ASSESSING THREATS TO NORTH AMERICAN BATS: IMPACTS OF WHITE-NOSE SYNDROME AND CLIMATE ON REPRODUCTION AND SURVIVAL

BY

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DISSERTATION

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Abstract

Many cave-hibernating North American bat species currently face the threat of extinction due to the newly emergent wildlife disease, white-nose syndrome (WNS). WNS is a fungal disease that has been causing catastrophic declines of bat populations in the eastern United States and Canada since it first emerged in 2006. The fungal pathogen, *Pseudogymnoascus destructans*, infects the wings, ears and nose of bats that hibernate in caves in winter while both cave temperatures and bats' body temperatures are low. The hibernating bats' immune systems do not respond to the infection, leading to wing damage, emaciation, depletion of fat stores, and often death. Infected bats that survive winter mount a vigorous immune response upon exiting hibernation. These bats typically clear the infection, regenerate wing tissue and survive. WNS mortality varies greatly by species. Some species have suffered greater than 90% population declines, while other species appear to have not declined at all.

Although researchers have made great strides in the last nine years in understanding WNS, there are still many unknowns. The vast majority of our knowledge of the effects of WNS comes from *M. lucifugus* because it is one of the most abundant North American species, and is also heavily affected by WNS (population declines > 90%). Aside from estimates of rates of population declines at hibernation sites, the effects of WNS on species other than *M. lucifugus* are not well resolved. In the next most abundant species, *E. fuscus*, estimates of population declines range from 0% to 40%. Wing damage had not been studied prior to WNS, making inferences about the relationship between wing damage and WNS difficult. The effects of WNS on reproduction are unknown. Population viability analyses of *M. lucifugus* determined that population growth is most influenced by survival of reproductive females, but the factors that

affect reproductive female survival remain unknown. Currently, the primary WNS population model assumes no effect on reproductive function due to lack of data on the subject. If WNS reduces reproductive output, this model will need to be adjusted to accurately project bat population growth in the post-WNS era.

Climate change is another factor that could affect the accuracy of models that project bats' likelihoods of persistence in the post-WNS era. There are few publications on the effects of a changing climate on North American cave-hibernating bats. Climate change has the potential to impact the persistence of cave-hibernating bat species, which have annual cycles of activity and hibernation that are precisely timed to coincide with the availability of their insect prey and temperatures that are conducive to reproduction. Published data on the effects of climate on bat survival are limited to a study of the little brown myotis (*Myotis lucifugus*) in New Hampshire and the big brown bat (*Eptesicus fuscus*) in Colorado. These studies indicate that *M. lucifugus* survival increases with high precipitation in the Northeastern United States, and *E. fuscus* survival decreases during drought periods in the Rocky Mountain region. This suggests an overall positive relationship between bat survival and precipitation, but the universality of this relationship is unclear without comparable data on multiple species from multiple regions. To my knowledge, Rick Adams's six-species Colorado study is the only published data of the effects of climate on reproduction in temperate-zone North American bats. He found that four of the six species had significantly lower proportions of reproductive females in drought years. Again, it is unclear how universally this applies to the other 42 bat species of the United States, and the extent to which it affects populations outside of the severely water-limited Rocky Mountain region.

My dissertation addresses aspects of the knowledge gaps described above. I conducted a survey of wing damage on bats that had been captured in Illinois prior to the arrival of WNS. I found that wing discoloration in particular is common among multiple species of bats. Additionally, I found that in *E. fuscus* wing discoloration increases in severity in early summer then decreases in severity in late summer, and also varies by year (possibly decreasing in drought years). I conducted a mark-recapture study of *M. lucifugus* and *E. fuscus* at a site in western Illinois to test predictions of trends in survival and reproduction in years that varied in temperature and precipitation. This study also modeled population-level effects on the two species as WNS entered the region. I found that reproduction decreased significantly in the drought year for both species, but did not find an effect of temperature or precipitation on survival rates. Survival rates for *M. lucifugus* dropped drastically in the presumed post-WNS year. There was no change in survival for *E. fuscus*, nor was there any significant difference in reproduction for either species in the presumed post-WNS year. I conducted an additional study of *M. lucifugus* at this site on the effects of annual spring temperature on parturition dates, and the effects of parturition date on maternal survival. I found that parturition dates occurred significantly earlier in the hottest year, but did not find an effect of parturition date on maternal survival. Finally, I conducted a study of the effects of WNS on *M. lucifugus* female fertility during hibernation, and estimated the impact of those effects on population growth rates. I found no effect of WNS on female fertility. My models demonstrated that even if fertility were reduced by 17% (the maximum included in the 95% confidence intervals of my results), post-WNS populations would not become extinct any sooner than they would if there were no effect of WNS on fertility. Unexpectedly, I observed that both infected and uninfected females had neutrophils (a white blood cell that responds to infection) present in their reproductive tracts

where sperm were present. This was surprising given that all published studies of the *P. destructans*-infected wing tissue in hibernating bats report an absence of neutrophils and other white blood cells.

The studies that I present in this dissertation contribute to our knowledge of WNS and bat conservation in several ways. I found that wing discoloration should not be interpreted as an indicator of WNS, and that researchers should anticipate changes in the severity of this type of damage from season-to-season and year-to-year. In a rare bit of good news from WNS studies, I found that the current WNS population model is accurate in terms of reproductive output: there is no effect of WNS on reproduction in hibernation, and no effect during the summer maternity season. However, I did find that reproduction drops in drought years for both species studied. This is similar to results from a previously published study in Colorado, and indicates that bat populations in both the Midwest and Rocky Mountain regions may face declines if the climate becomes hotter and drier in coming years. My results do not show evidence of reduced survival in drought years. This also comes as good news, because bat population growth is influenced more by survival than by reproduction. Additionally, I found a clue that may improve our understanding of bats' immune function during hibernation: Although hibernating bats do not mount an immune response to *P. destructans* infection, they are capable of immune cell recruitment. I look forward to investigating this apparent paradox in future studies.

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CHAPTER 1: GENERAL INTRODUCTION

Recently, with the sudden emergence of white-nose syndrome (WNS), researchers have been working at a rapid pace as the disease expands across the range of cave-hibernating bat species of North America. This work includes efforts to understand the etiology and impact of the disease, and also to gather basic data at the physiological and ecological level to provide a baseline reference that allows them to distinguish "normal" from "abnormal"

WNS was first documented at Howe Caverns in New York in winter 2006, and has since spread to a total of 5 provinces in Canada and 26 states in the United States, causing unprecedented declines in bat populations (Coleman and Reichard 2014; U.S. Fish and Wildlife Service 2016). The first few years of WNS rapidly identified basic facts about the disease. WNS only occurs in bat species that hibernate in caves, and is caused by a cold-adapted fungus, *Pseudogymnoascus destructans* (*Pd*), which infects bats' skin on the nose, muzzle, ears and wing membranes during winter hibernation (Blehert et al. 2009). *Pd* thrives at low temperatures (Gargas et al. 2009), making the cool skin of hibernating bats a welcome environment (Cryan et al., 2010). Additionally, torpid bats may have a decreased immune response (Cryan et al. 2010), creating an optimal growth medium for the cold-loving fungus. *Pd* infection frequently results in death, as evidenced by observations both in the field and in clinical trials (Frick et al. 2010a; Turner et al. 2011; Johnson et al. 2014). Symptoms of infection include emaciation, rapid depletion of fat stores, degradation of wing tissue and frequent arousal from torpor (Blehert et al. 2009; Meteyer et al. 2012; Reeder et al. 2012; Warnecke et al. 2012; Verant et al. 2014). *Pd*-infected bats are subject to numerous physiological stresses associated with dehydration, depletion of fat stores, shorter torpor bouts, and often death (Reeder et al. 2012; Warnecke et al. 2013; Verant et al. 2014). The depletion of fat stores is not simply a result of increased arousal

from torpor, and precedes symptoms like wing damage and frequent arousal by several weeks (Verant et al. 2014). There are usually no histological signs of inflammation in *Pd*-infected skin (Meteyer et al. 2009), but transcriptomic studies have found increased expression of genes associated with inflammation (Field et al. 2015). Histological and transcriptomic studies have both found a lack of leukocyte production in *Pd*-infected bats (Lorch et al. 2011; Field et al. 2015), lending support to the hypothesis that the immune system is downregulated during hibernation. When bats exit torpor, areas of *Pd* infection become areas of extensive inflammation with high densities of neutrophils (Meteyer et al. 2011). Bats that survive WNS and emerge in spring often clear the infection, allowing wings to heal and regain function over the course of several weeks (Fuller et al. 2011; Meteyer et al. 2011). However, some bats appear to experience an immune response after emerging from hibernation that is so aggressive that they perish in a manner similar to the fatal response of some AIDS patients following suppression of the HIV virus (Meteyer et al. 2012).

Researchers have proposed that wing damage could be a direct source of WNS-related mortality due to impaired flight for bats that have emerged from hibernation (Reichard and Kunz 2009), and also during hibernation by disrupting osmoregulation and other physiological functions (Cryan et al., 2010). Two recent studies have elaborated on the work of Reichard and Kunz, with noticeable differences in results (Francl et al., 2011; Fuller et al., 2011). Field studies have found a seasonal wing damage pattern at sites within WNS-affected areas in New England: the most severe damage occurring in May, then gradually tapers off over the course of the summer (Reichard and Kunz 2009; Fuller et al. 2011). There was no confirmation that these bats were infected with *Pd*, and there were no historical records of wing damage in bats, so the authors formed a scoring system called the wing damage index that would allow researchers to

begin documenting wing damage at field sites (Reichard and Kunz 2009). This scoring system was used by the authors of a more widespread study, who found no significant trend in seasonal wing damage patterns, possibly because some of their field sites were in areas where WNS was not yet established (Francl et al. 2011).

Bats' responses to *Pd* infection vary widely by species. For example, while *M. lucifugus* population declines hover around 91% (Turner et al. 2011), studies of *E. fuscus* populations estimate that they have either declined by a relatively modest 41% (Turner et al. 2011) or increased by 43% (Frank et al. 2014). There are no reports of any mortality to date in *Corynorhinus townsendii virginianus* despite extensive surveillance of this federally endangered species (Coleman and Reichard 2014). In Europe, where *Pd* is apparently endemic, cave-hibernating bat species harbor the fungus with no signs of mortality (Wibbelt et al. 2010). Extinction is a serious possibility for the most heavily affected bat species. A population model for the once common and widespread species, *Myotis lucifugus* , predicts it will be extinct in the eastern United States by 2026 (Frick et al. 2010a). So far, population growth models and viability analyses have only been done for *M. lucifugus* (Frick et al. 2010a). This analysis modeled population declines assuming that there is no effect of WNS on fecundity, because there are no data available on fertility and fecundity rates for *Pd*-infected bats (Frick et al. 2010a). The analysis includes several models that project rates of decline for *M. lucifugus* assuming that mortality ameliorates over time at a given WNS-affected site (Frick et al. 2010a). The researchers based the vital rates on mark-recapture data at a pre-WNS maternal colony, then adjusted those values theoretically based on count-data from hibernacula (Frick et al. 2010a; b). There have not yet been any post-WNS mark-recapture studies to compare to this theoretical model, so we do not yet have verification that *M. lucifugus* will be extinct in the projected time

frame. Around the epicenter of WNS in the northeastern United States, there are signs that some remnant bat populations are stabilizing (Reichard et al. 2014).

The results of these early studies also raised a long list of unanswered questions, including the following: How does the theoretical model of WNS-related *M. lucifugus* population decline compare to mark-recapture based field studies as WNS becomes established in the region? What do WNS population models look like for other cave-hibernating species? Does the impact of WNS on populations occur synchronously for all species in a community, or is there a lag in response between the first species infected and other sympatric species? Will WNS-related population declines be exacerbated by a changing climate? What type of wing damage is expected in the absence of WNS? Could the wing damage index developed by Reichard and Kunz be used as an indicator of *Pd* infection? Does *Pd* infection inhibit reproductive function? If it does, how do projections of WNS-related population declines change? Do the effects of WNS on reproduction occur during hibernation, when the disease is active, or does it cause delayed effects by leaving females in poor condition for pregnancy and nursing after spring emergence?

The effect of WNS on reproduction represents a major gap in the currently published literature. Several researchers have noted that effects of WNS on bats' reproductive processes have the potential to seriously impact the ability of bat populations to persist in the post-WNS era (Reichard and Kunz 2009; Frick et al. 2010b; Fuller et al. 2011; Jonasson and Willis 2011). Fortunately, there is a fairly substantial body of literature on the subject of bat reproduction, likely due to the uniqueness of the reproductive cycles of bats, particularly the hibernating species. Hibernating male and female bats have asynchronous reproductive cycles (Racey and Entwistle 2000). For example, female *M. lucifugus* have roughly five months after emergence

from hibernation to complete a pregnancy that lasts about two months, a nursing period that lasts about one month, and then spend the remaining time accumulating fat stores and returning to their hibernacula (Fenton and Barclay 1980; Kurta et al. 1989). Males spend the five month active season restoring their body condition after seven months of hibernation and producing sperm in preparation for mating, leaving them prepared to mate in autumn at the start of hibernation (Racey 1982). The unlikely solution to this problem is that the bats mate at the start of hibernation, the female stores the sperm in her reproductive tract, oogenesis proceeds to the point just before ovulation, then the process of fertilization is paused for roughly seven months until the female emerges from hibernation in spring (Wimsatt and Kallen 1957).

This unusual annual schedule presents the potential for problems for hibernating females, particularly in the age of WNS. They may be subject to reduced fertility if they are severely physiologically challenged during hibernation (Racey and Entwistle 2000). Females that emerge from hibernation in poor condition may not be able to successfully complete parturition and rearing of pups (Fuller et al. 2011). Even if *Pd*-infected females are just as fecund as their uninfected cohorts, there could still be negative repercussions. Pups that are born later are less likely to survive their first year, presumably because they have less time to store fat before hibernation begins (Frick et al. 2010b). WNS-positive females may need to use torpor more often during gestation, resulting in pups that are born later (Racey and Entwistle 2000), and consequent reduced juvenile survival. Additionally, maternal survival could also be reduced because mothers would have less time after weaning to store fat for hibernation. Population growth rates are most sensitive to adult female survival (Frick et al. 2010a), so this possibility could have profoundly negative effects on population viability.

In the following chapters, I will address many of these knowledge gaps. My PhD dissertation research began at an opportune time and place to investigate some of these questions. In April 2010 I met Illinois Department of Natural Resources Endangered Species Manager, Joe Kath, at the annual meeting of the Midwest Bat Working Group, and agreed to run PCR and histological tests for *P. destructans* in wing tissue samples he had collected from several hibernacula and maternal colonies in Illinois. I began conducting maternal colony field studies in spring 2011 and WNS was not detected at any Illinois sites until winter 2012-2013 (U.S. Fish and Wildlife Service 2016), thus my work consists of detailed population studies that include multiple years of data that pre-date WNS in the region. I conducted two field studies at Siloam Springs State Park in Adams County, Illinois, at a picnic shelter where maternal colonies of *M. lucifugus* and *E. fuscus* each inhabited different portions of the roof. One study compares the vital rates and population growth rates of the two species as *Pd* became established at hibernacula in the state; the second study tests the hypothesis that female *M. lucifugus* that give birth later are less likely to survive winter.

The first of these field studies followed the two species from 2011, when there were still no reports of WNS in Illinois, until 2014. The time frame included the hottest year on record in the region (2012) and also a year with one of the rainiest springs on record (2013), offering a unique opportunity to compare the effects of annual climate variation on the two species (*M. lucifugus* and *E. fuscus*) that were subject to identical weather patterns because they resided in the same building. This chapter also gives estimates of changes in vital rates and population growth rates in the early stages of the establishment of WNS in a naïve population. There are only a few published studies of these two species that use mark-recapture techniques, provide vital rate estimates, and use modern matrix-based population growth models (Frick et al. 2010b;

O'Shea et al. 2010, 2011). This chapter builds on those publications by allowing comparison of life-history variation in another region within their range. I categorized years as presumed pre- and post-WNS, and also according to annual spring weather. I created models of apparent adult and juvenile survival for both species using these weather categories as predictors in program MARK, then used Akaike Information Criterion (AIC) to determine model support (White and Burnham 1999). I estimated population growth for the two species under the different yearly conditions using the popbio program in R (Stubben and Milligan 2007).

The second of the two field studies followed *M. lucifugus* only from 2011 to 2014, investigating whether mothers that gave birth later had lower annual survival, and also investigating the effect of annual spring temperature on the timing of parturition.
When mother-pup pairs were known, I estimated parturition dates using regression equations of the growth rates of pups' wing bones. I estimated parturition dates of other mothers by assessing reproductive status and approximate fetal length at each recapture event, then calibrating those estimates to estimates of mothers of known pups. Using estimates of annual spring temperature from NOAA, I tested the hypothesis that parturition dates occurred earlier in the hottest year. I constructed models of apparent maternal survival in program MARK and used AIC to determine support for the hypothesis that early parturition mothers had higher survival rates (White and Burnham 1999).

I also conducted laboratory work in addition to my field research. These studies arose opportunistically from the availability of the preserved remains of bats from other institutions that had been collected for other purposes. For several years, bat specialists Joyce Hofmann and Jean Mengelkoch of the Illinois Natural History Survey had been storing the frozen carcasses of bats that they had identified by species, sex, and age following rabies testing by the Illinois

Department of Public Health. They kept a meticulous database that listed the date the bat was submitted for testing, details of the collection event, and geographic location. These data allowed me to investigate associations between pre-WNS wing damage and species, sex, age, season, year, and region of the state so I could detect patterns in normal variation in wing damage in the absence of WNS for comparison to the damage observed in areas affected by WNS. The other laboratory study used reproductive tissues from female *M. lucifugus* that I obtained from carcasses that were preserved during WNS-related research by DeeAnn Reeder at Bucknell University. This was a histological study that compared indicators of winter fertility in *P. destructans*-positive and *P. destructans*-negative *M. lucifugus* females.

Reichard and Kunz's (2009) paper that documented wing damage was one of the earlier papers that was published following the emergence of WNS. Researchers and wildlife managers were interested in the topic because there was speculation that wing damage may contribute directly to WNS mortality, and also because there were debates about whether it could be used as an on-site screening tool to identify WNS-positive bats (Francl et al. 2011). However, this possibility was hindered by the absence of any pre-WNS surveys of the normal range of variation in wing damage in a population. I conducted a pre-WNS survey using bats that had been captured for rabies testing in Illinois from 2006 to 2010, before the disease was detected in the state. I compared levels of wing damage between species, including bats that do not hibernate in caves. I also modeled wing damage as a function of sex, age, year, and season using *E. fuscus* (because that was the only species with a large enough sample size). I used AIC to choose the best models.

For my final chapter, I used histological techniques to test the hypothesis that *Pd* infection reduced female fertility in hibernating *M. lucifugus*. Female *M. lucifugus* store sperm in

the endometrium of the uterus near the junction of the oviduct, and maintain a single mature vesicular follicle (MVF, a.k.a. Graafian follicle) in one of their ovaries throughout hibernation (Guthrie 1933; Wimsatt and Kallen 1957). They ovulate only once per year, so they forfeit reproduction for the year if they cannot maintain both sperm and MVF until spring emergence from hibernation (Racey and Entwistle 2000). Potentially, the extreme physiological stress of WNS could disrupt maintenance of sperm and MVF storage. To test this, I compared reproductive tissues of *Pd*-positive and *Pd*-negative individuals. I then adjusted post-WNS population growth models to reflect the reduced fecundity predicted by my results to see how much this might contribute to WNS-related population declines.

The work presented here represents the first six years of my career as a mammalogist specializing in bats. In the following chapters I will present the results of my studies, which fill in some of the knowledge gaps identified above and also contribute a new list of questions to be addressed by future studies.

CHAPTER 2: TEMPORAL VARIATION IN BAT WING DAMAGE IN THE ABSENCE OF WHITE-NOSE SYNDROME[1]

Abstract

White-nose syndrome (WNS) is an emerging infectious wildlife disease that has killed over five million bats in the eastern United States since its discovery in winter 2006. The disease is associated with a cold-adapted fungus that infects bats during winter hibernation. Wing damage has been documented in bats with WNS and could become a useful screening tool for determining whether samples should be submitted for testing, but because there are no historical records of wing damage prior to the emergence of WNS, it is unknown what types of damage are specific to WNS. To address this knowledge gap, we inspected the wings of hundreds of bat carcasses collected in Illinois from 2005 to 2010, and used Akaike Information Criterion to evaluate general linearized models of the frequencies of different types of wing damage using age, sex, year and season as predictors in *Eptesicus fuscus*. Wing discoloration was best predicted by year and season. There were no clear predictors for other types of wing damage. We found that about one-fourth of all *E. fuscus* surveyed from this presumptive WNS-negative state had moderate or severe wing damage. We encourage further studies of the relationship between WNS and wing damage to better understand which types of damage are to be expected in the absence of WNS in susceptible species.

[1] This chapter appeared in its entirety in the Journal of Wildlife Diseases. Powers, L.E., J.E. Hofmann, J. Mengelkoch, and B. Magnus Francis. 2013. Temporal variation in bat wing damage in the absence of white-nose syndrome. 49(4) 946-954. J.E. Hofmann and J. Mengelkoch compiled demographic and geographic data. This article is printed with permission from the publisher and is available at www.jwildlifedis.org using DOI: 10.7589/2012-02-034

Introduction

White-nose syndrome (WNS) is an emerging infectious wildlife disease that has killed an estimated 5.7-6.7 million bats in the eastern United States and Canada since its discovery in winter 2006 (US Fish and Wildlife Service, 2012). The disease is associated with a cold-adapted fungus, *Geomyces destructans*, that infects the dermis of cave-hibernating bats during winter (Blehert et al., 2009). *G. destructans* grows optimally between 1-15 C (Gargas et al., 2009), which coincides with the body temperature of cave-hibernating bats that have entered torpor (Cryan et al., 2010). Torpid mammals may have a decreased immune response, and this is hypothesized to allow the fungus to grow unchecked (Cryan et al., 2010). Wing damage associated with WNS has been documented, and researchers have proposed that it could contribute directly to WNS-related mortality by impairing flight (Reichard and Kunz, 2009), disrupting osmoregulation or other physiological disruption (Cryan et al., 2010).

There is an interest in studying WNS-affected bats outside of the hibernation season when *G. destructans* infections are active, either to investigate the effects of the disease on bats that survived winter infection, or to monitor disease prevalence without disturbing bats during hibernation. Current methods of *G. destructans* detection include histological examination of wing tissue, wing punches or swabs for PCR testing, tape lifts for fungal morphological identification and swabs for fungal culturing (USGS National Wildlife Health Center, 2012; Dobony et al., 2011). These methods can be expensive and time-consuming, and in the case of histological examination it also requires euthanization of bats. To address these challenges, the USGS-National Wildlife Health Center (NWHC) limits the number of samples that may be submitted from a single site (USGS National Wildlife Health Center, 2012). The field researcher must therefore choose wisely when deciding which bats to include in the sample.

Wing damage has been documented (Meteyer et al., 2011) in spring and summer bat colonies in WNS-affected areas (Reichard and Kunz, 2009), and could be used as a post-hibernation screening tool for selecting bats that are more likely to test positive for *G. destructans.* Reichard and Kunz developed the Wing Damage Index (WDI) for basic field assessment of the severity of wing membrane damage (Reichard and Kunz, 2009). The NWHC currently includes WDI as one of the criteria that researchers may use to determine if they should submit tissue samples or euthanized animals for WNS testing (USGS National Wildlife Health Center, 2012). Bat wing damage has become an important topic post-WNS, both due to its potential as a screening tool, and because wing damage itself may contribute to WNS mortality (Cryan et al., 2010).

Two recent studies have elaborated on the work of Reichard and Kunz, with noticeable differences in results (Francl et al., 2011; Fuller et al., 2011). Fuller and colleagues conducted continued studies of Reichard and Kunz's New England maternal colonies and found that WDI was highest in early summer, with moderate to severe damage occurring in over 50% of bats in May, then significantly lower in early August (Reichard and Kunz, 2009; Fuller et al., 2011). Conversely, in a large scale mist-netting survey at locations spanning five WNS-positive states, Francl and colleagues found that moderate to severe damage was very rare, and the decrease in WDI over summer was the weak and not statistically significant (Francl et al., 2011). The reason for this disparity is unclear, but Francl and colleagues proposed that the colonies evaluated in the other studies were in a localized area heavily affected by WNS, resulting in a higher occurrence of wing damage. This statement is supported by their observation that WDI was higher in bats surveyed in areas closer to WNS-positive caves. However, wing damage has not been documented in WNS-negative bat populations, so the relationship between WNS and wing

damage remains uncertain. The ability to link WDI with the likelihood of *G. destructans* infection is limited because we do not know how much wing damage occurs in uninfected populations. The data presented here provide baseline reference for "typical" wing damage observed among presumed WNS-negative bat populations using the big brown bat, *Eptesicus fuscus*, as the model species.

Methods

We inspected 7,847 bat carcasses submitted for rabies testing in Illinois from 2005 and 2008-2010, then compared the frequencies of non-WNS wing damage types by month, year, sex and age group. Illinois is presumed to be WNS-negative because the Illinois Department of Natural Resources (IDNR) reports no detection of *G.destructans* in PCR tests and fungal cultures of wing punches and swabs collected at hibernacula and maternal colonies from 2010-2012 (J. Kath, pers. comm.). We had previously identified species, sex and age group for bats that had been tested for rabies by the Illinois Department of Public Health (IDPH), compiled this information into a database including collection date, location and exposure to humans or domestic animals, then stored them at -20 C. We sorted bats into two age groups (adults and juveniles) by examining the long bones of wings for epiphyseal-diaphyseal fusion (Brunet-Rossinni and Wilkinson, 2009). We clustered collection dates into five seasons: spring (15 April - 8 June), early summer (9 June - 18 July), late summer (19 July - 22 September), fall (23 September - 5 November), and hibernation (6 November - 14 April). These dates approximate the different activity periods of *E. fuscus* in Illinois, with spring representing the period when females are pregnant, early summer representing the period when females are nursing pups, late summer representing the period when juveniles are independent, and fall representing the

"swarming" period when bats leave their summer habitats and return to hibernacula (Hofmann, 2008).

We later thawed the bat carcasses, placed them on a light box to transilluminate the wing membranes, and characterized WDI as described by Reichard and Kunz (2009). We determined wing damage scores ranging from no damage (WDI = 0) to severe damage (WDI=3) for three categories: discoloration, holes and membrane loss (Fig. 2.1; Reichard and Kunz, 2009). Wing discoloration refers to areas of pallor where the color was lighter than surrounding membrane. Wing discoloration was scored as follows: five or fewer areas of discoloration = 0, from six areas of discoloration to 50% of membranes discolored =1, between 50-90% of membranes discolored =2, and greater than 90% of membrane discolored = 3 (Figs. 2.1 A-B). For holes, no holes or small pinholes = 0, holes less than 0.5 cm in diameter = 2, holes greater than 0.5 cm in diameter =3 (Figs. 2.1 A-B). For membrane loss, no loss = 0, tears at edge less than 1 cm = 2, and membrane loss greater than 1 cm from edge =3 (Fig. 2.1 C). The greatest of the three scores was taken as the composite score, WDI (Figs. 2.1 A-C; Reichard and Kunz 2009).

The majority of the bats we inspected were excluded from our study because they were damaged when captured or incurred damage from freezing and thawing. Freezing and thawing may cause skin to peel, so we did not assess two other types of documented wing damage: flaking forearm and necrotic tissue. These bats were still assessed for areas of discoloration, holes and membrane loss if these three types of damage occurred in isolation from areas of flaking and necrotic tissue. We excluded bats if they had areas of discoloration, holes or membrane loss that occurred within an area flaking or necrotic tissue. Bats that were reported as caught by pets, were dehydrated or decomposing, or were apparently damaged when captured (i.e. unhealed tears in wing membrane and/or unhealed broken limbs) were also excluded. There

is inherent uncertainty in determining if wing injuries occurred before or after capture. To maintain consistency in evaluating individuals, we classified areas of discoloration, holes or tears that had rounded borders as pre-mortem damage. We classified areas of discoloration, holes or tears that had jagged edges to be post-mortem, and did not include this damage in our analyses. Additionally, we excluded any areas of discoloration associated with peeling skin, with the assumption that the discoloration was the result of loss of layers of skin that may have resulted from freezing and thawing.

We chose bats previously collected for rabies testing in Illinois between 2005 and 2010 because they represent a large sample size of individuals that were very unlikely to have been previously exposed to *G. destructans*. However, there are inherent biases associated with our sample due to differences in collection and preservation methods, and also due to the artifacts of freezing tissues. The sample may over-estimate wing damage in natural bat populations due to possible damage during collection, rabies testing and freezing, and also because bats with wing damage may have had impaired flight that made them more likely to be caught and submitted for testing. We made every effort to minimize the biases of post-mortem damage using the criteria described in the previous paragraph. These efforts may have resulted in under-estimation of wing damage in natural populations, because some wing damage that occurred before collection may have erroneously been excluded as post-mortem.

Following the exclusion process, we compiled wing damage scores for a total of 1327 bat carcasses, including four cave-hibernating species (*Eptesicus fuscus*, n = 1108; *Myotis lucifugus*, n = 61; *M. septentrionalis*, n = 15; and *Perimyotis subflavus*, n = 1); and four species that do not hibernate in caves (*Lasionycteris noctivagans*, n= 72; *Lasiurus borealis*, n=50; and *L. cinereus*, n = 13; and *Nycticeius humeralis*, n = 7). The sample sizes were small for all species except *E.*

fuscus. We did not have individuals representing all five seasons for any species except *E. fuscus*, so our models include *E. fuscus* only. We modeled age, sex, season and year as predictors of wing damage using general linearized models (GLMs), then used Akaike information criterion (AIC) to select the best models (R Development Core Team, 2010; Pinheiro et al., 2011). We modeled the three damage types (discoloration, holes and membrane loss) and composite WDI separately to clarify which types of damage contribute to differences in WDI between the sexes, age groups, seasons and years. We evaluated the models by comparing ΔAIC scores, where models with ΔAIC < 2 were considered competitive, and compared the probability that any model can predict damage using Akaike weights (Burnham and Anderson, 2002).

Results

All species examined were observed to have individuals with WDI scores of 1 or 2, with the exception of *P. subflavus* in which only one specimen was evaluated. Individuals rating a WDI of 3 were observed in all species with a sample size greater than 50 (Fig. 2.2 A-B). We did not include species as a predictor in our models, because the sample size was small for all species except *E. fuscus*. The small samples of species other than *E. fuscus* do not represent all five seasons that we included in our study, so data depicted in Figure 2 may over-estimate or under-estimate typical wing damage in these species due to possible seasonal effects. We did not observe any bats with discoloration scores of 3.

In our GLMs of *E. fuscus*, AIC showed strong support for a single model of discoloration. The best model for discoloration included season (clustered into three groups: early summer; late summer; and a combined fall, winter, spring category) and year (clustered into two groups: 2005 and 2008-2010; Table 2.1). Akaike weights (ω) show support for only one

other model, and it was a similar model that held all five seasons as separate predictors and clustered years into the same two categories (2005 vs. 2009-2010; Table 2.1). However, this model is not competitive by our methods because the ΔAIC value is greater than 2. The proportion of moderate discoloration (discoloration = 2) was highest in early summer, lowest in late summer and intermediate in fall through spring (Fig. 2.3 A). By year, the proportion of moderate discoloration was lower in 2005 than in other years (Fig. 2.3 A).

All models for wing holes and membrane loss in *E. fuscus* were weakly supported by AIC. The four competing models for wing hole score included combinations of season, year and age (Table 2.1), but each of these models represents a modest improvement over the intercept-only model (ΔAIC scores from 0 - 1.7 for the top models vs. 2.3 for intercept-only; Table 2.1). Also, Akaike weights are low for all wing hole models (Table 2.1), indicating a low probability that any one model can be used to predict wing holes. The data show a slightly lower proportion of moderate and severe hole damage (scores of 2 and 3) in the two summer seasons relative to other seasons, and slightly higher proportion in 2005 relative to other years, but these differences are too modest to result in strongly predictive models (Fig. 2.3 B, Table 2.1). The difference between age groups was also very small (not shown). There is a similar lack of resolution between membrane loss models, where the top four models performed only slightly better than the intercept-only (null) model (ΔAIC scores from 0 - 1.4 for the top models vs. 2.2 for intercept-only; Table 2.1). Akaike weights are low for all membrane loss models and fail to resolve a single model with high probability. The data show that membrane loss is very rare, with very little difference between groups (Fig. 2.3 C). The difference between age groups was also very small (not shown). There were three competitive models for composite WDI scores, all of which included two seasonal groups (late summer vs. others), and which sometimes included year

(2005 vs. 2008-2010, or all four years as a separate parameter; Table 2.1). The ΔAIC scores show that all three models are much better than the intercept only, but only marginally better than two other models that cluster season into three groups (early summer vs. late summer vs. others; Table 2.1). Again, Akaike weights assign relatively small probabilities to several models. In sum, AIC analyses do not find strong support for any specific predictive models of wing holes, membrane loss or composite WDI in our *E. fuscus* sample using sex, age, season or year as predictors.

Discussion

In our study of bats collected in a presumptive WNS-negative area, we found all three of the wing damage types previously documented by researchers in WNS-positive areas. In particular, wing discoloration and holes are common, and not necessarily indicators of prior WNS infection. We observed bats with moderate to severe WDI scores that had been collected in Illinois in 2005 (Figure 1), two years before WNS was first documented in New York (Blehert et al. 2009). We also observed moderate to severe scores in species that do not hibernate in caves, and therefore are not known to be susceptible to WNS (Fig. 2.2 A). Field researchers should be aware that moderate and severe damage sometimes occur in the absence of WNS.

We found that moderate to severe wing damage in our Illinois sample was common. Overall, about one-quarter of the *E. fuscus* that we evaluated had a composite WDI of 2 or 3 (Fig. 2.2 B). In early summer, more than one-third of the *E. fuscus* we examined had composite WDI scores of 2 or greater (Fig. 2.3 D). Our observed frequency of moderate to severe WDI is somewhat greater on average than reported by Reichard and Kunz and by Fuller and colleagues (although in some weeks they reported WDI ≥ 2 for nearly 50% of bats surveyed), and much

higher than that reported by Francl, despite the fact that their studies were conducted in areas where WNS is widespread (Reichard and Kunz, 2009; Francl et al., 2011; Fuller et al., 2011). The modest differences between our sample and the two New England maternal colony studies could be explained by the inherent sampling biases of using bats collected statewide for rabies testing rather than bats mist-net at specific maternal colonies, or by inter-species variation in wing damage between *E. fuscus* and *M. lucifugus*. Although we could not include *M. lucifugus* in our models due to small sample size, we did observe a lower frequency of moderate to severe wing damage in our sample of 61 individuals (less than 15% - Fig. 2.2 B). However, this observation remains tentative, since we did not have *M. lucifugus* from all five seasons studied. Further studies of wing damage in *M. lucifugus* in WNS-free areas would determine if there is a true difference in WDI between these two species.

Inter-species differences cannot account for the large disparity between our results and those of Francl and colleagues. They observed WDI of 2 or 3 in less than 1% of all *E. fuscus* and less than 2% of all *M. lucifugus* (Francl et al., 2011). Their sample sizes were large (over 2700 *E. fuscus* and over 900 *M. lucifugus*), so the large difference in wing damage scores between their data and other studies cannot be dismissed on the basis of sample size. Francl's study surveyed damage in bats caught during mist-net surveys, while we studied bats that had been collected by IDPH for rabies testing. Our sample differs from the live-caught bats of Reichard, Fuller and Francl, because our bats were submitted from numerous sources to the IDPH and were subjected to rabies testing, and were stored in freezers before being examined for wing damage. Additional studies of wing damage in live bats caught in WNS-free regions will help clarify the extent to which the collection methods explain the observed differences. For this study, we excluded bats that were known to have been caught by animals and those that had unhealed fractures or wing

membrane tears, suggesting that they may have been damaged during capture. We expected this to result in underestimates of damage, since we would sometimes be excluding damage that occurred for reasons not related to capture. It is also possible that bats with wing damage are more likely to be captured and submitted for rabies testing, and that additional damage may occur in post-mortem handling and freezing, which would positively bias our data. Our analyses of damage by year indicate that damage did not substantially increase with time spent frozen: wing discoloration is lower in 2005 than in subsequent years (Fig. 2.3A), while holes, membrane loss and composite WDI show only a slightly higher scores in 2005 (Figs. 2.3B-D). The increases in these three scores were not sufficient to place 2005 as a predictor in all of their competitive models (Table 2.1).

Alternately, the disparity in observed wing damage between our study and Francl's could be due to inter-observer error. Since WDI was developed to be a simple scoring system for implementation in the field, it may be prone to subjective interpretation. For example, there may be high inter-observer error in what a given investigator estimates to be coverage of 50% of the wing area. In Figure 1A, the total area of wing discoloration is less than half the total area of the wing membrane, so we assigned it a score of 1. However, the areas of discoloration are distributed over more than 50% of the total wing membrane area, so another observer might assign a score of 2. Additionally, variation in lighting could result in large differences in observed damage. We had previously examined the bats in our study to identify species, sex and age for IDPH records, and were surprised to find so much damage when we later trans-illuminated the wing membranes using a light box for this study. Whatever the source of this disparity, the outcome of these differences are substantial: Francl and colleagues conclude that moderate to severe damage is very rare, while we find that bats with moderate to severe damage

are commonly found outside the known WNS range.

Of the three categories of damage we surveyed, wing discoloration was the only type that was predicted well using our parameters (Table 2.1). We found that wing discoloration had higher frequencies in early summer (9 June – 18 July) and lower frequencies in late summer (19 July – 22 September) relative to the other three seasons, and that the frequencies were lower in 2005 than in 2008-2010 (Fig. 2.3 A). Our sample size in fall (22 September – 5 November) was small (n = 45), so our reported frequencies of wing damage in this season are uncertain. This uncertainty can be seen in a second-place model with a low Akaike weight that is identical to the top model, except that it keeps all five seasons as separate parameters (Table 2.1). We found smaller differences between frequencies of damage by season and year for wing holes, membrane loss and composite WDI, which were not disparate enough to determine a best predictive model (Figs. 2.3 B-D). AIC ranks the best of set of predictive models but does not reject or accept models (Burnham and Anderson 2002). The numerous ΔAIC scores less than 2 and low Akaike weights of the models for holes, membrane loss and composite WDI demonstrate that our parameters are not reliable predictors of these categories of damage. WDI (a composite of wing discoloration, hole and membrane loss scores) is essentially a diluted version of the best model for discoloration. For example, the best model for discoloration included year as a predictor, with 2005 lower on average than other years (Fig. 2.3 A), but holes and membrane loss were slightly higher in 2005 than other years (Figs. 2.3 B-C), which when combined made WDI more similar across years (Fig. 2.3 D). Field researchers may find it helpful to make a distinction between the specific types of damage if using WDI to determine if bats should be submitted for testing. Based on our data, we would expect that in the absence of *G. destructans* more than 15% of *E. fuscus* in early summer have a score of 2 for discoloration

(Fig. 2.3 A), so there would be no reason to suspect anything abnormal in this case. However, it would be unusual if a similar proportion of *E. fuscus* had scores of 2 due to membrane loss (Fig. 2.3 C), or if any had a score of 3 for discoloration (Fig. 2.3 A).

We found that *E. fuscus* wing discoloration scores increased from spring (15 April through 8 June) to early summer (9 June through 18 July), then decreased from early summer to late summer (19 July through 22 September – Fig. 2.3 A). The general trend is consistent with WDI data for *M. lucifugus* from WNS-positive regions. Both field studies of wing damage conducted at the New England maternal colonies saw a similar increase and decrease in WDI over the maternal season (Reichard and Kunz, 2009; Fuller et al., 2011). Meteyer and colleagues noted that wing damage peaked on day 27 of their captive study of WNS-positive bats (Meteyer et al., 2011). They report collecting bats from hibernacula in May, which would make day 27 sometime in late May through June (Meteyer et al., 2011). However, these studies found that WDI peaked in late May through June, while we saw wing discoloration peak in late June to early July. In a more recent study, Meteyer presents data suggesting that damage increases in severity for weeks after *G. destructans* infected bats arouse from torpor due to a prolific inflammatory response that begins when the bat becomes euthermic (Meteyer et al., 2012). It is unclear if our observed seasonal increase in wing damage severity is also related to an inflammatory response, or what seasonal factor would trigger this response. It should also be noted that we very rarely observed damage as severe as the damage shown in Meteyer's paper (i.e. some wings with WDI=3 are more severely damaged than others - Meteyer et al., 2012).

While more research is needed to determine the causes of wing damage observed in the absence of WNS, our data demonstrate that wing damage in *E. fuscus* is relatively common and should not be viewed as a definitive indicator of previous *G. destructans* infection. In particular,

WDI scores may vary due to seasonal and annual fluctuations in wing discoloration. Also, wing holes were quite common in our sample, with 20% of *E. fuscus* in our study receiving a wing hole score of 2 or 3. These results underscore the need to follow NWHC submission guidelines that limit the number of bats euthanized for histological identification of *G. destructans*, whether bats are being submitted to NWHC or to other research facilities. The current scoring system for wing holes designated nearly 1 in 4 *E. fuscus* in our sample as moderately or severely damaged (i.e. scores of 2 or 3; Fig. 2.2 B). A minor alteration of the hole scoring rubric would adjust scores so that scores of 2 and 3 are less common in the absence of WNS. If 1-5 mm holes were given a score of 1 rather than 2, hole scores of 2 or greater in *E. fuscus* in our sample would drop from 20.0% to 3.4% (Fig. 2.2 B). This simple adjustment could help make the WDI scoring system more effective in distinguishing normal damage from abnormal damage in *E fuscus*. Studies of wing damage in other bat species would clarify whether this adjustment would be appropriate for bats in general, or if separate scoring systems should be implemented for the more abundant susceptible species.

Additional WDI analyses for other susceptible species in WNS-negative regions would be very helpful in determining if wing damage prevalence is similar to our observations of *E. fuscus*. Also, we were unable to document flaking or necrotic tissue in these frozen bat carcasses. Field data from live bats in WNS-negative regions would help determine if these other types of damage are specific to WNS and could be used more reliably as a pre-screening tool. The WDI scoring system is a potentially very useful tool for monitoring bat population health generally, and possibly for choosing which bats should be tested for *G. destructans* infection outside of the hibernation season. Further studies documenting wing damage in WNS-affected and unaffected areas will improve our ability to make this distinction. Until unambiguous indicators of WNS-

specific damage are found, field researchers should remain aware that wing damage may occur in the absence of *G. destructans*, and continue to follow NWHC guidelines that minimize the number of bats euthanized for WNS testing.

CHAPTER 3: EFFECTS OF WHITE-NOSE SYNDROME AND ANNUAL CLIMATE VARIATION ON REPRODUCTION AND SURVIVAL IN TWO COMMON NORTH AMERICAN CAVE-HIBERNATING BAT SPECIES

Abstract

Many hibernating bat species in North America are threatened by the disease white-nose syndrome (WNS). This includes two of the most common species: the little brown myotis (*Myotis lucifugus*), which has well-documented, severe population declines related to WNS; and the big brown bat (*Eptesicus fuscus*), which appears to be more resistant to WNS. Previous research suggests that the effects of a warming climate may add to the plight of these species by decreasing survival and reproductive rates. However, it is unclear how applicable these estimates are throughout their vast geographic ranges. We conducted a 4-year mark-recapture study at a site in western Illinois where *M. lucifugus* and *E. fuscus* maternal colonies are established in different portions of the same building. This site allowed for a natural experiment that compared the survival, fertility, and population growth rates of the two species experiencing the same annual weather patterns as WNS established itself in the region. We modeled apparent survival and estimated maternity for both species. We found that *M. lucifugus* had higher adult and juvenile survival rates and lower proportions of reproducing females than *E. fuscus*. The proportion of reproducing females was significantly lower for both species in the drought year. Survival and population growth rates were nearly constant from 2011-2014 for *E. fuscus* adults and juveniles. Survival and population growth rates dropped precipitously from 2013 to 2014 for *M. lucifugus* adults and juveniles, consistent with WNS-related declines. Our data support the prediction that drought negatively impacts reproduction for Midwestern bats. We did not find an effect of temperature or precipitation on survival for either species.

Introduction

Insectivorous bats are the primary predator of nocturnal flying insects, and therefore provide a valuable ecosystem service in agricultural landscapes. In the United States, bats save farmers an estimated $3.7 billion annually in pesticide application costs (Boyles et al. 2011). Unfortunately, many U.S. bat species, including two of the most abundant (little brown myotis, Myotis lucifugus and big brown bat, Eptesicus fuscus) are at risk due to a recently emerged disease called white-nose syndrome (WNS) (Coleman and Reichard 2014). Between 2006 and 2011, WNS killed at least 5.5 million bats in eastern North America (U. S. Fish and Wildlife Service 2012), and population models predict that the once common and widespread species M. lucifugus will be extinct in the eastern half of its range by 2026 (Frick et al. 2010a). The disease is caused by a cold-loving fungus, *Pseudogymnoascus destructans*, which infects bats during winter hibernation when body temperatures are low and there is an apparent reduction in immune response (Lorch et al. 2011), causing population losses often greater than 90% (Turner et al. 2011). There is currently no available treatment for the disease. At present, the most viable strategy for preventing widespread extinction is to ensure that bats have optimal spring and summer habitats that will maximize survival and reproduction during the active season, providing partial compensation for winter losses due to WNS. If we can slow population declines, perhaps bat populations can adapt before going extinct.

Vigorous research over the last nine years has demonstrated unequivocally the threat that WNS poses for bat populations, particularly in terms of survival rates for *M. lucifugus* populations in the northeastern United States (Turner and Reeder 2009; Frick et al. 2010b; Lorch et al. 2011; Wilder et al. 2011; Reeder et al. 2012). The outlook is less clear for other species, including *E. fuscus*. Researchers have prioritized studies of M. lucifugus because this species is

severely impacted by WNS (population declines averaging 91%;)(Turner et al. 2011), while *E. fuscus* is one of the least affected of the cave-hibernating species (population declines averaging 41% (Turner et al. 2011). However, the lesser impact of WNS on *E. fuscus* still presents the possibility of persistent population declines (i.e. ~40% losses are substantial). Conservation of *E. fuscus* populations could soon become a priority as other species are reduced to remnant populations and the bulk of the ecosystem services provided by insectivorous bats are shouldered by species that maintain relatively large population sizes. Unfortunately, there are only limited data on *E. fuscus* vital rates.

Climate change will also likely have some effect on these two species in coming decades, which could impact how populations adapt to a post-WNS environment (Adams 2010; Frick et al. 2010b). We expect that survival might be different following unusually warm years, however, based on observations from past studies, changes have been predicted in opposite directions. Juvenile female *M. lucifugus* in the northeastern United States that were born earlier had higher survival rates (Frick et al. 2010b). We predict that juvenile survival would be higher in hot years because dates of birth tend to occur earlier when temperatures are warmer (Ransome and McOwat 1994), and that adult female survival might also be higher because they may reap benefits of earlier weaning. However, warmer years are typically also drier. *E. fuscus* survival was lower during a drought year at a colony in Colorado (O'Shea et al. 2011). Data from a study of *M. lucifugus* in New Hampshire support this alternate prediction. Survival correlated positively with summer precipitation (Frick et al. 2010b), indicating that survival should be lower in years that are drier than average. We further predict that reproduction will be lower in drought years, based on the results of a study in Colorado that included six species, including *M.*

lucifugus and *E. fuscus* (Adams 2010). However, we make this prediction cautiously, as water is much more limited in Colorado than it is in western Illinois.

M. lucifugus and *E. fuscus* are both historically abundant North American cave-hibernating species with heavily overlapping ranges, but with different life-history traits (Fenton and Barclay 1980; Kurta and Baker 1990). In addition to providing vital rates and population projections that can be applied directly to management decisions, comparisons of the two species would improve our understanding of how ecological factors (disease and climate) reshape the survival and reproduction of two sympatric species that differ in body size, life span, and litter size (Fenton 1980, Kurta 1990). Despite being two of the most common bat species in North America, estimates of vital rates and modern estimates of population growth based on stage-structured matrix models are limited to one study of *M. lucifugus* in New Hampshire and two studies of *E. fuscus* in Colorado (Frick et al. 2010b; O'Shea et al. 2010, 2011). This limits our understanding of how local adaptation has shaped survival and fecundity throughout the diverse range of these species. This limitation is particularly problematic for our understanding of regional variation in the life-history characteristics of *E. fuscus*, which typically gives birth to a single offspring in the western portion of its range and two offspring in the eastern portion of its range (Kurta and Baker 1990).

We conducted a four-year mark-recapture study of *M. lucifugus* and *E. fuscus* maternal colonies in Illinois beginning in 2011 (one year before WNS was first documented in the state; Coleman and Reichard 2013). The study period included the hottest year on record in Illinois (2012) and one exceptionally rainy year (2013), giving us the opportunity to observe the effects of extreme annual climate trends on bats' vital rates. Our study was conducted at a site where a single structure houses maternal colonies for both species, providing a natural experiment in

which the two bat species were subject to identical annual weather fluctuations. Our purpose was to determine the relative impact of white-nose syndrome, spring temperature, and spring precipitation on survival, reproduction, and population growth in two cave-hibernating bat species native to Illinois: *M. lucifugus* and *E. fuscus*.

Methods

Field methods

This study of *M. lucifugus* and *E. fuscus* maternal colonies was conducted from 2011-2014 at Siloam Spring State Park (Adams County, Illinois). We followed annually published WNS decontamination protocols for all field studies (U.S. Fish and Wildlife Service 2012). All work was approved by the Institutional Animal Care and Use Committee at the University of Illinois (protocol 14076). We mist-netted immediately around the roosts every 2-4 weeks from early May through early August. We wing-banded females and juveniles with lipped metal bands with individual ID numbers (Porzana, Ltd., Icklesham, U.K.). Beginning in late May, we visited the sites roughly biweekly after dusk when mothers had emerged to forage. On these nights, we checked the roosts for new pups, then weighed, banded, and measured forelimbs of any new pups detected. We continued to mist-net outside the roost and recorded recaptures of previously banded bats through early August of each year for four years. We compiled regional precipitation and temperature data from a nearby weather station (Quincy-Baldwin Regional Airport) for each year as follows: mean spring temperature (average temperature from 1 March - 31 May), and total spring precipitation (total precipitation from 1 March - 31 May; NOAA 2014).

White-nose syndrome testing

During May, we took wing tissue biopsies from adult females for PCR testing for the presence of P. destructans using 3-mm tissue punches (Lorch et al. 2010). We placed tissue punches in 100% ethanol in gasketed microcentrifuge tubes on ice for transport to our lab, where they were stored at -20^{o} C until DNA extractions were completed. DNA was extracted from bat wing tissue samples, and PCR was conducted using custom primers and positive control DNA received from the United States Geological Survey-National Wildlife Health Center to test for the presence of *P. destructans* (Lorch et al. 2010). PCR products that produced a gel band roughly 624-bp long were sent for sequencing. The sequences were compared to the published diagnostic *P. destructans* sequence (GenBank accession no. FJ231098) using NCBI's BLAST (Altschul et al. 1990). Only sequences that were 100% matches to the GenBank sequence were considered PCR positive for *P. destructans* (Lorch et al. 2010).

Survival estimates

We recorded recapture events of individuals in subsequent years. We estimated apparent survival for adults and juveniles across years using Cormack-Jolly-Seber models in program MARK (White and Burnham 1999). For *M. lucifugus*, our total capture effort was greater in 2012 than 2013 or 2014 because we hand-captured adult females directly from the roost twice in May 2012 to collect wing punches. To account for this, we modeled recapture probability over time into two year groups: 2011-2012 vs. 2012-2013 and 2013-2014). Our capture efforts for *E. fuscus* were roughly equal each year, so we set recapture probabilities equal across all three intervals (2011-2012 = 2012-2013 = 2013-2014). We conducted bootstrap goodness-of-fit tests with 1000 replicates on the full model of each species, and applied c-hat corrections to models

that bootstraps found overdispersed (Cooch and White 2014). We observed much lower numbers of *M. lucifugus* in 2014 than in previous years, which led us to suspect the colony had increased over-winter mortality in 2013-2014, consistent with WNS. We therefore tested models that set apparent survival equal for the first two years (2011-2012 = 2012-2013 ≠ 2013-2014). We then ranked the models using Akaike Information Criterion (AIC), and considered any models with a $\Delta AICc \leq 2$ competitive (Hobbs and Hilborn 2006). We averaged the models according to their Akaike weights to get estimates of apparent survival for adult and juvenile females for both species across the three recapture intervals (2011-2012, 2012-2013, 2013-2014; Cooch and White 2014).

Our juvenile survival rates were prone to overestimation because our models estimated survival from the time that juveniles were banded until they were recaptured in subsequent years, and does not include mortality that occurred before banding. Population models combine survival estimates with estimates of fecundity, which were based on pregnancy assessment of adult females in our study. Thus, population models from our data set neglect prenatal declines in fecundity that occur after the mother has been assessed for pregnancy, and also neonate mortality that occurs between birth and the time that juveniles are banded. There is a dearth of data on early juvenile mortality (Barclay et al. 2004). To address this knowledge gap, we assumed that populations of both species were stationary ($\lambda = 1$) before WNS began affecting survival rates. We made this assumption based on annual counts of these colonies that indicate that populations have remained relatively stationary since 1996 (J. Kath, unpublished data). We then adjusted the post-banding juvenile survival rates accordingly by a factor that represented both fetal survival from the time the mother was assessed for pregnancy and neonate survival from birth until the juvenile was banded. We refer to the survival estimates from our models in

program MARK as "post-banding juvenile survival", and the adjusted estimates as "juvenile survival".

Reproduction and maternity

We defined maternity (M) as the product of: the proportion of females reproducing, litter size and the proportion of female offspring (which we assumed to be 0.5). To estimate the proportion of reproducing females in the population each year, we observed nipple morphology and palpated the abdomen to detect pregnancy in adult females (Racey et al. 2009). *E. fuscus* females in this part of their range typically give birth to two pups (Hofmann 2008). We could confirm the presence of two fetuses by palpating gravid females that were in mid to late pregnancy, but it can be difficult to determine the number by palpating in early pregnancy. We assumed that all pregnant *E. fuscus* were carrying two fetuses and all pregnant *M. lucifugus* were pregnant with one, based on published data on litter size in the Midwestern United States (Hofmann 2008). Thus, maternity was equal to one-half the proportion of reproducing females for *M. lucifugus*, and equal to the proportion of reproducing females for *E. fuscus*. These two measures of reproduction allow us to compare our results with previously published data. Specifically, our "proportion of reproducing females" is equivalent to "reproductive rates" reported by Frick and colleagues (2010b), and our "maternity" is equivalent to "fecundity" reported by O'Shea and colleagues (2011). We tested for differences in the proportion of reproducing females between species, and between drought and non-drought years using Fisher's exact tests.

Population modeling

We modeled population growth for *E. fuscus* and *M. lucifugus* using a two-stage Lefkovitch matrix using our survival and maternity estimates in the popbio package in R (Fig. 3.1) (Caswell 2001; Stubben and Milligan 2007). We used a female-only two-stage matrix (adults and juveniles), and maternities were estimated from a post-breeding census where juvenile females become pregnant in their first year. The popbio package calculates population growth rates (λ, finite rate of increase) as the dominant eigenvalue of the population matrix, with $\lambda > 1$ indicating annual population increases and $\lambda < 1$ indicating population decreases (Stubben and Milligan 2007). We constructed six stage-matrices to calculate estimates of population growth for the two species across the three recapture intervals: 2011 to 2012, 2012 to 2013, and 2013 to 2014.

Results

Ecological factors: White-nose syndrome, temperature and precipitation

All PCR test results for the presence of *P. destructans* were negative for both *M. lucifugus* and *E. fuscus*. Annual weather patterns varied greatly over the study period. The mean spring temperature (March-May) in 2012 was unusually warm (Table 3.1). Total spring precipitation (March-May) was much lower than average in 2012 and much higher than average in 2013 (Table 3.1). Roost temperatures varied predictably with ambient temperature, and were excluded from analyses (data not shown).

Vital rates and population growth rates

The proportion of reproducing females was high for *M. lucifugus* (0.81 - 0.96) and higher for *E. fuscus* (0.95 - 1.00) for all years from 2011-2014 (Fig. 3.2). The total proportion of *E. fuscus* reproducing over the four-year period was significantly higher than in *M. lucifugus* (*E. fuscus* mean = 0.98, *M. lucifugus* mean = 0.89; Fisher's exact test, $p < 0.001$, n = 505). The proportion of reproducing females was significantly lower in 2012 than in other years combined for both species (*M. lucifugus* 2012 = 0.81 vs. *M. lucifugus* mean for other years = 0.94, Fisher's exact test, $p = 0.011$, n = 282; *E. fuscus* 2012 = 0.95 vs. *E. fuscus* other years = 1.00, Fisher's exact test, $p = 0.021$, n = 223). Maternity estimates for *M. lucifugus* ranged from 0.41 in 2012 (presumed pre-WNS drought year) to 0.48 in 2014 (presumed post-WNS rainy year); estimates for *E. fuscus* ranged from 0.95 in 2012 to 1.00 (all other years).

The bootstrap goodness of fit test for *E. fuscus* indicated that our global model fit the data ($p = 0.67$, where p is probability of model's deviance given the data). Our data were underdispersed (c-hat = 0.82). There is no general consensus on use of a c-hat correction for underdispersed data so we did not apply a correction (Cooch and White 2014). *E. fuscus* models of apparent survival were not well resolved: six out of nine models were ranked as competitive (Table 3.2). The top model included only age as a predictor of survival (Table 3.2). This top model accounted for only 23% of total Akaike weights for all models, and was followed closely by our null model, which accounted for 17% of total Akaike (Table 3.2). The other four competitive models were as follows: (1) survival varied by year, with each year different (Akaike wt. = 0.15); (2) survival was higher in the drought year for juveniles only (Akaike wt. = 0.14); (3) survival was higher in the drought year for both adults and juveniles (Akaike wt. = 0.11); and (4) survival was higher in the post-WNS year (Akaike wt. = 0.10; Table 3.2). Model

averaged estimates of apparent survival indicated E. fuscus adult survival was between 0.66 – 0.70 with a mean of 0.69, and E. fuscus post-banding juvenile survival between 0.60 – 0.67 with a mean of 0.63 (Fig. 3.3). Based on the assumption that on average $\lambda \approx 1$, we estimated that the average offspring survival from maternal assessment to banding of juveniles is 0.5 for *E. fuscus*, giving an overall mean juvenile survival rate of 0.32.

When we created weighted averages of all models of *E. fuscus* survival, we found no difference *E. fuscus* adult survival ($\varphi_{A\ 2012\text{-}13} = 0.70$, $\varphi_{A\ 2013\text{-}14} = 0.70$; Fig. 3.3) and a small decrease in post-banding juvenile survival ($\varphi_{J\ 2012\text{-}13} = 0.67$, $\varphi_{J\ 2013\text{-}14} = 0.64$, Fig. 3.4) between 2012-2013 and 2013-2014, which followed years that were exceptionally dry and exceptionally rainy, respectively. These differences were well within the standard errors of the model estimates (Fig. 3.3), and AIC did not rank any of our models that included survival differences between years as high as the null and age-only models (Table 3.2). Using our estimates for survival and fecundity, population growth was lowest in 2011-2012 and highest in 2013-2014, but varied little from year-to-year for *E. fuscus* ($\lambda_{2011\text{-}12} = 0.960$, $\lambda_{2012\text{-}13} = 1.014$, $\lambda_{2013\text{-}14} = 1.020$; Table 3.1).

The bootstrap goodness of fit test for *M. lucifugus* indicated that our global model fit the data (p = 0. 26). Just as in our *E. fuscus* data set, these data were underdispersed (c-hat = 0.74) and we did not apply a correction. Captures of M. lucifugus were much lower in 2014 than in previous years. Our top-ranked model for M. lucifugus included an interaction between age and year group as predictors of survival and recapture probabilities, with equal survival probabilities for 2011-2012 and 2012-2013, and equal recapture probabilities for 2012-2013 and 2013-2014 (Akaike weight = 0.50; Table 3.3). Models that included differences in survival between years accounted for 100% of the Akaike weights (Table 3.3). Model-averaged estimates of apparent survival indicated *M. lucifugus* adult survival was approximately 0.80 in the first two years and

then declined to 0.36 in 2013-2014. *M. lucifugus* post-banding juvenile survival was approximately 0.67 in the first years but then declined to 0.18 in 2013-2014 (Fig. 3.4). Based on the assumption that on average $\lambda \approx 1$, we estimated that the average offspring survival from maternal assessment to banding of juveniles is 0.7 for *M. lucifugus*, giving an overall mean pre-WNS juvenile survival rate of 0.47, and an overall mean post-WNS juvenile survival rate of 0.13. Our estimates of *M. lucifugus* survival and fecundity for 2013-2014 yielded a presumed post-WNS population growth rate of 0.4440, which represents a sharp decline from our presumed pre-WNS population growth rate estimates ($\lambda_{2011\text{-}12} = 1.004$, $\lambda_{2012\text{-}13} = 0.993$; Table 4.1).

Discussion

Relationships between WNS, climate, and vital rates

The spring of 2013 was notable for its heavy rainfall, which was nearly double the historic average; the spring of 2012 was the hottest year on record, with exceptionally low precipitation (Table 3.1). We tested two predictions from previous studies of *M. lucifugus* and *E. fuscus*: (1) survival is positively correlated with precipitation; and (2) survival is positively correlated with temperature. Our models indicate that there was a slight increase in *E. fuscus* survival from the normal year (2011) to the drought year (2012), and virtually no change in survival from the drought year (2012) to the rainy year (2013). These annual differences were not sufficient to gain support for a year-based model (Table 3.2). Thus, our results do not support the prediction that *E. fuscus* survival decreases with decreased precipitation. This prediction was based on *E. fuscus* survival studies in Colorado (O'Shea et al. 2011), so the disparity in our results may reflect differences in the effect of drought that are dependent on regional climate

variation. Alternately, the relative constancy of *E. fuscus* survival that we observed across years may depict a tradeoff, where early birth dates in hot years promote higher winter survival rates by increasing the time available to store fat before hibernation, but the accompanying drought causes lower summer survival rates. The relative impact of these two potentially opposing forces could be clarified with additional studies of the effects of temperature on parturition dates, and the effect of parturition date on survival.

Our models indicate that there was a slight increase in *M. lucifugus* survival from the normal year (2011) to the drought year (2012), and a drastic drop in survival in the rainy year (2013). Previous studies of the effects of climate on *M. lucifugus* summer colonies found that survival increased with rainfall, and did not include survival rates nearly as low as ours for 2013-2014 in any of the 16 years of their study (Frick et al. 2010b). The severe declines we observed in *M. lucifugus* survival are consistent with population models of WNS (see discussion below).

We found that the proportion of reproducing females was significantly lower in the drought year for both species (Fig. 3.2). These results support previously published predictions that a drier, warmer climate will have a negative impact on reproduction in North American bats (Adams 2010). Our data indicate that the negative effects of drought on bat reproduction observed in the Rocky Mountain region also occur in the wetter Midwestern United States.

Species comparisons of vital rates

Bats represent anomalies in some respects of life-history theory because they are unusually long-lived and slow reproducing for their body size (Racey and Entwistle 2000). Even within the Order Chiroptera, some species' relationships buck typical life-history trends. The two sympatric vespertilionid species, *M. lucifugus* and *E. fuscus*, are an example of this: *M. lucifugus*

has smaller body mass, but lower litter sizes and longer maximum longevity relative to *E .fuscus* (Fenton and Barclay 1980; Kurta and Baker 1990). When studied in a common environment, we found that *M. lucifugus* has higher survival rates and lower maternity than *E. fuscus*. We expected lower fecundity for *M. lucifugus* because the litter size is smaller (Fenton and Barclay 1980; Kurta and Baker 1990). Our results support predictions from life-history theory that survival will be higher for species with lower fecundity. The difference in adult presumed pre-WNS survival between the two species is particularly strong ($\varphi_{A\ M\ lucifugus} = 0.79$, $\varphi_{A\ E\ fuscus} = 0.69$).

Regional comparison of *Myotis lucifugus* vital rates

Our survival estimates for *M. lucifugus* in western Illinois are higher than published estimates for *M. lucifugus* in New Hampshire ($\varphi_{j\ IL} = 0.46$ vs. $\varphi_{j\ NH} \approx 0.35$, $\varphi_{a\ IL} = 0.79$ vs. $\varphi_{a\ NH} \approx 0.73$; Frick et al. 2010b). When we compare our reproductive estimates to those from the New Hampshire study, our proportions of females reproducing are lower (IL mean = 0.89 vs. NH mean ≈ 0.95. Our mean proportion of reproducing females was nearly as low as the lowest rate in the 16-year New Hampshire study. This occurred in the last year of their study after WNS had been documented in the region, so they proposed that the drop in reproduction could have been a result of WNS despite a lack of survival declines that year (Frick et al. 2010b). Interestingly, we observed our lowest proportion of reproducing females the year before we observed drastic declines in survival rates that are typical of WNS. However, we also observed our lowest proportion of reproducing females for *E. fuscus* in that year with no subsequent decline in survival. This suggests to us that reduced reproduction in 2012 was related to the exceptionally hot, dry weather that both species experienced.

Regional comparison of *Eptesicus fuscus* vital rates

Our survival estimates for *E. fuscus* in western Illinois are lower ($\varphi_{j\ IL} = 0.32$ vs. $\varphi_{j\ CO} \approx 0.64$, $\varphi_{a\ IL} = 0.69$ vs. $\varphi_{a\ CO} \approx 0.79$) and maternity are higher ($M_{j\ IL} = 0.31$ vs. $M_{j\ CO} \approx 0.23$, $M_{a\ IL} = 0.67$ vs. $M_{a\ CO} \approx 0.42$) than published estimates for *E. fuscus* in the Rocky Mountain region of the United States (O'Shea et al. 2011). We expected maternity in Illinois to be higher because *E. fuscus* in this region typically have a litter size of two, whereas they typically have a litter size of one in the western portion of their range (Kurta and Baker 1990). When we compare our survival rates, it appears that juveniles in the eastern part of *E. fuscus*'s range experience the majority of the survival reduction. Our estimates provide a glimpse into the regional variation in vital rates for two common North American bat species, one of which is experiencing catastrophic declines from WNS. Our models for *E. fuscus* at our site did not find support for the hypothesis that they are also experiencing WNS-related declines. This could represent the species' resistance to the disease, or could occur because the *E. fuscus* colony at our site spends the winter at a different hibernaculum that does not yet have *P. destructans* present. This could be resolved with additional years of recapture and fecundity data at the site as WNS establishes itself in the area.

WNS and population growth rates

We did not detect the causative agent of WNS, *P. destructans*, in any of the Siloam Springs State Park samples we PCR tested from 2011-2014. Bats that survive winter infection with *P. destructans* appear to mount an immune response during the spring active season to clear infections (Meteyer et al. 2011). It is therefore possible that some of the bats in these two colonies could have been infected over winter, survived and cleared themselves of the fungal infection prior to PCR testing. We do not know where these colonies hibernate, so we have no

direct evidence of *P. destructans* exposure, but there are several hibernacula within 80 km of our site that tested positive for *P. destructans* or had confirmed cases of WNS, including one hibernaculum in Marion County, Missouri that tested positive for *P. destructans* in winter 2013-2014 and is less than 30 miles from our site (Coleman and Reichard 2014). While we have no evidence that WNS affected either of these populations during the study period from 2011-2014, we cannot exclude this possibility. Indeed this seems likely for the *M. lucifugus* colony, which showed sharp declines in apparent survival from 2013 to 2014 (Fig. 3.4). We know of no other reasonable explanation for this drastic decline outside of WNS.

Our models for *M. lucifugus* estimated a 54% decrease in survival for adult females and a 72% decrease in juvenile survival in the presumed post-WNS years. When we applied these estimates to population models, we saw a rapid decline in population growth between the presumed pre-WNS years and the presumed post-WNS year. It is important to recall that we deliberately set mean $\lambda = 1$ in the presumed pre-WNS years, based on the assumption that on average, the population had been experiencing neither net population losses nor population gains prior to the emergence of WNS. If the population had been experiencing net growth ($\lambda > 1$) prior to WNS, then our presumed post-WNS rate is an underestimate; if it had been experiencing a net decline ($\lambda < 1$), then our presumed post-WNS rate is an overestimate. Although a growth rate of 0.44 seems dismal, it is higher than published first-year post-WNS estimates of population growth at WNS-affected hibernacula (all first-year post-WNS $\lambda < 0.4$ with an average $\lambda \approx 0.15$; Frick et al. 2010a).

Conclusion

This study contributes to our general understanding of regional variation in vital rates and population growth rates by comparing two common North American bat species, *M. lucifugus* and *E. fuscus*, in a shared environment. It also yields mark-recapture-based estimates of changes in these rates as WNS enters the landscape at summer breeding colonies, which complement our understanding of the impact of WNS based on count-data at hibernacula. In the presumed post-WNS year, we observed greater survival reductions for *M. lucifugus* juveniles than for adults (Fig. 3.4) and no reductions in reproduction (Fig. 3.2). Additional years of field data at this site would clarify whether these trends are typical, and whether rates of decline persist at our estimate of 0.41, or if losses ameliorate as predicted in the model based on counts and banding studies at hibernacula (Frick et al. 2010a, Maslo et al. 2015). Continued studies would also allow us to assess whether the lack of *E. fuscus* declines that we observed were due to their resistance to WNS, or simply because this colony had not yet been exposed by the last year of our study.

Bat population ecology studies are hampered by the lack of published data on rates of miscarriage and neonatal mortality. We derived estimates of average offspring survival rates from assessment of pregnancy to banding for our *M. lucifugus* and *E. fuscus* maternal colonies based on estimates of fecundity and juvenile survival for both species and the assumption that mean $\lambda \approx 1$ prior to signs of effects of WNS. Our estimates of pre-banding juvenile survival rates of *M. lucifugus* and *E. fuscus*, respectively are 0.7 and 0.5. We observed significantly lower pregnancy rates for both *M. lucifugus* and *E. fuscus* in 2012, which was both a drought year and the hottest year on record (Fig. 3.2). Our modeling did not support predictions that survival was different in 2012 (neither the prediction that survival would be higher because births occur earlier in warmer years, nor the prediction that survival would be lower in drought years because

of lack of water and lower insect abundance; Tables 3.2 and 3.3). Previous work demonstrated that *M. lucifugus* juveniles that were born earlier had better survival rates (Frick et al. 2010b). Specific studies of the effects of spring temperature on parturition date and the impact of parturition date on *M. lucifugus* adult females and *E. fuscus* juvenile females would clarify how universal this effect is.

CHAPTER 4: RELATIONSHIP BETWEEN MATERNAL SURVIVAL AND TIMING OF PARTURITION IN *MYOTIS LUCIFUGUS*

Abstract

The little brown bat (*Myotis lucifugus*) faces possible extinction due to white-nose syndrome. Like other animals with slow life histories, *M. lucifugus* population growth is strongly affected by adult female survival. Unfortunately, we know very little about the factors that impact adult female survival in this species. Previous studies indicate that juvenile survival is lower for pups born later in the season, presumably because they have less time to store fat before hibernation. Studies of other vespertilionid bat species indicate females give birth earlier when temperatures are warmer. We predicted this would also be true for *Myotis lucifugus*. We hypothesized that mothers who give birth later would also have lower survival, and that the effect would be smaller in years with a longer active season. To test this hypothesis, we conducted a capture-recapture study at an *M. lucifugus* maternal colony from 2011 to 2014. We estimated parturition dates, banded and recaptured females, obtained temperature data for the active season each year. We used ANOVA to test the prediction that parturition dates were earlier in warmer years, and program MARK to model annual maternal survival, ranking models using Akaike Information Criterion. Our results yielded two top models: an intercept-only model, and a model in which parturition date was the sole predictor of maternal survival. The median parturition date was 10 days earlier in the warm year. Parturition does occur earlier in warmer years for *M. lucifugus*, concurring with studies of other hibernating bat species. We found no evidence that this results in higher maternal survival rates.

Introduction

The little brown myotis (*Myotis lucifugus*) has historically been one of the most common, widespread bat species in North America, but is projected to go extinct within the eastern half of its range by 2026 due to the newly emergent wildlife disease, white-nose syndrome (WNS)(Frick et al. 2010a). In the northeastern United States where the disease was first documented, field biologists are reporting early signs that some bat populations may be stabilizing, although this occurs after population reductions that often exceed 90% (Turner et al. 2011; Reichard et al. 2014). Currently there are no treatments available. Until an effective treatment for WNS is found, it will be critical to determine the non-WNS factors that have a large impact on bat population growth. Although this information is unlikely to prevent WNS-related bat population declines, it may provide critical information needed to develop management strategies that decrease the risk of extinction of populations that have stabilized in the aftermath of WNS.

Bats are long-lived mammals with slow reproductive rates (Wilkinson and South 2002). The species *M. lucifugus* has a maximum recorded lifespan of 34 years and produces only one offspring per year (Racey and Entwistle 2000). In mammal species with slow life-histories, population growth rates are typically most heavily impacted by adult female survival (Caswell 2001). Therefore, the best way to counteract WNS-related bat population declines is to identify factors other than WNS that impact adult female survival rates and develop wildlife management strategies that target these factors.

The annual cycles of adult female temperate zone bats are highly temporally constrained. In cooler climates within North America, bats hibernate more than half the year (Racey and Entwistle 2000). This leaves roughly five months for females to return to their summer colonies, successfully complete pregnancy, nursing, and weaning of a pup, then replenish fat stores before

returning to hibernacula in the fall (Racey 1982). Pregnancy in *M. lucifugus* females usually lasts about two months, but this schedule is variable (Fenton and Barclay 1980). Pregnant females will use daily torpor to conserve energy when temperatures are low, health is poor, or weather prohibits foraging (Racey and Entwistle 2000). Development arrests when the mother uses torpor, leading to longer gestation times (Racey 1973). Bat pups that are born later in the season can have lower survival in their first winter, presumably because they had less time to store fat before hibernation (Frick et al. 2010b). Thus, we could potentially increase survival of juveniles over their first winter by providing their mothers with warmer roosts during spring pregnancy. Unfortunately, juvenile survival has a relatively small impact on overall population growth (Caswell 2001), so if warmer roosts only increased juvenile survival without affecting maternal survival, the positive effects of warmer roosts might not be worth the effort (Table 4.1). However, if mothers that give birth earlier in the season also have higher survival in the following winter because they also benefit from extra time post-weaning to replenish fat stores, then this strategy could have a substantial impact on population growth (Table 4.1). Alternately, if mothers that give birth earlier have lower annual survival rates due to negative effects of being active during inclement spring weather, the overall effect on population growth would be negative, because the positive effects on juvenile survival would have less impact on population growth than the negative effects on maternal survival (Table 4.1). Clearly, we need a greater understanding of the effects of parturition date on maternal survival prior to experimentation with alteration of maternal roosts.

We collected field data that allowed us to estimate individual parturition dates and annual survival rates at a *M. lucifugus* maternal colony. With these data, we can determine whether annual maternal survival correlates with parturition date, and if so, whether the relationship is

negative or positive. We hypothesized that mothers that gave birth later should be subject to the same effects of late birth dates as their offspring, namely reduced time to build energy reserves for subsequent hibernation and consequently lower survival rates through the following winter. The active season (i.e. annual period when bats are not hibernating) is extended in warmer years, potentially providing bats with more nights to forage for insects and store fat prior to entering hibernation. Additionally, warmer temperatures can result in earlier parturition for other hibernating bats species (Racey 1973; Racey and Swift 1981; Ransome and McOwat 1994). We predicted that *M. lucifugus* females would give birth earlier on average during warmer years. Additionally, we tested two predictions of our hypotheses about the effects of late parturition: (1) survival decreases for *M. lucifugus* adult females that give birth later in the active season; and (2) the negative effect of late parturition on adult female survival is weaker (less negative) in warmer years.

Methods

Data Collection

We conducted a four-year study of a *M. lucifugus* maternal colony in west central Illinois (Adams County; 39.89 N, 90.94 W) from 2011-2014. Throughout spring and summer, we mist-netted bats directly outside their roost, wing-banded them, and assessed them for sex, age, and reproductive condition. To assess reproductive condition, we used nipple morphology to determine if a female was reproductive (pregnant, lactating or post-lactating) or non-reproductive, then palpated the abdomen for the presence of a fetus (Racey et al. 2009). Reproductive females with a discernible fetus were assumed to be in the last three-quarters of pregnancy. Reproductive females without a discernible fetus were assumed to be either in the

first quarter of pregnancy, or to have already given birth. We estimated gestation date for reproductive females with a discernible fetus based on measurements of the mother's abdomen at the widest width. These measurements were compared to abdominal measurements and associated stages of fetal development in gravid *M. lucifugus* females that had been collected for histological examination for *P. destructans* testing by Illinois Department of Natural Resources biologists in 2010. Females with nipple morphology indicating pregnancy but no discernible fetus were assumed to be 60-45 days from parturition. Females with nipple morphology indicating pregnancy and abdominal diameters less than 2.5 cm were estimated to be 45-30 days from parturition, those with diameters from 2.5 to 3.0 cm were estimated to be 30-15 days from parturition, and those with diameters ≥ 3.0 cm were estimated to be less than 15 days from parturition.

We obtained annual climate data from the National Climate Data Center for Quincy Baldwin Regional Airport, a weather station located near our field site, to get estimates of mean temperature during the active season (April-September) for each year that we collected parturition date data and also the historical average (NOAA 2014). The hottest year on record at our site was 2012, while 2011 and 2013 were nearer to average temperature. We expected that females would give birth earlier on average in the warm years based on previously published data (Racey 1973; Racey and Swift 1981).

Data Analysis

We used estimates of the juvenile's dates of birth to determine the first date of birth in a given year. Juveniles' dates of birth were estimated from metacarpal epiphyseal gap lengths, which yield birth date estimates with a margin of error of only a few days (Kunz et al. 2009).

When mother-pup pairs were observed, we used the pups' estimated date of birth as the mother's parturition date. We combined the reproductive condition data for recaptured females on each capture date to estimate parturition date. We excluded females if the parturition date estimates from all recapture events varied by more than 7 days, and also if data were insufficient to approximate parturition date (i.e. if there were no recaptures during a single breeding season, or if all captures occurred post-lactation). The parturition dates estimated by these methods do not give exact calendar dates, but do yield a small range of possible dates that consistently distinguish individuals that gave birth earlier from those that gave birth later, allowing comparison of the survival probabilities of individuals based on their parturition date relative to other individuals sampled. Thus, our models represent the relative parturition dates between individuals, and are not simply a function of the date an individual was captured.

We clustered parturition date data into two annual temperature groups: "average", which included 2011 and 2013; and "hot", which included 2012. We tested our prediction that parturition events would occur earlier on average in warmer years using a Mann-Whitney-Wilcoxon test ($\alpha = 0.05$) in R Statistical Environment (R Development Core Team 2015). We used program MARK to estimate apparent annual survival of female *M. lucifugus* that reproduced in 2011 (typical weather year at our site), 2012 (hottest year on record at our site), and 2013 (also a typical temperature year) (White and Burnham 1999; NOAA 2014). We constructed generalized linear models of apparent maternal survival then chose the best model using Akaike Information Criterion (AICc). We constructed a total of six models of apparent maternal survival. These include the intercept-only model, a parturition date model, a year-only model, a model that grouped average temperature years (2011 and 2013) in a separate category from the warm year (2012), a model in which parturition date and year interact, and a model

where parturition date and annual temperature category interacts. We considered any model with a $\Delta AIC < 2$ to be competitive (Hobbs and Hilborn 2006). For competitive models, we used the following criteria to interpret our results. Our first hypothesis is supported if the top AIC model included parturition date with a negative relationship between apparent survival parturition date (i.e. survival decreases for females that give birth later in the season). If the top model also includes an interaction between parturition date and year, and the relationship between parturition date and survival in the average year was more negative than in the average year, that would support both hypotheses (i.e. survival decreases for females that give birth later in the season, and the negative effect of giving birth late would be weaker in the warm year).

Results

The historical average temperature for the active season (April-September) at Quincy-Baldwin Regional Airport was 17.9 C. Active season temperatures were: 18.3 C in 2011, 20.3 in 2012, and 17.3 C in 2013. Our study included data on the reproductive phenology and apparent survival of 175 *M. lucifugus* females at a site in west central Illinois from 2011-2014. Our data set included estimates of parturition dates and recapture events for a total of 175 reproductive females, including 71 from the hot year and 104 from average temperature years. The median parturition date was earlier in the hot year than in the average years (06 June vs. 16 June; $W = 5452.5$, $p < 0.001$; Fig. 4.1).

There were two competitive models to predict apparent maternal survival (Table 4.2) with the intercept-only model ranking highest, followed by the model that included parturition date as its only predictor of apparent maternal survival (Table 4.2). The models are essentially ranked by number of parameters because deviance varied little between models (Table 4.2).

Thus, our results do not support our hypotheses that (1) maternal survival decreases for mothers with later parturition dates; and (2) the effect is less negative in warmer years. In this model, apparent maternal survival decreased from 0.84 on May 20th to 0.60 on July 9th (Fig. 4.2). The 95% confidence intervals at the beginning and end of the season are wide, demonstrating the uncertainty of the survival estimates that resulted in the ambiguous AIC rankings (Fig. 4.2).

Discussion

Our survival analysis does not support the hypothesis that *M. lucifugus* mothers giving birth earlier are more likely to survive to return the next year (Table 4.2). Parturition events were less numerous at the both beginning (May) and the end (July) of the reproductive period (Fig. 4.1), resulting in more uncertainty in the estimates of maternal survival at the extreme ends of the model (Fig. 4.2). Additional years of data would potentially provide narrower confidence intervals at each end of the season. Unfortunately, *M. lucifugus* abundance at the site began dropping precipitously in 2014 (Powers dissertation chapter 2). This is likely due to WNS, which was detected at hibernacula in three counties in Missouri that are within 50 miles of our site between winter 2009-2010 and winter 2013-2014 (Coleman and Reichard 2014). The total number of adult females (including non-reproductive females) captured dropped from 117 in 2013, to 46 in 2014 (Powers, unpublished data). Thus, we are unlikely to obtain the quantity of data needed for this species at this site.

Parturition did occur earlier in the hot year (2012) as predicted based on previously published studies (Fig. 4.1) (Racey 1973; Racey and Swift 1981; Ransome and McOwat 1994). The median parturition date in the warm year (2012) was 10 days earlier than the median parturition date in the average temperature years (2011 and 2013 – Fig. 4.1). The earliest births

also appear earlier in the season in the warm year: 21 May in the hot year; 29 May in the average years (Fig. 4.1). The year 2012 was the hottest on record for this site, so our distribution gives a rough approximation of the earliest possible births in the region (Adams County, Illinois; 39.89 N, 90.94 W). The latest births in 2012 were only 4 days earlier (04 July vs. 08 July), and the spread between the first and third quartiles is greater, indicating a wider distribution of parturition dates in the hot year (Fig. 4.1).

It is interesting that we observed a greater range in parturition dates in 2012. Previous studies of *M. lucifugus* maternal colonies have found that the first birth events occurred earlier and the period during which births occur lasted longer at lower latitudes (Fenton 1970; Humphrey and Cope 1976; Schowalter et al. 1979). In one respect, our observations are consistent with these studies because they found greater variance in parturition dates in lower latitude populations where temperatures are warmer, and observed greater variance in warmer years in our population. However, distributions of parturition dates in a population are presumably the result of selective pressure. It follows that populations at different latitudes have adapted to the regional climate such that lower latitudes allow births to occur later without fitness costs for mothers and their offspring. If a population is able to adjust both the median parturition date and the variance in response to annual climate variation, this suggests that environment has a stronger influence on reproductive phenology in *M. lucifugus* than heritability.

M. lucifugus populations will be forever changed by the impact of WNS. Wildlife managers, whether on the leading edge of the disease where they attempt to slow population declines or near the epicenter where they attempt to preserve remnant populations, face the massive challenge of preserving these populations. It would be extremely helpful to know if early parturition does increase maternal survival for *M. lucifugus* and other hibernating bat

species that are facing population declines, so conservationists could begin to develop strategies that fostered timely parturition. Although we no longer have the opportunity to conduct mark-recapture studies on large numbers of *M. lucifugus* at this site, we could repeat the study using another cave-hibernating species as a proxy. Although the specific values of vital rates vary between species (and indeed between regional populations, Powers dissertation chapter 2), general trends appear to hold among cave-hibernating species in how life-history characteristics respond to environmentally induced changes (Powers dissertation chapter 2; Ransome and McOwat 1994; Racey and Entwistle 2000; Frick et al. 2010b). We recommend additional studies of this kind.

CHAPTER 5: THE EFFECT OF *PSEUDOGYMNOASCUS DESTRUCTANS* INFECTION ON *MYOTIS LUCIFUGUS* FEMALE FERTILITY DURING HIBERNATION

Abstract

White-nose syndrome (WNS) is a disease caused by a cold-adapted fungus, *Pseudogymnoascus destructans* (*Pd*), which results in devastating population declines of North American cave-hibernating bats. Rates of decline at most sites are estimated by annual counts, so it is uncertain whether declines are due entirely to increased mortality or if reduced fecundity also occurs. Female little brown bats (*Myotis lucifugus*) store sperm and a single mature vesicular follicle (MVF, or Graafian follicle) throughout hibernation, and will not produce any offspring if storage fails before spring emergence. Bats with WNS become emaciated and dehydrated, which could compromise their ability to maintain the stored follicle and sperm. We conducted a histological study of reproductive tissues from 58 hibernating female *M. lucifugus*, including both *Pd*-positive and *Pd*-negative individuals, to determine if *Pd*-positive females would be less likely than *Pd*-negative females to maintain a MVF and stored sperm. We then modeled population growth assuming a 20% reduction in survival using published estimates of survival and fecundity, and estimates of fertility from our results. These estimates were used to model the effect of WNS-related fertility reduction on *M. lucifugus* population growth rates and time to extinction. All females in both groups had stored sperm. We found no significant difference in MVF presence (*Pd*-negative = 96%, *Pd*-positive = 92%). Our models of post-WNS population declines rates ranged from 0.658 to 0.636 assuming a 0% to 17% reduction in fertility, respectively. All of our models, which included a 20% reduction in survival and 0 – 17% reductions in fertility, projected quasi-extinction (reduction to 1% of original population size) of the population after 11 years. Notably, we observed neutrophils in the reproductive

tissues of all females from both groups. This was unexpected given the characteristic lack of neutrophilic response in hibernating Pd-infected bats. We conclude that *Pd* infection does not reduce fertility in hibernating female *M. lucifugus*, and that small fertility reductions (≤ 17%) are unlikely to affect population viability.

Introduction

White-nose syndrome is an emerging infectious wildlife disease that has caused precipitous population declines in several species of cave-hibernating bats, including the once common little brown myotis (*Myotis lucifugus*), in the northeastern United States and southeastern Canada (Frick et al. 2010a; Blehert 2012). The causative agent is a cold-adapted fungal pathogen, *Pseudogymnoascus destructans* (*Pd*), that infects the nose, muzzle, ears and wing membranes of torpid bats during winter, when both cave temperatures and bats' body temperatures are maintained at low temperatures that are ideal for proliferation of the fungus (Lorch et al. 2011) The etiology of the disease is not yet fully understood, but involves emaciation, rapid depletion of fat stores, degradation of wing tissue and frequent arousal from torpor (Blehert et al. 2009; Meteyer et al. 2012; Reeder et al. 2012; Verant et al. 2014). *Pd* infection often results in death of infected bats, as evidenced by observations both in the field and in clinical trials (Frick et al. 2010a; Turner et al. 2011; Johnson et al. 2014). However, the effect of *Pd* infection on reproductive function for individuals that survive remains unknown.

The unique reproductive cycle of female temperate zone bats may leave them vulnerable to reduced fertility if physiologically stressed during hibernation (Racey and Entwistle 2000). Hibernating bats such as *M. lucifugus* have a highly temporally-constrained annual reproductive cycle because the climate only allows them to be euthermic and maintain an active metabolism

for about 5 months per year (Fenton and Barclay 1980). During this period, pregnancy for *M. lucifugus* lasts about two months followed by about one month of nursing (Kurta et al. 1989). If fertilization occurs at the start of the active season, only about two months remain at the end of the season for mothers and offspring to store the fat needed before migration to the hibernation site. This timeline is complicated by male reproductive phenology: males devote their active-season energy budgets to spermatogenesis, making them prepared for mating in autumn at the start of hibernation rather than at spring emergence from hibernation (Racey 1982). The bat's female reproductive system has a remarkable adaptation to address this asynchrony: sperm from the male is stored at the uterotubal junction (the union of the oviduct and uterine horn) after mating in autumn, the ovary produces a mature vesicular follicle (MVF – a.k.a. Graafian follicle) in autumn and suspends its development for roughly seven months, when ovulation and fertilization will finally occur at spring emergence (Wimsatt and Kallen 1957)**.**

This ability to postpone fertilization is highly unusual among mammals, and reproductive biologists have long puzzled over the physiological mechanisms that make this possible (Guthrie 1933; Wimsatt and Kallen 1957; Wimsatt et al. 1966; Crichton 2000; Rasweiler and Badwaik 2000). One would expect that a system that pushes the limits of reproductive function so far from the norm could be highly vulnerable to any unusual physiological stress during the hibernation period. There is a tradeoff between survival and reproduction for female bats faced with increased metabolic energy demands during the hibernation period (Jonasson and Willis 2011). *Pd* infection causes severe physiological stress to hibernating bats, including hypovolaemia (reduced blood plasma volume), extracellular electrolyte depletion and respiratory acidosis, which results in frequent arousal from torpor and often eventually leads to death before spring emergence (Reeder et al. 2012; Warnecke et al. 2013; Verant et al. 2014). If any one of these

conditions affects the physiological processes that support sperm storage and the ability to maintain a suspended MVF in the ovary, then *Pd* infection will also result in infertility in hibernating females, rendering them incapable of reproduction for the entire year. From a conservation perspective, this represents a critical knowledge gap in our understanding of the impact of WNS on bat populations. If *Pd* infection does reduce bat fertility, population ecologists need estimates of the extent of this reduction to produce accurate population growth models, and reproductive biologists need to determine the point during the bat's reproductive cycle when fertility is reduced so they can make appropriate recommendations to wildlife managers. Once estimates of fertility rates in *Pd*-infected bats are available, these can be incorporated in fecundity estimates in post-WNS population matrices, which can be used to conduct population viability and sensitivity analyses to determine whether efforts to improve fertility will help ameliorate extinction (Maslo et al. 2015).

The study of the effects of *Pd* infection on female fertility presents WNS researchers with a moral dilemma. In order to definitely determine that a hibernating female bat has stored sperm and an MVF, the researcher must euthanize the bat and promptly preserve the organs for histological analysis before tissue degradation occurs. From a conservation perspective, this is problematic: bats have slow life-histories, and survival of reproductive adult females is typically the largest driver of population growth in animals with slow life-histories (Caswell 2001). Of course, conservationists stand no chance of stopping disease-induced population declines without controlled studies of disease dynamics that necessarily involve the afflicted species. However, researchers have an obligation to choose studies carefully, weighing the positive impact that the study is likely to contribute to our understanding of the disease against the negative impact of removing study subjects from the population (Reeder et al. 2016). In the case of studying the

effects of *Pd* infection on *M. lucifugus* female fertility, details of the species' life-history argue against terminating animals specifically to answer this question. We addressed this dilemma by: (1) limiting our histological study to reproductive tissues that had been collected and preserved at the end of other studies of the effects of WNS on survival; and (2) running basic population growth models to determine how much the observed reduction in fertility would impact population trajectories and time to extinction.

We conducted a histological analysis to test the hypotheses that *Pd* infected female *M. lucifugus* were less likely to maintain an MVF and stored sperm during hibernation. We simulated population growth using published data of *M. lucifugus* vital rates with and without our observed reduction in fertility, and estimated time to quasi-extinction to determine how much impact the observed fertility reduction would have on population dynamics. Previous WNS histological studies have reported no pathology of the following internal organs: heart, lungs, intestinal tract, liver, kidney (Blehert et al. 2009; Lorch et al. 2011). There are currently no publications specifically describing histological examination of reproductive organs in *Pd* infected bats, so we examined histological preparations of reproductive tracts of all individuals to identify any possible conditions associated with *Pd* infection.

Methods

Histological analysis

We collected whole female reproductive tracts from carcasses of 58 *M. lucifugus* females that had been previously preserved in either 10% neutral buffered formalin or 100% ethanol. Females were collected during the hibernation period and were histologically tested for *Pd* infection (Meteyer et al. 2009). All animal tissues used in this study were obtained following

previous WNS studies that had been conducted at Bucknell University in accordance with their ethical guidelines for animal research. We obtained tissues that were collected at the end of two types of studies that were combined for our analysis: (1) field studies where bats were collected from multiple hibernacula in Pennsylvania and Illinois between 8 February and 10 March 2011; and (2) a captive trial from 2011-2012 that included *Pd*-inoculated and uninoculated (control) females that were terminated at 3 weeks, 7 weeks, 11 weeks, and 15 weeks post-inoculation. Mating begins in autumn and continues into early winter for cave-hibernating bats (Guthrie 1933). Captive bats were collected from hibernacula in winter, and therefore were likely to have mated prior to entering captivity.

We processed whole female reproductive tracts (ovaries, oviducts, uteri, cervices and anterior portions of vaginae) using a Leica ASP 300 automated paraffin processor (Leica Microsystems, Nussloch, Germany), embedded them in paraffin using a Leica EG 1150H embedding station, and cut them into 6 µm thick serial sections using a Leica RM 2255 rotary microtome. We stained slides with hematoxalin and eosin. We created high-resolution digital images of the slides using a NanoZoomer Digital Slide Scanner (Hamamatsu Photonics, Hamamatsu, Japan), then checked for the presence of MVFs and stored sperm using NDPI software (Hamamatsu Photonics, Hamamatsu, Japan).

Previous studies prior to the emergence of WNS have shown that nearly all hibernating female *M. lucifugus* have an MVF and stored sperm present throughout hibernation (Guthrie and Jeffers 1938; Wimsatt and Kallen 1957; Buchanan 1987). We conducted one-sided Fisher's Exact tests (significance at $p < 0.05$) to determine whether *Pd*-positive females were less likely than *Pd*-negative to have: (1) sperm stored in uterotubal junctions (Fig. 5.1A), and (2) MVFs present in one of their ovaries (Fig 5.1B). We examined sections of the entire reproductive tract

(ovaries, oviducts, uteri, cervices and vaginae) of all individuals to determine if there were any consistent differences between *Pd*-negative and *Pd*-positive females. This survey of tissues included the hematoxalin and eosin slides we had prepared to search for stored sperm and MVFs, and additional periodic acid Schiff-stained slides that make *Pd* conidia visible if present (Meteyer et al. 2009).

Population modeling

The current model of post-WNS population declines holds fertility constant due to lack of information on the effects of *Pd* infection on fertility and fecundity (Frick et al. 2010a). We replicated a simplified version of the published model that estimated a 20% reduction in survival, including our observed reduction in fertility to estimate the overall effect on population growth trajectories. We modeled population growth (λ, where $\lambda = 1$ represents a stationary population) of a hypothetical population of 1,000 *M. lucifugus* experiencing a 20% reduction in survival over 50 years with demographic stochasticity and 500 replicates. We used a two-stage Lefkovich matrix and published estimates of *M. lucifugus* adult survival, juvenile survival, adult breeding probability, juvenile breeding probability, adult fertility and juvenile fertility (Fig. 5.2, Table 5.1) using the popbio package in R (Stubben and Milligan 2007; Frick et al. 2010a). We constructed a total of three models to demonstrate the impact of *Pd*-related fertility reductions on overall population growth: no reduction in fertility, our observed reduction in fertility, and the maximum reduction in fertility within our observed 95% confidence interval. We estimated time to quasi-extinction under each of these three scenarios, defining quasi-extinction as 10 or fewer individuals (1% of the initial population) using the popbio package in R (Stubben and Milligan

2007), to determine how much sooner extinction would occur in a WNS-affected population if female fertility is also affected.

Results

Histological analysis

We collected preserved reproductive tracts from a total of 58 *M. lucifugus* hibernating females. Seven females were excluded due to tissue degradation that made them unsuitable for histological analysis. All females had sperm stored at the uterotubal junction. An MVF was present in 22 of the 24 *Pd*-positive females and 26 of the 27 *Pd*-negative females. This represents a 4% reduction in fertility for female *M. lucifugus* with *Pd* infection, which was not statistically significant according to a one-sided Fisher's exact test ($p=0.455$, 95% CI = 0.172).

We did not observe any *Pd* conidia in any portion of the reproductive tract in any individual in this study. Additionally, we did not observe any histological conditions that were unique to *Pd*-positive individuals. A few females in each group had broken layers of stratum corneum associated with mucus secretions in the vaginal epithelium. All females in both groups had neutrophils present in uterine lumen (Fig. 5.3 A, gray arrow) and sometimes also in the superficial layers of the endometrium (Fig. 5.3 A, white arrow), uterotubal junction (Fig. 5.3 B, white arrow), and cervix (Fig. 5.3 C, white arrow), where they were frequently found engulfing sperm (Fig. 5.3 A-C).

Population modeling

We modeled a hypothetical *M. lucifugus* population experiencing a 20% decline in survival due to WNS under three scenarios that reflect the range of possible effects of *Pd*

infection on female fertility with: (1) no effect on fertility; (2) a 4% decrease in female fertility; and (3) a 17% decrease in female fertility (Table 5.1). Model 1 represents the null hypothesis, which is supported by the results of the Fisher's exact test. Model 2 represents our observed 4% decrease in fertility for *Pd*-positive females, although this difference was not great enough to be statistically significant. Model 3 represents the maximum decrease in fertility that falls within the 95% confidence interval of the binomial probability of our sample. Population growth rates varied little under these three scenarios. For model 1 (0% fertility reduction) $\lambda = 0.658$; for model 2 (4% fertility reduction) $\lambda = 0.652$; and for model 3 (17% fertility reduction) $\lambda = 0.636$. Estimated time to extinction was 11 years for all three models (Table 5.1, Fig. 5.4).

Discussion

We did not find a reduction in fertility for hibernating female *M. lucifugus* that were infected with *Pd*. Our study is the first to examine winter fertility rates, and our results are similar to WNS-related estimates of reproductive rates from previous studies in the field and in captivity. Frick and colleagues (2010b) conducted a 16-year study of a *M. lucifugus* summer maternal colony and observed a pregnancy rate that was 8% lower than the mean in the final year of the study after WNS arrived in the area (0.87 vs. 16-year mean = 0.95). Their 16-year mean pregnancy rate is similar to our observed fertility rate for *Pd*-negative females (0.95 vs. 0.96, respectively), but their observed rate in the post-WNS year is lower than in our *Pd*-positive groups (0.87 vs. 0.92, respectively). Meierhofer and colleagues measured reproductive success of *Pd*-infected and uninfected *M. lucifugus* females that had been kept captive for roughly five months of hibernation followed by six weeks in euthermic conditions (M.D. Meierhofer and D.M. Reeder pers. comm.). Defining reproductive success as survival of the offspring to six

weeks post-partum, they found that reproductive success was about 12% lower in *Pd*-infected females, but was not statistically significant. It is important to recognize that these studies represent different time frames in the reproductive cycle: ours was conducted late in hibernation before females have ovulated, Frick's was conducted in the spring after females had conceived and returned to their maternal colonies (Frick et al. 2010b), and Meierhofer's in summer after parturition had occurred (Meierhofer and Reeder pers. comm.). Bearing this in mind, comparison of these results suggests that the vast majority of females who maintain sperm and MVF throughout hibernation will conceive and maintain pregnancy into spring (i.e., our *Pd*-negative winter fertility ≈ 0.96 vs. Frick's spring pregnancy rate ≈ 0.95). It also suggests that *Pd*-infected females that survive hibernation may have difficulty conceiving and maintaining pregnancy during spring emergence and migration to maternal colonies (i.e., *Pd*-positive winter fertility ≈ 0.92 vs. presumed *Pd*-positive spring fertility ≈ 0.87). This is not unexpected given documentation of the extreme physiological stress that some *Pd*-positive bats experience when they emerge from hibernation and mount an immune response to *Pd*-infection (Meteyer et al. 2012).

There are striking differences in fertility rates and reproductive success rates across the three studies (in Frick's data and ours, fertility ranges from 87 – 96%; in Meierhofer's data, reproductive success ranges from 31 – 43%). There is a lack of published data on weaning success in vespertilionid bat populations (Barclay et al. 2004), so it is difficult to determine how much of this disparity is due to typical juvenile mortality between parturition and weaning, and how much is due to effects of captivity. However, field studies at pre-WNS *M. lucifugus* maternal colonies have reported capturing nearly half as many juvenile females as adult females, which is expected assuming 50:50 sex ratios of single offspring and nearly 100% of reproductive

females completing parturition (Humphrey and Cope 1976; Frick et al. 2010b). These studies have also found relatively low survival rates for juveniles over their first winter (Humphrey and Cope 1976; Frick et al. 2010b). Thus, the combined birth rates and winter survival rates observed in the field suggest that juvenile survival between birth and weaning is greater in the field than Meierhofer's group observed in captivity. Overall, the results of our study of reproductive function in *Pd*-infected female *M. lucifugus* was similar to that of Meierhofer and colleagues: we found a 4% reduction in winter fertility that was not statistically significant; they found a 12% reduction in summer reproductive success that was not significant. Although we found only a small, insignificant difference between infected and uninfected females, we cannot rule out the possibility that a decrease in winter fertility occurs later in hibernation than the time period covered in our study. When we combine our winter data, Frick and colleagues' spring data, and Meierhofer and colleagues' summer data, we have raw data that hint at a possible gradual decline in reproductive function for *Pd*-infected females from 4% in winter after mating, to 8% during pregnancy in spring, to 12% in summer when offspring are weaned. However, there are several reasons to caution against making these inferences: these studies compare results across captive and wild populations; Frick's post-WNS estimate is from a colony in a WNS-affected area, but individuals were not confirmed *Pd*-positive; and results for our study and for Meierhofer's group were not statistically significant. Additional field and captive studies of the reproductive rates of *Pd*-infected bats would greatly benefit our understanding of the impact of WNS on reproductive function throughout the year.

M. lucifugus female winter fertility was not significantly different between the *Pd*-positive group and the *Pd*-negative group. The lower boundary of the 95% confidence interval for these results represents a 17% reduction in fertility. We constructed models of WNS-related

population declines that assumed a 20% decrease in annual survival rates, and the range of possible fertility reductions according to our fertility study results (Table 5.1). Overall, we found relatively modest changes in population growth with these reductions in fertility (Fig. 5.4). Population growth rates dropped from a rapidly declining rate of $\lambda = 0.658$ with no decrease in fertility (model 1); to a nearly identical rate of $\lambda = 0.652$ with a 4% fertility reduction (model 2); and finally to a rate of $\lambda = 0.636$ for a 17% fertility reduction (model 3), which was the most extreme case included in the 95% confidence intervals of our results. When we used these population growth rates to estimate relative time to quasi-extinction in a hypothetical *M. lucifugus* population of 1000 individuals, the range of observed possible fertility reductions does not change the trajectory for the population (Fig. 5.4). Thus, although a larger sample size may have allowed us to pinpoint a modest reduction in winter fertility for *Pd*-positive *M. lucifugus* females that is statistically significant, it is unlikely that the actual fertility reduction would affect population growth strongly enough to change any management implications derived from current population models that assume there is no effect of *Pd*-infection on fertility. These results concur with a previous sensitivity analysis of post-WNS *M. lucifugus* populations that concluded that efforts to improve adult and juvenile survival would be more effective in mitigating population declines than efforts to improve fecundity (Maslo 2015). In addition to the marginal effect that our observed fertility reductions would have on population trajectories, there is one other consideration that lends support to our contention that small reductions in fertility are not particularly problematic. The tissues we studied came from terminal studies, thus we do not know how many of the *Pd*-positive females that were infertile would have survived winter to be able to reproduce.

Like other histological studies that probed organs outside of the wings and face of *Pd*-positive bats (Blehert et al. 2009; Lorch et al. 2011), we found no evidence of *Pd* growing in any of the female reproductive tissues. We found no remarkable characteristics that distinguished the tissues of the reproductive tracts of *Pd*-positive females from those of *Pd*-negative females. We did observe one characteristic of the uterus in both *Pd*-positive and *Pd*-negative females that differed from other WNS histological studies: neutrophils were present in the uterine lumen of all females included in the study (Fig. 5.3 A, gray arrow). Additionally, some individuals had neutrophils present in the endometrium (Fig. 5.3 A, white arrow), the uterotubal junction (Fig. 5.3 B, gray arrow), and the cervix (Fig. 5.3 C, white arrow). These neutrophils were frequently found surrounding sperm (Fig. 5.3). This contrasts with the absence of neutrophilic recruitment in the wing tissues of *Pd*-infected bats prior to emergence from hibernation that is characteristic of white-nose syndrome (Fig 5.3 D) (Meteyer et al. 2009). Histological studies of *Pd*-infected tissues in torpid bats have found that the sites of infection exhibit little or no inflammation and no leukocytes of any type, followed by extensive inflammation and presence of neutrophils in areas of *Pd*-infection after bats become euthermic (Meteyer et al. 2011). Gene expression studies of *Pd*-infected bats detected upregulation of genes associated with inflammatory response, but no increases in expression of genes associated with leukocyte production (Field et al. 2015). WNS researchers have proposed that this phenomenon is due to suppression of the immune response associated with lower metabolic function in hibernating bats (Meteyer et al. 2012; Cryan et al. 2013; Moore et al. 2013). Our sections of the uterus of hibernating *M. lucifugus* demonstrate that if it is correct that hibernating bats are unable to mount an immune response during hibernation, the lack of response only applies to areas of the body outside of the female reproductive system.

Female hibernating bats challenge our notion of the limits of mammalian reproduction in what appears to be a precarious solution to the challenge of being a slow-reproducing, strictly insectivorous animal in a temperate climate. Biologists have spent nearly a century trying to identify the distinct anatomy and physiology that allow these species to take the reproductive process to the brink of fertilization, then pause the process for roughly seven months to make parturition and nursing coincide with seasonal food availability (Guthrie and Jeffers 1938; Wimsatt and Kallen 1957; Wimsatt et al. 1966; Racey 1976; Buchanan 1987). These mechanisms are still not precisely understood, but clearly require little energetic input because they occur when metabolic rates run at a bare minimum (Carey et al. 2003). Previous research has demonstrated that *Pd* infection interferes with bats' normal physiological processes, including a two-fold increase in fat metabolism, as early as 67 days after initial infection before visible signs such as wing damage and frequent arousals occur (Verant et al. 2014). Yet our results show that, for the majority of *M. lucifugus* females, this disruption does not disable the processes involved in maintaining the MVF and stored sperm they need to conceive when hibernation ends. The results of our study underscore the extreme resilience of the winter reproductive cycle of hibernating bats.

CHAPTER 6: CONCLUSION

My dissertation research included a diverse array of field and laboratory techniques. This was born out of questions that called upon different subfields in biology to pull together a handful of the missing pieces in the puzzle of North American bat population declines. Here I give a brief summary of what I learned in each study, followed by a brief description of some future work I would like to do to follow up.

Wing damage in the absence of white-nose syndrome

In my chapter titled "Wing damage in the absence of white-nose syndrome", I modeled three types of the wing damage (discoloration, holes and membrane loss) and also the composite wing damage index score (WDI) observed in *Eptesicus fuscus* that were collected for rabies testing by the Illinois Department of Public Health. I constructed generalized linear models using the following predictors: sex, age, year, day of year, and season. Wing discoloration was the only type of wing damage that was strongly associated with any of the predictors. The top ranked model for wing discoloration according to Akaike Information Criterion (AIC) included year and season as predictors (Akaike weight = 0.786). In this model, wing discoloration had higher frequencies in early summer (9 June – 18 July) and lower frequencies in late summer (19 July – 22 September) than in the other three seasons. Also, frequencies of wing discoloration were lower in 2005 than in 2008-2010. For the remaining types of wing damage (holes, membrane loss, and composite score WDI) there were numerous competitive models, all with relatively low Akaike weights. About a quarter of all *E. fuscus* surveyed from this sample that pre-dates WNS had moderate or severe wing damage. However, this may over-represent wing damage in live populations because these bat carcasses had been frozen for several years. From these results, I

conclude that wing discoloration increases in severity in early summer, and also from year to year, even in the absence of WNS. Additionally, I conclude that wing discoloration, and possibly wing holes, are common in the absence of WNS and should not be interpreted as proof of WNS infection. This has become less of an issue in recent years as researchers have developed methods of PCR testing, and even the use of ultraviolet light, to detect *Pseudogymnoascus destructans* (*Pd*) infection (Muller et al. 2012; Turner et al. 2014).

Effects of white-nose syndrome and annual climate variation on reproduction and survival

In my chapter titled "Effects of white-nose syndrome and annual climate variation on reproduction and survival in two common North American cave-hibernating bat species", I estimated female juvenile survival, female adult survival, and fecundity for *E. fuscus* and *Myotis lucifugus* at a site in western Illinois where there were maternal colonies of both species occupying a single building. For four years from 2011 to 2014, I gathered mark-recapture and reproductive data, and collected wing tissue biopsies to PCR test for the presence of *Pd*. All of my PCR tests were *Pd*-negative, but they were collected in May, so it is possible that the bats had mounted an immune response upon emergence from hibernation and cleared the infection prior to testing (Fuller et al. 2011; Meteyer et al. 2011). I strongly suspect this is true based on the steep decline I observed in the *M. lucifugus* population between 2013 and 2014 ($\lambda_{2011\text{-}13}$ = 1.001, $\lambda_{2013\text{-}2014}$ = 0.444). This span of years included a wide range of spring weather patterns that I incorporated into my models of survival and fecundity. The hottest spring on record at my site occurred in 2012, and spring 2013 saw nearly double the average rainfall for the region.

Both species had significantly lower fecundities in 2012 than in other years (for *M. lucifugus* F_{2012} = 0.81 vs. other years; and for *E. fuscus* F_{2012} = 1.90 vs. other years). My

estimated fecundity for 2012 is considerably lower than the outlier year in a 16-year study of *M. lucifugus* in New Hampshire (F = 0.87) (Frick et al. 2010). This occurred in the last year of their study after WNS had just arrived in the area, and the authors proposed that it was possibly a result of WNS because it was so much lower than the mean fecundity for the 15 years that preceded it (15-year mean F = 0.96) (Frick et al. 2010). I find this an unlikely explanation for my results, because: (1) it affected both species even though I only observed WNS-suspect population declines in *M. lucifugus*, and (2) fecundities for both species returned to their expected rates in 2013 and 2014. Therefore, I think it is reasonable to propose that the exceptionally hot, drought conditions in 2012 drove the observed lower fecundities for *M. lucifugus* and *E. fuscus*.

None of my models of *E. fuscus* survival stood out as a clear favorite according to AIC. Even the model that only used age class as a predictor only fared slightly better than the null model (Akaike weights were 0.23 and 0.17, respectively). It would seem surprising that juvenile survival and adult survival would be similar ($\varphi_a = 0.69$; $\varphi_j = 0.64$), but this is an artifact of the sampling method used in bat mark-recapture studies. Survival estimates are made from the time the juvenile is banded which includes bats that range from days to weeks old. My best model for *M. lucifugus* survival included two parameters: age group and year group (Akaike weight = 0.50), where 2011-2012 and 2012-2013 were clustered together in a presumed pre-WNS year and 2013-2014 was the presumed post-WNS year. This is not surprising given the drastic decline of the *M. lucifugus* population in the final year of the study. Survival decreased much more for juveniles (72% decrease) than for adults (54% decrease).

Timing of parturition and maternal survival

In my chapter titled "The relationship between timing of parturition and maternal survival in *Myotis lucifugus*", I tested the predictions that mean parturition dates would occur earlier in 2012, which was the hottest year on record at my site, than in other years of my study. I also tested two related hypotheses: (2) females that gave birth earlier would be more likely to survive; and (3) maternal survival would be higher in 2012 than in other years. I found that median parturition date was 10 days earlier in 2012 than other years. I did not find support for my hypothesis that early parturition dates predicted higher maternal survival, nor did I find support for the hypothesis that maternal survival would be higher in the warmer year. Akaike weights were 0.48 for the intercept-only model, 0.22 for the parturition date only model. The second ranked parturition date-only model did predict that maternal survival declined with later parturition dates, but confidence intervals are wide at each end of the maternity season due to the scarcity of births occurring very early and very late in the season. Additional years of data would likely bring clarity, if it weren't for the fact that sightings of *M. lucifugus* have become increasingly rare since 2013.

***Pseudogymnoascus destructans* and *Myotis lucifugus* winter female fertility**

In my chapter titled "The potential for *Pseudogymnoascus destructans* to impact *Myotis lucifugus* populations by reducing female fertility during hibernation", I tested the hypothesis that *Pd* infection in a hibernating female bat would reduce the probability that she maintain the mature vesicular follicle (MVF) and stored sperm needed to become pregnant at the end of hibernation, then modeled population growth based on any observed declines in fertility using published vital rates for *M. lucifugus*. All females in both groups had stored sperm. There was no

significant difference between treatment groups for the presence of an MVF: 96% of *Pd*-negative females, and 92% of *Pd*-positive females ($p = 0.455$, $n = 51$, Fisher's exact test). Our sample size was relatively small (27 *Pd*-negative and 24 *Pd*-positive bats). The 95% confidence interval for the difference between treatment groups was 17%. We used published vital rates that assumed a 20% WNS-related reduction in survival rates for adults and juveniles to project population declines and estimate time to quasi-extinction with 0%, 4% and 17% reductions in fecundity to represent the full range of possible results from our fertility analysis. The population growth models predicted that a population of 1,000 bats would be virtually extinct in 11 years under any of these three scenarios. Thus, even though there is some uncertainty in the extent of fertility reduction for *Pd*-infected females, if any at all, there is no difference in outcome from a conservation perspective. The lack of effect of *Pd*-infection on female bat fertility is both surprising and not surprising. Although *Pd*-infection wreaks havoc on the basic physiological processes that are critical for survival during hibernation, the female reproductive cycle of cave-hibernating bats seems well-suited to standing up to seemingly impossible conditions.

Future directions

First and foremost, I would like to repeat my study of the relationship between parturition date and maternal survival in a species other than *M. lucifugus*. Ideally, it would be one that hopefully won't go extinct before I can gather enough data to test my hypotheses! I have plans to try this with data from the *E. fuscus* colony at Siloam Springs State Park. I was not aware that there was an *E. fuscus* maternal colony until after females had already given birth in summer 2011. As a result, I don't have sufficient reproductive data for females in 2011 to estimate

parturition dates. The *E. fuscus* colony is fairly small (approximately 70 females and their offspring), so two years of recapture data wouldn't be sufficient for the analysis. However, my former field assistant, Jeanette Bailey, collected data at the site last year as a student in Al Kurta's lab. I have asked her for her 2015 data, and will use this to replicate my study of *M. lucifugus*.

Jeanette also has recapture data for *M. lucifugus* from summer 2015. It's rather grim, with only 6 *M. lucifugus* females recaptured all season. Ideally, I would collect a few more years of data to get estimates of the rates of decline to see which of Winifred Frick's models best match my mark-recapture data. A few more years of data on *E. fuscus* would help clarify the relationship between drought and fecundity. Realistically, I don't see how I could do this while simultaneously running field studies at my new job in Pennsylvania, but I would enthusiastically share data and analyses with anyone willing to continue the field work!

In my study of survival rates at Siloam Springs, I had to estimate offspring mortality prior to banding by setting lambda equal to one and solving for an adjustment factor to my apparent juvenile survival rates for each species. I think it is a reasonable assumption that these populations, which have been present since at least the 1990's (when Joe Kath and Dean Corgiat of the Illinois Department of Natural Resources did mark-recapture work), have been stationary (no net growth or declines) over the years prior to the apparent arrival of WNS. However, my adjustment factors are very crude considering they correct for mortality that occurred before banding, and juveniles' ages at banding varied by weeks. I plan to review my data set to see if there are sufficient data to use age at banding as a predictor of apparent survival. I predict that older juveniles will have higher survival rates, based on the assumption that mortality is highest in the neonate period.

Finally, I would love to conduct gene expression studies to investigate how neutrophilic recruitment is so common in female reproductive tracts during hibernation but evidently not possible in *Pd*-infected wing tissue.

FIGURES AND TABLES

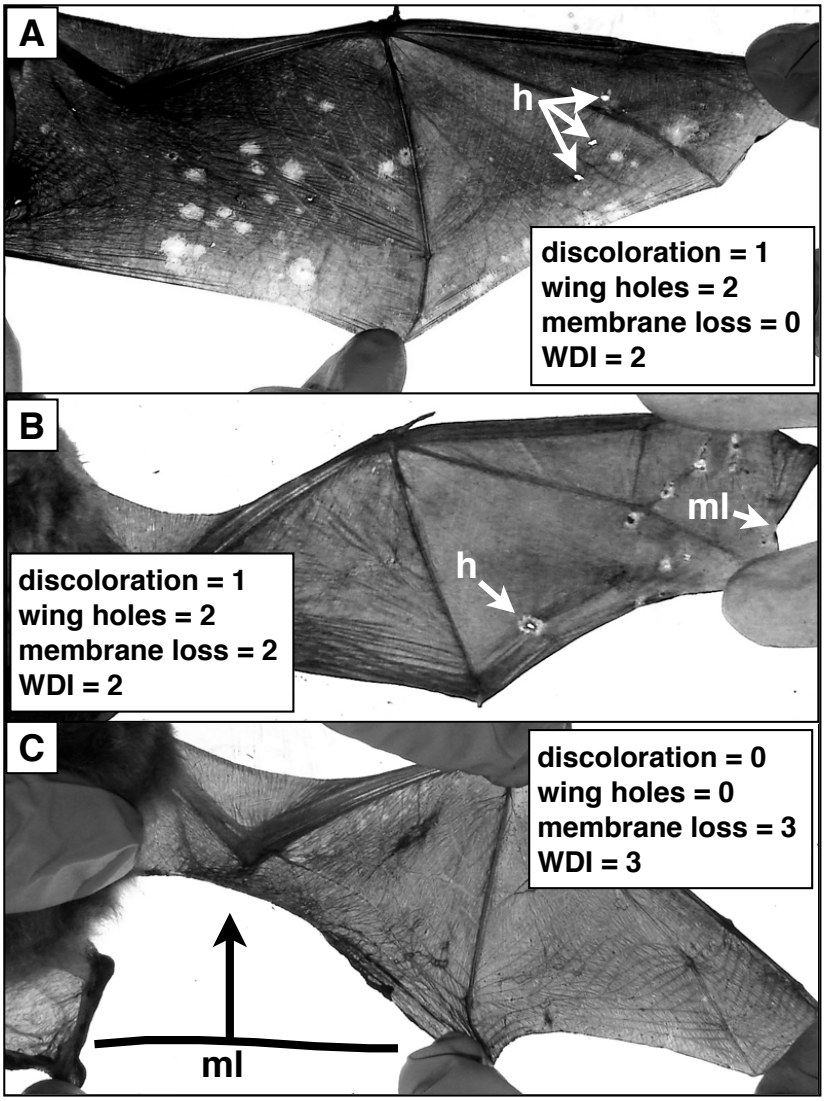

Figure 2.1. Three types of wing damage that contributed to wing damage index (WDI) scores in big brown bats (*Eptesicus fuscus*). A) Adult male collected June 5, 2010 with numerous wing discoloration areas covering less than 50% of the wing area and three 1 mm holes (arrows labeled "h"); wing discoloration score = 1, hole score = 2, membrane loss score = 0, WDI = 1. B) Adult male collected June 10, 2005 with greater than five areas of discoloration, but less than 50% of total membrane area discolored, one 2 mm hole (arrow labeled "h"), and one membrane loss lesion of less than 1 cm; wing discoloration score = 1, hole score = 2, membrane loss score = 2, WDI = 2. C) Adult male collected June 7, 2005 with membrane loss greater than 1 cm at trailing edge of plagiopatagium (indicated by arrow attached to line representing approximate boundary of wing before damage) and one area of discoloration; wing discoloration score = 0, hole score = 0, membrane loss score = 3, WDI = 3.

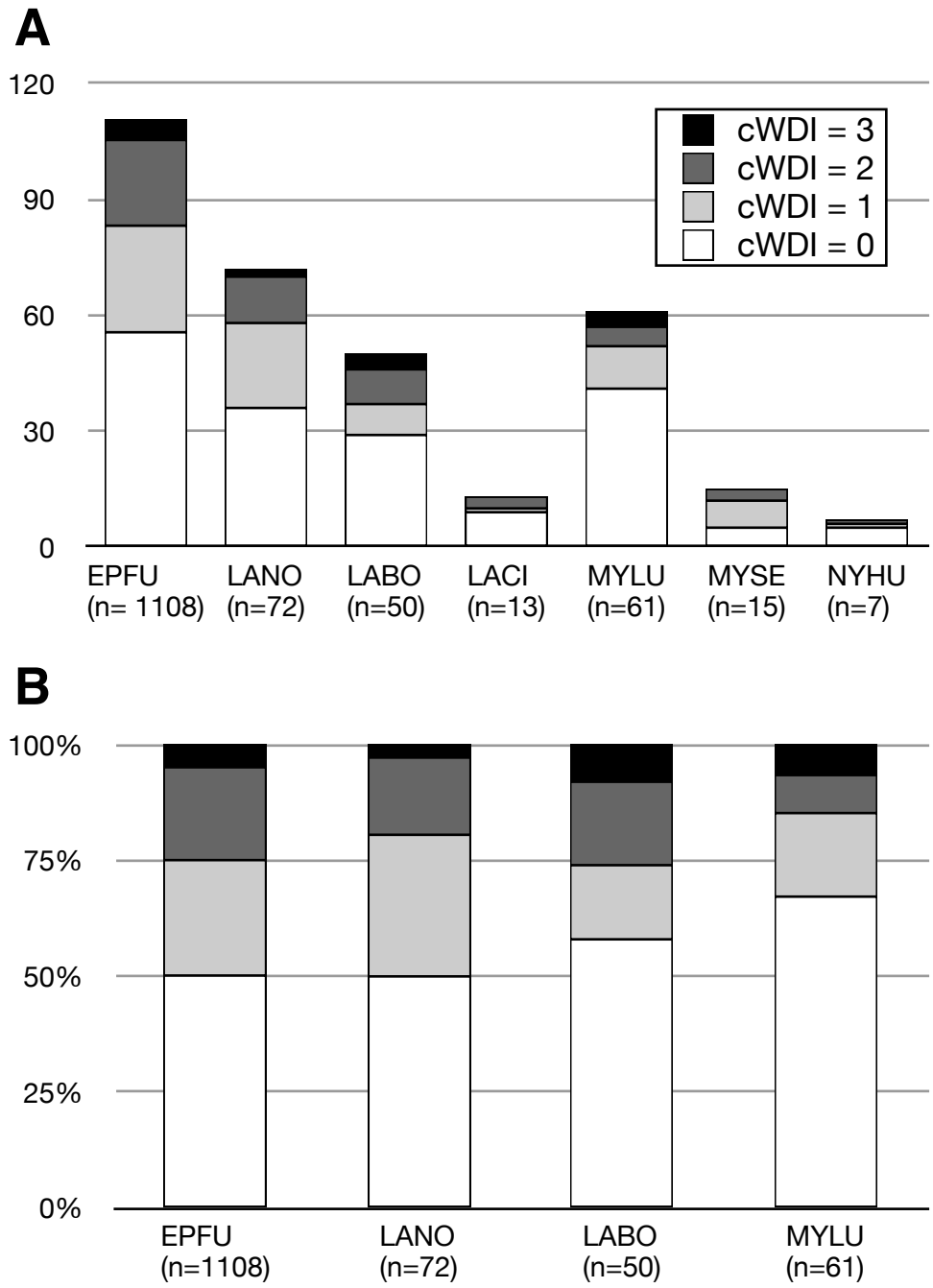

Figure 2.2. Frequency of wing damage index (WDI) for bat species surveyed. Species shown: *Eptesicus fuscus* (big brown bat, EPFU), *Lasionycteris noctivagans* (silver-haired bat, LANO), *Lasiurus borealis* (eastern red bat, LABO), *Lasiurus cinereus* (hoary bat, LACI), *Myotis lucifugus* (little brown myotis, MYLU), *M. septentrionalis* (northern long-eared myotis, MYSE) *Nycticeius humeralis* (evening bat, NYHU). A) WDI frequencies for seven species represented as absolute numbers; *E. fuscus* scaled 1:10. B) WDI frequencies for four species represented as percentages.

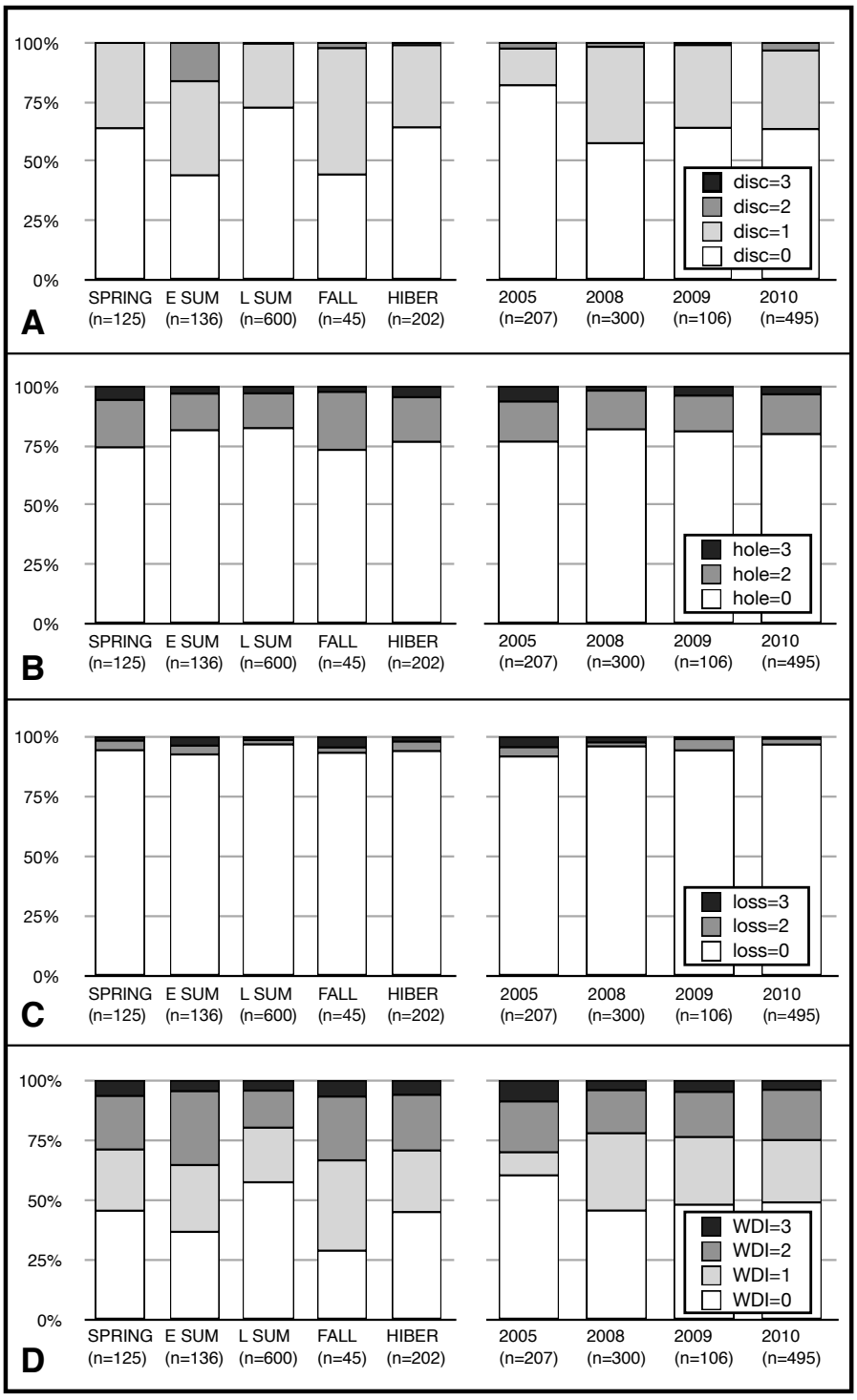

Figure 2.3. Frequencies of wing damage in the big brown bat (*Eptesicus fuscus*) by season and year. Seasons include: 15 April - 8 June (SPRING), 9 June - 18 July (E SUM), 19 July - 22 September (L SUM), 23 September - 5 November (FALL), and 6 November - 14 April (HIBER). A) Frequencies of wing discoloration scores (disc). B) Frequencies of wing hole scores (hole). C) Frequencies of membrane loss scores (loss). D) Frequencies of wing damage index (WDI).

Table 2.1. Akaike Information Criterion (AIC) scores for generalized linear models of three types of wing damage surveyed (discoloration, holes and membrane loss) and composite wing damage index (WDI). Additional model characteristics include: number of effects (K) and Akaike weights (ω). Competitive models shown in bold. Numbers in parentheses indicate the number of categories for each predictor. Only the top six models and the intercept-only model are shown.

Discoloration Models (dist=poisson):	n	K	AIC	ΔAIC	ω
Seas(3): ES v LS v other + Yr(2): 2005 v other	1108	7	1577.2	0	0.786
Seas(5) + Yr(2): 2005 v other	1108	9	2.6	-783.9	0.214
Seas(3) + Yr(4)	1108	9	23.3	-796.24	0
Age(2) + Seas(5) + Yr(4)	1108	13	1601.2	24	0
Seas(5) + Yr(4)	1108	11	1601.6	24.4	0
Age(2) + Sex(2) + Seas(5) + Yr(4)	1108	15	1603.1	25.9	0
Intercept only	1108	2	1672.6	95.4	0

Hole Models (dist=neg binomial):	n	K	AIC	ΔAIC	ω
Seas(2: sum v other)*	1108	4	1837.1	0	0.281
Seas(2: sum v other) + Age(2)	1108	6	1838.3	1.2	0.154
Seas(2: sum v other) + Yr(2: 2005 v other)	1108	6	1838.4	1.3	0.147
Age(2)	1108	4	1838.8	1.7	0.12
Intercept only	1108	2	1839.4	2.3	0.089
Yr(2: 2005 v other)	1108	4	1839.8	2.7	0.073
Yr(2: 2005 v other) + Age(2)	1108	6	1840.0	2.9	0.066

Membrane Loss Models (dist=neg binomial):	n	K	AIC	ΔAIC	ω
Seas(2): LS v other + Yr(2): 2010 v other	1108	6	605.2	0	0.287
Age(2) + Seas(2):LS v other + Yr(2): 2010 v other	1108	8	605.6	0.5	0.227
Seas(2): LS v other	1108	4	606.2	1.1	0.169
Yr(2): 2010 v other	1108	4	606.5	1.4	0.146
Intercept only	1108	2	607.4	2.2	0.094
Seas(2): LS v other + Yr(4)	1108	8	609.0	3.9	0.042
Age(2) + Seas(2):LS v other + Yr(4)	1108	10	609.6	4.4	0.032

Composite WDI Models (dist=poisson):	n	K	AIC	ΔAIC	ω
Seas(2): LS v other + Yr(2): 2005 v other	1108	6	2641.6	0	0.354
Seas(2): LS v other	1108	4	2642.9	1.3	0.185
Seas(2): LS v other + Yr(4)	1108	8	2643.0	1.4	0.176
Seas(3): ES v LS v other + Yr(4)	1108	9	2643.7	2.1	0.124
Seas(3): ES v LS v other	1108	5	2643.7	2.1	0.124
Seas(5) + Yr(4)	1108	7	2647.0	5.4	0.024
Intercept only	1108	2	2669.7	28.1	0

$$\begin{bmatrix} S_j * M_j & S_a * M_a \\ S_j & S_a \end{bmatrix}$$

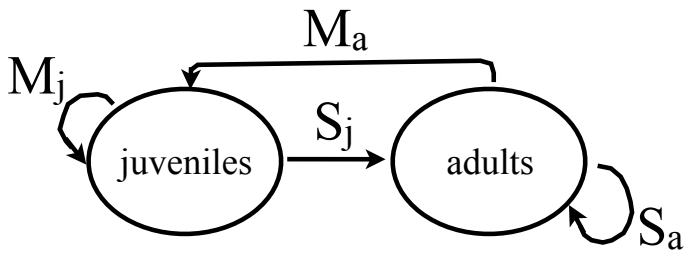

Figure 3.1. Two-stage Lefkovitch matrix (above) used to calculate population growth from vital rates based on the *Myotis lucifugus* and *Eptesicus fuscus* life cycle (below): Sa = adult survival, Sj = juvenile survival, Ma = adult maternity and Mj = juvenile maternity.

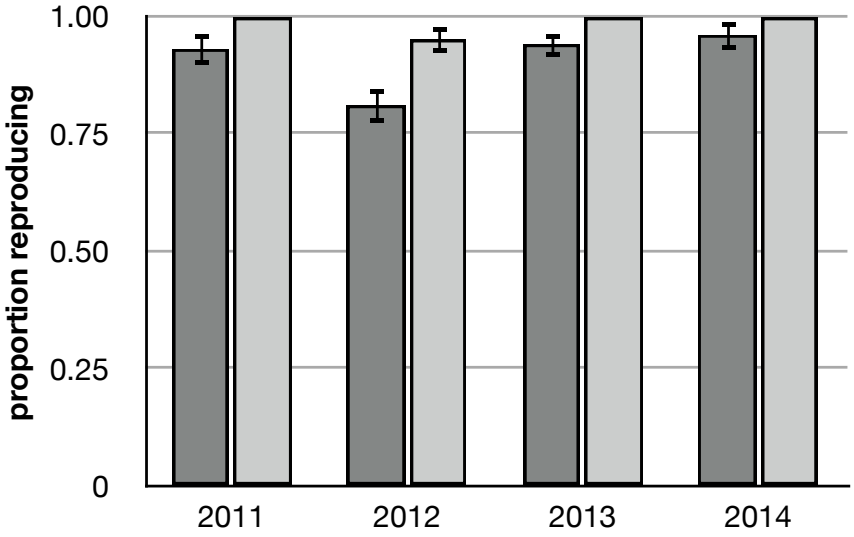

Fig. 3.2. Proportion of female bats reproducing at Siloam Springs State Park from 2011-2014. M. lucifugus females (dark gray): 2011 n = 65, 2012 n = 100, 2013 n = 71, 2014 n = 46; E. fuscus females (light gray): 2011 n = 15, 2012 n = 60, 2013 n = 68, 2014 n = 77. Bars represent standard errors. The proportion of females reproducing was significantly lower in 2012 than in other years for both species (*M. lucifugus*: Fisher's exact test, $p = 0.011$, n = 282; *E. fuscus*: Fisher's exact test, $p = 0.021$, n = 223). The proportion of *E. fuscus* reproducing during the 4-year study was significantly higher than in *M. lucifugus* (Fisher's exact test, $p < 0.001$, n = 505).

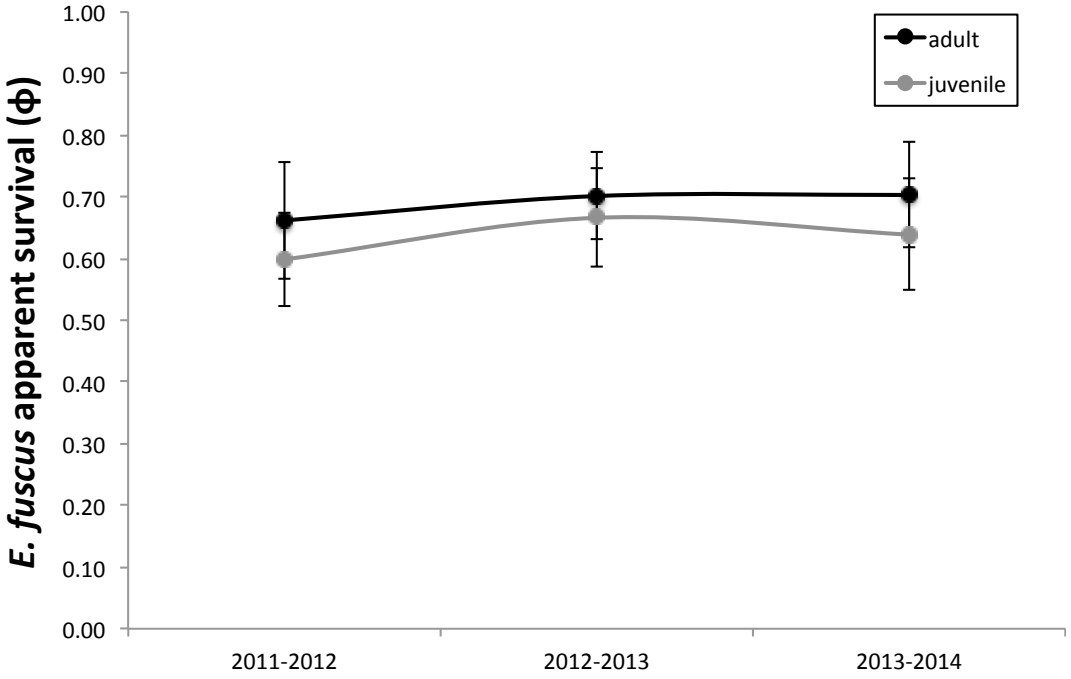

Fig. 3.3. Apparent survival of E. fuscus females at the Siloam Springs State Park maternal colony from 2011-2014. Adult survival estimates (black) ranged from 0.66 – 0.70 for all years; juvenile survival estimates (gray) ranged from 0.60 – 0.67 for all years. Adults: 2011 n = 15, 2012 n = 63, 2013 n = 71, 2014 n = 80; Juveniles: 2011 n = 48, 2012 n = 49, 2013 n = 40, 2014 n = 70. Bars represent standard errors from the mean.

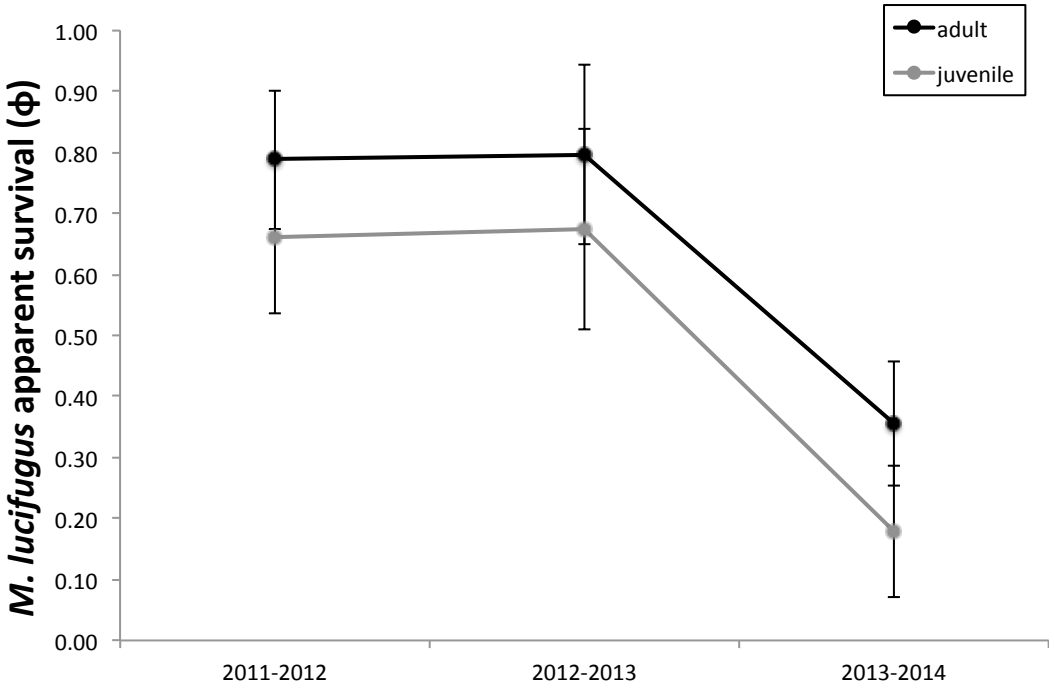

Fig. 3.4. Apparent survival of M. lucifugus females at the Siloam Springs State Park maternal colony from 2011-2014. Adult survival estimates (black) ranged from 0.79-0.80 in first two years then dropped to 0.36 in the presumed post-white-nose syndrome year (2013-2014); juvenile survival estimates (gray) ranged from 0.66-0.67 in first two years then dropped to 0.18 in the presumed post-white-nose syndrome year (2013-2014). Adults: 2011 n = 62, 2012 n = 134, 2013 n = 96, 2014 n = 45; Juveniles: 2011 n = 47, 2012 n = 79, 2013 n = 34, 2014 n = 8. Bars represent standard errors from the mean.

Table 3.1. Annual weather trends and bat colony vital rates at Siloam Springs State Park 2011 - 2013. T_spring = mean temperature from 1 March - 31 May; P_spring = total precipitation from 1 March - 31 May. "Average" represents the historical average for temperature and precipitation, and the 3-year average for vital rates. Data in bold italics represent departures from the average.

	2011	2012	2013	Average
Spring temperature (C)	12	***16***	10	11.2
Spring precipitation (cm)	23.1	***14.7***	***51.7***	27.8
E. fuscus adult survival ($\varphi_{a\ EPFU}$)	0.66	0.70	0.70	0.69
E. fuscus juvenile survival ($\varphi_{j\ EPFU}$)	0.30	0.33	0.32	0.32
M. lucifugus adult survival ($\varphi_{a\ MYLU}$)	0.79	0.80	***0.36***	0.65
M. lucifugus juvenile survival ($\varphi_{j\ MYLU}$)	0.46	0.47	***0.13***	0.35
E. fuscus proportion reproducing	1.00	***0.95***	1.00	0.98
M. lucifugus proportion reproducing	0.93	***0.81***	0.94	0.89
E. fuscus population growth (λ_{EPFU})	0.960	1.014	1.020	0.998
M. lucifugus population growth (λ_{MYLU})	1.004	0.993	***0.444***	0.814

Table 3.2. Models of *Eptesicus fuscus* apparent survival in west-central Illinois in 2011-2014 ranked by Akaike Information Criterion (AICc). Models included age and year (t) as predictors of apparent survival (Phi), with three types of model sets for years: "t", "yr 1,2 v. 3" and "yr 1,3 v. 2". "t" included each of the three recapture intervals separately (2011-2012, 2012-2013 and 2013-2014). The grouping "yr 1,2 v. 3" clustered years in two categories: presumed pre-white-nose syndrome (2011-2012 and 2012-2013) and presumed post-white-nose syndrome (2013-2014). The grouping "yr 1,3 v. 2" clustered years in two categories: average temperature years (2011-2012 and 2013-2014) and the hot year (2012-2013). Models also included age and year (t) as predictors of recapture probability (p), with t grouping years in two categories: high capture effort (2011-2012) and low capture effort (2012-2013 and 2013-2014). Phi(.) and p(.) indicate that survival and recapture parameters, respectively, were held constant. Competitive models in bold text.

Model	AICc	ΔAICc	AICc Wts.	Likelihood	Par.	Deviance
{Phi(age) p(.)}	**448.22**	**0**	**0.23**	**1.00**	**3**	**17.52**
{Phi(.) p(.)}	**448.75**	**0.53**	**0.17**	**0.77**	**2**	**20.10**
{Phi(t) p(.)}	**449.06**	**0.85**	**0.15**	**0.66**	**4**	**16.30**
{Phi(yr 1,3 v. 2*juv only) p(.)}	**449.12**	**0.91**	**0.14**	**0.64**	**4**	**16.36**
{Phi(yr 1,3 v. 2) p(.)}	**449.73**	**1.52**	**0.11**	**0.47**	**3**	**19.03**
{Phi(yr 1,2 v. 3) p(.)}	**449.90**	**1.68**	**0.10**	**0.43**	**3**	**19.20**
{Phi(yr 1,3 v. 2*age) p(.)}	451.20	2.99	0.05	0.22	5	16.36
{Phi(yr 1, 2 v. 3*age) p(.)}	451.75	3.54	0.04	0.17	5	16.91
{Phi(t*age) p(.)}	453.73	5.51	0.01	0.06	7	14.67

Table 3.3. Models of *Myotis lucifugus* apparent survival in west-central Illinois in 2011-2014 ranked by Akaike Information Criterion (AICc). Models included age and year (t) as predictors of apparent survival (Phi), with two sets of parameters for years: "t" and "yr 1,2 v. 3". "t" included each of the three recapture intervals separately (2011-2012, 2012-2013 and 2013-2014). The grouping "yr 1,2 v. 3" clustered years in two categories: presumed pre-white-nose syndrome (2011-2012 and 2012-2013) and presumed post-white-nose syndrome (2013-2014). Models also included age and year (t) as predictors of recapture probability (p), with t grouping years in two categories: high capture effort (2011-2012) and low capture effort (2012-2013 and 2013-2014). Phi(.) and p(.) indicate that survival and recapture parameters, respectively, were held constant. Competitive models in bold text.

Model	AICc	ΔAICc	AICc Wts.	Likelihood	Par.	Deviance
{Phi(yr 1,2 v. 3*age) p(t)}	**562.31**	**0**	**0.50**	**1.00**	**6**	**10.36**
{Phi(yr 1, 2 v. 3) p(t)}	564.64	2.33	0.16	0.31	4	16.79
{Phi(yr 1,2 v. 3*age) p(t*age)}	565.07	2.76	0.13	0.25	8	8.97
{Phi(t*age) p(t)}	566.26	3.95	0.07	0.14	8	10.16
{Phi(t) p(t)}	566.65	4.34	0.06	0.11	5	16.76
{Phi(yr 1, 2 v. 3) p(t*age)}	566.91	4.59	0.05	0.10	6	14.95
{Phi(t) p(t*age)}	568.90	6.58	0.02	0.04	7	14.88
{Phi(t*age) p(t*age)}	569.03	6.71	0.02	0.03	10	8.74
{Phi(.) p(t)}	575.67	13.36	0.00	0.00	4	27.82
{Phi(age) p(t*age)}	577.90	15.59	0.00	0.00	6	25.95
{Phi(.) p(t*age)}	580.43	18.12	0.00	0.00	5	30.54

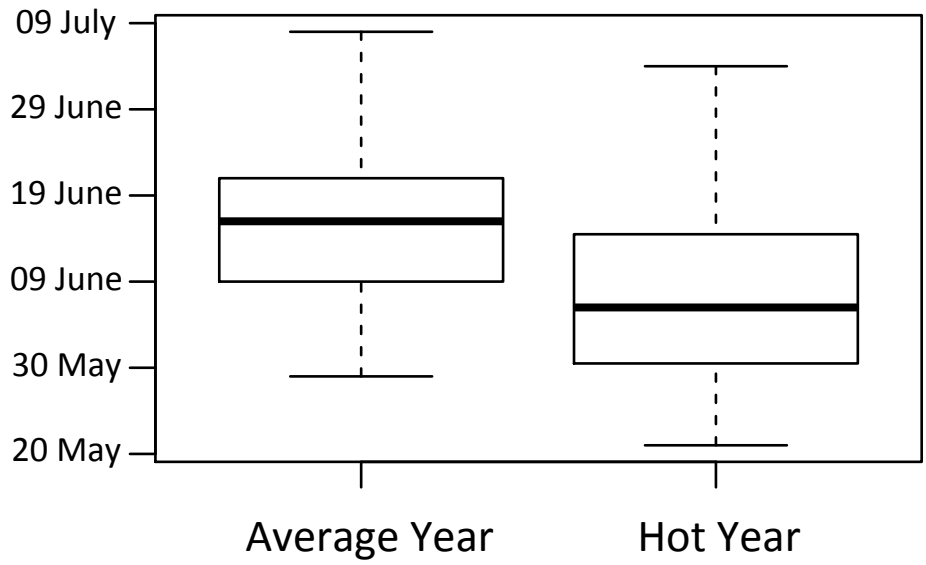

Figure 4.1. Box plot of estimated parturition dates for *Myotis lucifugus* females at a maternal colony in west-central Illinois in Average temperature years (2011 & 2013, n = 104) and a hot year (2012, n = 71). Thick horizontal lines represent the median; lower and upper sides of boxes represent the first and third quartiles, respectively; thin horizontal lines at ends of dashed vertical lines represent maxima and minima. The median parturition dates were: 06 June in the hot year and 16 June in average years. Mean temperatures from April-September (the approximate active season for *M. lucifugus*) were 0.4 C above average in 2011, 2.4 C above average in 2012, and 0.6 C below average in 2013.

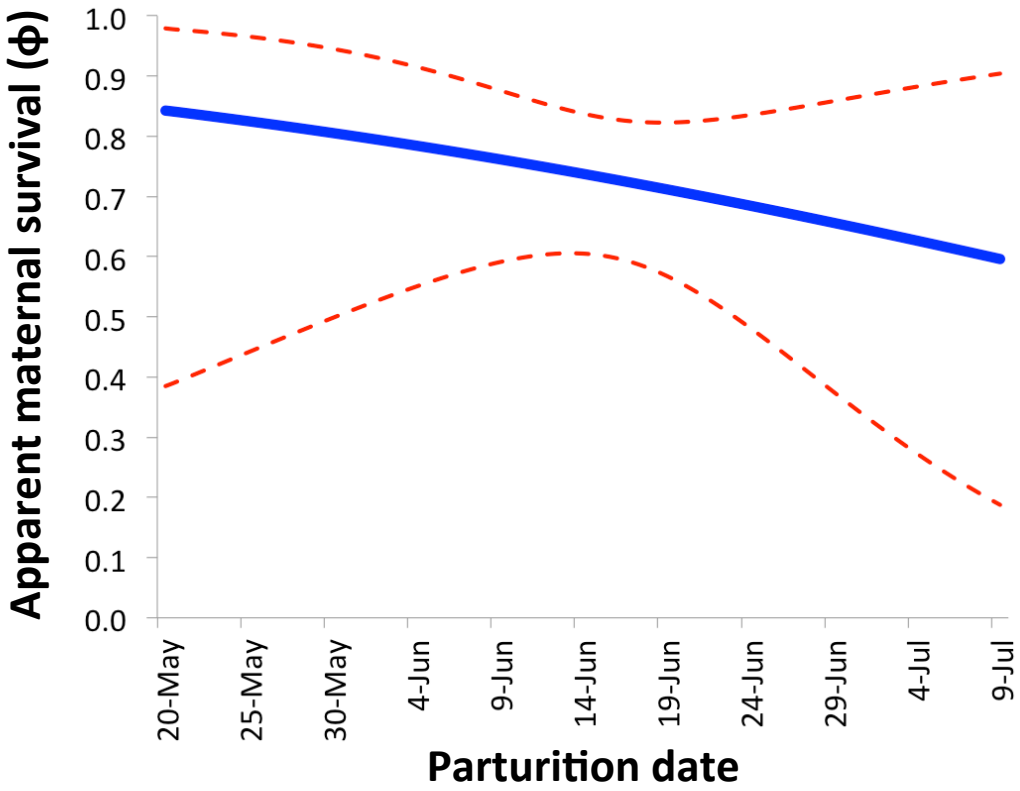

Figure 4.2. Modeled relationship between dates that *Myotis lucifugus* females give birth in years 2011-2013 at an Illinois maternal colony site, and probability of survival to the next year (solid blue line) with 95% confidence intervals (dashed red lines). Akaike Information Criterion ranked this model lower than the intercept-only model.

Table 4.1. Theoretical effects of early parturition on apparent survival of *Myotis lucifugus* mothers and their offspring, and the impact of these combined effects on population growth (λ). Juvenile survival has been shown to increase with earlier dates of birth, but the effect of juvenile survival on population growth is small. The effect of maternal survival on population growth is large, but the effect of early parturition on maternal survival is unknown. The net effect of early parturition on population growth would be: strongly positive if maternal survival increases with early parturition, slightly positive if there is no effect of parturition date on maternal survival, and negative if maternal survival decreases with early parturition.

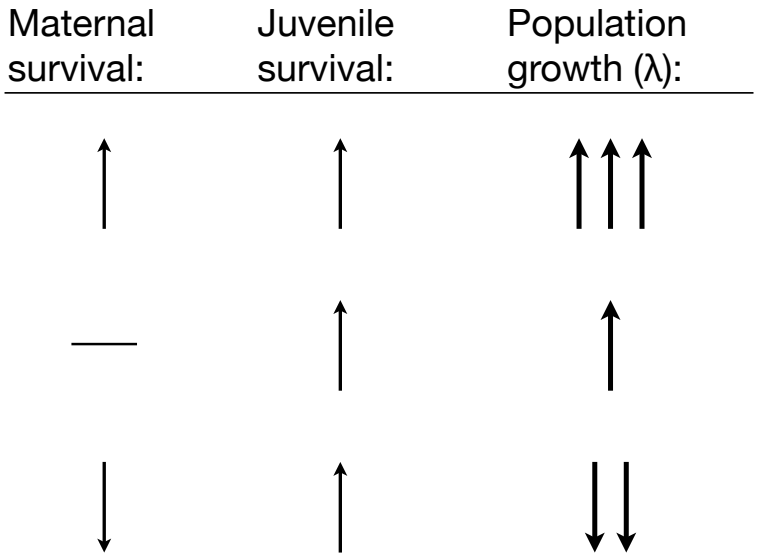

Table 4.2. Models of *Myotis lucifugus* apparent maternal survival in west-central Illinois in 2011-2014 ranked by Akaike Information Criterion (AICc). Models included estimated date the mother gave birth (Part Date), Year that the mother reproduced (2011, 2012 or 2013), Annual Temperature Category (2011 & 2013 = average; 2012 = warm). Competitive models in bold text.

Model	AICc	ΔAICc	AICc Weights	Likelihood	Parameters	Deviance
Intercept-only model	**299.06**	**0**	**0.48**	**1**	**1**	**297.04**
Part Date only	**300.61**	**1.55**	**0.22**	**0.46**	**2**	**296.56**
Annual Temp only	301.10	2.04	0.17	0.36	2	297.04
Year only	302.68	3.62	0.08	0.16	3	296.57
Part Date*Annual Temp	304.37	5.31	0.03	0.07	4	296.19
Part Date*Year	307.10	8.04	0.01	0.02	6	294.71

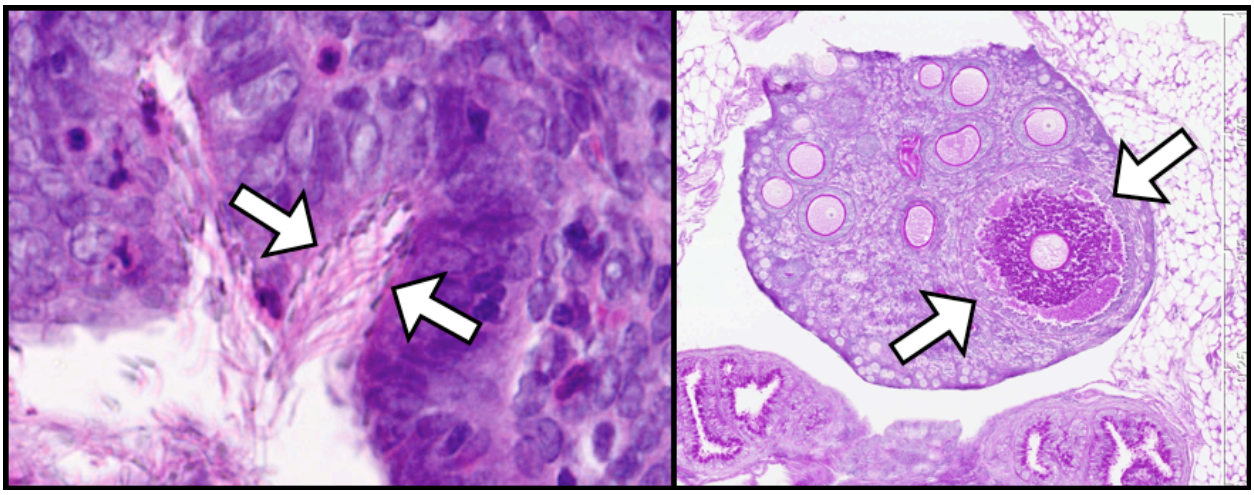

Figure 5.1. Histological indicators of female fertility in hibernating *Myotis lucifugus*. Figure 2A: sperm (indicated by white arrows) packed tightly against the columnar eptithelium at the junction between the uterus and oviducts (Hematoxalin and eosin, 40x). Figure 2B: a single mature vesicular follicle (MVF, indicated by white arrows) in one ovary is a sign that this female is capable of producing an offspring this year (Periodic Acid Schiff, 5x). Note the difference in size and morphology of the MVF compared to numerous smaller primary follicles present in the ovary, which will not be ovulated this year.

$$\begin{bmatrix} S_j * B_j * M_j & S_a * B_a * M_a \\ S_j & S_a \end{bmatrix}$$

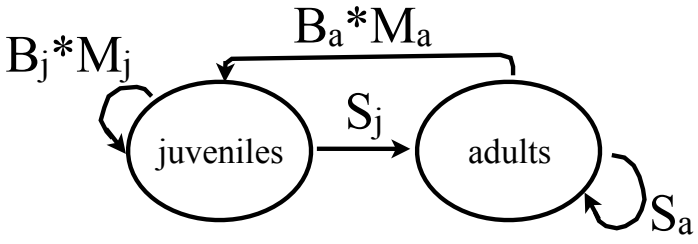

Figure 5.2. Two-stage Lefkovitch matrix (post-breeding census) used to model *Myotis lucifugus* population growth rates. Two-stage Lefkovitch matrix (above) used to calculate population growth from vital rates based on the *Myotis lucifugus* life cycle (below): Sa = adult survival, Sj = juvenile survival, Ba = adult probability of breeding, Bj = juvenile probability of breeding; Ma = adult fecundity and Mj = juvenile fecundity. Adapted from Frick, *et al*., 2010b.

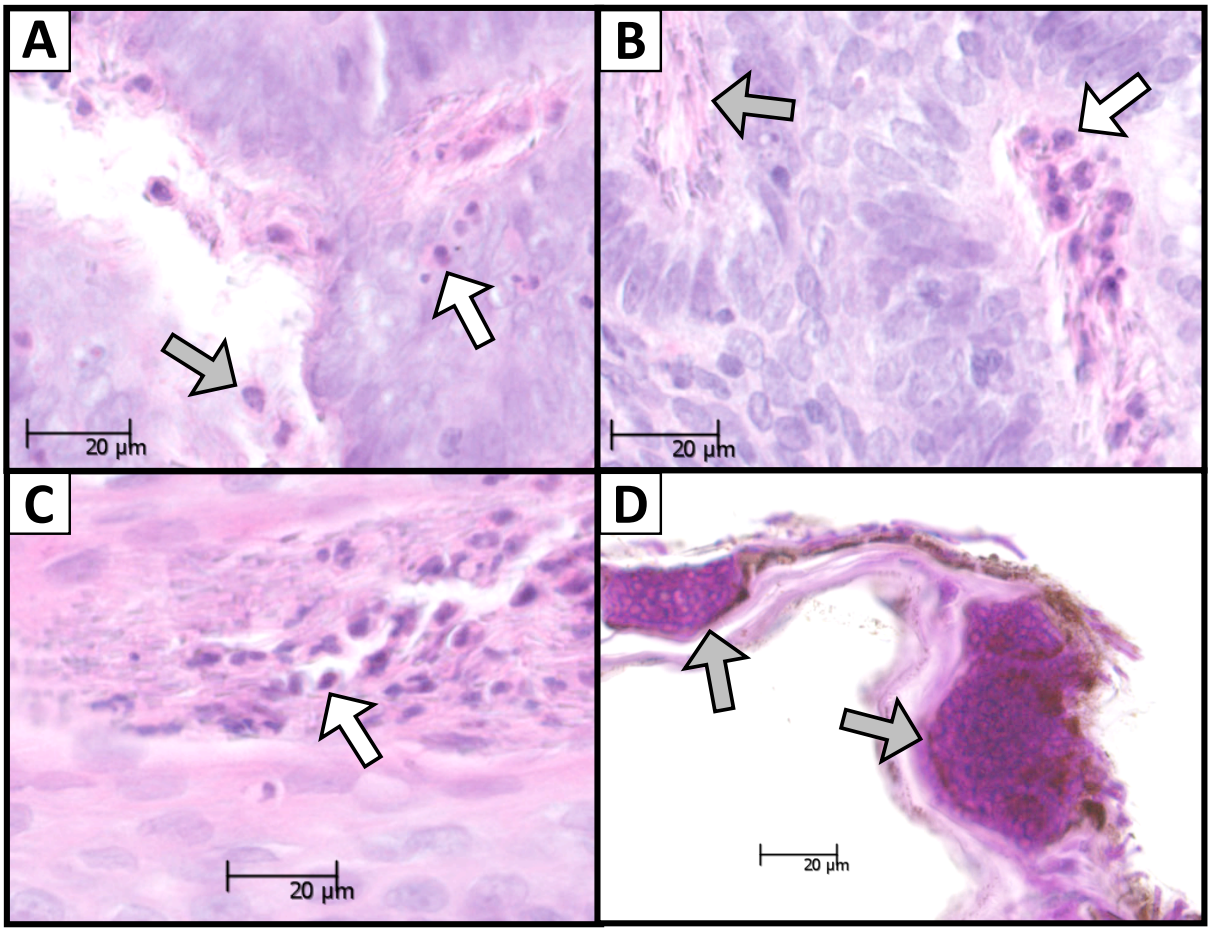

Figure 5.3. Neutrophils were found in various locations in the reproductive tracts of all hibernating female *Myotis lucifugus* studied, whether negative or positive for *Pseudogymnoascus destructans* (*Pd*), where sperm were the apparent targets. Figure 4A: neutrophils (cells with dark purple-stained multi-lobed nuclei and round, pink-stained cytosol) in the uterine lumen (gray arrow) and endometrium (white arrow) targeting sperm (structures with purple-stained arrow acrosomes and pink-stained flagella); hematoxalin and eosin (H&E). Figure 4B: neutrophils phagocytize sperm in one pocket of the uterotubal junction (white arrow) but not an adjacent one (gray arrow); H&E. Figure 4C: dense clusters of sperm and neutrophils (white arrow) in the cervix, H&E. Figure 4D: Dense clusters of pink-stained *Pd* (gray arrows) erode the epithelium of a bat's wing membrane with typical lack of neutrophilic recruitment; periodic acid Schiff.

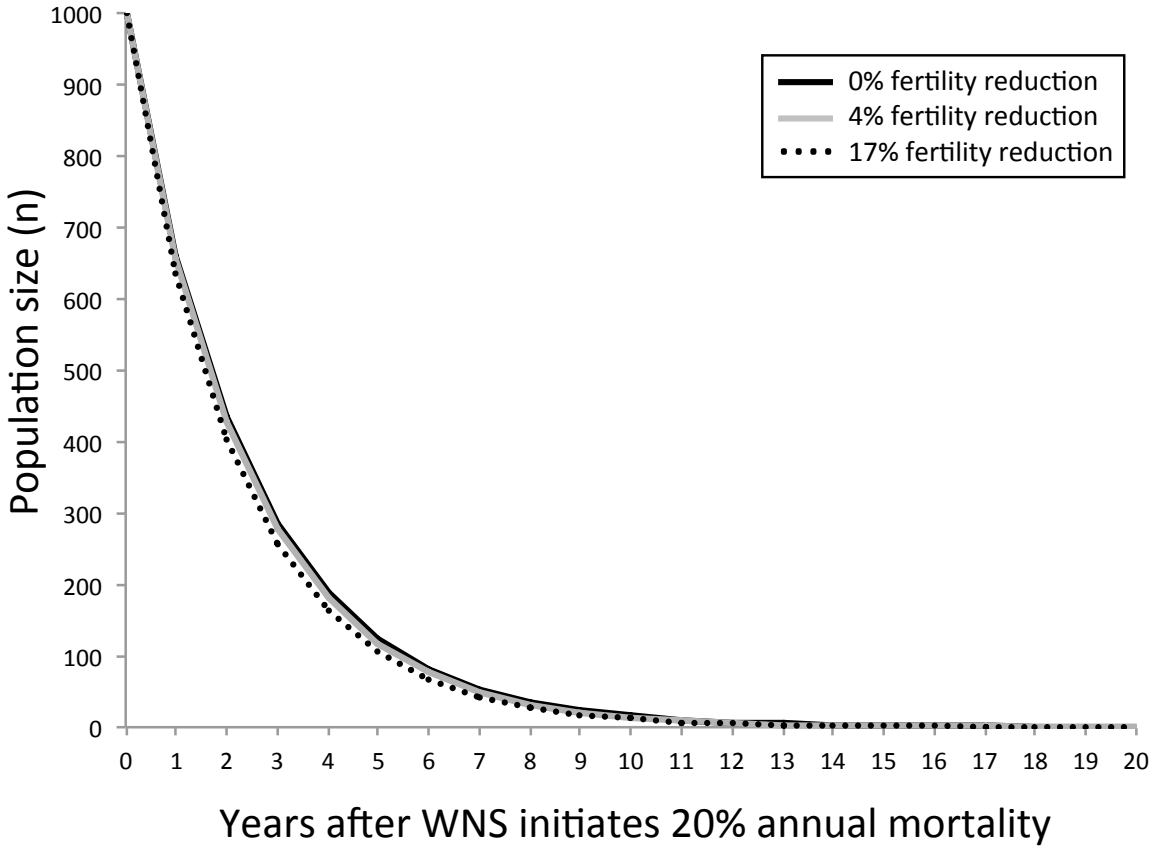

Figure 5.4. Projected population declines over 20 years of a hypothetical post-white-nose syndrome population of 1000 *Myotis lucifugus* with a 20% reduction in annual survival and a 0% (black line), 4% (gray line) or 17% reduction (dotted line) in fertility. Quasi-extinction (defined as 1% of the original population size) occurs in year 11 for all three modeled reductions in fertility.

Table 5.1. Vital rate estimates for a hypothetical population of 1,000 *Myotis lucifugus* experiencing a 20% reduction in survival and a 0%, 4% or 17% reduction in fertility. Estimates of survival, fecundity and breeding are mean values from pre-WNS data published in Frick, *et al.*, 2010b, with 0.20 deducted from both adult and juvenile survival. Maternity (M) is the proportion of female offspring per capita (estimated at 50% of offspring of both sexes).

	0% fertility reduction		4% fertility reduction		17% fertility reduction	
Parameter	adult	juvenile	adult	juvenile	adult	juvenile
Survival (S)	0.53	0.15	0.53	0.15	0.53	0.15
Breeding probability (B)	1.00	0.39	1.00	0.39	1.00	0.39
Fertility (F)	0.96	0.96	0.92	0.92	0.79	0.79
Maternity (M = 0.5*F)	0.48	0.48	0.46	0.46	0.40	0.40
Fecundity (Fec = S*B*M)	0.25	0.03	0.24	0.03	0.21	0.02

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