructans* prevalence and intensity for each species over time. To measure the change in prevalence or load over time, we used a modified time axis where 0 represented the first day of autumn swarm sampling and expressed time (in units of partial months). We examined differences in seasonal transmission (the change in prevalence over time) and changes in infection intensity as the fixed effect of time interacting with season (autumn, winter, spring) to estimate a slope (representing the transmission rate or fungal growth rate) and intercept for each season. We included time nested within site as a random effect to allow for variation among sites in transmission rate or fungal growth rate on bats.

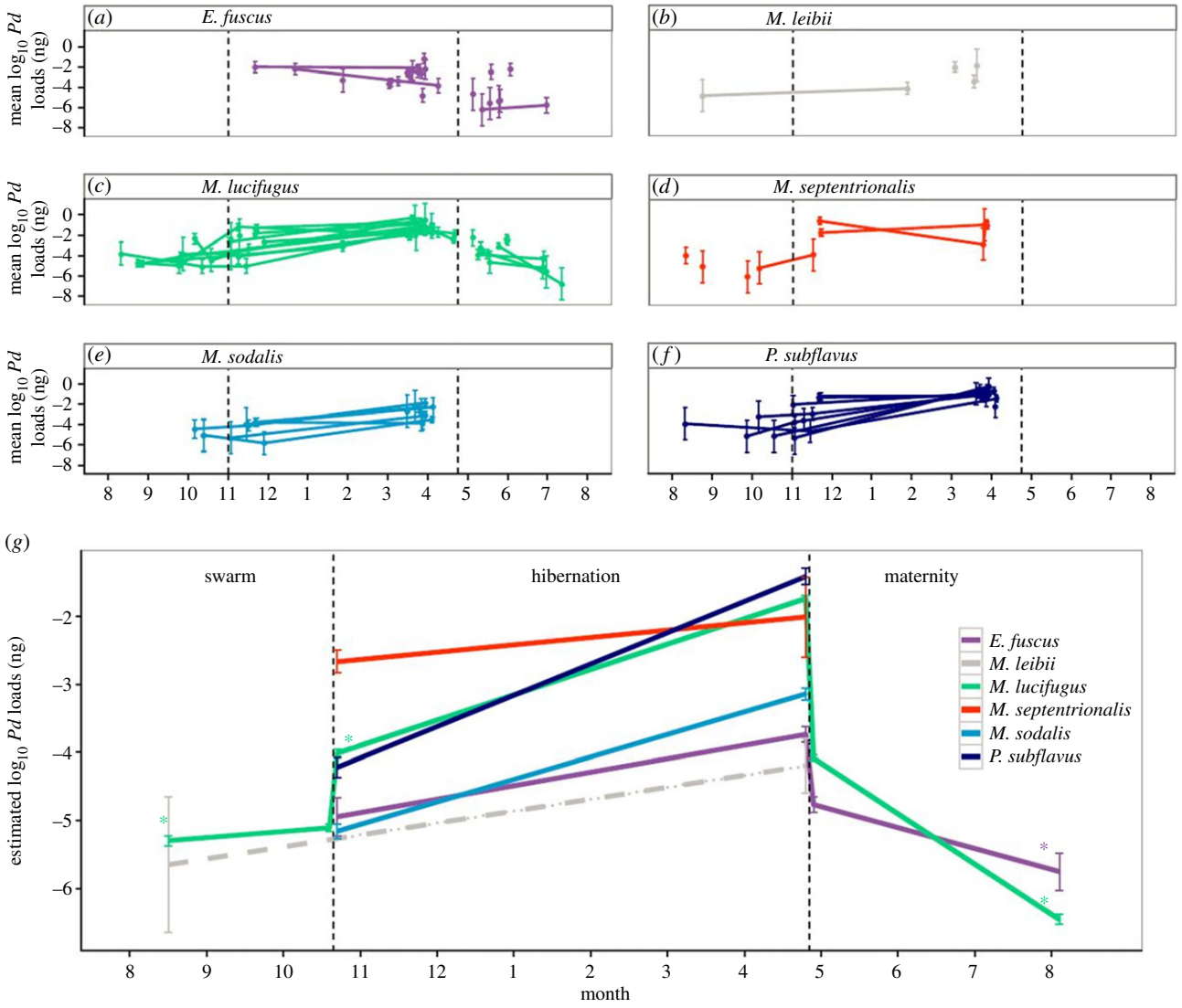

Figure 3. (*a–f*) Seasonal loads (±1 s.e.) of *Pseudogymnoascus destructans* for six species across all sites. Lines connect estimates from the same site. (*g*) Model predicted $\log_{10}$ *P. destructans* loads (± standard error of predicted mean) among species of *P. destructans* for six species from autumn to summer. Dashed lines indicate species that were poorly sampled across that season. Asterisks denote time points in which loads were significantly different ($p < 0.0001$ for all comparisons) than the previous season. Vertical dashed lines in all panels divide seasons (autumn swarm, winter hibernation, summer maternity). (Online version in colour.)

3. Results

We sampled a total of 1512 bats of six species at 20 hibernacula and 717 bats of two species at 10 maternity sites where *P. destructans* had been present for at least 1 year. Infection prevalence in early autumn when bats returned to infected hibernacula to swarm was between 5% and 50% for the six species (figure 2). Surprisingly, prevalence decreased during the autumn for *M. lucifugus* at all three sites where this species was sampled multiple times, and more limited data for other species also suggested a decline in prevalence during this season (figure 2 and the electronic supplementary material, table S1), despite high contact rate during promiscuous mating. By contrast, prevalence spiked when bats entered hibernation and was significantly higher than late autumn prevalence for all sampled species (figure 2*g*). During winter when bats were in hibernation, prevalence increased significantly for three species, *M. sodalis* (figure 2*e*), *P. subflavus* (figure 2*f*) and *E. fuscus* (figure 2*a*). For two other species, *M. lucifugus* and *M. septentrionalis*, prevalence was already nearly 100% in early hibernation and showed no change over time (figure 2*c*,*d*,*g* and the electronic supplementary material, table S1). In the sixth species, *M. leibii*, prevalence also increased significantly between autumn and late winter, but a lack of early winter samples from this relatively rare species prevented finer characterization of winter trends (figure 2*b* and the electronic supplementary material, table S1). During the summer, when bats in maternity colonies use torpor much less frequently and their body temperature is typically 15–20°C higher than the upper growth limit of *P. destructans*, prevalence of both species sampled, *M. lucifugus* and *E. fuscus*, decreased rapidly (figure 2*a*,*c* and the electronic supplementary material, table S1), and were not significantly different from zero by late summer (figure 2).

Loads of *P. destructans* on bats showed trends similar to prevalence patterns (figure 3). During the autumn, loads remained very low on all species. Loads increased significantly in most species during hibernation (figure 3*c*,*e*,*f*,*g* and the electronic supplementary material, table S2) and peaked on all species at the end of hibernation (figure 3). However, as soon as bats became active and migrated to maternity colonies, loads decreased substantially and fell to zero for most individuals by the end of summer (figure 3). Decreases in loads and prevalence over the summer did not parallel changes in colony size for *M. lucifugus*, which broadly overlapped and

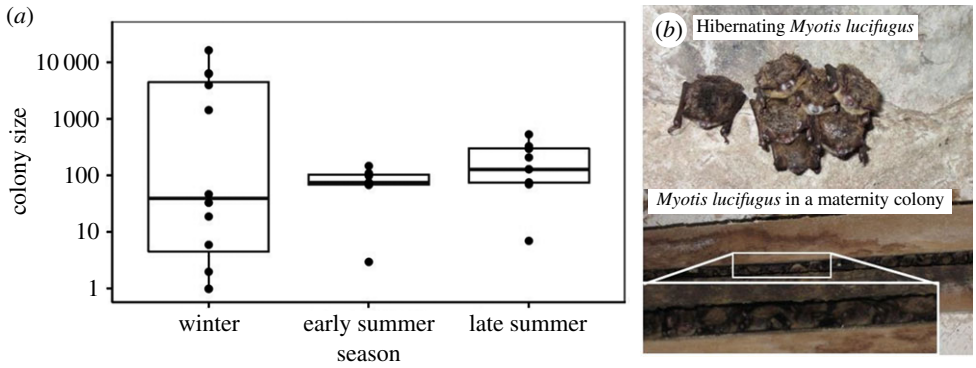

Figure 4. (*a*) Boxplot of colony sizes, on a log scale, of *M. lucifugus* at winter hibernacula and summer maternity colonies. (*b*) Photos of *M. lucifugus* roosting in groups in winter (top) and summer (bottom). (Online version in colour.)

were not significantly different between summer and winter (figure 4; $t_{19.21} = 0.46$, $p = 0.65$). Colony sizes at maternity sites grew to be approximately 2.2 times larger over the summer, suggesting that most active bats were clearing the pathogen rather than dying of latent infection, and some immigration occurred between the counts.

4. Discussion

The seasonal patterns of prevalence and loads of *P. destructans* were remarkably consistent for all six bat species, with a sharp increase in prevalence between autumn and early winter when bats began to hibernate and a peak in fungal load in late winter at the end of hibernation. In contrast to many other systems [2,15,16,39], we found no support for the hypothesis that birth pulses drive seasonality. Prevalence and intensity actually decreased precipitously in summer when all species gave birth and previously uninfected young of the year join the adult population. This suggests that an influx of susceptible individuals is not driving transmission dynamics. Furthermore, for *M. lucifugus*, transmission was unrelated to seasonal changes in colony size. Colony sizes among seasons broadly overlapped despite directionally opposite patterns of infection prevalence, suggesting that larger winter colony sizes are not the primary driver of differences in transmission among seasons. Although winter colonies affected by WNS decline over the winter, colony sizes would have to be 100 times higher in early winter to alter the qualitative relationship between seasonal colony size and transmission. Finally, although contact with contaminated hibernacula [29,30,40] in autumn initiated infection in bats, transmission and infection intensity remained low until bats increased prolonged torpor use [33], suggesting that habitat selection is not the primary factor driving disease dynamics. Furthermore, prevalence and loads decreased during the summer, suggesting that if summer maternity colony sites are infected, the routine use of body temperatures above the growth range of *P. destructans* probably prevented infection or growth.

Hibernation appeared to be the dominant factor determining transmission dynamics and pathogen growth. It was only after bats began to fully hibernate during the winter that transmission increased, and shortly thereafter nearly 100% of individuals became infected at many sites. Fungal loads also increased substantially with the onset of hibernation. The rapid increase in prevalence between late autumn and early hibernation could have been caused by the large increase in loads which increased infectiousness. Contact rates during hibernation are unknown, but males are known to mate with torpid females [33], and a combination of high infectiousness and moderately high contact rates could facilitate rapid transmission. Temperatures of bats during hibernation are approximately the same as ambient temperatures of hibernacula [41], and are within the range of temperatures that the pathogen can grow [28], resulting in explosive amplification of *P. destructans* on hibernating bats. In summary, the seasonality of *P. destructans* transmission appears to be driven by host physiology, specifically a sustained decrease in body temperature.

Changes in body temperature are also important for other diseases. Hibernation has been shown to be important for another pathogen of bats (rabies; [14]). However, in rabies, hibernation allows the virus to persist in a quiescent phase, whereas for WNS, hibernation increased both transmission among bats and pathogen replication on hosts. Host body temperature is also important in driving host impacts in the fungal pathogen of amphibians [42], *Batrachochytrium dendrobatidis*, highlighting a similarity between these important pathogens.

The timing of *P. destructans* transmission and increases in infection intensity probably maximize the impact of WNS on bat populations. Infection peaks when bat populations are near their annual minima, just prior to when females give birth, thereby reducing the reproductive population (figure 1). In addition, bats rely on colonial roosts for thermoregulatory benefits to both raise young in the summer and survive winter hibernation [43,44]. As a result, mortality is occurring at a time when bats may experience positive density dependence (i.e. Allee effects), meaning survival and reproduction would decrease with decreasing colony size [45]. Finally, transmission and disease-caused mortality are absent in late summer and early autumn, when density-dependent food limitation may be strongest because bats forage intensely to obtain enough food and fat to survive over winter [46]. Thus, the seasonal timing of transmission and pathogen growth probably results in nearly maximal disease impacts of this pathogen on bat populations and contributes to exceedingly high population declines across a wide region (more than 90% in several species; [25,26]). By contrast, the timing of peak disease mortality in many other systems often coincides with reproduction. For example, transmission and avian mortality from West Nile virus peaks in late summer and autumn, just after the seasonal birth pulse [47]. In this case, disease mortality reduces density-dependent regulation before populations reach minima overwinter.

The seasonality in *P. destructans* infection patterns, while leading to maximum disease impacts, probably reduces the rate of spatial spread of *P. destructans* [48] because of a mismatch between periods of high mobility and high pathogen

prevalence and load. Bats are highly mobile during the autumn when they travel among hibernacula to mate, and at the end of summer when they migrate from maternity sites to hibernacula [34,49]. However, pathogen loads and prevalence were relatively low during these periods. If infection loads and prevalence in autumn or late summer were at levels observed in winter, bats would be much more infectious, and spatial spread would probably be much faster. The high prevalence and infection load during winter make occasional movements among hibernacula during winter (either in winter or early spring) [49] potentially important in pathogen spread.

Although bats travel substantial distances from hibernacula to summer maternity sites [49,50], this is unlikely to facilitate spatial spread among hibernacula. While the high fungal loads and nearly 100% prevalence on bats at the end of winter facilitates rapid spread to their summer maternity sites, the high body temperatures and hot maternity roosts bats use during summer are too high for pathogen growth [28] and lead to bats clearing *P. destructans* infection from their skin. The combination of the seasonality of infection and the hot environments used by bats during the summer has probably slowed the geographical spread of *P. destructans* compared with pathogens where transmission peaks at the same time when animals are dispersing or migrating, such as West Nile virus [51] and avian influenza [4].

The seasonal patterns of transmission we have documented can be used to more effectively guide management interventions. When bats first become infected with *P. destructans*, loads, and therefore tissue invasion and damage, are relatively low. Therefore, applying treatments that reduce or clear infection during the autumn and early winter would be most effective for reducing transmission, impacts and spread to new sites. However, if treatments offer only short-term protection, our data suggest that treated bats will probably be rapidly re-infected upon return to natural environments owing to exceedingly high infection prevalence in other hosts. Our results also suggest that another management strategy, culling bats to remove infected individuals, would be ineffective during the winter, because nearly 100% of individuals are already infected by early hibernation. Finally, while rearing temperate bats in captivity is exceedingly challenging [52], if this strategy were attempted, capturing bats during late summer would maximize the fraction of uninfected bats that could be brought into captivity.

In the 7 years since the detection of WNS, bat populations have crashed to a small fraction of their former size [26], with several species at risk of extinction [25]. Our findings illustrate how the seasonality of transmission and infection intensity drives the impact of this deadly disease. Commonality across host physiology, specifically, hibernation for extended periods at temperatures that allow growth of the pathogen, has created a perfect storm, and led to the deaths of millions of individuals of multiple species. More broadly, our results demonstrate the importance of seasonal timing of infection in driving impacts and spread of emerging pathogens, and show how understanding seasonal patterns of transmission can provide critical information for mitigating the devastating impacts of wildlife disease.

Ethics statement. All research was conducted under protocol number 11-022 approved by the IACUC of Boston University.

Data accessibility. Data are deposited in Dryad Digital Repository [53]. Exact site locations are not disclosed to protect endangered species and landowners. Those interested in collaborations using these data should email corresponding author (klangwig@gmail.com) for additional information.

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