IMPACT OF WHITE-NOSE SYNDROME AND LOCAL CLIMATE

ON REPRODUCTIVE FEMALE BATS

IN THE SOUTHEASTERN UNITED STATES

By

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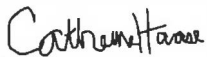Thesis Committee:

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Sarah Kathleen Krueger   05/06/2022

DEDICATION

To my sister, Caitlyn.

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ABSTRACT

Energetic trade-offs between hibernation and reproduction occur in hibernating bat species to ensure pups are born when forage availability is optimal, yet little is known about how disease impacts reproductive success and how these impacts may vary with local climate. White-nose syndrome (WNS) is an infectious disease that disrupts hibernation in bats, leading to premature exhaustion of fat stores. There is evidence of reproductive shifts in areas where WNS has devastated bat populations; however, current research has yet to assess these changes in response to winter duration or local climate. I compiled data from four states and used generalized linear mixed effects models to compare effects of WNS, winter duration, and local climate variables on the number of reproducing females for WNS-susceptible species (*Perimyotis subflavus* and *Myotis spp.*) and two species not affected by WNS (*Eptesicus fuscus* and *Lasiurus borealis*). I incorporated the effects of WNS in two ways: presence and absence of WNS, with presence dictated by year first observed, and year since WNS was reported. I predicted WNS susceptible species would see a decline in the number of reproductive females, with the effect exaggerated by longer winter durations and inadequate pre-hibernation climate variables. I found that the number of reproductive females in both WNS-susceptible species and species not affected by WNS was positively correlated with pre-hibernation local climate conditions conducive to foraging (number of summer days above 18°C); however, WNS-susceptible species experienced an overall decline with years since WNS. This overall negative trend of WNS-susceptible species may cause a shift in bat populations, which is critical to understanding the effects of disease on population growth through impacts on reproductive behavior.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I: LITERATURE REVIEW

Reproductive Ecology of Bats

During reproduction, mammals sequence through mating, gestation, parturition, lactation, post-lactating parental care, and post-parental care recovery (Gittleman and Thompson 1988). Lactation may be the most energetically costly stage due to the need to supply milk for offspring. The amount of fat needed for lactation can be severely influenced by lactation timing, litter size, and food consumption (Rogowitz 1996). Post-lactating care is also extremely costly due to the need to increase defense mechanisms to protect offspring and transport young when required. The increased energetic requirements of post-lactating care also depend on the offspring's body mass and relative mass of the mother (Rogowitz 1996).

Energetics of the reproductive cycle can vary among species. For example, larger litters or large offspring body mass require greater energy expenditure from the mother (Millar 1978; Gittleman and Thompson 1988). Künkele (2000) found that mother Guinea pigs (*Cavia spp.*) that produced larger litter sizes increased their daily caloric intake significantly during gestation to account for the increase in energy costs to produce more offspring. This study also found that the ability to convert energy into offspring tissue is directly affected by litter size, with median litter sizes having the highest efficiency rates of energy conversion (Künkele 2000). Research has also shown that males that have a larger body mass suffer greater energy expenditure during mating than those of a smaller body mass. For example, males of certain ungulate species decrease their food intake and losing up to 20% of their body mass during the breeding season due to spending more time and vitality trying to find a mate (Clutton-Brock and Iason 1986).

Generalizations about bat reproduction are hard to generate due to seasonal variations in food supply causing differences in reproductive cycles (Racey 1982). For example, temperate bats have been documented giving birth only during the summer months while other species

living in areas where insect density does not fluctuate seasonally, such as the tropics, have been seen going into heat repeatedly during the breeding season regardless of food availability (aseasonal polyestry; Fleming et al. 1972). Gestation duration is also extremely variable among species, with environmental factors such as temperature and precipitation driving length of gestation (Tuttle and Stevenson 1982). However, studies have indicated that almost all Neotropical bats have evolved to be seasonal breeders which allow for young to be weaned prior to the onset of the wet season (Bronson 2009).

Mating approach is also dependent on resource availability and energetics. Unlike most small mammals, bats are not described as "live- fast, die-young" strategists (Read and Harvey 1989; Racey and Entwistle 2000) due to their long lifespans, low litter size, and delayed sexual maturity (Racey 1982). This strategy in bats is also characterized by longer periods of gestation, lactation, and post-natal care, all to ensure the greatest chance of survival of pups (Racey and Entwistle 2000). Female bats have evolved different ways to ensure proper timing of birth such as delayed fertilization, delayed implantation, and delayed development. Delayed fertilization occurs when sperm is stored in the oviduct after copulation and thus fertilization does not occur until after hibernation (Bradley 2006). This strategy is seen in many temperate species in the family Vespertilionidae, the most common family of bats in the United States (Barclay et al. 2004). Delayed implantation occurs when fertilization commences immediately upon copulation but the fertilized egg does not implant in the uterus until after hibernation (Bradley 2006).

Hibernation Energetics

Hibernation is defined as a period of time a heterothermic mammal spends in a dormant state (Geiser 2004). Hibernation is characterized by periods of torpor and arousal, while torpor is the reduction in body temperature and metabolism and arousals are periods when an animal will

increase its body temperature and metabolism back up to euthermic levels (Rintoul and Brigham 2014). Most hibernators can reduce their body temperature from around 35-40°C by about 30°C to 5-10°C (Ruf and Geiser 2014), while some species such as the arctic ground squirrel *(Spermophilus parryii)* even reduce their body temperature by more than 40°C while in torpor (Buck and Barnes 2000). Hibernation is beneficial to bats and other mammals due to their need to generate heat internally and conserve energy and water during unfavorable seasonal variations in temperature and food shortages (Humphries et al. 2003). During hibernation, reduced metabolic rate associated with reduced body temperature can decrease energy requirements. It has been documented that bats can reduce their heart rate from 200-300 beats per minute to 10 beats per minute when in hibernation, which can reduce energy costs by about 98% (Boyles et al. 2007).

Hibernation is not a steady-state process. Though the exact mechanisms that drive arousals are unknown, long periods of decreased body temperatures and lowered metabolic rate can result in depletion of body water through evaporative water loss, accumulation of metabolic wastes, and decreased immune response (Czenze et al. 2017). Arousals are used to restore muscular function, excrete waste, and replenish water and energy stores during hibernation (Cryan et al. 2010); however, arousals can account for more than 85% of a bat's winter energy expenditure (Thomas et al. 1990). Although arousals are extremely energetically costly, many critical bodily processes can only be done when an animal is at euthermic levels. The most common hypothesis explaining the physiological benefits of arousals is the need to restore electrolyte balance and body water lost when in torpor (Thomas and Cloutier 1992). Another hypothesis is that individuals arouse due to their need to defecate (Thomas and Cloutier 1992)

and maintain adequate blood glucose concentrations for organ and nervous system function (Galster and Morrison 1970).

Hibernation duration is primarily based on seasonal temperatures and responses to a decrease in food supply. While some mammalian species hibernate every winter, regardless of external conditions (obligate hibernators), most enter hibernation as a response to colder weather and a reduction in food resources (facultative hibernators; Chayama et al. 2016). Hibernation can last days, weeks, or even months depending on species and climate. For example, the Belding's ground squirrel (*Spermophilus beldingi*) can hibernate for up to 8 months while living at high altitudes in California (Frank 2011). Due to this long hibernation duration, it is thought that about two-thirds of all juvenile Belding ground squirrels and about one-third of adults do not survive the winter because their fat reserves do not last the entire duration of their hibernation period (Frank 2011).

Hibernation also leads to a reduction in the reproductive cycle, which is why many hibernators use delayed reproduction strategies. Hibernation during the reproductive period (i.e. copulation before hibernation but fertilization after) can decrease the costs of reproduction in animals that rely on insectivorous, seasonal food availability (McAllan and Geiser 2014). Delayed reproduction grants an extension of the reproductive period, allowing offspring to be born when food supplies are optimal in the spring. For example, bats in the family Vespertilionidae mate in the fall before many insect species die off from winter and when male sperm production is at its peak (Racey and Entwistle 2000). This timing also allows pups to maximize food availability during the timing of birth, thus increasing their chances of survival (Humphries et al. 2003).

White-nose Syndrome

White-nose syndrome (WNS) is an emergent infectious disease characterized by the novel white fungus *Pseudogymnoascus destructans* and leads to mortality in bats due to disruption of hibernation behavior (Blehert et al. 2009). WNS was first documented in a cave in New York in 2006 after appearing to arrive overseas from Europe (Blehert et al. 2009). It is thought to have been introduced to North America by humans visiting caves and transporting the fungus on their equipment (Zukal et al. 2016).

Although the exact mechanism in which *P. destructans* impacts hibernation behavior is still unclear, it has killed millions of hibernating bats since its first detection in North America. The fungus grows on the muzzle, ears, and wings of bats during hibernation and causes ulcerations and skin irritations to the wing tissue (Lorch et al. 2011). This breakdown of wing tissue causes the bat to arouse more frequently, expend critical body fat, and experience dehydration and starvation from lack of resources during the winter (Ehlman et al. 2013). Since the arrival of WNS, bats have been seen leaving hibernaculum prematurely when food supplies are limited during the winter months, leaving bats to starve from lack of resources (Bernard and McCracken 2017). This interruption from homeostasis associated with the wing damage from *P. destructans* is the main reason why WNS is fatal (Cryan et al. 2010).

Currently, in North America, 12 bat species have been confirmed with WNS and the causative fungus has been found on 6 additional species. North American bat species with diagnostic symptoms include big brown bat (*Eptesicus fuscus*), cave bat (*Myotis velifer*), Eastern small-footed bat (*Myotis leibii*), fringed bat (*Myotis thysanodes*), gray bat (*Myotis grisescens*), Indiana bat (*Myotis sodalis*), little brown bat (*Myotis lucifugus*), long-legged bat (*Myotis volans*), Northern long-eared bat (*Myotis septentrionalis*), Western long-eared bat (*Myotis evotis*), tri-

colored bat (*Perimyotis subflavus*), and Yuma bat (*Myotis yumanensis*). Bats species on which *P. destructans* has been detected but no diagnostic sign of WNS has been documented include: Eastern red bat (*Lasiurus borealis*), Mexican free-tailed bat (*Tadarida brasiliensis*), Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), silver-haired bat (*Lasionycteris noctivagans*), Townsend's big-eared bat (*Corynorhinus townsendii*), Virginia big-eared bat (*C. townsendii virginianus*), Ozark big-eared bat (*C. townsendii ingens*), and Western small-footed bat (*Myotis ciliolabrum*; *White-nose Syndrome Response Team*).

Status of Bats in the United States

There are more than 45 species of bats found in the United States (O'Shea and Bogan 2003). Six species have been declared endangered under the U.S. Endangered Species Act of 1973 (ESA), and listed as federally endangered by the U.S. Fish and Wildlife Service (Table 1.1; *White-nose Syndrome Response Team*).

Declining bat populations due to the disturbance of caves and habitat alteration have been a focus of increasing conservation concern since the 1970s. Since species like the gray bat (*M. grisescens*) aggregate in caves year-round (Tuttle 1979), these human-driven cave disturbances had lasting effects on population numbers with some disappearing entirely. Today, bats still face threats from hibernaculum disturbance, habitat loss and fragmentation, climate change, and WNS (O'Shea et al. 2016). Unusual and unprecedented variations in seasonal weather patterns make it difficult for bats to adjust their behaviors to suit the changing environment (O'Shea et al. 2016). Migratory and tree-roosting species are particularly affected by wind turbines. Wind turbine blades can easily be mistaken for adequate roosting sites and bats can be injured while finding a place to rest while migrating (Horn et al. 2008). Declines in insect abundance from pesticide use and intensive farming practices have also led to declines in bat populations in the U.S (Torquetti

et al. 2021). White-nose syndrome may be the current leading cause of bat mortality in the United States. Since the detection of *P.destructans* in 2006, bat declines exceeding 75% have been recorded at surveyed hibernacula (Blehert et al. 2009).

Table 1.1. Species of bats in the U.S. were declared as endangered under the U.S. Endangered Species Act of 1973 (*White-nose Syndrome Response Team*).

Species	Year listed
Florida bonneted bat (*Eumops floridanus*)	2013
Mexican long-nosed bat (*Leptoncyteris nivalis*)	1988
Gray bat (*Myotis grisescens*)	1976
Ozark big-eared bat (*Corynorhinus townsendii ingens*)	1979
Virginia big-eared bat (*Corynorhinus townsendii virginianus*)	1979
Indiana bat (*Myotis sodalis*)	1967

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CHAPTER II: IMPACT OF WHITE-NOSE SYNDROME AND LOCAL CLIMATE ON

REPRODUCTIVE FEMALE BATS IN THE SOUTHEASTERN UNITED STATES

Introduction

Reproduction is a critical phase in the life cycle of all species on Earth. In sexually

reproducing species, proper timing of birth is crucial to the success of a population (Hernández-

Aguilar et al. 2020). To maximize fitness, timing parturition to coincide with peak food resource

availability ensures adequate energetic resources for raising offspring. For example, the African

buffalo (*Synerus caffer*) synchronizes the time of birthing to periods when protein content is

abundant in forage. Researchers believe this strategy is triggered by environmental cues such as

rainfall and sprouting of seedlings and saplings (Ryan et al. 2007). Many other mammalian

species have evolved strategies to delay reproduction to wait out unfavorable conditions for

sustaining newborns, such as lack of food, to ensure the success of their young (Orr and Zuk

2013). These strategies are driven by the physiology of the mother, and include delayed

fertilization through sperm storage (Racey 1973), delayed implantation of the zygote (Birkhead

and Møller 1993), and delayed development of young (Racey 1982).

Delayed reproduction allows for trade-offs of energetic needs with other key elements of

a species' life cycle. For example, hibernation is a vital period in the life cycle of many

mammals that require energy-saving strategies to maximize fitness over periods of food scarcity,

such as winter (Geiser and Ruf 1995). Mammals use delayed reproduction to allow for

hibernation over energetically expensive periods while timing parturition outside of these

periods. Hibernation is characterized by periods of torpor and arousal, while torpor is the short-

term reduction in body temperature and metabolism (Rintoul and Brigham 2014). Mating

generally occurs during periods of peak male fitness, which often coincides right before

hibernation while males are accumulating fat stores; thus females can delay reproduction through sperm storage or embryonic diapause to allow for hibernation and time parturition outside of this energetically expensive period (Racey and Entwistle 2000; Pfeiffer and Mayer 2013).

Delayed reproduction through hibernation is a common strategy of many temperate bat species in the family Vespertilionidae (Orr and Zuk 2014). Female temperate bats either delay fertilization or implantation to reduce the energetic impacts of winter with hibernation and to maximize food availability during the timing of birth (Humphries et al. 2003). This ensures pups are born when forage availability is optimal, and provides enough time for pups to reach maximum adult weight before going into hibernation the following winter (Racey 1973). Local climate can also greatly influence the success of reproduction and winter survival of temperate bats because they rely on sufficient insect abundance to create fat stores (Bronson 2009; Adams 2010; Sherwin et al. 2013). For example, Burles et al. (2009) found that *Myotis lucifugus* experienced delays in reproductive timing in 1999 when the study area was experiencing an increase in cool, wet weather from El Niño. This research and other studies have shown that low ambient temperatures increase the energetic costs of maintaining a normal body temperature and may result in prolonged gestation and lower reproductive success (Burles et al. 2009).

Though much research has determined the impacts of food resource availability and climate on delayed reproduction (Bronson 2009; Burles et al. 2009; Adams 2010; Frick et al. 2010; Pfeiffer and Mayer 2013; Sherwin et al. 2013; O'Keefe et al. 2019), little is known about this response with the increased energetic costs of disease. For example, white-nose syndrome (WNS), a devastating disease of North American hibernating bat species, increases energetic costs over hibernation through disruption of the torpor-arousal cycle, leading to loss of fat stores and high rates of mortality (Lorch et al. 2011; Ehlman et al. 2013; Bernard and McCracken

2017). Changes in body composition such as decreased fat mass, elevation of CO_2 levels in the blood, dehydration, and increased fat consumption have been documented in bats during the stages of WNS (Verant et al. 2014). These physiological changes can result in respiratory acidosis, hyperkalemia, and reduction of fat stores, possibly stimulating arousal from hibernation (Jonasson and Willis 2011; Verant et al. 2014). Due to the increased energetic requirements over hibernation in WNS-affected bats, there has been evidence of bats postponing or completely forgoing fertilization (Frick et al. 2010; Jonasson and Willis 2011). Fertilization at a later date has resulted in shifts in the peak proportion of pregnant females to later in the summer in areas where WNS has devastated susceptible populations (Francl et al. 2012). These shifts can be problematic to WNS-susceptible populations if juveniles lack adequate time to gain fat stores before hibernation the following winter.

Since its detection in New York in 2006 (Blehert et al. 2009), WNS has been confirmed in 37 states and seven Canadian provinces (Figure 2.1; *White-nose Syndrome Response Team*). WNS has been confirmed in twelve North American bat species and the causal fungus, *Pseudogymnoascus destructans*, has been found on an additional six species. Susceptibility to WNS differs due to differences in body size, sociability during hibernation, hibernaculum microclimate, and migration patterns during winter (Langwig et al. 2012; Grieneisen et al. 2015; Haase et al. 2021). Small-bodied bats such as *Perimyotis subflavus* (tri-colored bat) have high evaporative water loss and often hibernate in caves that sustain optimal temperatures for *P. destructans* growth (2.5-15.8° C; Johnson et al. 2016; Bernard and McCracken 2017). Large-bodied bats such as *Eptesicus fuscus* (big brown bat) are resistant to mass mortality even though the typically roost in caves because they tend to have higher fat stores due to their large body size (Johnson et al. 2016). Unlike *P. subflavus* and *E. fuscus, Lasiurus borealis* (eastern red bat)

typically roost in trees and are therefore less likely to be exposed to *P. destructans* or hibernate in temperatures that offer optimal fungal growth (Mager and Nelson 2001; Limpert et al. 2007).

The objective of this study was to assess how WNS, winter duration, and local climate impact the reproduction of female bats in the southeastern United States. With variability in local and regional susceptibility to WNS, it is important to assess the combined impacts of hibernation behavior, local climate, and winter duration on energetics of reproduction. Winter duration and local climate conditions before hibernation can greatly influence the survival rates of overwintering bats (Ehlman et al. 2013; Hranac et al. 2021). Here, I will use data from multiple states across the southeast to test competing hypotheses of impacts on the number of reproductive females in bat populations in respect to WNS. First, I hypothesize that the impacts of WNS on susceptible species will be modulated by winter duration. Longer winters mean more time in hibernation requiring greater fat stores and may result in increased energetic stress with WNS, resulting in less reproduction. Shorter winters allow more time spent foraging to build up those fat stores and could reduce the impacts of WNS. Therefore, I predict that there will be a negative relationship between winter duration and the number of reproductive females post-hibernation in WNS-susceptible species. Alternatively, I hypothesize that reproductive potential is dependent on pre-hibernation foraging throughout spring, summer, and fall to enhance fattening. Thus, I predict there will be a positive relationship between pre-hibernation climate conditions that are conducive to foraging and the number of reproductive females the following year. Additionally, I propose two competing hypotheses of how WNS will impact the number of reproductive females. First, I expect there will be clear differences between the pre-and post-WNS abundance of reproductive individuals. Alternatively, I suspect there will be local adaptation in the years since WNS was confirmed. If this hypothesis holds true, I would expect

that years since WNS will better explain variation in reproductive females compared to pre-or post-WNS exposure. Finally, I expect that there has been an increase in capture effort since the onset of WNS in each state.

Methods

Study Area

I collected data from Fort Campbell Army Installation on the border of Kentucky and Tennessee in 4 counties (Stewart and Montgomery counties in Tennessee and Trig and Christian counties in Kentucky; Figure 2.2). Fort Campbell is located on approximately 105,000 acres of forests, native grasslands, wetlands, and agriculture fields. The installation has approximately 68,000 acres of military training areas and approximately 27,000 acres of range and impact zones. Fort Campbell's natural resources are currently managed according to an Integrated Natural Resources Management Plan prepared by the installation's fish and wildlife program's directorate of public works. This plan manages wetlands, rare plants, game and non-game fish and wildlife, riparian zones, outdoor recreation, wildfire, or prescribed fire, migratory birds, and endangered species (Fort Campbell Fish Wildlife 2020). Fort Campbell was the first army installation to report WNS in February 2012. WNS was found in four underground bunkers on bats that were heavily clustered. Fort Campbell currently contains two endangered bat species, *Myotis sodalis* and *Myotis grisescens,* and the threatened *Myotis septentrionalis*. Fort Campbell has two other bat species that have been petitioned for federal protection: *P. subflavus* and *M. lucifugus* (*White-nose Syndrome Response Team*).

Data Collection

I captured bats during two summers (July - August 2020, May-August 2021) from 51 sites on Fort Campbell. To determine sample sites, I first deployed ultrasonic detection devices

(Titley Scientific Anabat Express Passive Bat Detector, Zero Crossing) to assess bat species presence. Ultrasonic bat detectors convert echolocation signals to frequencies that humans can hear and record on an SD card. After deployment, I offloaded, digitized, and analyzed the data using Kaleidoscope Pro Analysis Software (Wildlife Acoustics, Inc.) to determine species presence. I determined site occupancy (> 50 calls per night) for each species based on individual bat call characteristics (Balantic and Donovan 2019).

Once species presence was acoustically determined at a site, I surveyed bats using mist-netting. Bats were captured using Avinet Inc., 38 mm mesh 2.6 x 2.6, 4, 6, 9, and 12 m mist-nets placed across sources of water, old fire breaks, or roads that act as flight corridors. The number of nets placed at each site were dependent upon water presence and presentable flight corridors. I opened nets at sundown to prevent unwanted capture of birds and checked each net approximately every ten minutes for 3-4 h. Once captured in a net, I extracted each bat by hand and placed it into a brown paper bag for processing. I identified species, sex, and reproductive status. Pregnant females were defined by palpation of the abdomen and lactating and post-lactating were determined by the condition of the mammary glands (Racey 1968). All bats were handled in compliance with the Austin Peay State University Institutional Animal Care and Use Committee (IACUC 20.003R) and following Association of Mammalogists Care and Use guidelines (Sikes and Mammalogists 2016). I worked under a Tennessee Wildlife Resources Agency issued state scientific collection permit (#2314), Kentucky Department of Fish and Wildlife Resources issued state educational wildlife collection permits (#SC2011006, #SC2111014), and federally issued United States Fish and Wildlife Service Endangered Species permits (#TE80381A-2, #TE62026D-0, and #ES62026D-1).

To supplement my field data, I compiled additional data from four southeastern states (Figure 2.3) from published (Bernard et al. 2017; Rojas et al. 2017; O'Keefe et al. 2019) and unpublished regulatory surveys (Fort Campbell Fish & Wildlife; Kentucky Fish & Wildlife; Georgia Department of Natural Resources; North Carolina Wildlife Resources Commission). A total of 7,678 reproductive female bats from 9 species in 267 counties were collected from 1989-2020. Only those individuals that had clearly defined reproductive conditions were included in the analyses. I calculated the sampling effort by summarizing the total number of net nights per county per year (O'Keefe et al. 2019). I obtained WNS determination status records from the US Fish and Wildlife Service for all known records of WNS-positive and WNS-negative counties for each state (www.whitenosesyndrome.org). I then calculated the years since WNS for each county by counting the number of years since WNS was first documented.

I calculated the mean, maximum, and minimum elevation for each county with capture data using a digital elevation model (DEM) provided by Wang et al (2016). I calculated a suite of summer, winter, spring, and autumn severity metrics (Table 2.1) for each county (Bilotta et al. 2015) with the ClimateNA v5.10 software package (available at http://tinyurl.com/ClimateNA), based on methodology described by Wang et al. (2016). Using methods described by Hranac et al. (2021), I calculated the mean predicted winter duration for each county based on latitude, elevation, and the number of days in frost.

I used a principal component analysis to find the key combinations of climate variables from the previous year (pre-hibernation) that described most of the variation in the data (>90%; Manly 1994; Júnior and Nóbrega 2018). Those components with eigenvalues > 1 were then used as covariates in my statistical models described below (Jackson 1993). I interpreted the

components by selecting the predictor variables with the highest eigenvectors ($>|0.40|$)

associated with each component (Peres-Neto et al. 2005).

Statistical Analyses

To test my predictions, I developed a suite of generalized linear mixed models using a

Poisson distribution with a log-link with the number of reproductive females as the response

variable. I ran the same suite of models for three species groups: susceptible southeastern species

(*Myotis austroriparius, M. grisescens, Myotis leibii, M. lucifugus, M. septentrionalis, M. sodalis,*

and P. subflavus; Ingersoll et al. 2013), *E. fuscus*, and *L. borealis*. I assume the group of

susceptible species would respond in the same direction to the explanatory variables, but that the

two non-susceptible species would not. *L. borealis* exclusively roost in trees during the winter,

and thus rarely are exposed to WNS (Johnson et al. 2016; Jorge et al. 2021). *E. fuscus*, however,

is exposed to WNS, yet does not seem to experience the same population declines as other cave-

hibernating bat species due to potential factors such as larger body size and relative fat mass

(Frank et al. 2014).

I incorporated the effects of WNS in each model in two ways: first as presence and

absence of WNS, with presence dictated by year first observed in the county in which that bat

was surveyed, and second, year(s) since WNS was reported, to determine if local adaptation

would occur. To measure the relative importance of WNS, two models for each species group

incorporated WNS as a fixed effect to predict the number of reproductive females. I also

incorporated the effects of local climate in two ways: first as a winter severity metric of winter

duration (predicted days in winter per county) and second as principal components for pre-

hibernation climate variables per county. To include variation associated across years and

counties, I incorporated year and county as random effects in all models. I also included

sampling effort (log[capture nights/year]) as a fixed effect in all models to account for increases in bat survey efforts post-WNS. To determine the influence of WNS on sampling effort, I ran a generalized linear model of sampling effort (number of capture nights per year) against years since WNS with a Gaussian distribution and an identity-link.

I checked for overdispersion, which is common in Poisson regression, using the X^2 approximation of the residual variance (Zuur et al. 2009). Models that had a c_t ratio <1 were overdispersed. To account for overdispersion, I compared models for each species group using second-order Akaike's Information Criterion ($\Delta AICc_c$; Akaike 1973) and used the lowest $\Delta AICc$ values to designate the top models to assess my hypotheses. I compared models within < 2 $\Delta AICc_c$ using model weights and reported adjusted standard errors. I calculated the R^2 values to assess the variance of the number of reproductive females explained by the fixed effects and total, selected model for each species group. I conducted all statistical analyses in R v4.01 (R Core Development Team 2020) using the R package *nlme* (Pinheiro et al. 2021).

Results

I found that WNS susceptible species (*M. austroriparius, M. grisescens, M. leibii, M. lucifugus, M. septentrionalis, M. sodalis,* and *P. subflavus*) experienced an overall 73.35% decline in the number of reproductive females post-WNS (pre-WNS: 3,115 reproductive females, post-WNS: 830 reproductive females; Table 2.2). The total number of pregnant WNS susceptible individuals decreased by 65.84% post-WNS (pre-WNS: 1452 pregnant females, post-WNS: 496 pregnant females). Lactating females susceptible to WNS also declined considerably by 63.94% post-WNS (pre-WNS: 1772 lactating females, post-WNS: 639 lactating females). The total number of post-lactating females decreased by 68.03% after the detection of WNS (pre-WNS: 1545 post-lactating females, post-WNS: 494 post-lactating females).

E. fuscus and *L. borealis* also declined in the number of reproductive females post-WNS (pre-WNS: 2,813 reproductive females, post-WNS: 932 reproductive females; Table 2.2); however, both non-susceptible species comprised the majority of reproductive females post-WNS (52.89%), suggesting a less devastating loss of non-susceptible reproductive females. The total number of pregnant *L. borealis* decreased by 61.22% (pre-WNS: 214 pregnant females, post-WNS: 83 pregnant females) while the total number of pregnant *E. fuscus* decreased by 78.63% (pre-WNS: 379 pregnant females, and post-WNS: 81 pregnant females). Lactating *L. borealis* and *E. fuscus* also declined post-WNS (pre-WNS: 566 lactating *L. borealis* females and 585 lactating *E. fuscus* females, and post-WNS: 260 lactating *L. borealis* females and 213 lactating *E. fuscus* females). WNS also decreased the number of post-lactating *E. fuscus* and *L. borealis* females (pre-WNS: 396 post-lactating *L. borealis* females and 762 post-lactating *E. fuscus* females, and post-WNS: 180 post-lactating *L. borealis* females and post-lactating *E. fuscus* females).

Mean survey effort was higher post-WNS (1181.35 ± 201.37 SD capture nights per year) than pre-WNS (700.04 ± 415.90 SD capture nights per year) and increased as the years since WNS also increased ($\beta = 38.84 \pm 1.18$ SE, p-value < 0.001; Table 2.3; Figure 2.4).

The first two principal components had eigenvalues > 1 and were used as covariates in my statistical models (PC1=1.75 and PC2=1.55; Table 2.4; Figure 2.5). Independent variables for principal component 1 (PC1) with the highest eigenvectors included mean summer relative humidity ($>|0.44|$) and mean spring relative humidity ($>|0.46|$; Table 2.5). Independent variables for principal component 2 (PC2) with the highest eigenvectors included number of summer days above 18°C ($>|0.43|$) and mean annual temperature ($>|0.49|$; Table 2.5). PC1 and PC2 retained 61% of the variances contained in the data (Figure 2.6).

My prediction of a decrease in the number of reproductive females in WNS-susceptible

species and the impact of winter duration was not supported by the best model. However, there

was an impact of pre-hibernation climate conditions as I suspected. The models containing

covariates for the years since WNS was first detected and pre-hibernation climate conditions

(principal components 1 and 2) had better fits for *Myotis spp*. (AICc = 13879.42, *LL* = -6933.71,

K = 6, w_t = 0.94) and *L. borealis* (AICc = 5739.30, *LL* = -2863.65, *K* = 6, w_t = 0.96) than models

containing other climate variables (mean winter duration) or WNS alone; however, the model

containing only years since WNS had a better fit for *E. fuscus* (AICc = 4209.91, *LL* = -2100.96,

K = 4, w_t = 0.39; Table 2.6). As I expected given my predictions, year since WNS was a better fit

to the data than just pre/post-WNS; there were large differences in ΔAICc values between the

suite of models (Table 2.6). The best models for all species groups were overdispersed (c_t >1)

and thus AICc was used to compare models.

For susceptible species (*Myotis* spp.), I found that the number of reproductive females

increased as pre-hibernation climate variables (PC2) increased (β = 0.07 ± 0.02 SE, p = 0.001;

Figure 2.6). Contrary to my prediction, however, there was not a decline in the number of

reproductive females as the years since WNS increased (β = 0.02 ± 0.01, p = 0.28; Figure 2.6).

There was not a relationship between sampling effort by year on the number of reproductive

females when considering the other variables (β = -0.02 ± 0.04, p = 0.65). Around 88% of the

number of reproductive female *Myotis spp.* were explained by the fixed effects (years since

WNS and PC2; R^2 = 0.88).

For non-susceptible species, my predictions were correct: I found that *L. borealis* showed

a positive relationship in the number of reproductive females and years since WNS (β = 0.04 ±

0.01, p = 0.02). Reproductive females also increased with warmer conditions pre-hibernation as

expected (PC1: $\beta = -0.06 \pm 0.03$, p = 0.04; PC2: $\beta = 0.09 \pm 0.03$, p = 0.002; Figure 2.7).

Sampling effort by year was not found to be a significant fixed effect ($\beta = -0.05 \pm 0.06$, p = 0.35). Around 85% of the number of reproductive female *L. borealis* were explained by the fixed effects (years since WNS and PC1 and PC2; $R^2 = 0.85$).

Additionally, my predictions regarding *E. fuscus* were not supported; there was no clear top model for this species. The top two models, including year since WNS and year since WNS with pre-hibernation climate, were only 0.1 ΔAICc apart and were close in model weights ($w_t = 0.39$ and $w_t = 0.37$, respectively; Table 2.6). However, there were no significant impacts of year since WNS in either model ($\beta = 0.01 \pm 0.02$, p = 0.57; $\beta = 0.02 \pm 0.02$, p = 0.40, respectively) nor pre-hibernation climate on the number of reproductive females ($\beta = 0.05 \pm 0.03$, p = 0.05). Although there was no significance in the direction of the effect, around 84% of the variation associated with the number of reproductive females of *E. fuscus* was explained by years since WNS ($R^2 = 0.84$; Figure 2.8).

Discussion

My results demonstrate that changes in southeastern bat populations have occurred following the arrival of WNS (Figure 2.9). Prior to the onset of WNS, *M. lucifugus*, *M. septentrionalis,* and *P. subflavus* were considered abundant and stable; however, my research and complementary studies have recorded these species with significantly lower capture rates post-WNS, suggesting dramatic declines in population sizes (Ford et al. 2011; Francl et al. 2012; Pettit and O'Keefe 2017; Thalken et al. 2018; Nocera et al. 2019). This decrease of interspecific competition has allowed for other species, such as *E. fuscus* and *L. borealis*, to increase their population size, therefore creating a shift in the bat community (Francl et al. 2012; Deeley et al. 2021).

My results suggest that there are energetic trade-offs between successful reproduction and WNS. I found that WNS susceptible species (*M. austroriparius, M. grisescens, M. leibii, M. lucifugus, M. septentrionalis, M. sodalis,* and *P. subflavus*) experienced a decline in the number of reproductive females post-WNS. This study and other published research studies (Barclay et al. 2004; Francl et al. 2012; Johnson et al. 2021) agree with the idea that female bats allocate their limited resources to self-preservation when the probability of successful reproduction is low, and higher energetic costs are required to protect their own survival. WNS-induced fat depletion could force female bats to miscarry pups or prolong the development of young (Pettit and O'Keefe 2017). Delaying the development of young until after hibernation is incredibly risky. If pups are born later in the summer, it is likely they will not have enough time to build up proper fat stores before going into their first hibernation, thus decreasing their probability of survival over winter. Further evidence that WNS may be delaying reproduction is needed to determine the relevance of this phenomenon. I suggest future studies that aim at studying the mechanisms of WNS that can lead to offspring loss.

My findings suggest that we will continue to observe changes in bat populations in the southeast. For example, while I did not find an increase in the number of reproductive *E. fuscus* females post-WNS, I did observe a positive influence of years since WNS (Figure 2.8), suggesting that *E. fuscus* are not directly benefiting from the arrival of WNS but rather the decline of other species. In addition, *L. borealis* showed a positive relationship in the number of reproductive females and years since WNS (Figure 2.7), giving further evidence to support the idea that species less susceptible to the effects of WNS will experience population increases overtime (Deeley et al. 2021; Johnson et al. 2021). Bats that do not hibernate in caves where temperatures are optimal for the growth of *P. destructans* and have a larger body size are

typically less susceptible to WNS. Higher body fat when going into hibernation, a quality that both *E. fuscus* and *L. borealis* share, can directly contribute to the success of overwinter survival (Haase et al. 2020). For example, Cheng et al. (2019) found that higher body fat observed in persisting bats would reduce WNS mortality by an average of 64%. These results suggest that increases in pre-hibernation fat storage have reduced the impacts of WNS in some bat populations, and management strategies should aim at directly increasing pre-hibernation food supply and foraging habitat (Bernard et al. 2017; Cheng et al. 2019).

My long-term dataset provides evidence that pre-hibernation climate, specifically favorable summer weather conditions, greatly influences the reproduction of both WNS-susceptible species and species not susceptible to WNS. Environmental conditions including summer and spring relative humidity, number of summer days above 18°C, and mean annual temperature had the highest eigenvalues associated with each pre-hibernation principal component (PC1 and PC2; Table 2.4). My results showed that the number of reproductive females increased with warmer climate conditions during the pre-hibernation period. In general, my findings did not indicate that successful reproduction is driven by winter duration, but rather that temperate-zone bats rely on optimal foraging conditions before going into hibernation, and efficiency is increased by higher annual temperatures. Higher summer and spring relative humidity directly impact prey availability. If prey is abundant prior to going into hibernation, it is more likely that bats will build up prime fat stores, therefore increasing their potential overwinter survival if affected by WNS.

My findings conclude that successful reproduction is likely in places that are generally warmer and wetter, suggesting that mortality could be related to drier and colder climates. Warmer temperatures can be associated with earlier arousals from torpor, followed by earlier

births as described above. This shift in the timing of births may allow for more time for individuals to find mates and gain critical fat stores before hibernation (Francl et al. 2012; Sherwin et al. 2013). Although I found that higher annual temperatures were positively associated with the number of reproductive females, future climate extremes could negatively impact population dynamics by creating unstable weather and inadequate habitat (Lučan et al. 2013). Body temperatures above 41℃ are lethal for most mammals, including bats (Luo et al. 2021; Reher and Dausmann 2021). If ambient temperatures continue to rise due to climate change, roost selection and migration patterns may have to shift, and if bats are unsuccessful in changing their behavior in order to migrate for higher temperatures, they will likely experience increased mortality. Finding suitable habitat may become more difficult for many individuals, and therefore summer and winter range shifts may change accordingly (Loeb and Winters 2013).

Prior to the onset of WNS, survey efforts in the form of both mist-netting and acoustics remained relatively low (Ford et al. 2011; Francl et al. 2012; Nocera et al. 2019). Due to changes in research objectives, post-WNS efforts increased substantially (Balzer et al. 2021; Deeley et al. 2021; Figure 2.4). I included survey effort (log[capture nights/year]) as a covariate; however, effort was summarized by county per year (O'Keefe et al. 2019), creating an assumption that there was equal survey effort across the county, which we know is not the case. Additionally, I classified counties for each state by WNS-positive or WNS-negative, assuming that all bats captured in a county that was WNS-positive were at least showing signs of infection. I considered it unlikely that bats in WNS-positive counties would not at least be positive for *P. destructans*, and therefore contagious to others. I highly recommend future studies to give specific attention to the disproportion in survey effort and WNS status of individuals.

This study concluded that both WNS and local climate impact the reproduction of southeastern bat species. Using data spanning from 1989-2020, I was able to assess if disease is affecting the success of reproduction and how this success may vary with local climate. My results demonstrated the importance of summer habitat and forage ability with the number of reproductive WNS-susceptible females increasing as pre-hibernation climate variables increased. Although winter duration did not have a significant impact on reproductive females, changes in winter duration and annual fluctuations in weather due to climate change could indirectly affect prey abundance, thus signifying a need for further research into the topic. Regardless, non-susceptible species will probably continue to increase in population size due to decreased interspecific competition, causing a shift in bat populations. Continued conservation of all southeastern bat species, regardless of WNS status, is critical in maintaining the biodiversity of our ecosystem.

Figure 2.1. Map of current white-nose syndrome (WNS) spread in the United States impacting

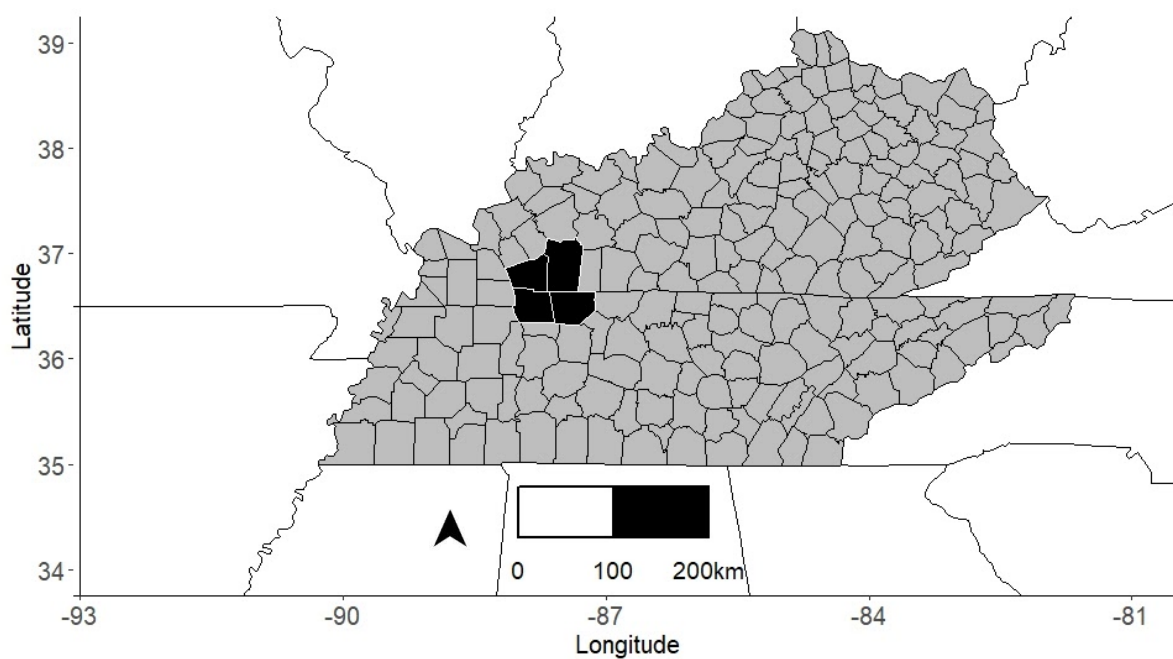bat species (indicated by black fill). Modified from www.whitenosesyndrome.org.

Figure 2.2. Field data were collected in 4 counties in Kentucky (Trigg and Christian counties) and Tennessee (Stewart and Montgomery counties) from 15 May – 15 August in 2020-2021.
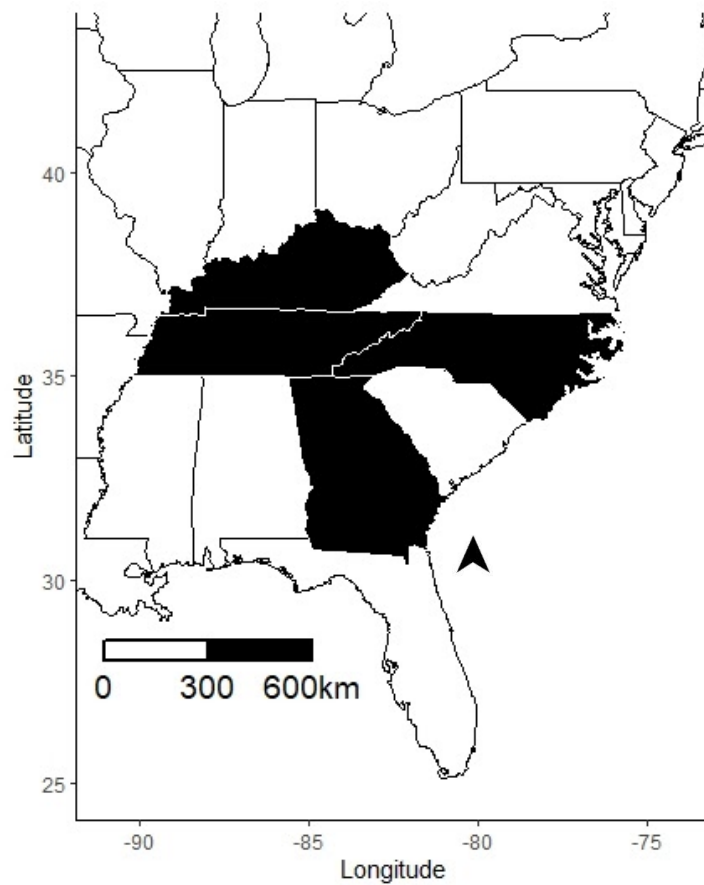
Figure 2.3. Map of southeastern United States. Data were collected across 267 counties in

Kentucky, Tennessee, North Carolina, and Georgia.

Table 2.1. Winter (December [prev. yr for an individual year], January, and February), spring (March, April, and May), summer (June, July, and August), autumn (September, October, and November), and annual severity metrics calculated per county for Tennessee, North Carolina, Georgia, and Kentucky.

Season	Variable
Summer	Number of days below 0°C
	Number of days above 18°C
	Mean relative humidity (%)
Winter	Number of days below 0°C
	Number of days above 18°C
	Precipitation as snow (mm)
	Mean relative humidity (%)
Spring	Number of days below 0°C
	Number of days above 18°C
	Mean relative humidity (%)
Autumn	Number of days below 0°C
	Number of days above 18°C
	Precipitation as snow (mm)
	Mean relative humidity (%)
Annual	Temperature (°C)
	Precipitation (mm)
	Precipitation as snow (mm)
	Relative humidity (%)
	Mean winter duration (days)
	Maximum winter duration (days)
	Minimum winter duration (days)
	Number of frost-free days

Table 2.2. Total number of reproductive females for each bat species captured from 1989-2020 in the southeastern United States before the discovery of white-nose syndrome (pre-WNS) and after the discovery of white-nose syndrome (post-WNS).

Scientific name	Common name	Pre-WNS	Post-WNS
Myotis austroriparius	Southeastern myotis	115	9
Myotis grisescens	Gray bat	184	117
Myotis leibii	Small-footed bat	151	44
Myotis lucifugus	Little brown bat	443	105
Myotis septentrionalis	Northern long-eared bat	1023	174
Myotis sodalis	Indiana bat	603	203
Perimyotis subflavus	Tri-colored bat	596	178
Eptesicus fuscus	Big brown bat	1628	468
Lasiurus borealis	Eastern red bat	1185	464

Table 2.3. Total mean and standard deviation of capture nights per year for all states (Tennessee, Kentucky, North Carolina, Georgia) after white-nose syndrome (WNS) was first detected.

Years since WNS	Mean net nights per year	Standard deviation
1	921.60	65.70
2	1033.18	69.09
3	1096.93	83.79
4	1171.38	102.14
5	1297.93	103.99
6	1365.90	120.07
7	1451.00	89.22
8	1518.86	61.04
9	1522.48	42.15

Table 2.4. Eigenvalues and proportion of total variance explained by each axis derived from a principal component analysis of pre-hibernation climate data for Tennessee, North Carolina, Georgia, and Kentucky.

Axis	Eigenvalues	Proportion (%)	Cumulative proportion
PC1	1.7522	0.3411	0.3411
PC2	1.5498	0.2669	0.6080
PC3	0.9568	0.1017	0.7097
PC4	0.8551	0.0812	0.7910
PC5	0.7761	0.0669	0.8579
PC6	0.7189	0.0574	0.9153
PC7	0.5873	0.0383	0.9536
PC8	0.5115	0.0291	0.9827
PC9	0.3945	0.0173	1.0000

Table 2.5. Eigenvectors associated with each pre-hibernation climate variable for Tennessee, North Carolina, Georgia, and Kentucky for each principal component.

| Pre-hibernation climate variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Number of frost-free days	0.3390	-0.2721	0.4633	-0.0500	0.3760	-0.4621	0.4100	-0.2461	0.1019
Number of summer days above 18°C	0.1713	0.4362	-0.1167	-0.6856	0.1544	-0.2947	-0.0270	0.2835	-0.3241
Number of spring days above 18°C	0.2866	0. 3213	-0.2312	0.5598	0.4940	-0.2047	-0.3829	-0.0545	-0.1112
Number of spring days below 0°C	-0.2154	-0.4636	-0.3477	-0.1610	0.4602	-0.0857	-0.1180	0.4330	0.4140
Mean annual temperature (°C)	0.2506	0.4967	0.1453	-0.0136	0.1481	0.4232	0.2594	0.1866	0.6050
Summer mean relative humidity (%)	0.4410	-0.2602	0.1330	0.2633	-0.1522	0.1426	0.1417	0.6910	-0.3313
Spring mean relative humidity (%)	0.4614	-0.1332	0.1232	-0.1716	0.3983	-0.1989	0.6086	-0.0362	0.3934
Autumn mean relative humidity (%)	0.3693	-0.2885	-0.1223	-0.2957	0.3108	0.6190	-0.1353	-0.3527	-0.2357
Mean annual precipitation (mm)	0.3473	-0.0463	-0.7270	0.0432	-0.2804	-0.1683	0.4412	-0.1752	0.1209
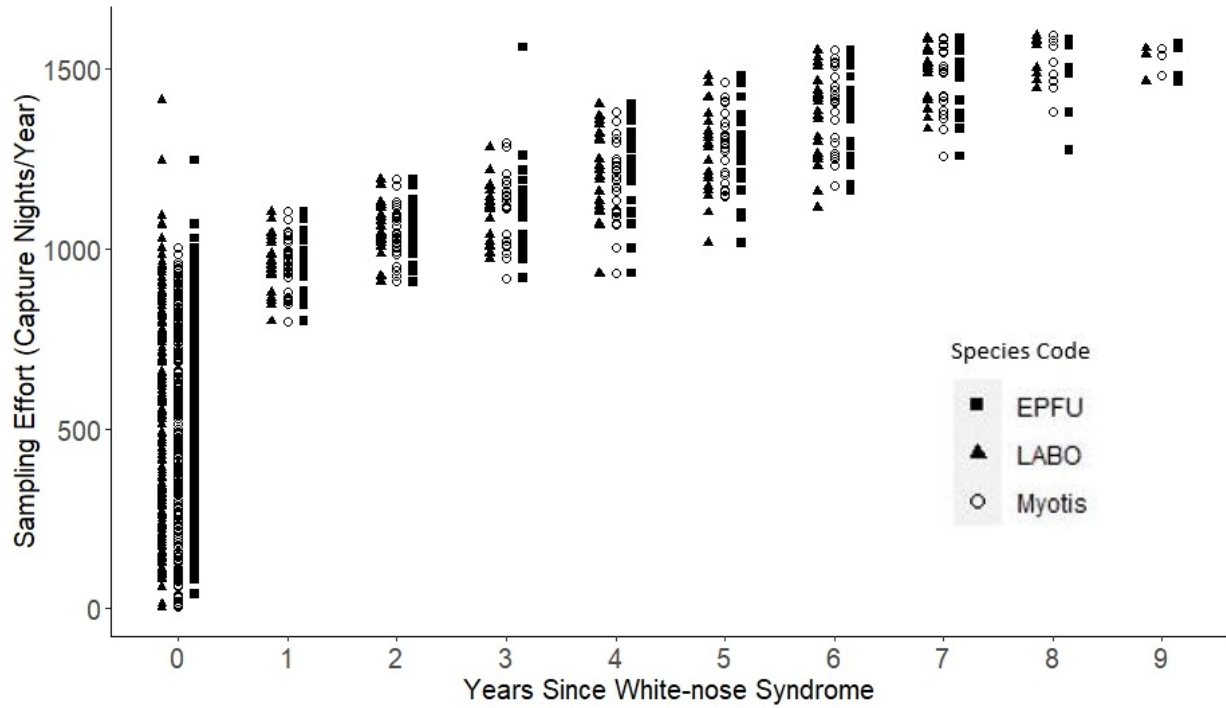
Figure 2.4. Sampling effort (summarized by county and state) for *Eptesicus fuscus* (EPFU)*,*

Myotis spp. (*Myotis austroriparius, M. grisescens, M. leibii, M. lucifugus, M. septentrionalis, M.*

sodalis, and Perimyotis subflavus) and *Lasiurus borealis* (LABO) with year since white-nose
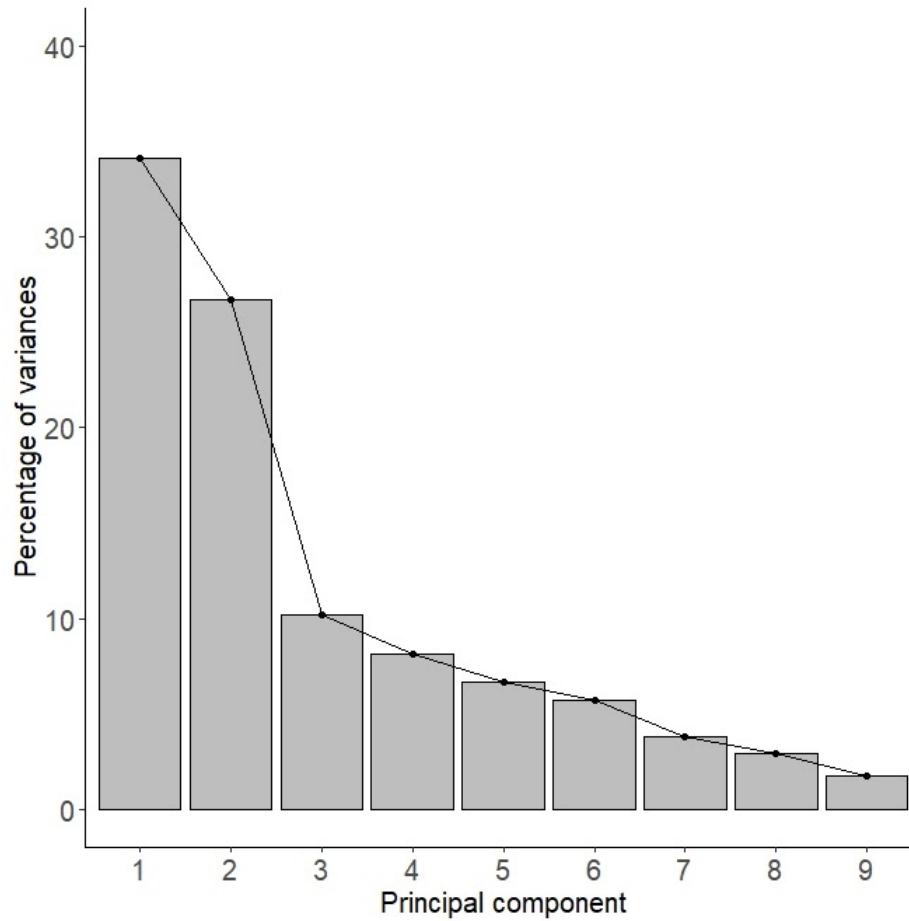
syndrome.

Figure 2.5. Eigenvalues to show the percentage of explained variances for each principal component. 61% of the variances contained in the data are retained by the first two principal components.

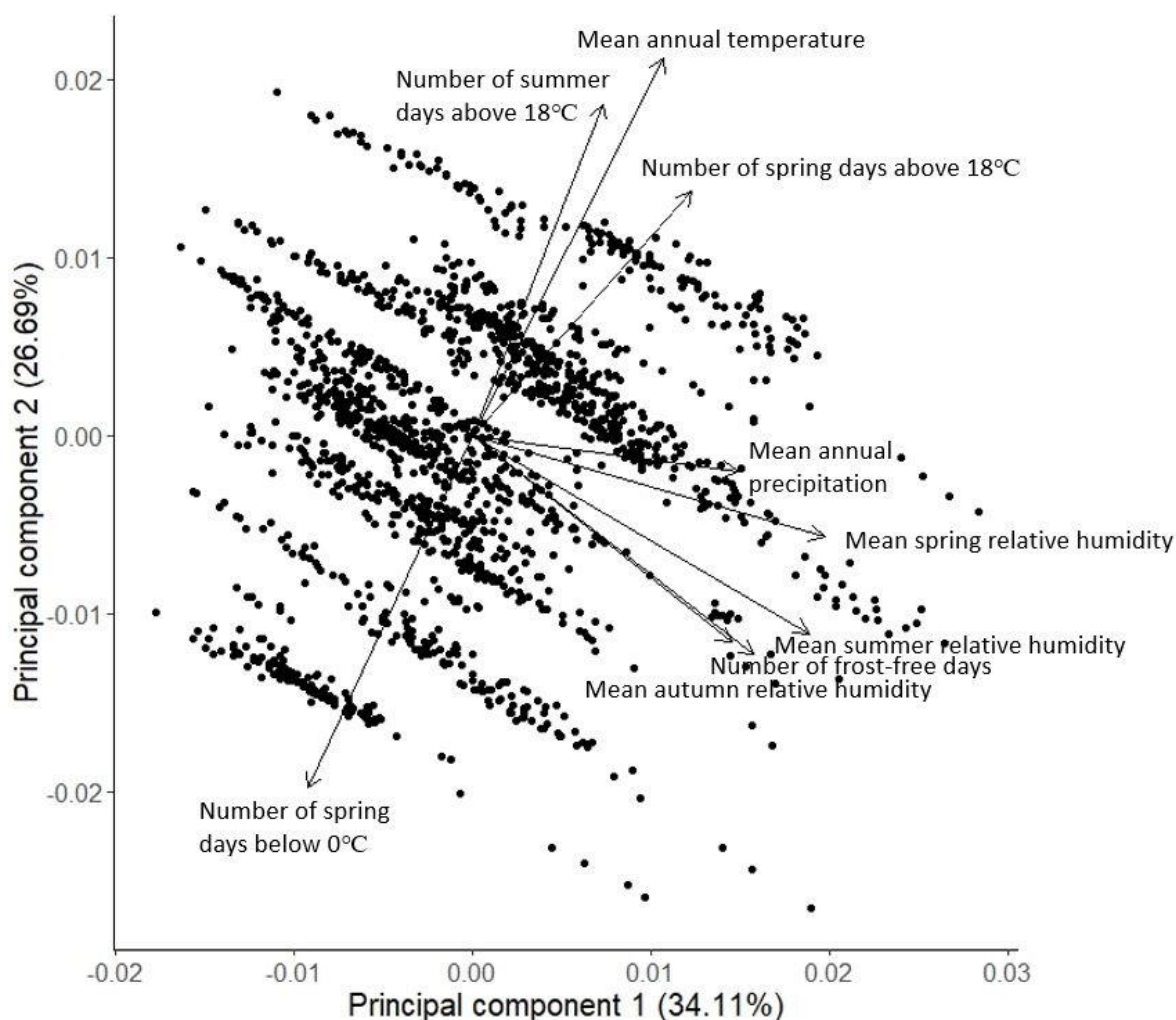Figure 2.6. Factor loadings for the first two principal components of the ClimateNA

environmental variables for North America. Identifiers of the variables: Mean annual

temperature, number of summer days above 18°C, number of spring days above 18°C, mean

annual precipitation, spring mean relative humidity, summer mean relative humidity, autumn

mean relative humidity, number of frost-free days, and number of spring days below 0°C.
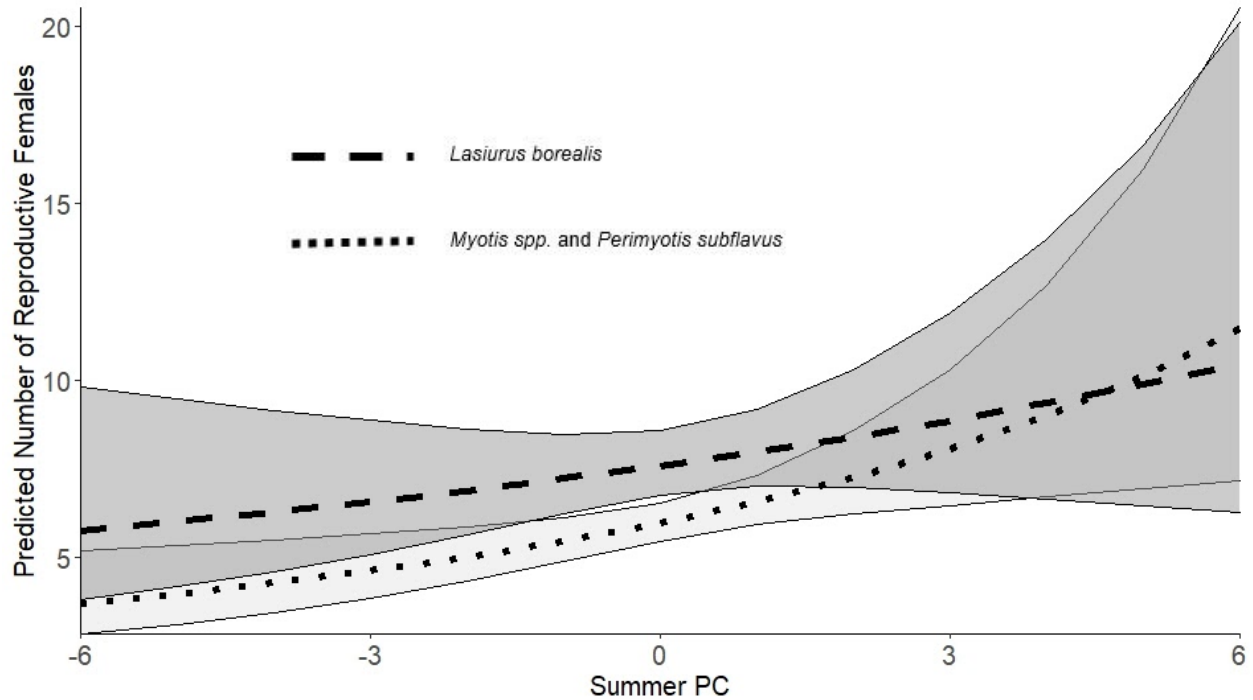
Figure 2.7. Predicted number of reproductive female *Myotis spp.* (*Myotis austroriparius, M. grisescens, M. leibii, M. lucifugus, M. septentrionalis, M. sodalis,* and *Perimyotis subflavus*) and *Lasiurus borealis* against the principal components of pre-hibernation climate variables. The year since white-nose syndrome was confirmed was set at 5 and survey effort (log[capture nights/year]) was set at the mean for that year. Bands represent 95% confidence intervals.
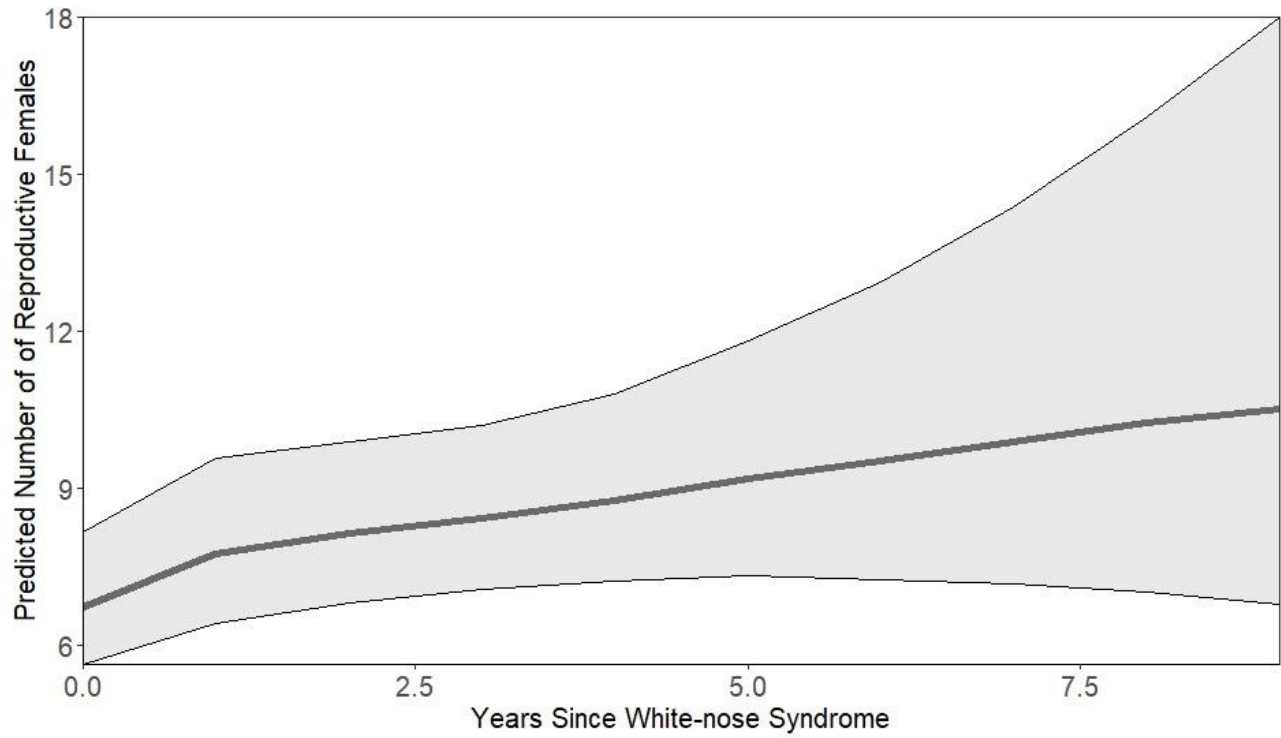
Figure 2.8. Predicted number of reproductive female *Eptesicus fuscus* against year since white-nose syndrome was confirmed with survey effort (log[capture nights/year]) set at the mean. Band represents 95% confidence intervals.

Figure 2.9. Proportion of reproductive females (*Eptesicus fuscus, Myotis spp.* [*Myotis austroriparius, M. grisescens, M. leibii, M. lucifugus, M. septentrionalis, M. sodalis, and Perimyotis subflavus*] and *Lasiurus borealis)* captured per survey night (summarized by county and state) for each year since white-nose syndrome was first detected in that county.

Table 2.6. Model covariates for reproductive condition of southeastern bat species (*Myotis spp*. and *Perimyotis subflavus*, *Lasiurus borealis*, and *Eptesicus fuscus*) with Akaike information criterion for over-dispersed data (ΔAICc), log-likelihood values (*LL*), number of parameters (*K*), and AICc weights (w_t). All models had a fixed effect of survey effort (log[capture nights/year]) and random effects of year and county.
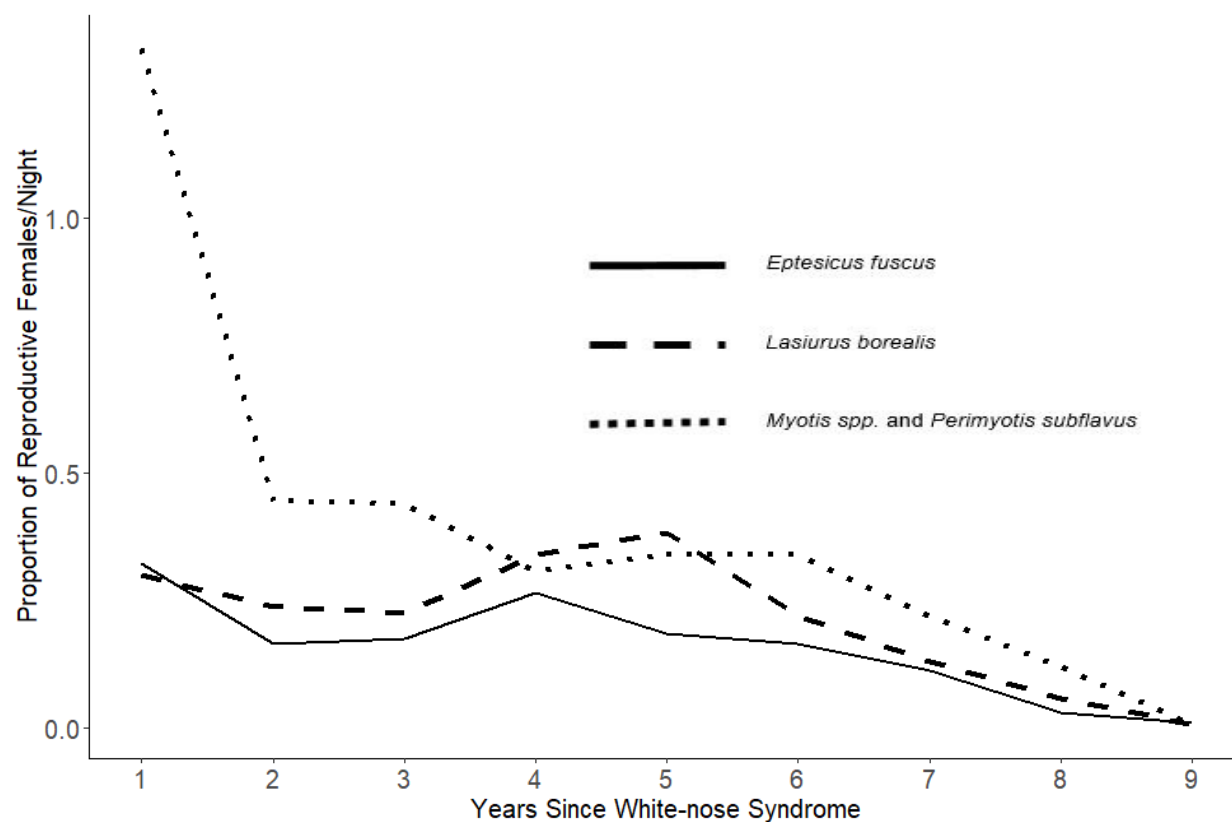
Model	*Myotis spp*. and *Perimyotis subflavus*				*Lasiurus borealis*				*Eptesicus fuscus*			
	ΔAICc	*LL*	K	w_t	ΔAICc	*LL*	K	w_t	ΔAICc	*LL*	K	w_t
Years since WNS + local climate	0.00	6933.71	6	0.95	0.00	2863.65	6	0.96	0.14	2099.01	6	0.37
Years since WNS[b]	6.65	-6939.04	4	0.03	7.87	2869.64	4	0.02	0.00	2100.96	4	0.39
Years since WNS + mean winter duration	7.51	-6938.47	5	0.02	7.95	2868.60	5	0.02	1.00	2100.45	5	0.24
WNS	11254.56	12570.19	9	0.00	5548.22	5636.38	9	0.00	5265.42	4731.86	9	0.00
WNS + mean winter duration	11278.72	12570.00	10	0.00	5551.45	5635.66	10	0.00	5268.46	4730.54	10	0.00
WNS + local climate	11278.96	12556.00	11	0.00	5552.03	5632.75	11	0.00	4728.89	4728.89	11	0.00
Mean winter duration	11280.56	12585.52	4	0.00	5585.87	5660.24	4	0.00	4735.19	4735.19	4	0.00
Local climate	11299.61	12574.08	5	0.00	5589.14	5657.61	5	0.00	4732.67	4732.67	5	0.00

[a]Denotes top model for *Myotis spp*. and *Perimyotis subflavus, Lasiurus borealis*

[b]Denotes top model for *Eptesicus fuscus*

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