BASELINE DATA ON OVERWINTERING BATS AND ROOSTS IN TEXAS

A Dissertation

by

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ABSTRACT

New or introduced infectious diseases can have detrimental effects on wildlife populations, including reduction of population size of some species, and even extinction. White-nose syndrome (WNS), a bat disease caused by a fungus (*Pseudogymnoascus destructans*), has led to substantial declines in population size of some bat species in eastern North America. As a result of the spread of *P. destructans* in Texas, WNS was documented in Texas in spring 2020. To investigate site-specific landscape and environmental predictors of bat presence and abundance, I collected data on two WNS-affected species (tri-colored bat (*Perimyotis subflavus*) and cave myotis (*Myotis velifer*)). I collected data from 450 sites (145 caves and 305 culverts) from January–March 2016, November–February 2016–2017, and October–March 2017–2018/2018–2019. Furthermore, I used data collected from 86 hibernacula (55 caves, 30 culverts, and 1 tunnel) to investigate site-specific landscape and environmental predictors of *P. destructans* presence. Using zero-inflated Poisson regressions, I found that predictors of bat presence and abundance differed between bat species at the state level. Tri-colored bat abundance increased as slope, elevation and distance to nearest water source decreased, as well as sites with easterly aspects. Cave myotis abundance increased as slope, elevation, and distance to water increased, as canopy cover decreased, and at sites with easterly aspects. As tri-colored bats and cave myotis were only present in particular ecoregions, I conducted individual logistic regressions using data from these ecoregions for each species to determine site-specific landscape and environmental factors predicting bat presence. My analyses of combined presence/absence data for the ecoregions in which each species was documented suggested that there were no site-specific landscape and environmental predictors of tri-colored bats and cave myotis. However, individual analyses for each ecoregion where tri-colored bats were present or where cave myotis were present suggest that there are differences in predictors based on location within the state (i.e., ecoregion). My research will aid in the management of WNS-afflicted bat species by better understanding their overwintering distribution. Further, this baseline data will play a critical role in developing management plans for both biologists and landowners.

DEDICATION

In memory of my mother—who from a young age instilled within me a passion for wildlife—I dedicate this to you.

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CHAPTER I

INTRODUCTION TO INFECTIOUS DISEASE

Infectious disease emergence can cause declines in numbers of free-ranging wildlife populations (e.g., Thorne and Williams 1988, Young 1994, Laurenson et al. 1998) and even extinction (McCallum and Dobson 1995, Daszak et al. 2000, McCallum 2008, Kilpatrick et al. 2010). Impairments to the health of animals as a result of disease threaten biodiversity (e.g., Jones 1982, Scott 1988, Daszak et al. 2000, McLean 2007:263–267) and ecosystem function and services (e.g., Parker et al. 1999, Mack et al. 2000, Bohlen et al. 2004). These threats can result in the reduction in welfare and survivorship of animals (Cunningham et al. 2017). Introductions of disease into animal populations have led to declines in population size as illustrated by Ebola outbreaks in African apes (Leroy et al. 2004), canine distemper in Serengeti lions (Roelke-Parker et al. 1996), multiple pathogens that affect amphibian populations (Daszak et al. 1999, Pounds et al. 2006), botulism in waterfowl (Smith 1982:97–119) and rabies in foxes (Anderson et al. 1981, Murray et al. 1986).

There are various factors that lead to the emergence of infectious diseases (e.g., population growth, travel, migration; Morse 1995, Deem et al. 2001, Friend et al. 2001). One of the main causes of invading and emerging disease is the increase in frequencies of international travel, transporting people, animals, animal products, and pathogens across the world (e.g., McLean 2007:262–263). Increases in travel can increase the risk of disease traveling from one continent to another, as was the case with the emergence of the Severe Acute Respiratory Syndrome (SARS) epidemic (Cunningham 2005) and coronavirus disease (COVID-19) epidemic (Guo et al. 2020, Lu et al. 2020, Shereen et al. 2020, Sohrabi et al. 2020). Furthermore, intermixing wildlife species from all over the world in wildlife markets combined with close contact with humans and domestic animals provide opportunities for disease transmission and emergence of new diseases (McLean 2007:262–263).

Although some introductions are unsuccessful, others are able to survive in the new environment, infect a susceptible host, cause disease, become established, and become a major disease of animal health concern (Wilson 1995, Blackburn et al. 2011, Cunningham et al. 2017). Hantavirus, a disease with Korean origin, is transmitted by rodents through urine, droppings, or saliva (Lee et al. 1978), and is a respiratory disease which only affects humans. West Nile virus, indigenous to Africa, Asia, Europe, and Australia, emerged in the United States in 1999 and was thought to have spread either by mosquito or bird (Campbell et al. 2002). West Nile virus is a disease that affects both humans (Centers for Disease Control and Prevention 1999) and wildlife (e.g., birds; Campbell et al. 2002) and thus is an excellent example of how international travel and human encroachment of wildlife habitat can transmit diseases to previously unaffected regions and species.

One such taxon that has been affected by the transmission of disease is bats (Gargas et al. 2009, Lorch et al. 2011). In addition to disease, there are other factors that influence bat fatality, including extermination, vandalism at roost sites, pesticide poisoning, and wind energy (Arnett 2005, GAO 2005, Johnson 2005, Fiedler et al. 2007, Schmidly and Bradley 2016:111–112). As a result of the aforementioned factors and others, researchers report substantial reductions in the size of bat populations worldwide.

Several species are near extinction, whereas others are already extinct (Schmidly and Bradley 2016:111). Small litter sizes (typically 1), and the gregarious nature of many bat species facilitates slow population growth and disease transmission, making them vulnerable to extinction (Kunz and Lumsden 2003:49–54, Schmidly and Bradley 2016:111).

A number of North American bat species are currently at risk of white-nose syndrome (WNS). WNS is a disease caused by the cold tolerant fungus, *Pseudogymnoascus destructans*, formerly known as *Geomyces destructans* (Blehert et al. 2009, Gargas et al. 2009, Lorch et al. 2011, Warnecke et al. 2012, Minnis and Lindner 2013). The fungus is believed to have been introduced from Europe into North America (Leopardi et al. 2015), with the first documentation of the disease occurring in North America in Howes Cave near Albany, New York in February 2006 (Blehert et al. 2009, Turner and Reeder 2009). Since the first documentation of WNS in the United States, the disease has spread north into Canada and westward across the United States (Foley et al. 2011, Coleman and Reichard 2014, Lorch et al. 2016) resulting in the loss of some bat populations (Frick et al. 2010).

The fungus grows on the wings, ears, and muzzles of hibernating bats, invading the dermal layer of the wing tissue, resulting in visible tissue damage (Lorch et al. 2011, Warnecke et al. 2012) and dehydration (Cryan et al. 2013). In addition, bats affected by WNS arouse frequently from winter torpor resulting in depletion of fat reserves and death (Blehert et al. 2009, Boyles and Willis 2010, Reeder et al. 2012, Warnecke et al. 2012).

The temperature range for *P. destructans* growth is 3.0° C to 19.7° C (Verant et al. 2012). Though there is no established relative humidity range for growth of *P. destructans*, research suggests that growth of *P. destructans* is similar between 70.5–96.5% (Marroquin et al. 2017). Hibernating bats tend to select roost locations with temperatures between 3.0° C and 15.0° C, which allows for energy conservation during hibernation (McNab 1982:163–165, Nagel and Nagel 1991, Brack 2007, Foley et al. 2011). Hibernating bat species differ in their selection of microclimate within a roost to conserve energy and reduce the effects of evaporative water loss (EWL; Twente 1955, Davis 1970:265–300, Webb et al. 1995). The range in hibernation temperatures likely impact winter survival of bats and the fitness of survivors' post-hibernation as they fall within the growth range of *P. destructans* (Cryan et al. 2010).

Transmission of *P. destructans* occurs via bat-to-bat contact (Zimmerman 2009, Lorch et al. 2011) and can occur via substrate-to-bat, with some evidence suggesting that humans can transport the fungus from infected to uninfected sites on clothing and equipment (Crawley 2009, Turner and Reeder 2009). Additionally, cave sites within the known range of WNS have tested positive for *P. destructans* (Linder et al. 2010). Currently, the fungus affects twelve species of hibernating insectivorous bats (big brown bat, *Eptesicus fuscus*; cave myotis, *Myotis velifer*; eastern small-footed bat, *M. leibii*; gray bat, *M. grisenscens*; Indiana bat, *M. sodalis*; little brown bat, *M. lucifugus*; long-legged bat, *M. volans*; Northern long-eared bat, *M. septentrionalis*; western long-eared bat, *M. evotis*; southeastern myotis, *M. austroriparius*; tri-colored bat, *Perimyotis subflavus*; and Yuma bat, *M. yumanensis*), with detection of *P. destructans* without histological confirmation of WNS in eight additional species (eastern red bat, *Lasiurus* *borealis*; Mexican free-tailed bat, *Tadarida brasiliensis*; Rafinesque's big-eared bat, *Corynorhinus rafinesquii*; silver-haired bat, *Lasionycteris noctivicans*; Townsend's big-eared bat, *C. townsendii*; Virginia big-eared bat, *C. townsendii virginianus*; Ozark big-eared bat, *C. townsendii ingens*, and western small-footed bat, *M. ciliolabrum*) (USFWS 2019).

There are 33 known species of bats found in Texas (Ammerman et al. 2012). At of the onset of my study, four of these hibernating bat species found in Texas were known to be susceptible to WNS, including the big brown bat (*Eptesicus fuscus*), tri-colored bat (*Perimyotis subflavus*), northern long-eared myotis (*M. septentrionalis*), and the southeastern myotis (*M. austroriparius*) (USFWS 2016). Additionally, swab samples from cave myotis (*M. velifer*), Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), Townsend's big-eared bat (*Corynorhinus townsendii*) and the eastern red bat (*Lasiursus borealis*) tested positive for *P. destructans*, but were not histologically confirmed positive for WNS (USFWS 2016). As the distribution of some eastern and western bat species in North America overlap in Texas, this region could be a possible bridge for WNS to spread further west and south.

Indeed, WNS was documented in Texas for the first time in spring 2020 (TPWD 2020), with the first documentation of *P. destructans* in Texas in 2017 (TPWD 2017). Unfortunately, there is little information known about the environmental conditions in potential bat hibernacula (e.g., caves, culverts) throughout the state of Texas, and how this may contribute to the spread of *P. destructans* through the state. Similarly, there is a lack of understanding of the natural histories of hibernating bats in Texas and there remains a need to fully investigate and characterize species and their overwintering habitats (Ammerman et al. 2012:1–224). The U.S. Fish and Wildlife Service (USFWS) identified surveillance of conditions within hibernacula as a necessary step in forecasting locations potentially suitable for the occurrence of *P. destructans* (USFWS 2011). Therefore, it is necessary to collect data on the composition of bats and their winter hibernacula in order to establish baseline information prior to the potential development of WNS in Texas (Hayman et al. 2016).

Although researchers are conducting individual studies on bats in Texas, none have completed a comprehensive statewide assessment. I used data collected from 450 sites (145 caves and 305 culverts) from January–March 2016, November–February 2016–2017, October-March 2017–2018, and October–March 2018–2019 located across 10 Texas Level III ecoregions to investigate site-specific landscape and environmental predictors of tri-colored bat presence and abundance and cave myotis bat presence and abundance. I also looked at site-specific landscape and environmental predictors of *P. destructans* presence. My research will aid in the management of WNS-afflicted bat species by better understanding their overwintering distribution, as well as aid in understanding characteristics of sites positive for *P. destructans*.

CHAPTER II

THE INFLUENCE OF SITE-SPECIFIC LANDSCAPE AND ENVIRONMENTAL CHARACTERISTICS ON OVERWINTERING POPULATIONS OF CAVE MYOTIS AND TRI-COLORED BATS

Summary

Worldwide, researchers report substantial reductions in abundance of bat populations and, in some case, extirpation from large parts of the range and extinction. There are several factors driving reductions in bat populations, one of which is disease. White-nose syndrome (WNS), an infectious disease, affects populations of some North American bat species, and has resulted in the reduction of many bat populations in Canada and the eastern United States. *Pseudogymoascus destructans*, the causative agent of WNS, was recently documented in Texas as a result of the continued spread. Two species known to be affected by WNS are the tri-colored bat (*Perimyotis subflavus*) and cave myotis (*Myotis velifer*). Tri-colored bats occur throughout eastern and central United States, as far west as New Mexico. In contrast, cave myotis are found in the southwestern and south-central United States. Both species occur in Texas, creating a bridge for the disease to continue to spread west in the United States. In the winter both tri-colored bats and cave myotis occupy subterranean roost sites in Texas, increasing their potential susceptibility to WNS. I used data collected from 450 sites located across Texas (145 caves and 305 culverts) from January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019 to investigate site-specific landscape and environmental predictors of tri-colored bat and cave myotis

presence and abundance. Using zero-inflated Poisson regression analyses, I found that predictors of tri-colored bat presence and abundance differed from those of cave myotis. My analysis suggested that abundance of tri-colored bats increased as slope, elevation and distance to nearest water source decreased, and at sites with easterly aspects. Finally, my analysis suggested that abundance of cave myotis increased as slope, elevation, and distance to water increased, as canopy cover decreased, and at sites with easterly aspects. Because tri-colored bats and cave myotis were primarily found in three ecoregions (Edwards Plateau, East Central Texas Plains, South Central Plains) and two ecoregions (Edwards Plateau, Southwestern Tablelands) respectively, I combined presence/absence data for the ecoregions in which each species was found, and investigated how site-specific landscape and environmental predictors influence presence of each species using logistic regression analyses. Furthermore, I ran individual logistic regression analyses on each ecoregion in which the species was found for each species to determine how site-specific landscape and environmental predictors predict tri-colored bat and cave myotis presence at the ecoregion level. My analyses of combined presence/absence data for the ecoregions in which each species was documented suggested that there were no site-specific landscape and environmental predictors of tri-colored bats and cave myotis. However, my analyses of individual ecoregions suggested ecoregion-specific predictors of tri-colored bat and cave myotis presence. My results regarding relationships between presence of tri-colored bats and cave myotis suggest that identifying potential hibernacula using site-specific landscape and environmental predictors may be best effective if tailored to individual ecoregions. This should be true throughout the range for both species.

Introduction

Wildlife-habitat relationships have been the subject of human interest for centuries (Morrison et al. 2006:3–14). Understanding the factors (e.g., biological, environmental) that drive animal species distribution is critical for implementing effective biological conservation efforts. As studies on wildlife-habitat relationships provide information on habitat, and in some cases animal demographics, this research is particularly important in light of recent declines in the distribution and abundance of many animal populations (e.g., Bonebrake et al. 2010).

One such taxon that has been affected by the transmission of disease are bats (Gargas et al. 2009, Lorch et al. 2011). In addition, there are other factors that influence bat fatality, including extermination, vandalism at roost sites, pesticide poisoning, and wind energy (Arnett 2005, GAO 2005, Johnson 2005, Fiedler et al. 2007, Schmidly and Bradley 2016:111–112). As a result of the aforementioned factors and others, researchers report substantial reductions in the size of bat populations worldwide. Several species are near extinction, whereas others are already extirpated (Schmidly and Bradley 2016:111). Small litter sizes (typically 1), and the gregarious nature of many bat species facilitates slow population growth and disease transmission, making them vulnerable to extinction (Kunz and Lumsden 2003:49–54, Schmidly and Bradley 2016:111).

White-nose syndrome (WNS), an infectious disease, affects populations of some North American bat species (Foley et al. 2011, Langwig et al. 2015, Leopardi et al. 2015). WNS is caused by the cold-tolerant fungus, *Pseudogymnoascus destructans* (Blehert et al. 2009, Lorch et al. 2011, Warnecke et al. 2012). Although considered cold-tolerant, the temperature range for *P. destructans* growth is broad, ranging from 3.0° C to 19.7° C (Verant et al. 2012), and overlapping temperatures commonly selected by hibernating bats (between 3.0° C and 15.0° C, McNab 1982:163–165, Nagel and Nagel 1991, Brack 2007, Foley et al. 2011).

WNS differentially impacts hibernating bat species, with mortality varying substantially within and between sites (Langwig et al. 2012). Some of these species, such as the little brown bat (*Myotis lucifugus*) and tri-colored bat (*Perimyotis subflavus*) are greatly afflicted with population declines greater than 90% within the first year of WNS, while others, such as the big brown bat (*Eptesicus fuscus*), had minimal population declines post-WNS (Turner et al. 2011). Myotis species in particular experienced greater population declines in comparison to non-myotis species (with the exception of tri-colored bats) within the first year of WNS (Turner et al. 2011).

The fungal causative agent of WNS is believed to have been introduced from Europe into North America (Leopardi et al. 2015), with the first documentation of the disease occurring in North America in Howes Cave near Albany, New York in February 2006 (Blehert et al. 2009, Turner and Reeder 2009). Since the first documentation of WNS in the United States, the disease has spread north into Canada and westward across the United States, with the disease documented as far west as Washington state (Foley et al. 2011, Coleman and Reichard 2014, Lorch et al. 2016, Figure 1). Although it had not yet been documented in Texas in winter 2015, the proximity of the fungus to Texas was concerning as both eastern and western North American bat species occur in Texas thereby providing a potential bridge for WNS to spread farther west and south. Because Texas is on the leading edge of the movement west of *P. destructans*, and because species from both eastern and western United States overlap in Texas, this region represents an important point of focus to suppress the spread of the disease.

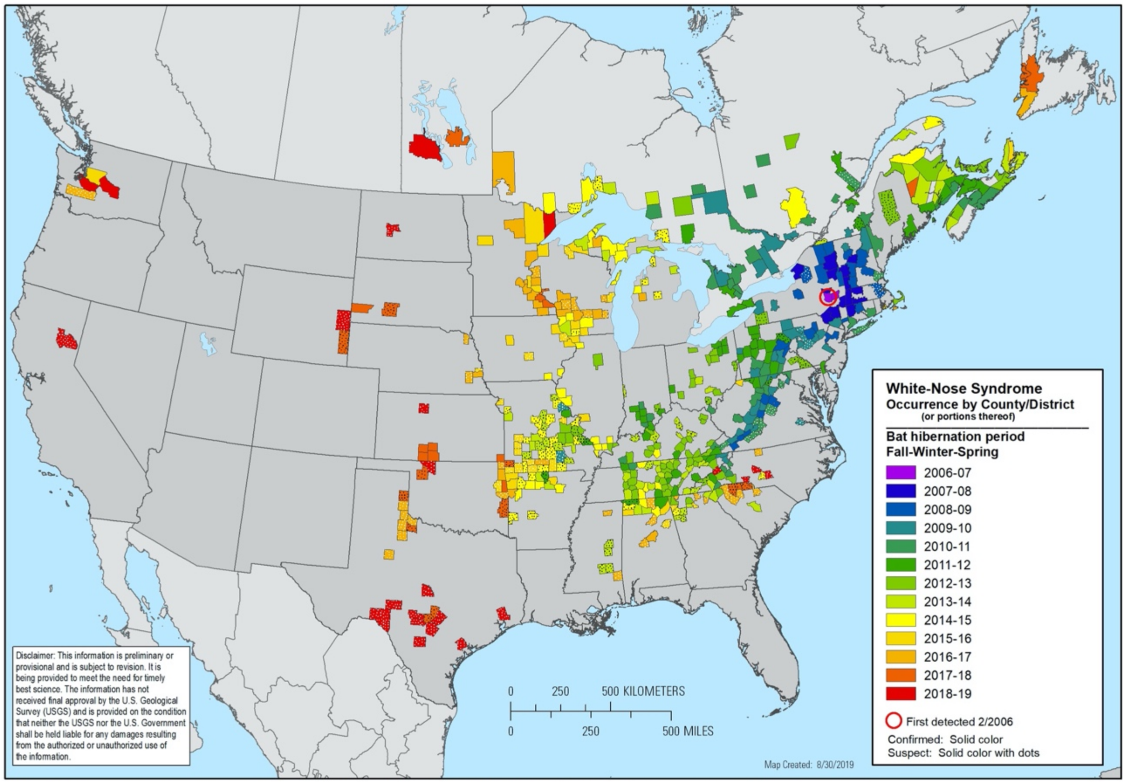


Figure 1. Map of bat white-nose syndrome occurrence by county/district as of 30 August 2019 (White-nose syndrome occurrence map – by year, 2019). Reprinted.

Four species in Texas had known susceptibility to WNS in 2015, including the big brown bat, southeastern myotis (*M. austroriparius*), northern long-eared bat (*M. septentrionalis*) and tri-colored bat (USFWS 2016). Although wrongly identified as being positive for the fungus in 2010 in Oklahoma (ODWC 2010), cave myotis (*M. velifer*) were considered a species likely to be susceptible to the disease because of how other myotis species were impacted by WNS in the northeast and due to their hibernation behaviors (e.g., clustering, Lanwig et al. 2012). Understanding that WNS differentially impacts species, with myotis species and tri-colored bats being more impacted than others (Turner et al. 2011), it is important to focus efforts on tri-colored bats and cave myotis in Texas, as opposed to other myotis species not known to be affected by WNS. Although northern long-eared bats are known to be affected by WNS, only a single specimen of this species was documented in Texas (Ammerman et al. 2012:106–107). Southeastern myotis are also known to be affected by WNS in northern states, however their winter roost selection in Texas (i.e., predominantly roosting in trees in the winter, Ammerman et al. 2012:92) and their active winter behavior (suggestive of a flexible hibernation strategy) in the coastal plains (e.g., Florida, Humphrey and Gore 1992:338–339) suggests that the effects of WNS in Texas would be less than those of the cave-dwelling tri-colored bat and cave myotis.

Once a common bat species in eastern North America, with a distribution as far west as New Mexico (Fujita and Kunz 1984), the tri-colored bat has declined in regional relative abundance by an estimated 30% to greater than 70%, primarily as a result of WNS (Foley et al. 2011, Ingersoll et al. 2013, Langwig et al. 2015). As a result of region-wide population declines, tri-colored bats are currently being reviewed for listing under the Endangered Species Act by the United States Fish and Wildlife Service (USFWS 2017). Cave myotis are another common temperate bat species found in central and western North America, as well as Central America. Unlike the tri-colored bat, cave myotis were only recently documented with *P. destructans* in 2014 (ODWC 2010), with the first bat confirmed with WNS in Kansas in 2018 (KDWPT 2018). This is likely a result of the spread of the fungus from eastern North America, where cave myotis are not found, to western North America.

Both cave myotis and tri-colored bats roost in natural (e.g., caves) and artificial (e.g., culverts) roosts during winter months (e.g., Raun and Baker 1958, Walker et al. 1996, Sandel et al. 2001, Claire et al. 2018, Meierhofer et al. 2019b). The selection of hibernacula by cave-dwelling bat species is critical for overwinter survival. Choice of hibernacula by bats may be influenced by a number of factors, including roost dimensions, thermoregulatory benefits, availability and abundance of alternative roosts, and surrounding landscape characteristics (Kunz 1982:1–55, Adam and Hays 2000, Lance et al. 2001, Briggler and Prather 2003, Meierhofer et al. 2019b). Choice of hibernacula by bats is important for overwinter survival, and may also affect potential susceptibility to WNS.

As a result of the continued spread of *P. destructan*s, tri-colored bats and cave myotis tested positive for the fungus in Texas in spring 2017 (TPWD 2017) and are now at risk of potentially developing WNS. Although any animal that comes into contact with *P. destructans* should test positive given the mode of transmission (i.e., bat-to-bat, substrate-to-bat), there are several reasons why only certain bat species are testing positive for *P. destructans*. First, it is easier to monitor the spread of *P. destructans* in common bat species with large distributions such as the cave myotis. Thus, it is more likely to document *P. destructans* in these more common species than for rare species. Second, WNS is known to only affect hibernating bat species (Blehert et al. 2009). As such, the focus of *P. destructans* and WNS-monitoring efforts focuses on subterranean roost locations, and in particular, those that contain species previously known to be affected by WNS. As a result, species that roost primarily in trees and other non-subterranean roost locations (e.g., red bat (*Lasiursus borealis*)) are not being surveyed for *P. destructans* to the extent that other subterranean roosting species are. Third, monitoring efforts for *P. destructans* focused in areas near the first site of documentation in New York. As such, bat species only found in the western United States (e.g., California myotis, *M. californicus*) have not yet been the focus of testing for *P. destructans*. Finally, access to potential roosting locations may not be permitted, reducing the potential monitoring efforts in regions predominantly privately owned, such as Texas. Monitoring known hibernacula sites that are easily accessible is both time and cost-effective, and as such, bat of certain species might not be tested for *P. destructans*.

Determining site-specific landscape and environmental characteristics predictive of presence and abundance of tri-colored bats and cave myotis in Texas is important because, in a state as large as Texas (within which over 95% of land is privately owned), identifying easily observable landscape and environmental characteristics associated with hibernacula allows researchers and wildlife managers to systematically select sites that may be more likely to have tri-colored bats and/or cave myotis present. Conditions that drive the presence of these overwintering bat species may also overlap optimal conditions for *P. destructans*, thus leading to the eventual development of WNS. Although the distributions of both tri-colored bats and cave myotis are known, much of the information used to delineate their distributions in Texas results from data gathered during summer months with minimal data collection occurring during winter months. Given that there are limited data on overwintering tri-colored bat and cave myotis populations, and the threat of the development of WNS in Texas, additional data are needed to determine the factors that are likely to predict the presence of these two species.

My goal was to understand the site-specific landscape and environmental factors that predict the presence and abundance of tri-colored bats and cave myotis in Texas. As there are species-specific differences in behavior and ecology between tri-colored bats and cave myotis (Ammerman et al. 2012:111–116,161–166), it is fair to assume that there will be differences in predictors of hibernacula use between these two species. As such, my objective was to quantify the site-specific landscape and environmental factors that best predict presence and abundance of tri-colored bats and cave myotis in Texas. By knowing the predictors of presence and abundance for these species, biologists and managers will be able to identify potential regions of Texas to best monitor for, and manage for these WNS-susceptible species prior to the potential invasion of WNS. These same predictors can be used in other parts of the species ranges, thus identifying regions at risk for WNS invasion for focused monitoring and mitigation efforts beyond Texas.

Methods

Study Area

Texas is diverse, compromising 12 Level III ecoregions, which exhibit variation in landscape, and environment (Griffith et al. 2004, Griffith et al. 2007, USEPA 2013). I focused my sampling efforts to the northern and eastern regions of Texas, which was closest to the known location of WNS positive sites (Heffernan 2015), and expanded out across the state. I conducted surveys across the following 10 Texas Level III ecoregions: Central Great Plains (6,807 km^2), Chihuahuan Deserts (91,576 km^2), Cross Timbers (51,917 km^2), East Central Texas Plains (54,774 km^2), Edwards Plateau (74,964 km^2), High Plains (84,829 km^2), South Central Plains (63,670.22 km^2), Southwestern Tablelands (60,314 km^2), Texas Blackland Prairies (43,382 km^2), and Western Gulf Coastal Plain (60,752 km^2) (Figure 2).

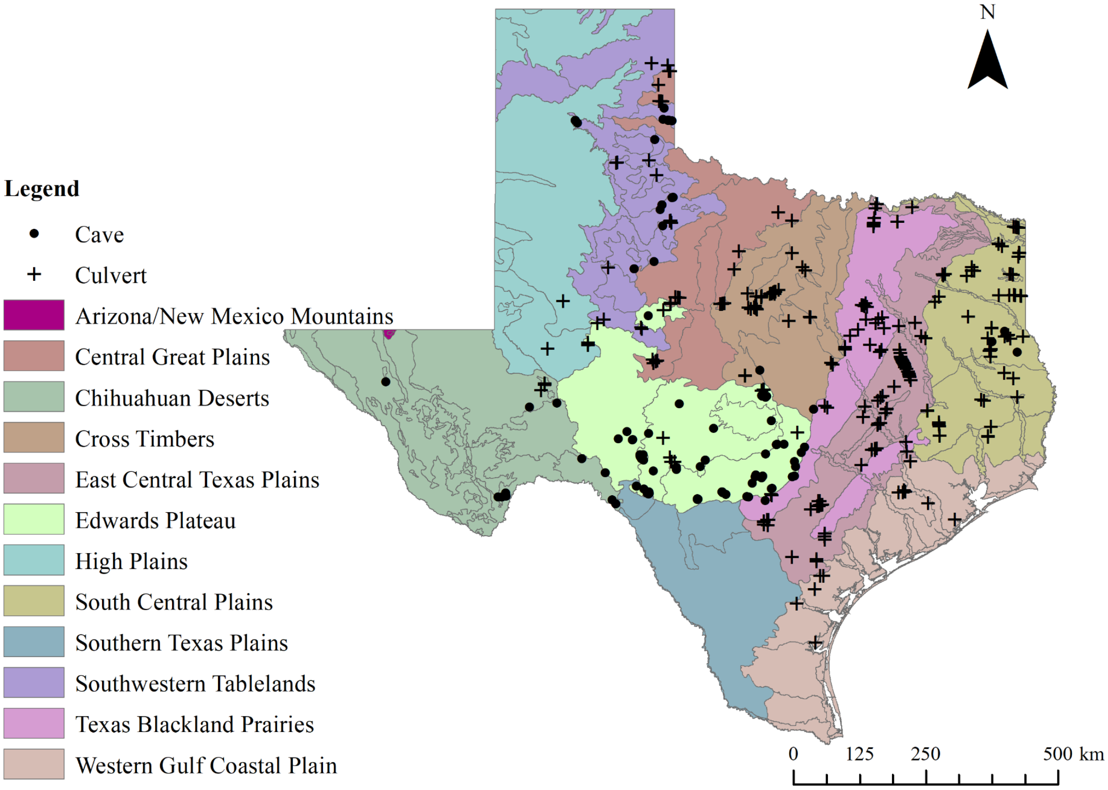


Figure 2. Distribution of 450 subterranean sites (145 caves and 305 culverts) surveyed for overwintering tri-colored bats (*Perimyotis subflavus*) and cave myotis (*Myotis velifer*) within their known range from January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019 across 10 Level III Texas.

Site Selection

I used the Generalized Random Tessellation Stratified (GRTS) design (Stevens and Olsen 2004) of the North American Bat Monitoring Program (NABat) to establish a stratified random sampling approach to select culverts for sampling across Texas. NABat developed a multi-purpose sampling frame consisting of 10 x 10 km (100 km^2) grid cell sample units that are the focal analytical unit for regional and range-wide assessments. These grid cells provided a standardized sampling system and allowed data collected to link back to the NABat database. I randomly selected grid cells within each Level III ecoregion. Furthermore, I monitored sites occupied by bats once every year when feasible whereas I did not revisit unoccupied sites (i.e., sites with no signs of bats, guano, or roost stains.

After the selection of an initial set of potential sampling grid cells, I screened each grid cell for the presence of potential bat roosts (i.e., culverts and caves) using ArcGIS 10.4.1 (ESRI 2015) and Google Earth Pro 7.3.2 (Google 2018). I obtained information from the Texas Speleological Survey (TSS) database on the presence of and types of caves. Texas Department of Transportation (TxDOT) provided a comprehensive list of all culverts for Texas. I used Google Earth to identify other structures not within the TSS database or TxDOT database. I attempted to gain access to sites within each grid cell initially selected. However, I did not always gain access at site(s) within each grid cell as access was not always granted by landowners due to safety concerns or because it was not physically possible to survey the site (e.g., the opening to the cave was too small for a person to enter). To avoid potential bias, I continued to the next random site for sampling. Thus, I only sampled all potential sites within some grid cells. Additionally, I visited any known, historic roost/hibernacula within an ecoregion to ensure I did not exclude potentially important sites through our random selection of cells. I gathered information about historic roost/hibernacula from information noted in the TSS database, from Bat Conservation International (BCI), and from public knowledge.

Data Collection

I conducted surveys for overwintering tri-colored bats and cave myotis from January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019. As some sites were resurveyed whereas others were not (either due to access or because it was the last year of field work), I retained data from the last survey for each location resulting in 450 sites (145 caves and 305 culverts). I visually surveyed 145 caves and 305 culverts for overwintering tri-colored bats and cave myotis. At each site visited, I recorded whether tri-colored bats and cave myotis were present (yes or no), and conducted a complete census by visually surveying all caves and culverts for presence and abundance of bats. If I was unable to count bats individually (i.e., in the case of large clusters of cave myotis), I estimated the total count of bats by counting the number of bats in a square foot and multiplying that over the total area of the cluster. To reduce disturbance, I collected all data without any animal handling. To my knowledge, no bats left the site during survey efforts. I was confident in my identification abilities as all bats documented were morphologically distinct within their range. I decontaminated all equipment and persons between surveys and sites following the most updated WNS decontamination protocol (USFWS 2016).

I used 30-meter resolution data layers pulled into ArcGIS 10.4.1 (ESRI 2015) to obtain site-specific landscape and environmental data for all surveyed sites. I obtained aspect, slope, and elevation data from Digital Elevation Models (DEM) in ArcGIS 10.4.1. I obtained data on percent canopy cover, minimum temperature, and maximum temperature from the National Land Cover Database (NLCD). I calculated Euclidean distance to nearest permanent water source from each site using the Euclidean distance tool in ArcGIS. I chose Euclidean distance because some species select roosts close to water, and this method can provide insight on animal-landscape associations (Ormsbee and McComb 1998, Sedgeley and O'Donnell 1999, Boonman 2000, Conner et al. 2003). From the temperature layers, I used the Raster Calculator in ArcGIS to calculate mean minimum and maximum temperature and associated standard deviations for each month of the survey period (January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019). I calculated the sine and cosine of aspect to transform them into north/south and east/west components (Jenness 2007). I then extracted data based on the latitude and longitude of each surveyed site (Table 1). Research on bats followed ASM guidelines (Sikes et al. 2016) and was approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC 2015-0296).

Table 1. Descriptions, units of measure, and means (min–max) of site-specific landscape and environmental measures obtained from the coordinates of all sites surveyed for bats for analysis.

Variable	Definition	Mean (min–max)
Slope	Slope of survey site; degrees	4.0 (0.0–44.4)
Elevation	Elevation of survey site; m	331.2 (3.0–1814.0)
Distance to water	Euclidean distance to nearest water source; m	678.0 (0.0–4742.4)
Canopy	Canopy cover; %	29.7 (0.0–98.0)
Mean maximum temperature	Mean maximum monthly temperature for survey months, ºC	61.4 (51.6–82.6)
Mean maximum temperature (standard deviation)	Associated standard deviation of mean maximum temperature for survey months	1.4 (0.3–4.1)
Mean minimum temperature	Mean minimum monthly temperature for survey months, ºC	36.5 (24.6–59.6)
Mean minimum temperature (standard deviation)	Associated standard deviation of mean minimum temperature for survey months	1.3 (0.2–3.8)
Sine aspect	East-west component	-0.5 (-1.0–1.0)
Cosine aspect	North-south component	0.5 (-1.0–1.0)

Data Analysis

I conducted all analyses in R 3.4.1 (R Core Team 2018). I tested collinearity of site-specific landscape and environmental factors discussed above using variance inflation factor (VIF). Variance inflation factors suggested that the variables mean minimum and maximum temperature and associated standard deviations showed multicollinearity (VIF > 10) (Meyers 1990:131–132). Thus, to transform the site-specific landscape and environmental independent factors into a smaller number of uncorrelated factors, and to standardize all factors before analysis, I used a Principal Components Analysis (PCA) using the princomp function in the ‘MASS’ package in R

(Ripley et al. 2011). PCA is a factor model in which Principal Components (PCs) are based on summarizing the total variance through which the process usually yields smaller, more compact number of output components (Boslaugh 2013:291). I selected created PCs based on the following two criteria: the PC had an eigenvalue > 1 and the PC contained independent factors with loadings greater than 0.364 (Fields et al. 2012:762,767).

For analysis of abundance data for tri-colored bats and cave myotis, I analyzed all 450 surveyed sites. Prior to analysis, I tested data for zero-inflation using a simple score test (van den Broek 1995) for excess zeros using the zero.test function in R. Abundance data (i.e., total count data) were zero-inflated ($P < 0.001$) and overdispersed. Thus, I analyzed data for both tri-colored bats and cave myotis using a zero-inflated Poisson regression because it is used for count data that exhibit overdispersion and excess zeros (Borcard et al. 2011:269, Lambert 1992). In order to approximate the proportion of variance explained by each model, I calculated a *pseudo* R^2 value using the deviance of the null model minus the deviance of the candidate model divided by the deviance of the null model (Zuur et al. 2009:218).

For analysis of presence/absence data for tri-colored bats and cave myotis, I focused initially on ecoregions where each species was present, removing ecoregions with only absence data. I chose to remove ecoregions where only absence data were collected as these data may not reflect true absence within their range. As such, I combined presence/absence data for the ecoregions in which each species were primarily found (tri-colored bats: Edwards Plateau, East Central Texas Plains, South Central Plains, and cave myotis: Edwards Plateau, Southwestern Tablelands) and investigated how PCs predict the presence of each species using logistic regression using the 'glm' function in package 'MASS' in R (Ripley et al. 2011). Logistic regression is used when the outcome variable is dichotomous; in this case, presence/absence of each bat species (tri-colored bat or cave myotis) (Fields et al. 2012:313). Furthermore, as not all ecoregions contained tri-colored bats and/or cave myotis, I ran individual logistic regression analyses on each ecoregion for each species to determine how PCs predict tri-colored bat and cave myotis presence. It is important to look at individual occupied ecoregions as each ecoregion exhibits variation in landscape, and environment (Griffith et al. 2004, Griffith et al. 2007, USEPA 2013), and thus may have different predictors driving presence of bat species. I calculated estimated odds ratio for each factor, which indicated the change in the probability of tri-colored bat occupancy or cave myotis occupancy in sites that would result from a one-unit change in the value of the indicated variable. I used McFadden's *pseudo* R^2 test to check the model's overall goodness-of-fit (Smith and McKenna 2013). I evaluated the reliability and validity of my models as fair ($0.50 < AUC \leq 0.75$), good ($0.75 < AUC \leq 0.92$), very good ($0.92 < AUC \leq 0.97$), or excellent ($0.97 < AUC \leq 1.00$) based on the value of AUC (Hosmer et al. 2013:161). I considered *P*-values of < 0.05 significant for all tests.

Results

Description of Principal Components Retained for Analysis in the Zero-inflated Poisson Regression Analyses and Logistic Regression Analyses

PC1 explained 31.1% variance and described low external site temperatures, with negative loadings for the mean maximum and minimum temperatures and their associated standard deviations (Table 2). PC2 explained 19.4% variance and described the topography of the site, being positively loaded by slope, elevation, and distance to water. PC3 explained 12.2% variance and was positively loaded by canopy cover. PC4 explained 10.8% and described sites with an easterly aspect, being positively loaded by the sine of aspect. The cumulative percentage explained by the four selected PCs described 73.4% of the variance in the data (Table 2).

Table 2. Principal components analysis on all 10 factors to produce principal components (PCs) that account for site-specific landscape and environmental characteristics associated with tri-colored bat (*Perimyotis subflavus*) and cave myotis (*Myotis velifer*) presence and abundance. Values represent the loadings (or the percent of variance in the PC explained by the factor) for each of the 10 environmental factors. Bold values represent factors with loadings greater than 0.364 (Fields et al. 2012:767). — indicates that the variable did not load on the PC.

	PC1	**PC2**	**PC3**	**PC4**
Slope (%)	—	**0.548**	0.314	—
Elevation (m)	0.203	**0.542**	-0.304	—
Euclidean distance to water (m)	—	**0.564**	0.135	0.123
Canopy cover (%)	—	—	**0.687**	-0.280
Mean max temp. (° C)	**-0.492**	0.142	0.175	0.111
Mean max temp. S.D.	**-0.483**	0.130	-0.327	-0.140
Mean min temp. (° C)	**-0.497**	—	0.265	0.120
Mean min temp. S.D.	**-0.478**	0.114	-0.331	-0.152
Sine of Aspect	—	—	—	**0.836**
Cosine of Aspect	—	0.166	—	0.361
% variance explained	31.1%	19.4%	12.2%	10.8%

Results of Site Surveys and Zero-inflated Poisson Regression for Tri-colored Bats and Cave Myotis Abundance for All Data

I found 91 of 450 sites (20.2%, 54 culverts and 37 caves) occupied by tri-colored bats (Figure 3) and 28 of 450 sites (6.2%, 25 caves and 3 culverts) occupied by cave myotis (Figure 4).

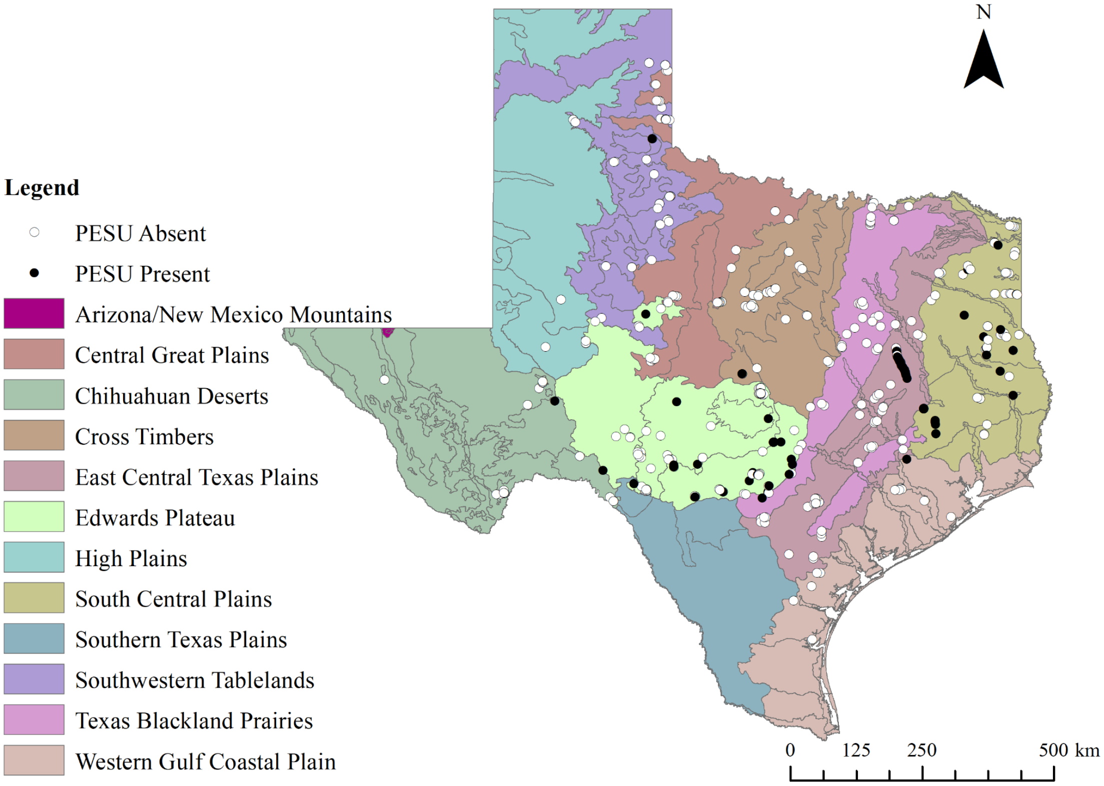


Figure 3. Distribution of 450 subterranean sites (145 caves and 305 culverts) surveyed for tri-colored bats (*Perimyotis subflavus*) within the known range from January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019 across 10 Level III Texas ecoregions. PESU = tri-colored bat.

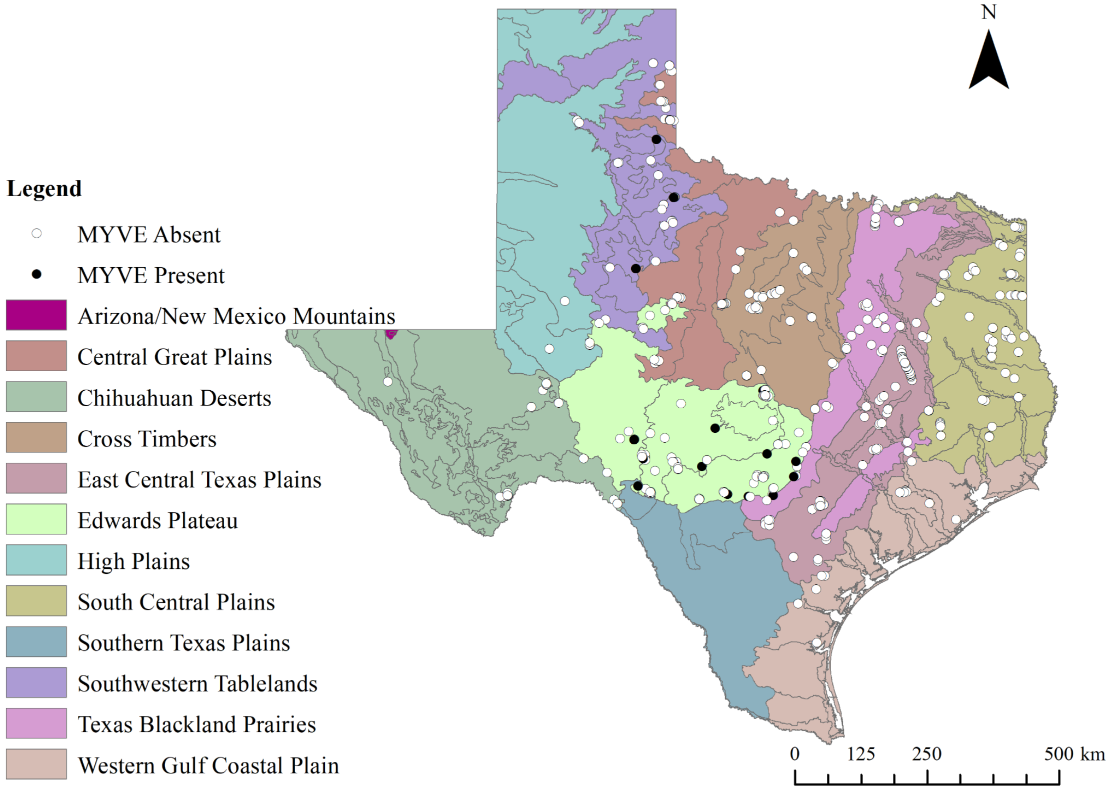


Figure 4. Distribution of 450 subterranean sites (145 caves and 305 culverts) surveyed for cave myotis (*Myotis velifer*) within the known range from January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019 across 10 Level III Texas ecoregions. MYVE = cave myotis.

Across the sites where tri-colored bats were present, I counted 5,002 overwintering tri-colored bats ($\bar{x} + SD = 12.86 \pm 84.39$, range = 1,130). All PCs were predictive of tri-colored bat abundance. However, PC2 (slope, elevation, and distance to water) and PC4 (aspect) were the driving predictors of the model because of the relative coefficient size.

Results of the zero-inflated Poisson model suggest that as slope, elevation, distance to water (PC2) decreased by one unit, tri-colored bat abundance increased by

0.47 (Table 3). My results also suggest that tri-colored bat abundance increased by 0.36 as site aspect became more easterly (PC4).

Table 3. Results of the zero-inflated Poisson regression analysis for tri-colored bat (*Perimyotis subflavus*) presence and abundance across 10 Level III Texas ecoregions. Table contains principal components (variable: PCs), coefficients, standard errors (*SE*), *z* values and associated *P*s. PESU = tri-colored bat. Significant predictors ($P < 0.05$) are in bold.

	Variable	**Coefficient**	***SE***	***Z* value**	***P***
PESU Abundance	PC1	0.09	0.01	9.38	**< 0.05**
	PC2	-0.47	0.02	-25.52	**< 0.05**
	PC3	-0.07	0.01	-4.87	**< 0.05**
	PC4	0.36	0.02	22.04	**< 0.05**

Table 4. Results of the zero-inflated Poisson regression analysis for cave myotis (*Myotis velifer*) abundance across 10 Level III Texas ecoregions. Table contains principal components (variable: PCs), coefficients, standard errors (*SE*), *z* values and associated *P*s. MYVE = cave myotis. Significant predictors ($P < 0.05$) are in bold.

	Variable	**Coefficient**	***SE***	***Z* value**	***P***
MYVE Abundance	PC1	-0.01	0.01	-1.02	0.31
	PC2	0.32	0.02	13.79	**< 0.05**
	PC3	-0.42	0.02	-20.90	**< 0.05**
	PC4	0.52	0.02	26.64	**< 0.05**

Across the sites where cave myotis were present, I counted 3,640 overwintering cave myotis ($\bar{x} + SD = 11.59 \pm 70.36$, range = 699). Results suggested that cave myotis abundance increased by 0.32 as slope, elevation, and distance to water (PC2) increased by one unit (Table 4). Results also suggested as canopy cover (PC3) decreases one unit, cave myotis abundance increases by 0.42. My results also suggested that cave myotis abundance increased as site aspect became more easterly (PC4). The model explained approximately 21% of the deviation in cave myotis presence and abundance (*pseudo* $R^2 = 0.21$).

Logistic Regression Analyses on Tri-colored Bat and Cave Myotis Presence/Absence for Only Ecoregions in which Species were Present

I found 82 of 249 sites (32.9%, 29 caves and 53 culverts) occupied by tri-colored bats across the East Central Texas Plains, Edwards Plateau, and South Central Plains (Figure 3). Results suggested that no PCs predicted tri-colored bat presence (all Ps > 0.05, *pseudo* $R^2 = 0.02$). The AUC score of the model was .45 indicating a fair ability to discriminate between presence and absence of bats.

I found 27 of 154 sites (17.5%, 25 caves and 2 culverts) occupied by cave myotis across the Edwards Plateau and Southwestern Tablelands (17.5%, 25 caves and 2 culverts) (Figure 4). Results suggested that no PCs predicted cave myotis presence (all Ps > 0.05, *pseudo* $R^2 = 0.03$). The AUC score of the model was .47 indicating a fair ability to discriminate between presence and absence of bats.

Logistic Regression Analysis on Tri-colored Bat and Cave Myotis Presence/Absence for Individual Ecoregions in which Species were Present

I found 34 of 87 sites (39.1%, 0 caves and 34 culverts) occupied by tri-colored bats across the East Central Texas Plains (Figure 3). Results suggested as temperature increases by one unit (PC1, $P < 0.05$), and as slope, elevation, and distance to water increased by one unit (PC2, $P < 0.05$), probability of detecting tri-colored bat presence increased by 0.49 and 41.71 respectively (*pseudo* $R^2 = 0.29$, Table 5). Results further suggested that as canopy cover decreased by one unit (PC3, $P < 0.05$), and as aspect became more easterly (PC4, $P < 0.05$), probability of detecting tri-colored bat presence increased by 0.44 and 4.24 respectively (*pseudo* $R^2 = 0.29$, Table 5). However, based on coefficient sizes, slope, elevation, and distance to water (PC2) and aspect (PC4) were likely the driving predictors of detecting tri-colored bats (Table 5). The AUC of the model was .15, indicating a fair ability to discriminate between presence and absence of bats.

I found 29 of 105 sites (27.6%, 27 caves and 2 culverts) occupied by tri-colored bats across the Edwards Plateau (Figure 3). Results suggested that as slope, elevation, and distance to water decreased by one unit (PC2, $P < 0.05$), probability of detecting tri-colored bats increased by 0.54 (*pseudo* $R^2 = 0.08$, Table 6). The AUC score of the model was .44 indicating a fair ability to discriminate between presence and absence of bats.

I found 19 of 57 sites (33.3%, 2 caves and 17 culverts) occupied by tri-colored bats across the South Central Plains (Figure 3). Results suggested that as slope, elevation, and distance to water increased by one unit (PC2, $P = 0.05$), probability of detecting tri-colored bats increased by 13.97 (*pseudo* $R^2 = 0.13$, Table 7). The AUC score of the model was .43 indicating a fair ability to discriminate between presence and absence of bats.

Table 5. Results of a logistic regression analysis for tri-colored bat (*Perimyotis subflavus*) occupancy of sites within the East Central Texas Plains ecoregion. Table contains principal components (variable: PCs), coefficients, standard errors (*SE*), odds ratio, odds ratio 95% confidence interval, and associated *P*s. *The estimated odds ratio indicates the change in the probability of tri-colored bat presence at sites resulting from a 1-unit change in the value of the indicated variable. Significant predictors ($P < 0.05$) are in bold.

Variable (Principal Components)	**Coefficient**	**SE**	**Odds ratio**	**Odds ratio 95% C.I.***		***P***
				Lower	**Upper**	
PC1	-0.70	0.20	0.49	0.31	0.72	**< 0.05**
PC2	3.73	0.95	41.71	7.64	332.28	**< 0.05**
PC3	-0.83	0.38	0.44	0.20	0.89	**< 0.05**
PC4	1.44	0.41	4.24	2.07	10.42	**< 0.05**

Table 6. Results of a logistic regression analysis for tri-colored bat (*Perimyotis subflavus*) occupancy of sites within the Edwards Plateau ecoregion. Table contains principal components (variable: PCs), coefficients, standard errors (*SE*), odds ratio, odds ratio 95% confidence interval, and associated *P*s. *The estimated odds ratio indicates the change in the probability of tri-colored bat presence at sites resulting from a 1-unit change in the value of the indicated variable. Significant predictors ($P < 0.05$) are in bold.

Variable (Principal Components)	**Coefficient**	**SE**	**Odds ratio**	**Odds ratio 95% C.I.***		***P***
				Lower	**Upper**	
PC1	-0.06	0.15	0.94	0.69	1.24	0.67
PC2	-0.61	0.22	0.54	0.34	0.82	**< 0.05**
PC3	0.36	0.24	1.43	0.91	2.31	0.13
PC4	0.08	0.23	1.09	0.69	1.71	0.72

Table 7. Results of a logistic regression analysis for tri-colored bat (*Perimyotis subflavus*) occupancy of sites within the South Central Plains ecoregion. Table contains principal components (variable: PCs), coefficients, standard errors (*SE*), odds ratio, odds ratio 95% confidence interval, and associated *P*s. *The estimated odds ratio indicates the change in the probability of tri-colored bat presence at sites resulting from a 1-unit change in the value of the indicated variable. Significant predictors ($P < 0.05$) are in bold.

Variable (Principal Components)	**Coefficient**	**SE**	**Odds ratio**	**Odds ratio 95% C.I.***		***P***
				Lower	**Upper**	
PC1	-0.32	0.29	0.73	0.39	1.26	0.28
PC2	2.64	1.03	13.97	2.19	132.60	**< 0.05**
PC3	0.01	0.41	1.01	0.45	2.29	0.97
PC4	0.29	0.36	1.34	0.66	2.80	0.42

Of the 105 sites surveyed for cave myotis across the Edwards Plateau, I found 14 sites (13.3%, 2 caves and 12 culverts) occupied by cave myotis (Figure 4). Results of the logistic regression suggest that as temperatures decreases by one unit (PC1, $P < 0.01$), probability of detecting cave myotis increases by 1.76 (*pseudo* $R^2 = 0.15$, Table 8). The AUC score of the model was .26 indicating a fair ability to discriminate between presence and absence of bats.

Of the 49 sites surveyed for cave myotis across the Southwestern Tablelands, I found 13 sites (26.5%, 13 caves and 0 culverts) occupied by cave myotis (Figure 4). Results of the logistic regression suggest that as canopy cover increases by one unit (PC3, $P < 0.05$), probability of detecting cave myotis increases by 2.86 (*pseudo* $R^2 = 0.11$, Table 9). The AUC score of the model was .32 indicating a fair ability to discriminate between presence and absence of bats.

Table 8. Results of a logistic regression analysis of cave myotis (*Myotis velifer*) occupancy of sites within the Edwards Plateau ecoregion. Table contains principal components (variable: PCs), coefficients, standard errors (*SE*), odds ratio, odds ratio 95% confidence interval, and associated *P*s. *The estimated odds ratio indicates the change in the probability of cave myotis presence at sites resulting from a 1-unit change in the value of the indicated variable. Significant predictors ($P < 0.05$) are in bold.

Variable (Principal Components)	**Coefficient**	**SE**	**Odds ratio**	**Odds ratio 95% C.I.***		***P***
				Lower	**Upper**	
PC1	0.56	0.18	1.76	1.24	2.61	**< 0.05**
PC2	0.37	0.26	1.45	0.87	2.46	0.15
PC3	0.05	0.30	1.05	0.58	1.91	0.86
PC4	-0.25	0.33	0.78	0.39	1.46	0.44

Table 9. Potential determinants of cave myotis (*Myotis velifer*) occupancy of sites within the Southwestern Tablelands ecoregion. Table contains principal components (variable: PCs), coefficients, standard errors (SE), odds ratio, odds ratio 95% confidence interval, and associated *P*s. *The estimated odds ratio indicates the change in the probability of cave myotis presence at sites resulting from a 1-unit change in the value of the indicated variable. Significant predictors ($P < 0.05$) are in bold.

Variable (Principal Components)	**Coefficient**	**SE**	**Odds ratio**	**Odds ratio 95% C.I.***		***P***
				Lower	**Upper**	
PC1	0.10	0.29	1.11	0.59	1.94	0.73
PC2	-0.01	0.66	0.99	0.27	3.69	0.99
PC3	1.05	0.52	2.86	1.14	9.09	**< 0.05**
PC4	-0.11	0.37	0.90	0.43	1.86	0.77

Discussion

As hypothesized, my research shows that site-specific landscape and environmental characteristics of caves and culverts influence tri-colored bat and cave myotis presence and abundance, and that predictors for presence and abundance differ between species. Furthermore, these differences were also apparent for ecoregion-specific analyses.

Factors Influencing Tri-colored Bat Presence and Absence

Analyzing my data on East Central Texas Plains, I found that the probability of predicting tri-colored bat increased by 1 as canopy cover decreased by one unit. These results complimented previous research that found that occurrence of tri-colored bats decreased with forest cover (Starbuck et al. 2015). Similarly, Ford and colleagues (2006) found that tri-colored bats were more likely to be found in open areas. Caves generally are found in areas of both open and closed vegetation, but culverts are often found in highly urbanized areas, with reduced vegetation around the culverts. Indeed, 54 of the 91 sites where I documented tri-colored bats were culverts and all sites surveyed within East Central Texas Plains were culverts. Therefore, location of sites surveyed within the state (i.e., East Central Texas Plains ecoregion), and the type of site (i.e., cave versus culvert) were not mutually exclusive and thus were driving factors for the results of my analyses. Alternatively, reduced vegetation around sites may make them easier to locate, enter, and navigate.

In addition to the influence of canopy on predicting tri-colored bat presence in the East Central Texas Plains, the probability of tri-colored bat presence increased by 42 when slope, elevation, and distance to water increased by one unit. The probability of tri-colored bat presence increased by 4 when aspects became more easterly. However, based on coefficient sizes, increased slope, elevation, and distance to water and easterly aspects are likely the driving predictors of tri-colored bat presence in the East Central Texas Plains, with findings driven by the type and location of sites occupied by tri-colored bats. Specifically, I only surveyed for tri-colored bats in culverts in the East Central Texas Plains as no known caves exist within that ecoregion due to the lack of karst, or other parent material, from which caves are formed, or mines. The culverts in which I primarily found tri-colored bats roosting were along a highway that runs north to south, and most culverts were located with entrances orientated east to west. Elevation had little variation within this region. Furthermore, as culverts assist in drainage by allowing water to flow under roads, distance to an external water source may not be as important when water is present within these culverts. Although previous research has indicated that proximity to water can influence tri-colored bat presence (Ford et al. 2006), the GIS layer from which distance to water was calculated only considered large, permanent water sources and did not include ephemeral or small water sources (e.g., streams and ponds). Indeed, the role of large water sources may not be as important in some regions where small or seasonal water sources may act as a source of water for bat species. For instance, because culverts assist in drainage, culvert-roosting tri-colored bats have a local water source readily accessible, thus potentially rendering large water sources as less important. Indeed, East Central Texas Plains receives on average 28–40 inches of rain annually, and with the dense clay pan under the soil (Griffith et al. 2007), this water may collect in ephemeral pools near and around culverts. As such, I think the purported influence of slope, elevation, distance to water, and aspect may be an artefact of the highway direction and location (Meierhofer et al. 2019b). Similarly, individual analysis of tri-colored bats in the South Central Plains suggested that increased slope, elevation, and distance to water increased the probability of presence of tri-colored bats in the South Central Plains. Indeed, the increased distance to permanent water sources does not take into consideration ephemeral water sources, which may be common in an ecoregion which receives 40–58 inches of mean annual rainfall (Griffith et al. 2007). Again, many of the sites surveyed within this ecoregion were culverts (3 caves versus 54 culverts), which are associated with water, and elevation and slope for sites surveyed within this ecoregion had little variation.

Contrary to the predictors of presence for tri-colored bats in the East Central Texas Plains and South Central Plains, individual analysis of tri-colored bats in the Edwards Plateau suggested that the probability of presence increased by 1 with a decrease in slope, elevation, and distance to water by one unit. It is known that tri-colored bats are commonly found in regions associated with low elevations (Ammerman et al. 2012:162). Although the winter activity of tri-colored bats in Texas is unknown, shorter distances to water may be important for overwinter survival if these bats are foraging on the landscape. The Edwards Plateau is in central Texas where temperatures are mild in comparison to ecoregions farther north (Griffith et al. 2004). Indeed, Perry (2013) reported mean annual surface temperatures (MAST) in Texas ranging from 12.0° C to greater than 21.0° C, exceeding 10° C (temperatures below which are optimal for hibernation) suggesting that some bats may not be hibernating in southern regions like Texas. If tri-colored bats are foraging in Texas in winter as a result of the mild winters and warm MAST (Perry 2013) a reduced distance to water may be important for foraging and drinking (Krusic et al. 1996, Brooks 2009).

Factors Influencing Tri-colored Bat Abundance

Results of my state-wide analysis suggested that as slope and elevation decrease by one unit, tri-colored bat abundance increases by 1. This supports previous research findings as tri-colored bats are commonly found in regions in bottomland streams and forest flyways, which are associated with low elevations (Ammerman et al. 2012:162). Similar to tri-colored bat presence, abundance was driven by location within the state based on parent soil material for formation of caves and location of roads for culverts. I documented the largest colonies of tri-colored bats in culverts in east Texas (Meierhofer et al. 2019b), a region in which slope and elevation are low compared to central Texas. Thus, these culvert sites affected the results of my analysis. Another potential reason why more tri-colored bats were found in areas of low slope and elevation is foraging behavior. If tri-colored bats actively forage on the landscape in winter in Texas, as has been recorded in other states (e.g., Grider et al. 2016), tri-colored bats may be selecting for regions of low elevation as insect abundance increases at low elevations (Grindal and Brigham 1999). However, foraging behavior may also be influenced by roost availability and location (Kunz 1982:42–43, Brigham 1991).

I found that tri-colored bat abundance increased by 1 as distance to water decreased by one unit. Water is an important resource for survival, and aggregations of tri-colored bats may select for sites near water for overwinter survival. Indeed, some caves are formed by the dissolution of limestone by water, often containing water within or nearby (Perry 2013), and culverts are associated with drainage, often built in creek beds. During a study of tri-colored bat presence during summer months, Ford and colleagues (2006) found the probability of detecting tri-colored bats increased in areas closer to a water source. If tri-colored bats are active on the landscape in Texas as a result of the mild winters and warm (> 10° C MAST, Perry 2013), or during arousals from torpor (Davis 1970:265–300), a reduced distance to water may be important for foraging during winter months (Krusic et al. 1996, Brooks 2009).

Results of my analysis of tri-colored bat abundance further suggested that tri-colored bat abundance increased by 1 as aspects of sites became more easterly. Although these findings are consistent with other studies (Briggler and Prather 2003; Meierhofer et al. 2019b), this finding may be a result of the large populations of tri-colored bats occupying culverts along a road running north to south with east/west entrances (Meierhofer et al. 2019b). Thus, these results may be a result of the structural differences with the sites (e.g., culverts) rather than preference for the feature (i.e., east aspect).

Factors Influencing Cave Myotis Presence and Absence

I found that the probability of detecting a cave myotis in the Edwards Plateau increased by 2 as temperature decreases by one unit. Cave myotis are commonly found in cold temperatures, ranging between 5 to 20° C (Webb et al. 1995, Meierhofer et al. 2019a). Perry (2013) suggested that temperatures less than 10° C are most suitable for hibernation. Thus, selection of sites where temperatures are suitable for maintaining metabolic rates optimal for energy conservation is important for bat survival (Geiser 2004).

I found that the probability of detecting a cave myotis in the Southwestern Tablelands increased by 3 as canopy cover increased by one unit. While foraging, cave myotis generally fly close to, or in areas of, dense vegetation (Fitch et al. 1981). Although foraging may not occur often during winter months, the ability to forage may be important in regions where winters are mild, such as in Texas. Alternatively, hibernacula may be selected with increased canopy cover to provide close foraging opportunities for spring emergence from hibernation when bats need to replenish their reserves (Ammerman et al. 2012:22). Furthermore, forest cover may reduce MAST (Blackwell et al. 1980), thus reducing surface temperatures and in doing so, reduce internal hibernacula temperatures. Indeed, cave myotis are sometimes found in cold temperatures during winter dependent upon their location (Webb et al. 1995; Meierhofer et al. 2019a).

Factors Influencing Cave Myotis Abundance

Results of my statewide analysis suggested that cave myotis abundance increased by 1 with a one unit decrease in canopy cover. The model explained 21 percent of the deviation in cave myotis abundance, however, the amount of variance in the data explained by canopy cover was 12.2 percent. Although forest cover may reduce MAST (Blackwell et al. 1980) thereby providing sites with temperatures suitable for hibernation (< 10° C, Perry 2013), the amount of variance explained by reduced canopy cover was small. Similar to tri-colored bats, cave myotis may also be selecting caves in areas of reduced vegetation as they may be easier to locate and access.

Similar to tri-colored bat abundance increased at sites with more easterly aspects, results from my analysis suggested that cave myotis abundance increased by 1 as aspect becomes more easterly. Cave myotis may be selecting for sites with easterly aspects because of availability (i.e., available sites had entrances with easterly aspects) or other features that the site may provide regardless of aspect (e.g., increased airflow, length). Alternatively, there may be differences in the thermal mass of these occupied sites, whereby having an eastern aspect may provide suitable internal microclimates for bats during the winter. Further research should investigate whether aspect is a biologically relevant predictor of cave myotis abundance or if it is a byproduct of the sites available and surveyed.

Contrary to a reduction in distance to water predicting the probability of occurrence of cave myotis, my state-wide model found that as distance to water increased by one unit, cave myotis abundance increased by 1. Unfortunately, minimal information exists on hibernation ecology of cave myotis, but it is suggested that cave myotis select hibernacula that have high humidity and/or standing water in Texas (Tinkle and Patterson 1965), suggesting the importance of ephemeral water sources. Thus, large sources of water, such as those considered in this study, may not be as important for this species when small sources of water may be readily accessible. Alternatively, Kunz (1974) found that for summer colonies, colony size affected dispersal distances with cave myotis dispersing farther at night to forage to reduce intraspecific competition. Although a summer study, this may apply to winter in Texas where MAST is warm (> 10° C, Perry 2013), which may result in increased winter activity. Indeed, Tinkle and Patterson (1965) noted that cave myotis in north Texas moved frequently within and between sites during winter months. Thus, cave myotis in Texas use roosts of certain characteristics that occur across various environmental conditions.

My study included several potential limitations. Survey date may have influenced the presence and abundance of bats within hibernacula. A study conducted by Sandel and colleagues (2001) noted varying number of tri-colored bats occupying culverts during winter months, with greater abundance of bats between December and January. Similarly, cave myotis have been document to vary in population size within a winter period and across years (Tinkle and Patterson 1965, Claire et al. 2018). Due to logistical constraints, I was unable to conduct all surveys between December and January within the same month, potentially resulting in some bias during survey. Another potential factor that affected the resulting models was the inclusion of historic cave roost sites within analyses. Unfortunately, the initial lack of knowledge of the distribution of caves throughout the state of Texas limited my ability to randomly select sites for survey from those present. As such, inclusion of all sites where access was gained was important to increase the likelihood of having sites occupied by bats for analyses. Finally, another caveat of the study was initial variable selection. Although all variables were selected for their biological relevance to bats and roosts, these variables were gathered across a broad region, and in some instances, averaged for a given month of survey. This, in turn, may have affected the results of the analyses. For example, the variable of temperature was gathered as mean monthly minimum and maximum as surveys of potential bat hibernacula occurred across the span of several months. However, minimal fluctuations (e.g., daily fluctuations) in temperature may impact presence of bats (e.g., Tinkle and Patterson 1965, Sandel et al. 2001). Despite the limitations, increased data collection at new sites and repeat surveys within a shorter period of time may improve model output. Indeed, increasing site surveys will also allow for the ability to analyze the impact of different site-specific landscape and environmental factors on bat presence at different site types.

Management Recommendations and Future Research

Understanding site-specific landscape and environmental predictors of tri-colored bat and cave myotis presence/absence and abundance is essential for identifying regions in Texas to better focus survey efforts, and identify important colonies for continued monitoring and management efforts. With the continued spread of *P. destructans* and WNS, biologist and land managers are limited by time and financial constraints, and thus must identify ways in which they can focus WNS monitoring and mitigating efforts. My results suggested that there are regional and species-specific differences in factors predicting presence/absence of tri-colored bats and cave myotis, and as such, efforts should be focused on using these site-specific landscape and environmental predictors to best focus survey efforts. The factors reported above may indeed influence tri-colored bat and cave myotis presence/abundance, but there may be additional factors not included in this study that affect tri-colored bat and cave myotis presence/abundance as well (e.g., internal hibernacula characteristics; alternative landscape characteristics). In fact, the *pseudo* R^2 values for my models were low, indicating limited explanatory power. Furthermore, it may be beneficial to analyze factors that predict presence of individual bat species based on site type (i.e., cave versus culvert), which may improve the overall model fit. As discussed in my study, culverts are associated with, for example, water, and were all located within the same ecoregion. Therefore, it may be advantageous to identify whether factors differ between site types for the same species. Indeed, the structural and environmental characteristics that predicted presence of tri-colored bats at culverts differed from those that predicted presence of the same species at caves (Meierhofer et al. 2019b, M. B. Meierhofer unpublished data). As Texas is diverse, compromising 12 Level III ecoregions, which exhibit variation in landscape, and environment (Griffith et al. 2004, Griffith et al. 2007, USEPA 2013), it is feasible that factors predicting species presence will vary across other ecoregions for these species, and for other species, and may indeed depend on the type of site. Therefore, my results present a foundation from which further research can explore site-specific factors driving individual species presence within ecoregions.

CHAPTER III

SITE-SPECIFIC LANDSCAPE AND ENVIRONMENTAL FACTORS PREDICTING PRESENCE OF *PSEUDOGYMNOASCUS DESTRUCTANS*, THE CAUSATIVE AGENT OF WHITE-NOSE SYNDROME, IN TEXAS

Summary

Infectious disease emergence can cause declines in numbers of free-ranging animal populations and even extinction. Although some exotic diseases are unsuccessful, others are able to survive in the new environment and become a major disease of animal health concern. White-nose syndrome (WNS) is an example of a disease that differentially affects populations of some North American bat species. Since its first documentation in New York, the fungal causative agent, *Pseudogymnoascus destructans*, has spread into Canada and the United States. In spring 2017, *P. destructans* was documented on cave myotis (*Myotis velifer*), tri-colored bats (*Perimyotis subflavus*), and Townsend's big-eared bats (*Corynorhinus townsendii*) in caves located in six north Texas counties. As a result, WNS may expand in Texas. To identify suitable sites for *P. destructans*, I conducted surveys between January and March 2016, December and March 2016–2017, November and March 2017–2018, and December and March 2018–2019 at 86 hibernacula (55 caves, 30 culverts, and 1 tunnel) across six Level III ecoregions (Chihuahuan Deserts, East Central Texas Plains, Edwards Plateau, South Central Plains, Southwestern Tablelands, and Texas Blackland Prairies) in Texas. I compared site-specific landscape and environmental factors of sites where I documented

P. *destructans* on bats to sites where I did not document the fungus on bats using a logistic regression analysis. Results of the logistic regression suggested that there were no predictors of presence of *P*. *destructans*. One principal component (PC2), which described distance to water, elevation, and low temperatures, approached significance. However, the model only described 6% of the overall variance in predicting *P*. *destructans* presence. Continued monitoring efforts are needed to document the continued spread of *P*. *destructans* and monitor for the potential development of WNS in Texas.

Introduction

Infectious disease emergence can cause declines in numbers of free-ranging animal populations (e.g., Thorne and Williams 1988, Young 1994, Laurenson et al. 1998) and even extinction (e.g, Daszak et al. 2000, McCallum and Dobson 1995, McCallum 2008, Kilpatrick et al. 2010). Impairments to the health of animals as a result of disease threaten biodiversity (e.g., Jones 1982, Scott 1988, Daszak et al. 2000, McLean 2007:263–267) and ecosystem function and services (e.g., Parker et al. 1999, Mack et al. 2000, Bohlen et al. 2004). These threats can result in the reduction of animals (e.g, Cunningham et al. 2017). Introductions of disease into wildlife populations have led to declines in population size as illustrated by Ebola outbreaks in African apes (Leroy et al. 2004), canine distemper in Serengeti lions (Roelke-Parker et al. 1996), and rabies in foxes (Anderson et al. 1981, Murray et al. 1986). There are various factors that lead to the emergence and spread of infectious diseases (e.g., population growth, habitat destruction, international travel; Deem et al. 2001, Friend et al. 2001, McLean 2007:262–263).

Although some invasive diseases are unsuccessful, others are able to survive in the new environment, infect a susceptible host, cause disease, become established, and become a major concern for animal health (Wilson 1995, Blackburn et al. 2011, Cunningham et al. 2017). One such example is white-nose syndrome (WNS). WNS caused by the cold-adaptive fungus, *Pseudogymnoascus destructans* (Blehert et al. 2009, Lorch et al. 2011, Warnecke et al. 2012), which differentially affects populations of some North American bat species (Foley et al. 2011, Langwig et al. 2015, Leopardi et al. 2015). The first documentation of the disease occurred in New York in February 2006 (Blehert et al. 2009, Turner and Reeder 2009). WNS has since spread north into Canada and the United States, with the farthest western documentation of WNS in Washington state (Foley et al. 2011, Coleman and Reichard 2014, Lorch et al. 2016, Figure 5). Transmission occurs via bat-to-bat contact (Zimmerman 2009, Lorch et al. 2011) and can occur via substrate-to-bat, with some evidence suggesting that humans can transport the fungus from infected to uninfected sites on clothing and equipment (Crawley 2009, Turner and Reeder 2009). The fungus grows on the wings, ears, and muzzles of hibernating bats, invading the dermal layer of the wing tissue, resulting in visible tissue damage (Lorch et al. 2011, Warnecke et al. 2012). In addition to damaged wing membranes, bats affected by WNS arouse more frequently from winter torpor, suffer depletion of fat reserves, and can die (Blehert et al. 2009, Boyles and Willis 2010, Reeder et al. 2012, Warnecke et al. 2012). WNS differentially impacts hibernating bat species, with mortality varying substantially within- and between sites (Langwig et al. 2012). Although most known WNS positive sites are caves and mines, some roosting bats using artificial structures (e.g., tunnels and culverts) have also tested positive for *P. destructans* (USFWS 2014, TPWD 2018).

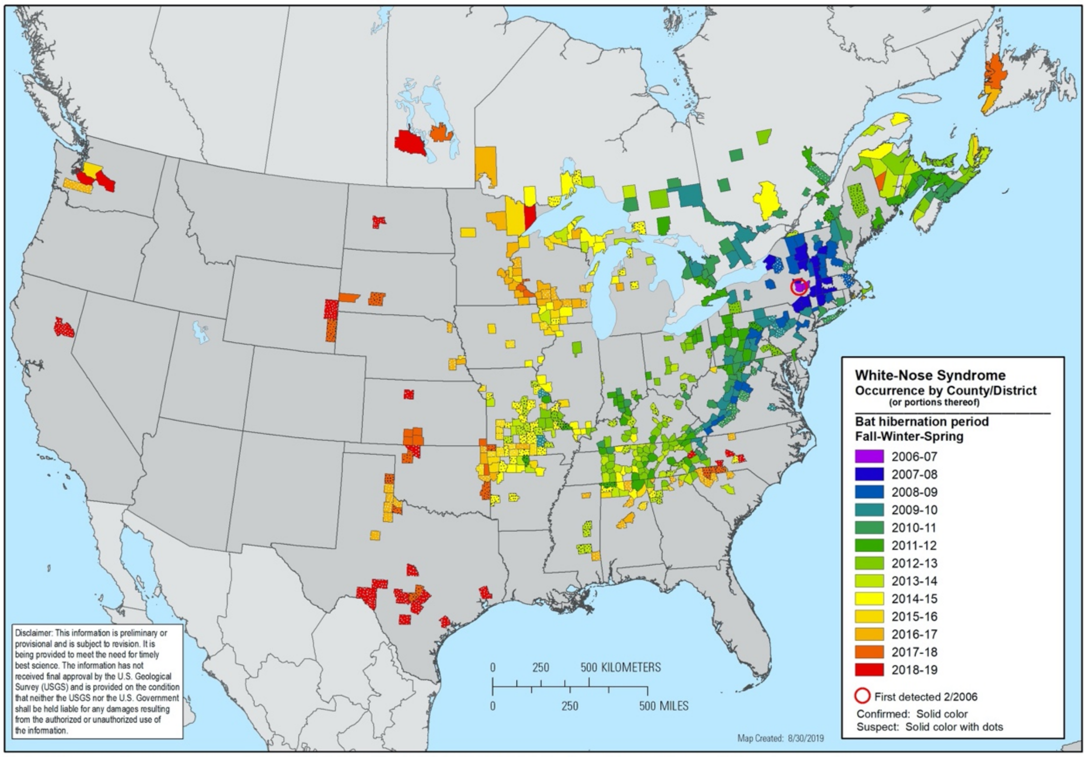


Figure 5. Map of bat white-nose syndrome occurrence by county/district as of 30 August 2019 (White-nose syndrome occurrence map – by year, 2019). Reprinted.

The temperature range for *P. destructans* growth is 3.0° C to 19.7° C (Verant et al. 2012). Though there is no established relative humidity range for growth of *P.*

destructans, research suggests that growth of *P. destructans* is similar between 70.5–96.5% (Marroquin et al. 2017). Hibernating bats tend to select roost locations with temperatures between 3.0° C and 15.0° C, which allows for energy conservation during hibernation (McNab 1982:163–165, Nagel and Nagel 1991, Brack 2007, Foley et al. 2011). Hibernating bat species differ in their selection of microclimate within a roost to conserve energy and reduce the effects of evaporative water loss (EWL; Twente 1955, Davis 1970:265–300, Webb et al. 1995). The range in hibernation temperatures likely impact winter survival of bats and the fitness of survivors' post-hibernation if they fall within the growth range of *P. destructans* (Cryan et al. 2010).

Currently, the fungus affects twelve species of hibernating bats in North America (big brown bat, *Eptesicus fuscus*; cave myotis, *Myotis velifer*; eastern small-footed bat, *M. leibii*; gray bat, *M. grisenscens*; Indiana bat, *M. sodalis*; little brown bat, *M. lucifugus*; long-legged bat, *M. volans*; Northern long-eared bat, *M. septentrionalis*; western long-eared bat, *M. evotis*; southeastern myotis, *M. austroriparius*; tri-colored bat, *Perimyotis subflavus*; and Yuma bat, *M. yumanensis*), with detection of *P. destructans* without histological confirmation of WNS in eight additional species (eastern red bat, *Lasiurus borealis*; Mexican free-tailed bat, *Tadarida brasiliensis*; Rafinesque's big-eared bat, *Corynorhinus rafinesquii*; silver-haired bat, *Lasionycteris noctivicans*; Townsend's big-eared bat, *C. townsendii*; Virginia big-eared bat, *C. townsendii virginianus*; Ozark big-eared bat, *C. townsendii ingens*, and western small-footed bat, *M. ciliolabrum*) (USFWS 2019).

Recently, WNS was documented in Texas in spring 2020, with presence of the fungus in several counties (TPWD 2020). Therefore, there is a need to understand and identify site-specific landscape and environmental factors that predict presence of the fungus to best manage for WNS-susceptible individuals, and mitigate WNS. My goal was to identify the site-specific landscape and environmental factors that predict presence of *P*. *destructans* in order to better focus monitoring and management efforts. My research will aid our ability to identify regions of Texas suitable for *P*. *destructans*. This information will improve management strategies for WNS-threatened species, and assist with continued monitoring efforts of these WNS-threatened species.

Methods

Study Area

Texas is diverse, compromising 12 Level III ecoregions which exhibit variation in landscape, and environmental conditions (Griffith et al. 2004, Griffith et al. 2007, USEPA 2013). I focused my sampling efforts to the northern and eastern regions of Texas, which were closest to the known location of WNS positive sites (Heffernan 2015), and expanded out across the state. I conducted surveys across the following six Texas Level III ecoregions: Chihuahuan Deserts (91,576 km^2), East Central Texas Plains (54,774 km^2), Edwards Plateau (74,964 km^2), South Central Plains (63,670.22 km^2), Southwestern Tablelands (60,314 km^2), and Texas Blackland Prairies (43,382 km^2) (Figure 6).

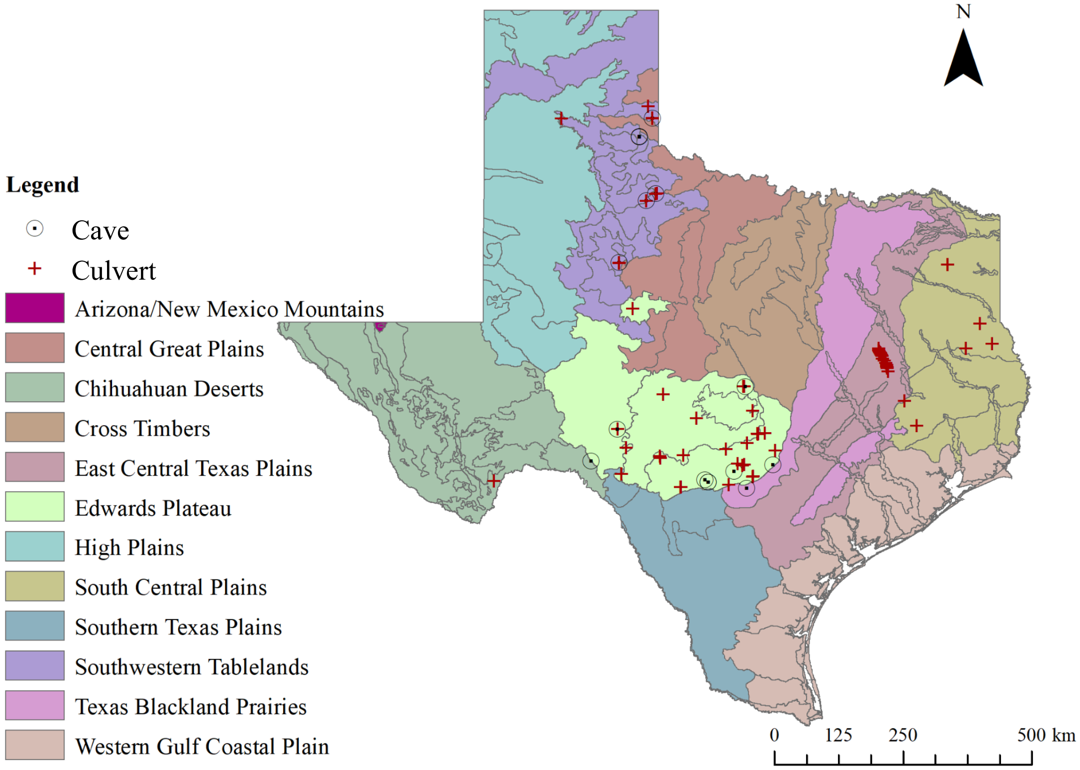


Figure 6. Distribution of 86 bat hibernacula (55 caves, 30 culverts, and 1 tunnel) across six Level III ecoregions in Texas (Chihuahuan Deserts, East Central Texas Plains, Edwards Plateau, South Central Plains, Southwestern Tablelands, and Texas Blackland Prairies) surveyed for bats for *Pseudogymnoascus destructans* from January and March 2016, December and March 2016–2017, November and March 2017–2018, and December and March 2018–2019.

Site Selection

I used the Generalized Random Tessellation Stratified (GRTS) design (Stevens and Olsen 2004) of the North American Bat Monitoring Program (NABat) to establish a stratified random sampling approach to select sites for sampling across Texas. NABat developed a multi-purpose sampling frame consisting of 10 x 10 km (100 km^2) grid cell sample units that are the focal analytical unit for regional and range-wide assessments.

These grid cells provided a standardized sampling system and allowed data collected to link back to the NABat database. I randomly selected grid cells within each Level III ecoregion. Because the focus of my project is WNS, I focused my sampling efforts in the more eastern and northern ecoregions (Figure 2). Furthermore, I monitored sites occupied by bats once every year when feasible whereas I did not revisit unoccupied sites (i.e., sites with no signs of bats, guano, or roost stains.

After the selection of the initial set of potential sampling grid cells, I screened each grid cell for the presence of potential bat roosts (i.e., culverts and caves) using ArcGIS 10.4.1 (ESRI 2015) and Google Earth Pro 7.3.2 (Google 2018). I obtained information from the Texas Speleological Survey (TSS) database on the presence of and types of caves. Texas Department of Transportation (TxDOT) provided a comprehensive list of all culverts for Texas. I used Google Earth to identify other structures not within the TSS database or TxDOT database. I attempted to gain access to sites within each grid cell initially selected. However, I did not always gain access at site(s) within each grid cell as access was not always granted by landowners due to safety concerns or because it was not physically possible to survey the site (e.g., the opening to the cave was too small for a person to enter). To avoid potential bias, I continued to the next random site for sampling. Additionally, I visited any known, historic roost/hibernacula to which I was granted access within an ecoregion to ensure I did not exclude potentially important sites through our random selection of cells. I gathered information about historic roost/hibernacula from information noted in the TSS database, from Bat Conservation International (BCI), and from public knowledge.

Data Collection

I conducted surveys between January and March 2016, December and March 2016–2017, November and March 2017–2018, and December and March 2018–2019 at 86 hibernacula (55 caves, 30 culverts, and 1 tunnel) across six Level III ecoregions (Chihuahuan Deserts, East Central Texas Plains, Edwards Plateau, South Central Plains, Southwestern Tablelands, and Texas Blackland Prairies) in Texas (Figure 6).

I visually surveyed roost locations for overwintering bats to test bats for presence of *P. destructans*. I collected swab samples from bats following protocols from the University of California in Santa Cruz (UCSC), the National Wildlife Health Center (NWHC) (U.S. Geological Survey National Wildlife Health Center 2016), and from a protocol I modified using the UCSC protocol as a foundation for my protocol. My sampling protocol differed from that of UCSC and NWHC in that it was not constrained by the bat species swabbed and the number of bats (e.g., >10 bats or at least 5 bats per species per UCSC protocol) per site swabbed. Therefore, results are not biased by these differing protocols. I thoroughly monitored sites in order to document the first probable *P. destructans*-infected site: by limiting swabbing to sites with particular species and to sites with at least 10 bats, I would not have conducted widespread monitoring. These samples contributed to the national surveillance effort at the NWHC, characterized existing microfauna on bat wings (NWHC), and contributed to a comprehensive study of WNS transmission (UCSC). I sent all bat swabs to their respective offices (NWHC or Northern Arizona University (NAU)), and samples collected with my kits were sent to NAU.

I used 30-meter resolution data layers (ArcGIS 10.4.1; ESRI 2015) to obtain site-specific landscape and environmental data for all surveyed sites. I obtained aspect, slope, and elevation data from Digital Elevation Models (DEM) in ArcGIS 10.4.1. I obtained data on percent canopy cover, minimum temperature, and maximum temperature from the National Land Cover Database (NLCD). I calculated Euclidean distance to nearest permanent water source from each site using the Euclidean distance tool in ArcGIS. I chose Euclidean distance because some species select roosts closer to water, and this method can provide insight on animal-landscape associations (Ormsbee and McComb 1998, Sedgeley and O'Donnell 1999, Boonman 2000, Conner et al. 2003). From the temperature layers, I used the Raster Calculator in ArcGIS to calculate mean minimum temperature, mean maximum temperature, and associated standard deviations for each month of the survey period (January–March 2016, November–February 2016–2017, October–March 2017–2018, and October–March 2018–2019). I calculated the sine and cosine of aspect to decompose them into east/west and north/south components (Jenness 2007). I then extracted data based on the latitude and longitude of each surveyed site (Table 10). Research on bats followed ASM guidelines (Sikes et al. 2016) and was approved by the Texas A&M Institutional Animal Care and Use Committee (IACUC 2015-0296).

Table 10. Descriptions, units of measure, and means (min–max) of site-specific landscape and environmental measures obtained from the coordinates of all sites surveyed for *Pseudogymnoascus destructans* for analysis.

Variable	Definition	Mean (min–max)
Slope	Slope of survey site; degrees	6.4 (0.6–34.9)
Elevation	Elevation of survey site; m	353.9 (77.0–886.0)
Distance to water	Euclidean distance to nearest water source; m	779.3 (108.2–4742.4)
Canopy	Canopy cover; %	35.2 (0.0–90.0)
Mean maximum temperature	Mean maximum monthly temperature for survey months, ºC	61.2 (51.6–73.7)
Mean maximum temperature (standard deviation)	Associated standard deviation of mean maximum temperature for survey months	1.4 (0.5–3.2)
Mean minimum temperature	Mean minimum monthly temperature for survey months, ºC	36.1 (24.9–45.9)
Mean minimum temperature (standard deviation)	Associated standard deviation of mean minimum temperature for survey months	1.3 (0.2–3.5)
Sine aspect	East-west component	0.1 (-1.0–1.0)
Cosine aspect	North-south component	-0.0 (-1.0–1.0)

Data Analysis

I conducted all analyses in R 3.4.1 (R Core Team 2018). I tested collinearity of site-specific landscape and environmental factors discussed above using variance inflation factor (VIF). Variance inflation factors suggested that the variables mean minimum and maximum temperature and associated standard deviations showed multicollinearity (VIF > 10) (Meyers 1990:131–132). Thus, to transform site-specific landscape and environmental factors into a smaller number of uncorrelated factors, and to standardize all factors before analysis, I used a Principal Components Analysis (PCA) using the princomp function in the ‘MASS’ package in R (Ripley et al. 2011). PCA is a factor model in which Principal Components (PCs) are based on summarizing the total variance through which the process usually yields smaller, more compact number of output components (Boslaugh 2013:291). I selected PCs based on the following two criteria: the PC had an eigenvalue > 1 and the PC contained independent factors with loadings greater than 0.364 (Fields et al. 2012:762, 767).

For analysis of presence of *P. destructans*, I analyzed 86 surveyed sites. I retained data from only one survey per location for analysis. For sites where *P. destructans* was documented, I retained the first survey year the site was positive, and for sites where *P. destructans* was not documented, I retained the final survey. I performed a logistic regression analysis to investigate if PCs predicted presence of *P. destructans* using the ‘glm’ function in package ‘MASS’ in R (Ripley et al. 2011). Logistic regression analysis is used when the outcome variable is dichotomous; in this case, presence/absence of *P. destructans* (Fields et al. 2012:313). I calculated estimated odds ratio for each factor which indicated the change in the probability of *P. destructans* presence in sites that would result from a one-unit change in the value of the indicated variable. I used McFadden’s *pseudo* R^2 test to check the model’s overall goodness-of-fit (Smith and McKenna 2013). I evaluated the reliability and validity of the model as fair ($0.50 < AUC \leq 0.75$), good ($0.75 < AUC \leq 0.92$), very good ($0.92 < AUC \leq 0.97$), or excellent ($0.97 < AUC \leq 1.00$) based on the value of AUC (Hosmer et al. 2013:161). I considered *P*-values of < 0.05 significant for all tests.

Results

Description of Principal Components Retained for Analysis in the Logistic Regression Analysis

PC1 explained 32.4% of the variance and described high external site temperature, being positively loaded by the mean maximum temperature, mean minimum temperature, and their associated standard deviations. PC2 explained 19.2% of the variance and described low temperature and topography of the site, being positively loaded by elevation and distance to water, and negatively loaded by minimum temperature. PC3 explained 15.0% of the variance and was positively loaded by slope and canopy cover. PC4 explained 10.8% of the variance and described sites with an easterly aspect, being positively loaded by the sine of aspect. The cumulative percentage explained by the four selected PCs described 77.4% of the variance in the data (Table 11).

Logistic Regression Analysis on Pseudogymnoascus destructans Presence/Absence

I found 15 sites (17.4%, 15 caves) with bats positive for *P. destructans* (Figure 7). Results suggested that no PCs were predictive of *P. destructans* presence (Table 12, Figure 8). However, PC2 approached significance ($P = 0.054$, Table 12). The model explained approximately 6% of the deviation in *P. destructans* presence ($pseudo\ R^2 = 0.06$). The AUC score of the model was .35 indicating a fair ability to discriminate between presence and absence of *P. destructans*.

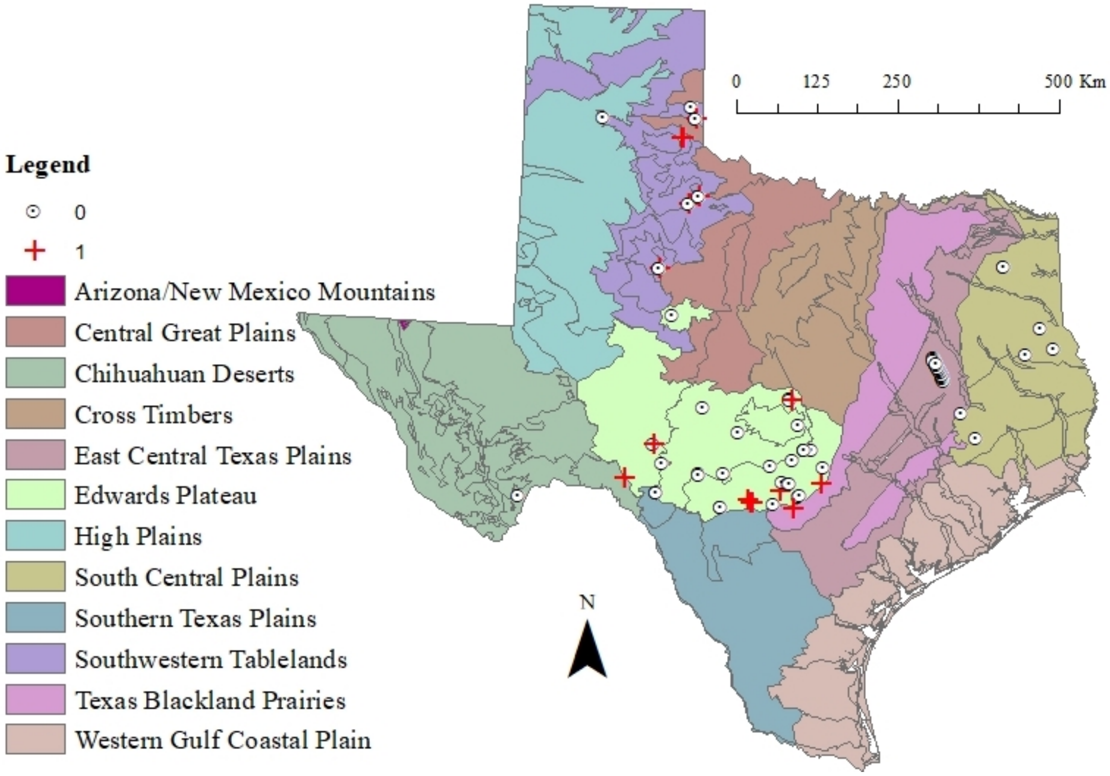


Figure 7. Distribution of 86 bat hibernacula (55 caves, 30 culverts, and 1 tunnel) across six Level III ecoregions in Texas (Chihuahuan Deserts, East Central Texas Plains, Edwards Plateau, South Central Plains, Southwestern Tablelands, and Texas Blackland Prairies) surveyed for bats for *Pseudogymnoascus destructans* from January and March 2016, December and March 2016–2017, November and March 2017–2018, and December and March 2018–2019. Bullseye indicates absence of *P. destructans*. Plus symbol indicates presence of *P. destructans*.

Table 11. Principal components analysis on 10 factors to produce principal components (PCs) that account for site-specific landscape and environmental characteristics associated with sites occupied by bats with and without *Pseudogymnoascus destructans*. Values represent the loadings (the percent of variance in the PC explained by the factor) for each of the 10 environmental factors. Bold values represent factors with loadings greater than 0.364 (Fields et al. 2012:767).

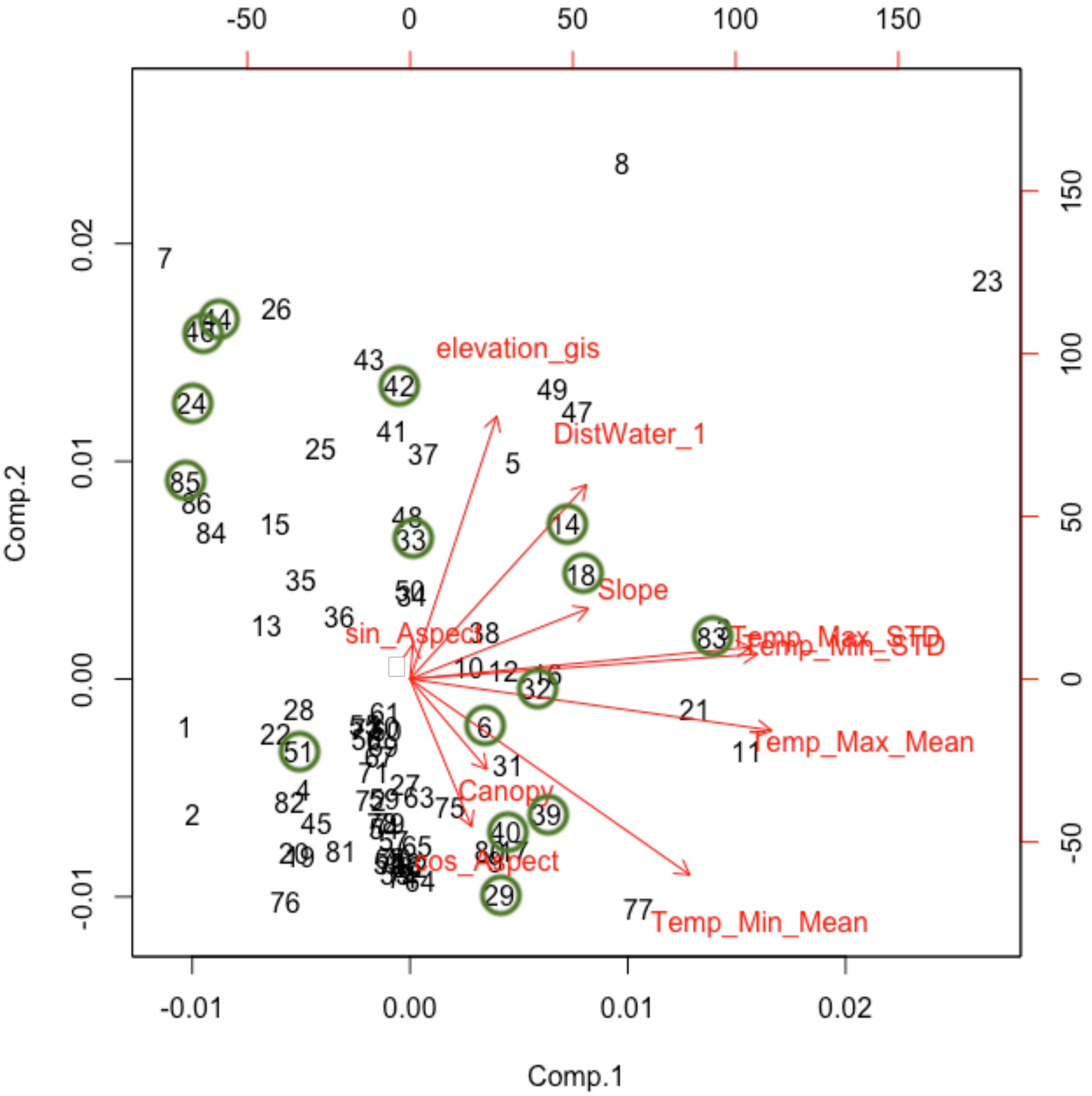
	PC1	**PC2**	**PC3**	**PC4**
Slope (%)	0.246	0.164	**0.501**	
Elevation (m)	0.120	**0.610**	0.211	
Euclidean dist. to water (m)	0.243	**0.451**		-0.165
Canopy cover (%)	0.106	-0.209	**0.665**	
Mean max temp. (° C)	**0.499**	-0.119		-0.137
Mean max temp. S.D.	**0.470**		-0.332	0.142
Mean min temp. (° C)	**0.386**	**-0.455**		-0.133
Mean min temp. S.D.	**0.479**		-0.320	0.113
Sine of Aspect				**0.907**
Cosine of Aspect		-0.342	0.168	0.269
% variance explained	32.4%	19.2%	15.0%	10.8%

Table 12. Results of logistic regression analysis for *Pseudogymnoascus destructans* presence on bats in hibernacula within six Level III Texas ecoregions (Chihuahuan Deserts, East Central Texas Plains, Edwards Plateau, South Central Plains, Southwestern Tablelands, and Texas Blackland Prairies). Tablelands ecoregion. Table contains principal components (variable: PCs), coefficients, standard errors (SE), odds ratio, odds ratio 95% confidence interval, and associated *P*s. *The estimated odds ratio indicates the change in the probability of bat presence at sites resulting from a 1-unit change in the value of the indicated variable. Significant predictors ($P < 0.05$) are in bold.

Variable (Principal Components)	**Coefficient**	**SE**	**Odds ratio**	**Odds ratio 95% C.I.***		***P***
				Lower	**Upper**	
PC1	0.05	0.14	1.05	0.78	1.40	0.72
PC2	0.38	0.20	1.46	0.99	2.16	0.05*
PC3	0.12	0.24	1.12	0.69	1.78	0.63
PC4	0.23	0.29	1.26	0.73	2.26	0.42

*Significance value (*P*) for PC2 approached significance with a *P* value of 0.054.

Figure 8. Biplot of PC1 and PC2, the scores of the samples, and the loadings of the variables. Numbers represent cave sites surveyed for *Pseudogymnoascus destructans*. Circled numbers represent sites where *P. destructans* was documented. Biplot scaled by 2 for clarity.



Discussion

Although there were no significant site-specific landscape or environmental predictors that were significant in my analysis, I documented *P. destructans* in 15 sites. One factor though, which described distance to water, elevation, and low temperatures, approached significance. These factors explained 19.2% of the variance within the component. However, the model has little predictive power, explaining only 6% of the overall deviation in *P. destructans* presence.

Spatial heterogeneity and winter duration have been suggested as key environmental co-variates to consider when modeling the spread of WNS (Maher et al. 2012) and may be important when understanding the spread of the fungal causative agent. However, understanding the spread of *P. destructans* may only require knowing where bats and roosts co-occur as *P. destructans* is primarily spread from bat-to-bat (Zimmerman 2009, Lorch et al. 2011). Although primarily spread by bats, there is the potential for local movement of the fungus by other animals and humans as well (Crawley 2009, Turner and Reeder 2009). Given this knowledge, the fungus may in fact be documented in any location where bats are present at a given time during the year. Therefore, presence of potential subterranean roosts (e.g., karst regions, location of culverts), and where these regions co-occur with factors that drive bat presence within ecoregions, is important to consider when identifying regions to monitor for *P. destrutans* and WNS.

Presence of the fungus itself may have few environmental constraints, as the fungus has been documented both in winter and summer months and is known to persist in cave environments in the absence of bat hosts (Lorch et al. 2013). Any human or bat that comes into contact with the fungus in one location could then transport it to another location. Thus, the fungus could be found in any environment. Indeed, Reynolds and colleagues (2015) found that *P. destructans* can proliferate in cave sediment incubated at 10 °C and 95% humidity, and that caves with high organic detritus would increase WNS infection rates. Furthermore, their model also suggests that environmental growth of *P. destructans* would allow for the persistence of the pathogen within hibernacula for decades. Although the fungal spores can persist in these environments year-round, the growth of the fungus is restricted by temperature (Verant et al. 2012). As such, environmental persistence of the fungus in environments in the absence of bats can be hindered by both limited nutrients and when temperatures are not suitable for growth.

My study did include potential limitations which may have resulted in a limited number of sites documented with *P. destructans*. Due to time constraints, I only swabbed up to 25 bats per site, and only focused on sampling bats and not sampling sediment and substrate. In sites with large abundances of bats, this limited sampling strategy may have resulted in false negatives. Additionally, research has shown that *P. destructans* can persist in environments in the absence of bats (Lorch et al. 2013). As such, increased sampling effort may increase the likelihood of documenting *P. destructans*, and the addition of samples may have further improved the overall model. Further, there were discrepancies with PCR results between the National Wildlife Health Center and Dr. Foster's lab at Northern Arizona University. Unfortunately, this was only recently investigated within the last year of surveys. As such, any statewide analyses should consider using one lab for all swab analyses to reduce the potential for differences in lab results. Finally, another caveat of the study was initial variable selection. Unfortunately, little is known about potential landscape and environmental predictors of *P. destructans* presence. However, it is known that the fungus is spread bat-to-bat (Zimmerman 2009, Lorch et al. 2011), and so all variables were selected for their biological relevance to bats and roosts. Unfortunately, selecting variables important to bats may have affected the results of the analyses as the fungus can persist in an environment without the presence of bats (Lorch et al. 2013). As such, my model could be improved with increased sampling at sites both with and without bats, a consideration of other potential variables that may affect presence of the fungus, and also with having one lab analyze samples to ensure consistency.

Although I did document *P. destructans* in 15 sites, 71 sites with bats returned negative results over the course of four years. However, I predict that these sites will test positive in subsequent years given the mode of dispersal of *P. destructans*. Langwig and colleagues (2015) found that hibernation was one important factor determining transmission dynamics of *P. destructans*. Specifically, temperatures within caves monitored for *P. destructans* fell within the range of temperatures that the pathogen can grow (Verant et al. 2012), resulting in increased amplification of *P. destructans* on hibernating bats (Langwig et al. 2015). Although temperatures within sites in Texas fall within the growth range of the fungus (Meierhofer et al. 2019a), the fluctuations in temperature may reduce the amplification of *P. destructans*. Thus, invasion of the fungus into new regions in Texas may be delayed as a result of low fungal loads. Future research should investigate whether fluctuations in temperature affect the amplification of *P. destructans* in southern regions of the United States to better understand invasion dynamics.

Monitoring efforts should continue in regions of known subterranean sites, and where these sites co-occur with predictors of bat species presence. This will allow for more strategic survey efforts in areas of the highest probability of finding bats that may be in contact with *P. destructans*, and thus at risk of WNS infection. Once sites are identified, sites with large populations of species known to be affected by WNS, such as those documented within culverts (Meierhofer et al. 2019b), should be of primary focus for WNS monitoring efforts.

CHAPTER IV

CONCLUSIONS

My broad-scale study quantitatively demonstrated that there are site-specific landscape and environmental predictors of presence and abundance of tri-colored bat and cave myotis in Texas during the winter, and that these predictors varied depending on the geographic scale (statewide versus ecoregion). Although distributions of bat species are known for Texas' ecoregions (Ammerman et al. 2012:11), it was important to investigate the distribution of species affected by WNS during winter months to best identify potential hibernacula for *Pseudogymnoascus destructans* and white-nose syndrome (WNS) monitoring efforts. My study further indicated that identifying predictors of presence and abundance for species may also be best identified based on the site type (e.g., cave, culvert). For example, I found that within the East Central Texas Plains, tri-colored bats were only found occupying culverts. Thus, results from my analysis for predicting presence of tri-colored bats within the East Central Texas Plains ecoregion are specific to culvert structures. Within ecoregions where multiple roost site types exists, predictors of presence for the bat species of interest may be different. Thus, research should focus on identifying predictors of presence not only at the ecoregion scale, but also based on site type to best identify potential hibernacula sites.

Unfortunately, my study did not determine site-specific landscape or environmental predictors of *P. destructans* presence. Although one factor, describing distance to water, elevation, and low temperatures, approached significance, the model only described 6% of the overall deviation in *P. destructans* presence. However, this study provided some insight into how to improve monitoring efforts for the fungal pathogen in Texas. Although any animal that comes into contact with *P. destructans* should test positive given the mode of transmission, there are several reasons why only certain bat species are testing positive for *P. destructans*. First, it is easier to monitor the spread of *P. destructans* in common bat species with large distributions such as the cave myotis. Thus, it is more likely to document *P. destructans* in these more common species than for threatened, rare (commonly undocumented) species (e.g., northern long-eared bat (*Myotis septentrionalis*)). Second, WNS is known to only affect hibernating bat species (Blehert et al. 2009). As such, the focus of *P. destructans* and WNS-monitoring efforts focuses on subterranean roost locations, and in particular, those that contain species previously known to be affected by WNS. As a result, species that roost primarily in trees and other non-subterranean roost locations (e.g., red bat (*Lasiursus borealis*)) are not being surveyed for *P. destructans* to the extent that other subterranean roosting species are. Third, monitoring efforts for *P. destructans* focused in areas near the first site of documentation in New York. As such, bat species only found in western United States (e.g., California myotis, *M. californicus*) have not yet been the focus of testing for *P. destructans*. Finally, access to potential roosting locations may not be permitted, reducing the potential monitoring efforts in regions predominantly privately owned, such as Texas. Monitoring known hibernacula sites that are easily accessible is both time and cost-effective, and as such, bats of certain species might not be tested for *P. destructans*.

Although *P. destructans* was documented in spring 2017 in Texas (TPWD 2017), WNS was only recently documented on cave myotis in Texas in spring 2020 (TPWD 2020). There may be several reasons why WNS has only recently developed in Texas, such as invasion dynamics of *P. destructans* and hibernation ecology. Although this information is known from northern regions of North America, limited research exists for more southern regions. One study by Grider and colleagues (2016) documented winter activity of bats affected by WNS, such as the tri-colored bat, in North Carolina. Their results suggested that bats in southern regions sustain more consistent year-round activity, which could result in less mortality associated with WNS (Frick et al. 2010, Grider et al. 2016). Although activity of bats at southern regions and invasion dynamics of *P. destructans* may affect the development and spread of WNS in Texas, this should not minimize the need for continued survey efforts to better understand the threat of WNS to bat species in Texas.

In conclusion, my results suggested that focusing on factors that predict individual species presence should be determined based upon ecoregions and the type of site. Specifically, within an ecoregion, the factors predicting the probability of presence of a species occupying caves may differ than the factors predicting the probability of occupancy of culverts (Meierhofer et al. 2019b, M. B. Meierhofer unpublished data). Furthermore, although I did not determine factors predicting presence of the fungal causative agent of WNS, my study highlighted the pitfalls of monitoring for *P. destrucans* in regions where the fungus is likely to be documented. Future studies should examine the differences in predictors across site types for various bat species, and apply that knowledge to the distribution of potential roosting sites (e.g., overlap predictors of species presence with areas of karst to identify regions where bats may co-occur with caves). Conducting this research at the ecoregion scale will effectively allow biologists and managers to identify regions in which to focus efforts for monitoring for *P. destructans* and managing of species threatened by WNS in a state as large as Texas.

LITERATURE CITED

Anderson, R. M., H. C. Jackson, R. M. May, and A. M. Smith. 1981. Population dynamics of fox rabies in Europe. Nature 289:765–771.

Ammerman, L. K., C. L. Hice, D. J. Schmidly, and C. Brown. 2012. Bats of Texas. Texas A&M University Press, College Station, Texas, USA.

Arnett, E. B. 2005. Relationships between bats and wind turbines in Pennsylvania and West Virginia: an assessment of bat fatality search protocols, patterns of fatality, and behavioral interactions with wind turbines. A final report submitted to the Bats and Wind Energy Cooperative. Bat Conservation International, Austin, Texas, USA. <http://batsandwind.org/pdf/postconpatbatfatal.pdf>.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome: an emerging fungal pathogen? Science 323:5911.

Blackburn, T. M., P. Pyšek, S. Bacher, J. T. Carlton, R. P. Duncan, V. Jarošík, J. R. U. Wilson, and D. M. Richardson. 2011. A proposed unified framework for biological invasions. Trends in Ecology and Evolution 26:333–339.

Blackwell, D. D., J. L. Steele, and C. A. Brott. 1980. The terrain effect on terrestrial heat flow. Journal of Geophysical Research 85:4757–4772.

Bohlen, P. J., S. Scheu, C. M. Hale, M. A. McLean, S. Migge, P. M. Groffman, and D. Parkinson. 2004. Non-native invasive earthworms as agents of change in northern temperate forests. Frontiers in Ecology and the Environment 22:427–35.

Bonebrake, T. C., J. Christensen, C. L. Boggs, and P. R. Ehrlich. 2010. Population decline assessment, historical baselines, and conservation. Conservation Letters 3:371–378.

Boonman, M. 2000. Roost selection by noctules (*Nyctalus noctula*) and Daubenton's bats (*Myotis daubentonii*). Journal of Zoology (London) 251:385–389.

Borcard, D., F. Gillet, and P. Legendre. 2011. Numerical ecology with R. Springer, New York, New York, USA.

Boslaugh, S. 2013. Statistics in a nutshell. 2nd edition. O'Reilly Media, Sebastopol, California, USA.

Boyles, J. G., and C. K. R. Willis. 2010. Could localized warm areas inside old caves reduce mortality of hibernating bats affected by white-nose syndrome? Frontiers in Ecology and the Environment 8:92–98.

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

Briggler, J. T., and J. W. Prather. 2003. Seasonal use and selection of caves by the Eastern Pipistrelle bat (*Pipistrellus subflavus*). The American Midland Naturalist 149:406–412.

Brooks, R. T. 2009. Habitat-associated and temporal patterns of bat activity in a diverse forest landscape of southern New England, USA. Biodiversity and Conservation 18:529–545.

Campbell, G. L., A. A. Marfin, R. S. Lanciotti, and D. J. Gubler. 2002. West Nile virus. Infectious Diseases 2:519–529.

Centers for Disease Control and Prevention. 1999. Update: West Nile-like viral encephalitis–New York. Morbidity and Mortality Weekly Report 48:845–849.

Claire, W., L. S. Loucks, J. B. Shaw, J. W. Evans, K. E. Gillies, and M. A. Caywood. 2018. Variation in the number of hibernating cave myotis (*Myotis velifer*) in western Oklahoma and northwest Texas caves prior to the arrival of white-nose syndrome. Southwestern Naturalist 63:124–132.

Coleman, J. T. H., and J. D. Reichard. 2014. Bat white-nose syndrome in 2014: a brief assessment seven years after discovery of a virulent fungal pathogen in North America. Outlooks on Pest Management 25:374–377.

Conner, L. M., M. D. Smith, and L. W. Burger Jr. 2003. A comparison of distance-based and classification-based analysis of habitat use. Ecology 84: 526–531.

Crawley, C. 2009. Scientists tackle the mystery of white-nose syndrome in bats. National Institute for Mathematical and Biological Synthesis. <http://www.eurekalert.org/pub_releases/2009-06/nifm-stt060409.php>. Accessed 1 December 2016.

Cryan P. M., C. U. Meteyer, J. G. Boyles, J. G., and D. S. Blehert. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology 8:1–8.

Cryan, P. M., C. U. Meteyer, D. S. Blehert, J. M. Lorch, D. M. Reeder, G. G. Turner, J. Webb, M. Behr, M. Verant, R. E. Russell, and K. T. Castle. 2013. Electrolyte depletion in white-nose syndrome bats. Journal of Wildlife Diseases 49:398–402.

Cunningham, A. A. 2005. A walk on the wild side – emerging wildlife diseases. British Medical Journal 331:1214–1215.

Cunningham, A. A., P. Daszak, and J. L. N. Wood. 2017. One Health, emerging infectious disease and wildlife: two decades of progress? Philosophical Transactions of the Royal Society B 372:2016167.

Daszak, P., L. Berger, A. A. Cunningham, A. D. Hyatt, D. E. Green, and R. Speare. 1999. Emerging infectious diseases and amphibian population declines. Emerging Infectious Diseases 5:735–748.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife – threats to biodiversity and human health. Science 287:443–449.

Davis, W. H. 1970. Hibernation: ecology and physiological ecology. Pp. 265–300 *in* W. A. Wimsatt, editor. Biology of bats. Academic Press, New York, New York, USA.

Deem, S. L., W. B. Karesh, and W. Weisman. 2001. Putting theory into practice: wildlife health in conservation. Conservation Biology 15:1224–1233.

ESRI. 2015. Arc GIS. Ver. 10.4.1. Environmental System Research Institute, Inc. Redlands, California, USA.

Fiedler, J. K., T. H. Henry, R. D. Tankersley, and C. P. Nicholson. 2007. Results of bat and bird mortality monitoring at the expanded Buffalo Mountain windfarm, 2005. Tennessee Valley Authority, Knoxville, Tennessee, USA. <https://docs.wind-watch.org/fiedler2007-batbirdmortality2005.pdf>.

Fields, F., J. Miles, and Z. Field. 2012. Discovering statistics using R. Sage Publishing, Los Angeles, California, USA.

Fitch, J. H., K. A. Shump, Jr., and A. U. Shump. 1981. *Myotis velifer*. Mammalian Species 149:1–5.

Foley, J., D. Clifford, K. Castle, P. M. Cryan, and R. S. Ostfeld. 2011. Investigating and managing the rapid emergence of white-nose syndrome, a novel fatal, infectious disease of hibernating bats. Conservation Biology 25:223–231.

Ford, W. M., J. M. Menzel, M. A. Menzel, J. W. Edwards, and J. C. Kilgo. 2006. Presence and absence of bats across habitat scales in the Upper Coastal Plain of South Carolina. Journal of Wildlife Management 70:1200–1209.

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

Friend, M., R. G. McLean, and F. J. Dein. 2001. Disease emergence in birds: challenges for the twenty-first century. The Auk 118:290–303.

Fujita, M. S., and T. H. Kunz. 1984. *Pipistrellus subflavus*. Mammalian Species 228:1–6.

GAO [U.S. Government Accountability Office]. 2005. Wind power: impacts on wildlife and government responsibilities for regulating development and protecting wildlife. U.S. Government Accountability Office, Washington D.C., Maryland, USA.

Gargas A., M. T. Trest, M. Christensen, T. J. Volk, and D. S. Blehert. 2009. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108:147–154.

Geiser, F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. Annual Review of Physiology 66:239–274.

Google. 2018. Google Earth Pro. Ver. 7.3.2. Google LLC. Mountain View, California, USA.

Grider, J. F., A. L. Larsen, J. A. Homyack, and M. C. Kalcounis-Rueppell. 2016. Winter activity of coastal plain populations of bat species affected by white-nose syndrome and wind energy facilities. PLOS One 11: e0166512.

Griffith, G. E., S. A. Bryce, J. M. Omernik, J. A. Comstock, A. C. Rogers, B. Harrison, S. L. Hatch, and D. Bezanson. 2004. Ecoregions of Texas. Corvallis, Oregon, USA: U.S. Environmental Protection Agency.

Griffith, G. E., S. A. Bryce, J. M. Omernik, and A. Rodgers. 2007. Ecoregions of Texas. Austin, Texas, USA: Texas Commission on Environmental Quality.

Grindal, S. D., and R. M. Brigham. 1999. Impacts of forest harvesting on habitat use by foraging insectivorous bats at different spatial scales. Ecoscience 6:25–34.

Guo, Y.-R., Q.-D. Cao, Z.-S. Hong, Y.-Y. Tan, S.-D. Chen, H.-J. Jin, K.-S. Tan, D.-Y. Wang, and Y. Yan. 2020. The origin, transmission and clinical therapies of coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Military Medical Research 7:11.

Hayman, D. T. S., J. R. C. Pulliam, J. C. Marshall, P. M. Cryan, and C. T. Webb. 2016. Environment, host, and fungal traits predict continental-scale white-nose syndrome in bats. Science Advances 2:e1500831.

Heffernan, L. 2015. Bat white nose syndrome occurrence by county/district. <https://www.whitenosesyndrome.org/static-spread-map/november-16-2015>. Accessed 1 December 2015.

Hosmer, D. W., Jr., S. Lemeshow, and R. X. Sturdivant. 2013. Applied logistic regression. John Wiley and Sons, New York, New York, USA.

Humphrey, S. R., and J. A. Gore. 1992. Southeastern brown bat. Pages 335–342 *in* S. R. Humphrey, editor. Rare and endangered biota of Florida. Volume 1. Mammals. University Press of Florida, Gainesville, Florida, USA.

Ingersoll, T. E., B. J. Sewall, and S. K. Amelon. 2013. Improved analysis of long-term monitoring data demonstrates marked regional declines of bat populations in the eastern United States. PLOS One 8: e65907.

Jenness, J. 2007. Some thoughts on analyzing topographic habitat characteristics. <http://www.jennessent.com/downloads/topographic_analysis_online.pdf>. Accessed 28 June 2019.

Johnson, G. D. 2005. A review of bat mortality at wind-energy developments in the United States. Bat Research News 46:45–49.

Jones, D. M. 1982. Conservation in relation to animal disease in Africa and Asia. Symposia of the Zoological Society of London 50:271–285.

KDWPT [Kansas Department of Wildlife, Parks and Tourism]. 2018. First cave myotis bat with white-nose syndrome found in Kansas. <https://ksoutdoors.com/KDWPT-Info/News/Weekly-News/5-29-18/First-Cave-Myotis-Bat-With-White-nose-Syndrome-Found-In-Kansas>. Accessed 8 April 2019.

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

Krusic, R. A., M. Yamasaki, C. D. Neefus, and P. J. Pekins. 1996. Bat habitat use in White Mountain National Forest. Journal of Wildlife Management 60:625–631.

Kunz, T. H. 1974. Feeding ecology of a temperate insectivorous bat (*Myotis velifer*). Ecology 55:693–711.

Kunz, T. H. 1982. Roosting ecology. Pages 1–55 *in* T. H. Kunz, editors. Ecology of Bats. Plenum Press, New York, New York, USA.

Kunz, T. H., and L. F. Lumsden. 2003. Ecology of cavity and foliage roosting bats. Pages 680–743 *in* T. H. Kunz and M. B. Fenton, editors. Bat ecology. University of Chicago Press, Chicago, Illinois, USA.

Lambert, D. 1992. Zero-inflated Poisson regression, with an application to defects in manufacturing. Technometrics. 34:1–14.

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

Langwig, K. E., J. R. Hoyt, K. L. Parise, J. Kath, D. Kirk, W. F. Frick, J. T. Foster, and A. M. Kilpatrick. 2015. Invasion dynamics of white-nose syndrome fungus, Midwestern United States, 2012-2014. Emerging Infectious Diseases, 21:1023–1026, doi:10.3201/eid2106.150123.

Laurenson, K., C. Sillero-Zubiri, H. Thompson, F. Shiferaw, S. Thirgood, and J. Malcolm. 1998. Disease as a threat to endangered species: Ethiopian wolves, domestic dogs and canine pathogens. Animal Conservation 1:273–280.

Lee, H. W., P. W. Lee, and K. M. Johnson. 1978. Isolation of the etiologic agent of Korean Hemorrhagic fever. Journal of Infectious Diseases 137:298–308.

Leopardi, S., D. Blake, and S. J. Puechmaille. 2015. White-nose syndrome fungus introduced from Europe to North America. Current Biology 25:R217–R219.

Leroy, E. M., P. Rouquet, P. Formenty, S. Souquière, A. Kilbourne, J-M Froment, M. Bermejo, S. Smit, W. Karesh, R. Swanepoel, S. R. Zaki, and P. E. Rollin. 2004.

Multiple Ebola virus transmission events and rapid decline of central African wildlife. Science 303:387–390.

Lorch J. M., C. U. Meteyer, M. J. Behr, J. G. Boyles, P. M. Cryan, A. C. Hicks, A. E. Ballmann, J. T. H. Coleman, D. N. Redell, D. M. Reeder, and D. S. Blehert. 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. Nature 480:376–378.

Lorch, J. M., L. K. Muller, R. E. Russell, M. O'Connor, D. L. Linder, and D. S. Blehert. 2013. Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the eastern United States. Applied and Experimental Microbiology 79:1293–1301.

Lorch J. M., J. M. Palmer, D. L. Linder, A. E. Ballmann, K. G. George, K. Griffin, S. Knowles, J. R. Huckabee, K. H. Haman, C. D. Anderson, P. A. Becker, J. B. Buchanan, J. T. Foster, and D. S. Blehert. 2016. First detection of bat white-nose syndrome in western North America. American Society of Microbiology 1:e00148–16.

Lu, H., C. W. Stratton, and Y.-W. Tang. 2020. Outbreak of pneumonia of unknown etiology in Wuhan, China: The mystery and the miracle. Journal of Medical Virology 92:401–402.

Mack, R. N., D. Simberloff, W. M. Lonsdale, H. Evans, M. Clout, and F. A. Bazzaz. 2000. Biotic invasions: causes, epidemiology, global consequences, and control. Ecological Applications 1100:689–710.

Maher, S. P., A. M. Kramer, J. Tomlin Pulliam, M. A. Zokan, S. E. Bowden, H. D. Barton, K. Magori, and J. M. Drake. 2012. Spread of white-nose syndrome on a network regulated by geography and climate. Nature Communications 3:1306.

Marroquin, C. M., J. O. Lavine, and S. T. Windstam. 2017. Effect of humidity on development of *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome. Northeastern Naturalist 24:54–64.

McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology and Evolution 10:190–194.

McCallum, H. 2008. Tasmanian devil facial tumour disease: lessons for conservation biology. Trends in Ecology and Evolution 23:631–637.

McLean, R. G. 2007. The introduction and emergence of wildlife diseases in North America. Pages 261–278 *in* T. E. Fulbright and D. G. Hewitt, editors. Wildlife science: linking ecological theory and management applications. CRC Press, Boca Raton, Florida, USA.

McNab, B. K. 1982. Evolutionary alternatives in the physiological ecology of bats. Pages 151–200 *in* T. H. Kunz, editor. Ecology of bats. Plenum Press, New York, New York, USA.

Meierhofer, M. B., J. S. Johnson, S. J. Leivers, B. L. Pierce, J. W. Evans, and M. L. Morrison. 2019a. Winter habitats of bats in Texas. PLOS One 14:e0221590.

Meierhofer, M. B., S. J. Leivers, R. R. Fern, L. K. Wolf, J. H. Young Jr., B. L. Pierce, J. W. Evans, M. L. Morrison. 2019b. Structural and environmental predictors of presence and abundance of tri-colored bats in Texas culverts. Journal of Mammalogy 100:1274–1281.

Meyers, R. 1990. Classical and modern regression with application. 2nd edition. Boston, Massachusetts, USA.

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

Morrison, M. L., B. G. Marcot, and R. W. Mannan. 2006. Wildlife-habitat relationships: concepts and applications. 3rd edition. Island Press, Washington, D. C., USA.

Morse, S. S. 1995. Factors in the emergence of infectious diseases. Emerging Infectious Diseases 1:7–15.

Murray, J. D., E. A. Stanley, and D. L. Brown. 1986. On the spatial spread of rabies among foxes. Proceedings of the Royal Society of London B. 229:111–150.

Nagel, A., and R. Nagel. 1991. How do bats choose optimal temperatures for hibernation? Comparative Biochemistry and Physiology Part A: Physiology 99:323–326.

ODWC [Oklahoma Department of Wildlife Conservation]. 2010. Bat fungus documented in Oklahoma. <https://caves.org/WNS/Oklahoma_Press_Release.pdf>. Accessed 1 December 2015.

Ormsbee, P. C., and W. C. McComb. 1998. Selection of day roosts by female long-legged myotis in central Oregon Cascade Range. Journal of Wildlife Management 62:596–603.

Parker, I. M., D. Simberloff, W. M. Lonsdale, K. Goodell, M. Wonham, P. M. Kareiva, M. H. Williamson, B. Von Holle, P. B. Moyle, J. E. Byers, and L. Goldwasser. 1999. Impact: toward a framework for understanding the ecological effects of invaders. Biological Invasions 11:3–19.

Perry, R. W. 2013. A review of factors affecting cave climate for hibernating bats in temperate North America. Environmental Reviews 21:28–39.

Pounds, J. A., M. R. Bustamante, L. A. Coloma, J. A. Consuegra, M. P. L. Fogden, P. N. Foster, E. L. Marca, K. L., Masters, A. Merino-Viteri, R. Puschendorf, S. R. Ron, G. A. Sánchez-Azofeifa, C. J. Still, and B. E. Young. 2006. Widespread amphibian extinctions from epidemic disease driven by global warming. Nature 439:161–167.

Raun, G. G., and J. K. Baker. 1958. Some observations of Texas cave bats. Southwestern Naturalist 3:102–106.

Reeder, D. M., C. L. Frank, G. G. Turner, C. U. Meteyer, A. Kurta, E. R. Britzke, M. E. Vodzak, S. R. Darling, C. W. Stihler, A. C. Hicks, and R. Jacob. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. PLOS One 7:e38920.

Reynolds, H. T., T. Ingersoll, and H. A. Barton. 2015. Modeling the environmental growth of *Pseudogymnoascus destructans* and its impact on the white-nose syndrome epidemic. Journal of Wildlife Diseases 51:318–331.

Ripley, B., D. Bates, K. Hornik, A. Gebhardt, and D. Firth. 2011. Package 'MASS'. <cran.r-project.org/ web/packages/MASS>. Accessed 1 July 2019.

Roelke-Parker, M. E., L. Munson, C. Packer, R Kock, S. Cleaveland, M. Carpenter, S. J. O'Brien. A. Pospischil. R. Hofmann-Lehmann, H. Lutz, G. L. Mwamengele, M. N. Mgasa, G. A. Machange, B. A. Summers, and M. J. Appel. 1996. A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). Nature 379:441–445.

Schmidly, D. J., and R. D. Bradley. 2016. The Mammals of Texas. 7th ed. The University of Texas Press, Austin, Texas, USA.

Scott, M. E. 1988. The impact of infection and disease on animal populations: implications for conservation. Conservation Biology 2:40–56.

Sedgeley, J. A. and C. F. J. O'Donnell. 1999. Factors influencing roosts cavity selection by a temperate rainforest bat (*Chalinolobus tuberculatus*, Vespertilionidae) in New Zealand. Journal of Zoology (London), 249:437–446.

Shereen, M. A., S. Khan, A. Kazmi, N. Bashir, and R. Siddique. 2020. COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. Journal of Advanced Research 24:91–98.

Smith, G. R. 1982. Botulism in waterfowl. Pages 97–119 *in* M. A. Edwards and U. McDonnell, editors. Animal disease in relation to conservation. Academic Press, London, England.

Smith, T. J., and C. M. McKenna. 2013. A comparison of logistic regression pseudo R^2 indices. Multiple Linear Regression Viewpoints 32:17–26.

Sohrabi, C., Z. Alsafi, N. O'Neill, M. Khan, A. Kerwan, A. Al-Jabir, C. Losifidis, and R. Agha. 2020. World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). International Journal of Surgery 76:71–76.

Starbuck, C. A., S. K. Amelon, and F. R. Thompson III. 2015. Relationships between bat occupancy and habitat and landscape structure along a savanna, woodland, forest gradient in the Missouri Ozarks. Wildlife Society Bulletin 39:20–30.

Stevens, D. L., and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. Journal of the American Statistical Association 99:262–278.

Thorne, E. T., and E. S. Williams. 1988. Disease and endangered species: the black-footed ferret as a recent example. Conservation Biology 2:66–74.

Tinkle, D. W., and I. G. Patterson. 1965. A study of hibernating populations of *Myotis velifer* in northwestern Texas. Journal of Mammalogy 46:612–633.

TPWD [Texas Parks and Wildlife]. 2017. Fungus that causes white-nose syndrome in bats detected in Texas. <https://tpwd.texas.gov/newsmedia/releases/?req=20170323c>. Accessed 29 November 2018.

TPWD [Texas Parks and Wildlife]. 2018. Fungus causing white-nose syndrome spreads into central Texas.

<https://tpwd.texas.gov/newsmedia/releases/?req=20180404a>. Accessed 29 November 2018.

TPWD [Texas Parks and Wildlife]. 2020. White-nose syndrome confirmed in bats in Texas. <https://tpwd.texas.gov/newsmedia/releases/?req=20200305a>. Accessed 7 May 2020.

Turner, G. G., and D. M. Reeder. 2009. Update of white-nose syndrome in bats. Bat Research News 50:47–53.

Turner, G. G., D. M. Reeder, and J. T. H. Coleman. 2011. A five-year assessment of mortality and geographic spread of white-nose syndrome in North America and a look to the future. Bat Research News 52:13–27.

Twente, J. W. 1955. Some aspects of habitat selection and other behavior of cavern-dwelling bats. Ecology 36:706–732.

USEPA [U.S. Environmental Protection Agency]. 2013. Level III and IV ecoregions of the continental United States. U.S. EPA, National Health and Environmental Effects Research Laboratory, Corvallis, Oregon, Map scale 1:3,000,000. <http://www.epa.gov/wed/pages/ecoregions/level_iii_iv.htm>. Accessed 1 December 2016.

USFWS [U.S. Fish and Wildlife Service]. 2011. A national plan for assisting states, federal agencies, and tribes in managing white-nose syndrome in bats. <https://www.whitenosesyndrome.org/>. Accessed 1 December 2016.

USFWS [U.S. Fish and Wildlife Service]. 2014. Fungus that causes deadly bat disease discovered in Mississippi. <https://caves.org/WNS/Mississippi%20Release.pdf>. Accessed 27 February 2019.

USFWS [U.S. Fish and Wildlife Service]. 2016. Bats affected by WNS. <https://www.whitenosesyndrome.org/static-page/bats-affected-by-wns>. Accessed 1 January 2016.

USFWS [U.S. Fish and Wildlife Service]. 2017. Endangered and threatened wildlife and plants; 90-Day findings for five species, Vol. 82, No. 243. December 2017. <https://www.gpo.gov/fdsys/pkg/FR-2017-12-20/pdf/2017–27389.pdf>. Accessed 25 April 2018.

USFWS [U.S. Fish and Wildlife Service]. 2019. Bats affected by WNS. <https://www.whitenosesyndrome.org/static-page/bats-affected-by-wns>. Accessed 29 July 2019.

van den Broek, J. 1995. A score test for zero inflation in a Poisson distribution. Biometrics 51:738–743.

Verant, M. L., J. G. Boyles, W. Waldrep, Jr., G. Wibbelt, and D. S. Blehert. 2012. Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. PLOS One 7:e46280.

Walker, C. W., J. K. Sandel, R. L. Honeycutt, and C. Adams. 1996. Winter utilization of box culverts by vespertilionid bats in southeast Texas. Texas Academy of Science 48:166–168.

Warnecke, L., J. M. Turner, T. K. Bollinger, J. M. Lorch, V. Misra, P. M. Cryan, G. Wibbelt, D. S. Blehert, and C. K. R. Willis. 2012. Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. PNAS 109:6999–7003.

Webb, P. I., J. R. Speakman, and P. A. Racey. 1995. Evaporative water loss in two sympatric species of vespertilionid bat, *Plecotus auritus* and *Myotis daubentonii*: relation to foraging mode and implications for roost site selection. Journal of Zoology 235:269–278.

White-nose syndrome occurrence map – by year. 2019. Data last updated 8/30/2019. <https://www.whitenosesyndrome.org/static-page/wns-spead-maps>. Accessed 5 May 2020.

Wilson, M. E. 1995. Travel and the emergence of infectious diseases. Emerging Infectious Diseases 1:39–46.

Young, T. P. 1994. Natural die-offs of large mammals: implications for conservation. Conservation Biology 8:410–418.

Zimmerman, R. 2009. Biologists struggle to solve bat deaths. Science 324:1134–1135.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York, New York, USA.