UNIVERSITY OF CALIFORNIA

SANTA CRUZ

DETERMINING THE DRIVERS OF SPECIES AND POPULATION EXTINCTION IN THE EMERGING INFECTIOUS DISEASE OF BATS, WHITE-NOSE SYNDROME

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

By

Kate E. Langwig

June 2015

The Dissertation of Kate E. Langwig
is approved:

_____
Professor A. Marm Kilpatrick, Chair

_____
Professor Bruce Lyon

_____
Professor Winifred F. Frick

_____
Professor Stephan Munch

_____
Tyrus Miller
Vice Provost and Dean of Graduate Studies

Table of Contents

Content Pages

Acknowledgements and Dedication

I would like to dedicate this work to my mother, Ellen Dorchester Langwig. Thank you for all of your sacrifices, for always being my rock, and for believing in me. You gave me the freedom to become whatever I wanted, although I'm sure you're relieved I chose to be a scientist instead of a cat.

The text of this dissertation includes reprints of the following previously published material:

Langwig, K. E., W. F. Frick, J. T. Bried, A. C. Hicks, T. H. Kunz, and A. Marm Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecology Letters ***15****:1050-1057.*

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. Foster, and A. M. Kilpatrick. 2015. Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. Proceedings of the Royal Society B: Biological Sciences ***282****.*

The candidate led the research, performed the analyses, and wrote the papers listed above, with input and from all co-authors.

Abstract

Kate E. Langwig

Determining the drivers of species and population extinction in the emerging infectious disease of bats, white-nose syndrome

Emerging infectious diseases pose a key threat to wildlife, and the number of disease emergence events is increasing. Despite the importance of disease in wildlife conservation, understanding the drivers of population and species extinction from disease has not been tested in an empirical framework. My research incorporates empirical and theoretical approaches to understand factors that influence pathogen transmission and disease impacts. Here, we focus on the emerging fungal disease of bats, white-nose syndrome, which has caused widespread declines in bat populations across Eastern North America. Our findings highlight the importance of social behavior, microclimate conditions, and seasonality in driving impacts from this disease. We find that while seasonal transmission is broadly similar across species, winter differences in pathogen growth drive variation in species impacts from disease. Species appear to have different transmission mechanism which influences the likelihood they will persist in the face of white-nose syndrome. We also identify a species, the Northern long-eared bat, which is likely to go extinct if rapid management action is not taken. These data provide critical information needed to manage wildlife disease epidemics, enabling management action prior to species extinction.

Introduction

Emerging infectious diseases are a key threat to wildlife, and the number of disease emergence events are increasing (Daszak et al. 2000, Jones et al. 2008). Wildlife disease has caused multiple species extinctions, made formerly widespread and abundant species rare, and has changed the structure of ecological communities (Smith et al. 2006, Skerratt et al. 2007, Frick et al. 2010, Langwig et al. 2012). Impacts from emerging infectious disease are determined by transmission of the pathogen, and species susceptibility to disease given exposure (Kilpatrick et al. 2013). Non-density dependent transmission, and reservoirs may exacerbate disease impacts and drive populations and species to extinction (de Castro and Bolker 2005). Despite the importance of these factors in contributing to extinction from disease, few have been tested empirically, and never in combination. Most importantly, whether species are at risk of extinction due to disease has rarely been assessed prior to the loss of the species, and so management action to save species imperiled by disease is rarely taken (Langwig et al. 2015).

White-nose syndrome (WNS) is a recently emerged infectious disease that threatens several species of hibernating bats with extinction (Langwig et al. 2012, Frick et al. 2015). The disease caused by the fungal pathogen, *Pseudogymnoascus destructans* (Blehert et al. 2008, Warnecke et al. 2012), formerly *Geomyces destructans* (Minnis and Lindner 2013). *Pseudogymnoascus destructans* is a cutaneous fungal infection that causes severe damage to wing tissue and increases torpor arousal frequency during hibernation (Reichard and Kunz 2009, Warnecke et

al. 2012). Disruption of blood physiology, dehydration, and depletion of fat reserves may ultimately result in the morbidity and mortality caused by WNS (Warnecke et al. 2013). *Pseudogymnoascus destructans* grows optimally between 7-14°C (Verant et al. 2012), causing severe mortality during winter hibernation when bats roost at cool temperatures (Lorch et al. 2011).

Several unique characteristics of bats may have contributed to the exceedingly high mortality observed in several bat species. Pre-WNS colony sizes varied by four orders of magnitude (Langwig et al. 2012), and large colony sizes of bats may have facilitated rapid spread of the disease across eastern North America (Wilder et al. 2011). Some bat species are highly social, forming large clusters during hibernation, while other species most commonly roost singly or in small groups. During the winter, bats of several species may roost together in a single hibernaculum, but then migrate to species and sex segregated colonies during the summer (Barbour and Davis 1969). *Pseudogymnoascus destructans* is able to persist for long periods of time in the absence of bats (Hoyt et al. 2014) in hibernacula sediments (Lorch et al. 2013). This variation in sociality, contact with potential reservoirs, and magnitude of disease impacts provides a powerful opportunity to empirically test theoretically-proposed factors influencing transmission, impacts, and extinction in a multi-host pathogen system, including social behavior, climate, and biotic and environmental reservoirs.

Social behavior of hosts can greatly influence spread of a pathogen through a community of hosts. For pathogens where infected hosts infect the same number of

individuals regardless of population size (often termed "frequency-dependent transmission" or "non-density dependent transmission"), host extinction is more likely because pathogens will continue to be transmitted at low population densities (Getz and Pickering 1983, Lockhart et al. 1996). Frequency-dependent transmission is thought to occur when hosts seek out each other, either to mate, or to aggregate in social groups. The resulting contact can maintain high transmission despite population declines (Anderson and May 1991, Lloyd-Smith et al. 2005, Nunn et al. 2008, McCallum et al. 2009). If social behavior among hosts carries risks of lethal disease, then these risks may potentially outweigh the benefits of sociality. Small bodied mammals like bats have to maintain a high mass specific metabolic rate to keep their body temperature stable, and roosting with many individuals can help to defray these energetic costs. However, in WNS, there may be strong selective pressure from the disease for bats to reduce social group sizes, although doing this during hibernation may come at a high physiological costs (Boyles et al. 2008).

Impacts of disease are also influenced by climate, which can affect both the susceptibility of hosts as well as pathogen transmission. Climatic conditions can influence transmission by affecting persistence or growth of the pathogen (Shaman et al. 2010), and can influence host physiology or immune function and thereby host susceptibility (Raffel et al. 2013). Environmental conditions that physiologically favor hosts may also favor growth of the pathogen, as is the case in *P. destructans* which grows best at the cool temperatures at which bats hibernate to save energy during the winter (Verant et al. 2012).

Seasonal changes in climate and habitat use by hosts may influence both the environmental conditions experienced by the pathogen and host, as well as induce changes in host population sizes and contact rates as animals migrate to new foraging grounds, breed, or give birth (Altizer et al. 2006). Seasonal timing of epidemics is frequently associated with an influx of susceptible individuals into the host-pathogen system through births (George et al. 2011). Seasonal epidemics may also be driven by contact with environmental reservoirs, as seasonal changes in use of habitats by hosts can influence host contact with infective environmental stages. Presence of environmental reservoirs can substantially influence disease dynamics by providing a constant source of reinfection and therefore increase likelihood of host extinction (de Castro and Bolker 2005) as infection continues even as hosts become rare.

The diverse ecological and evolutionary pressures that drive disease dynamics make predicting general factors influencing disease impacts and species extinction difficult to discern and test in an empirical framework. As pathogens exploit new ecological niches, they may take advantage of new growing conditions, new hosts, and new routes of transmission. Host responses to pathogen invasion may also vary among host species and populations, and across space. Disease dynamics may drive hosts extinct before evolution can occur, or because demographic or genetic factors cause hosts to spiral down the extinction vortex (Courchamp et al. 1999). In my dissertation I empirically test theoretical predictions of how pathogens impact host populations and species to identify species that are at greatest risk of extinction and guide management action to prevent the loss of species.

Literature Cited

Altizer, S., A. Dobson, P. Hosseini, P. Hudson, M. Pascual, and P. Rohani. 2006. Seasonality and the dynamics of infectious diseases. Ecology Letters **9**:467-484.

Anderson, R. M. and R. M. May. 1991. Infectious diseases of humans: Dynamics and control. Oxford University Press, London.

Barbour, R. and W. Davis. 1969. Bats of America. The University Press of Kentucky, Lexington, KY, USA.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2008. Bat white-nose syndrome: an emerging fungal pathogen? Science **323**:227.

Boyles, J., J. Storm, and V. Brack. 2008. Thermal benefits of clustering during hibernation: a field test of competing hypotheses on Myotis sodalis. Funct Ecol **22**:632 - 636.

Courchamp, F., T. Clutton-Brock, and B. Grenfell. 1999. Inverse density dependence and the Allee effect. Trends in Ecology & Evolution **14**:405-410.

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.

Frick, W. F., J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, and T. H. Kunz. 2010. An emerging disease causes regional population collapse of a common north american bat species. Science **329**:679-682.

Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B. A. Nickel, K. E. Langwig, J. T. Foster, K. E. Barlow, T. Bartonička, D. Feller, and A. J. Haarsma. 2015. Disease alters macroecological patterns of North American bats. Global Ecology and Biogeography.

George, D. B., C. T. Webb, M. L. Farnsworth, T. J. O'Shea, R. A. Bowen, D. L. Smith, T. R. Stanley, L. E. Ellison, and C. E. Rupprecht. 2011. Host and viral ecology determine bat rabies seasonality and maintenance. Proceedings of the National Academy of Sciences.

Getz, W. M. and J. Pickering. 1983. Epidemic models: thresholds and population regulation. The American Naturalist **121**:892-898.

Hoyt, J. R., K. E. Langwig, J. Okoniewski, W. F. Frick, W. B. Stone, and A. M. Kilpatrick. 2014. Long-term persistence of Pseudogymnoascus destructans, the causative agent of white-nose syndrome, in the absence of bats. EcoHealth:1-4.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-993.

Kilpatrick, A. M., R. J. Peters, A. P. Dupuis Ii, M. J. Jones, P. Daszak, P. P. Marra, and L. D. Kramer. 2013. Predicted and observed mortality from vector-borne disease in wildlife: West Nile virus and small songbirds. Biological Conservation **165**:79-85.

Langwig, K. E., W. F. Frick, J. T. Bried, A. C. Hicks, T. H. Kunz, and A. Marm Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecology Letters **15**:1050-1057.

Langwig, K. E., J. Voyles, M. Q. Wilber, W. F. Frick, K. Murray, B. M. Bolker, J. P. Collins, J. R. Hoyt, C. Willis, W., , T. L. Cheng, M. Fisher, D. Lindner, H. I. McCallum, R. Puschendorf, E. B. Rosenblum, M. Toothman, C. J. Briggs, and A. M. Kilpatrick. 2015. Context dependent conservation responses to wildlife disease. Frontiers in Ecology and the Environment.

Lloyd-Smith, J. O., P. C. Cross, C. J. Briggs, M. Daugherty, W. M. Getz, J. Latto, M. S. Sanchez, A. B. Smith, and A. Swei. 2005. Should we expect population thresholds for wildlife disease? Trends in Ecology & Evolution **20**:511-519.

Lockhart, A. B., P. H. Thrall, and J. Antonovics. 1996. Sexually transmitted disease in animals: ecological and evolutionary implications. Biological Reviews **71**:415-471.

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.

McCallum, H., M. Jones, C. Hawkins, R. Hamede, S. Lachish, D. L. Sinn, N. Beeton, and B. Lazenby. 2009. Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease-induced extinction. Ecology **90**:3379-3392.

Minnis, A. M. and D. L. Lindner. 2013. Phylogenetic evaluation of Geomyces and allies reveals no close relatives of Pseudogymnoascus destructans, comb. nov., in bat hibernacula of eastern North America. Fungal Biology **117**:638-649.

Nunn, C., P. Thrall, K. Stewart, and A. Harcourt. 2008. Emerging infectious diseases and animal social systems. Evolutionary Ecology **22**:519-543.

Raffel, T. R., J. M. Romansic, N. T. Halstead, T. A. McMahon, M. D. Venesky, and J. R. Rohr. 2013. Disease and thermal acclimation in a more variable and unpredictable climate. Nature Climate Change **3**:146-151.

Reichard, J. and T. Kunz. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (Myotis lucifugus). Acta Chiropterol **11**:457 - 464.

Shaman, J., V. E. Pitzer, C. Viboud, B. T. Grenfell, and M. Lipsitch. 2010. Absolute humidity and the seasonal onset of influenza in the continental United States. Plos Biology **8**.

Skerratt, L. F., L. Berger, R. Speare, S. Cashins, K. R. McDonald, A. D. Phillott, H. B. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. Ecohealth **4**:125-134.

Smith, K. F., D. F. Sax, and K. D. Lafferty. 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology **20**:1349-1357.

Verant, M. L., J. G. Boyles, W. Waldrep, G. Wibbelt, and D. S. Blehert. 2012. Temperature-Dependent Growth of Geomyces destructans, the Fungus That Causes Bat White-Nose Syndrome. Plos One **7**.

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**.

Wilder, A. P., W. F. Frick, K. E. Langwig, and T. H. Kunz. 2011. Risk factors associated with mortality from white-nose syndrome among hibernating bat colonies. Biology Letters **7**:950-953.

Ecology Letters, (2012) doi: 10.1111/j.1461-0248.2012.01829.x

LETTER

Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome

Kate E. Langwig,[1,2]* Winifred F. Frick,[2] Jason T. Bried,[3] Alan C. Hicks,[4] Thomas H. Kunz[1] and A. Marm Kilpatrick[2]

Abstract

Disease has caused striking declines in wildlife and threatens numerous species with extinction. Theory suggests that the ecology and density-dependence of transmission dynamics can determine the probability of disease-caused extinction, but few empirical studies have simultaneously examined multiple factors influencing disease impact. We show, in hibernating bats infected with *Geomyces destructans*, that impacts of disease on solitary species were lower in smaller populations, whereas in socially gregarious species declines were equally severe in populations spanning four orders of magnitude. However, as these gregarious species declined, we observed decreases in social group size that reduced the likelihood of extinction. In addition, disease impacts in these species increased with humidity and temperature such that the coldest and driest roosts provided initial refuge from disease. These results expand our theoretical framework and provide an empirical basis for determining which host species are likely to be driven extinct while management action is still possible.

Keywords

Adaptive management, climate change, conservation, density-dependent transmission, disease ecology, emerging infectious disease, endangered species, frequency-dependent transmission, *Geomyces destructans*, myotis, white-nose syndrome.

Ecology Letters (2012)

INTRODUCTION

Novel pathogens introduced to naïve host communities can have devastating effects on wildlife populations, drive species to extinction and thereby decrease biodiversity (Daszak *et al.* 2000; Smith *et al.* 2006). However, the impact of multi-host pathogens differs substantially, with some species declining to extinction whereas others suffer little mortality (Riper *et al.* 1986; Harvell *et al.* 1999; Lips *et al.* 2006; LaDeau *et al.* 2007), and some may even benefit from disease-caused reductions in competitors or predators (Whitlaw & Lankester 1994). Variation in behavioural characteristics among species can lead to differences in exposure which, combined with variation in susceptibility to mortality from a disease, influence population-level impacts (Loehle 1995; Altizer *et al.* 2003; Lloyd-Smith *et al.* 2004; LaDeau *et al.* 2007; Nunn *et al.* 2008). The environment can also mediate disease impacts through direct influences on pathogen growth and persistence, or indirect effects on host physiology and behaviour (Kilpatrick *et al.* 2010; Shaman *et al.* 2010) Previous studies that have examined initial or long-term impacts of disease typically have focused on a single host (Packer *et al.* 1999; Hochachka & Dhondt 2000) or have analysed either host or environmental factors but rarely both (Dwyer *et al.* 1990; Hudson *et al.* 1998; Lips *et al.* 2006; LaDeau *et al.* 2007; McCallum *et al.* 2009). This limits strong inference about factors influencing disease-caused extinction.

Theory suggests that the scaling of pathogen transmission with population size can determine whether or not a pathogen drives a host extinct. If transmission increases with the density of hosts, there may be a threshold density below which the pathogen will die out and the host may persist (McCallum *et al.* 2001; Fenton *et al.* 2002; de Castro & Bolker 2005; Lloyd-Smith *et al.* 2005). In contrast, for pathogens where infected hosts infect the same number of individuals regardless of population size (often termed 'frequency-dependent transmission'), host extinction is more likely because pathogens will continue to be transmitted at low population densities (Getz & Pickering 1983; Lockhart *et al.* 1996). Frequency-dependent transmission is more likely if infectious contacts occur when hosts seek each other out, either to mate, or to aggregate in social groups. These behaviours contact can maintain high transmission despite population declines (Anderson & May 1991; Lloyd-Smith *et al.* 2005; Nunn *et al.* 2008; McCallum *et al.* 2009). Empirically testing how sociality influences disease impact would ideally examine population declines due to a single pathogen in a community of hosts that co-occur in the same sites, but differ in social aggregation.

White-nose syndrome (WNS) is an emerging infectious disease caused by *Geomyces destructans*, a fungus in the family Myxotrichaceae (Blehert *et al.* 2008; Lorch *et al.* 2011) that was likely recently introduced from Europe (Puechmaille *et al.* 2011; Warnecke *et al.* 2012). In North America, *G. destructans* is known to cause severe mortality in one formerly common bat species (Frick *et al.* 2010), and infect

[1]*Center for Ecology and Conservation Biology, Department of Biology, Boston University, Boston, MA, 02215, USA*
[2]*Department of Ecology and Evolutionary Biology, University of California, Santa Cruz, CA, 95064, USA*
[3]*Department of Zoology, Oklahoma State University, Stillwater, OK, 74078, USA*
[4]*New York State Department of Environmental Conservation, Endangered Species Unit, Albany, NY, 12233, USA*
**Correspondence: E-mail: klangwig@bu.edu*

at least six species of hibernating bats (Table S1) (Cryan *et al.* 2010). WNS is characterised by lesions on flight membranes of bats (Meteyer *et al.* 2009) which may disrupt patterns of torpor (Warnecke *et al.* 2012) or critical physiological processes and possibly result in death by starvation or dehydration (Cryan *et al.* 2010).

The six species of bats known to be infected with Gd co-occur in the same sites, and vary substantially in abundance and sociality. Average population sizes for colonies (population and colony size are used interchangeably hereafter) of these six species during hibernation vary across four orders of magnitude (Table S1). Cluster sizes within hibernacula (groups of bats in contact with one another) can also vary by two orders of magnitude among species (Barbour & Davis 1969). A key question is whether contact and transmission rates among bats increase with colony size (i.e. are density-dependent) or whether social clustering of individuals into one or more tightly packed groups in some species might result in similar contact rates in large and small colony sizes (Nunn & Altizer 2006; Streicker *et al.* 2012). Clustering with a fixed number of neighbours in gregarious species is likely to result in elevated and constant transmission in highly gregarious species and may lead to populations declining to extinction (Lockhart *et al.* 1996; de Castro & Bolker 2005). In contrast, for species that are less likely to form clusters in hibernacula, contact and transmission among bats is predicted to be lower in smaller populations and decrease as populations decline. As a result, disease is less likely to cause extinction in these species (Lockhart *et al.* 1996; Castro & Bolker 2005).

Susceptibility to mortality from WNS, given exposure, may also be influenced by microclimate effects on host-pathogen interactions. *G. destructans* shows increasing growth across the range of hibernacula temperatures in the northeast USA, 0–15 °C (Gargas *et al.* 2009) and like many other fungi, likely grows better under more humid conditions. Across the same temperature range, host immune function, which is greatly reduced during hibernation (Moore *et al.* 2011), would be predicted to increase. Thus, dryer sites would be hypothesised to have lower disease impacts and increasing roost site temperature may increase or decrease WNS impacts depending on whether host or pathogen processes dominate.

Here we examine how colony size, sociality and environmental conditions (temperature and humidity) drive patterns of disease impact. We do so by quantifying the population growth rates of 120 populations of six species of bats in multi-host communities at 37 sites in the northeastern United States before and after the arrival of *G. destructans*. We examine both spatial patterns of population declines and how they scale with colony size, and temporal changes in clustering and population growth rates as species decline. Finally, we investigate how microclimates at roost sites in hibernacula influence the population growth rate of two declining species.

MATERIALS AND METHODS

Hibernacula surveys

Hibernacula in the New York, Vermont, Connecticut, and Massachusetts were surveyed by trained biologists from state natural resources departments between 1 December and 10 April in some years from 1979 to 2010 (Fig. S1). Visual counts were conducted during hibernacula visits and photographs were used to enhance survey accuracy. Data on clustering behaviour of the two gregarious species, little brown myotis (*Myotis lucifugus* LeConte) and Indiana myotis (*Myotis sodalis* Miller and Allen), were collected in New York by state researchers in an opportunistic subset of 45 populations prior to WNS detection, and during all (23) census counts beginning in 2009.

Determining the first year of WNS at a site

White-nose syndrome usually causes aberrant behaviour of bats during hibernation, including bats prematurely staging at hibernacula entrances, failure of bats to arouse normally in response to disturbance, and diurnal and mid-winter emergence of bats. We used the best available estimates of year of WNS detection based on reports of bats emerging onto the landscape in close proximity to hibernacula, and surveys of hibernacula entrances for bat carcasses. However, sites may have been infected with *G. destructans* prior to detection of disease when sites were not surveyed every year.

We determined the sensitivity of our results to uncertainty in the year of WNS detection by performing analyses with three sets of arrival years: the most likely year using the information described above, the latest year of WNS detection, determined by the first year a hibernacula survey was conducted and symptoms of WNS were present, and the earliest possible year of WNS detection. For this last estimate a site was considered infected in a year if the distance to the presumed site of introduction (Howes Cave) was less than the distance from Howes cave to the furthest site known to be infected. Results were qualitatively similar among all three sets of analyses so we present only the results for the most likely year.

Estimating pre-WNS growth

We calculated population trends prior to WNS infection using an average of 9.2 (range: 4–22) hibernacula surveys prior to WNS. Because counts were not conducted in consecutive years, we used a regression technique to estimate the log population growth rate. Here, the dependent variable, y_i, and the independent variable, x_i, are given by:

$$y_i = \frac{\ln\left(\frac{N_{i+1}}{N_i}\right)}{\sqrt{t_{i+1} - t_i}} \; and \; x_i = \sqrt{t_{i+1} - t_i} \qquad (1)$$

where i is an index for the hibernacula counts, t_i gives the year of count i, and N_i is the count in year i. The slope of the regression of y_i vs. x_i (with the regression forced through the origin) estimates the log population growth rate, $\ln(\lambda)$ (Morris & Doak 2002).

Scaling of WNS impacts with colony size among species

We estimated the population growth rate, λ, for each population of each species with counts both before and after WNS detection. We used the single most recent pre-WNS census as a proxy for colony size prior to onset of WNS infection, which was an average of 3.7 (range: 1–9) years before WNS detection. Our results were qualitatively similar if we excluded the two sites that were surveyed 9 years before WNS detection. For sites where the first post-WNS count was more than 1 year after WNS detection we calculated the average yearly population growth rate, λ, following the arrival of WNS by adjusting for the number of years between WNS detection at a hibernacula, and the post-WNS census via:

$$\lambda = \sqrt[t_x]{\left(\frac{N_i}{N_{i-1}}\right)} \quad (2)$$

Here, N_i is the first count post-WNS detection and count, N_{i-1} is the most recent prior count before WNS detection, and t_x is the number of years between the first post-WNS detection survey and the year prior to WNS detection. These values of λ use just two population counts and represent a single estimate of population growth rate pre- and post-WNS detection for each population. They are thus distinct from the estimates of pre-WNS population growth rates described above which use multiple counts pre-WNS detection. We used this approach because, for many populations, there was only a single count post-WNS detection and thus alternate approaches (e.g. segmented regression) would lack degrees of freedom to yield improved slope estimates over those given by eqn 2.

Statistical analysis

We examined the scaling of population declines with population size using mixed-effects generalised linear models of population growth rate, λ, with a gamma distribution and the canonical inverse link using function glmmPQL in package MASS (Venables & Ripley 2002) in R v2.15 (R Development Core Team 2012). In these mixed-effects models we treated site as a random effect, and species and $\log_{10}$ population size prior to WNS detection as fixed effects. We added one to zero values of N_i because gamma distributions must be positive. Adding other fixed values or a small fraction of the pre-WNS count produced qualitatively identical results. We tested for spatial autocorrelation using Moran's I and found no significant correlations (all $P > 0.2$).

For sites where we had counts from several years' post-WNS detection, we also analysed temporal variation in the rate of decline since WNS detection. Residuals from this analysis were not temporally autocorrelated ($P > 0.05$).

We examined changes in roosting behaviour (the fraction of bats roosting singly) pre- and post-WNS detection for two species where data were available, little brown myotis and Indiana myotis. We used a mixed-effects generalised linear model of the number of bats roosting alone with a binomial distribution, and the canonical logit link using function *lmer* in package lme4 (Bates *et al.* 2011).

We analysed the influence of temperature on WNS declines of Indiana and little brown myotis among hibernacula (data were unavailable for other species), and relative humidity for Indiana myotis (relative humidity data were unavailable for little brown myotis). We used linear regression on $\log_{10}$ transformed population growth rate (results were qualitatively identical using a generalised linear model with a gamma distribution on untransformed population growth rates). We measured relative humidity and temperature in hibernacula every 3 h between 1 December and 15 April using Hobo loggers (Onset Corporation, Bourne, MA, USA) or iButtons (Maxim Inc., Sunnyvale, CA, USA) that were placed on walls at roosting locations of each species. We could not include temperature and humidity in the larger analyses described above, because microclimates of roost locations differ among species and are poorly correlated with above ground measurements.

RESULTS

Impacts of WNS on the host community

Prior to WNS emergence, all species were increasing significantly in abundance (Fig. 1a, Fig. S2, all $P < 0.05$), although confidence intervals for λ for individual populations often overlapped 1 (Table S2). A single species, little brown myotis, dominated pre-WNS hibernacula communities (Fig. S3).

After WNS detection, population growth rates varied significantly among species, with four species declining significantly and two species with log-population growth rates that were not significantly different from 0 (Fig. 1b). For all six species, the growth rates following WNS detection were significantly lower than the pre-WNS population trend, and 32 of the 120 bat populations became locally extinct (Fig. 1, Fig. S2). WNS arrived at sites across a 4 year span (2007–2010; Figs S1 and S2), and population growth rates during the same year were significantly lower at sites where WNS was present than unaffected sites in the same region (Generalised linear mixed-effects model with a gamma distribution and inverse link of population growth rate with site as random effect and species, year and WNS presence as fixed effects: WNS effect 1.28 ± 0.35, $P = 0.0007$), suggesting that declines were more likely due to WNS than other regional factors such as weather.

The scaling of declines with population size

For all species, population growth rates were unrelated to total colony size, summed across all species (all $P > 0.2$). However,

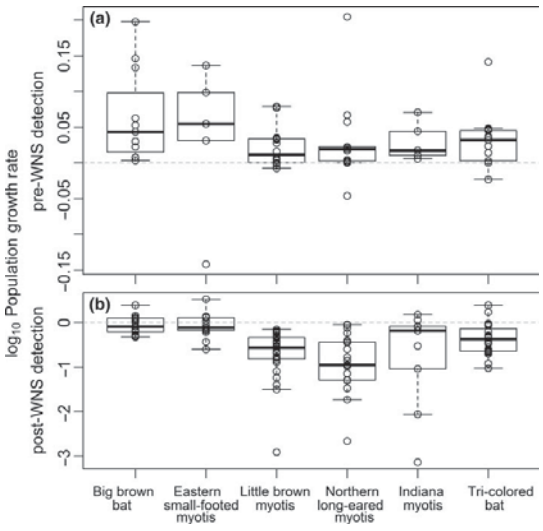

Figure 1 Population growth rates of bats pre- and post-WNS detection. (a) Box plot of $\log_{10}$ population growth rates of six hibernating bat species (a) prior to and (b) after WNS detection. The bold line indicates the median, the box encompasses the 25–75th percentiles of the data, and the whiskers extend to points within 1.5 times the inter-quantile range. The dotted grey line indicates stability and growth rates above/below 0 indicate growing/declining populations.

within-species density-dependent declines were apparent in two of the six species. Both tri-coloured bats (*Perimyotis subflavus* F. cuvier) and northern long-eared myotis (*Myotis septentrionalis* Trouessart), frequently roost solitarily or in small groups within hibernacula (Barbour & Davis 1969). In these species, declines were larger in larger pre-WNS populations (Fig. 2; Table S3), and relationships were strongest and significant only for asymptotic functions of log (colony size) (all linear relationships: $P > 0.2$). The x-intercepts of the fitted relationships imply that populations of tri-coloured bats would be expected to stabilise at an average of ~6 bats per hibernacula, but populations of northern long-eared myotis are predicted to go extinct (Fig. 2). In the other four species population growth rates were unrelated to conspecific pre-WNS population size. Of these four, the two declining species, little brown and Indiana myotis, are highly gregarious and roost in large tightly packed aggregations (Barbour & Davis 1969). In these species declines were equally severe in populations spanning four orders of magnitude, consistent with frequency-dependent transmission (Table S3, Fig. 2), and suggesting that these species might be driven to extinction by WNS.

We also examined the influence of pre-WNS population size of all other species on post-WNS population growth rates of a focal species. All but one of these 30 relationships were non-significant (all $P > 0.05$), and the single significant relationship (tri-coloured bat declines were more severe where Indiana myotis were more abundant) was relatively weak (coef. ± SE: 0.47 ± 0.21; $P = 0.04$) compared with the conspecific slope (Table S3), suggesting that this correlation may have been simply due to chance.

Temporal trends in populations and communities

Temporal analyses of population trends were consistent with predictions based on the spatial patterns of density dependence for three of the four impacted species (Figs 2 and 3). The rate of decline of tri-coloured bat populations decreased with time and populations stabilised at much lower levels 3–4 years post-WNS detection, as would be predicted if transmission were density-dependent. In contrast to this pattern of stabilisation/persistence, but also in agreement with predictions based on spatial patterns (the negative x-intercept for this species in Fig. 3), 14 populations of northern

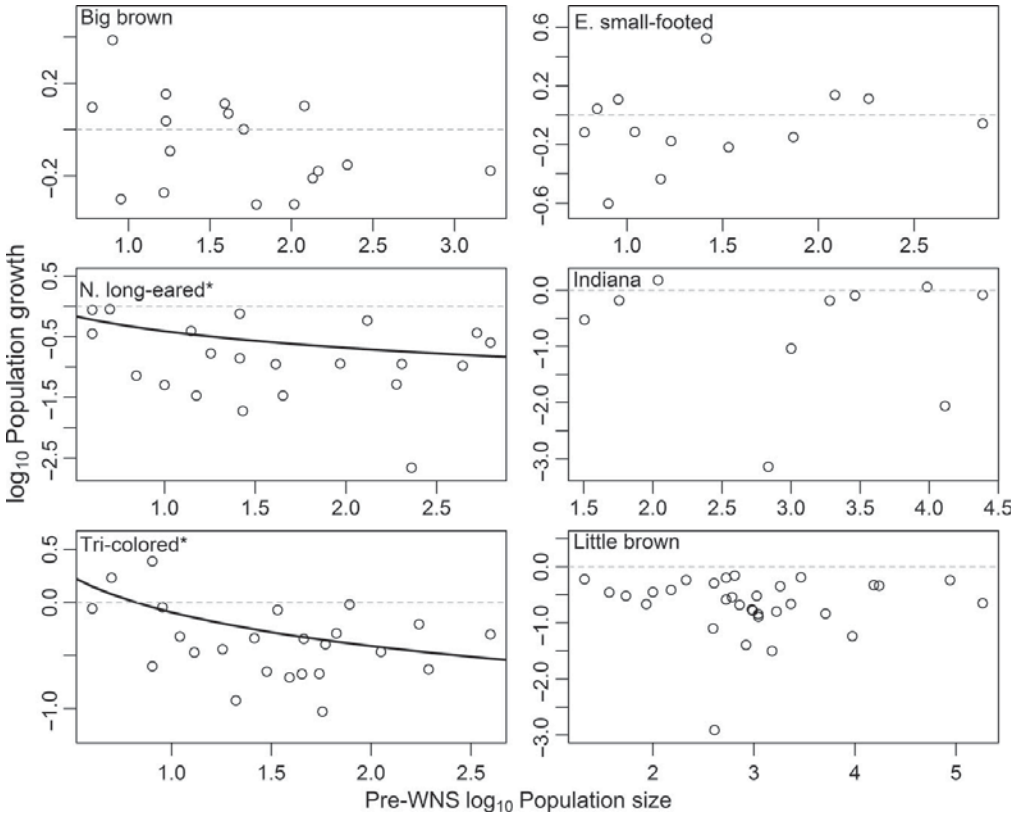

Figure 2 The influence of pre-WNS population size on population growth rate following WNS detection. Fitted lines and asterisks (*) following species names identify species in which pre-WNS population size was significantly negatively correlated with population growth rates following WNS detection. The curves show the fitted relationships, which are linear on the inverse scale used in the generalised linear model.

long-eared myotis became locally extinct within 2 years after WNS detection and no population remained after 5 years (Fig. 3). Population growth rates of Indiana myotis (which exhibited no evidence of density-dependent declines), showed little evidence for reduced declines over time (Fig. 3, Table S4). Somewhat surprisingly, declines of the fourth impacted species, little brown myotis, attenuated significantly over time with most remaining populations reaching stability within 4 years of WNS detection (Fig. 3; Table S4), despite no spatial evidence of density-dependent declines (Fig. 2).

Amelioration of declines in little brown myotis and the contrast with continuing declines in Indiana myotis may have been related to greater changes in social behaviour in little brown myotis following declines. Prior to WNS detection, both these species hibernated almost entirely in clustered aggregations (Fig. 4; fraction roosting individually: little brown myotis 1.16% ± 1.1%; Indiana myotis 0.29% ± SE 0.12%). After WNS detection, a significantly higher fraction of populations of both species roosted individually (little brown myotis: 44.5% ± 9.42%; Indiana myotis 9.6% ± 6.1%), but Indiana myotis, which continued to decline, remained far more social (Fig. 4; Table S5). For both species, the number of bats roosting singly after WNS detection was 17 times greater at each site than before WNS detection, despite greatly reduced population sizes, implying that individual bats changed clustering behaviour, rather than disease simply eliminating all but singly roosting individuals.

Overall, the differential impacts of WNS on different species resulted in changes in bat community composition pre- and post-WNS detection with the two least-impacted species, big brown bats (*Eptesicus fuscus* Palisot de Beauvois) (Wilcoxon signed rank test, $P < 0.001$) and eastern small-footed myotis (*Myotis leibii* Audubon and Bachman) ($P = 0.008$) making up significantly larger percentages of hibernating bat colonies post-WNS (Fig. S3).

Environmental influences

Across sites within a species, population growth rate of Indiana myotis post-WNS detection decreased with the relative humidity at hibernation sites within a hibernacula, but was unrelated to temperature (Fig. 5a: univariate linear regression for relative humidity coefficient ± SE: −0.18 ± 0.060; $P = 0.024$; Fig. 5b: coefficient for temperature in a multiple regression model with relative humidity: −0.17 ± 0.13; $P = 0.23$) and was uncorrelated with total pre-WNS population size or any two-way interaction terms (all $P > 0.05$). For populations of little brown myotis, which roosted across a larger

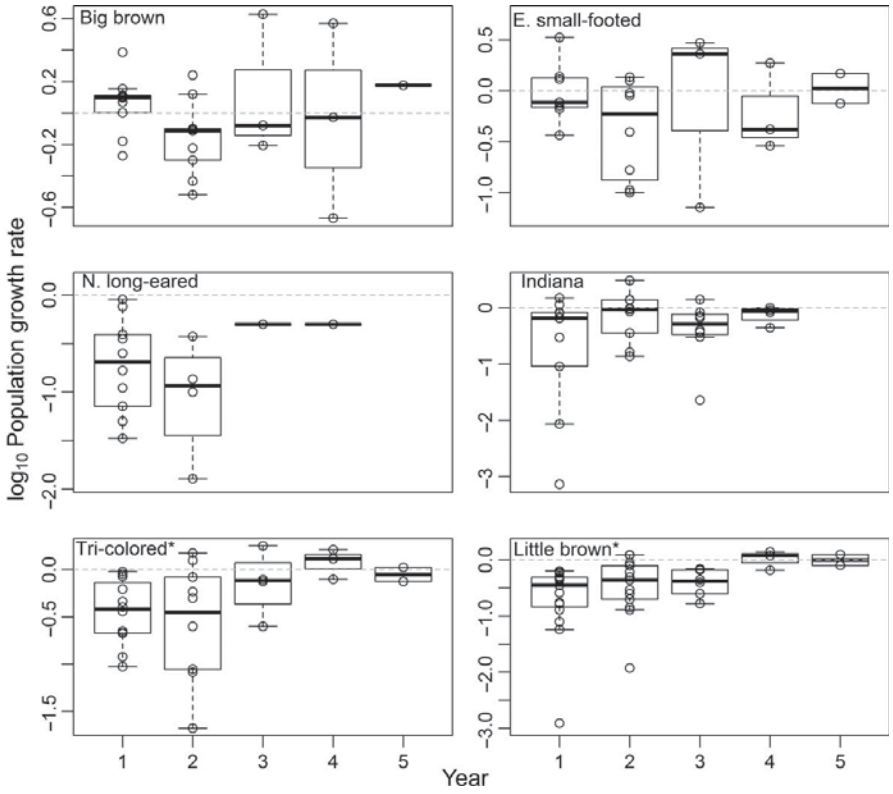

Figure 3 Population trends for six bat species in the 5 years post-WNS detection. An asterisk (*) following the species name denotes species in which population growth rates increased significantly with years since WNS detection. Boxplot details are described in Fig. 1.

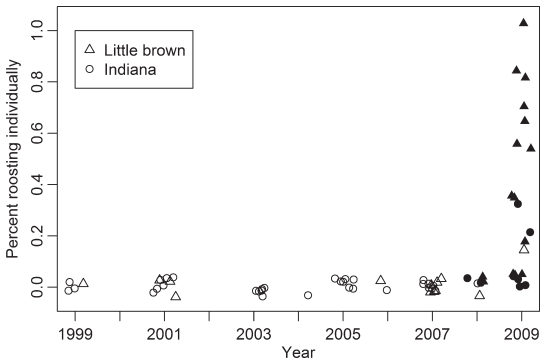

Figure 4 Clustering behaviour of little brown and Indiana myotis in hibernacula before (open symbols) and after (filled symbols) WNS detection. Points show the fraction of each population of each species roosting individually. A small amount of random variation was added to each point to show overlapping symbols.

range of temperatures, the effect of microclimate temperature was stronger and statistically significant (Fig. 5c; coef. ± SE: −0.099 ± 0.034, $P = 0.017$). As for Indiana myotis, population growth rates at this subset of sites were unrelated to total pre-WNS population size or interaction with temperature (both $P > 0.05$). In summary, populations of both species in the coolest and driest hibernacula were stable in the first year after WNS detection (Fig. 5).

DISCUSSION

In the past three decades a number of pathogens have invaded new regions and caused declines across entire communities of hosts (Riper *et al.* 1986; Lips *et al.* 2006; LaDeau *et al.* 2007). An outstanding question is which factors determine whether or not disease will cause extinctions, and which populations or species will persist? Although theory has identified several potentially important factors (Castro & Bolker 2005), empirical analyses of disease impacts on multiple host species infected with the same pathogen and varying in sociality are absent, despite the importance of assessing the risk of extinction for effective conservation (Martin *et al.* 2012).

We have shown that differences in sociality can influence the impacts of disease on populations. Declines were higher in larger winter colonies of two solitary species, northern long-eared myotis and tri-coloured bats (Fig. 2). These species rarely form large clusters (Barbour & Davis 1969) and, as a result, contact among individuals of these species would be expected to increase with colony size, resulting in density-dependent transmission. Saturating functions of density were a better fit to the declines for both species than linear functions suggesting that increases in contact rate asymptote with colony size as suggested by theory (McCallum *et al.* 2001). In contrast, in little brown and Indiana myotis, which clustered in tight aggregations during hibernation prior to WNS detection (Davis & Hitchcock 1965; Thomson 1982), we found that declines were equally severe across a large range of colony sizes. This suggests that clustering behaviour facilitated high transmission regardless of colony size, with infected individuals having approximately the same number of contacts in small colonies as they did in larger populations. This pattern is consistent with transmission being frequency-dependent, which conflicts with expectations of how populations transmit non-sexually transmitted pathogens and puts these species at risk of extinction. In these analyses, we implicitly assumed that transmission of Gd occurred directly from bat-to-bat, or if indirect transmission (e.g. bat – substrate – bat) happens, that it was proportional to conspecific density, as might be the case if contact with individual surfaces was species-specific.

The unexpected change we observed in social behaviour following WNS detection (Fig. 4) reveals how altered social aggregation can allow a species to persist, and suggests that theoretical predictions using a static scaling of transmission with host density may need revision. An increase in the number and fraction of little brown myotis roosting individually after populations declined likely results in each bat having fewer neighbours during hibernation and lower pathogen exposure. It is worth noting that the impact of WNS on this species was still severe, with populations stabilising at only 2–20% of the pre-WNS population size. The smaller changes in sociality observed in Indiana myotis apparently were not large enough to reduce transmission and disease impact to allow for populations to stabilise, and this puts this species at a high risk of extinction.

We found little support for total colony size or the abundance of individual heterospecifics as significant predictors of declines. This likely resulted in part from the fact that the species with density-dependent declines (tri-coloured bats and northern long-eared myotis) were never dominant at sites (Fig. S3). Nonetheless, it suggests that the total number of individuals within a hibernaculum is not determining transmission intensity, and that interactions among species are playing a relatively minor role in transmission. We caution that this analysis is purely observational and based on population trends rather than infection data, and thus should be treated as a hypothesis to be tested with data on the infectiousness of each species, and while accounting for other factors, such as environmental conditions.

Our results demonstrate how environmental conditions can modulate disease impacts. We found that declines in Indiana myotis were greater under more humid conditions, which suggest that growth of the fungus, and either intensity or prevalence of infections may be higher in more humid conditions. We also found that for little brown myotis declines were higher in hibernacula with higher temperatures. This suggests that, for this species, increased pathogen growth observed in the lab across the range of temperatures measured in hibernacula, 3–15 °C (Fig. 5) (Gargas *et al.* 2009; Chaturvedi *et al.* 2010), is more important than increases in host immune function, if any. It is possible that the lower declines observed in Indiana myotis compared to little brown myotis may be partly due to the cooler temperatures where Indiana myotis hibernate (Table S1; Fig. 5).

In the four decades prior to WNS detection, bat populations were growing at an average of 8% per year. WNS has reversed this trend and changed the composition of bat communities. Our findings illustrate how among-species variation in sociality, the scaling of declines with colony size and dynamic changes in clustering behaviour influence long-term persistence of species suffering from disease. Geographical variation in sociality and population size, that is widespread in bats and other species (Barbour & Davis 1969; Nunn & Altizer 2006), combined with changes in behaviour in response to disease (Funk *et al.* 2009), will modulate impacts as pathogens spread following introduction. More broadly, our results highlight key factors that can determine the impact of a pathogen on a community of co-occurring hosts, and provide an empirical basis for assessing risk of extinction from disease.

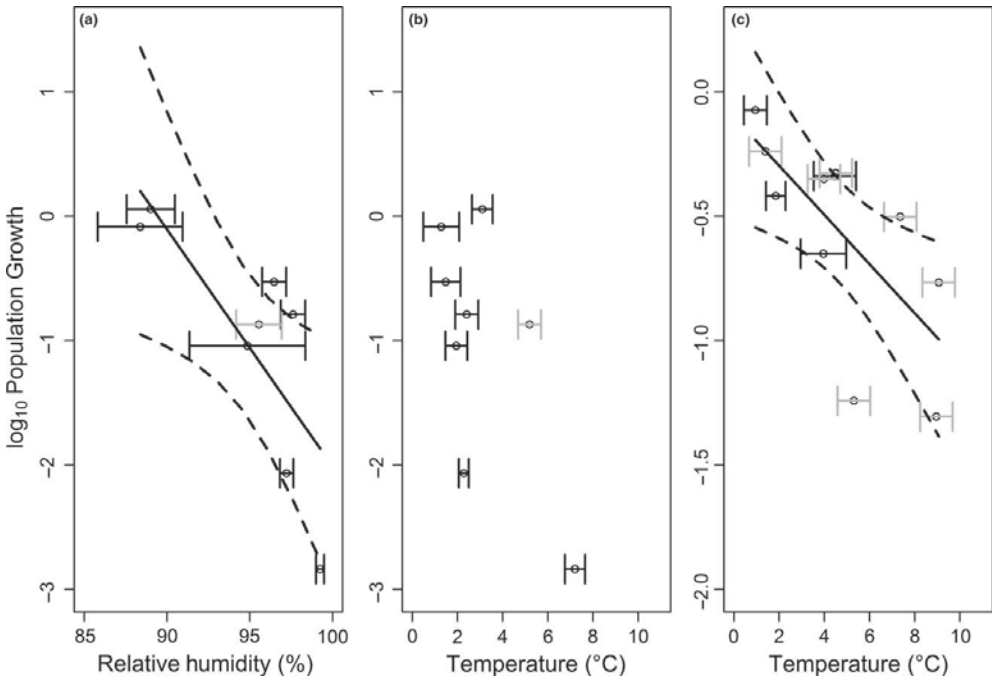

Figure 5 Influence of environmental factors on declines post-WNS detection. (a) Relative humidity and (b) Mean temperature of Indiana myotis roosts at seven hibernacula in New York State. (c) Mean temperature of little brown myotis roosts from 11 sites in New York State. Black error bars show standard error and grey bars show mean standard error estimated from all points.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (DGE-0741448 to KEL, DEB-1115895 to THK, WFF, and AMK, and EF-0914866 to AMK), Bat Conservation International, and Federal Aid and Wildlife Restoration Grant WE-1730-G.

We thank Scott Darling, Carl Herzog, Ryan von Linden, Amanda Bailey, Kathleen O'Conner, Ryan Smith, Tom French, Christina Kocer, and the many individuals that assisted with counts of bats at hibernacula over the past 30 years. We thank Ben Bolker for his enlightening discussion.

AUTHOR CONTRIBUTIONS

All authors conceived of and designed the study. ACH and KEL collected the data. KEL, WFF, JTB and AMK analysed the data. KEL, WFF and AMK wrote the paper. All authors contributed to revising the manuscript.

REFERENCES

Altizer, S., Nunn, C.L., Thrall, P.H., Gittleman, J.L., Antonovics, J., Cunningham, A.A. *et al.* (2003). Social organization and parasite risk in mammals: integrating theory and empirical studies. *Annu. Rev. Ecol. Evol. Syst.*, 34, 517–547.

Anderson, R.M. & May, R.M. (1991). *Infectious Diseases of Humans: Dynamics and Control.* Oxford University Press, London.

Barbour, R. & Davis, W. (1969). *Bats of America.* The University Press of Kentucky, Lexington, KY.

Bates, D., Maechler, M. & Bolker, B. (2011). Lme4: Linear Mixed-Effects Models Using S4 Classes. R Package Version 0.999375-42. Available at: http://CRAN.R-project.org/package=lme4. Last accessed 20 May 2012.

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L. *et al.* (2008). Bat white-nose syndrome: an emerging fungal pathogen? *Science*, 323, 227.

de Castro, F. & Bolker, B. (2005). Mechanisms of disease-induced extinction. *Ecol. Lett.*, 8, 117–126.

Chaturvedi, V., Springer, D.J., Behr, M.J., Ramani, R., Li, X.J., Peck, M.K. *et al.* (2010). Morphological and molecular characterizations of psychrophilic fungus Geomyces destructans from New York bats with white nose ssyndrome (WNS). *PLoS One*, 5, e10783. DOI: 10.1371/journal.pone.0010783.

Cryan, P., Meteyer, C., Boyles, J. & Blehert, D. (2010). Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biol.*, 8, 135.

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

Davis, W.H. & Hitchcock, H.B. (1965). Biology and migration of the bat, *Myotis lucifugus*, in New England. *J. Mammal.*, 46, 296–313.

Dwyer, G., Levin, S.A. & Buttel, L. (1990). A simulation-model of the population-dynamics and evolution of Myxomatosis. *Ecol. Monogr.*, 60, 423–447.

Fenton, A., Fairbairn, J.P., Norman, R. & Hudson, P.J. (2002). Parasite transmission: reconciling theory and reality. *J. Anim. Ecol.*, 71, 893–905.

Frick, W.F., Pollock, J.F., Hicks, A.C., Langwig, K.E., Reynolds, D.S., Turner, G. G. *et al.* (2010). An emerging disease causes regional population collapse of a common North American bat species. *Science*, 329, 679–682.

Funk, S., Gilad, E., Watkins, C. & Jansen, V.A.A. (2009). The spread of awareness and its impact on epidemic outbreaks. *Proc. Natl. Acad. Sci. USA*, 106, 6872–6877.

Gargas, A., Trest, M.T., Christensen, M., Volk, T.J. & Bleher, D.S. (2009). *Geomyces desctructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon*, 108, 147–154.

Getz, W.M. & Pickering, J. (1983). Epidemic models: thresholds and population regulation. *Am. Nat.*, 121, 892–898.

Harvell, C.D., Kim, K., Burkholder, J.M., Colwell, R.R., Epstein, P.R., Grimes, D.J. *et al.* (1999). Emerging marine diseases - climate links and anthropogenic factors. *Science*, 285, 1505–1510.

Hochachka, W.M. & Dhondt, A.A. (2000). Density-dependent decline of host abundance resulting from a new infectious disease. *Proc. Nat. Acad. Sci.*, 97, 5303–5306.

Hudson, P.J., Dobson, A.P. & Newborn, D. (1998). Prevention of population cycles by parasite removal. *Science*, 282, 2256–2258.

Kilpatrick, A.M., Briggs, C.J. & Daszak, P. (2010). The ecology and impact of chytridiomycosis: an emerging disease of amphibians. *Trends Ecol. Evol.*, 25, 109–118.

LaDeau, S.L., Kilpatrick, A.M. & Marra, P.P. (2007). West Nile virus emergence and large-scale declines of North American bird populations. *Nature*, 447, 710–713.

Lips, K.R., Brem, F., Brenes, R., Reeve, J.D., Alford, R.A., Voyles, J. *et al.* (2006). Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Natl. Acad. Sci. USA*, 103, 3165–3170.

Lloyd-Smith, J.O., Getz, W.M. & Westerhoff, H.V. (2004). Frequency-dependent incidence in models of sexually transmitted diseases: portrayal of pair-based transmission and effects of illness on contact behaviour. *Proc. R. Soc. Lond. B. Biol. Sci.*, 271, 625–634.

Lloyd-Smith, J.O., Cross, P.C., Briggs, C.J., Daugherty, M., Getz, W.M., Latto, J. *et al.* (2005). Should we expect population thresholds for wildlife disease? *Trends Ecol. Evol.*, 20, 511–519.

Lockhart, A.B., Thrall, P.H. & Antonovics, J. (1996). Sexually transmitted disease in animals: ecological and evolutionary implications. *Biol. Rev.*, 71, 415–471.

Loehle, C. (1995). Social barriers to pathogen transmission in wild animal populations. *Ecology*, 76, 326–335.

Lorch, J.M., Meteyer, C.U., Behr, M.J., Boyles, J.G., Cryan, P.M., Hicks, A.C. *et al.* (2011). Experimental infection of bats with Geomyces destructans causes white-nose syndrome. *Nature*, 480, 376–378.

Martin, T.G., Nally, S., Burbidge, A.A., Arnall, S., Garnett, S.T., Hayward, M.W. *et al.* (2012). Acting fast helps avoid extinction. *Conserv. Lett.*, DOI: 10.1111/j.1755-263X.2012.00239.x.

McCallum, H., Barlow, N. & Hone, J. (2001). How should pathogen transmission be modelled? *Trends Ecol. Evol.*, 16, 295–300.

McCallum, H., Jones, M., Hawkins, C., Hamede, R., Lachish, S., Sinn, D.L. *et al.* (2009). Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease-induced extinction. *Ecology*, 90, 3379–3392.

Meteyer, C.U., Buckles, E.L., Blehert, D.S., Hicks, A.C., Green, D.E., Shearn-Bochsler, V. *et al.* (2009). Histopathologic criteria to confirm white-nose syndrome in bats. *J. Vet. Diagn. Invest.*, 21, 411–414.

Moore, M.S., Reichard, J.D., Murtha, T.D., Zahedi, B., Fallier, R.M. & Kunz, T. H. (2011). Specific alterations in complement protein activity of little brown myotis *Myotis lucifugus* hibernating in white-nose syndrome affected sites. *PLoS One*, 6, e27430.

Morris, W.F. & Doak, D.F. (2002). *Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis*. Sinauer Associates, Inc., Sunderland, MA.

Nunn, C.L. & Altizer, S. (2006). *Infectious Diseases in Primates: Behavior, Ecology, and Evolution*. Oxford University Press, New York.

Nunn, C., Thrall, P., Stewart, K. & Harcourt, A. (2008). Emerging infectious diseases and animal social systems. *Evol. Ecol.*, 22, 519–543.

Packer, C., Altizer, S., Appel, M., Brown, E., Martenson, J., O'Brien, S.J. *et al.* (1999). Viruses of the Serengiti: patterns of infection and mortality in African lions. *J. Anim. Ecol.*, 68, 1161.

Puechmaille, S.J., Frick, W.F., Kunz, T.H., Racey, P.A., Voigt, C.C., Wibbelt, G. *et al.* (2011). White-nose syndrome: is this emerging disease a threat to European bats? *Trends Ecol. Evol.*, 26, 570–576.

R Development Core Team (2012). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing Vienna, Austria.

van Riper, C., van Riper, S.G., Goff, M.L. & Laird, M. (1986). The epizootiology and ecological significance of malaria in Hawaiian land birds. *Ecol. Monogr.*, 56, 327–344.

Shaman, J., Pitzer, V.E., Viboud, C., Grenfell, B.T. & Lipsitch, M. (2010). Absolute humidity and the seasonal onset of influenza in the continental United States. *PLoS Biol.*, 8, e1000316. DOI: 10.1371/journal.pbio.1000316.

Smith, K.F., Sax, D.F. & Lafferty, K.D. (2006). Evidence for the role of infectious disease in species extinction and endangerment. *Conserv. Biol.*, 20, 1349–1357.

Streicker, D.G., Recuenco, S., Valderrama, W., Benavides, J.G., Vargas, I., Pacheco, V., Condori, R.E.C., Montgomery, J., Rupprecht, C.E., Rohani, P. & Altizer, S. (2012). Ecological and anthropogenic drivers of rabies exposure in vampire bats: implications for transmission and control. *Proc. R. Soc. B*, DOI: 10.1098/rspb.2012.0538.

Thomson, C.E. (1982). Myotis sodalis. *Mamm. Species*, 163, 1–5.

Venables, W.N. & Ripley, B.D. (2002). *Modern Applied Statistics With S*, 4th edn. Springer, New York.

Warnecke, L., Turne, J.M., Bollinger, T.K., Lorch, J.M., Misrae, V., Cryan, P.M. *et al.* (2012). Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proc. Natl. Acad. Sci. USA*, 109, 6999–7003.

Whitlaw, H.A. & Lankester, M.W. (1994). A retrospective evaluation of the effects of parelaphostrongylosis on moose populations. *Can. J. Zool.*, 72, 1–7.

SUPPORTING INFORMATION

Additional Supporting Information may be downloaded via the online version of this article at Wiley Online Library (www.ecologyletters.com).

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organised for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Editor, Kevin Lafferty
Manuscript received 16 April 2012
First decision made 8 May 2012
Manuscript accepted 5 June 2012

PROCEEDINGS B

rspb.royalsocietypublishing.org

Research

Cite this article: Langwig KE *et al*. 2015 Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proc. R. Soc. B* **282**: 20142335. http://dx.doi.org/10.1098/rspb.2014.2335

Received: 21 September 2014
Accepted: 6 November 2014

Subject Areas:
ecology, health and disease and epidemiology

Keywords:
seasonality, emerging infectious disease, fungal pathogen, white-nose syndrome, *Myotis lucifugus*, hibernation

Author for correspondence:
Kate E. Langwig
e-mail: klangwig@gmail.com

Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2014.2335 or via http://rspb.royalsocietypublishing.org.

THE ROYAL SOCIETY PUBLISHING

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

Kate E. Langwig[1], Winifred F. Frick[1], Rick Reynolds[2], Katy L. Parise[3], Kevin P. Drees[3], Joseph R. Hoyt[1], Tina L. Cheng[1], Thomas H. Kunz[3], Jeffrey T. Foster[4,5] and A. Marm Kilpatrick[1]

[1]Department of Ecology and Evolutionary Biology, University of California, EE Biology/EMS, Santa Cruz, CA 95064, USA
[2]Virginia Department of Game and Inland Fisheries, 517 Lee Highway, Verona, VA 24482, USA
[3]Department of Biology, Boston University, Boston, MA 02215, USA
[4]Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ 86011-4073, USA
[5]Department of Molecular, Cellular and Biomedical Science, University of New Hampshire, Durham, NH 03824, USA

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

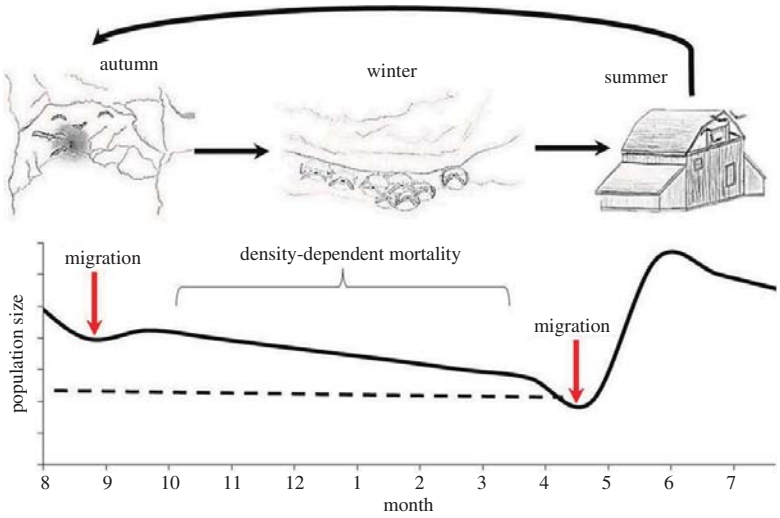

Figure 1. Seasonal life-history patterns of temperate hibernating bats and hypothesized trends in population size in the absence of disease. In the summer, female bats form single-species maternity colonies in human structures, trees or rock crevices, where they give birth to two (*Eptesicus fuscus*) 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

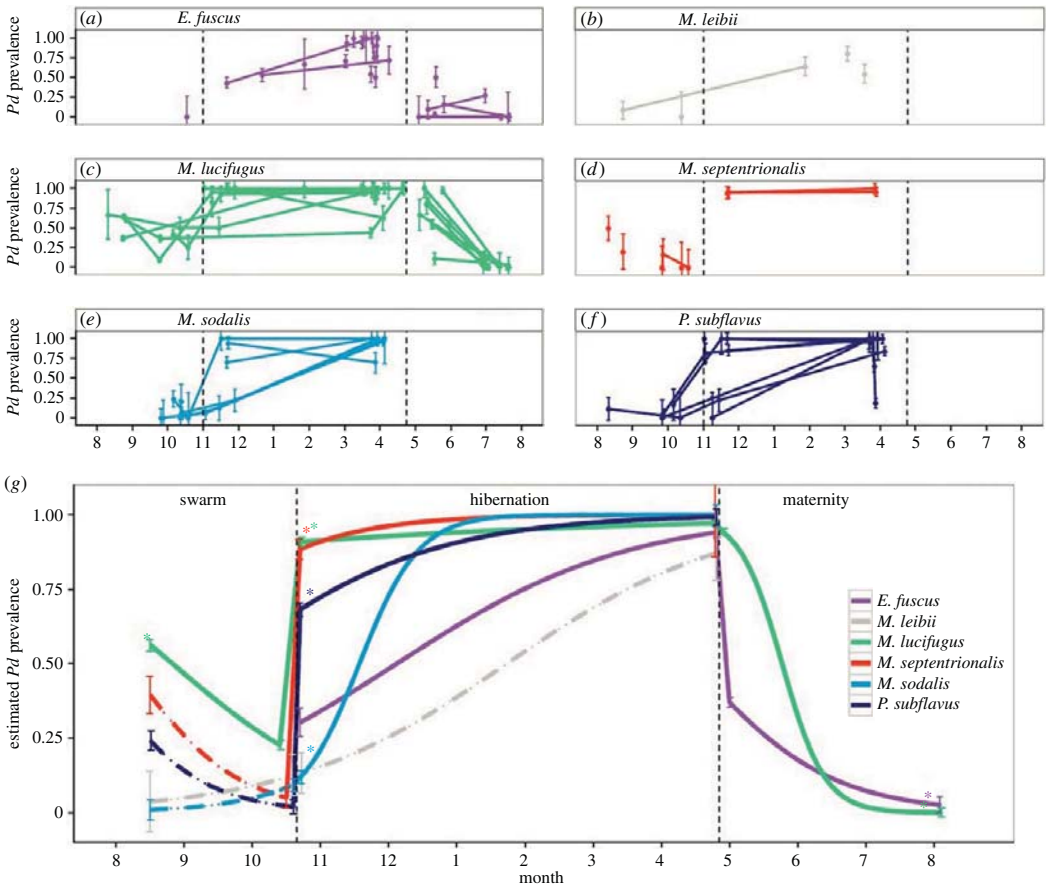

Figure 2. (*a–f*) Seasonal prevalence (±1 s.e.) of *Pseudogymnoascus destructans* for six species across all sites. Lines connect estimates from the same site. (*g*) Model predicted prevalence (± standard error of predicted mean) of *P. destructans* for six species from autumn to summer. Dashed lines 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.

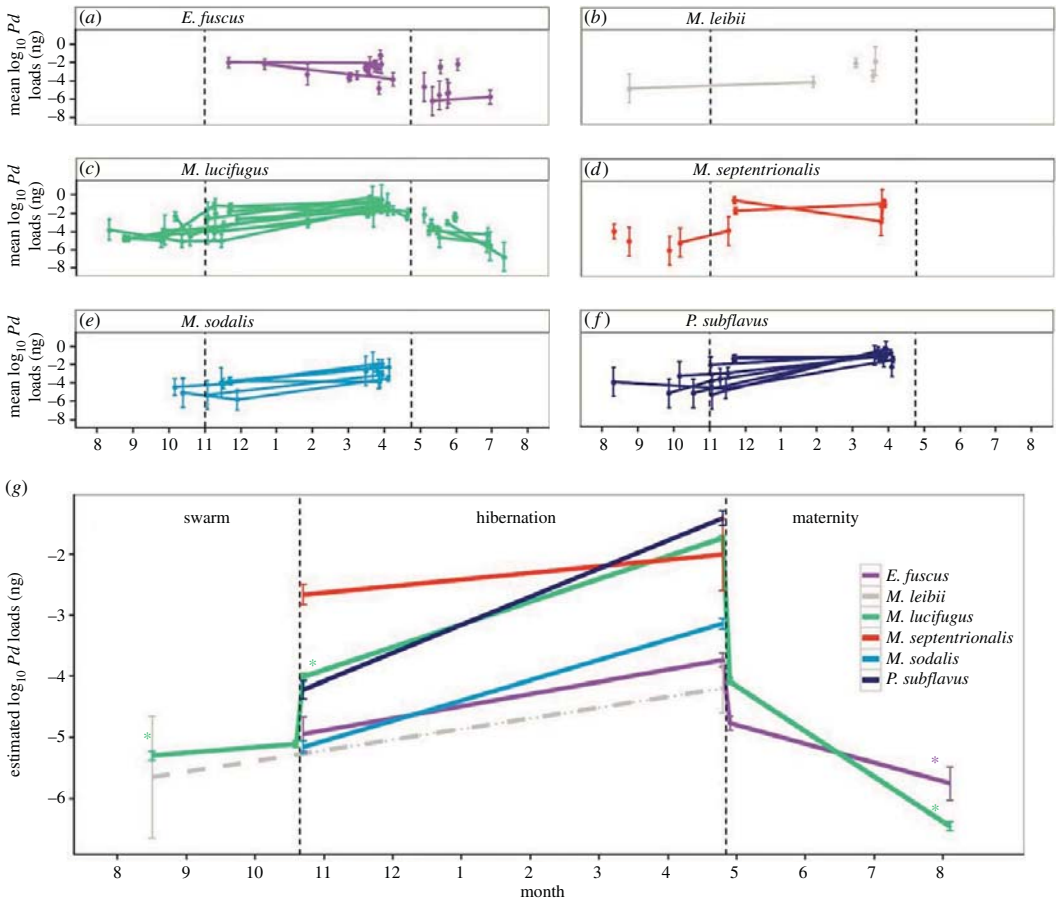

Figure 3. (*a–f*) Seasonal loads (±1 s.e.) of *Pseudogymnoascus destructans* for six species across all sites. Lines connect estimates from the same site. (*g*) Model predicted $\log_{10}$ *P. destructans* loads (± standard error of predicted mean) among species of *P. destructans* for six species from 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

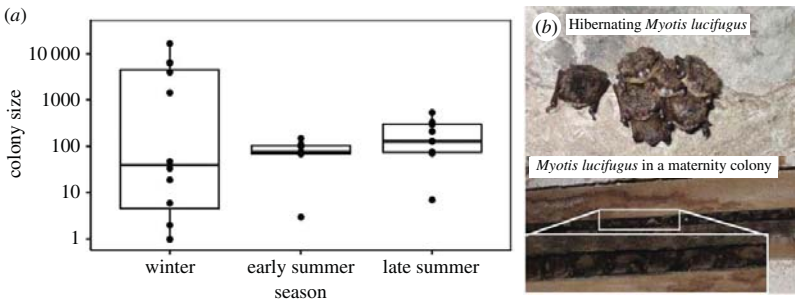

Figure 4. (*a*) Boxplot of colony sizes, on a log scale, of *M. lucifugus* at winter hibernacula and summer maternity colonies. (*b*) Photos of *M. lucifugus* roosting in groups in winter (top) and summer (bottom). (Online version in colour.)

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.

Acknowledgements. We thank the numerous personnel that have contributed to collecting data for this work, in particular Wil Orndorff, Karen Powers, Ryan von Linden, Ashley Banks, Elizabeth Braun de Torrez, Joy Collins and Amanda Hunt. We also thank the numerous land owners who permitted access to these sites, and the state agencies that assisted in coordination (NY, VA, IL, NH, VT and MA.) We thank Maya McCrea for contributed artwork. We thank Bruce Lyon, Stephan Munch and two semi-anonymous reviewers for helpful comments on the manuscript.

Funding statement. This work was supported by the National Science Foundation (DGE-0741448 to K.E.L., DEB-1115895 to T.H.K., W.F.F., J.T.F. and A.M.K.), Bat Conservation International and the National Geographic Society.

References

1. Altizer S, Bartel R, Han BA. 2011 Animal migration and infectious disease risk. *Science* **331**, 296–302. (doi:10.1126/science.1194694)
2. Altizer S, Dobson A, Hosseini P, Hudson P, Pascual M, Rohani P. 2006 Seasonality and the dynamics of infectious diseases. *Ecol. Lett.* **9**, 467–484. (doi:10.1111/j.1461-0248.2005.00879.x)
3. Hochachka WM, Dhondt AA. 2000 Density-dependent decline of host abundance resulting from a new infectious disease. *Proc. Natl Acad. Sci. USA* **97**, 5303–5306. (doi:10.1073/pnas.080551197)
4. Kilpatrick AM, Chmura AA, Gibbons DW, Fleischer RC, Marra PP, Daszak P. 2006 Predicting the global spread of H5N1 avian influenza. *Proc. Natl Acad. Sci. USA* **103**, 19 368–19 373. (doi:10.1073/pnas.0609227103)
5. Altizer S, Hochachka WM, Dhondt AA. 2004 Seasonal dynamics of mycoplasmal conjunctivitis in eastern North American house finches. *J. Anim. Ecol.* **73**, 309–322. (doi:10.1111/j.0021-8790.2004.00807.x)
6. Rappole JH, Derrickson SR, Hubalek Z. 2000 Migratory birds and spread of West Nile virus in the Western Hemisphere. *Emerg. Infect. Dis.* **6**, 319–328. (doi:10.3201/eid0604.000401)
7. Cooley HS, Wielgus RB, Koehler GM, Robinson HS, Maletzke BT. 2009 Does hunting regulate cougar populations? A test of the compensatory mortality hypothesis. *Ecology* **90**, 2913–2921. (doi:10.1890/08-1805.1)
8. Singer FJ, Harting A, Symonds KK, Coughenour MB. 1997 Density dependence, compensation, and environmental effects on elk calf mortality in Yellowstone National Park. *J. Wildl. Manage.* **61**, 12–25. (doi:10.2307/3802410)
9. Kistner EJ, Belovsky GE. 2014 Host dynamics determine responses to disease: additive vs. compensatory mortality in a grasshopper–pathogen system. *Ecology* **95**, 2579–2588. (doi:10.1890/13-0969.1)
10. Bjornstad ON, Finkenstadt BF, Grenfell BT. 2002 Dynamics of measles epidemics: estimating scaling of transmission rates using a time series SIR model. *Ecol. Monogr.* **72**, 169–184. (doi:10.2307/3100023)
11. Dhondt AA, States SL, Dhondt KV, Schat KA. 2012 Understanding the origin of seasonal epidemics of mycoplasmal conjunctivitis. *J. Anim. Ecol.* **81**, 996–1003. (doi:10.1111/j.1365-2656.2012.01986.x)
12. Nunn CL, Altizer S. 2006 *Infectious diseases in primates: behavior, ecology, and evolution*. New York, NY: Oxford University Press.
13. Hamede RK, Bashford J, McCallum H, Jones M. 2009 Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in

social behaviour and its implications for transmission of devil facial tumour disease. *Ecol. Lett.* **12**, 1147–1157. (doi:10.1111/j.1461-0248.2009.01370.x)

14. George DB, Webb CT, Farnsworth ML, O'Shea TJ, Bowen RA, Smith DL, Stanley TR, Ellison LE, Rupprecht CE. 2011 Host and viral ecology determine bat rabies seasonality and maintenance. *Proc. Natl Acad. Sci. USA* **108**, 10 208–10 213. (doi:10.1073/pnas.1010875108)
15. Hosseini PR, Dhondt AA, Dobson A. 2004 Seasonality and wildlife disease: how seasonal birth, aggregation and variation in immunity affect the dynamics of *Mycoplasma gallisepticum* in house finches. *Proc. R. Soc. Lond. B* **271**, 2569–2577. (doi:10.1098/rspb.2004.2938)
16. Martinez-Bakker M, Bakker KM, King AA, Rohani P. 2014 Human birth seasonality: latitudinal gradient and interplay with childhood disease dynamics. *Proc. R. Soc. B* **281**, 20132438. (doi:10.1098/rspb.2013.2438)
17. Breban R, Drake JM, Stallknecht DE, Rohani P. 2009 The role of environmental transmission in recurrent avian influenza epidemics. *PLoS Comput. Biol.* **5**, e1000346. (doi:10.1371/journal.pcbi.1000346)
18. Rohani P, Breban R, Stallknecht DE, Drake JM. 2009 Environmental transmission of low pathogenicity avian influenza viruses and its implications for pathogen invasion. *Proc. Natl Acad. Sci. USA* **106**, 10 365–10 369. (doi:10.1073/pnas.0809026106)
19. Shaman J, Pitzer VE, Viboud C, Grenfell BT, Lipsitch M. 2010 Absolute humidity and the seasonal onset of influenza in the continental United States. *PLoS Biol.* **8**, e1000316. (doi:10.1371/journal.pbio.1000316)
20. Hillgarth N, Ramenofsky M, Wingfield J. 1997 Testosterone and sexual selection. *Behav. Ecol.* **8**, 108–109. (doi:10.1093/beheco/8.1.108)
21. Mougeot F, Irvine JR, Seivwright L, Redpath SM, Piertney S. 2004 Testosterone, immunocompetence, and honest sexual signaling in male red grouse. *Behav. Ecol.* **15**, 930–937. (doi:10.1093/beheco/arh087)
22. Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, Wibbelt G, Blehert DS, Willis CKR. 2012 Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proc. Natl Acad. Sci. USA* **109**, 6999–7003. (doi:10.1073/pnas.1200374109)
23. Lorch JM *et al*. 2011 Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* **480**, 376–378. (doi:10.1038/nature10590)
24. Blehert DS *et al*. 2008 Bat white-nose syndrome: an emerging fungal pathogen? *Science* **323**, 227. (doi:10.1126/science.1163874)
25. Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Kilpatrick AM. 2012 Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecol. Lett.* **15**, 1050–1057. (doi:10.1111/j.1461-0248.2012.01829.x)
26. Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH. 2010 An emerging disease causes regional population collapse of a common North American bat species. *Science* **329**, 679–682. (doi:10.1126/science.1188594)
27. Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, Wibbelt G, Willis CKR. 2013 Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. *Biol. Lett.* **9**, 20130177. (doi:10.1098/rsbl.2013.0177)
28. Verant ML, Boyles JG, Waldrep W, Wibbelt G, Blehert DS. 2012 Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. *PLoS ONE* **7**, e46280. (doi:10.1371/journal.pone.0046280)
29. Lorch JM, Lindner DL, Gargas A, Muller LK, Minnis AM, Blehert DS. 2013 A culture-based survey of fungi in soil from bat hibernacula in the eastern United States and its implications for detection of *Geomyces destructans*, the causal agent of bat white-nose syndrome. *Mycologia* **105**, 237–252. (doi:10.3852/12-207)
30. Lorch JM, Muller LK, Russell RE, O'Connor M, Lindner DL, Blehert DS. 2013 Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the eastern United States. *Appl. Environ. Microbiol.* **79**, 1293–1301. (doi:10.1128/aem.02939-12)
31. Hoyt J, Langwig K, Okoniewski J, Frick W, Stone W, Kilpatrick AM. In press. Long-term persistence of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, in the absence of bats. *EcoHealth* (doi:10.1007/s10393-014-0981-4)
32. Barbour R, Davis W. 1969 *Bats of America*. Lexington, KY: The University Press of Kentucky.
33. Thomas DW, Fenton MB, Barclay RMR. 1979 Social behavior of the little brown bat, *Myotis lucifugus*. I. Mating behavior. *Behav. Ecol. Sociobiol.* **6**, 129–136. (doi:10.1007/BF00292559)
34. Kunz TH, Lumsden L. 2003 Colony size. In *Bat ecology* (eds TH Kunz, MB Fenton), pp. 49–56. Chicago, IL: University of Chicago Press.
35. Shelley V, Kaiser S, Shelley E, Williams T, Kramer M, Haman K, Keel K, Barton HA. 2013 Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of white-nose snydrome (WNS). *J. Cave Karst Stud.* **75**, 1–10. (doi:10.4311/2011lsc0249)
36. Muller LK, Lorch JM, Lindner DL, O'Connor M, Gargas A, Blehert DS. 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. (doi:10.3852/12-242)
37. Bates D, Maechler M, Bolker B. 2011 lme4: linear mixed-effects models using S4 classes. R package version 0999375-42 http://CRAN.R-project.org/package=lme4
38. R Development Core Team 2012 *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.
39. Pathak AK, Boag B, Poss M, Harvill ET, Cattadori IM. 2011 Seasonal breeding drives the incidence of a chronic bacterial infection in a free-living herbivore population. *Epidemiol. Infect.* **139**, 1210–1219. (doi:10.1017/s0950268810002311)
40. Lindner DL, Gargas A, Lorch JM, Banik MT, Glaeser J, Kunz TH, Blehert DS. 2011 DNA-based detection of the fungal pathogen *Geomyces destructans* in soils from bat hibernacula. *Mycologia* **103**, 241–246. (doi:10.3852/10-262)
41. Thomas D. 1995 The physiological ecology of hibernation in vespertilionid bats. *Symp. Zool. Soc. Lond.* **67**, 233–244.
42. Raffel TR, Romansic JM, Halstead NT, McMahon TA, Venesky MD, Rohr JR. 2013 Disease and thermal acclimation in a more variable and unpredictable climate. *Nat. Clim. Change* **3**, 146–151. (doi:10.1038/nclimate1659)
43. Altringham JD. 2011 *Bats: from evolution to conservation*, 2nd edn. New York, NY: Oxford University Press.
44. Boyles J, Storm J, Brack V. 2008 Thermal benefits of clustering during hibernation: a field test of competing hypotheses on *Myotis sodalis*. *Funct. Ecol.* **22**, 632–636. (doi:10.1111/j.1365-2435.2008.01423.x)
45. Courchamp F, Clutton-Brock T, Grenfell B. 1999 Inverse density dependence and the Allee effect. *Trends Ecol. Evol.* **14**, 405–410. (doi:10.1016/S0169-5347(99)01683-3)
46. Kunz TH, Wrazen JA, Burnett CD. 1998 Changes in body mass and fat reserves in pre-hibernating little brown bats (*Myotis lucifugus*). *Ecoscience* **5**, 8–17.
47. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. 2006 West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biol.* **4**, 606–610. (doi:10.1371/journal.pbio.0040082)
48. Maher SP, Kramer AM, Pulliam JT, Zokan MA, Bowden SE, Barton HD, Magori K, Drake JM. 2012 Spread of white-nose syndrome on a network regulated by geography and climate. *Nat. Commun.* **3**, 1306. (doi:10.1038/ncomms2301)
49. Norquay KJO, Martinez-Nunez F, Dubois JE, Monson KM, Willis CKR. 2013 Long-distance movements of little brown bats (*Myotis lucifugus*). *J. Mammal* **94**, 506–515. (doi:10.1644/12-mamm-a-065.1)
50. Matheson AL, Campbell KL, Willis CKR. 2010 Feasting, fasting and freezing: energetic effects of meal size and temperature on torpor expression by little brown bats *Myotis lucifugus*. *J. Exp. Biol.* **213**, 2165–2173. (doi:10.1242/jeb.040188)
51. Kilpatrick AM, LaDeau SL, Marra PP. 2007 Ecology of West Nile virus transmission and its impact on birds in the Western Hemisphere. *Auk* **124**, 1121–1136. (doi:10.1642/0004-8038(2007)124[1121:eownvt]2.0.co;2)
52. Racey PA. 1970 The breeding, care and management of vespertilionid bats in the laboratory. *Lab Anim.* **4**, 171–183. (doi:10.1258/002367770781071635)
53. Langwig KE *et al*. 2015 Hibernation drives seasonal dynamics of a fungal disease. Data from: Dryad Digital Repository. (doi:10.5061/dryad.k4h77)

Title: **Infection intensity determines host impacts from fungal disease**

Authors: Kate E. Langwig[a*], Winifred F. Frick[a], Katy L. Parise[b], Joseph R. Hoyt[a], Kevin P. Drees[b,c], Thomas H. Kunz[d], Jeffrey T. Foster[b,c] A. Marm Kilpatrick[a]

Author affiliations:

[a]*Department of Ecology and Evolutionary Biology, University of California, EE Biology/EMS, Santa Cruz, CA 95064;*518.928.9714, klangwig@gmail.com*

[b]*Center for Microbial Genetics and Genomics, Northern Arizona University, Flagstaff, AZ 86011-4073, USA*

[c]*Department of Molecular, Cellular & Biomedical Science, University of New Hampshire, Durham, NH 03824, USA*

[d]*Department of Biology, Boston University, Boston, MA 02215*

Keywords: emerging infectious disease, microparasite, macroparasite, multi-host pathogen, wildlife disease, *Myotis lucifugus*, white-nose syndrome, fungal pathogen

Abstract:

Disease plays an important role in structuring species communities, including driving some species toward extinction, while others suffer relatively little impact. White-nose syndrome (WNS), an emerging fungal disease in bats, has decimated populations of some species while sympatric and taxonomically related species have experienced little effect. Why disease impacts vary among host species remains poorly understood for many multi-host pathogens, including WNS. We analyzed data on infection prevalence, intensity, and environmental factors to determine how variation in infection dynamics among sympatric host species influenced severity of WNS population impacts. Intense winter transmission resulted in almost uniformly high infection prevalence, whereas infection intensity varied over three orders of magnitude among species, and explained 97% of the variation among species in disease impacts. Infection intensity was also correlated with roosting temperatures of bats during hibernation, with bats roosting at warmer temperatures having more intense infections and higher WNS impact. We found evidence of a threshold of infection intensity above which mortality occurs that was similar for multiple species. More broadly, these results show how behavioral differences – in this case microclimate preferences - that may have been previously adaptive, are deleterious after the introduction of a new pathogen. Management to reduce intensity rather than exposure may be an effective way of reducing disease impact and preventing extinctions.

Significance Statement. While the role of infectious disease in structuring species communities through variable impacts among hosts is undebated, the causes of this variation are poorly understood. We show that in the emerging fungal disease of bats, white-nose syndrome, variation in population impacts among hosts is driven by differences in intensity of fungal infections. Infection intensity is, in turn, driven by differences in microclimate preferences among bat species, and there is a threshold of maximum fungal intensity at which mortality occurs. Managing infectious diseases, particularly in wildlife, can be extremely challenging, but for white-nose syndrome, reducing infection intensity may be an efficacious and easier way to reduce population impacts.

Text:

Emerging infectious diseases are an important threat to wildlife populations (Daszak et al. 2000). Increases in human trade and travel over the last fifty years have driven increases in disease emergence events, and introduction of generalist pathogens threaten many wildlife populations (Jones et al. 2008). Generalist pathogens are capable of infecting multiple host species which has led to devastation of populations (Lips et al. 2006, Frick et al. 2010), species extinctions (Smith et al. 2006, Skerratt et al. 2007), and cascading effects on communities and ecosystems (Whitlaw and Lankester 1994, Holdo et al. 2009). Changes in communities and ecosystems are frequently caused by the variability in population impacts of multi-host pathogens, with some species declining to extinction whereas others suffer little

mortality (van Riper et al. 1986, Harvell et al. 1999, Lips et al. 2006, LaDeau et al. 2007, Langwig et al. 2012). Understanding the mechanisms that drive variation in species impacts can help to reduce impacts, prevent species extinctions, and thus minimize ecosystem effects.

The impact of a disease on a population is the product of the fraction of the population infected multiplied by the fraction of infected individuals that die or fail to reproduce from disease, directly, or indirectly and disease management can target either or both of these components (Wobeser 2002, LaDeau et al. 2007, Kilpatrick et al. 2013). Reducing transmission by reducing contacts among individuals is often very difficult, whereas reducing mortality of infected individuals is sometimes more feasible. Reducing mortality can be done through direct treatment, or by altering environmental conditions that influence pathogen growth or host defenses. Treatment can sometimes reduce transmission by reducing infectiousness (Cohen et al. 2011), and environmental manipulations may also reduce transmission by altering growth and survival of the pathogen on or outside the host (Shaman et al. 2010). However, disentangling which factors are most important in determining disease impacts, and which will be most effective for disease management, can be challenging, and requires understanding interactions between hosts, the pathogen, and the environment.

Multi-host fungal pathogens that have recently emerged to threaten multiple species with extinction (Fisher et al. 2012) are particularly emblematic of the

interplay between host, pathogen and the environment, because many of them survive outside the host for long periods, or they infect hosts with variable body temperatures and are therefore strongly affected by environmental conditions. For example, for chytridomycosis, a fungal disease of amphibians, high environmental temperatures reduce mortality in amphibian populations, with highest mortality in cool, high-elevation areas (Kilpatrick et al. 2010). At the extreme, amphibians living in hot springs were uninfected, despite widespread infection of surrounding populations, because the warm water temperatures either impeded fungal growth, killed free-living stages, enhanced host immune function, or some combination of all three (Schlaepfer et al. 2007) .

White-nose syndrome (WNS), caused by the fungal pathogen *Pseudogymnoascus destructans* (Lorch et al. 2011, Warnecke et al. 2012), is a recently emerged fungal disease that has caused widespread mortality in many communities of hibernating bats and is predicted to drive several species extinct (Langwig et al. 2012, Frick et al. 2015). Bats first become infected when they return to hibernacula in the fall, and both transmission and fungal growth on bats occurs primarily during winter once bats lower their body temperature and begin to hibernate (Langwig et al. 2015a). Mortality from this disease differs substantially among species and populations, even if they occur at the same sites, with some species declining more than 90% in the first year following WNS detection, whereas population growth in other species only decreased 8% (Langwig et al. 2012).

Here, we examine how differences in exposure, infection intensity, and environmental factors determine disease impacts in six species of bats. The relatively high infection prevalence (>50%) observed in many populations of all six species (Langwig et al. 2015a) suggests that variation among species in mortality after infection may be especially important in determining population impacts. Although the exact mechanism by which infection with *P. destructans* leads to death is unknown, tissue damage from fungal invasion is thought to set off a cascade of physiological disruptions (Warnecke et al. 2013, Verant et al. 2014), which eventually lead to death approximately 70-120 days after infection (Warnecke et al. 2012). Mortality in another fungal disease, chytridiomycosis, occurred when infection intensity reached a threshold (Vredenburg et al. 2010), suggesting that variation in pathogen growth on hosts might be a key driver of mortality. *Pseudogymnoascus destructans* growth increases with temperature across the range of hibernation temperatures used by these bats (approximately 1-12° C) (Webb et al. 1996, Brack 2007, Verant et al. 2012). Thus, we hypothesize that species that roost at warmer temperatures will have higher fungal loads, and suffer higher mortality and impact from WNS. To test these predictions and hypotheses we compared patterns of infection prevalence and intensity to differences in species impacts, and then examined links between microclimate temperatures used by bats and infection intensity.

Results

We sampled 1,314 bats of six species in 21 hibernation sites across New York, Virginia, Massachusetts, Vermont and Illinois. In early winter, infection prevalence varied from 20% to 90% among species and was significantly correlated with impacts, measured as the difference in population growth rate before and in the first year after detection of WNS (Fig. 1A). Loads (infection intensity) on infected bats varied almost two orders of magnitude among species at the beginning of hibernation and were even more strongly correlated with impacts (Figure 1B). By late hibernation, prevalence of *P. destructans* had increased to >80% for five of six species and was no longer significantly correlated with WNS impacts (Figure 1C). In contrast, *P. destructans* loads varied over three orders of magnitude among species and estimated loads in late hibernation were very strongly correlated with WNS impacts (Figure 1D), and neither early or late prevalence added explanatory power to this model (both $P > 0.45$).

Species' estimated fungal loads at the end of hibernation increased with roosting temperatures (Figure 2). The species with the highest *P. destructans* loads, the Northern long eared bat, roosted at temperatures, on average, 6° Celsius warmer than the Eastern small-footed bat which had loads that were 100-fold lower. Hibernacula temperatures varied substantially among colonies, with the best fitting model of roosting temperature including an interaction between species and site.

Fungal loads increased during winter but appeared to reach a maximum threshold (Figure 3, Figure 4). The distributions of loads in early hibernation were consistently less skewed (closer to 0) for most species than distributions of fungal

loads during late hibernation, which were negatively skewed for the three highly impacted species. The skewness of these distributions suggests a maximum fungal load threshold above which no fungal loads are detected (Figure 3, Supplemental Table 1). Across 19 colonies, of five species, the increase in infection intensity was negatively correlated with mean fungal loads in early hibernation (Figure 4). Average loads in colonies that were already high in early hibernation showed little increase over winter, whereas colonies with low average loads of -5 log ng increased 1 log or 10-fold each month during hibernation. The best fitting model included an interaction between species and early hibernation *P. destructans* loads. Comparisons of intercepts and coefficients suggest that the saturation in fungal growth with increasing early hibernation loads was essentially identical for little brown and tricolored bats (Supplemental Table 2). In contrast, increases in fungal loads were lower for Indiana bats (but data were insufficient to accurate estimate the saturation threshold).

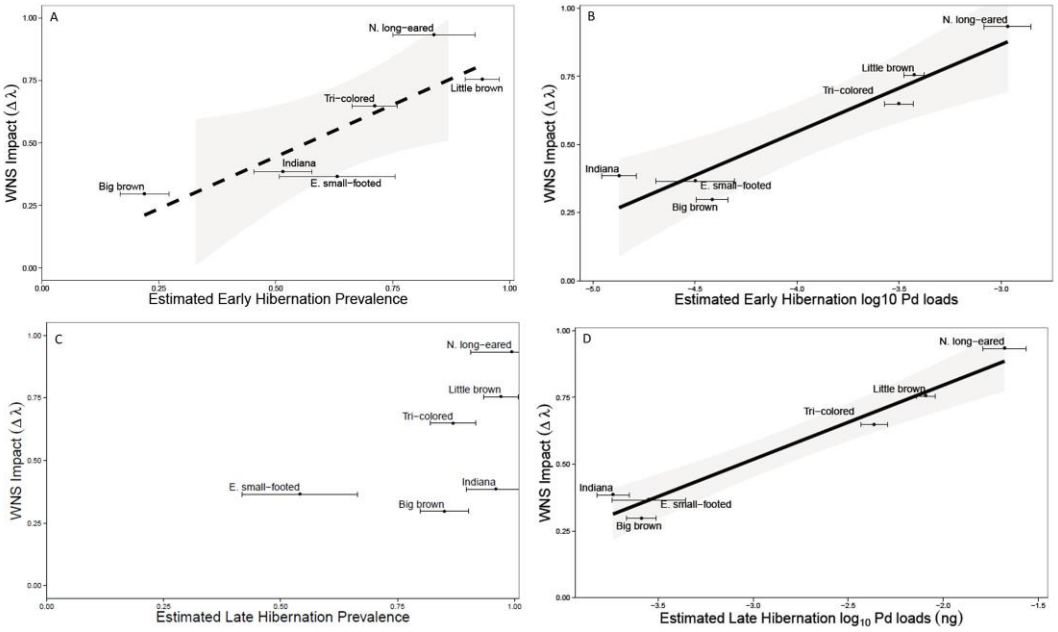

Figure 1. Impact of white-nose syndrome, measured as the change in population growth rates (λ) following the detection of WNS, and infection prevalence (A, C) and intensity (B,D) of *P. destructans* for six bat species. Error bars show +/- standard error of predicted mean. Lines denote significant relationships, with dashed lines indicating relationships significant only in univariate regressions. Infection prevalence during early hibernation was significantly correlated with WNS impacts (intercept: -0.09; slope: 1.04± 0.034; t = 3.03; P = 0.039; R^2 = 0.70), as were early hibernation loads (intercept: 1.81, slope: 0.03.2± 0.036, t=8.90, P=0.005, R^2=0.95). Late hibernation prevalence was high, and not significantly predictive of WNS impacts (intercept: -0.22, slope: 0.92 ± 0.5, t=1.82 , P=0.14, R^2=0.45) whereas late hibernation loads were highly correlated with WNS impacts (intercept: 1.33, slope: 0.27 ± 0.021, t=13.3, P=0.0002, R^2=0.98).

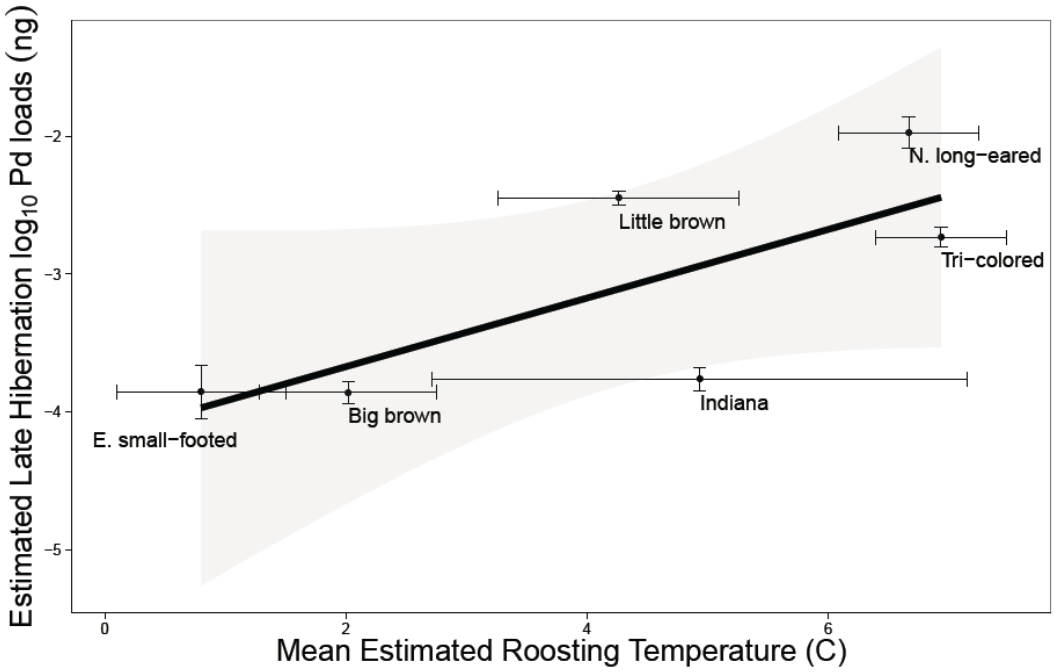

Figure 2. Estimated *P. destructans* loads in late hibernation and hibernation roosting temperature for six bat species. Data shown include only those individuals in which roost temperatures were collected (n = 419). Roosting temperature was significantly positively correlated with late hibernation loads (intercept: -4.23; slope: 0.27 ± 0.12, one-tailed P=0.04, t=2.34, R^2=0.575762)

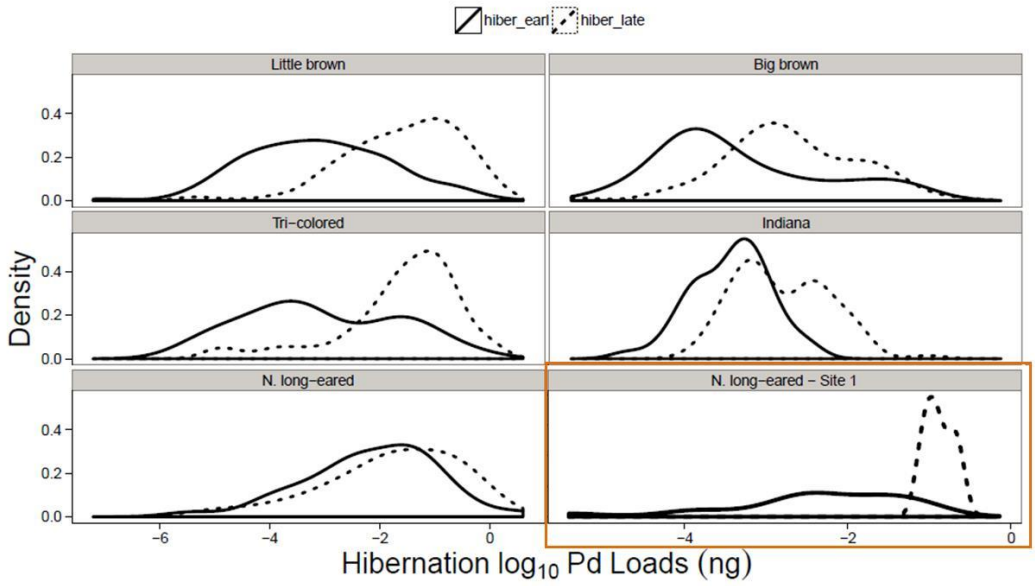

Figure 3. Distributions of fungal loads on bats in early (solid line) and late hibernation (dashed line). Fungal loads in highly impacted species were all negatively (left) skewed at the end of hibernation. At some sites with exceedingly high mortality of Northern long-eared bats (99% decline over the winter), distributions were narrower and heavily skewed by the end of hibernation (orange panel).

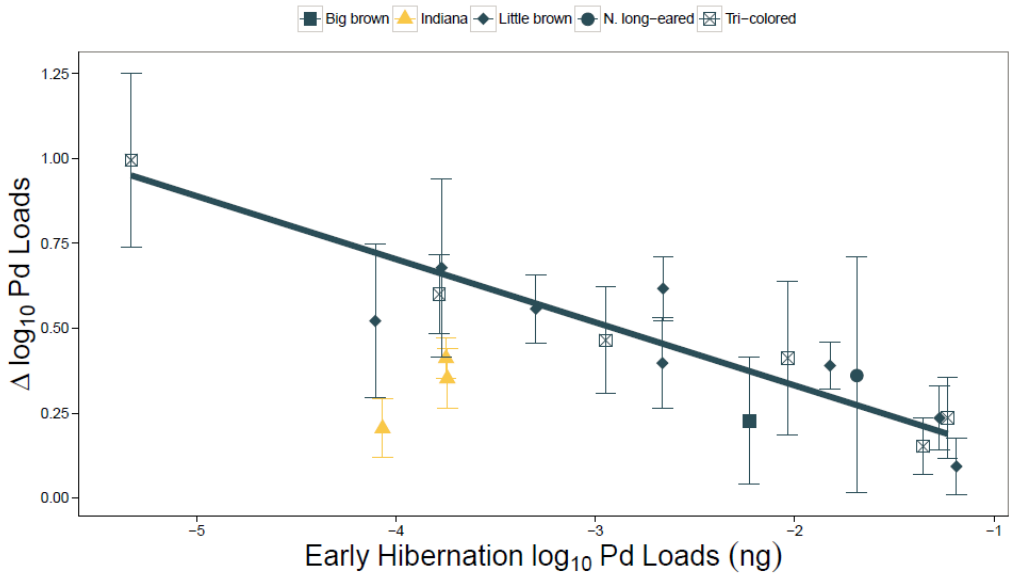

Figure 4. Change in infection intensity over time of *P. destructans* plotted against infection intensity at the beginning of hibernation for five species of bats in 19 colonies. Colonies included in the analysis had an early hibernation prevalence greater than 50%. Sample sizes of *M. septentrionalis* were low due to high mortality. The best fitting model included an interaction between species and early hibernation *P. destructans* loads, and comparisons of intercepts and coefficients suggest that the saturation in fungal growth with increasing early hibernation loads was similar for little brown and tricolored bats, the best sampled species.

Discussion

The emergence of WNS in North America has altered bat communities on a continental scale, by decimating some species while others are far less affected (Langwig et al. 2012, Frick et al. 2015). Our results suggest that differences in

impacts are driven by differential infection intensity and fungal growth among species. Differences in timing of infection may result in significant differences in exposure and therefore infection prevalence at the beginning of hibernation, but as the fungus begins to proliferate, microclimate preferences among species result in differential fungal growth and infection intensity, and this leads to widely varying population impacts of white-nose syndrome. In contrast, infection prevalence was extremely high in all species at the end of hibernation which may result from widespread environmental contamination (Frick et al. submitted; Langwig, Hoyt, et al. In Press), and high contact rates among individuals (Hoyt et al, in prep).

The differences in infection intensity among species are more likely due to temperature-dependent fungal growth than to differences in bat immune function (Verant et al. 2012). Warmer roosting temperatures, up to 13°C, increase fungal growth rates, whereas the increase in bats' immune function with temperature (Bouma et al. 2010, Bouma et al. 2011, Moore et al. 2013) would likely decrease fungal loads. Our finding that bats roosting at warmer temperatures had higher fungal loads suggest that temperature-dependent pathogen growth likely overwhelmed any differences in immune function, and suggest that during hibernation bats are essentially Petri dishes incubating at different temperatures.

The increase in WNS impact with roosting temperature suggests that although preferences for higher roosting temperatures in some bat species may have been beneficial prior to the arrival of WNS, they became highly maladaptive after *P. destructans* was introduced. However, there was considerable variation in roosting

temperature within each species (Figure 2) and intense mortality from WNS is likely selecting for individuals that roost at cooler locations. If preference for roosting temperature is a heritable trait, WNS mortality could drive the evolution of a change in behavior, as well as select for genes associated with surviving colder winter roosting temperatures. Future studies should examine changes in the roosting behavior of bats as populations decline and possibly stabilize or recover.

Our results indicate that there is a threshold of infection intensity above which loads do not increase further. One explanation for this pattern is that as loads on individual bats reach the threshold, they die from disease associated pathology, which has been suggested for *Batrachochytrium dendrobatidis*, a fungal pathogen of amphibians (Vredenburg et al. 2010). The relationship between increases in loads over winter and loads in early hibernation were similar for little brown bats and tricolored bats suggesting that they share a common threshold. The skewness of load distributions for Northern long-eared bats suggests a threshold present in this species as well, and the negative skew of the distribution in early hibernation suggests that many bats may already be near a maximum load threshold, thus explaining the exceedingly high mortality in this species. However, it is also possible that the smaller increase in fungal loads in colonies of bats that begin winter at higher infection intensity is simply due to density-dependent fungal growth on individual bats. Quantifying fungal loads over time from infection to mortality in an experimental infection study or on banded bats, and could determine which mechanism is resulting in the threshold of fungal loads we observed.

Understanding the drivers of variation in WNS impacts can be used to more effectively guide management interventions. Cool and drier hibernacula appear to serve as refugia from disease (Langwig et al. 2012). Managers should consider manipulating hibernacula entrances to create cooler sites or restricting access to the warmer and wetter portions of hibernacula. These alterations could provide roosting conditions that promote lower infection loads and increase chances of survival. Successful environmental manipulation would provide a long-term solution for management of WNS, whereas chemical or biological treatments that are not self-replicating will require continual reapplication making long-term management on a broad scale challenging (Langwig et al. 2015b). Some hibernacula contain tens of thousands of bats of multiple species, and making sites cooler could save these diverse populations and help maintain genetic diversity.

The relationship between hibernation roosting temperature and infection intensity could potentially be used to predict impacts to Western bat species where *P. destructans* has not yet arrived. For example, our analysis would suggest that *Myotis ciliolabrum*, which frequently hibernates below 3°C (Holloway and Barclay 2001), would be predicted to suffer much lower impacts from WNS than eastern *Myotis* species which hibernate at warmer temperatures. However, predictions should be made with caution because cave obligate species such as *Corynorhinus townsendii virginianus* appear to suffer little impacts from WNS despite roosting at warmer temperatures (Coleman and Reichard 2014).

For WNS, and a growing number of emerging pathogens (Briggs et al. 2010), infection intensity is an important determinant of disease impacts. Management to reduce intensity rather than exposure may be an effective way of reducing mortality from disease and preventing species extinctions. Reducing transmission in wildlife pathogens can be exceedingly difficult (Wobeser 2002), particularly in the face of biotic and abiotic reservoirs, which also increase the likelihood of host extinction (de Castro and Bolker 2005). WNS has not yet caused a global extinction of a bat species, and management actions to save bats threatened with extinction are still possible. Our results indicate that reducing infection intensity on bats is likely sufficient to stave off mortality, and prevent species extinction.

Methods

Field sampling and analysis

We sampled bats at 21 hibernacula in New York, Vermont, Massachusetts, Virginia, and Illinois. We sampled bats twice per hibernation season (November/December, and March/early April) in sites where *P. destructans* had been detected at least one year previously. One to six bat species were present in each hibernacula, including the following species: little brown myotis (*Myotis lucifugus*), Northern long-eared myotis (*Myotis septentrionalis*), Eastern small-footed myotis (*Myotis leibii*), Indiana myotis (*Myotis sodalis*), Tri-colored bat (*Perimyotis subflavus*), and the big brown bat (*Eptesicus fuscus*). We followed field hygiene

protocols in accordance with United State Fish & Wildlife Service WNS Decontamination Guidelines, and individual state recommendations. All research was conducted under protocol #11-022 approved by the IACUC of Boston University and protocol #Frickw1106 approved by the IACUC of the University of California, Santa Cruz.

We used epidermal swab sampling to determine infection prevalence and infection intensity of *P. destructans*. We sampled bats by dipping a sterile polyester swabs into sterile water to moisten, and rubbing the swab five times across the forearm and muzzle of a bat. Swabs were stored in RNAlater® for preservation until extraction. We recorded skin temperature of approximately half of sampled bats using an infrared laser thermometer at time of sampling. We tested samples for *P. destructans* DNA using real-time PCR (Muller et al. 2013) and quantified infection intensity based on the cycle threshold (C_t) value to estimate a fungal load on each bat, with a cut-off of 40 cycles. Quantification of serial dilutions of the DNA from 10 ng to 1000 fg resulted in C_t scores ranging from 17.33 to 30.74 and a quantification relationship of $C_t = -3.348*\log_{10}(P.destructans[ng]) + 22.049$, $r^2 = 0.986$.

Statistical Analyses

To test hypotheses of how prevalence and loads influenced WNS impacts, we used generalized linear mixed models (glmm) with site as a random effect and species interacting with date of sampling as fixed effects (function *glmer* in package lme4 (Bates et al. 2011) in R v3.02 (R Development Core Team 2012)) to predict *P.*

destructans prevalence and infection intensity for each species. We calculated a predicted prevalence and $\log_{10}$ loads of each species on December 1st and March 1st from the glmm model described above in order to standardize early and late hibernation time points because prevalence and loads increase over winter and bats were sampled at different times (Langwig et al. 2015a). We quantified WNS impact using the change in median population growth rate, $\Delta\lambda$, between the year before and after the first year of WNS detection, based on a previous analyses of the six species of hibernating bats common in the northeastern USA (Langwig et al. 2012). We examined the effect *P. destructans* prevalence and loads on WNS impacts using phylogenetic regression (Blomberg et al. 2003) in MATLAB (v. R2013).

We estimated a mean roosting temperature for each of the six species by using an interactive model of site and species, and averaging the mean predicted temperatures from the regression. We re-calculated average loads for each species using only data for the individuals for which roosting temperature data were also collected. We then used phylogenetic regression to examine the effect of species roosting temperature on predicted log transformed *P. destructans* loads.

To examine patterns in the increase in fungal loads over the winter, we calculated skew and of the infection intensities for each species in early and late hibernation. We then examined how loads increased in individual colonies of each species as a function of the early hibernation loads. We used linear models of site interacting with species to estimate the change in *P. destructans* loads at sites over the

winter, and compared these with the mean starting loads of *P. destructans* in each colony.

Acknowledgements

We thank R. Reynolds, W. Orndorf, K. Powers (VA), S. Darling (VT), C. Herzog, R. von Linden, K. O' Conner (NY), D. Kirk, J. Kath (IL), and T. Cheng. This work was supported by the National Science Foundation (DGE-0741448 to K.E.L., and DEB-1115895 to T.H.K., W.F.F., J.T.F., and A.M.K., Bat Conservation International, and the National Geographic Society.

Literature Cited

Bates, D., M. Maechler, and B. Bolker. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. **http://CRAN.R-project.org/package=lme4**.

Blomberg, S. P., T. Garland, and A. R. Ives. 2003. Testing for phylogenetic signal in comparative data: behavioral traits are more labile. Evolution **57**:717-745.

Bouma, H., H. Carey, and G. Kroese. 2010. Hibernation: the immune system at rest? J Leukocyte Biol **88**:619 - 624.

Bouma, H. R., F. G. M. Kroese, J. W. Kok, F. Talaei, A. S. Boerema, A. Herwig, O. Draghiciu, A. Buiten, A. H. Epema, A. van Dam, A. M. Strijkstra, and R. H. Henning. 2011. Low body temperature governs the decline of circulating lymphocytes during hibernation through sphingosine-1-phosphate. Proceedings of the National Academy of Sciences of the United States of America **108**:2052-2057.

Brack, V. 2007. Temperatures and locations used by hibernating bats, including Myotis sodalis (Indiana bat), in a limestone mine: implications for conservation and management. Environmental Management **40**:739-746.

Briggs, C. J., R. A. Knapp, and V. T. Vredenburg. 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proceedings of the National Academy of Sciences **107**:9695-9700.

Cohen, M. S., Y. Q. Chen, M. McCauley, T. Gamble, M. C. Hosseinipour, N. Kumarasamy, J. G. Hakim, J. Kumwenda, B. Grinsztejn, and J. H. Pilotto. 2011. Prevention of HIV-1 infection with early antiretroviral therapy. New England Journal of Medicine **365**:493-505.

Coleman, J. T. H. and J. D. Reichard. 2014. Bat white-nose syndrome in 2014: A brief assessment seven years after the discovery of a virulent fungal pathogen in North America. Outlooks on Pest Management.

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.

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., J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, and T. H. Kunz. 2010. An emerging disease causes

regional population collapse of a common north american bat species. Science **329**:679-682.

Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B. A. Nickel, K. E. Langwig, J. T. Foster, K. E. Barlow, T. Bartonička, D. Feller, and A. J. Haarsma. 2015. Disease alters macroecological patterns of North American bats. Global Ecology and Biogeography.

Harvell, C. D., K. Kim, J. M. Burkholder, R. R. Colwell, P. R. Epstein, D. J. Grimes, E. E. Hofmann, E. K. Lipp, A. D. M. E. Osterhaus, R. M. Overstreet, J. W. Porter, G. W. Smith, and G. R. Vasta. 1999. Emerging marine diseases - climate links and anthropogenic factors. Science **285**:1505-1510.

Holdo, R. M., A. R. E. Sinclair, A. P. Dobson, K. L. Metzger, B. M. Bolker, M. E. Ritchie, and R. D. Holt. 2009. A disease-mediated trophic cascade in the serengeti and its implications for ecosystem C. Plos Biology **7**.

Holloway, G. I. and R. M. R. Barclay. 2001. Myotis ciliolabrum. Mammalian Species:1-5.

Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-U994.

Kilpatrick, A. M., C. J. Briggs, and P. Daszak. 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends in Ecology & Evolution **25**:109-118.

Kilpatrick, A. M., R. J. Peters, A. P. Dupuis Ii, M. J. Jones, P. Daszak, P. P. Marra, and L. D. Kramer. 2013. Predicted and observed mortality from vector-borne disease in wildlife: West Nile virus and small songbirds. Biological Conservation **165**:79-85.

LaDeau, S. L., A. M. Kilpatrick, and P. P. Marra. 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature **447**:710-713.

Langwig, K. E., W. F. Frick, J. T. Bried, A. C. Hicks, T. H. Kunz, and A. Marm Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecology Letters **15**:1050-1057.

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. Foster, and A. M. Kilpatrick. 2015a. Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. Proceedings of the Royal Society B: Biological Sciences **282**.

Langwig, K. E., J. Voyles, M. Q. Wilber, W. F. Frick, K. Murray, B. M. Bolker, J. P. Collins, J. R. Hoyt, C. Willis, W., , T. L. Cheng, M. Fisher, D. Lindner, H. I. McCallum, R. Puschendorf, E. B. Rosenblum, M. Toothman, C. J. Briggs, and A. M. Kilpatrick. 2015b. Context dependent conservation responses to wildlife disease. Frontiers in Ecology and the Environment.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proceedings of the National Academy of Sciences of the United States of America **103**:3165-3170.

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.

Moore, M. S., J. D. Reichard, T. D. Murtha, M. L. Nabhan, R. E. Pian, J. S. Ferreira, and T. H. Kunz. 2013. Hibernating Little Brown Myotis (Myotis lucifugus) Show Variable Immunological Responses to White-Nose Syndrome. Plos One **8**.

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.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Schlaepfer, M. A., M. J. Sredl, P. C. Rosen, and M. J. Ryan. 2007. High prevalence of Batrachochytrium dendrobatidis in wild populations of lowland leopard frogs Rana yavapaiensis in Arizona. EcoHealth **4**:421-427.

Shaman, J., V. E. Pitzer, C. Viboud, B. T. Grenfell, and M. Lipsitch. 2010. Absolute humidity and the seasonal onset of influenza in the continental United States. Plos Biology **8**.

Skerratt, L., L. Berger, R. Speare, S. Cashins, K. McDonald, A. Phillott, H. Hines, and N. Kenyon. 2007. Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. EcoHealth **4**:125-134.

Smith, K. F., D. F. Sax, and K. D. Lafferty. 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conservation Biology **20**:1349-1357.

van Riper, C., S. G. van Riper, M. L. Goff, and M. Laird. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs **56**:327-344.

Verant, M. L., J. G. Boyles, W. Waldrep, G. Wibbelt, and D. S. Blehert. 2012. Temperature-Dependent Growth of Geomyces destructans, the Fungus That Causes Bat White-Nose Syndrome. Plos One **7**.

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.

Vredenburg, V. T., R. A. Knapp, T. S. Tunstall, and C. J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences **107**:9689-9694.

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**.

Webb, P. I., J. R. Speakman, and P. A. Racey. 1996. How hot is a hibernaculum? A review of the temperatures at which bats hibernate. Canadian Journal of Zoology-Revue Canadienne De Zoologie **74**:761-765.

Whitlaw, H. A. and M. W. Lankester. 1994. A retrospective evaluation of the effects of parelaphostrongylosis on moose populations. Canadian Journal of Zoology **72**:1-7.

Wobeser, G. 2002. Disease management strategies for wildlife. Revue Scientifique Et Technique De L Office International Des Epizooties **21**:159-178.

Conclusions and Significance

This research has advanced our understanding of factors that influence pathogen transmission, and provided novel information on how behavioral and environmental factors determine disease impact. Broadly, by merging theory with empirical data, we have begun to test predictions about how disease drives impacts and extinction, and provide critical information needed to manage wildlife disease epidemics.

Given the devastating impacts of white-nose syndrome on bat populations, management intervention will be critical in preventing extinction of species imperiled by this disease. We predict that the Northern long-eared bat is likely to be driven extinct by this disease. This species suffers high initial mortality, (Langwig et al. 2012), and has contact with persistent environmental reservoirs (Hoyt et al. 2014, Langwig et al. 2015), and while having evidence of density-dependent declines, stabilizes at very low population sizes. We have observed extirpation of this species from many sites in the northeastern U.S. (Langwig et al. 2012), and across much of eastern North America (Frick et al. 2015), and population collapse to a few individuals in recently invaded sites in the Midwestern United States (Langwig, in press.) The last remaining significant populations of this species in the U.S. are in the upper peninsula of Michigan where WNS arrived in 2014.

Our findings were key in the decision by the United State Fish and Wildlife Service to propose listing the Northern long-eared bat under the Federal Endangered Species Act, with a final decision expected in April 2015. Our data strongly support

this decision, and provide strong evidence that this species will likely vanish if management action is not taken.

In addition to providing critical information needed to assess the likelihood of species extinction, our data also provide important information on disease dynamics that can be used to strategize management interventions. Our data strongly support the importance of species microclimates in determining impacts of disease on populations (Langwig et al. 2012) and among species (Langwig et al, In Review). These data suggest that microclimate manipulation is a promising strategy for reducing WNS impacts on bat populations. By making warm, wet hibernacula cooler or drier by opening entrances, or providing chimneys for cold air, sites could be kept cooler to reduce impacts. Unlike many treatments, the effects of habitat manipulation to reduce disease impacts would be long lasting. Although the initial time and investment costs could be high in some cases, there is still a potential savings against approaches that would need constant yearly reapplication (e.g. vaccines and chemical treatments). Hibernacula are frequently altered by human activities that likely influence the temperature and humidity of these environments (e.g. digging out caves filled in by glaciers for recreation, commercial mining, installation of gates), and therefore there is some precedent for this action. While this strategy is not without risk, given that overall population declines from this disease frequently exceed 95%, habitat manipulation has potential to save thousands of bats from WNS, and preserve genetically viable populations.

Our data also suggest that there may be potential to reduce infections and yearly epidemics by decreasing *P. destructans* in the environment (Langwig et al. 2015). This intervention will likely be most successful in the first year of infection, before contamination of the environment is widespread (Langwig et al., in press). Reducing environmental contamination, may reduce early winter transmission from the environment to bats, resulting in delayed infection, and lower infection intensity on bats at the end of winter (Langwig et al, in Review). By keeping infection intensity from reaching a threshold, reducing fungal loads in the environmental may reduce impacts to bat populations.

Future Directions

Recently, several emerging infectious diseases have been predicted to cause host extinctions in several taxa (e.g. in amphibians, marsupials, and bats) (Mitchell et al. 2008, McCallum et al. 2009, Frick et al. 2010, Vredenburg et al. 2010), but several years subsequent to disease invasion, populations of these affected species remain in some regions (Briggs et al. 2010, Kilpatrick et al. 2010, Hamede et al. 2012, Langwig et al. 2012). Understanding the drivers of disease dynamics during and after pathogen invasion can provide valuable clues about factors that may result in population persistence, and enable more robust predictions about which populations may survive pathogen invasion. Several years after the first detection of *P. destructans*, colonies of the little brown bat in some of the oldest infected hibernacula are persisting and appear to be stabilizing: population growth rates appear stable, and are slightly increasing in some sites, but bat densities are greatly reduced. These populations have

persisted at different sizes, with some sites less 5% of the original population size, whereas others are 20-30% (Langwig et al. 2012; Jeremy Coleman, NASBR 2011; Carl Herzog, WNS Symposium 2012). Although it is encouraging that some remnant populations are persisting, the mechanisms allowing bats to survive in the presence of *P. destructans* remain unknown.

Several likely mechanisms may result in potential stabilization of bat populations. First, favorable environmental conditions at sites, or in a subset of microclimates available in sites, may provide disease refugia to surviving individuals. Second, if transmission of *P. destructans* is density-dependent, then as bat densities decline, contacts among bats or with the environment also decrease, resulting in reduced infection rates and/or fungal inoculum loads. Third, if bats have become resistant or tolerant to infection, bats would have lower WNS mortality due to traits that allow them to either resist infection, or cope with infection. Examining the role of these factors in driving population stability of bats is an important next step in broadening our predictions about which species and populations are likely to persist in the face of WNS and which management strategies are likely to be most effective to prevent species extinction and aid in population recovery.

Literature Cited

Briggs, C. J., R. A. Knapp, and V. T. Vredenburg. 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proceedings of the National Academy of Sciences **107**:9695-9700.

Frick, W. F., J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, and T. H. Kunz. 2010. An emerging disease causes regional population collapse of a common north american bat species. Science **329**:679-682.

Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B. A. Nickel, K. E. Langwig, J. T. Foster, K. E. Barlow, T. Bartonička, D. Feller, and A. J. Haarsma. 2015. Disease alters macroecological patterns of North American bats. Global Ecology and Biogeography.

Hamede, R., S. Lachish, K. Belov, G. Woods, A. Kreiss, A.-M. Pearse, B. Lazenby, M. Jones, and H. McCallum. 2012. Reduced Effect of Tasmanian Devil Facial Tumor Disease at the Disease Front Reducción del Efecto de la Enfermedad de Tumores Faciales del Demonio de Tasmania en el Frente de la Enfermedad. Conservation Biology **26**:124-134.

Hoyt, J. R., K. E. Langwig, J. Okoniewski, W. F. Frick, W. B. Stone, and A. M. Kilpatrick. 2014. Long-term persistence of Pseudogymnoascus destructans, the causative agent of white-nose syndrome, in the absence of bats. EcoHealth:1-4.

Kilpatrick, A. M., C. J. Briggs, and P. Daszak. 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. Trends in Ecology & Evolution **25**:109-118.

Langwig, K. E., W. F. Frick, J. T. Bried, A. C. Hicks, T. H. Kunz, and A. Marm Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecology Letters **15**:1050-1057.

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. Foster, and A. M. Kilpatrick. 2015. Host and

pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. Proceedings of the Royal Society B: Biological Sciences **282**.

McCallum, H., M. Jones, C. Hawkins, R. Hamede, S. Lachish, D. L. Sinn, N. Beeton, and B. Lazenby. 2009. Transmission dynamics of Tasmanian devil facial tumor disease may lead to disease-induced extinction. Ecology **90**:3379-3392.

Mitchell, K. M., T. S. Churcher, T. W. J. Garner, and M. C. Fisher. 2008. Persistence of the emerging pathogen Batrachochytrium dendrobatidis outside the amphibian host greatly increases the probability of host extinction. Proceedings of the Royal Society B: Biological Sciences **275**:329-334.

Vredenburg, V. T., R. A. Knapp, T. S. Tunstall, and C. J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences **107**:9689-9694.