

Bats and Disease: Behavioral and Community
Responses of Southern Bat Populations during the
White-nose Syndrome Epizootic

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Riley Fehr Bernard
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DEDICATION

To Charles and Eleanor Bernard and Albert and Marcia Kassel.

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ABSTRACT

This dissertation investigates regional differences in the behavior and activity of bats in eastern North America during the white-nose syndrome epizootic, specifically in the understudied region of the Southeastern United States. An introductory section provides a brief review of the history of white-nose syndrome, an emerging infectious disease in bats, and its introduction into North America. Chapter one provides the first documented evidence of bat activity outside of hibernacula throughout winter. The research presented in chapter two attempts to explain the variation in load and prevalence of *P. destructans* among species, sites and between years. Finally, chapter three examines the differences in the species affected by white-nose syndrome in the Southeast, as well as the regional variation in the timing and severity of decline in bat communities during summer. A conclusion section at the end of this dissertation summarizes the main findings and provides directions for future research.

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INTRODUCTION

Emerging infectious diseases (EIDs) in wildlife are listed among the top causes of global species extinctions and pose a significant threat to biodiversity, environmental health and ecosystem services (Daszak et al., 2000; Smith et al., 2006). EIDs are bacterial, fungal, or viral diseases that have evolved from a known infection, spread to a new location or host, or recently increased in incidence (Daszak et al., 2000; Plowright et al., 2008). Recent outbreaks of pathogenic fungi have caused collapses in many amphibian (*Batrachochytrium dendrobatidis*), invertebrate (*Nosema* species in bees), mammalian (*Pseudogymnoascus destructans* in bats) and reptilian (*Fusarium solani* in sea turtles) populations (Fisher et al., 2012). Emerging fungal diseases have the propensity to be more deadly than viral and bacterial diseases due to their ability to persist in the environment outside of a host, thrive across broad spatial and temporal ranges, and have high rates of virulence in many naïve hosts (Fisher et al., 2012; Venesky et al., 2013).

To date, there are an estimated 1.5 million fungal species (Hawksworth, 2001), of which only a few hundred are known to be pathogenic to mammals (Garcia-Solache and Casadevall, 2010). Even fewer have the propensity to cause systemic fungal diseases within mammals, which are likely due to physiological adaptations such as mammalian endothermy, homeothermy, and fever (Casadevall, 2005; Robert and Casadevall, 2009). Fungal infections (mycoses) in mammals do not typically lead to mortality, except in cases involving immunosuppression initiated by age or other pathogens (Cafarchia et al., 2012; MacPhee and Greenwood, 2013). However, a newly described disease

in bats has changed how scientists view fungal diseases in mammals, specifically those that perform seasonal hibernation.

There are two primary groups of mycoses that can cause systemic infections in mammals. Dimorphic systemic mycoses, such as histoplasmosis (*Histoplasma capsulatum*) and blastomycosis (*Blastomyces dermatitidis*), are fungal infections that can overcome the physiological and cellular defenses of healthy mammalian hosts (Cafarchia et al., 2012). Infection by these mycoses most commonly occurs through inhalation or ingestion of fungal spores (Brandt and Warnock, 2007; Cafarchia et al., 2012). The creation of toxic microbial byproducts or antigens that elicit harmful immune responses are two primary ways mycoses can cause damage to the host system (Casadevall and Pirofski, 1999). Opportunistic systemic mycoses, however, are fungal infections that occur almost exclusively in hosts with suppressed immune systems. During winter, bats reduce their metabolic rate and body temperature, thereby lowering their immune system (Melvin and Andrews, 2009), becoming an optimal host for infection by opportunistic mycoses.

White-nose syndrome (WNS), characterized by the psychrophilic (cold-loving) fungus *Pseudogymnoascus destructans* (formerly known as *Geomyces destructans*; Lorch et al., 2011; Minnis and Lindner, 2013), has decimated hibernating bat populations throughout northeastern North America (Blehert et al., 2009; Wibbelt et al., 2010; Lindner et al., 2011). Initially detected in a single cave in upstate New York during the winter of 2006, WNS has spread to more than half of the US states and Canadian provinces (Figure 1). Phylogenetic

comparisons of fungal isolates collected from hibernacula in eastern North America and Europe confirmed *P. destructans* as a non-native, invasive pathogen (Minnis and Lindner, 2013; Leopardi et al., 2015). Increased variation observed across these European isolates demonstrate a long-term persistence of *P. destructans* in Europe (Leopardi et al., 2015). Thus far, the occurrence of *P. destructans* has been confirmed in six European countries, however, the full distribution of the fungus remains unknown (Paiva-Cardoso et al., 2014). Recent evidence suggests Western Europe (Belgium, France and Germany) as the most likely origin for North American *P. destructans* (Leopardi et al., 2015).

Pseudogymnoascus destructans has been documented on twelve of the 45 North American bat species (Foley et al., 2011; Bernard et al., 2015), including three endangered species (*Corynorhinus townsendii virginianus*, *Myotis grisescens* and *M. sodalis*). Six species (*Eptesicus fuscus*, *M. lucifugus*, *M. leibii*, *M. septentrionalis*, *M. sodalis*, and *Perimyotis subflavus*) have experienced population losses due to WNS, with some *M. lucifugus* populations in the Northeast experiencing declines of 90 – 100% within the first year of infection (Frick et al., 2010; Langwig et al., 2012). Currently, *M. septentrionalis* are being reviewed for listing under the US Endangered Species Act, with two additional species (*M. lucifugus* and *P. subflavus*) proposed for review. In December 2014, three bat species were added to the Canadian Species at Risk Act due to WNS-related population decline.

While knowledge regarding the complete pathogenesis of WNS is still emerging, the fungus is known to colonize the muzzle, ears, and wings of torpid

bats during hibernation, penetrate the epidermis, and cause ulcerations and necrotic tissue (Blehert et al., 2011). These disturbances cause bats to arouse more frequently and consume critical body reserves needed to survive the winter (Storm and Boyles, 2010; Warnecke et al., 2012, 2013). Infected bats suffer from increased dehydration during hibernation, which leads to an imbalance in key electrolytes, such as sodium and chloride (Cryan et al., 2013). Even bats with mild infections of *P. destructans* suffer from respiratory acidosis, which is an accumulation of CO₂ within the body, leading to an organisms' bodily fluids becoming increasingly acidic (Verant et al., 2014). While 11 European bat species have been identified with diagnostic symptoms of WNS, none have suffered from the high rates of mortality observed in North America (Puechmaille et al., 2011).

Increased fungal growth, ulcerated lesions and changes in behavior are most detectable during mid-hibernation (January – March) when external cave temperatures and abundance of prey are lowest (Foley et al., 2011). Aberrant behaviors of infected bats documented in Northeastern North America include increased flight within the hibernaculum, flying during daylight or in cold-weather, colliding with stationary objects, choosing maladaptive microclimates such as regions at or near the cave entrance, increased thirst, and reduced clustering behavior (Cryan et al., 2010; Blehert et al., 2011; Turner et al., 2011; Langwig et al., 2015). Lab studies indicate that once a bat is infected with *P. destructans*, torpor bouts shorten, clustering during hibernation lessens, and mortality will occur within 2 – 3 months of initial infection (Warnecke et al., 2012).

White-nose syndrome was first recorded in Tennessee during winter 2010 on three bat species (*M. lucifugus*, *M. septentrionalis*, and *P. subflavus*; Samoray, 2011). During winter 2012 – 2013, bats in Great Smoky Mountain National Park (GRSM) began to exhibit abnormal behavior, such as daytime and cold-weather activity and lethargic and labored flight, similar to what has been seen in the Northeast. Between December 2012 and March 2013, 23 bats were found hanging dead on cave entrances or on the ground and unable to fly. All bats collected were submitted for testing to the National Park Service Wildlife Health Branch (Ft. Collins, CO) and identified as WNS positive (Carr et al., 2014). By the end of winter 2014, nearly half of the counties in Tennessee were positive for WNS (Figure 2; Flock, 2014). Populations of *M. septentrionalis* and *P. subflavus*, once common in Tennessee, declined substantially in several major hibernacula, and over 80% of the *M. sodalis* known to hibernate within GRSM are thought to have succumbed to the disease.

Due to the variation in morbidity and mortality, and regional differences in bat community composition, there is a great need to understand the winter behavior of bats in regions outside of Northeastern North America. Winter activity during warm nights in the Southeast may slow the cause of death by affording bats an extra energy pathway that northern populations of bats do not have. Episodic feeding behavior during winter would allow bats to raise their body temperature, which could both activate the bats' immune systems and retard fungal growth, resulting in repeat low-level exposures to the pathogen. Evidence from rabies in bats (Turmelle et al., 2010), as well as other host-pathogen

systems (Dimitrov et al., 2008), indicate that host immunity is a likely consequence of repeated low-level exposure to pathogens. This dissertation will address the following questions: 1) How does the behavior and activity of winter bat populations vary by region? 2) Does the variation in winter behavior alter susceptibility and infection by disease? 3) Does *P. destructans* affect bats differently in the Southeast? 4) Are bat populations in the Southeast experiencing the same rates of WNS-related decline as in the Northeast? 5) Does the severity and timing of population declines due to WNS vary regionally?

CHAPTER 1: WINTER ACTIVITY AND THE PROGRESSION OF WHITE-NOSE SYNDROME IN BATS OF THE SOUTHEASTERN UNITED STATES

This chapter is slightly modified from a paper in review:

Bernard, RF & McCracken GF. *In Review*. Winter activity and the progression of white-nose syndrome in bats of the Southeastern United States. *Animal Behaviour*.

My consistent use of “we” throughout this chapter is in reference to my co-author, Gary McCracken and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) measurement of acoustic data and statistical analysis, (3) gathering and interpretation of the relevant literature, and (4) all of the writing.

Abstract

Understanding the winter behavior of bats in temperate North America can provide insight into how bats react to perturbations caused by weather or disease. Over the course of three winters (2011 – 2013) we collected acoustic data and captured bats outside of five hibernacula in Tennessee, United States. During this time, *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, became established in hibernacula throughout the region, allowing us to track disease-related changes in the winter behavior of ten bat species. We determined that bats in the region are active during winter regardless of disease. We recorded activity outside of hibernacula at temperatures as low as -13°C. Although bat activity was best determined by a combination of variables, the strongest factor was mean daily temperature ($R^2 = 0.2838$, $F_{1,1473} = 583.82$, $P < 0.0001$). Bats that left the hibernacula earlier in evening had lower body condition than those that left two to four hours after sunset ($F_{7,125} = 22.794$, $P < 0.0001$). As time since *P. destructans* detection at a site increased, we saw a rise in the number of daytime and cold temperature flights ($F_{5,2085} = 137.76$, $P < 0.0001$). Through the use of passive acoustic

monitoring and monthly captures, we were able to determine that winter activity was driven by both ambient temperature and the presence of *P. destructans*.

Introduction

Winter activity is documented for several species of North American bats that use roost sites with variable microclimates such as foliage, bark, or man-made structures (Boyles et al., 2006). However, information on winter behavior of North American cave-roosting bats is based largely on studies at latitudes above 40°N, or in desert regions of the southwestern United States (US; Boyles et al. 2006). Previous studies of winter activity of bats in Ontario, New England, Indiana, and Missouri found that individuals arousing during winter months mostly flew within the hibernaculum, and rarely left the cave (Griffin, 1945; Whitaker, Jr. and Rissler, 1992; Boyles et al., 2006). These findings reinforce the assumption that bats enter hibernation in the fall and are not observed on the landscape until mid-spring. Prior to the white-nose syndrome (WNS) disease epidemic, it was suggested that bats leave hibernacula in winter infrequently, and do so to switch roosts or in search of water or food (Boyles et al., 2006). However, as WNS became established in the Northeast, behaviors such as daytime and cold-weather flight during winter became indicative of infection. To date, no studies have looked at the winter behavior of bats in the lower latitudes of the southeastern US, where warmer temperatures and available insects may allow for sustained activity through winter.

The importance of understanding the winter behavior of bats in the US has become better appreciated due to WNS. First identified in February 2006 in a cave in New York, this disease has spread across eastern North America as far west as Missouri, south into Mississippi and north into Canada. To date, WNS has killed several million bats of seven hibernating species (USFWS 2014). Mortality as high as 90 – 100% has been documented at some northeastern hibernacula, leading to possible regional extinction of several once common species (Frick et al., 2010; Langwig et al., 2012). The highest rates of morbidity and mortality at sites in the Northeast have been documented following the second and third winters after initial visual detection of the fungus (Titchenell, 2012; Knudsen et al., 2013).

White-nose syndrome is caused by the psychrophilic (cold-tolerant) fungus, *Pseudogymnoascus destructans* (Lorch et al., 2011; Minnis and Lindner, 2013), a non-native pathogen from Europe (Wibbelt et al., 2010; Puechmaille et al., 2011). *Pseudogymnoascus destructans* invades the muzzle, wings and tail membrane of bats during torpor when their immune systems are suppressed (Blehert et al., 2009). Invasion of the wing tissue by *P. destructans* can cause dehydration, electrolyte imbalance, and increased arousal frequency leading to critical loss of fat stores needed to survive hibernation (Cryan et al., 2010; Reeder et al., 2012; Warnecke et al., 2012, 2013; Verant et al., 2014). Changes in winter behavior associated with WNS have been documented, with bats roosting in exposed regions of cave entrances or leaving hibernacula during the day, and flying during cold winter nights (Foley et al., 2011; Carr et al., 2014). To

date, seven bat species have been confirmed with the disease via histopathology, with five additional species confirmed to carry *P. destructans* DNA (Meteyer et al. 2009; USFWS 2014; Bernard et al. 2015).

Tennessee is among several southeastern states that contain hibernacula used by endangered species, including *Myotis sodalis* and *M. grisescens*.

Pseudogymnoascus destructans was first detected in Tennessee during winter 2009 – 2010 at the largest Indiana bat hibernacula in the state (Samoray, 2011; Carr et al., 2014). By winter 2013 – 2014, 44% of the counties in Tennessee (n = 42/95) were deemed WNS-positive; with several high priority Indiana bat hibernacula experiencing declines in bat populations (Flock 2014). Although declines due to WNS have not been confirmed in gray bats, they are thought to be vulnerable to the disease. *Myotis grisescens* are known to hibernate in eight caves in Alabama, Arkansas, Kentucky, Missouri, and Tennessee (USFWS 1982, 1997). In-vitro growth curves suggest that *P. destructans* may reproduce more quickly in cave environments that maintain more moderate temperatures of 10 – 15°C in winter (Verant et al., 2012), which could result in increased virulence in southern hibernacula.

In this study we address the following questions. 1) Are bats active outside of hibernacula during winter? If so, 2) is this activity restricted to bats hibernating in WNS-affected sites? 3) What are the temperature or temporal correlates for when bats are active during winter? 4) Do patterns of activity vary by species? 5) Does body condition change in relation to how long a hibernaculum is WNS-positive? Passive acoustic monitoring and bat captures during winter outside of

caves allowed us to address these questions without adding additional stress to bats within hibernacula.

Materials and Methods

Study Area

We monitored bat activity at three hibernacula in middle and eastern Tennessee during January – May 2012 (year one), and five hibernacula during October – May 2012 – 2013 (year two) and 2013 – 2014 (year three; Figure 3). Blount cave is the largest known *M. sodalis* hibernaculum in Tennessee, with an estimated 9,500 individuals in winter 2012 – 2013 (Flock, 2013). Hawkins and Warren caves are two of the largest *M. grisescens* hibernacula in the state and also contain small populations of *M. sodalis*. Campbell and White caves contain *Corynorhinus rafinesquii*, *Eptesicus fuscus*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, and *M. sodalis* (Holliday, 2012). Recent hibernacula counts estimated 150,000 and 500,000 endangered *M. grisescens* at Hawkins and Warren caves respectively. White cave contained approximately 700 *M. grisescens*. Four bat species, including 9,800 *M. sodalis* hibernated in Blount cave, and approximately six species of bat ($n = < 10,000$ individuals) were counted during hibernacula surveys at Campbell cave (Samoray 2011; Flock 2013).

Data Collection: Acoustic Activity

We deployed ultrasonic bat detectors near the entrance of each hibernaculum. Acoustic data were recorded in zero-crossing mode onto 4Gb

compact flash (CF) cards (year one) or 32Gb secure digital (SD) cards (years two and three). Detectors were placed approximately 25 – 40 m from each cave entrance. During year one, we used three Anabat II detectors with CF ZCAIM's (Titley Electronics, Ballina, New South Wales, Australia) at Blount, Campbell and Warren caves. We housed microphones in waterproof "bat hats" with reflector plates (Arnett and Hayes, 2006) created in-house and secured on PVC pipes approximately 2 m above ground level. We enclosed all electronic equipment (Anabat II detector, ZCAIM and two 12-volt batteries in parallel) in large utility boxes (Duluth Trading Company, Belleville, Wisconsin, USA) located at the base of the PVC pipe.

Due to technical difficulties with aging Anabat II and ZCAIM units, we transitioned to SM2Bat+ detectors (Wildlife Acoustics, Maynard, Massachusetts, USA) in years two and three. Detectors were redeployed at Blount, Campbell and Warren caves and added to White and Hawkins caves. SM2Bat+ detectors were secured to a 30.5 x 20 cm piece of plywood and attached to a 5 cm diameter PVC pipe using U-bolts. The SMX-US microphone was attached to a 1 m cord and fastened at a 45° angle to the top of the PVC pipe. The SM2Bat+ detectors were powered by a 6-volt external battery enclosed in utility boxes at the base of the entire unit.

Because WNS-affected bats in the Northeast are often active during daytime, we recorded bat acoustic activity and temperature 24 hours/day. We replaced data cards and batteries monthly. Acoustic data were collected from January 4 – May 30, 2012 (Blount, Campbell & Warren caves; n = 361 acoustic

days), October 4, 2012 – May 7, 2013 (Blount, Campbell, Hawkins, Warren & White caves; $n = 1,004$ acoustic days), and at these same caves October 2, 2013 – May 13, 2014 ($n = 1,001$ acoustic days). During the three winters, we conducted acoustic monitoring on 538 days at Blount cave, 498 days at Campbell cave, 384 days at Hawkins cave, 543 days at Warren cave and 403 days at White cave. Acoustic monitoring for the last two years of the study was from the end of fall swarming through spring emergence. Temperature was recorded at each site at 15 minute intervals using HOBO U-series data loggers with an accuracy of ± 0.21 °C (Onset Computer Corporation, Bourne, Massachusetts, USA). Each temperature meter was 2.5 m above the ground and 10 – 15 m from the acoustic detector.

Data Collection: Bat Captures

During years two and three, we captured bats at each cave site once each month using 6-, 9-, and 12-meter mist nets (Avinet, Dryden, NY; 75/2 mesh size, 2.6 meters high, 4 shelves, black polyester for bats). We deployed nets for a total of 343.5 hours over both winters. Each site had designated equipment to prevent the spread of *P. destructans* among sites. Mist nets were deployed 30 minutes before civil sunset and remained open for five hours; until we captured 30 bats, or until the temperature dropped below freezing (0 °C). Bats were held individually in paper bags and placed in a large insulated box with four hand-warmers (HotHands[®], Dalton, GA) for 30 – 60 minutes prior to processing. We identified each captured bat to species and recorded reproductive condition, forearm

length (mm), weight (g), wing-damage index (WDI); and attempted to collect feces from each bat. Wing Damage was classified from 0 to 3, where WDI score = 0 indicates no obvious scarring or discoloration on the membranes, WDI score = 1 denotes light damage covering less than 50% of the membranes, WDI score = 2 indicates moderate damage greater than 50% of the wing membrane covered in scar tissue, and WDI score = 3 signifies heavy damage with deteriorated wing membranes and necrotic tissue (Reichard and Kunz, 2009). We also illuminated bat wings with ultraviolet (UV) light to check for lesions indicative of WNS. Under UV light, damage caused by *P. destructans* infiltration will fluoresce yellow-orange (UV-positive), allowing for confirmation of WNS infection without submitting the bat for histology confirmation (Turner et al., 2014a). To assess overall health of each bat, we calculated body condition indices (BCI) by dividing weight by forearm length (Speakman and Racey, 1986). All species, except *Lasiurus borealis*, were banded with either 2.4 mm or 2.9 mm identification bands and released on site.

Data Analysis

Acoustic bat activity was quantified as the number of files or “bat passes” recorded per 24-hour period. We defined a “bat pass” as a file containing a search-phase echolocation sequence of ≥ 2 echolocation pulses (Gannon et al., 2003). This metric is an index of overall activity and does not correlate to the number of individual bats actively flying outside of the cave (Kunz et al., 2007; Schwab and Mabee, 2014). All call files were analyzed in AnalookW (version

3.9c) using anti-noise filters. Filters were used to eliminate files that contained calls with less than 2 pulses, calls with short duration (< 4ms) or low frequency noise (i.e. wind, insect noise, or rain). Once all noise files were removed, each file was manually checked to determine if the remaining call files fit the “bat pass” criteria. Because of difficulties in distinguishing the closely similar calls of several *Myotis* species (Barclay, 1999; Britzke et al., 2011) and due to distortion of the acoustic signals resulting from the proximity of detectors to cave walls and large trees, species identification of each call was not attempted for this study.

We used generalized linear mixed models (GLMM) using the package lme4 (version 1.1-7) in R (version 3.1.0; R Core Development Team, 2014) to test for differences in bat acoustic activity among years using daily and hourly “bat pass” totals. We chose a mixed model to account for repeated sampling at the same sites throughout the season and over the years. We used negative binomial GLMMs to account for over-dispersion within the data. We set site as a random effect, with the fixed effects as year, season, Julian day (day of the year), time of day (day or night), hour (0:00 to 23:00 hrs), mean daily temperature (24-hour period), temperature at time of emergence (dusk), relative humidity, moon illumination, and year since *P. destructans* confirmation at each cave. Year since *P. destructans* confirmation was based on the detection of fungal DNA on epidermal swab samples or histology of voucher specimens (USGS National Wildlife Health Center 2013). We used one-way ANOVAs to test for differences in activity patterns among species, sites and years; as well as to test for differences in body condition among species, month, site, and year since *P.*

destructans confirmation. T-tests were used to determine whether winter activity and body condition varied between males and females. We used contingency tables (X^2 test) to test for differences in bat captures per site based on year since confirmation of *P. destructans*.

Results

Acoustic Activity

We obtained useable acoustic data for 88.4%, or 2,091 of 2,366 detector nights. Loss of data resulted from malfunctioning Anabat/ZCAIM units, SM2Bat+ external AC adaptor failure and a microphone cord failure. Our recordings contain a total of 566 000 bat passes with bat activity recorded on 1,695 of the 2,091 recording days. Mean activity at sites varied considerably (Table 1), presumably due to differences in species compositions and population sizes at each location. Daily bat activity was best predicted ($w_i = 1.00$, $F_{1,7} = 12.75$, $P < 0.0001$) by the full model comprised of pooled data across all sites and seasons (Table 2). Hourly bat activity was best described ($w_i = 1.00$, $F_{1,3} = 817.45$, $P < 0.0001$) by the model that included the interaction of time of day (day or night classification) with mean hourly temperature and year since *P. destructans* was confirmed (Table 3).

Bat calls were recorded on nights where the temperature at emergence was below 0°C on 23 nights at Blount cave, 27 nights at Campbell cave, eight nights at Hawkins cave, three nights at Warren cave, and three nights at White cave. The lowest temperature at which bats were detected was -13°C ($N = 2$

calls) and bats were not detected on nights when the temperature at emergence was below -8°C ($N = 49$ nights). Normal bat activity was driven by mean temperature ($R^2 = 0.2838$, $F_{1,1473} = 583.82$, $P < 0.0001$). The highest mean numbers of nightly calls were recorded during year one, with a reduction in total calls recorded in subsequent years (Table 1). There was an increase in daytime activity at Blount cave in year two, two years post-WNS confirmation, followed by a significant decline in total acoustic activity by the end of year three. However, at all sites nighttime acoustic activity always exceeded daytime activity. The highest number of bat calls recorded per day (mean $\pm$ SE; $1,252.53 \pm 40.16$ bat calls; $F_{5,1874} = 133.91$, $P < 0.0001$) were recorded at caves that were *P. destructans* negative during the first year of monitoring (Campbell and Warren caves). Four caves experienced significant decreases in mean nightly bat calls each year following *P. destructans* confirmation ($F_{2, 39\ 845} = 1,051.63$, $P < 0.0001$, Table 1). However, nightly bat activity at Hawkins cave increased significantly between years one and two ($t_{140.7} = 4.77$, $P < 0.0001$), despite similar mean nightly temperatures for both years (Table 1).

Bat Captures

We captured a total of 947 individuals of 10 species (Table 4). These included 648 males, 297 females and two *M. leibii* of unknown sex due to escape. Almost all bats captured ($N = 936$) hibernate in caves; however, we also captured eight *Lasiurus borealis* and three *Lasionycteris noctivagans*; both migratory species that do not typically use caves as hibernacula. Netting

attempts were most successful on mild nights where mean temperatures ranged between 11 – 15 °C. Eastern red bats were captured on nights when average temperatures during the five hours following emergence were above 11 °C, with all other species captured as low as 8 °C and one *M. grisescens* captured on a night where the mean nightly temperature was 0 °C. Across all seasons, we captured the most bats in October ($N = 190$) and April ($N = 222$), the first and last months of the hibernation period. More bats were captured during winter 2012 – 2013 (year two; $N = 531$) than in winter 2013 – 2014 (year three; $N = 416$). We also found a significant decline in the number of individuals captured at a site as the time since confirmation of *P. destructans* increased ($\chi^2_{16} = 1387.01$, $P < 0.0001$). Most bats captured during the study period ($N = 871$) had a WDI = 0. Sixty-four individuals (6.75%) scored a WDI = 1, with the remaining individuals ($N = 10$; 1.15%) scoring WDI = 2 – 3. Of these 10 bats, four were *M. grisescens*, four were *M. septentrionalis*, and two were *Perimyotis subflavus* (Reboredo-Segovia et al. unpublished).

Across all seasons, body condition (BCI) was highest in October, declined during December – February, and began to increase during March and April, the last two months of hibernation ($F_{6, 312} = 42.9653$, $P < 0.0001$; Figure 4). There were no significant differences in mean BCI between males and females. Mean BCI for all species captured during January through April of year three were significantly higher than for the same months in year two ($t_{940} = 7.69$, $P < 0.0001$; Figure 4). Bats captured in the first 2 hours after sunset had the lowest BCI on average, with those captured several hours after emergence having higher mean

BCI ($F_{7,125} = 22.794$, $P < 0.0001$). Individuals that provided fecal samples had a higher average BCI than those that did not defecate while in captivity ($t_{940} = 3.57$, $P = 0.0004$). All 290 fecal pellets collected contained recently consumed prey (as described by Dunbar et al. 2007). There was no significant difference between mean BCI and WDI of bats captured during the study. Body condition varied in relation to how long a site was *P. destructans* positive ($F_{4, 937} = 12.87$, $P < 0.001$). Bats captured at caves positive for two years had the highest mean BCI (0.222 ± 0.005 g/mm, $N = 107$), followed by sites positive for one year ($N = 269$), three years ($N = 206$), and four years ($N = 51$). Bats captured at caves that had yet to be confirmed, exhibited the lowest mean BCI (0.182 ± 0.003 g/mm, $N = 309$). Individuals identified as UV-negative ($N = 477$) had higher mean BCI than UV-positive bats ($N = 68$; $t_{137.8} = -7.31$, $P < 0.0001$).

Discussion

This is the first study to demonstrate that bats are active throughout winter in the southeastern US. Activity throughout winter was not restricted to bats in WNS-infected caves; however, increases in activity associated with low temperatures and diurnal flight were attributed to the disease. Variation in body condition across all species was found throughout a capture session, across seasons and in relation to how long a hibernaculum was *P. destructans* positive.

Winter activity

Hibernating mammals arouse periodically throughout winter to maintain physiological balance (Willis, 1982), however, in northern latitudes they rarely leave the hibernacula (Whitaker, Jr. and Rissler, 1992; Schwab and Mabee, 2014). Czenze et al. (2013) noted that *M. lucifugus* in Canada (53°N 99°W) entered a hibernaculum in mid-September and remained there until mid-May, showing no signs of emergence. Nevertheless, moderate bat activity has been reported throughout winter in some high latitude regions that experience mild oceanic climates (Park et al., 2000; Hope and Jones, 2012; Burles et al., 2014). Continental and Great Lake effects during winter in the Northeast, where WNS has caused the most dramatic population declines, generally result in severe weather. We recorded and captured bats that were taking advantage of more mild southern climates, and fecal samples from bats captured outside of caves verify that bats were leaving the hibernacula to forage. We captured bats representing every species of cave-dwelling bat known to hibernate in the region, as well as tree and foliage-roosting species, demonstrating that it is not only the latter that remain active during winter (Boyles et al. 2006). Both cave and tree or foliage roosting species captured were active throughout winter at a range of temperatures, indicating that bats over-wintering in the Southeast exhibit different behaviors than northern populations (Boyles et al., 2006). This is an important factor for understanding if there are latitudinal differences in WNS susceptibility and mortality (Bernard et al. unpublished).

We found evidence for a strong correlation of temperature with winter activity of bats in all three years at all study caves. Winter temperatures at each site varied significantly during the study period with the warmest temperatures recorded in year one. While activity levels were impacted by temperature, they were confounded by disease and species. The apparent effects of WNS are seen in the seasonal activity for the species most affected by WNS (i.e. *M. lucifugus*, *M. septentrionalis*, and *P. subflavus*), however, changes in acoustic activity at caves dominated by *M. grisescens*, which are as yet little affected, were linked to temperature, not disease.

In the Northeast, peak mortality occurred 2 – 3 years after the first detection of WNS infections, with populations of *M. lucifugus* and *P. subflavus* experiencing slower rates of population decline 3 – 4 years post-WNS detection (Langwig et al., 2012). In the Southeast, however, significant declines were not documented until year three of this study, which was nearly five years after the first confirmed WNS infections. By the second year of monitoring, bats hibernating at caves with large populations of WNS-affected species exhibited expected signs of disease-related behavioral changes. Bats at Blount cave were recorded flying during the day, with the number of calls recorded in April of year two equivalent to mid-hibernation levels, indicating reduced activity during spring emergence. In October of year three, bats at Blount Cave were recorded at similar numbers to the previous year, however, activity dropped off significantly by January, with no bats recorded at the cave after February. Over 80% of the bats captured at Blount cave in March and April of year three had visible signs of

WNS, including wing damage or ultraviolet (UV) fluorescence (Turner et al., 2014a) and lower than average body condition. At Campbell cave, the total number of bats captured decreased significantly by the third year of sampling, with an increase in the number of tri-colored bats found roosting outside of the cave. Our data did not show the effects of WNS seen in the Northeast in years 2 – 3 until 4 – 5 years after the disease was detected in the Southeast. Therefore, hibernating bat populations in the Southeast may continue to experience delayed declines caused by WNS due to regional differences in climate-related life history traits such as shorter hibernation periods (Sherwin et al., 2013), abbreviated torpor bouts (Brack Jr. and Twente, 1985; Twente et al., 1985; Jonasson and Willis, 2012), and foraging opportunities throughout winter.

Winter activity and capture rates documented at Hawkins, Warren and White caves, which are dominated by *M. grisescens*, remained consistent throughout our study to pre- or early WNS-detection years. We credit the decreases in acoustic activity during the second and third years of sampling to colder temperatures rather than to the effects of WNS. *Myotis grisescens* are the largest bodied Myotine species in eastern North America and hibernate in clusters of 100 000 to over one million individuals (USFWS 2009). Living in large colonies is expected to promote density-dependent transmission of the pathogen through increased contact rates (Ryder et al., 2007; Langwig et al., 2012), however, *M. grisescens* are currently surviving hibernation without any obvious declines due to WNS (Flock 2014; USFWS 2014). Studies examining load and prevalence of *P. destructans* on hibernating bat populations have found that less

than 20% of *M. grisescens* sampled are infected with the fungus (Bernard et al. unpublished; Janicki et al. unpublished). *Myotis grisescens* are the only eastern *Myotis* species to roost in caves year round (Decher and Choate, 1995). They preferentially hibernate at temperatures as low as 1 – 9°C (Tuttle, 1976; Tuttle and Kennedy, 2005) and roost in dense clusters of more than 1,800 bats per m² (Gore, 1992). Although WNS has been confirmed in *M. grisescens*, we believe their large body size and relaxed energetic constraints for hibernating through shorter winters may prevent *P. destructans* from reproducing at disease inducing levels. While these theories have yet to be investigated, they could provide insight as to why *M. grisescens* populations are so far persisting during the WNS epizootic.

Bat captures and body condition

We captured twice as many males as females flying outside of each hibernaculum. This is likely due to disproportionate sex ratios of bats within hibernacula (Whitaker and Gummer, 2000; Parsons et al., 2003). Previous studies suggest sex-ratios at winter sites are biased towards males at more northern roosts and become more evenly distributed the farther south a hibernacula is located, with sites in Florida reported to have an equal distribution of males and females (Davis, 1959; Tinkle and Milstead, 1960; Elder and Gunier, 1978). An alternative explanation for sex-bias in captures of bats active in winter is the “thrifty female hypothesis” (Jonasson and Willis 2011). This theory suggests that adult females minimize energy loss by relying more on deep torpor

during hibernation, whereas adult males have more energy to expend and rely less on torpor. The capture of more active males than females is consistent with this idea. However, we did not detect any difference in body condition between males and females, nor did we find any seasonal patterns in the rate of decline in BCI for males versus females, as would be expected if females were remaining in caves to preserve energy. The “thrifty female hypothesis” was proposed for bats in hibernacula at higher latitudes and may function best where winters are more severe and intermittent foraging is not feasible. Although not significant due to low sample size, we saw a more even distribution of male and female *C. rafinesquii*, which use abbreviated torpor during hibernation and continue to forage throughout winter (Johnson et al., 2012). We also saw a more even distribution of male and female *L. borealis* and *L. noctivagans*, which hibernate in more thermally unstable environments. We interpret these observations as suggesting that both sexes of these species are similarly active in winter in foraging or searching for alternative roosts.

Conclusions

We quantified the winter activity of 10 species of bats at five hibernacula in the southeastern US prior to and during the WNS disease epidemic. Bats are active outside of hibernacula and feed on insects throughout winter regardless of disease, with activity strongly correlated with temperature. Activity during daytime and sub-freezing temperatures increased following confirmation of *P. destructans* at each hibernacula, suggesting that in the Southeast vulnerable bat species

exhibit similar behaviors comparable to those seen at WNS infected hibernacula in the Northeast. The onset of aberrant behavior and mortality occurred 4 – 5 years after WNS confirmation, which was later than documented in the North. We found regional differences in the bat faunas affected by WNS. Caves dominated by *M. septentrionalis*, *M. sodalis* and *P. subflavus* displayed increased aberrant behaviors and reduced activity towards the end of year three, whereas populations of *M. grisescens* persisted. We also identified differences in how bats prepare for winter, with individuals in the Southeast entering hibernation with lower mean BCI than bats studied in the Northeast. Mild winter climates and available insects allow for shorter hibernation and continued foraging, likely reducing the demands of fat storage prior to winter. These regional differences in bat behavior provide better understanding of adaptive variation in bats to climatic perturbations and diseases, which are relevant to how bat populations across North America will react to infection by *P. destructans*.

CHAPTER 2: WINTER ACTIVITY OF BATS AND WHITE-NOSE SYNDROME FUNGAL INFECTIONS IN THE SOUTHEASTERN UNITED STATES

This chapter is slightly modified from a paper that will be submitted:

Bernard, R. F., J. T. Foster, E. V. Willcox, K. L. Parise, & G. F. McCracken. Winter activity of bats and white-nose syndrome fungal infections in the Southeastern United States.

My consistent use of “we” throughout this chapter is in reference to my co-authors, Jeffery Foster, Katy Parise, Emma Willcox, Gary McCracken and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) statistical analysis of the dataset, (3) gathering and interpretation of the relevant literature, and (4) all of the writing.

Abstract

Emerging infectious diseases (EIDs) in wildlife have increased over several decades and threaten global biodiversity and conservation. White-nose syndrome (WNS) in bats is the most recent EID to affect wildlife in North America. In the Northeast, winter behavior of bats, such as flying during the day or in cold weather, has been attributed to WNS. The winter activities of bats in the southeastern United States, however, where winters are warmer, have received little attention. The goals of this study are to determine if winter activity results from the presence of *Pseudogymnoascus destructans*, the causative pathogen of WNS, and to investigate how pathogen load and prevalence varies by species, site, and over time. We collected epidermal swab samples from active bats captured outside of hibernacula in Tennessee during winters 2012 – 2013 and 2013 – 2014. Approximately 46% of the bats were *P. destructans* positive, showing that bats in the Southeast remained active throughout winter; regardless of the presence of *P. destructans*. *Myotis septentrionalis* had the highest fungal load among infected individuals, whereas *M. lucifugus* had the

highest infection prevalence of all species sampled. Less than 18% of all *M. grisescens* captured had detectable *P. destructans* DNA on their forearms and muzzle. Hibernacula with large populations of *M. grisescens* had lower fungal loads than sites used by other species, however, we did not find a significant difference in the mean load per species when comparing between *M. grisescens* and non-*M. grisescens* sites. We found that pathogen load and prevalence were higher on bats captured during winter 2012 – 2013, indicating that fungal loads on bats did not increase the longer a site was *P. destructans* positive. Repeated low-dose exposure, mild temperatures and availability of prey during winter in the Southeast may provide a regional refuge for surviving bat populations.

Introduction

Emerging infectious diseases (EIDs) in wildlife pose an increasing threat to global biodiversity and conservation (Daszak et al., 1999, 2000). A significant proportion of these EIDs are the result of “pathogen pollution”, the introduction of novel pathogens into naïve wildlife populations from humans and livestock (Daszak et al., 2000; Rachowicz et al., 2005). Two prominent examples of pathogen pollution are the African rinderpest panzootic of the 1880’s and amphibian chytridiomycosis. Both diseases caused mass mortality, with rinderpest killing 90% of Kenya’s buffalo population, resulting in downstream effects on predator populations and ecosystem health (Daszak et al., 2000). Chytridiomycosis is known to infect over 50% of all amphibian species and can kill 80% of the population within 4 – 5 months after its introduction (Mendelson et

al., 2006). Loss of species from EIDs such as these, are devastating to global biodiversity, and secondary effects due to these losses are ecologically harmful (Daszak et al., 2000; Fisher et al., 2012).

A recent EID to affect North American wildlife is white-nose syndrome (WNS), a rapidly spreading disease of cave hibernating bats. White-nose syndrome is caused by the psychrophilic (cold-loving) fungus *Pseudogymnoascus destructans*, which was first documented in February 2006 at a single cave in upstate New York (Blehert et al., 2009, 2011). This invasive pathogen, which originated in Europe (Warnecke et al., 2012), has since spread to more than half of the US states and Canadian provinces and has killed upwards of 5 – 6 million bats (U.S. Fish and Wildlife Service, 2014). Currently, six bat species are experiencing population losses due to WNS, with once abundant species threatened with regional extinction (Frick et al., 2010; Turner et al., 2011; Langwig et al., 2012; U.S. Fish and Wildlife Service, 2014). Population declines and the loss of bat species due to WNS are likely to have major ecological and economic consequences, with expected increases in crop and forest pest populations (Kunz et al., 2011).

Pseudogymnoascus destructans colonizes the cutaneous membranes of the muzzle, ears, wings and tail of bats, eroding the epidermis and invading the underlying skin and connective tissue (Meteyer et al., 2009). Once invasion occurs, *P. destructans* disrupts critical physiological functions such as cutaneous respiration, blood circulation, and water balance (Cryan et al., 2010; Willis et al., 2011; Warnecke et al., 2013; Verant et al., 2014). These physiological changes

cause bats to arouse from torpor more frequently, resulting in the premature depletion of energy reserves needed for hibernation (Warnecke et al., 2012). WNS-infected bats also exhibit aberrant behavior in winter, including movement from thermally stable cave environments to locations near the cave entrance, daytime emergence, and flying in cold winter temperatures (Blehert et al., 2011; Turner et al., 2011; Foley et al., 2011). In the Northeast, where winters are severe and prey is limited, bats flying outside during hibernation are likely infected with WNS. However, bats in the southeastern U.S. are known to leave hibernacula to feed on warm winter nights, suggesting winter activity in the South is not necessarily a consequence of disease (Bernard and McCracken, in review). Winter foraging may provide bats hibernating in southern latitudes with energy not available to more northern bat populations. Episodic feeding during winter will also raise body temperatures, which could activate the immune system, retard fungal growth on bats, and result in repeated low-level exposures to the pathogen. Evidence from rabies in bats (Turmelle et al., 2010), as well as other host-pathogen systems (Dimitrov et al., 2008; Dobony et al., 2011), demonstrates that host immunity can result from repeated low-level exposure to pathogens. Although it is established that bats in the Southeast are often active throughout winter, the possible effects of this activity on the epizootiology of WNS are unknown. This study is the first to examine fungal load and prevalence on bats captured outside of hibernacula during winter.

In this study we assess the prevalence and fungal load on 10 species of bats that are active during winter in Tennessee. We collected epidermal swab

samples for real-time polymerase chain reaction (PCR) analysis of fungal DNA to rapidly detect and quantify *P. destructans* on active bats (Muller et al., 2013), and employed ultraviolet light to identify lesions and ulcerations caused by infiltration of *P. destructans* into wing and tail membranes (Turner et al., 2014). Our goals are to determine if winter activity is driven by the presence of *P. destructans*, and to examine the relationships between winter behavior, body condition and species differences in the load and prevalence of WNS fungal infections on bats in the Southeastern US. We tested the following hypotheses: 1) bats from sites that are WNS-positive for longer periods have higher load and increased prevalence compared to bats from more recently infected sites; 2) fungal load and prevalence will be higher on small-bodied cave hibernating species, such as *Myotis lucifugus*, *M. septentrionalis* and *Perimyotis subflavus*, than on larger bodied species, such as *Eptesicus fuscus* and *M. grisescens*; 3) bats with high loads of *P. destructans* will have low body condition and evidence of epidermal erosion as detected by ultraviolet fluorescence.

Methods

We conducted our study at the entrances of five hibernacula in Tennessee from October – April 2012 – 2013 and 2013 – 2014 (Figure 3). The National Park Service (NPS), Tennessee Wildlife Resources Agency (TWRA), and The Nature Conservancy (TNC) manage four of the five hibernacula used in this study; the fifth cave is privately owned. Blount cave (NPS) is the largest known endangered *M. sodalis* hibernacula in the state of Tennessee, with an estimated population of

9,500 individuals in February 2013 (Flock, 2013). Small numbers of *M. lucifugus* and *P. subflavus* also occur in Blount cave. Hawkins (TWRA) and Warren (TNC) caves are two of the largest endangered *M. grisescens* hibernacula in the state, with estimated populations of 150,000 and 400,000 *M. grisescens*, respectively. Both caves also contain small numbers of *M. sodalis* during winter (Holliday, 2012). Campbell (private) and White (TWRA) caves contain populations of *M. leibii*, *M. lucifugus*, *M. septentrionalis*, and *M. sodalis*, with less than 1,000 individuals in each cave (Samoray, 2011). Blount and Hawkins caves were confirmed *P. destructans* positive in the winters of 2009 – 2010 and 2010 – 2011, respectively, with all other sites confirmed by winter 2012 – 2013 (Holliday, 2012).

We captured bats at each site once a month using mist nets (Avinet, Inc., Dryden, NY; mesh diameter: 75/2, 2.6m high, 4 shelves, 6-12m wide). Site-specific single-, double- and triple-high nets were deployed 30-minutes before civil sunset at cave entrances and along corridors within 100m of the cave. We kept the nets open for five hours or until we captured 30 bats, and closed them when temperatures dropped below 0 °C. After capture, individual bats were placed in paper bags and held for 30 – 60 minutes in an insulated box with four hand-warmers (HotHands[®], Dalton, GA). Endangered *M. grisescens* and *M. sodalis* were held for a maximum of 30 minutes. We recorded species, reproductive condition, forearm length (mm), weight (g), mite load (Zahn and Rupp, 2004) and wing-damage index (WDI, (Reichard and Kunz, 2009)); and collected epidermal swab samples from each bat. We also examined bats for

WNS-related fluorescence by transilluminating the wings with UV light (wavelengths 385 – 390 nm, Turner et al., 2014). If Pd has infiltrated the skin, lesions will fluoresce yellow-orange under UV illumination (Turner et al., 2014). All cave-roosting species were banded with either 2.4mm or 2.9mm numbered, lipped alloy forearm bands (Porzana, Ltd., UK) and released at the point of capture.

Fungal samples were collected using sterile epidermal swabs dipped in sterile deionized water and rubbed on the bat's forearm and muzzle five times each (USGS National Wildlife Health Center, 2013). Swabs were placed in RNALater[®] Tissue Stabilization Solution (Life Technologies) and stored at -4 °C. We extracted fungal DNA from each swab sample using Qiagen DNeasy 96 Blood & Tissue kits (Qiagen Inc., Valencia, CA). All samples, as well as 16 negative control wells, were tested for the presence of *P. destructans* using a real-time PCR assay targeting the IGS region of the rRNA gene complex (Muller et al., 2013). All plates were run in duplicate to minimize false positive detections for the fungus. Any reaction that crossed the threshold baseline in less than 40 cycles was considered positive for *P. destructans* DNA and the average fungal DNA concentration in nanograms (ng) was calculated in each sample based on the cycle threshold value and a standard curve (Muller et al., 2013).

We followed field decontamination protocols in accordance with the United States Fish and Wildlife Service WNS Decontamination Guidelines and recommendations by the state of Tennessee (Shelley et al., 2013). All capture and handling techniques were approved by the University of Tennessee Institute

of Animal Care and Use (IACUC 2026-0514) and consistent with the guidelines issued by the American Society of Mammalogists (Sikes et al., 2011). We obtained both federal (USFWS TE-71613A) and state (TWRA 3716 and Tennessee Department of Environment and Conservation [TDEC] 2011-031) permits to capture and handle bats at winter hibernacula for this study.

Fungal load data (ng) were log transformed prior to analyses to meet assumptions of normality and homogeneity of variance. We examined spatial and temporal differences in fungal load and prevalence of *P. destructans* among species and seasons using one-way or nested ANOVAs and *t* tests in R (version 3.1.2; www.r-project.org). Infection prevalence was calculated by dividing the total number of infected individuals by the total number of individuals captured during the same time period. We calculated the body condition index (BCI) of each bat as a proxy for overall health by dividing weight by forearm length (Speakman and Racey, 1986). We excluded *Corynorhinus rafinesquii*, *Lasiurus borealis*, and *Lasionycteris noctivagans* from the comparative analyses due to small sample size. All means are reported $\pm$ standard error.

Results

We captured and swabbed 871 bats of 10 species (593 males, 276 females, 2 unknown due to escape). Of these, 409 individuals were positive for *P. destructans* DNA by real-time PCR analysis (Table 5). At least one individual from all species captured was *P. destructans* positive, including two *C. rafinesquii*, two *L. borealis* and one *L. noctivagans* (Bernard et al., 2015).

Over 51% of the bats captured ($N = 245/480$) during winter 2012 – 2013 were *P. destructans* positive, whereas only 41.6% of the bats ($N = 163/391$) were positive for *P. destructans* in winter 2013 – 2014. Fungal loads were significantly higher during the first year of sampling (0.010ng versus 0.003ng, respectively; $t_{315.7} = -3.91$, $p < 0.0001$). Excluding species with low sample size, there were significant differences in the monthly load of *P. destructans* among species ($F_{6, 397} = 62.82$, $p < 0.0001$). However, when comparing years, we found no significant difference in monthly loads of *P. destructans* among species. Fungal loads were lowest when bats entered hibernation in October and peaked for most species in February (Figure 5). Thereafter, *P. destructans* loads on six of the seven species declined through the end of the hibernation period in April. However, mean fungal load on *P. subflavus* continued to increase through the end of hibernation, reaching levels twice as high as those recorded in December. *Myotis septentrionalis* had the highest mean load (0.0178 ± 0.0017 ng), with one individual having a load of 2.30 ng, whereas *M. grisescens* had the lowest mean fungal load of all species sampled ($1.72 \times 10^{-4} \pm 0.0026$ ng). Infection prevalence varied among species, with large-bodied species, such as *E. fuscus* and *M. grisescens*, experiencing the lowest rates of prevalence (Figure 6).

Male *M. grisescens* had higher mean body condition than females (males: 0.223 g/mm, females: 0.216 g/mm; $t_{234.2} = 2.44$, $p < 0.0152$), while female *E. fuscus* had significantly higher body condition than males (males: 0.358 g/mm, females: 0.401 g/mm; $t_{33.6} = -2.55$, $p < 0.0153$). Body condition did not differ between males and females for the other five species. Mean body condition of *P.*

destructans negative bats ($0.220\text{g/mm} \pm 0.002$) was significantly higher than *P. destructans* positive bats ($0.170\text{g/mm} \pm 0.003$; $t_{865} = -14.12$, $p < 0.0001$; Figure 7). Within infected individuals, body condition decreased as a function of increased fungal load ($R^2 = 0.151$, $F_{1,404} = 72.092$ $p < 0.0001$). Body condition was highest at the onset of hibernation (October and November) and reached its lowest values during mid-hibernation (January and February), when fungal loads were highest (Figure 8). By the end of hibernation, mean body condition increased as the mean loads of *P. destructans* decreased.

We examined 481 bats for WNS-related fluorescence. Ultraviolet (UV) fluorescence was observed on bats with varying degrees of damage; from small pin-sized lesions to large coalescing regions of fluorescence and infiltration corresponding with increased pathogen loads. No bats captured during early hibernation (October and November) were found UV positive. The highest percent of bats found UV positive were captured during mid-hibernation (December = 35.9%, January = 29.5%), when body condition declined. Of all the individuals examined under UV, 66 were both positive for *P. destructans* and UV, with only two UV detections later classified as false-positives. A total of 181 individuals were positive for *P. destructans* but UV negative, whereas 232 bats were both negative for *P. destructans* and UV. Bats that were positive for both *P. destructans* and UV had higher fungal loads than individuals that were determined *P. destructans* positive but UV negative ($t_{201.8} = -8.72$, $p < 0.0001$). Ultraviolet negative individuals had higher body condition indices than bats positive for UV ($t_{154.3} = -6.11$, $p < 0.0001$), corresponding with comparisons

between mean fungal load and body condition. We found strong differences in pathogen load, prevalence and UV confirmation per site, with pathogen load highest at sites that did not contain *M. grisescens* ($t_{192.2} = -8.61$, $p < 0.0001$). Other bat species captured at *M. grisescens* sites had similar fungal loads as bats captured at non-*M. grisescens* sites. Of the individuals that were positive for UV, eight were captured at *M. grisescens* hibernacula, whereas the remaining individuals ($N = 57$) were from caves that were dominated by *M. septentrionalis*, *M. sodalis*, and *P. subflavus*.

Discussion

This is the first study to examine the prevalence and fungal load of *P. destructans* on bats active outside of caves during winter. Bats were captured leaving caves to forage when temperatures at emergence were at or above freezing (Bernard and McCracken, in review). Of the bats sampled, less than half were positive for the fungus, indicating that activity during winter in the Southeast is not indicative of disease. However, towards the end of our study, we saw an increase in the number of bats captured prior to sunset (Bernard and McCracken, in review). Whereas increases in unusual winter behavior associated with WNS in the Northeast were documented within the first two years of infection (Frick et al., 2010; Turner et al., 2011; Langwig et al., 2012), changes in the winter activity of bats in the Southeast were not evident until 3 – 4 years post-WNS confirmation (Carr et al., 2014, Bernard and McCracken, in review) .

Contrary to our prediction, both pathogen load and prevalence were lower in the second year of the study than in the first year on five of the seven bat species we captured. By the second year, all caves had been positive for at least two years. Similar trends have been documented after the arrival of *Batrachochytrium dendrobatidis*, the etiological agent of chytridiomycosis in frogs. Fungal loads in naïve frog populations increased precipitously in the first year, causing a rapid rise in infection intensity and prevalence in densely populated habitats (Briggs et al., 2010). As the fungal load on infected frogs increased, many populations suffered from high rates of mortality. However, the survival of infected individuals led to pathogen endemism and population persistence. A similar dynamic could be occurring in WNS infected hibernacula. Individuals with high pathogen loads perish in the first year, perhaps leaving individuals with lower fungal loads to return to the hibernacula each winter. Repeated low-dose exposure to *P. destructans*, mild winters and episodic feeding may allow for persistence of bat populations hibernating in the Southeast. Although we did not recapture any bats, recent evidence in the Northeast, as well as the marked decrease in fungal load over the course of our study, suggests some populations of bats have the ability to persist and reproduce regardless of continued exposure to WNS (Dobony et al., 2011; Reichard et al., 2014).

The infection prevalence and loads varied considerably among species, with all small-bodied species, except *M. leibii*, having higher fungal loads and increased rates of infection than in big brown and gray bats. Small, solitary bats,

such as *M. septentrionalis* and *P. subflavus*, were documented previously with significantly higher fungal loads than similar sized colonial species (Turner et al., 2012, Langwig et al., 2012) suggesting *P. destructans* spreads via density-dependent transmission in these species as cluster size increases (Langwig et al., 2012). In contrast, in our study, *M. lucifugus* and *M. sodalis*, which cluster in tight aggregations, were documented with fungal loads and prevalence similar to solitary species. Therefore, transmission of the pathogen among colonial species may be a function of the frequency of infected individuals within the cluster, rather than the increase in cluster size (Begon et al., 2002; Langwig et al., 2012).

Interestingly, *M. grisescens*, which exclusively hibernate in caves in the Midwest and southeastern US, had the lowest fungal loads and prevalence of *P. destructans* among all species sampled. In the Northeast, disease impacts on *M. lucifugus*, *M. septentrionalis* and *P. subflavus* increased with higher humidity and temperature within roosts, such that individuals sampled in the coldest and driest roosts had significantly lower fungal loads (Langwig et al., 2012). *Myotis grisescens*, however, hibernate in populations ranging from 100,000 to 1,500,000 individuals (U.S. Fish and Wildlife Service, 1982) in cold air traps varying from 1 – 9 °C (U.S. Fish and Wildlife Service, 2009), the lowest growth temperatures recorded for *P. destructans* (Verant et al., 2012). As of this writing, *M. grisescens* have yet to experience any WNS-related declines and appear to have consistent numbers at hibernation sites within Tennessee. Although the *M. grisescens* we captured have been identified with secondary fungal infections, skin discoloration, and or substantial tissue loss, we have yet to identify why the

species is surviving WNS. Several behavioral traits, such as preferred microclimates within hibernacula, sustained activity and foraging throughout winter (Bernard and McCracken, in review), and year-round cave use (Tuttle, 1976; Stevenson and Tuttle, 1981), may enable the species to prevent or minimize the colonization of Pd during torpor.

When all seven species were combined statistically, mean fungal load was highest during mid-hibernation, the coldest period of the year. In the Northeast, fungal loads were highest towards the beginning of spring, when bats first emerge from hibernation to prepare for migration and reproduction (Langwig et al., 2015). Although bats remain active throughout winter in the Southeast, the bats we captured during mid-hibernation, which had low mean body condition and increased wing damage, were likely attempting to replenish fat stores lost to the effects of WNS. *Perimyotis subflavus*, however, continued to experience an increase in fungal load through the end of hibernation, which could be attributed to the preferred microclimate (11 – 23 °C) of the species during hibernation (Fujita and Kunz, 1984; Briggler and Prather, 2003; Perkins, 2011). Alternatively, in-vitro growth curves suggest that *P. destructans* may reproduce more quickly in cave environments that maintain more moderate temperatures of 10 – 15 °C in winter (Verant et al., 2012), which could result in increased virulence in southern hibernacula, and therefore lead to a peak in fungal load during the coldest periods of winter.

Throughout our study, we found that the body conditions for *P. destructans* positive individuals were significantly lower than for individuals negative for the fungus. Variation in body condition among infected and uninfected individuals could be explained in two ways. First, bats entering hibernation with low fat reserves are more prone to infection due to the inability to sustain normal hibernation physiology and behavior. Pre-hibernation energy reserves must be large enough to allow for the prolonged torpor bouts necessary to survive low temperatures and insufficient food resources, while also enabling individuals to have enough energy to prepare for spring emergence when migration and reproduction occur (Humphries et al., 2003). Low fat stores and interrupted torpor bouts during this critical period may reduce the immune response necessary to combat WNS infection, leading to increased mortality in spring (Meteyer et al., 2012; Moore et al., 2013). Alternatively, bats may be entering hibernation with adequate energy reserves, however, individuals with high fungal loads are suffering from increased arousals during hibernation, leading to the loss of critical fat reserves necessary to survive winter and ward off infection. Increased arousal frequency due to infection by *P. destructans* has been well documented, with alterations in torpor bouts occurring within 35 days post inoculation (Warnecke et al., 2012), likely before invasion of the skin membranes.

In our study, body condition remained high during October and November and declined significantly by December, approximately 61 days into hibernation,

which corresponded with the increase in mean fungal loads and first sighting of UV fluorescence on bats. The mean body condition for all bats sampled was lowest during mid-hibernation, when loads of *P. destructans* peaked and external cave temperatures were coldest. Mean body condition began to increase towards the end of hibernation, likely as a function of the rise in ambient temperatures and prey availability. This increase in body condition corresponded with a significant decline in the mean fungal load of bats sampled in February and April. Only a small proportion of the individuals examined under UV were positive for *P. destructans* and also showed signs of tissue intrusion and increased wing damage. The remaining individuals that were *P. destructans* positive did not show signs of WNS infiltration under UV, indicating that bats confirmed with *P. destructans* are experiencing declines in body condition prior to suffering from WNS. Species, such as *M. septentrionalis*, with high rates of prevalence and fungal loads, were consistently found with the largest regions of fluorescence and wing damage, indicating high rates of tissue intrusion. Whereas, *M. grisescens*, which have low loads and prevalence, were found with substantial discoloration, wing damage and tissue loss unrelated from WNS.

Emerging infectious disease like WNS, are increasingly detrimental to environmental health and sustainability. North American bat populations are experiencing biodiversity loss due to WNS through the depopulation of naïve bat hosts, and will likely see chronic population depression with the long-term persistence of *P. destructans* within cave environments. Our findings illustrate how winter activity can drive the variations seen in pathogen load and infection

intensity among species and within sites, specifically in the understudied region of the Southeast. By understanding the species-specific dynamics of *P. destructans* within active winter populations, management strategies, such as regional area closures and bio-control methods can be implemented more effectively. In the Southeast, mitigation measures may work best when targeting hibernacula with small-bodied bats such as *M. lucifugus*, *M. septentrionalis*, and *P. subflavus*. Our results also demonstrate that the time since *P. destructans* confirmation at a site is critical in trying to mitigate biodiversity loss. Infection prevalence and load were highest during the first year of invasion; therefore mitigation measures should be applied at the beginning of hibernation within the first year of disease confirmation. Finally, our study indicates that some populations are persisting regardless of repeated exposure to *P. destructans*. Although the region is currently experiencing WNS-related mortality within highly affected species, mild temperatures and the persistent availability of prey during winter may allow for the Southeast to be a refuge for surviving bat populations.

CHAPTER 3: EFFECTS OF WHITE-NOSE SYNDROME ON BAT COMMUNITIES IN THE SOUTHEASTERN UNITED STATES

This chapter is slightly modified from a paper in review:

Bernard, R. F. and G. F. McCracken. *In Review*. Effects of White-Nose Syndrome on Bat Communities in the Southeastern United States. *Royal Publishing Open Science*.

My consistent use of “we” throughout this chapter is in reference to my co-author, Gary McCracken and myself. I was the primary contributor to this work, which involved the following tasks: (1) development of project design and all data collection, (2) measurement of acoustic data and statistical analysis, (3) gathering and interpretation of the relevant literature, and (4) all of the writing.

Abstract

Previous studies in northeastern North America have examined the effects of winter mortality from WNS on bat communities during summer, documenting up to a 73% decline in *Myotis spp.* within 1 – 2 years after WNS confirmation. However, to date, no studies have examined the effects of WNS on summer bat communities in the South, where winter habitats are warmer and species composition is more diverse. We recorded bat activity to monitor the community composition of bats in Tennessee from June – August in 2012 – 2014. In contrast to the Northeast, where *Myotis lucifugus* and *Lasiurus cinereus* are the dominant species, the community composition of bats in the Southeast was dominated by *Eptesicus fuscus* and *L. cinereus*, with *Myotis spp.* making up less than 12% of all bat passes recorded. We documented a 33% decline in the relative acoustic activity of WNS-affected species (*E. fuscus*, *Myotis spp.*, and *P. subflavus*). However, this decline was not evident until the 4th summer following the detection of WNS in the region, and was less severe than declines documented in the North. *Myotis grisescens*, a species not present in the North, was the dominant *Myotis spp.* recorded, and increased in acoustic abundance

throughout the study. While WNS-related mortality in the Southeast led to changes in the composition of summer bat communities, the species affected and the severity and timing of those declines were significantly different when compared to mortality events in the Northeast.

Introduction

Populations of several bat species in northeastern North America have declined dramatically since the discovery of white-nose syndrome (WNS) in New York during winter 2006. Recent estimates suggest WNS has killed approximately 5 – 6 million cave-dwelling bats over the last 8 years (Titchenell, 2012; U.S. Fish and Wildlife Service, 2014), with some populations exhibiting 90 – 100% declines (Frick et al., 2010; U.S. Fish and Wildlife Service, 2014). Seven species have been confirmed with the disease (*Eptesicus fuscus*, *Myotis grisescens*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, *M. sodalis*, and *Perimyotis subflavus*), with the most serious losses seen in *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* (U.S. Fish and Wildlife Service, 2014).

The psychrophilic (cold-thriving) fungus *Pseudogymnoascus destructans*, the causative agent of WNS, is endemic to Europe, where it does not cause large-scale mortality (Wibbelt et al. 2010, Lindner et al. 2011, Lorch et al. 2011). Throughout its newly invaded range, signs of WNS, such as white fungal growth on the muzzle, ears, tail and wing membranes (Blehert et al., 2009), tend to manifest during the coldest months of winter when most population surveys and disease surveillance of cave-roosting bats occur. During hibernation, the fungus

invades the hair follicles and skin glands of bats when their immune systems are suppressed during torpor, leading to the invasion of underlying connective tissue and capillaries, severely eroding the wing and tail membranes (Meteyer et al., 2009). As a consequence of disturbance during torpor, bats may not survive hibernation due to loss of stored fat and physiological stress. Mass mortality also occurs soon after emergence from hibernation due to the sudden increase in a bats' immune response upon returning to sustained euthermia (Francl et al., 2011; Reeder et al., 2012; Meteyer et al., 2012) and post-emergence wing damage (Fuller et al., 2011; Meteyer et al., 2012). Due to state and federal restrictions limiting the number of visits to caves during winter and because of post-hibernation mortality, declines in susceptible bat populations are difficult to assess from hibernacula counts alone (Ford et al., 2011).

In addition to counts within hibernacula, acoustic surveys of activity in summer have proven valuable for documenting declines in bat populations and resulting shifts in community compositions (Rodhouse et al., 2011; Ford et al., 2011). In Massachusetts and New York, acoustic surveys have documented substantial declines in the abundance of cave hibernating species in the first and second summers following the detection of WNS, with shifts in acoustic abundances favoring tree-roosting species and larger-bodied bats (Brooks, 2011; Dzal et al., 2011; Ford et al., 2011). These acoustic data are consistent with observations that large-scale mortality of the most sensitive bats species in the Northeast occurs within two years of confirmation of WNS (Frick et al., 2010; Turner et al., 2011; Langwig et al., 2012).

White-nose syndrome was first documented in Tennessee during winter 2009 – 2010 and was confirmed in 46 of the 95 counties in the State by winter 2013 – 2014 (Flock, 2014). However, mortality and unusual winter behavior attributed to WNS were not documented until February 2014 at 8 caves in 6 counties (Carr et al. 2014, Bernard and McCracken in review, Cory Holliday pers. comm.), nearly five winters after WNS was confirmed in the region (Flock 2014). Here we investigate whether the community composition of bats active in summer in Tennessee show similar shifts as observed in the Northeast. Due to regional differences in the impacts of WNS on bats (Carr et al. 2014, Bernard and McCracken in review), we test the following hypotheses about shifts in composition of bat communities in summer in the Southeast: 1) acoustic activity of WNS-affected species will decline as WNS-related mortality increases at regional hibernacula; 2) due to regional differences in susceptible bat fauna, impacts of WNS will differ among species shared between regions and include species not present in the North; however, 3) compared to patterns observed in the Northeast we expect these effects to be revealed 4 – 5 years after the fungus was confirmed in the region.

Methods

Acoustic monitoring was initiated three years after *P. destructans* was confirmed in Tennessee, and before detection of bat mortality due to WNS (Carr 2014; Flock 2014). SM2Bat+ ultrasonic bat detectors (Wildlife Acoustics, Inc., Concord, MA) were stationed at five sites; two locations in Jefferson Co.

(36°6'14.7"N 83°37'40.8"W and 36°6'14.6"N 83°37'21.4"W) and three locations in Knox Co. (35°57'27.5"N 83°51'51.3"W, 35°57'9.9"N 83°52'83.5"W and 35°57'27.7"N 83°55'32.9"W) near Knoxville, Tennessee, USA from June 3 – August 31, 2012, June 1 – August 29, 2013 and June 1 – August 31, 2014. Detector locations included riparian, forested and urban landscapes to account for differences in habitat use by bat species (Hayes, 2000; Rodhouse et al., 2011; Skalak et al., 2012). Habitat types were not included in the final analysis. Bat detectors were programmed to record every night in full-spectrum, WAC-0 compression mode, onto 32GB SD-cards from 30-minutes before civil sunset to 30-minutes after civil sunrise. Units were checked every 10 – 30 days depending upon battery capacity. When detectors were on private property or buildings, we used external AC-adaptors (Wildlife Acoustics, Inc.) connected to rechargeable sealed lead-acid batteries [6-volt 58.0 Amp. Hrs., Power-Sonic Corp., San Diego, CA). Bat detectors using external batteries were checked every 30 days to change batteries and SD cards. Detectors deployed in public parks or high traffic areas were programmed to use 4-internal D-cell batteries and checked every 10 – 12 days to change batteries and SD cards. Microphones were replaced each season to minimize differences in detectability due to loss in sensitivity.

Data were downloaded using a SD-card reader and converted from .WAC files to .WAV files using wac2wav converter software (v. 3.2.7, Wildlife Acoustics, Inc.). To process .WAC files, we chose an 8-second split trigger to separate bat passes and selected the SMX-US filter to scrub all files. Noise files and poor quality bat passes were removed during conversion. Converted .WAV files were

analyzed using the SonoBat™ (v. 3.06 KY-TNvi, Arcata, CA) SM2 batch compensation processing tool for automated identification of each call file. SonoBat™ is a full-spectrum discriminant function analysis identification software which confirms species IDs using 76 call parameters (i.e. maximum frequency, frequency at knee, duration, etc). Prior to analysis, we confirmed that all classification preference settings were consistent between every batch analysis. All settings either met or exceeded recommended values with the discriminant probability threshold for classification set at 0.95, and the acceptable call quality at 0.80. Automated identification of echolocation calls allows for increased efficiency in processing large quantities of data, while minimizing operator bias, however, accuracy of species identification can vary from 60 – 95% (Parsons and Jones, 2000; Redgwell et al., 2009; Armitage and Ober, 2010; Britzke et al., 2011). Therefore, only call files that met the requirements for classification in Sonobat™ and were consistent among all identification categories (“by vote”, “by consensus”, and “mean classification”) were included in the final analysis.

Bat calls were normalized as the total number of “bat passes” recorded per hour. A bat pass was defined as a file containing a search-phase echolocation sequence of ≥ 2 echolocation pulses (Gannon et al., 2003). This metric is an index of overall activity and does not measure the number of individual bats actively flying in the area (Kunz et al., 2007; Schwab and Mabee, 2014). Activity levels per species were compared between years and WNS vulnerability status (as per www.whitenosesyndrome.org) using one-way ANOVAs and Tukey’s HSD post hoc tests ($\alpha = 0.05$) in R (Version 3.1.0, R

Development Core 2014). We classified WNS vulnerability status as: WNS-affected species (*E. fuscus*, *M. grisescens*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, *M. sodalis*, and *P. subflavus*) and unaffected species (*Corynorhinus rafinesquii*, *Lasiurus borealis*, *L. cinereus*, *Lasionycteris noctivagans*, *Nycticeius humeralis*, and *Tadarida brasiliensis*). Due to low sample size and the challenge of accurately classifying *Myotis* bats to species (Weller et al., 2007; Walters et al., 2012), we grouped all *Myotis* spp. together for most comparative analyses. We downloaded daily weather data for Knoxville from Weather Underground (www.wunderground.com) and compared mean temperature and precipitation for each month across all years.

Results

Over the three summers, we collected 6,245 hours of acoustic data, totaling 114,602 individual call files. We identified 32,989 bat passes to species (Table 6). WNS-affected species experienced significant declines in activity from 2013 to 2014 ($F_{2,4383} = 11.76$, $p < 0.0001$), with a decline of 33.5% over the entire study period (Figure 9). Summer activity of unaffected species increased by 64% from 2012 to 2014 ($N = 4,094$ bat passes in 2012 versus $N = 7,346$ bat passes in 2014), with the most significant increase in relative activity observed in 2014 ($F_{2,4383} = 15.42$, $p < 0.0001$; Figure 9). Acoustic activity of *E. fuscus* was nearly identical in 2012 and 2013, but declined significantly in 2014 ($F_{2,4383} = 18.66$, $p < 0.0001$). Summer activity of *Myotis* spp. varied significantly throughout the study ($F_{2,4383} = 5.61$, $p = 0.0037$). A post-hoc Tukey's HSD test indicated that the

number of bat passes identified as *Myotis* were significantly higher in 2014 than in 2012 and 2013, with no significant difference between mean numbers of *Myotis* calls in 2012 and 2013. Although not significant due to low sample size, the abundance of bat passes attributed to *M. lucifugus* and *M. sodalis* declined each year of the study, whereas the acoustic abundance of *M. septentrionalis* increased (Table 6). Calls identified as those of *M. grisescens* comprised between 75 and 82% of those classified as *Myotis*, and showed no consistent shift in abundance over the years of the study. The acoustic abundance of *C. rafinesquii*, *L. borealis* and *L. noctivagans* did not vary significantly over the course of the study, even though all three species have been documented with the fungus at local hibernacula (Bernard et al., 2015). The apparent absence of impact of WNS on these species is consistent with previous studies (Dzal et al., 2011; Johnson et al., 2012). Interestingly, there was an increase in bat passes identified as *T. brasiliensis*, a species not historically resident in the area.

We found no significant difference in mean precipitation over the course of the study. Mean temperature did not vary significantly during June and August across all years, however, there was a significant difference in mean daily temperature in July ($F_{2,90} = 22.16$, $p < 0.0001$), with July 2012 significantly warmer on average than both 2013 and 2014.

Discussion

The bat communities we investigate in the Southeast comprise of the same species present in infected regions in the Northeast, plus four additional

species including *C. rafinesquii*, *M. grisescens*, *N. humeralis* and *T. brasiliensis*. While the two regions share many species, there are substantial differences in the regional abundance of these species. Prior to WNS, bat populations in the Northeast were dominated by *M. lucifugus*, with the calls of that species accounting for 72% to 82% of all bat activity (Brooks, 2011; Dzal et al., 2011). In contrast, the pre-WNS acoustic activity in our study was dominated by *E. fuscus*, *L. cinereus* and *M. grisescens*, with the calls of these three species accounting for 38.6%, 13.4%, and 9.2%, respectively, of all bat passes recorded. In Tennessee, all *Myotis spp.*, combined, comprised less than 12% of identified bat passes, and of these, 77.3% were identified as *M. grisescens*, a species not present in the Northeast.

Brooks (2011) observed a 72.2% reduction in the acoustic activity of *Myotis spp.* between pre-WNS and post-WNS surveys in New England, corresponding to the 73% decline of *Myotis* populations within 2 years of when WNS was documented in regional hibernacula (Frick et al., 2010). Our results reveal that the impacts of WNS on summer activity and community composition of bats in the Southeast were not evident until the 4th summer post-detection; and at that time, the effects were not as severe as in the Northeast. In addition, in our study, two different species, *E. fuscus* and *P. subflavus*, drove the observed decrease in acoustic abundance of WNS-affected species. While the abundance of calls attributed to *M. lucifugus* and *M. sodalis* declined over the course of our study, we did not observe an overall decline in *Myotis spp.* because of the large numbers of calls identified as *M. grisescens* (Table 6). The decline in acoustic

activity of WNS-affected species in 2014 coincided with the 80% decline in *M. sodalis* and 40% decline in both *M. lucifugus* and *P. subflavus* reported from cave surveys during winter 2013 – 2014 at hibernacula in Great Smoky Mountains National Park (Carr et al., 2014, William Stiver, pers. comm.). At this writing, other hibernacula in the Southeast have yet to record comparable losses. Notably, this includes no reductions of numbers of *M. grisescens* in hibernacula (Cory Holliday, pers. comm.). Therefore, in contrast to the Northeast, *E. fuscus* and *P. subflavus* appear to be the species most affected by WNS in our region (Flock, 2014; William Stiver, pers. comm.).

Previous studies that examined the impact of WNS on summer bat communities in the Northeast have documented increases in the relative numbers of bat passes for tree-roosting and migratory species (Brooks 2011; Dzal et al., 2011; Ford et al., 2011). Shifts in the relative abundance of bat passes are expected with the decline of WNS-affected species. However, in Massachusetts and New York, Dzal et al. (2011) and Ford et al. (2011) also report increases in the total activity of unaffected species. Ford et al. (2011) suggested that competitive release resulting from the loss of WNS-affected bats could be responsible for the observed increase in absolute number of those species that are not impacted by WNS. We observed similar patterns, with an increase in both the relative activity and total numbers of bat passes of unaffected bats. A possible alternative hypothesis to competitive release is that prior to the impacts of WNS, low flying, clutter-adapted bats, such as *Myotis spp.* and *P. subflavus* that are most affected by the disease, were recorded in

apparently greater abundance due to their proximity to the detectors and intensity of echolocation calls (Murray et al., 1999). However, our data show that WNS-affected and unaffected species were often recorded at the same time. Thus, we reject this “acoustic proximity” hypothesis and suggest that reduced competition from WNS-affected species may have resulted in a numeric response of some unaffected species. Specifically, between 2012 and 2014, the increase in acoustic activity of unaffected species, was due largely to *L. cinereus* and *T. brasiliensis* (Table 6). *Lasiurus cinereus* is an endemic species in the region that may be benefiting from the loss of WNS-affected species. *Tadarida brasiliensis*, on the other hand, is an abundant bat in southern latitudes that has recently expanded its range into the greater Tennessee Valley (McCracken et al. unpublished). At this time it is not possible to assess if the increased abundance of *T. brasiliensis* is due to competitive release caused by the loss of WNS-affected bat species, because of climate change, or because of some combination of factors.

Our study highlights regional differences in the impact of WNS-related mortality on widespread bat species, and the impacts of disease on regional bat communities. We found that the most noticeable declines attributed to WNS in Tennessee were of *E. fuscus* and *P. subflavus*, species that have not experienced substantial population losses in the Northeast (Brooks, 2011; Dzal et al., 2011; Ford et al., 2011). Although census counts during winter can more effectively track changes in population size for more gregarious species, such as *M. grisescens*, *M. lucifugus*, and *M. sodalis*, population losses of more cryptic or

solitary cave species such as *E. fuscus* and *P. subflavus* are more difficult to assess. We propose that monitoring summer habitats is a valuable method for identifying how bat species assemblages are being affected by WNS as *P. destructans* continues to spread throughout North America.

CONCLUSIONS

Chapter 1: Winter activity and the progression of white-nose syndrome in bats of the Southeastern United States

- Ten species of bat remained active throughout winter in the Southeastern United States, with their activity correlated with mean daily temperature and temperature at emergence. Bats left hibernacula on warm winter nights to search for available prey and were often successful in their attempt to replenish energy stores as determined by fecal samples.
- Aberrant behavior associated with white-nose syndrome, such as cold weather activity and daytime flights became apparent 4 to 5 years after initial confirmation of *Pseudogymnoascus destructans* in hibernacula. Excluding *Myotis grisescens* dominated caves; hibernacula containing *Myotis spp.* and *Perimyotis subflavus* experienced the most significant change in behavior and body condition due to disease.
- Although WNS has been confirmed in *M. grisescens*, we believe their large body size and relaxed energetic constraints for hibernating through shorter winters may prevent *P. destructans* from reproducing at disease inducing levels. While these theories have yet to be investigated, they could provide insight as to why *M. grisescens* populations are so far persisting during this epizootic.
- Bats hibernating in southern latitudes entered hibernation with lower mean body condition than reported for bats in the Northeast, suggesting regional differences in climate drive variations in the behavioral responses of bats

during winter. Mild winter climates and available resources allow for shorter hibernation, thus reducing the demands of increased fat storage prior to hibernation.

Chapter 2: Variation in load and prevalence of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, on active bats during winter in the Southeastern United States

- This chapter confirms for the first time that the activity of bats during winter in the Southeast is not necessarily due to white-nose syndrome. Over 50% of the bats captured in winter were not found with detectable *P. destructans* DNA, indicating that they were emerging from caves for other reasons, likely warm temperatures and foraging.
- The total number of bats positive for *P. destructans* decreased each year, with fungal loads significantly higher during the first year of sampling. Repeated low-dose exposure to *P. destructans*, mild winters and episodic feeding may allow for some populations of bats hibernating in the Southeast to survive infection by *P. destructans*. If reproduction within survivors is occurring, species with ranges extending into the hardest hit regions in the North may repopulate habitats devastated by white-nose syndrome.
- Small-bodied bats, such as *M. lucifugus*, *M. septentrionalis* and *P. subflavus*, had the highest fungal loads and prevalence of all species sampled. *Myotis grisescens*, however, had the lowest load and

prevalence. This evidence may help identify target areas for possible bio-control and mitigation methods. By focusing on hibernacula with a higher diversity of species, managers may minimize the number of species lost within the region.

- *Myotis grisescens*, which hibernate in colonies of 10s to 100s, of thousands have yet to experience declines caused by white-nose syndrome. *Myotis grisescens* cohabitate in hibernacula with highly susceptible species such as *M. septentrionalis* and *M. sodalis*, but maintain low loads and prevalence of *P. destructans*. Additional research focusing on species-specific behaviors and habitat-use may help identify the mechanisms enabling *M. grisescens* to minimize infection by *P. destructans* and thrive during this epizootic.

Chapter 3: Effects of white-nose syndrome on bat communities in the Southeastern United States

- Changes in summer bat communities in the Southeast were not evident until the 4th summer following confirmation of *P. destructans* in regional hibernacula. This contrasts with impacts seen the first and second year after confirmation of the fungus in northeastern hibernacula. The decline in WNS-affected species correlated with the behavioral changes and mortality documented within Great Smoky Mountains National Park during winter 2013 – 2014.

- As seen in the Northeast, the decline in WNS-affected species was associated with increased acoustic activity of unaffected species in the Southeast. This shift in acoustic abundance may be due to the competitive release from the loss of once dominant species.
- The decline in acoustic activity in WNS-affected species was largely driven by the decrease in acoustic activity of *E. fuscus* and *P. subflavus*. Observed declines in the activity of several *Myotis spp.* were masked due to the abundance of *M. grisescens*, a dominant species in Southern latitudes.
- Increased acoustic activity of unaffected species was driven by an increase in the acoustic abundance of *Lasiurus cinereus* and *Tadarida brasiliensis*. Whereas *L. cinereus* are regionally endemic, *T. brasiliensis*, a species common in southern latitudes, are currently expanding their range northward. The timing of this expansion coincides with the white-nose syndrome epizootic, but the presence and increase in this species may be due to other factors such as climate change.

Directions for future research

Several areas for future research arise from this dissertation. While my research documents the variation in winter behavior and susceptibility of active bats in the Southeast, no one has looked at the habitat use of cave-roosting bats outside of hibernacula during winter. Annual hibernacula surveys have indicated a number of bats banded during this study were found roosting within the same

caves they were captured, suggesting some long-term survival within affected populations. Due to the low recapture rate of bats, it is difficult to determine how often bats arouse from torpor under natural conditions or leave caves during the hibernation period. Through the use of temperature-sensitive radio transmitters or Passive Integrated Transponder (PIT) tags, researchers could answer questions that focus on the activity of bats outside of caves during winter in the Southeast. Specifically, researchers could look at how often wild bats arouse from torpor; how long their euthermic periods are; if they leave the cave during each arousal period; how often they leave the cave throughout winter; how long they are outside of the hibernaculum; if arousal periods and cave departures correlate with periods of high fungal loads or low body condition; track how far they fly away from the hibernaculum during foraging bouts; how long each foraging bout lasts; and if they use nocturnal roosts during winter foraging. By attempting to answer these research questions, biologists will be able to understand species-specific behaviors that will inform managers when or where to initiate habitat regulations or mitigation measures.

Another area for future research is through long-term acoustic monitoring. Our research identified regional differences in the impact of WNS-related mortality on widespread bat species, as well as the variation in the impacts of disease on regional bat communities. Although census counts during winter can more effectively track changes in population size for more gregarious species, population losses of more cryptic or solitary cave species are more difficult to assess. Long-term acoustic monitoring of both summer habitats and winter

hibernacula is a valuable method for identifying how bat species assemblages are being affected by WNS and which species may be experiencing the most significant declines due to disease.

LIST OF REFERENCES

- ARMITAGE, D. W., AND H. K. OBER. 2010. A comparison of supervised learning techniques in the classification of bat echolocation calls. *Ecological Informatics* 5: 465–473.
- ARNETT, E. B., AND J. P. HAYES. 2006. Patterns of pre-construction bat activity at a proposed wind facility in south-central Pennsylvania. 46 pp.
- BARCLAY, R. M. R. 1999. Bats are not birds: A cautionary note on using echolocation calls to identify bats: A comment. *Journal of Mammalogy* 80: 290–296.
- BEGON, M., M. BENNETT, R. G. BOWERS, N. P. FRENCH, S. M. HAZEL, AND J. TURNER. 2002. A clarification of transmission terms in host-microparasite models: numbers, densities and areas. *Epidemiology and Infection* 129: 147–153.
- BERNARD, R. F., J. T. FOSTER, E. V. WILLCOX, K. L. PARISE, AND G. F. MCCracken. 2015. Molecular detections of the causative agent of white-nose syndrome on Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) and two species of migratory bats in the Southeastern USA. *Journal of Wildlife Diseases* 51: 000–000.
- BLEHERT, D. S., A. C. HICKS, M. J. 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. 2009. Bat white-nose syndrome: an emerging fugal pathogen? *Science* 323: 227.
- BLEHERT, D. S., J. M. J. M. LORCH, A. E. BALLMANN, P. M. CRYAN, AND C. U. METEYER. 2011. Bat white-nose syndrome in North America. *In* *Fungal diseases: An emerging threat to human, animal and plant health*. The National Academies Press, Washington, D.C. pp. 167–176.
- BOYLES, J. G., M. B. DUNBAR, AND J. O. WHITAKER JR. 2006. Activity following arousal in winter in North American vespertilionid bats. *Mammal Review* 36: 267–280.
- BRACK JR., V., AND J. W. TWENTE. 1985. The duration of the period of hibernation of three species of vespertilionid bats. I. Field studies. *Canadian Journal of Zoology* 63: 2952–2954.
- BRANDT, M. E., AND D. W. WARNOCK. 2007. Histoplasma, Blastomyces, Coccidioides, and other dimorphic fungi causing systemic mycoses, 9th edition. *In* *Manual of clinical microbiology*. P. R. Murray and E. J. Baron (eds.). ASM Press, Palo Alto, CA. pp. 1857–1873.

- BRIGGLER, J. T., AND J. W. PRATHER. 2003. Seasonal use and selection of caves by the eastern pipistrelle bat (*Pipistrellus subflavus*). *American Midland Naturalist* 149: 406–412.
- BRIGGS, C. J., R. A. KNAPP, AND V. T. VREDENBURG. 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. *PNAS* 107: 9695–9700.
- BRITZKE, E. R., J. E. DUCHAMP, K. L. MURRAY, R. K. SWIHART, AND L. W. ROBBINS. 2011. Acoustic identification of bats in the eastern United States: A comparison of parametric and nonparametric methods. *The Journal of Wildlife Management* 75: 660–667.
- BROOKS, R. T. 2011. Declines in summer bat activity in central New England 4 years following the initial detection of white-nose syndrome. *Biodiversity and Conservation* 20: 2537–2541.
- BURLES, D. W., M. B. FENTON, R. M. R. BARCLAY, R. M. BRIGHAM, AND D. VOLKERS. 2014. Aspects of the winter ecology of bats on Haida Gwaii, British Columbia. *Northwestern Naturalist* 95: 289–299.
- CAFARCHIA, C., K. EATWELL, D. S. JANSSON, C. U. METEYER, AND G. WIBBELT. 2012. Other fungal infections. *In* *Infectious Diseases of Wild Mammals and Birds in Europe*. D. Gavier-Widen, A. Meredith, and J. P. Duff (eds.). John Wiley & Sons, Inc., Somerset, NJ. pp. 466–475.
- CARR, J. A., R. F. BERNARD, AND W. H. STIVER. 2014. Unusual bat behavior during winter in Great Smoky Mountains National Park. *The Southeastern Naturalist* 13: 18–21.
- CASADEVALL, A. 2005. Fungal virulence, vertebrate endothermy, and dinosaur extinction: Is there a connection? *Fungal Genetics and Biology* 42: 98–106.
- CASADEVALL, A., AND L. PIROFSKI. 1999. Host-pathogen interactions: Redefining the basic concepts of virulence and pathogenicity. *Infection and Immunity* 67: 3703–3713.
- CRYAN, P. M., C. U. METEYER, D. S. BLEHERT, J. M. LORCH, D. M. REEDER, G. G. TURNER, J. WEBB, M. J. BEHR, M. VERANT, R. E. RUSSELL, AND K. T. CASTLE. 2013. Electrolyte depletion in white-nose syndrome bats. *Journal of Wildlife Diseases* 49: 398–402.
- CRYAN, P. M., C. U. METEYER, J. G. BOYLES, AND D. S. BLEHERT. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biology* 8: 135.

- CZENZE, Z. J., A. D. PARK, AND C. K. R. WILLIS. 2013. Staying cold through dinner: cold-climate bats rewarm with conspecifics but not sunset during hibernation. *Journal of Comparative Physiology B* 183: 859–66.
- DASZAK, P., L. BERGER, A. A. CUNNINGHAM, A. D. HYATT, D. E. GREEN, AND R. SPEARE. 1999. Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5: 735–48.
- DASZAK, P., A. A. CUNNINGHAM, AND A. D. HYATT. 2000. Emerging infectious diseases of wildlife: threats to biodiversity and human health. *Science* 287: 443–449.
- DAVIS, W. H. 1959. Disproportionate sex ratios in hibernating bats. *Journal of Mammalogy* 40: 16–19.
- DECHER, J., AND J. R. CHOATE. 1995. *Myotis grisescens*. *Mammalian Species*: 1–7.
- DIMITROV, D. T., T. G. HALLAM, C. E. RUPPRECHT, AND G. F. MCCracken. 2008. Adaptive modeling of viral diseases in bats with a focus on rabies. *Journal of Theoretical Biology* 255: 69–80.
- DOBONY, C. A., A. C. HICKS, K. E. LANGWIG, R. I. VON LINDEN, J. C. OKONIEWSKI, AND R. E. RAINBOLT. 2011. Little brown myotis persist despite exposure to white-nose syndrome. *Journal of Fish and Wildlife Management* 2: 190–195.
- DZAL, Y. A., L. P. MCGUIRE, N. VESELKA, AND M. B. FENTON. 2011. Going, going, gone: the impact of white-nose syndrome on the summer activity of the little brown bat (*Myotis lucifugus*). *Biology Letters* 7: 392–4.
- ELDER, W. H., AND W. J. GUNIER. 1978. Sex ratios and seasonal movements of gray bats (*Myotis grisescens*) in Southwestern Missouri and adjacent states. *American Midland Naturalist* 99: 463–472.
- 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 Publishing Group. Nature* 484: 186–94.
- FLOCK, B. 2013. Tennessee Bat Population Monitoring and White Nose Syndrome Surveillance. 17 pp.
- . 2014. Bat population monitoring and White-nose Syndrome surveillance. 15 pp.

- FOLEY, J., D. CLIFFORD, K. CASTLE, P. M. CRYAN, AND R. S. OSTFELD. 2011. Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. *Conservation biology* 25: 223–231.
- FORD, W. M., E. R. BRITZKE, C. A. DOBONY, J. L. RODRIGUE, AND J. B. JOHNSON. 2011. Patterns of acoustical activity of bats prior to and following white-nose syndrome occurrence. *Journal of Fish and Wildlife Management* 2: 125–134.
- FRANCL, K. E., D. W. SPARKS, V. BRACK, AND J. TIMPONE. 2011. White-nose syndrome and wing damage index scores among summer bats in the northeastern United States. *Journal of Wildlife Diseases* 47: 41–48.
- 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: 679–82.
- FUJITA, M. S., AND T. H. KUNZ. 1984. *Pipistrellus subflavus*.
- FULLER, N. W., J. D. REICHARD, M. L. NABHAN, S. R. FELLOWS, L. C. PEPIN, AND T. H. KUNZ. 2011. Free-ranging little brown myotis (*Myotis lucifugus*) heal from wing damage associated with white-nose syndrome. *EcoHealth* 8: 154–62.
- GANNON, W. L., R. E. SHERWIN, AND S. HAYMOND. 2003. On the importance of articulating assumptions when conducting acoustic studies of habitat use by bats. *Wildlife Society Bulletin* 31: 45–61.
- GARCIA-SOLACHE, M. A., AND A. CASADEVALL. 2010. Global warming will bring new fungal diseases for mammals. *mBio* 1: e00061–10.
- GORE, J. A. 1992. Gray bat, *Myotis grisescens*. In *Rare and endangered biota of Florida*. Vol. 1: Mammals. S. R. Humphrey (ed.). University Press of Florida, Gainesville. pp. 63–70.
- GRIFFIN, D. R. 1945. Travels of banded bats. *Journal of Mammalogy* 26: 15–23.
- HAWKSWORTH, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* 105: 1422–1432.
- HAYES, J. P. 2000. Assumptions and practical considerations in the design and interpretation of echolocation-monitoring studies. *Acta Chiropterologica* 2: 225–236.

- HOLLIDAY, C. 2012. White-Nose Syndrome Disease Surveillance and Bat Population Monitoring Report A Report of the Tennessee WNS Response Cooperators. 1-10 pp.
- HOPE, P. R., AND G. JONES. 2012. Warming up for dinner: torpor and arousal in hibernating Natterer's bats (*Myotis nattereri*) studied by radio telemetry. *Journal of Comparative Physiology B* 182: 569–78.
- HUMPHRIES, M. M., D. W. THOMAS, AND D. L. KRAMER. 2003. The role of energy availability in mammalian hibernation: a cost-benefit approach. *Physiological and Biochemical Zoology* 76: 165–179.
- JOHNSON, J. S., M. J. LACKI, S. C. THOMAS, AND J. F. GRIDER. 2012. Frequent arousals from winter torpor in Rafinesque's big-eared bat (*Corynorhinus rafinesquii*). *PLoS ONE* 7: e49754.
- JONASSON, K. A., AND C. K. R. WILLIS. 2011. Changes in body condition of hibernating bats support the thrifty female hypothesis and predict consequences for populations with white-nose syndrome. *PLoS ONE* 6: e21061.
- , AND ———. 2012. Hibernation energetics of free-ranging little brown bats. *The Journal of Experimental Biology* 215: 2141–9.
- KNUDSEN, G. R., R. D. DIXON, AND S. K. AMELON. 2013. Potential spread of white-nose syndrome of bats to the Northwest: epidemiological considerations. *Northwest Science* 87: 1–37.
- KUNZ, T. H., E. B. ARNETT, B. M. COOPER, W. P. ERICKSON, R. P. LARKIN, T. J. MABEE, M. L. MORRISON, M. D. STRICKLAND, AND J. M. SZEWCZAK. 2007. Assessing impacts of wind-energy development on nocturnally active birds and bats: A guidance document. *The Journal of Wildlife Management* 71: 2449–2486.
- KUNZ, T. H., E. BRAUN DE TORREZ, D. BAUER, T. LOBOVA, AND T. H. FLEMING. 2011. Ecosystem services provided by bats. *Annals of the New York Academy of Sciences* 1223: 1–38.
- 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 of London B* 282: 20142335.

LEOPARDI, S., D. BLAKE, AND S. J. PUECHMAILLE. 2015. White-nose syndrome fungus introduced from Europe to North America. *Current Biology* 25: R217–R219.

LINDNER, D. L., A. GARGAS, J. M. LORCH, M. T. BANIK, J. GLAESER, T. H. KUNZ, AND D. S. BLEHERT. 2011. DNA-based detection of the fungal pathogen *Geomyces destructans* in soils from bat hibernacula. *Mycologia* 103: 241–6.

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 Publishing Group. Nature* 480: 376–8.

MACPHEE, R. D. E., AND A. D. GREENWOOD. 2013. Infectious Disease, Endangerment, and Extinction. *International Journal of Evolutionary Biology* 2013: 1–9.

MELVIN, R. G., AND M. T. ANDREWS. 2009. Torpor induction in mammals: recent discoveries fueling new ideas. *Trends in Endocrinology and Metabolism* 20: 490–8.

MENDELSON, J. R., K. R. LIPS, R. W. GAGLIARDO, G. B. RABB, J. P. COLLINS, J. E. DIFFENDORFER, P. DASZAK, R. I. D, K. C. ZIPPEL, D. P. LAWSON, K. M. WRIGHT, S. N. STUART, C. GASCON, H. R. DA SILVA, P. A. BURROWES, R. L. JOGLAR, E. LA MARCA, S. LÖTTERS, L. H. DU PREEZ, C. WELDON, A. HYATT, J. V. RODRIGUEZ-MAHECHA, S. HUNT, H. ROBERTSON, B. LOCK, C. J. RAXWORTHY, D. R. FROST, R. C. LACY, R. A. ALFORD, J. A. CAMPBELL, G. PARRA-OLEA, F. BOLANOS, AND E. AL. 2006. Confronting amphibian declines and extinctions. *Science* 313: 48.

METEYER, C. U., D. BARBER, AND J. N. MANDL. 2012. Pathology in euthermic bats with white nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. *Virulence* 3: 583–8.

METEYER, C. U., E. L. BUCKLES, D. S. BLEHERT, A. C. HICKS, D. E. GREEN, V. SHEARN-BOCHSLER, N. J. THOMAS, A. GARGAS, AND M. J. BEHR. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 21: 411–414.

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. Elsevier Ltd. Fungal Biology 117: 638–649.

- 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: e58976.
- MULLER, L. K., J. M. LORCH, D. L. LINDNER, M. O'CONNOR, A. GARGAS, AND D. S. BLEHER. 2013. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. Mycologia 105: 253–9.
- MURRAY, K. L., E. R. BRITZKE, B. M. HADLEY, AND L. W. ROBBINS. 1999. Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. Acta Chiropterologica 1: 105–112.
- PAIVA-CARDOSO, M. D. N., F. MORINHA, P. BARROS, H. VALE-GONÇALVES, A. C. COELHO, L. FERNANDES, P. TRAVASSOS, A. S. FARIA, E. BASTOS, M. SANTOS, AND J. A. CABRAL. 2014. First isolation of *Pseudogymnoascus destructans* in bats from Portugal. European Journal of Wildlife Research.
- PARK, K. J., G. JONES, AND R. D. RANSOME. 2000. Torpor, arousal and activity of hibernating greater horseshoe bats (*Rhinolophus ferrumequinum*). Functional Ecology 14: 580–588.
- PARSONS, K. N., G. JONES, I. DAVIDSON-WATTS, AND F. GREENAWAY. 2003. Swarming of bats at underground sites in Britain: Implications for conservation. Biological Conservation 111: 63–70.
- PARSONS, S., AND G. JONES. 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. The Journal of Experimental Biology 203: 2641–56.
- PERKINS, C. E. 2011. Microclimate and bat occupation trends at Gorman cave, Colorado Bend State Park, San Saba county, Texas durin 2010-2011. 28 pp.
- PLOWRIGHT, R. K., S. H. SOKOLOW, M. E. GORMAN, P. DASZAK, AND J. E. FOLEY. 2008. Causal inference in disease ecology: investigating ecological drivers of disease emergence. Frontiers in Ecology and the Environment 6: 420–429.
- PUECHMAILLE, S. J., G. WIBBELT, V. KORN, H. FULLER, F. FORGET, K. MÜHLDORFER, A. KURTH, W. BOGDANOWICZ, C. BOREL, T. BOSCH, T. CHEREZY, M. DREBET, T. GÖRFÖL, A.-J. HAARSMA, F. HERHAUS, G. HALLART, M. HAMMER, C. JUNGSMANN,

- Y. LE BRIS, L. LUTSAR, M. MASING, B. MULKENS, K. PASSIOR, M. STARRACH, A. WOJTASZEWSKI, U. ZÖPHEL, AND E. C. TEELING. 2011. Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. PLoS ONE 6: e19167.
- RACHOWICZ, L. J., J.-M. HERO, R. A. ALFORD, J. W. TAYLOR, J. A. T. MORGAN, V. T. VREDENBURG, J. P. COLLINS, AND C. J. BRIGGS. 2005. The novel and endemic pathogen hypotheses: Competing explanations for the origin of emerging infectious diseases of wildlife. Conservation Biology 19: 1441–1448.
- REDGWELL, R. D., J. M. SZEWCZAK, G. JONES, AND S. PARSONS. 2009. Classification of echolocation calls from 14 species of bat by support vector machines and ensembles of neural networks. Algorithms 2: 907–924.
- REEDER, D. M., C. L. FRANK, G. G. TURNER, C. U. METEYER, A. KURTA, E. R. BRITZKE, M. E. VODZAK, S. R. DARLING, C. W. STIHLER, A. C. HICKS, R. JACOB, L. E. GRIENEISEN, S. A. BROWNLIE, L. K. MULLER, AND D. S. BLEHER. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. PLoS ONE 7: e38920.
- REICHARD, J. D., N. W. FULLER, A. B. BENNETT, S. R. DARLING, M. S. MOORE, K. E. LANGWIG, E. D. PRESTON, S. VON OETTINGEN, C. S. RICHARDSON, AND D. S. REYNOLDS. 2014. Interannual survival of *Myotis lucifugus* (Chiroptera: Vespertilionidae) near the epicenter of white-nose syndrome. Northeastern Naturalist 21: N56–N59.
- REICHARD, J. D., AND T. H. KUNZ. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropterologica 11: 457–464.
- ROBERT, V. A., AND A. CASADEVALL. 2009. Vertebrate endothermy restricts most fungi as potential pathogens. The Journal of Infectious Diseases 200: 1623–1626.
- RODHOUSE, T. J., K. T. VIERLING, AND K. M. IRVINE. 2011. A practical sampling design for acoustic surveys of bats. The Journal of Wildlife Management 75: 1094–1102.
- RYDER, J. J., M. R. MILLER, A. WHITE, R. J. KNELL, AND M. BOOTS. 2007. Host-parasite population dynamics under combined frequency- and density-dependent transmission. Oikos 116: 2017–2026.
- SAMORAY, S. 2011. White-nose Syndrome monitoring and bat population survey of hibernacula in Tennessee. 35 pp.

- SCHWAB, N. A., AND T. J. MABEE. 2014. Winter Acoustic Activity of Bats in Montana. *Northwestern Naturalist* 95: 13–27.
- SHELLEY, V., S. KAISER, E. SHELLEY, T. WILLIAMS, M. KRAMER, K. HAMAN, K. KEEL, AND H. BARTON. 2013. Evaluation of strategies for the decontamination of equipment for *Geomyces destructans*, the causative agent of the white-nose syndrome (WNS). *Journal of Cave and Karst Studies* 75: 1–10.
- SHERWIN, H. A., W. I. MONTGOMERY, AND M. G. LUNDY. 2013. The impact and implications of climate change for bats. *Mammal Review* 43: 171–182.
- SIKES, R. S., W. L. GANNON, AND T. A. C. AND U. C. OF THE A. S. OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 92: 235–253.
- SKALAK, S. L., R. E. SHERWIN, AND R. M. BRIGHAM. 2012. Sampling period, size and duration influence measures of bat species richness from acoustic surveys. *Methods in Ecology and Evolution* 3: 490–502.
- 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.
- SPEAKMAN, J. R., AND P. A. RACEY. 1986. The influence of body condition on sexual development of male brown long-eared bats (*Plecotus auritus*) in the wild. *Journal of Zoology London* 210: 515–525.
- STEVENSON, D. E., AND M. D. TUTTLE. 1981. Survivorship in the endangered gray bat (*Myotis grisescens*). *Journal of Mammalogy* 62: 244–257.
- STORM, J. J., AND J. G. BOYLES. 2010. Body temperature and body mass of hibernating little brown bats *Myotis lucifugus* in hibernacula affected by white-nose syndrome. *Acta Theriologica* 56: 123–127.
- TINKLE, D. W., AND W. W. MILSTEAD. 1960. Sex ratios and population density in hibernating *Myotis*. *American Midland Naturalist* 63: 327–334.
- TITCHENELL, M. 2012. White-nose syndrome: A deadly disease of bats. The Ohio State University Extension: 5.
- TURMELLE, A. S., L. C. ALLEN, F. R. JACKSON, T. H. KUNZ, C. E. RUPPRECHT, AND G. F. MCCracken. 2010. Ecology of rabies virus exposure in colonies of Brazilian free-tailed bats (*Tadarida brasiliensis*) at natural and man-made roosts in Texas. *Vector-Borne and Zoonotic Diseases* 10: 165–175.

- TURNER, G. G., C. U. METEYER, H. BARTON, J. F. GUMBS, D. M. REEDER, B. OVERTON, H. BANDOUCHOVA, T. BARTONIČKA, N. MARTÍNKOVÁ, J. PIKULA, J. ZUKAL, AND D. S. BLEHERT. 2014. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases* 50.
- TURNER, G. G., D. M. REEDER, AND J. T. H. COLEMAN. 2011. A five-year assessment of mortality and geographic spread of White-nose Syndrome in North American bats and a look to the future. *Bat Research News* 52: 13–27.
- TURNER, J. M., L. WARNECKE, A. WILCOX, D. BALOUN, T. K. BOLLINGER, V. MISRA, AND C. K. R. WILLIS. 2014b. Conspecific disturbance contributes to altered hibernation patterns in bats with white-nose syndrome. *Elsevier B.V. Physiology & behavior*: 3–5.
- TUTTLE, M. D. 1976. Population ecology of the gray bat (*Myotis grisescens*): factors influencing growth and survival of newly volant young. *Ecology* 57: 587–595.
- TUTTLE, M. D., AND J. KENNEDY. 2005. Field guide to eastern cave bats. Bat Conservation International, Austin, TX, 41 pp.
- TWENTE, J. W., J. TWENTE, AND V. BRACK JR. 1985. The duration of the period of hibernation of three species of vespertilionid bats. II. Laboratory studies. *Canadian Journal of Zoology* 63: 2955–2961.
- U.S. FISH AND WILDLIFE SERVICE. 1982. Gray bat recovery plan. 1-143 pp.
- . 1997. Gray Bat, *Myotis grisescens*. In *Threatened and Endangered Species*. pp. 1–2.
- . 2009. Gray Bat (*Myotis grisescens*) 5-Year Review□: Summary and Evaluation. 1-34 pp.
- . 2014. White-Nose Syndrome: The devastating disease of hibernating bats in North America.
- USGS NATIONAL WILDLIFE HEALTH CENTER. 2013. Bat White-nose Syndrome (WNS)/Pd surveillance submission guidelines.
- VENESKY, M. D., T. R. RAFFEL, T. A. MCMAHON, AND J. R. ROHR. 2013. Confronting inconsistencies in the amphibian-chytridiomycosis system: implications for disease management. *Biological Reviews* 89: 477–483.

- 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: e46280.
- VERANT, M. L., C. U. METEYER, 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.
- WALTERS, C. L., R. FREEMAN, A. COLLEN, C. DIETZ, M. BROCK FENTON, G. JONES, M. K. OBRIST, S. J. PUECHMAILLE, T. SATTLER, B. M. SIEMERS, S. PARSONS, AND K. E. JONES. 2012. A continental-scale tool for acoustic identification of European bats. Journal of Applied Ecology 49: 1064–1074.
- 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. PNAS 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: 20130177.
- WELLER, T. J., S. A. SCOTT, T. J. RODHOUSE, P. C. ORMSBEE, AND J. M. ZINCK. 2007. Field identification of the cryptic vespertilionid bats, *Myotis lucifugus* and *M. yumanensis*. Acta Chiropterologica 9: 133–147.
- WHITAKER, J. O., AND S. L. GUMMER. 2000. Population structure and dynamics of big brown bats (*Eptesicus fuscus*) hibernating in buildings in Indiana. American Midland Naturalist 143: 389–396.
- WHITAKER, JR., J. O., AND L. J. RISSLER. 1992. Winter activity of bats at a mine entrance in Vermillion County, Indiana. American Midland Naturalist 127: 52–59.
- WIBBELT, G., A. KURTH, D. HELLMANN, M. WEISHAAR, A. BARLOW, M. VEITH, J. PRÜGER, T. GÖRFÖL, L. GROSCHE, F. BONTADINA, U. ZÖPHEL, H. P. SEIDL, AND D. S. BLEHERT. 2010. White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe. Emerging Infectious Diseases 16: 1237–43.
- WILLIS, C. K. R., A. K. MENZIES, J. G. BOYLES, AND M. S. WOJCIECHOWSKI. 2011. Evaporative water loss is a plausible explanation for mortality of bats from white-nose syndrome. Integrative and comparative biology 51: 364–73.

- WILLIS, J. S. 1982. The mystery of the periodic arousal. *In* Hibernation and torpor in mammals and birds. C. P. Lyman, J. S. Willis, A. Malan, and L. C. W. Wang (eds.). Academic Press, New York, NY. pp. 92–103.
- ZAHN, A., AND D. RUPP. 2004. Ectoparasite load in European vespertilionid bats. *Journal of Zoology* 262: 383–391.

APPENDIX

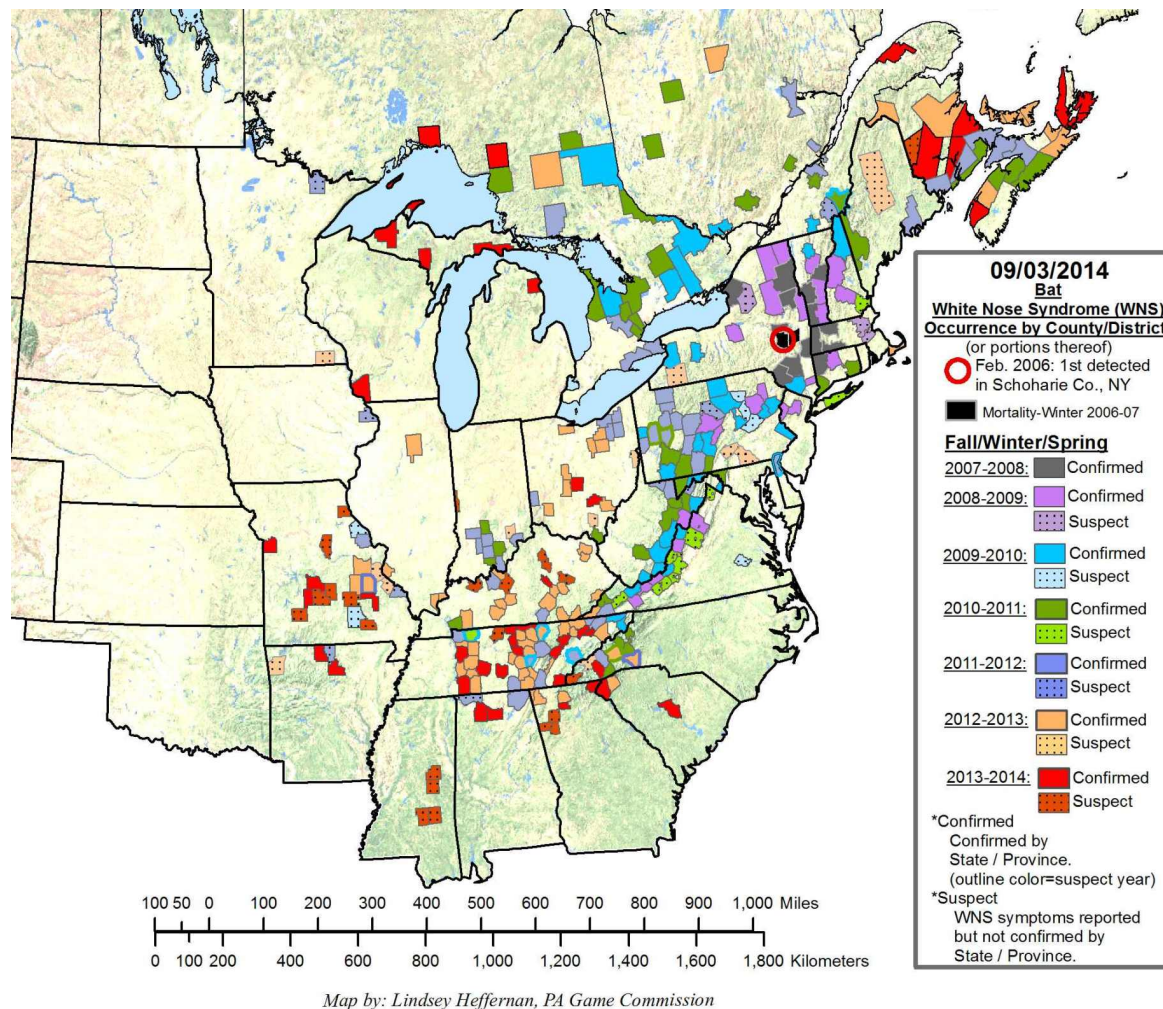


Figure 1. Distribution of white-nose syndrome and *Pseudogymnoascus destructans* in North America. Map created by the Pennsylvania Game Commission and distributed on September 3, 2014 by the United States Fish and Wildlife Service (www.whitenosesyndrome.org).

2014 White Nose Surveillance

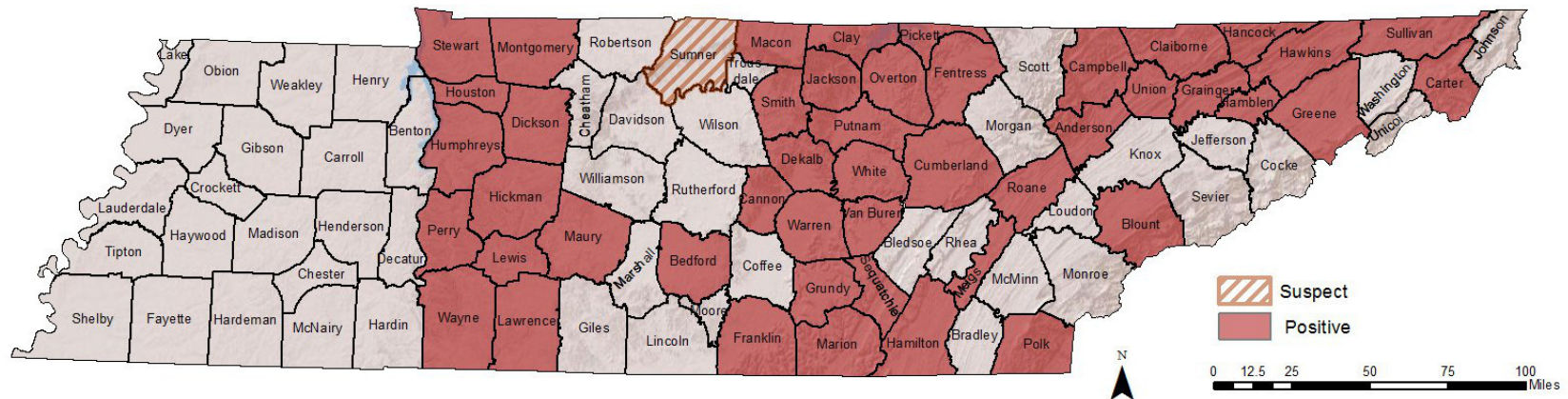


Figure 2. Distribution of white-nose syndrome in Tennessee. Map created by the Tennessee Wildlife Resources Agency and distributed by the Tennessee Bat Working Group (www.tnbgw.org).

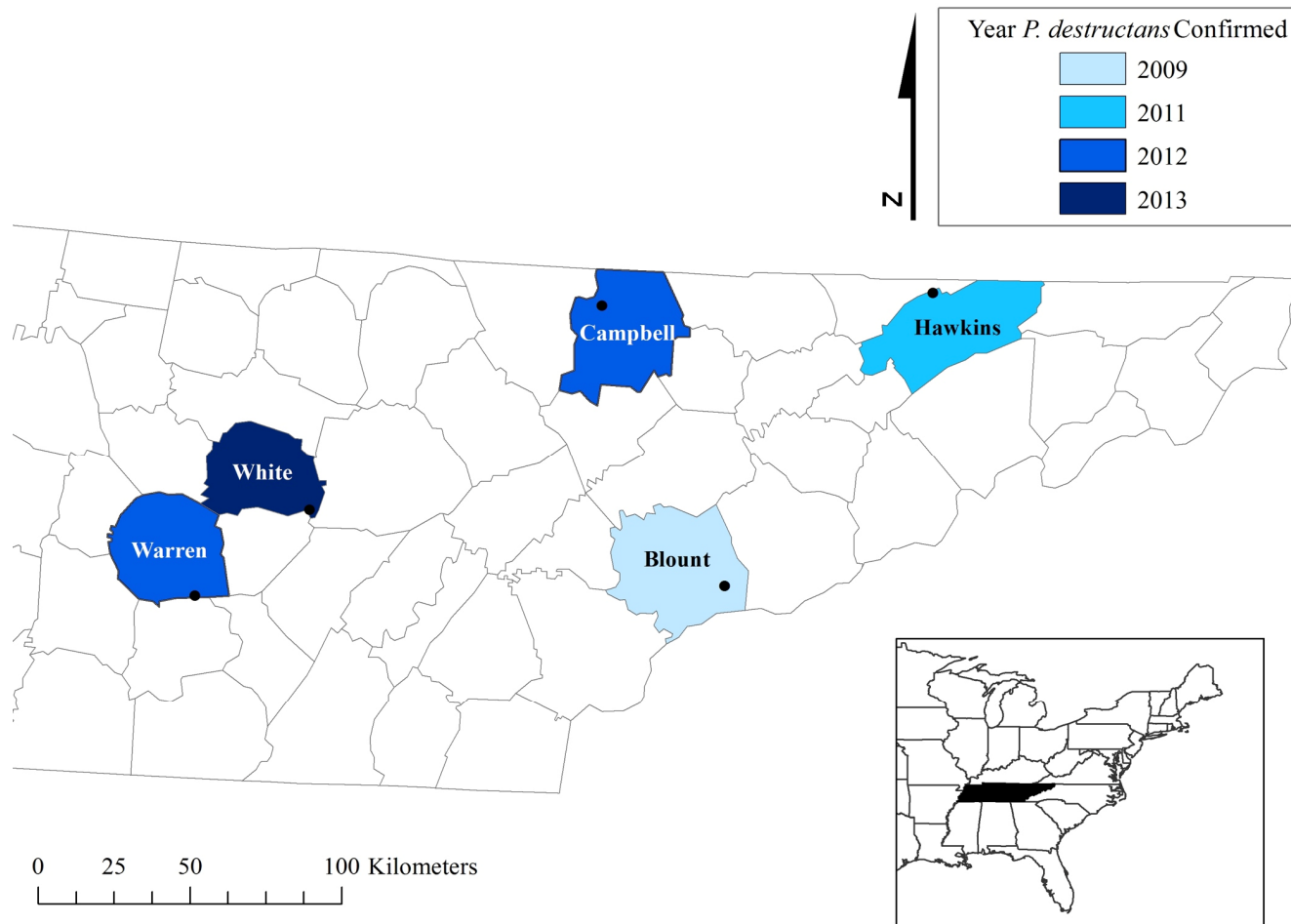


Figure 3. Cave locations within county boundaries in Tennessee, United States. Color-coding of counties corresponds to the year *Pseudogymnoascus destructans* was confirmed using either Real-Time PCR (Muller et al. 2013) or histopathological (Meteyer et al 2009) methods.

Table 1. Mean bat calls $\pm$ SE and mean temperature ($^{\circ}\text{C}$) $\pm$ SE per site from January through April 2012 (* indicates truncated season) and October through April 2012 – 2013 and 2013 – 2014. Bat calls and temperature are separated by time of day. Activity occurring between sunrise and sunset was classified as Day, with activity from sunset to sunrise as Night. Hawkins and White caves were not added to the sampling regime until winter 2012 – 2013. Mean activity at Blount cave during winter 2013 – 2014 is high due to the number of calls recorded during October 2013 (†). The temperature meter at White cave malfunctioned in winter 2012 – 2013.

Cave	Time of Day	2011 – 2012*		Winter Survey Year 2012 – 2013		2013 – 2014	
		Mean Bat Calls	Mean Temp.	Mean Bat Calls	Mean Temp.	Mean Bat Calls	Mean Temp.
Blount	Day	3.55 $\pm$ 0.34	15.03 $\pm$ 0.18	5.34 $\pm$ 0.29	8.95 $\pm$ 0.18	0.15 $\pm$ 0.07 †	11.67 $\pm$ 0.19
	Night	21.74 $\pm$ 0.84	9.42 $\pm$ 0.17	6.69 $\pm$ 0.27	4.41 $\pm$ 0.16	8.64 $\pm$ 0.96 †	6.48 $\pm$ 0.16
Campbell	Day	19.88 $\pm$ 3.16	No temp meter	0.15 $\pm$ 0.02	8.49 $\pm$ 0.16	1.63 $\pm$ 0.11	9.34 $\pm$ 0.21
	Night	131.56 $\pm$ 8.33	No temp meter	11.22 $\pm$ 0.75	6.25 $\pm$ 0.12	17.67 $\pm$ 0.71	5.47 $\pm$ 0.15
Hawkins	Day			0.50 $\pm$ 0.21	11.02 $\pm$ 0.21	0.50 $\pm$ 0.09	13.44 $\pm$ 0.26
	Night			7.54 $\pm$ 0.80	4.08 $\pm$ 0.13	29.95 $\pm$ 1.37	4.83 $\pm$ 0.19
Warren	Day	7.39 $\pm$ 0.88	No temp meter	0.63 $\pm$ 0.10	10.65 $\pm$ 0.24	1.38 $\pm$ 0.25	5.57 $\pm$ 0.26
	Night	87.13 $\pm$ 2.73	No temp meter	24.34 $\pm$ 0.96	7.33 $\pm$ 0.19	18.35 $\pm$ 1.15	5.23 $\pm$ 0.21
White	Day			0.25 $\pm$ 0.06	Meter broke	0.10 $\pm$ 0.01	5.81 $\pm$ 0.17
	Night			7.78 $\pm$ 0.44	Meter broke	2.48 $\pm$ 0.18	5.20 $\pm$ 0.14

Table 2. ANOVA comparisons of GLMM model outputs for daily acoustic activity of bats in Tennessee during winters 2011 – 2012, 2012 – 2013, and 2013 – 2014. Akaike information criterion (AIC) values were used to identify the best-fit model. Models receiving substantial empirical support ($\Delta\text{AIC} \leq 2.0$) are in bold face type. (*) Indicates interaction between two variables and (+) indicates additive effects.

Model	df	AIC	BIC	χ^2	χ^2 df	Pr > χ^2	Δi	wi
<i>Julian Day and Mean Daily Temperature</i>								
Julian Day * Avg Temp + Yr Pd Confirmed + Avg RH + Moon Illumination	11	10619.9	10674.3	3275.6	1	$<2 \times 10^{-16}$	1100.6	9.77×10^{-240}
Julian Day * Avg Temp + Yr Pd Confirmed + Avg RH	10	11005.5	11055.3	3366.4	1	$<2 \times 10^{-16}$	1486.2	1.98×10^{-323}
Julian Day * Avg Temp + Yr Pd Confirmed	9	13835.5	13882.0	0.0	1	1	4316.2	0
Julian Day * Avg Temp	6	13947.8	13978.8	551.0	1	$<2 \times 10^{-16}$	4428.5	0
Julian Day * Avg Temp + Yr Pd Confirmed + Moon Illumination	10	13354.9	13406.2	0.0	0	1	3835.6	0
Julian Day * Avg Temp + Moon Illumination	7	13458.4	13494.3	0.0	1	1	3939.1	0
Julian Day * Avg Temp + Avg RH + Moon Illumination	8	10693.3	10732.8	3319.3	1	$<2 \times 10^{-16}$	1174.0	1.17×10^{-255}
Julian Day * Avg Temp + Avg RH	7	11086.7	11121.5	2371.7	0	$<2 \times 10^{-16}$	1567.4	0
<i>Julian Day and Mean Daily Temperature (No interaction term)</i>								
Julian Day + Avg Temp + Yr Pd Confirmed + Avg RH + Moon Illumination	10	10654.7	10704.1	2700.2	0	$<2 \times 10^{-16}$	1135.4	2.77×10^{-247}
Julian Day + Avg Temp + Yr Pd Confirmed + Avg RH	9	11042.4	11087.3	2793.0	0	$<2 \times 10^{-16}$	1523.1	0
Julian Day + Avg Temp + Yr Pd Confirmed	8	13875.2	13916.5	0.0	0	1	4355.9	0
Julian Day + Avg Temp	5	13961.5	13987.3				4442.2	0
Julian Day + Avg Temp + Yr Pd Confirmed + Moon Illumination	9	13392.3	13438.4	0.0	0	1	3873.0	0
Julian Day + Avg Temp + Moon Illumination	6	13471.9	13502.7	476.0	0	$<2 \times 10^{-16}$	3952.6	0
<i>Julian Day and Temperature at Emergence</i>								
Julian Day * Emerg. Temp. + Yr Pd Confirmed + Avg RH + Moon Illumination	11	9519.3	9572.3	1100.7	0	$<2 \times 10^{-16}$	0.0	1.0
Julian Day * Emerg. Temp. + Yr Pd Confirmed + Avg RH	10	9838.3	9886.8	816.4	0	$<2 \times 10^{-16}$	319.0	5.22×10^{-70}
Julian Day * Emerg. Temp.	6	14491.3	14522.8	0.0	0	1	4972.0	0
Julian Day * Emerg. Temp. + Avg RH + Moon Illumination	8	9574.0	9612.5	4301.2	0	$<2 \times 10^{-16}$	54.7	1.31×10^{-12}
Julian Day * Emerg. Temp. + Avg RH	7	9896.7	9930.7	1190.0	0	$<2 \times 10^{-16}$	377.4	1.09×10^{-82}
Julian Day * Emerg. Temp + Moon Illumination	7	14010.6	14047.1	0.0	0	1	4491.3	0
<i>Julian Day and Temperature at Emergence (No interaction term)</i>								
Julian Day + Emerg. Temp. + Yr Pd Confirmed + Avg RH + Moon Illumination	10	9528.3	9576.5	310.0	0	$<2 \times 10^{-16}$	9.0	1.08×10^{-20}
Julian Day + Emerg. Temp. + Yr Pd Confirmed + Avg RH	9	9847.3	9890.9	3545.0	0	$<2 \times 10^{-16}$	328.0	5.87×10^{-72}
Julian Day + Emerg. Temp. + Yr Pd Confirmed	9	14369.9	14417.2	0.0	0	1	4850.6	0
Julian Day + Emerg. Temp.	5	14496.8	14523.1	0.0	0	1	4977.5	0
Julian Day + Emerg. Temp. + Yr Pd Confirmed + Moon Illumination	10	13893.5	13945.7	0.0	0	1	4374.2	0
Julian Day + Emerg. Temp. + Moon Illumination	6	14015.6	14046.9	475.7	0	$<2 \times 10^{-16}$	4496.3	0
Julian Day + Emerg. Temp + Avg RH	6	9901.5	9930.7	4114.1	0	$<2 \times 10^{-16}$	382.2	9.70×10^{-84}

Table 3. ANOVA comparisons of GLMM model outputs for hourly acoustic activity of bats in Tennessee during winters 2011 – 2012, 2012 – 2013, and 2013 – 2014. Akaike information criterion (AIC) values were used to identify the best-fit model. Models receiving substantial empirical support ($\Delta\text{AIC} \leq 2.0$) are in bold face type. (*) Indicates interaction between two variables and (+) indicates additive effects.

Model	df	AIC	BIC	χ^2	χ^2 df	Pr > χ^2	Δi	w_i
<i>Time of Day and Mean Hourly Temperature</i>								
Time of Day * Mean Temp. + Yr Pd Confirmed	10	130819.0	130904.0	8036.7	1	<2.2 x 10⁻¹⁶	0.0	1.0
Time of Day * Mean Temp.	6	133435.0	133487.0	6076.3	1	<2.2 x 10 ⁻¹⁶	2616.3	0
<i>Time of Day and Mean Hourly Temperature (No interaction term)</i>								
Time of Day + Mean Temp. + Yr Pd Confirmed	9	132275.0	132352.0	6179.1	1	<2.2 x 10 ⁻¹⁶	1456.2	6.08 x 10 ⁻³¹⁷
Time of Day + Mean Temp.	5	134445.0	134488.0				3625.9	0
<i>Time of Day and Year since Pd (No interaction term)</i>								
Time of Day + Yr Pd Confirmed	8	138453.0	138521.0	1001.9	0	<2.2 x 10 ⁻¹⁶	7633.3	0
<i>Sample Season and Mean Hourly Temperature</i>								
Season * Mean Temp.	8	139454.0	139523.0	10.6	2	4.9 x 10 ⁻³	8306.3	0
<i>Sample Season and Mean Hourly Temperature (No interaction term)</i>								
Season + Mean Temp.	6	139461.0	139512.0	0.0	0	1.0	8641.8	0
<i>Hour and Mean Hourly Temperature</i>								
Hour * Mean Temp. + Yr Pd Confirmed	10	138470.2	138555.4				7651.2	0
Hour * Mean Temp.	6	139125.0	139177.0	0.0	0	1.0	8306.3	0
<i>Hour and Mean Hourly Temperature (No interaction term)</i>								
Hour + Mean Temp. + Yr Pd Confirmed	9	138854.0	138931.0	0.0	0	1.0	8034.7	0
Hour + Mean Temp.	5	139510.0	139552.0	0.0	0	1.0	8690.6	0

Table 4. Total bat captures per species for winter 2012 – 2013 and 2013 – 2014. Nets were deployed for a total of 343.5 hours (winter 2012 – 2013 = 166.25 net hours, winter 2013 – 2014 = 177.25 net hours) at a capture rate of approximately 2.75 bats per net hour. *Indicates one MYLE from each year escaped prior to collecting any biometric information, including sex.

Species	Winter 2012/13			Winter 2013/14		
	Females	Males	Total	Females	Males	Total
CORA	1	3	4	2	3	5
EPFU	7	13	20	6	13	19
LABO	1	2	3	2	1	3
LANO	0	0	0	1	2	3
MYGR	31	133	164	54	128	182
MYLE	17	35	52*	10	40	51*
MYLU	7	5	12	3	16	19
MYSE	39	103	142	24	32	56
MYSO	25	27	52	6	39	45
PESU	33	49	82	8	25	33

Species acronym code: CORA = *Corynorhinus rafinesquii*; EPFU = *Eptesicus fuscus*; LABO = *Lasiurus borealis*; LANO = *Lasionycteris noctivagans*; MYGR = *Myotis grisescens*; MYLE = *Myotis leibii*; MYLU = *Myotis lucifugus*; MYSE = *Myotis septentrionalis*; MYSO = *Myotis sodalis*; PESU = *Perimyotis subflavus*

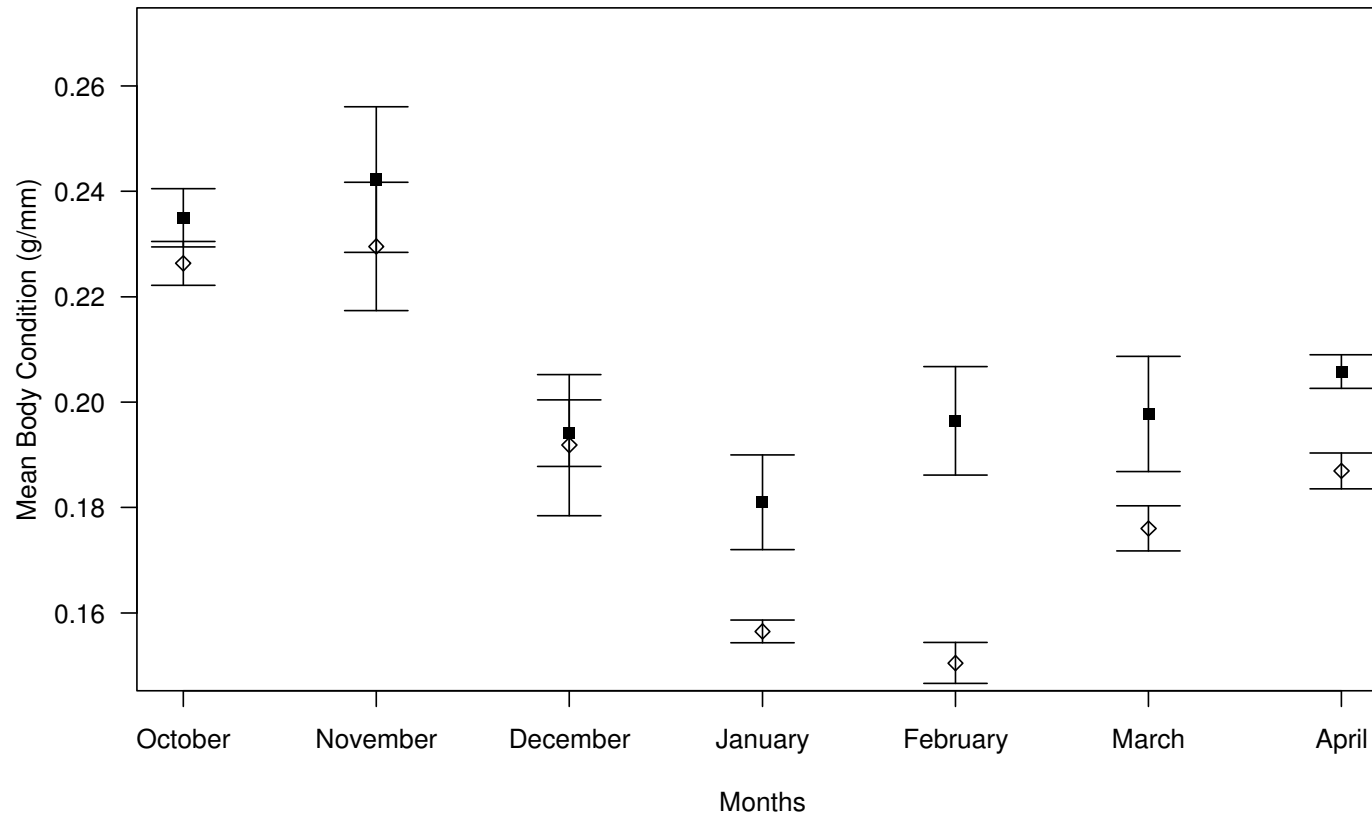


Figure 4. Mean body condition (g/mm) of bats captured outside of five caves in Tennessee. Bats captured during winter 2012 – 2013 (solid squares) had significantly lower body condition from mid- to late-hibernation (January through April) when compared to bats captured during the same time period winter 2013 – 2014 (open diamonds).

Table 5. Total bats captured and swabbed at five caves in Tennessee during winters (October – April) 2012 – 2013 and 2013 – 2014. Tallies are provided for the total number of individuals determined positive (Pd+) or negative (Pd-) for *P. destructans* through Real-Time PCR analysis. *Data for these species are explained further in Bernard et al. 2015.

Species	Winter 2012 – 2013			Winter 2013 – 2014		
	Pd+	Pd-	Total	Pd+	Pd-	Total
CORA*	1	2	3	1	4	5
EPFU	8	11	19	8	11	19
LABO*	2	0	2	0	3	3
LANO*	0	0	0	1	2	3
MYGR	24	139	163	35	147	182
MYLE	21	23	44	24	27	51
MYLU	11	1	12	14	5	19
MYSE	85	30	115	45	10	55
MYSO	38	11	49	14	8	22
PESU	55	18	73	21	11	32

Species acronym code: CORA = *Corynorhinus rafinesquii*; EPFU = *Eptesicus fuscus*; LABO = *Lasiurus borealis*; LANO = *Lasionycteris noctivagans*; MYGR = *Myotis grisescens*; MYLE = *Myotis leibii*; MYLU = *Myotis lucifugus*; MYSE = *Myotis septentrionalis*; MYSO = *Myotis sodalis*; PESU = *Perimyotis subflavus*

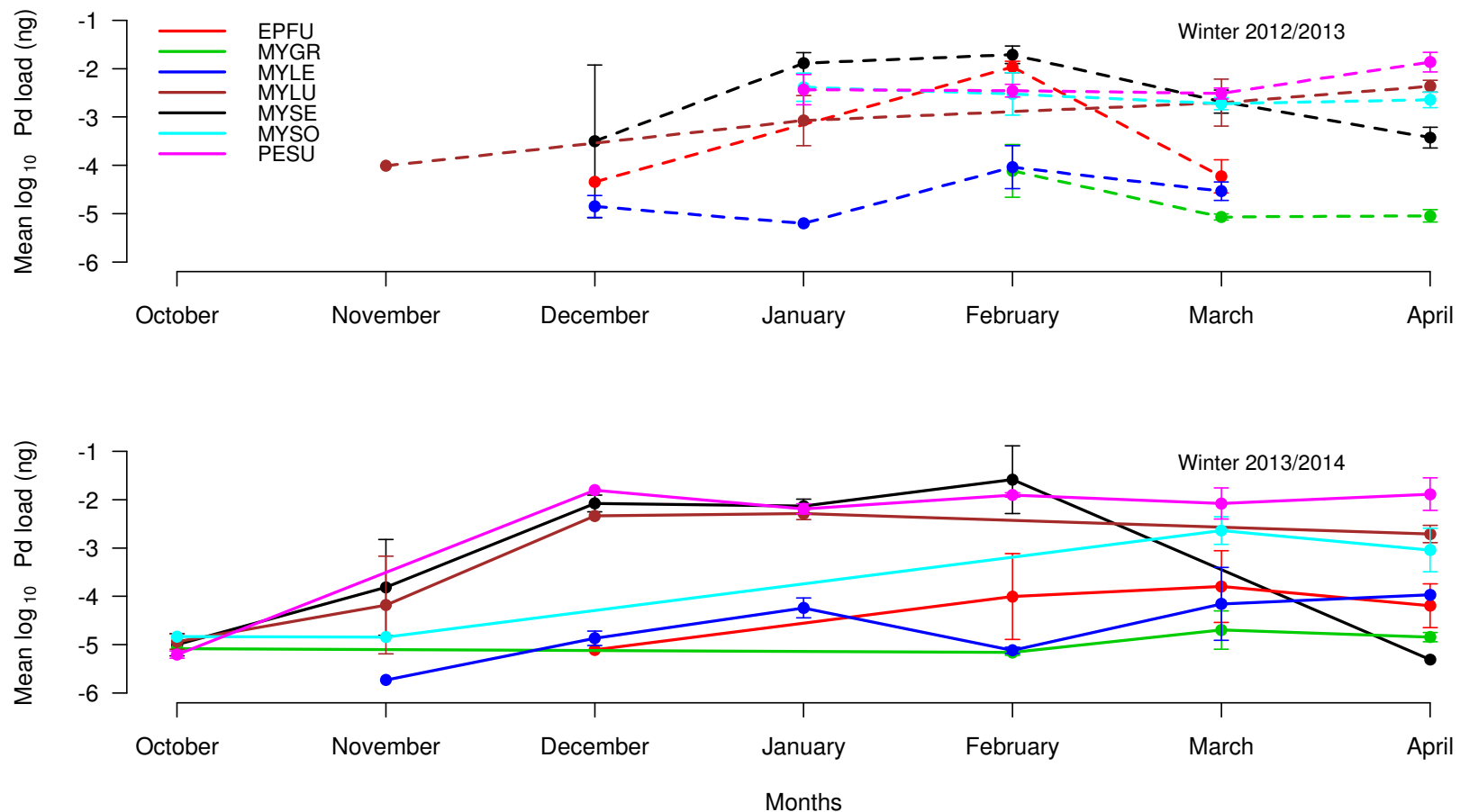


Figure 5. Mean load ($\pm$ SE) of *P. destructans* (Pd) per month for seven species captured at five sites in Tennessee during October – April 2012 – 2013 (top panel) and 2013 – 2014 (bottom panel). Circles indicate months where Pd+ individuals were sampled. Species acronym code: EPFU = *Eptesicus fuscus*; MYGR = *Myotis grisescens*; MYLE = *Myotis leibii*; MYLU = *Myotis lucifugus*; MYSE = *Myotis septentrionalis*; MYSO = *Myotis sodalis*; PESU = *Perimyotis subflavus*.

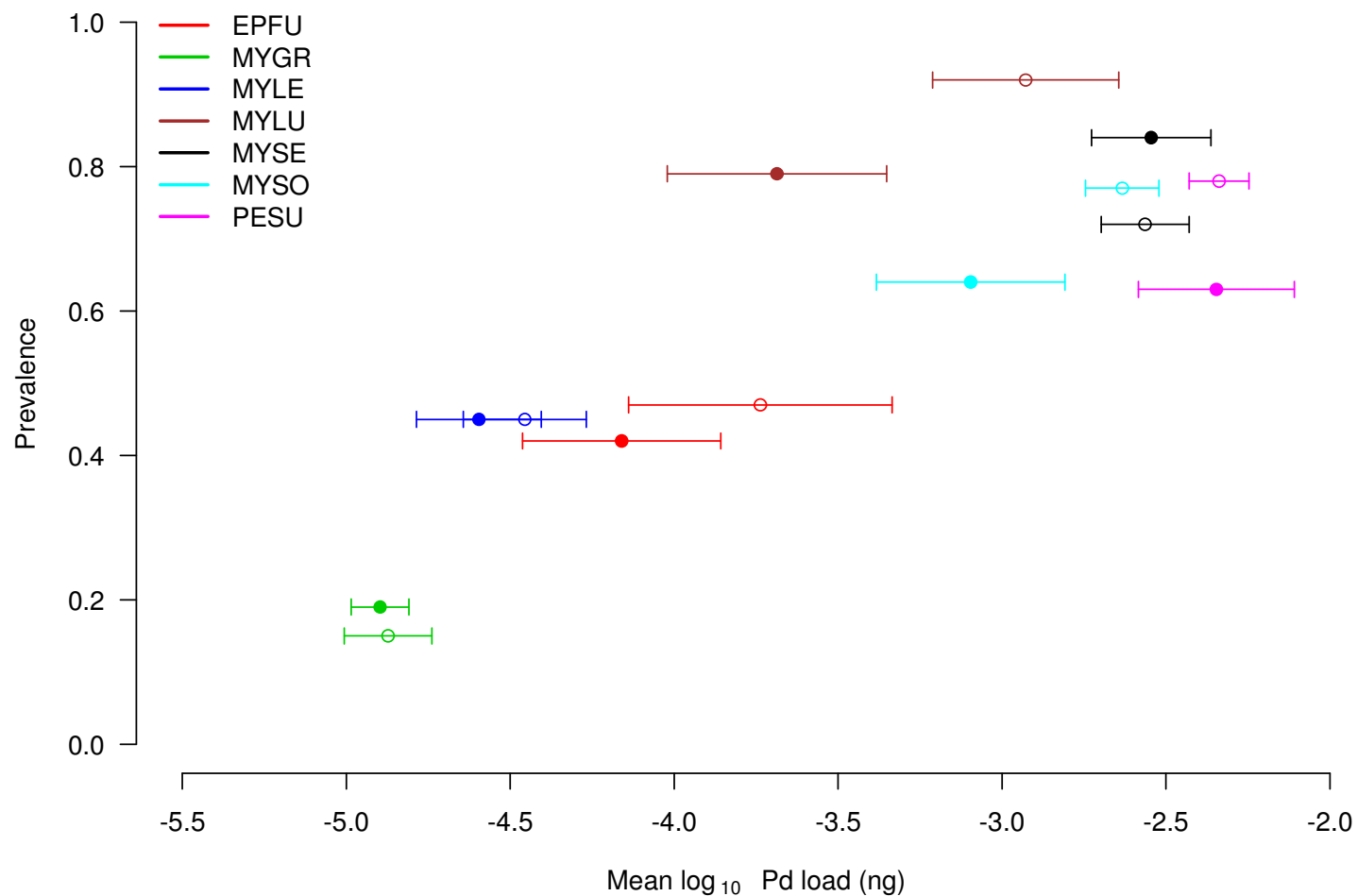


Figure 6. Infection prevalence (# infected individuals/total # individuals sampled) and mean load of *P. destructans* ($\pm$ SE) for seven bat species captured at five hibernacula in Tennessee during winters 2012 – 2013 (open circles) and 2013 – 2014 (solid circles).

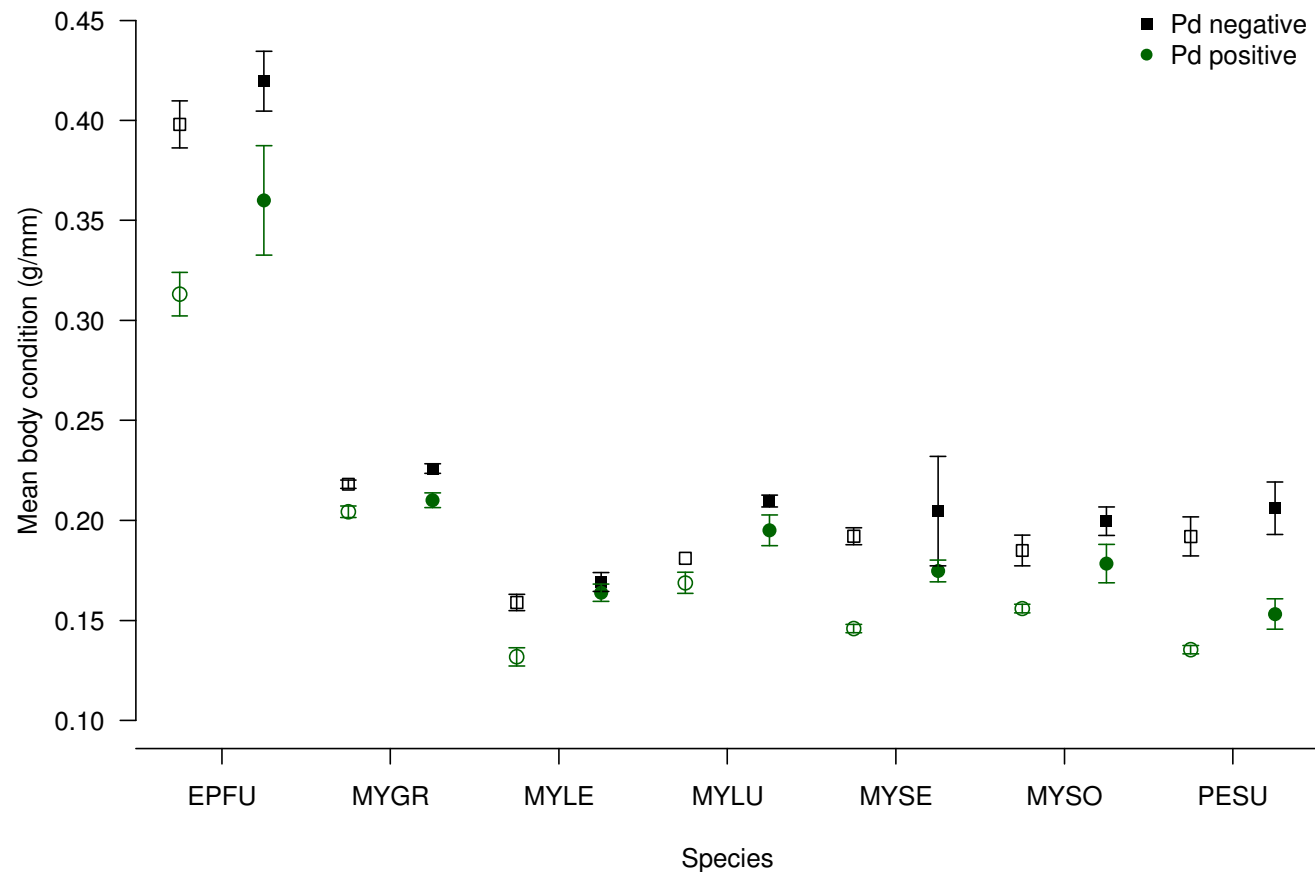


Figure 7. Mean body condition (g/mm) of *P. destructans* positive (Pd+) and negative (Pd-) bats for seven species captured at five sites in Tennessee during winters 2012 – 2013 (open symbols) and 2013 – 2014 (closed symbols). Mean body condition for Pd- (black squares) and Pd+ (green circles) individuals are significantly different for all species. Error bars are one standard error from the mean. The large variation in EPFU is due to the low sample size of that species for both years.

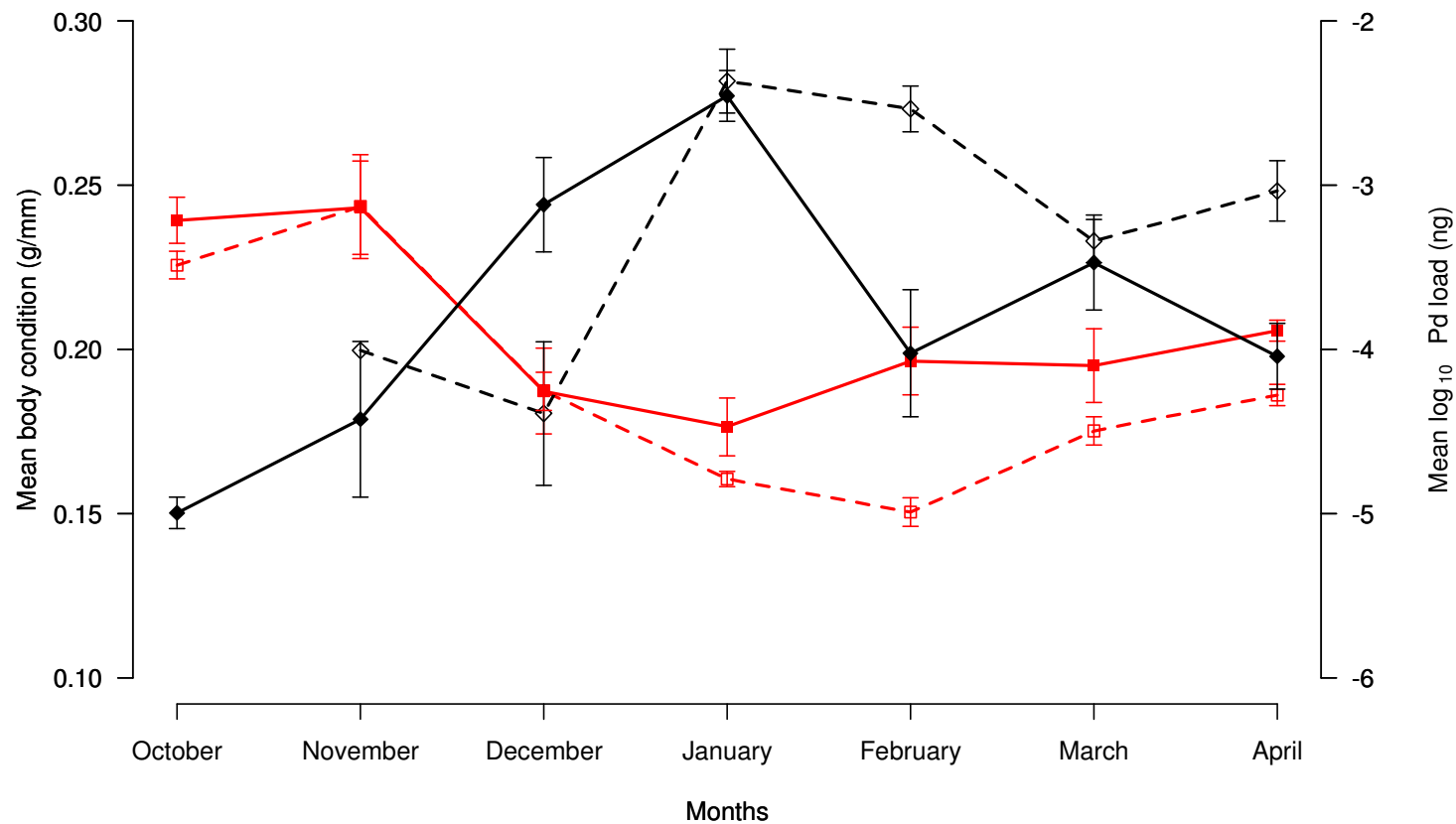


Figure 8. Seasonal variation in body condition (g/mm) and fungal load (ng) of all individuals captured at five sites in Tennessee during October 2012 – April 2013 (open symbols, dashed lines) and October 2013 – April 2014 (solid symbols, solid lines). Mean body condition (red squares) is measured on the primary Y-axis and mean fungal load (black diamonds) is measured on the secondary Y-axis. Error bars are one standard error from the mean. Missing data for October 2012 indicates no Pd+ bats were captured that month.

Table 6. Relative number (% bat passes per species) of echolocation passes recorded per species from the beginning of June through the end of August 2012, 2013, and 2014. Mean bat passes recorded per species per hour are in parentheses. N equals the total number of hourly recordings analyzed. Only known identifications were included in this table. *Species listed as endangered under the U.S. Endangered Species Act.

Species	Year			
	2012 N = 2158	2013 N = 1724	2014 N = 2363	All Years N = 6245
<i>Corynorhinus rafinesquii</i>	1.60 (0.09)	1.70 (0.08)	1.70 (0.09)	1.65 (0.08)
<i>Eptesicus fuscus</i>	44.30 (2.42)	52.40 (2.51)	24.50 (1.34)	38.60 (2.04)
<i>Lasiurus borealis</i>	11.70 (0.64)	9.50 (0.45)	10.80 (0.59)	10.80 (0.57)
<i>Lasiurus cinereus</i>	4.30 (0.23)	10.20 (0.49)	23.80 (1.30)	13.41 (0.71)
<i>Lasionycteris noctivagans</i>	7.00 (0.38)	6.50 (0.31)	6.14 (0.33)	6.51 (0.34)
<i>Myotis grisescens</i> *	9.30 (0.51)	7.10 (0.34)	10.41 (0.57)	9.21 (0.49)
<i>Myotis leibii</i>	0.22 (0.01)	2.08 (0.10)	2.96 (0.16)	1.80 (0.09)
<i>Myotis lucifugus</i>	1.26 (0.07)	0.05 (0.002)	0.16 (0.008)	0.52 (0.03)
<i>Myotis septentrionalis</i>	0.08 (0.004)	0.02 (0.001)	0.25 (0.01)	0.13 (0.007)
<i>Myotis sodalis</i> *	0.40 (0.02)	0.20 (0.01)	0.17 (0.01)	0.25 (0.01)
<i>Nycticeius humeralis</i>	4.44 (0.24)	1.06 (0.05)	2.40 (0.13)	2.78 (0.15)
<i>Perimyotis subflavus</i>	9.70 (0.53)	2.05 (0.09)	4.80 (0.26)	5.90 (0.31)
<i>Tadarida brasiliensis</i>	5.80 (0.32)	7.10 (0.34)	12.00 (0.66)	8.60 (0.45)
Total number of bat passes recorded	11781	8264	12944	32989

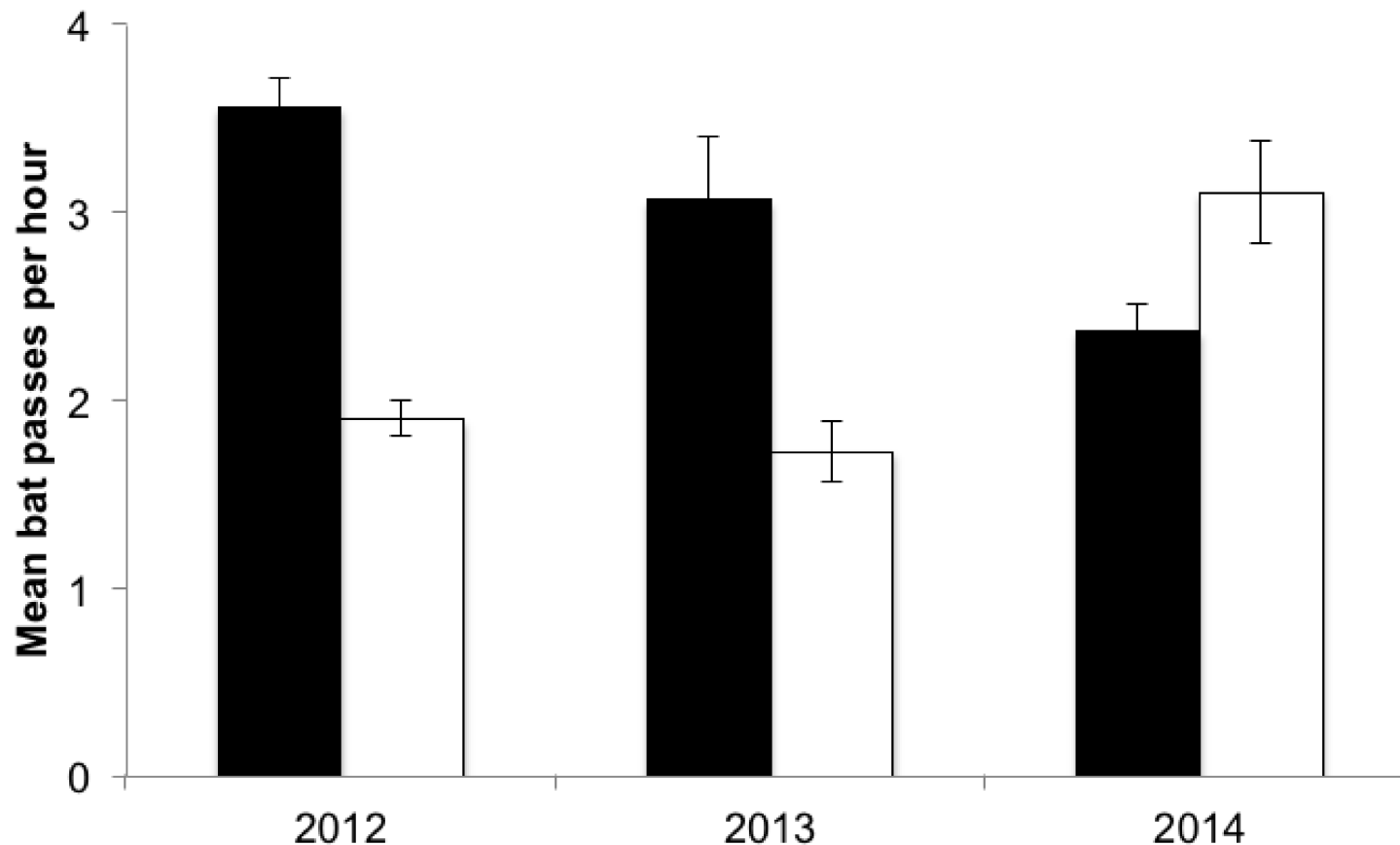


Figure 9. Mean bat passes recorded per hour at five detector (N = 5) sites in East Tennessee from the beginning of June through the end of August 2012, 2013, and 2014. Acoustic abundance of WNS-affected species (*E. fuscus*, *M. grisescens*, *M. leibii*, *M. lucifugus*, *M. septentrionalis*, *M. sodalis*, and *P. subflavus*; black bars) decreased over the course of the study, while acoustic abundance of unaffected species (*C. rafinesquii*, *L. borealis*, *L. cinereus*, *L. noctivagans*, *N. humeralis*, and *T. brasiliensis*; white bars) increased significantly in summer 2014. Error bars are one standard error from the mean.

VITA

Riley Fehr Bernard was born in Silverton, Oregon on May 21, 1985. She graduated from Silverton High School and went on to pursue her Bachelor's degree in Environmental Studies at Linfield College in McMinnville, Oregon. Riley completed her Bachelors of Science degree in 2007 and worked in the Natural Resources Department of the Confederated Tribes of the Grand Ronde in Grand Ronde, Oregon for a year. In 2008, she entered the Tropical Conservation Biology and Environmental Science Master's of Science program at the University of Hawaii at Hilo to study the dietary overlap of the invasive coquí frog and Hawaiian hoary bat. After completing her Master's degree, Riley entered the doctoral program in the Department of Ecology and Evolutionary Biology at the University of Tennessee to pursue research on the winter activity of bats and the progression of white-nose syndrome in Tennessee.