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Use of highway culverts, box bridges, and caves by winter-roosting bats in Mississippi

By

Jessica Brown Katzenmeyer

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Wildlife and Fisheries Science
in the Department of Wildlife, Fisheries, and Aquaculture

Mississippi State, Mississippi

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Use of highway culverts, box bridges, and caves by winter-roosting bats in Mississippi

By

Jessica Brown Katzenmeyer

Approved:

Jeanne C. Jones
(Major Professor)

Bruce D. Leopold
(Committee Member)

W. Daryl Jones
(Committee Member)

Kevin M. Hunt
(Graduate Coordinator)

Andrew J. Kouba
Department Head

George M. Hopper
Dean
College of Forest Resources

Name: Jessica Brown Katzenmeyer

Date of Degree: May 6, 2016

Institution: Mississippi State University

Major Field: Wildlife and Fisheries Science

Major Professor: Dr. Jeanne C. Jones

Title of Study: Use of highway culverts, box bridges, and caves by winter-roosting bats in Mississippi

Pages in Study: 70

Candidate for Degree of Master of Science

White-nose Syndrome (WNS) has caused declines in bat populations in many areas of North America. To understand bat use and fungus presence in caves and culverts in Mississippi, I recorded bat species and abundance in these sites, roosting site characteristics, and incidence of WNS in selected caves and culverts used by bats. Sixteen caves and 214 culverts were surveyed from November-March 2010-2015. Five bat species were detected, and tricolor bats (*Perimyotis subflavus*) and southeastern myotis (*Myotis austroriparius*) were most abundant. Over five years, 3,789 roosting bats were recorded in caves and 16,812 were detected in culverts. I found significant relationships between bat numbers in culverts and microclimate conditions, dimensions, and proximity to public lands ($P < 0.03$). This study can help biologists with prioritization of protection and monitoring of culvert and cave roost sites and provide a greater understanding WNS incidence in these sites.

DEDICATION

This thesis is dedicated to my incredibly supportive husband, Zachary F. Katzenmeyer.

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CHAPTER I

INTRODUCTION TO STUDY

Introduction and Literature Review

Composing one-fifth of the mammalian population worldwide, there are 18 recognized families of bats encompassing 202 genera and 1,116 species (Simmons 2005). Approximately 25% of native bat species of North America are now threatened (IUCN 2010). Of the 15 species of bats found in Mississippi, 6 are species of concern, and 3 species are endangered. These species include Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), southeastern myotis (*Myotis austroriparius*), silver-haired bat (*Lasionycteris noctivagans),* hoary bat (*Lasiurus cinereus*), northern yellow bat (*Lasiurus intermedius*), little brown bat (*Myotis lucifugus*), northern long-eared bat (*Myotis septentrionalis*), gray bat (*Myotis grisescens*) and Indiana bat (*Myotis sodalist*; IUCN 2010). Population declines of many species of bats in the Southeast are due to anthropogenic changes, such as closure of caves, conversion of bottomland hardwood forests for agriculture or timber production uses, siltation and drainage of riparian areas, and accumulations of pesticides (Kunz and Pierson 1994).

Recent spread of disease has also contributed to decline in bat populations. White-nose syndrome (WNS) is associated with a fungus (*Pseudogymnoascus destructans*) that grows on the skin of hibernating bats causing premature awakenings, abnormal behavior, loss of critical fat reserves, and ultimately, mortality (Blehert et al.

2009). Current bat population surveys in areas of disease outbreak in the eastern United States suggest the possibility of a 75% population decline (Blehert et al. 2009). Prior to this study, limited information was published on incidence of the fungus in roosting sites of bats in the Gulf Coastal Plains of the U.S. However, monitoring for presence of the fungus has recently been funded through a National Science Foundation grant (US Fish and Wildlife Service 2014).

Declines in bat populations have been associated with economic and ecological impacts (Fujita and Tuttle 1991). Bats fill vital ecological niches in many different ecosystems. Some species of bats prey on great numbers of insects that cause damage to timber and agricultural resources. Whitaker (1995) found that big brown bats (*Eptesicus fuscus*) feed on a variety of insects that are often carriers of many different plant and animal diseases. In addition, bats in forested ecosystems are important seed dispersers and plant pollinators (Fujita and Tuttle 1991, Fleming and Estrada 2012).

Adequate roosting sites are key life requirements for survival of all bat species in North America (Barclay and Kurta 2007). Bats typically occupy roost sites over 60% of a 24-hour period; therefore, roost habitat availability, selection, and quality are important components of bat ecology (Barclay and Kurta 2007). Roost sites are important because of their role in regulation of metabolism, social interactions, dormancy, reproduction, flight behavior, and avoidance of predation risks (Kunz 1982, Barclay and Kurta 2007). Roost site selection varies among bat species across their ranges (Harvey et al. 2006). Certain bats roost in caves, culverts, cisterns, abandoned buildings, and under bridges in many areas of their range (Clark 1990, Cochran 1999, Lance et al. 2001, Harvey et al. 2006, Trousdale et al. 2011). Habitat characteristics that may influence use of roost sites by bats include presence of surface water, such as wetlands, rivers, or streams (Rice 1957). Varieties of cavities are used as winter hibernacula, and these roost sites may offer stable and desirable temperature and relative humidity conditions, as well as protection from predators (Trousdale et al. 2008). Winter roost sites are particularly important to survival, because bats must undergo thermoregulation at a time of decreased food availability (Speakman and Thomas 2003). Additionally, most bats enter torpor during winter roosting, and in this state of low activity, they are more vulnerable to predators and diseases (Bouma et al. 2010, Estók et al. 2009).

Natural caves and underground mine shafts often exhibit stable interior microclimates and internal structure features that attract roosting bats (Harvey et al. 2006). Many bat species of concern roost in caves including gray bat, Indiana bat, southeastern myotis, little brown bat, and northern long-eared bat (Kunz 1982). One study reported that tri-colored bats (*Perimyotis subflavus*) were observed using caves in Arkansas with occupancy rates ranging from 34-40% during winter and spring surveys (Briggler and Prather 2003). Caves occurring in Mississippi could potentially serve as important roost sites for bats, and surveys conducted since 2010 by wildlife biologists have revealed presence of winter-roosting bats in several Mississippi caves (Pers. Comm. Kathy Shelton, Mississippi Department of Wildlife, Fisheries and Parks). Caves in Mississippi may be associated with ceremonial and burial mounds of North American Indians. Caves associated with these mounds seldom contain speleothems and most exhibit wet and muddy bottom substrates with restricted entrance pathways (Knight et al. 1974). To date, 47 caves have been studied in Mississippi, and these caves display speleothem, stalactite, and stalagmite formations (Knight et al. 1974, Moore 2006). In addition to caves, abandoned mines can serve as roosting sites for selected bat species with some mines being occupied by >1 million bats (www.batcon.org). Three bat species including gray, northern long-eared, and tri-colored bats, have been reported to roost in abandoned silica and chalk mines in northeastern Mississippi (White 1960, Best and Caesar 2000). However, little is known about use of caves and cave-like structures, such as mines, by winter-roosting bats in Mississippi.

Landscape conditions may play a role in roost site selection by bats (Clark 1990, Sealander and Heidt 1990). For example, bottomland hardwood forests provide important roost and foraging sites for many bat species of the southeastern United States (Clark 1990, Sealander and Heidt 1990, Cochran 1999, Menzel et. al 2001, Mirowsky et al. 2004). Also, interspersion of good foraging areas near quality roosting sites may influence roost use by bats (Clark 1990, Sealander and Heidt 1990). Loss of mature hardwood forests since European settlement may be associated with loss of natural roost sites, such as old growth trees with internal cavities (Clark 1990). At the time of European settlement, the Mississippi Alluvial Valley consisted of ten million hectares of bottomland hardwood forests. Today less than half of the historical forest cover remains with 87% of that loss attributed to conversion for agriculture (Tiner 1984, Hefner and Brown 1985). Flood control projects following the floods of 1912, 1913, 1916, and 1927 lowered the risk of flooding on millions of hectares of land and catalyzed site conversion of bottomland hardwood forests to agricultural land uses (U.S. Department of the Interior 1988, Newling 1990). Remaining bottomland hardwood forests are severely fragmented, and many of the residual patches are in a degraded condition due to poor timber management practices (Rudis 1995). Impacts resulting for this type of habitat loss could be associated with use of human-made structures by roosting bats (Trousdale et al. 2011). However, a greater understanding of use of human-constructed structures by roosting bats is needed to fully understand associated conservation implications for bats and human-bat interactions (Keeley and Tuttle 1999, Bach et al. 2004, Trousdale et al. 2008).

Underpasses, including culverts and bridges, are extremely common within the landscape and are frequently used by certain bat species (Keeley and Tuttle 1999, Bach et al. 2004, Boonman 2011). Culverts provide adequate roosting conditions, because they generally have reduced light, stable microclimates, and are associated with surface water of streams or canals that drain through them. These structures could be critical habitat for species that historically relied on natural roosts, and some species may seasonally depend on anthropogenic roosts in landscapes with limited natural roosting alternatives (Trousdale et al. 2008). Behavioral adaptations of bats relative to culvert and bridge use could be related to multiple factors including degree of structural stability, thermoregulatory benefits, availability and abundance of alternative roosts, and surrounding habitat (Kunz 1982, Lance et al. 2001). According to Boonman (2011), dimensions of underpasses were the most significant factor associated with use by roosting bats with height being the most significant factor. Adam and Hayes (2000) reported that bats used larger bridges with larger dimensions, because of factors such as increased surface area for roosting, better accessibility, greater solar-thermal stability, and greater protection from predators. Keeley and Tuttle (1999) found that 94% of bats using bridges and culverts were crevice-dwelling bats, showing evidence that availability of crevices in culverts and bridges may also be a factor in use. However, more information is needed on factors that influence use of box bridges and culverts by roosting bats in North America (Keeley and Tuttle 1999, Trousdale and Beckett 2004, Trousdale et al. 2011).

Justification

Studies that have investigated bat use of culverts and box bridges have contributed to our understanding of roosting behaviors of selected bat species. However, to date, studies have been localized in their scope. Currently, there is an extreme lack an understanding of roosting ecology of bats in culverts and box bridges along highway corridors in Mississippi. However, due to the need for this data, bat biologists have been collecting data on this usage since 2010. Bat biologists cooperating in this long term study include professionals of the Mississippi Bat Working Group, Mississippi Department of Wildlife, Fisheries and Parks, Department of Wildlife, Fisheries and Aquaculture of Mississippi State University, and U.S.D.I. Fish and Wildlife Service.

In an effort to summarize these data and contribute to this effort, I have conducted the following study. This study was comprised of several tiers of data collection, summary, and analyses including the following:

1. Summary and analysis of data collected by biologists from 2010 through 2015 on bat occupancy and characteristics of culverts and box bridges,
2. Repeated surveys in 2015 of a subset of culvert and box bridges from 2010-2014 culvert and box bridge population, and
3. Numbers of bats recorded using selected caves in Mississippi as winter roost sites.

In addition to numbers and species of roosting bats, I reported cave, culvert, and box bridge characteristics, selected landscape features associated with these sites, temperature regimes within culverts and box bridges, year to year trends in bat numbers in selected individual culverts, and results of testing for WNS.

Objectives

1. Record and report abundance and species of bats detected in highway culverts and box bridges in Mississippi during November through mid-March, 2010-2015.
2. Measure internal characteristics, ambient air temperatures, and selected landscape conditions associated with surveyed culverts and box bridges and estimate relationships of these metrics with numbers of roosting bats.
3. Report numbers of bats and bat species detected in Mississippi caves from 2010-2015, and graphically compare detected numbers over this study period, and
4. Report results of testing for white-nose fungus (*Pseudogymnoascus destructans*) in surveyed culverts, box bridges, and caves and assess potential for negative impacts to bats based on recorded temperature regimes and numbers of roosting bats.

CHAPTER II

CAVE USE BY WINTER-ROOSTING BATS IN MISSISSIPPI

Introduction

Currently fifteen bat species have been reported to occur in Mississippi including gray bat (*Myotis grisescens*), Indiana bat (*Myotis sodalist*), Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), southeastern myotis (*Myotis austroriparius*), silver-haired bat (*Lasionycteris noctivagans*), hoary bat (*Lasiurus cinereus*), northern yellow bat (*Lasiurus intermedius*), little brown bat (*Myotis lucifugus*), northern long-eared bat (*Myotis septentrionalis*), big brown bat (*Eptesicus fuscus*), eastern red bat (*Lasiurus borealis*), seminole bat (*Lasiurus seminolus*), tricolor bat (*Perimyotis subflavus*), Brazilian free-tailed bat (*Tadarida barsiliensis*), and evening bat (*Nycticeius humeralis*) (Kennedy et al. 1974, Jones and Carter 1989). Nine of these bat species may use caves for roosting. Of these nine species, six are endangered species or species of special concern. These species include gray bat, Indiana bat, northern long-eared bat, Rafinesque's big-eared bat, southeastern myotis, and little brown bat (IUCN 2010).

Caves are important roosting sites for many bat species, and therefore, may play an important role in bat conservation. Some of the largest bat colonies reported in North America are found in caves, such as Bracken Cave, Texas (McCracken 1986). Caves are roosting sites that enable bats to regulate metabolism, interact socially, initiate flight behavior, reproduce, and avoid predators (Kunz 1982, Barclay and Kurta 2007). Because of their structure and subsurface locations, caves may offer stable and desirable temperature and humidity regimes for roosting bats (Wilkinson and South 2002). Greater microclimate stability, limited disturbance, and protection from predators may be factors involved in greater longevity reported for cave-roosting bats (Wilkinson and South 2002).

Caves in the southeastern U.S. are often associated with parent materials, such as limestone or consolidated rocks, or mountainous topography (Knight et al. 1974, Moore 2006). In southern states, such as South Carolina, Tennessee, Texas, and Florida, studies have reported use of caves by over 12 species of bats (Rice 1957, Hall and Wilson 1966, LaVal 1970, Clark et al. 1975, Humphrey 1978, McCracken 1986, Ludlow and Gore 1997, Gore and Hovis 1992, Menzel et al. 2003). In Mississippi, 47 caves have been recorded that could be used by roosting bats. These caves exhibit formations of speleothem, stalactite, and stalagmite, exhibit wet and muddy bottom substrates, and typically have restricted entrance access (Knight et al. 1974, Moore 2006). Some caves in Mississippi are remnants of ceremonial Native American burial grounds or are of karst decent, specifically limestone eroded by dissolution (Moore 2006). In addition to caves, bats may also roost in abandoned mines (Harvey et al. 1991). In northeastern Mississippi, gray, northern long-eared, and tricolor bats have been reported to roost in an abandoned silica and chalk mine in Tishomingo County (White 1960, Best and Caesar 2000). Due to morphology and subsurface positions, these mines are similar to naturally formed caves and may exhibit similar internal microclimate conditions (White 1960). To date, limited information is available on use of caves by winter roosting bats in Mississippi.

Objectives of this portion of the study were as follows:

1. Report numbers of bats and bat species detected in 16 Mississippi caves during winters of 2010-2015, and graphically compare detected numbers during this study period.
2. Estimate associations between bat numbers roosting in caves and proximity to selected landscape features, such as public forest lands, streams, and rivers and cave length.

Methods

Sixteen caves were surveyed at least one time during the study period - November-March of 2010-2015. Surveyed caves were located in nine counties in Mississippi: Attala, Franklin, Jasper, Neshoba, Smith, Tippah, Tishomingo, Union, and Wayne. Twelve caves were surveyed once during the study period, and four caves were surveyed twice during the same season (November-March) of 2011-2012.

Surveys were conducted by inspection of internal cave ceilings and sides during daylight hours on days in which ambient air temperature was $< 10^{o}$ C. Inspections were accomplished by walking silently through caves with head lamp and recording number of roosting bats observed. Roosting bats were not disturbed or handled during surveys. The following information was recorded for each bat observed: identification to species, location of roosting bat, and condition of bat including visible lesions, injuries, or fungal growth (Blehert et al. 2009). During each survey, total number of roosting bats and species detected was recorded. For caves surveyed twice in the same season, total number of bats detected in each survey event was recorded and average number of bats detected during the two survey periods was calculated and reported.

Associations between numbers of roosting bats of each species and proximity to landscape variables were analyzed using ArcMap GIS Version 10 (Environmental Systems Research Institute, Inc., Redlands, California) and nonparametric statistical methods (Zar 1999). Locations of caves were obtained using a handheld Global Positioning System unit, then overlain on a base map created by the Mississippi Automated Resource Information System (MARIS). The following landscape variables were identified: Wildlife Management Areas, National Wildlife Refuges, State Parks, National Parks, National Forests, major rivers, and streams. Distance (km) from each cave location to the closest public land area and water body or waterway categories were measured in ArcMap using the measure tool (ArcMap Version 10, Figure 2.1). Coordinates of one cave ("Waddell Cave") in this study were not recorded and therefore, bat numbers and associations of this cave were not tested.

Dimensions of surveyed caves were difficult to find in the literature. Knight et al. (1974) and Moore (2006) describe the lengths of selected caves in Mississippi (Table 2.1). In comparing dimensions found in the two studies, I determined that structural characteristics of these caves changed over time due to hydrology, sediment build up, and erosion (Moore 2006). Therefore, I used lengths measured by Moore (2006) of mutual caves surveyed (Table 2.1).

Cave locations were subdivided into three groups based on their geographic location across the state: north, central, and south Mississippi based on Stewart (2003). This regional classification resulted in the following: six caves in Tippah, Tishomingo, and Union counties were in the northern region; two caves in Attala and Neshoba counties were in the central region, and eight caves in Jasper, Franklin, Smith, and Wayne counties were in the southern region (Stewart 2003).

Statistical Analyses

The Shapiro-Wilk test (Zar 1999) was used to estimate normality of the data and showed that bat count data exhibited a non-normal distribution ($P \leq 0.001$). Therefore, Spearman's nonparametric correlation analysis was used to estimate relationships between numbers of detected bats and landscape variables: distance to public lands, rivers, and streams and length of caves (from cave opening to farthest internal vertical cave wall (Zar 1999). A P-value of < 0.05 was considered significant for all tests (Zar 1999). Statistical analyses were performed using SAS for Windows 8.0 Version 9.4 (SAS Institute, Cary, NC).

Results

Populations of southeastern myotis and tricolor bats were detected in the 16 surveyed caves. Total bat numbers detected in caves during the five-year period ranged from 99 to 1,527 (Table 2.2; Figure 2.2). Numbers of detected southeastern myotis in all caves during the study period ranged from 0 to 1,500 (Table 2.2; Figure 2.2). Numbers of tricolor bats in caves ranged from 27 to 772 (Table 2.2; Figure 2.2). Mean numbers of bats derived from pooled data in all caves within each of the 3 regions yielded mean numbers of bat detections ranging from 6.47 (± 1.53) bats in northern Mississippi to 153.83 (± 66.56) in south Mississippi (Table 2.3; Figure 2.3). In 69% of surveyed caves (n=11), only tricolor bats were detected (Table 2.2). The greatest number of bats (n=1,527) were identified throughout all regions in November-March of 2010-2011, with southeastern myotis being the most numerous bats detected (Figure 2.1). The greatest numbers of southeastern myotis were detected in caves of southern Mississippi (Table 2.3; Figure 2.3). However, tricolor bats were more numerous and more commonly detected in caves of northern Mississippi (Table 2.3; Figure 2.3).

Spearman's nonparametric correlation analysis showed no relationship between numbers of bats in caves and distances to public lands ($r = 0.27$, $P < 0.08$), distances to rivers ($r = 0.18$, $P < 0.24$), distances to streams ($r = -0.28$, $P < 0.06$), and lengths of caves ($r = 0.13$, $P < 0.62$; Table 2.4; Table 2.5).

Discussion

Conservation of caves and protection of caves from human disturbance has been reported to be important in bat conservation due to their requirements of stable microclimates and secluded habitats in which to roost and rear young (Hutson et al. 2001). Furthermore, disturbance and collapse of caves have been major causes of decline in populations of bats across the world, making the identification, research, and protection of cave roosting sites vitally important for bat conservation (Hutson et al. 2001). Species of bats that may use caves in the southeastern U.S. and in Mississippi include gray bat, Indiana bat, Rafinesque's big-eared bat, southeastern myotis, little brown bat, northern long-eared bat, big brown bat, tricolor bat, and Brazilian free-tailed bat (Harvey 1991, Gore and Hovis 1994, Ludlow and Gore 1997, Briggler and Prather 2003, Menzel et al. 2003). Of these species, I detected two species roosting in surveyed caves of Mississippi: southeastern myotis and tricolor bats. Three species of federally-listed bats, gray, Indiana, and northern long-eared bats, typically use caves as roost sites

(Humphrey 1978, Tuttle 1979, Broders et al. 2006). However, these species were not detected in caves surveyed in this study.

Prior to this study, localized studies had investigated cave use by roosting bats, but investigations of cave use by winter-roosting bats had not been accomplished statewide. This study reports numbers and species of bats in 16 of the 47 known caves in southern, northern, and central Mississippi. Best and Caesar (2000) studied six caves and one abandoned mine in Tishomingo County and four caves in Union county of northeast Mississippi and found that roosting bats were present in two surveyed caves. Similar to this study, tricolor bats were reported by Best and Caesar (2000) in sampled caves of northeast Mississippi. However, Best and Caesar (2000) also reported big brown bats roosting in surveyed caves, a species which was not encountered in caves of this study. Similar findings were reported by Trousdale and Beckett (2002) who detected tricolor and southeastern myotis bats roosting in two caves of southern Mississippi, Eucutta and William's Caves (also referred to as Pitts' Cave). In their study, they detected three southeastern myotis and one tricolor in Eucutta Cave and five southeastern myotis and one tricolor in William's Cave. In my study, I found greater numbers of both bat species in both of these caves. In 2013-2014 and 2014-2015 surveys of Eucutta Cave, numbers of tricolor bats ranged from 320 to 330 and numbers of southeastern myotis ranged from 41 to 70. In 2013-2014 and 2014-2015 surveys of William's Cave, numbers of tricolor bats ranged from 53 to 226 and one southeastern myotis was detected. William's Cave was not surveyed in this study previous to the winter season of 2013-2014. Differences in numbers of roosting bats in the caves in this study and Trousdale and Beckett (2002) could be related to different seasons of surveys and greater survey coverage of cave interior in this study the second season due to accessibility. Trousdale and Beckett (2002) conducted surveys during August and September; whereas, surveys of this study were conducted during winter. It is possible that these caves hold greater numbers of winter-roosting bats compared to summer and maternal roosting bats.

Roth (2014) observed maternity colonies consisting of 2,700 southeastern myotis in Waddell Cave and 10,000 southeastern myotis in William's Cave during summer surveys of 2011 and 2012. Numbers of roosting bats during breeding season were greater than those of winter-roosting bat numbers detected during my study. Similar to Trousdale and Beckett (2002), my findings indicated that numbers of bats using selected caves as winter hibernacula in Mississippi may be less than numbers of maternity colonies of those caves. Also, numbers of bats using caves as roost sites during winter were typically less than those reported by studies in other southern states. For example, large numbers of southeastern myotis have been found using caves as hibernacula in Kentucky and Florida (Rice 1957, Barbour and Davis 1969). Harvey et al. (1991) reported 3,000 southeastern myotis in caves of western Kentucky. Hoffmeister (1989) found southeastern myotis forming multiple clusters of 8 to 120 bats on cave ceilings in Indiana. Other studies conducted in the southeastern U.S. indicated that some caves support large maternity colonies. For example, Gore and Hovis (1992) reported maternity colonies of up to 100,000 adult southeastern myotis in limestone sink caves of Florida. Similarly, Menzel et al. (2003) reported that southeastern myotis commonly roosted in limestone sinks in Orangeburg County, South Carolina.

Parent material, cave morphology, and substrates may be related to roosting bat numbers in caves of Mississippi. For example, only 12 counties in Mississippi are located within limestone outcrop regions, and caves of this parent material and origin are typically uncommon across the state's landscape (Moore 2006). Ten counties containing thirteen caves do not fall within the limestone outcrop regions, and these caves are reported to have pseudokarst features (Moore 2006). Calcote Branch Cave, Kosciusko Cave, and Nanih Waiya Cave are categorized as pseudokarst caves. A pseudokarst cave has characteristics, such as closed depressions, reduced surface water or stream occurrence, and is produced by processes other than dissolving of rock, resulting in muddy walls rather than consolidated stone or rock (Neuendorf et al. 2005). These caves exhibited roosting bats during this study with numbers ranging from 0 to 42 over the study period, and although roosting bats were present, larger numbers (>100 roosting individuals) were not detected. I submit that features and decent of pseudokarst caves of Mississippi may be less suitable for large colonies of winter-roosting bats based on descriptions by Neuendorf et al. (2005).

Unlike pseudokarst caves of central Mississippi, north Mississippi caves were comprised of limestone outcroppings, walls, and ceilings (Rice 1957). These caves exhibited lowest numbers of winter-roosting bats during the study period with 123 tricolor bats being detected. Other studies which have investigated roosting bats in caves with rock or stone structure have reported that tricolor and southeastern bats were the primary bat species detected roosting in caves during winter study periods in Minnesota, West Virginia, South Carolina, Maryland, and Pennsylvania (Swanson and Evans 1936, Davis 1966, Golley 1966, Raesly and Gates 1987). Other reasons for lower numbers of roosting bats detected in north Mississippi may be landscape location and regional forest cover types. Although I did not measure forest type composition within the regions of

Mississippi over the study period, bat numbers in caves may be attributed to the differences in these landscape conditions. For example, southern Mississippi consists of more evergreen, softwood forests whereas northern Mississippi consist of greater concentrations of hardwood and mixed hardwood forests (Collins et al. 2005, Mississippi Forest Inventory Database 2006 unpublished data, Mississippi State University). Tricolor and southeastern myotis bats also use basal cavities of hardwoods as roosting sites, and therefore, are often associated with hardwood forest cover types (Frost et al. 1986). Differences in numbers of roosting bats between southern and northern Mississippi may also be associated with different latitudinal temperatures and proximity of physiographic regions, such as the Appalachian Plateau, where caves are more commonly available on the landscape (McCoy and Connor 1980).

Human disturbance in roosting sites can have deleterious effects on bats. In a study by Johnson et al. (1998), human disturbance contributed to early arousal of bats out of hibernation, leading to weight loss and lower survival. In the least disturbed cave of Johnson's study, there was less weight loss in roosting bats and greater numbers of bats detected. My detection of tricolor bats in Poole Cave could be related to this species wider range of tolerance to human disturbance and more generalist life requirements compared to other cave-roosting bat species (Russ and Montgomery 2002, Davidson-Watts et. al 2006).

I did not detect associations between roosting bat numbers in caves and length of caves; however, I was unable to validate lengths and other morphometric features reported by Moore (2006), and based on examination of historical cave morphology reported for Mississippi caves, these features may change over time due to cave-ins, wall collapse, and erosion (Table 2.1). Also, sample sizes of caves surveyed may have hampered my abilities to draw strong inferences concerning associations between roosting bat numbers and cave morphology.

Sampling greater number of caves and including surveys during winter, spring, and summer would have provided more inferential strength in this study. For example, I submit that numbers and species richness of bats detected during this study may have been related to sampling intensity of caves. I recommend that annual surveys of each cave with repeated surveys during each season should be conducted to provide stronger inferences concerning numbers of roosting bats within a specific season, seasonal variation in numbers of roosting bats, and importance of caves as maternity roosts. Also, sampling of a greater proportion of caves in Mississippi would have potentially provided more information on winter-roosting bat communities. This study included surveys of 33% of Mississippi's caves and inclusion of a greater number of caves in roosting surveys is recommended for future studies. However, increasing sampling intensity will require a dedicated source of funding for travel and human resource budgets.

The U.S. Fish and Wildlife Service provides a survey protocol for the Indiana bat that could also be implemented in Mississippi in the future. The protocol identifies cave characteristics unsuitable to bats: a) <1 horizontal opening < 15.24 cm in diameter with little airflow detected, b) it is a vertical channel < 0.305 m in diameter, c) horizontal passage is < 15.24 m with no fissures for bat access, d) opening shows evidence of flooding or has collapsed, and e) an opening < 1 year old due to changes in landscape features (fws.org). Therefore, suitable caves can be better identified and less costly. This report focuses on capturing bats to better assess population numbers and provides summer and fall capture protocol, but does not include winter surveying, signifying that no mist-netting should be performed during hibernation. However, it later states that similar to trapping, duration of monitoring should be a minimum 5 hours and begin half an hour before sunset recording numbers of bats and possible changes in bat numbers. Also, bat acoustic surveys should be implemented to better identify species of bats using caves.

Table 2.1 Reported lengths (m) of six caves surveyed in winter-roosting bat study in Mississippi from 2010 through 2015.

Cave Name and County of Location	Cave Length (Moore 2006)	Cave Length (Knight et al. 1974)
Grubbs's Dry Cave, Union County	67.84 m	300.00 m
Muddy Ridge Cave, Tippah County	64.40 m	105.00 m
William's Cave aka "Pitt's Cave", Wayne County	420.00 m	4020.00 m
Triple H Cave, Wayne County	176.90 m	480.00 m
Waddell Cave, Smith County	235.00 m	Not reported

(Knight et al. 1974, Moore 2006)

Table 2.2 Total species and numbers of bats detected during diurnal roost surveys of caves in Mississippi during November – March 2010-2011 through November – March 2014 - 2015.

Cave Name, County	Species and Numbers of Bats Detected During Diurnal Roost Surveys of Caves									
	Winter 2010 - 2011		Winter 2011-2012		Winter 2012-2013		Winter 2013-2014		Winter 2014-2015	
	Tricolor Bat [a]	SE Myotis [a]	Tricolor Bat	SE Myotis	Tricolor Bat	SE Myotis	Tricolor Bat	SE Myotis	Tricolor Bat	SE Myotis
Belding's Cave, Jasper Co.	0	0	0	0	17	0	46	0	23	0
Calcote Branch Cave, Franklin Co.	0	0	0	0	0	0	24	0	0	0
Eucutta Cave, Wayne Co.	0	0	0	0	0	0	330	70	320	41
Grubb's Dry Cave, Union Co.	0	0	0	0	2	0	3	0	0	0
Holder's Cave, Jasper Co.	0	0	0	0	7	0	0	0	0	0
Kosciusko Cave, Attala Co.	0	0	4	0	0	0	0	0	0	0
Lamar Graham Cave, Wayne Co.	0	0	0	0	0	0	20	0	0	0
Land of Caves #1 Lower Chamber, Union Co.	5	0	3.5[b]	0	0	0	0	0	0	0
Land of Caves #1 Upper Chamber, Union Co.	15	0	13[b]	0	28	0	7	0	0	0
Land of Caves #3, Union Co.	5	0	2.5[b]	0	9	0	5	0	0	0
Muddy Ridge Cave, Tippah Co.	0	0	1.5[b]	0	0	0	0	0	0	0
Nanih Waiya Cave, Neshoba Co.	0	0	42	0	34	0	27	1	20	0

Table 2.2 (Continued)

William's Cave aka "Pitt's Cave", Wayne Co.	0	0	0	0	0	0	53	1	226	1
Poole Cave, Tishomingo Co.	1	0	0	0	2	0	0	0	0	0
Triple H Cave, Wayne Co.	0	0	0	0	0	0	127	1	183	1
Waddell Cave, Smith Co.	1	1,500	30	0	0	0	69	447	0	0
Total Number of Bats	27	1,500	96.5	0	99	0	711	520	772	43
Total Number of Bats of Both Species	1,527		117		99		1,231		815	

[a] Tricolor Bat (*Perimyotis subflavus*); SE Myotis - Southeastern Myotis (M*yotis austroriparius*)

[b] Number of bats detected in two surveys of same cave were averaged and number reported represents mean number of bats detected in two surveys.

Table 2.3 Numbers of bats detected in caves during winter-roosting surveys of 2010-2015 by geographic region of Mississippi.

Region of Mississippi	Number of Caves Surveyed	Total No. of Bats Detected	Mean No. (± SE)	Range in No. of Bats Detected during Surveys
North [a]	6	123	6.47 (±1.53)	1 - 28
Central [b]	2	128	21.33 (±11.55)	1 - 42
South [c]	8	3,538	153.83 (±66.56)	1- 1,500
Total	16	3,789	78.94 (±57.51)	1 – 1,500

[a] North Region includes Tippah County, Tishomingo County, and Union County.
[b] Central Region includes Attala County and Neshoba County.
[c] South Region includes Franklin County, Jasper County, Smith County and Wayne County.

Table 2.4 Spearman's nonparametric correlation results for selected landscape variables and cave length and total number of bats detected in winter-roost surveys during November- March 2010-2015 in Mississippi.

Response Variable		Distance to Public Land	Distance tc Rivers	Distance to Streams	Cave length
Total No. of Bats	*r*	0.27	0.18	-0.28	0.13
	P	0.08	0.24	0.067	0.62
Range		0.10 km- 29.765 km	0.465 km- 24.537 km	0.067 km- 3.052 km	64.40 m- 420.00 m

Spearman Correlation Coefficients; Prob > |r| under H_0: Rho=0

Table 2.5 Distance (km) and length (m) metrics of features associated with Mississippi caves surveyed for roosting bats from 2010-2015.

Landscape Variable	Minimum	Maximum	Mean (± SE)
Distance to Public Lands [a]	0.10 km	29.755 km	13.89 km (±1.136)
Distance to Rivers [b]	0.465 km	24.526 km	6.66 km (±0.936)
Distance to Streams [c]	0.06 km	3.05 km	1.36 km (±0.165)
Length of Cave	64.40 m	420.00 m	192.83 m (±31.086)

[a] Public Lands include Wildlife Management Areas, National Wildlife Refuges, State Parks, National Parks, and National Forests.

[b] Rivers – ≥ 5 Order Lotic System (MARIS 1983).

[c] Stream – 1st-4th Order Lotic System (MARIS 2000).

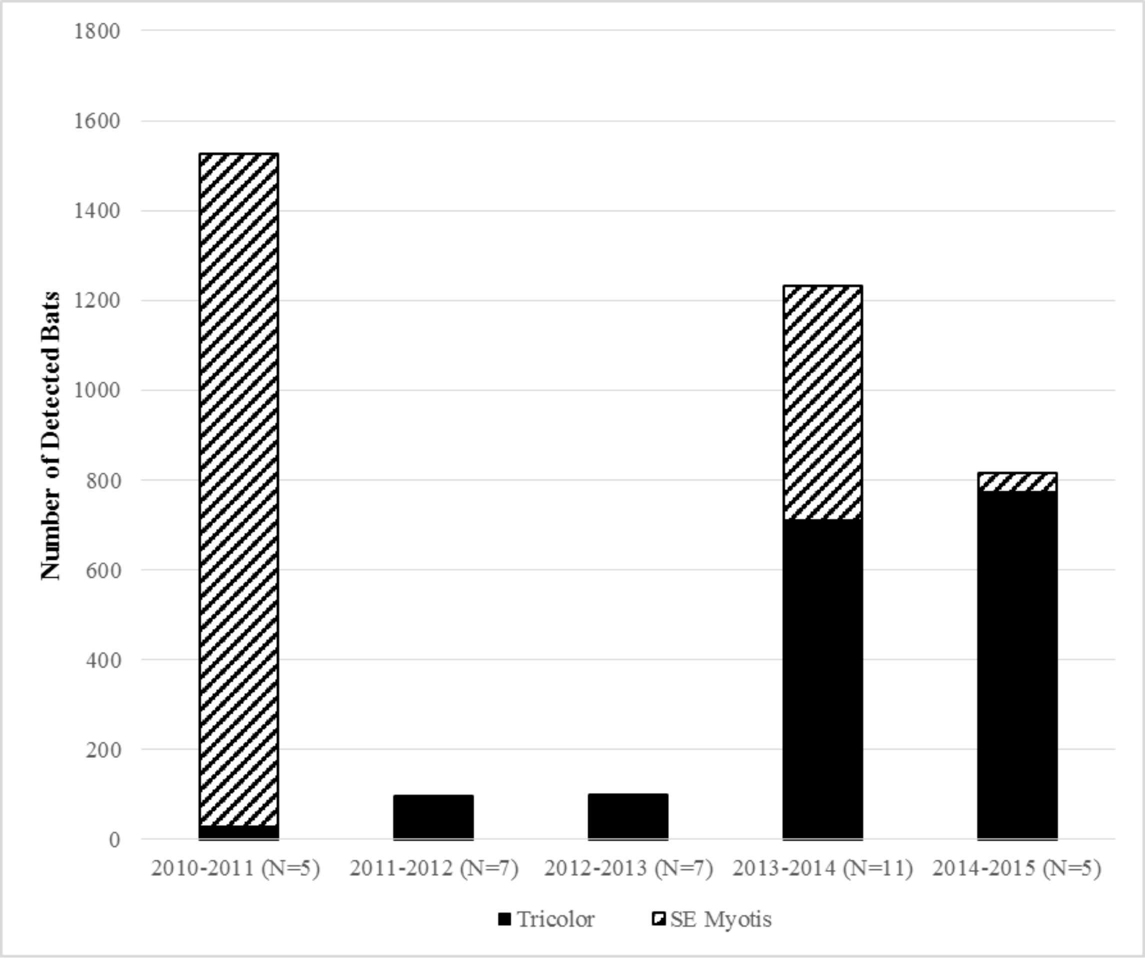


Figure 2.1 Total number of bats and species of bats detected during diurnal roost surveys of caves in Mississippi during November through March, 2010 through 2015

(Tricolor bat – Perimyotis subflavus and SE Myotis - Myotis austroripiarius).

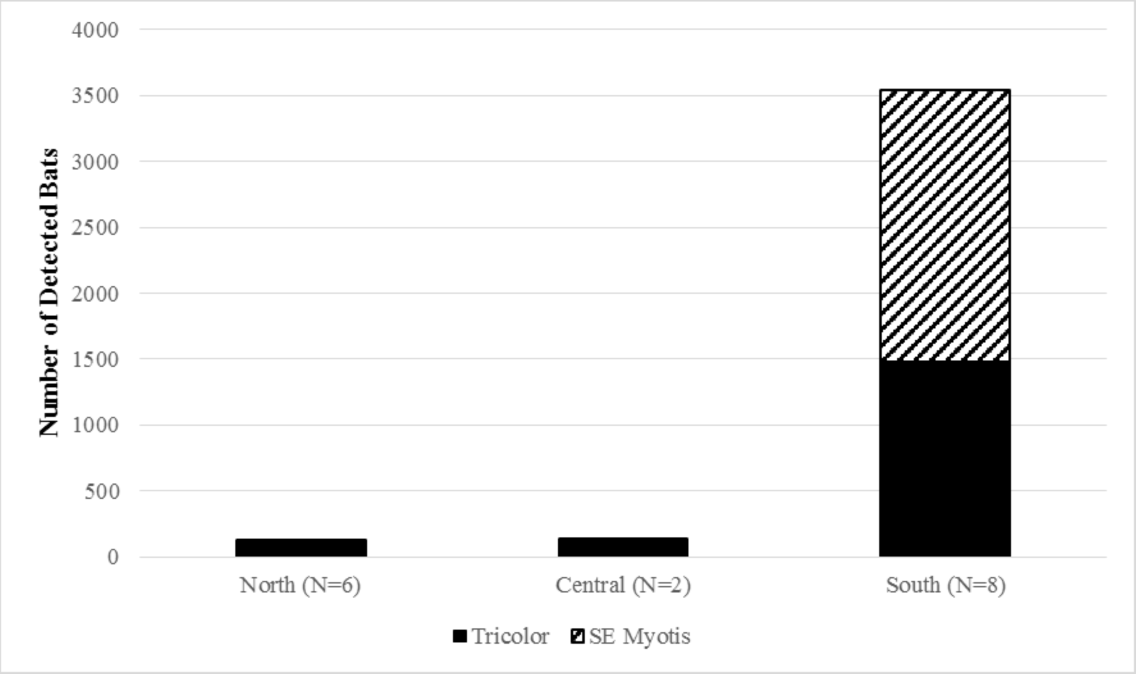


Figure 2.2 Total number and species of roosting bats detected during November through March of 2010-2015 in caves in Mississippi subdivided by geographic region.

CHAPTER III

USE OF HIGHWAY CULVERTS AND BOX BRIDGES AS WINTER-ROOSTING SITES BY BATS IN MISSISSIPPI

Introduction

Many studies have recorded the use of culverts and bridges by wildlife (Cavallaro et al. 2005). Culverts and box bridges are structures used as highway and road underpasses that aid in prevention of roadway flooding and erosion (MDOT 2004). These structures may be used as movement corridors and cover by wildlife. In some states of the United States, culverts and bridges of roadways have been constructed or modified to improve wildlife underpasses to reduce traffic-related mortality (Jackson 1996, Ruediger 2001).

Culverts have been used by many bat species in the United States. Over 50% of bat species indigenous to Mississippi may use culverts as roost sites (LaVal 1967, Kennedy et al. 1974, Jones and Carter 1989, Humphrey and Gore 1992, Keeley and Tuttle 1999, Trousdale and Beckett 2004, Bender et al. 2010). These include bat species of conservation concern, such as gray bat (*Myotis grisescens*), Indiana bat (*Myotis sodalis*), Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), southeastern myotis (*Myotis austroriparius*), and little brown bat (*Myotis lucifugus*), and more common species, such as Brazilian free-tailed bat (*Tadarida brasiliensis*), big brown bat (*Eptesicus fuscus*), and tricolor bat (*Perimyotis subflavus*) (LaVal 1967, Humphrey and

Gore 1992, Keeley and Tuttle 1999, Trousdale and Beckett 2004, Bender et al. 2010). Culverts may serve as critical habitat for certain bat species that historically relied on natural roosting sites in landscapes with limited roosting habitat (Lance et al. 2001, Trousdale et al. 2008). Use of these anthropogenic sites as roosts may be related to dimensions of the structure, thermoregulatory benefits, availability and abundance of alternative roosts, and surrounding landscape characteristics (Kunz 1982, Lance et al. 2001).

Culverts and bridges particularly those made of concrete, have been recognized across the United States as roosting sites for a number of insectivorous bats (Walker et al. 1996, Keeley and Tuttle 1999, Sandel et al. 2001, Trousdale and Beckett 2004). Increased information on bat use of culverts and features of used culverts could assist in conservation planning for culvert-roosting bats. Also, this information could be used to change future construction of these sites to aid in conservation of imperiled bat species that roost in culverts. Although maternity roost studies have been conducted in south Mississippi, limited published information was available on use of culverts by roosting bats in Mississippi during winter.

Objectives of this portion of the study were:

1. Report numbers of bats and bat species detected in 214 Mississippi culverts during winters of 2010-2015, and graphically compare detected numbers during this study period,
2. Report numbers of bats and bat species detected in 39 Mississippi culverts during winter of 2015 repeated surveys, and graphically compare detected numbers during this study period, and

3. Measure dimension characteristics, ambient air temperatures, and distance to public lands and major rivers in surveyed culverts and estimate relationships of these metrics with numbers of roosting bats.

Methods

All Surveyed Culverts

This study was conducted in 27 counties throughout Mississippi from November - March of 2010-2015. Diurnal roost surveys were conducted in 214 concrete culverts and box bridges during the study period. Structures included in my study were defined as follows. A culvert was defined as "any structure, not classified as a bridge, which provides an opening under the roadway." [Mississippi Department of Transportation (MDOT) 2004]. Box bridges were defined as "a box culvert having a clear distance between inside face of end supports exceeding 6.67 m measured along the centerline of the roadway." (MDOT 2004). Most culverts and box bridges included in this study were surveyed once during the November – March periods of 2010 -2015, except for 39 culverts that were surveyed twice in 2015.

One culvert ("Louisville Culvert") was surveyed throughout all seasons, with repeated surveys during winters of 2011-2012, 2013-2014, and 2014-2015. For this culvert, mean numbers of bats per sample year were reported for years in which repeated surveys/season were accomplished. For those with one survey those numbers were graphically represented.

Inspections were accomplished during daylight hours by walking silently through culverts and recording number of roosting bats using a digital hand counter and head lamp. Roosting animals were not disturbed or handled during culvert surveys. At each site the following information was recorded for each bat observed: identification to species, location of roosting bat (wall/ceiling or crevice of wall or ceiling), and condition of bat including visible lesions, injuries, or fungal growth (Blehert et al. 2009).

The following data on culvert characteristics was recorded: culvert dimensions- height, width, and length (Figure 3.1), presence of crevices within culvert interiors, external and internal ambient air temperatures, and external and internal relative humidity. Culvert dimensions were measured using carpenter rulers and 100-m tapes. Ambient air temperature and relative humidity outside culverts and within culvert interiors near center points were measured using a Kestrel 3000.

2015 Survey Culverts

From the afore-indicated sample population (N=214), a subset population of 39 culverts were selected for repeated surveys during January – March, 2015. Numbers of detected bats in previous year surveys and sizes of culverts were criteria used to include culverts targeted for resurvey in 2015. Size criteria was based on a minimal size required for safe access and inspection through upright posture of observers as follows: >1.7 m in height, > 1.5 m in width, and water depths < 0.5 m. Culverts were stratified according to numbers of roosting bats detected in 2013 and 2014 survey years as follows: a) absence of roosting bats, b) 1-10 roosting bats, c) 11-50 roosting bats, and d) > 50 roosting bats. Sites in the existing database that met criteria of size and bat numbers occurred along Interstate 55 in Carroll, Grenada and Montgomery Counties; State Highways 25 in Rankin, Oktibehha, and Winston Counties, State Highway 45 in Noxubee County, and State Highway 84 in Adams, Franklin, and Lincoln Counties; State Highway 61 in Adams County, and Artesia Road in Oktibbeha County, MS.

I used the same methods for bat surveys and recording of culvert metrics and conditions as used in surveys of 2014 culverts as afore-described. Additional data recorded in culverts surveyed in 2015 included number of internal chambers, range in surface water features (flowing or pooled water and water depth), and bottom substrate characteristics.

Associations between numbers of roosting bats of each species and proximity to landscape variables were analyzed using ArcMap GIS Version 10 (Environmental Systems Research Institute, Inc., Redlands, California). Locations of culverts were obtained using a handheld Global Positioning System unit, then overlain on a base map created by the Mississippi Automated Resource Information System (MARIS). The following landscape variables were identified: Wildlife Management Areas, National Wildlife Refuges, State Parks, National Parks, National Forests, and major rivers (1st order lotic systems). Distance (km) from each culvert location to the closest public land area and major rivers were measured in ArcMap using the measure tool (ArcMap Version 10).

Statistical Analyses

Similar analysis approaches were used for both populations of surveyed culverts: 204 culverts surveyed from 2010 through 2015 and 39 culverts selected for repeated surveys in January-March 2015.

I used Shapiro-Wilks test to test normality of data, including numbers of detected bats and culvert dimension measurements, (length, width, and height). These analyses indicated that my data was non-normal ($P \leq 0.001$). Therefore, I used Spearman's nonparametric correlation rank to examine associations between numbers of bats and dimensions of culverts (Zar 1999).

For comparisons of roosting bat numbers within different lengths of culverts surveyed in 2010-2015, I subdivided culverts according to the following length categories based on findings of Keeley and Tuttle (1999): a) ≤33.3 m, b) 33.4-99.7 m, c) ≥ 99.8 m. Because number of culverts surveyed in 2010-2015 greatly exceeded a sample size ≥ 25 (N=214), I used parametric statistical analysis methods (Moore et al. 2014). I used Analysis of Variance (ANOVA) to test if numbers of detected bats differed in the three different culvert length categories. Total number of bats and the association of position (wall or crevice) was also tested using ANOVA. A P-value < 0.05 was considered significant for all tests (Zar 1999). Statistical analyses were performed using SAS for Windows 8.0 Version 9.4 (SAS Institute, Cary, NC).

In testing associations between numbers of roosting bats and culvert metrics and conditions for data collected in 2015 on 39 culverts, I used Shapiro-Wilks to test for normality. Results of this analysis indicated that data exhibited non-normal distribution characteristics ($P \leq 0.001$; Zar 1999). Therefore, Spearman's nonparametric correlation analysis was used to investigate potential relationships between culvert conditions and metrics and numbers of detected bats (Zar 1999). For 24 culverts surveyed in 2015 with correctly georeferenced locations, associations between roosting bat numbers and distance to public lands and major rivers were evaluated using Spearman's nonparametric correlation analysis (Zar 1999).

Results

All Surveyed Culverts

Out of the 214 culverts surveyed during the study period, 111 (52%) were used by winter-roosting bats. Five different species were detected including Rafinesque's big-eared bat, southeastern myotis, big brown bat, tricolor bat, and Brazilian free-tailed bat. Bats that could not be identified due to obstruction of sight were categorized as "unknown" species. Across all surveyed culverts, total numbers of bats detected per culvert ranged from 0 to 927 with a mean of 27.80 (± 4.92) per inspected culvert. Tricolor bats were the most commonly detected species occurring in 72 of 111 culverts used by bats. Numbers of tricolor bats roosting in all culverts surveyed ranged from 0 to 927 roosting bats with a mean of 81.73 (±15.14) per culvert. The least commonly detected species was Rafinesque's big-eared bats which were detected in 9 culverts. Numbers of Rafinesque big-eared bats in all surveyed culverts ranged from 0 to 1 in all culverts. A mean of 1.0 (±0.25) Rafinesque's big-eared bat per culvert was estimated in the 9 culverts in which this species was detected. Southeastern myotis were detected in 66 culverts, and their numbers ranged from 0 to 109 with a mean of 7.23 (±1.43) in occupied culverts. Numbers of Brazilian free-tail bats ranged from 0 to 600 with a mean of 550 (±50.00), and this species was detected in 2 culverts. Numbers of big brown bats ranged from 0 to 8 with a mean of 1.63 (±0.14). Big brown bats were detected in 50 culverts.

Ranges in numbers of bats detected within culverts of three length categories were as follows: a) 0 – 11 bats in < 33.3-m culverts, b) 0 – 24 bats in 33.4 - 99.7- m culverts, and c) 0 – 901 bats in > 99.8-m culverts (Table 3.1; Figure 3.2). Analysis of variance testing indicated that > 99.8 m culverts supported greater numbers of roosting bats than other length categories *(F* = 5.45, *df* = 2; 201, *P* < 0.005). All five species detected during the entire study (2010-2015) were detected in culverts of 33.4-99.7 m lengths and ≥ 99.8 m length. The greatest proportion of bats were found in culverts of ≥ 99.8 m in length, which is also where the least common bat of this study, Rafinesque's big-eared bat, was detected. Only tricolor bats were detected in culverts of ≤ 33.3 m in length (Table 3.1; Figure 3.2).

Spearman's nonparametric correlation analysis showed a moderate positive relationship between numbers of bats roosting in culverts and length (*r* = 0.56, *P* ≤ 0.001; Table 3.3). Culvert dimensions ranged from 9.14 m to 701.04 m in length. Culvert widths ranged from 0.91 m to 6.71 m, and a weak inverse relationship was detected between bat numbers and culvert width (*r* = -0.10, *P* < 0.02; Table 3.3). Culvert heights ranged from 0.61 m to 7.01 m (Table 3.3), and no association was detected between this metric and numbers of roosting bats (*r* = 0.0006*, P* < 0.99; Table 3.3).

Location of roosting bats were recorded at the time of detection which included wall/ceiling or within crevice of culvert wall/ceiling. Comparisons of numbers of bats detected by roosting site location revealed that a greater number of bats were detected on culvert walls and ceilings than within crevices (*F* = 6.64*, df* = 1; 326*, P* < 0.0104). Numbers of bats detected on wall and ceiling locations ranged from 1 to 866 with a mean of 52.23 (±14.77). Numbers of roosting bats detected in crevices ranged from 1 to 600 with a mean of 16.74 (±4.65).

Spearman's nonparametric correlation analysis showed a moderate inverse relationship between numbers of bats roosting in culverts and outside ambient air temperatures (r= -0.24, $P \leq 0.001$) and central interior ambient air temperatures (r = -0.14, $P < 0.015$). Outside air temperatures ranged from 2.6˚C to 27˚C, and central interior air temperatures ranged from 4.5˚C to 26.7˚C (Table 3.2). I detected no relationship between numbers of bats in culverts and outside relative humidity (r = -0.07, $P < 0.22$) and central interior relative humidity (r = -0.02, $P < 0.73$; Table 3.2).

2015 Surveyed Culverts

Of the 39 surveyed culverts, 27 (69%) were used by roosting bats in at least one of the two survey periods. The same five species that were detected in culverts from 2010-2014 were also detected in culverts surveyed in 2015. The species detected most often was the tricolor bat with number of detections ranging from 0 to 866 with a mean of 45.39 (±16.26).

After separating the surveys into January-early February and late February-March survey periods, ANOVA comparisons of numbers of roosting bats detected within the two survey period revealed no significant differences in numbers of roosting bats between the two surveys (F = 0.16, df = 1; 148, $P < 0.70$; Figure 3.4). Spearman's nonparametric correlation analysis showed a moderate relationship between numbers of bats roosting in culverts and all dimensions as follows: culvert length (r = 0.34, $P < 0.0002$, width (r = - 0.29, $P < 0.0014$), and height (r = -0.21, $P < 0.026$; Table 3.4).

Over 56% of culverts exhibited presence of crevices in walls and ceilings. Location of roosting bats were recorded at the time of detection either on wall/ceiling or within crevice of culvert wall/ceiling. Comparisons of numbers of detected bats detected by roosting site location revealed no association between bats roosting on walls/ceilings than within crevices (F = 1.75, df = 1; 137, $P < 0.188$, N=39). Numbers of bats detected on culvert walls and ceilings ranged from 1 to 866 with a mean of 53.47 ($\pm$23.71); whereas, bats found roosting in crevices ranged from 1 to 600 with a mean of 23.87 ($\pm$9.76).

Measurement of surface water and bottom substrate characteristics in culverts revealed that approximately 95% of surveyed culverts exhibited standing or flowing water during 2015 survey periods and water depths were typically < 0.5 m. Also, all surveyed culverts exhibited concrete bottoms. Deposition of sediment or gravel over concrete bottoms was present in $> 90\%$ of surveyed culverts. Deposited alluvium in culvert bottoms included mixtures of gravel, sand, loam-clay-sand soils, and depths of depositions were < 0.30 in all culverts surveyed during 2015. Of the 2015 culvert population, one culvert exhibit > 2 chambers, 12 exhibited two chambers and 26 exhibited one chamber.

During surveys of culverts, outside air temperatures ranged from 3.3˚C to 25.3˚C (Table 3.5). Spearman's nonparametric correlation analysis showed a moderate inverse relationship between numbers of bats roosting in culverts and outside ambient air temperatures ($r = -0.44$, $P \leq 0.001$). I detected no relationship between numbers of bats in culverts and outside-culvert, relative humidity ($r = -0.19$, $P < 0.13$), internal-culvert humidity ($r = -0.24$, $P < 0.06$), and internal culvert temperatures ($r = -0.15$, $P < 0.24$; Table 3.5).

Spearman's nonparametric correlation analysis showed a moderate inverse relationship between numbers of roosting bats in culverts and box bridges and distances (km) from public lands ($r = -0.44$, $P < 0.03$; Table 3.6). I detected no relationship between numbers of bats in culverts and box bridges and distances to major rivers (r = 0.16, $P < 0.45$; Table 3.6).

Discussion

Bat Use of Culverts

My study reported that 16,812 individual detections of 5 bat species were recorded in concrete culverts and box bridges in Mississippi from November through March over a 5-year period. Findings of my study indicated that bats use roadway box bridges and culverts as winter roosting sites, and this roosting behavior occurs throughout Mississippi. My findings are similar to those of LaVal (1967), Humphrey and Gore (1992), Keeley and Tuttle 1999, Trousdale and Beckett (2004), and Bender et al. (2010). Culverts, particularly concrete ones, have been reported as winter hibernacula for many bat species including gray bat, Indiana bat, Rafinesque's big-eared bat, southeastern myotis, little brown bat, Brazilian free-tailed bat, big brown bat, and tricolor bat (LaVal 1967, Humphrey and Gore 1992, Keeley and Tuttle 1999, Trousdale and Beckett 2004, Bender et al. 2010). Culverts provide adequate roosting conditions, because they generally have reduced light, stable microclimates, and are associated with surface water of streams or canals that drain through them (Humphrey and Gore 1992). Surface water may attract bats due to influence of water on microclimate conditions, greater availability of flying insect prey, and foraging flight paths over streams (Humphrey and Gore 1992). Stable microclimate conditions may also attract bats and concrete culverts may exhibit good conditions for roosting and in-torpor bats. For example, concrete culverts have been reported to absorb heat from solar radiation during the day and retain that amount of heat into the night, providing temperature stability in these sites for roosting bats (Perlmeter

1996). During my study, the five detected species were Rafinesque big-eared bat, southeastern myotis, Brazilian free-tailed bat, big brown bat and tricolor bat. Two federally listed bats gray and Indiana bat, typically use culverts as roost sites (Barbour and Davis 1969). However, records for these species are rare in Mississippi (USFWS 2012), and these species were not detected in culverts surveyed in this study. Rafinesque big-eared bats and southeastern myotis are “species of concern” listed by Mississippi Natural Heritage Program (www.mdwfp.com/seek-study/heritage-program). Detection of these species during winter in my study and findings of Trousdale and Beckett (2004) who reported maternity colonies of Rafinesque’s big-eared bats in concrete bridges in southern Mississippi provide support for concept that culverts and bridges can provide roosting sites for rare bat species. Furthermore, these structures may be important roost sites in regions where natural roost sites no longer occur or are rare on the landscape (Trousdale and Beckett 2004).

Prior to this study, little was known about culvert use by winter-roosting bats in Mississippi. Most research to date has focused on spring surveys of bridges for detection of maternal colonies, such as Trousdale and Beckett (2004). My study represents the first effort toward gaining a greater understanding of winter-roosting bats and their use of concrete culverts and box bridges throughout the state. Research conducted in other states has reported that bats use bridges and roadway culverts and box bridges as roost sites. Keeley and Tuttle (1999) reported approximately 4,250,000 bats of 24 species roost in over 200 highway structures throughout the United States. Selected species seem to be attracted to human-made structures. For example, LaVal (1976) reported that southeastern myotis roosted in culverts, buildings, and bridges throughout its range.

Also, Humphrey and Gore (1992) reported use of bridges by southeastern myotis in Florida. In Oregon, Adam and Hayes (2000) reported that big brown bats, Rafinesque big-eared bats, and *Myotis* spp. used concrete bridges as night-roosts. Additionally, bridges and culverts may be important as maternal colony roost sites for some species. For example, Davis and Cockrum (1963) reported that maternity colonies of big brown bat, Brazilian free-tailed bat, pallid bat (*Antrozous pallidus*), and *Myotis* spp. were detected in bridges of Arizona. My findings were similar to those of Bender et al. (2010) who reported that southeastern myotis, big brown, and Brazilian free-tailed bats used culverts and bridges in Alabama. Research conducted on use of culverts by roosting bats in the Southeast and in other regions of the United States supports hypotheses that suggest the culverts can be important roost sites for selected species of bats during winter and early spring.

In both culvert populations of this study, length was the most significant structural dimension associated with use of culverts by winter-roosting bats. Culverts longer in length yielded greater numbers of bats. Culverts measuring ≥ 99.8 m in length exhibited the greatest number of bat detections during the study (Table 3.1; Figure 3.2). These results were similar to findings of Keeley and Tuttle (1999) who reported culverts and bridges ≥ 100 m were best suited for bat use. In both populations of this study, culvert width was slightly inversely related to bat numbers indicating that lesser widths were associated with greater numbers of roosting bats. For culverts re-surveyed in 2015, similar associations were detected with culvert height with greater numbers of bats being associated with reduced heights. However, no association between bat numbers and culvert height was detected in all culverts surveyed from 2010 – 2015. Also, criteria placed on inclusion of culverts in sample populations for this study required that culverts be at least 2 m in height. Therefore, interpretation of my findings of a negative association between bat numbers heights of culverts should consider that minimal height of culverts in this study were about 2 m. Associations between numbers of roosting bats and culvert dimensions could be related to multiple factors including microclimate stability and thermoregulatory benefits due to stable ambient air temperatures, less wind and airflow draft, and less light illumination (Kunz 1982). In my study, I submit that greater culvert lengths and heights of at least 2 m may have provided greater surface area for roosting bats and better accessibility as reported by Adam and Hayes (2000). In their study, Adam and Hayes (2000) reported that bats used larger bridges with larger dimensions potentially due to increased surface area for roosting, better accessibility, and greater protection from predators.

Keeley and Tuttle (1999) found that 94% of bats using bridges and culverts were detected in crevices, showing evidence that availability of crevices in culverts and bridges may also be a factor in use. However, I detected greater numbers of roosting bats on culvert walls and ceilings than within crevices of culverts. Greater numbers of bats were found on the wall versus crevices throughout the study. Of total number of bat detections, 69.7% were roosting on walls and ceilings and 30.3% were roosting in crevices. Underestimation of bats roosting in crevices could have occurred in my study due to lack of crevice accessibility by observers and lack of visibility into deep, narrow crevices of culvert walls and ceilings. Also, in crevices that supported large numbers of roosting bats (> 50 individuals), observers were often able to view individuals roosting closest to the crevice opening, and visibility of bats roosting behind others deeper into crevices may have gone undetected. Also, in my sample population of culverts, at least five culverts supported > 100 bats and most of these bats were detected on walls and ceilings of culverts. Therefore, greatest congregations of winter-roosting bats in most culverts of this study were detected on walls and ceilings as opposed to within culvert crevices. Choice of roosting sites within culverts may vary with season, however. For example, surveys of maternal colonies might reveal different roost site selection.

Outside and interior ambient air temperatures were inversely related to total numbers of roosting bats. With lower outside and interior ambient air temperatures, more roosting bats were detected. I found no relationships of roosting bat numbers with outside and interior relative humidity measurements. Reduced air temperatures outside and inside culverts during winter was potentially associated with greater numbers of roosting bats entering states of torpor during periods of colder temperatures (McNab 1982). Wojciechowski et al. (2006) reported that most bat species begin to enter winter dormancy and torpor at temperatures of 0-20˚C. Analysis of bat counts for 2015 revealed a significant inverse association between outside air temperature and numbers of roosting bats. However, no association was found between numbers of roosting bats and interior temperature, outside relative humidity, and interior relative humidity.

Keeley and Tuttle (1999) describe the minimum needs of day-roosting bats that use highway bridges and culverts as follows; 1) location in relatively warm geographic regions, 2) made of concrete, 3) between 1.5 and 3 m tall and 100 m or more long, 4) openings protected from high winds, 5) not susceptible to flooding, 6) inner areas relatively dark with roughened walls or ceilings, and 7) crevices, imperfections, or swallow nests. Larger culverts and box bridges in my study exhibited most of these characteristics. Flooding of > ½ of culvert height was never recorded in my study culverts. Streams (< 3rd order) and drainages ran through most of my culverts and water depths appeared to remain < ½ of interior culvert height even during periods of flooding.

Characteristics recommended by Keeley and Tuttle (1999) are included by The Arizona Department of Transportation in environmental impact statements for assessing impacts of bridge and culvert renovation on roosting bats. Some state transportation departments are integrating bat management techniques into maintenance schedules of bridges, box bridges, and culverts. However, < 1% of bridges and box culverts in the U.S. have the afore-listed characteristics and retrofitting may be accomplished with minimal costs to the tax payer (Keeley and Tuttle 1996). The Texas Bat Abode and the Oregon Wedge have been used in retrofitting culverts. The Texas Bat Abode can house thousands of bats and provides crevices for roosting. The Oregon Wedge provides a single crevice that can house several hundreds of bats. Both of these have proven successful in attracting greater numbers of roosting bats (Keeley 1998, Keeley and Tuttle 1999).

Culvert Microclimates and Potential for White-nose Syndrome

Outbreaks of White-nose Syndrome (WNS) have been reported most often for northern states in the U.S. Large congregations of bats in caves and this pathogen's adaptations to cold temperatures are reported as reasons for the rapid spread of the fungus and the impacts to bat populations in the north and northeastern U.S. (Blehert et al. 2009). Also, awakening and exhaustion associated with manifestation of disease symptoms causes starvation and tissue damage (Blehert et al. 2009, Meteyer et al. 2009). At optimal temperatures (12.5-15.8˚C), hyphae of *Pseudogymnoascus destructans* grow and spread across the surface of the skin creating lesions and eroding and replacing skin structures, such as sebaceous glands, hair follicles, and apocrine glands (Cryan et al. 2010).

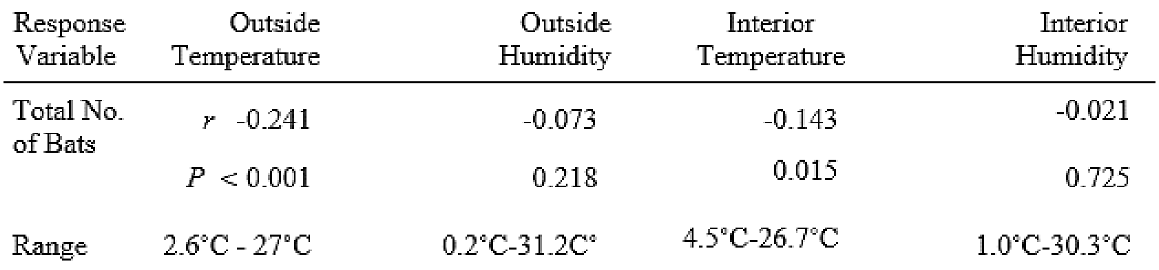
In 33% (n=71) of surveyed culverts, the internal temperature at culvert center ranged from 12.5-15.8˚C, the optimal temperature range for *Pseudogymnoascus destructans.* Four of the culverts that exhibited optimal temperatures of WNS supported > 100 roosting bats per survey. In these culverts with large bat numbers, WNS could potentially cause mortality if temperatures continue to remain within adequate ranges for fungal growth, spread, and infection of bats. Therefore, I suggest monitoring of bat numbers and surveys to detect WNS fungus in these culverts. Because of negative impacts of human disturbance to roosting bats and the possibility of translocation of WNS to roosting sites, I recommend closure of these culverts to the public and to educational groups. For individuals conducting monitoring, I suggest following the decontamination protocol set by whitenosesyndrome.org (Version 06.25.2012). This protocol is currently being used by biologists of Mississippi Department of Wildlife, Fisheries, and Parks who are monitoring bat numbers and conducting WNS testing.

I recommend future "resurvey" of culverts that supported bats during 2010-2015 to assess potential changes in bat numbers. These culverts should also be tested for presence of WNS fungus on substrates and bats over time. To gain a better understanding of seasonal use of culverts by bats, I also recommend repeated surveys in each culvert during different seasons: winter – early spring, mid spring to mid-summer; and late summer through fall.

Table 3.1 Numbers of bats detected in culverts of three length categories during winter-roost surveys of 2010-2015 in Mississippi.

Culvert Length Category	Number of Culverts Surveyed	Total No. Detected	Mean No. (± SE) Detected	Range in No. of Bats Detected
< 33.3 m	32	14.33	0.45 (±0.35)	0 - 11
33.4 - 99.7m	110	257.80	2.34 (± 0.41)	0 – 23.67
> 99.8m	62	2,555.35	41.22 (± 18.02)	0 – 900.6
All Culverts	204	2,827.00	13.86 (± 5.6)	0 – 900.6

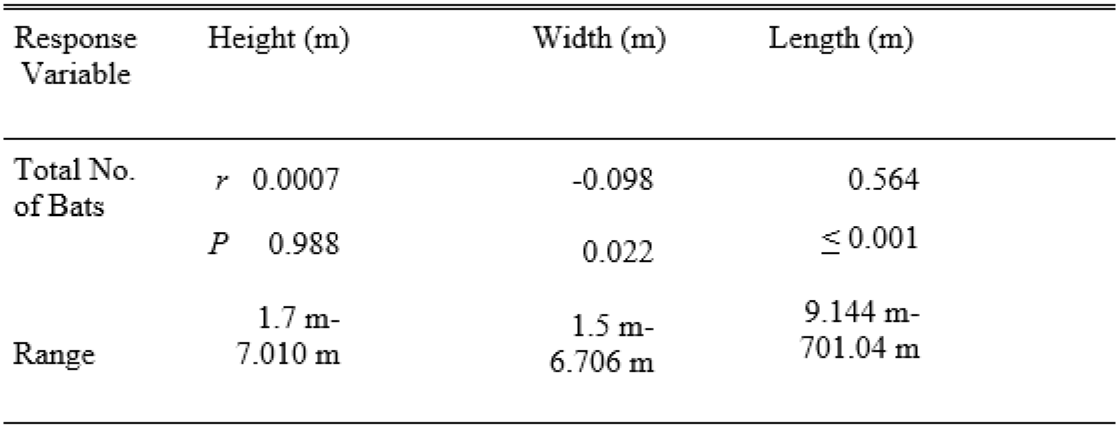
Table 3.2 Spearman's nonparametric correlation results for associations between ambient air temperature and humidity measurements and total number of bats detected in winter-roost surveys (November-March) 2010-2015 in Mississippi.



Response Variable		Outside Temperature	Outside Humidity	Interior Temperature	Interior Humidity
Total No. of Bats	r	-0.241	-0.073	-0.143	-0.021
	P	< 0.001	0.218	0.015	0.725
Range		2.6°C - 27°C	0.2°C-31.2C°	4.5°C-26.7°C	1.0°C-30.3°C

Spearman Correlation Coefficients; Prob > |r| under H_0: Rho=0

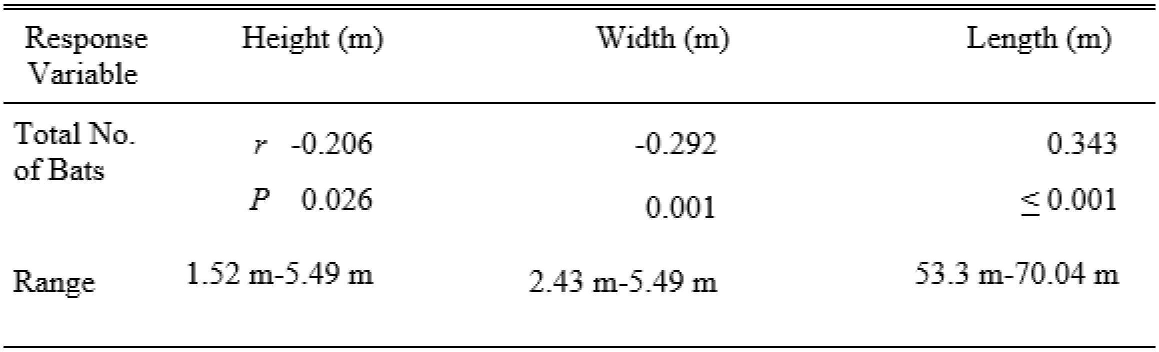
Table 3.3 Spearman's nonparametric correlation results for associations between dimension measurements of culverts and total number of bats detected in winter-roost surveys during November-March 2010-2015 in Mississippi.



Response Variable		Height (m)	Width (m)	Length (m)
Total No. of Bats	*r*	0.0007	-0.098	0.564
	P	0.988	0.022	≤ 0.001
Range		1.7 m-7.010 m	1.5 m-6.706 m	9.144 m-701.04 m

Spearman Correlation Coefficients; Prob > |r| under H_0: Rho=0

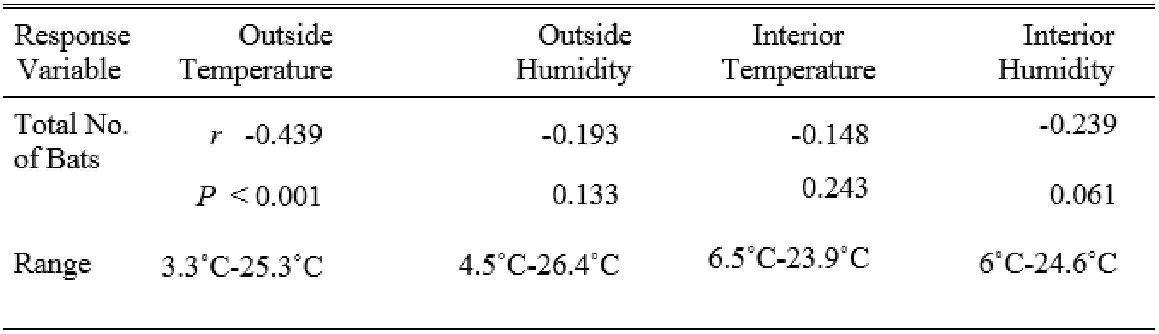
Table 3.4 Spearman's nonparametric correlation results for associations between dimension measurements of culverts and total number of bats detected in winter-roost surveys (January-March) 2015 in Mississippi



Response Variable		Height (m)	Width (m)	Length (m)
Total No. of Bats	*r*	-0.206	-0.292	0.343
	P	0.026	0.001	≤ 0.001
Range		1.52 m-5.49 m	2.43 m-5.49 m	53.3 m-70.04 m

Spearman Correlation Coefficients; Prob > |r| under H_0: Rho=0

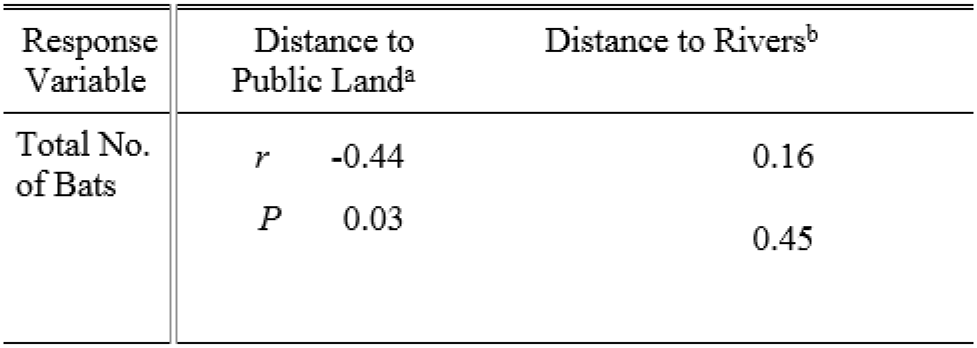
Table 3.5 Spearman's nonparametric correlation results for ambient air measurements and total number of bats detected in winter-roost surveys during January-March 2015 in Mississippi.



Response Variable		Outside Temperature	Outside Humidity	Interior Temperature	Interior Humidity
Total No. of Bats	*r*	-0.439	-0.193	-0.148	-0.239
	P	< 0.001	0.133	0.243	0.061
Range		3.3˚C-25.3˚C	4.5˚C-26.4˚C	6.5˚C-23.9˚C	6˚C-24.6˚C

Spearman Correlation Coefficients; Prob > |r| under H_0: Rho=0

Table 3.6 Spearman's nonparametric correlation results for selected landscape variables surrounding culverts and total number of bats detected in winter-roost surveys during November- March 2015 in Mississippi.



Response Variable		Distance to Public Land[a]	Distance to Rivers[b]
Total No. of Bats	*r*	-0.44	0.16
	P	0.03	0.45

Spearman Correlation Coefficients; Prob > |r| under H_0: Rho=0

[a] Public Lands include Wildlife Management Areas, National Wildlife Refuges, State Parks, National Parks, and National Forests.

[b] Rivers – $\geq$ 5th Order Lotic System (MARIS 1983).

Figure 3.1 Measurement trajectories to estimate dimensions of culverts and box bridges in Mississippi study to estimate numbers and species of winter-roosting bats from 2010 through 2015.

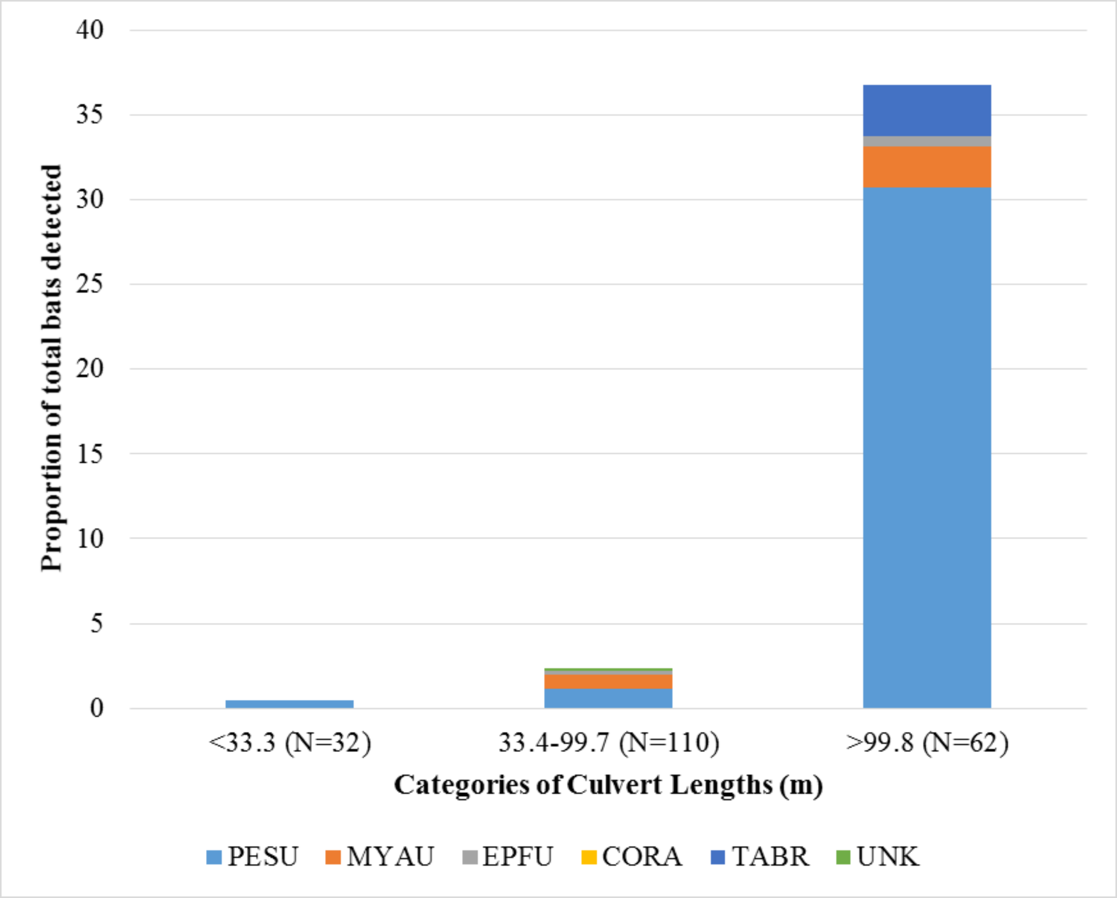


Figure 3.2 Proportion of average number of different bat species detected in three different length categories of surveyed culverts throughout Mississippi

(N= 204;PESU – Perimyotis subflavus, MYAU –Myotis austroriparius, EPFU – Eptesicus fuscus, CORA- Corynorhinus rafinesquii , TABR- Tadarida brasiliensis and UNK- Unknown).

[a] N=number of surveys per season.

[b]Average number of bats = total number counted over all repeated surveys ÷ number of surveys.

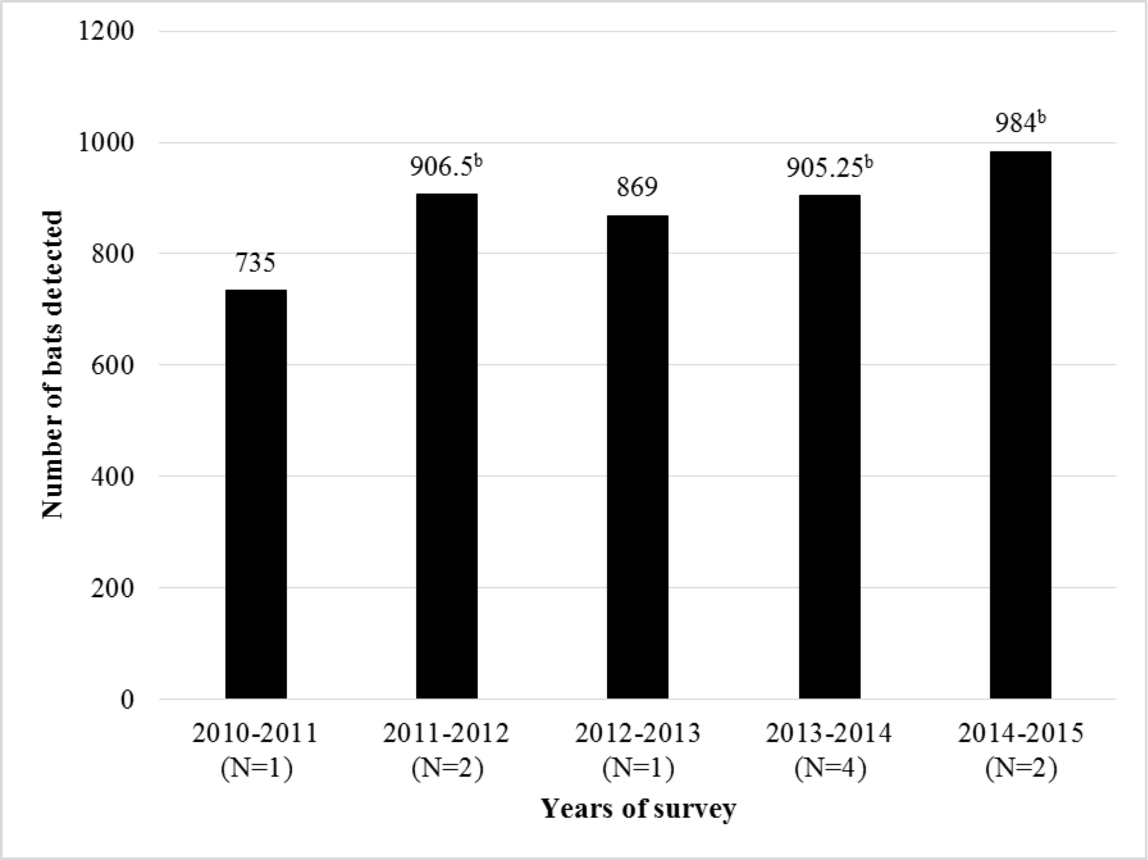


Figure 3.3 Total number of bats detected in culvert located on Highway 25, Winston County, MS during November - March 2010-2015.

[a] N=number of surveys per season.

[b] Average number of bats = total number counted over all repeated surveys ÷ number of surveys.

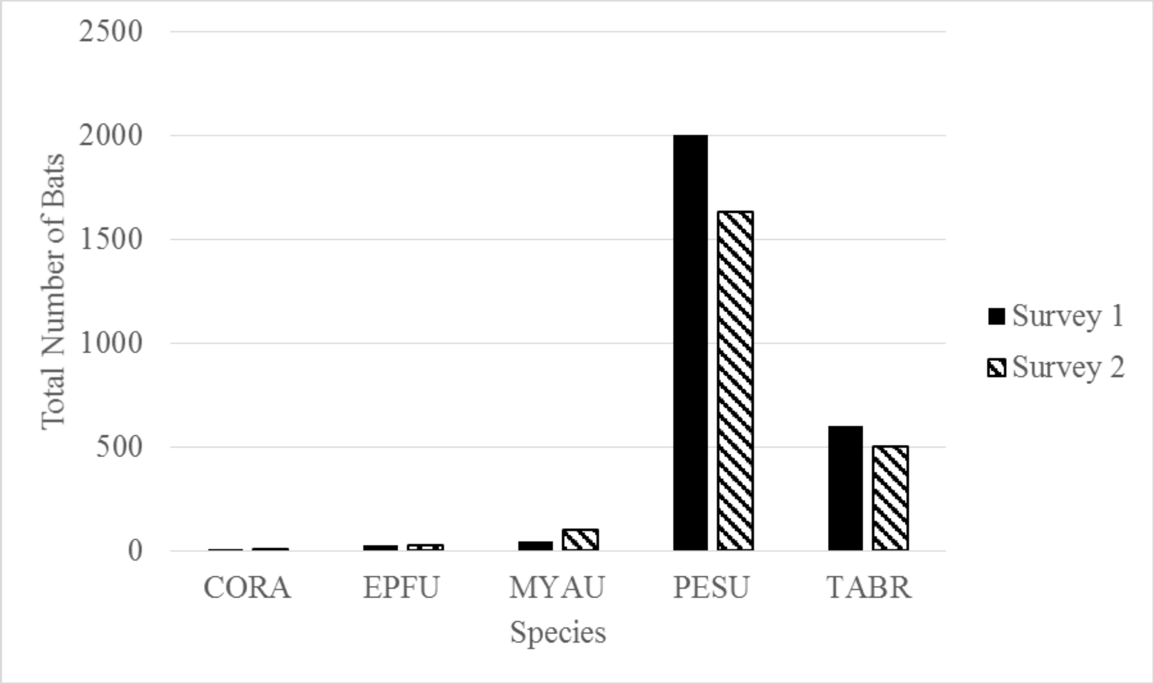


Figure 3.4 Total number of bats and species detected over two survey periods in 2015 in culverts of Mississippi.

(CORA- Corynorhinus rafinesquii ,EPFU – Eptesicus fuscus, MYAU –Myotis austroriparius, and PESU – Perimyotis subflavus, TABR- Tadarida brasiliensis).

CHAPTER IV

TESTING OF WHITE-NOSE FUNGUS (*PSEUDOGYMNOASCUS DESTRUCTANS*) IN SURVEYED CULVERTS, BOX BRIDGES, AND CAVES

Introduction

Disease was not considered to be a contributing factor of decline in bats until the emergence of the fungus *Pseudogymnoascus destructans* in winter 2005-2006 in Howes Cave, New York (Rice 1957, Bigler et al. 1975, Jones and Suttkus 1975, Foley et al. 2011). This pathogen is the etiologic agent of the fatal disease, White-nose Syndrome (WNS). Since 2006, WNS has been reported as a major source of decline in hibernating populations of insectivorous bat, resulting in mortality levels of 90-100% in some infected hibernacula (Hallam and McCracken 2010). This fungus grows at temperatures 12.5 to 15.8˚ C and > 90% relative humidity, conditions similar to bat hibernacula and bodies of hibernating bats (Cryan et al. 2010). Transmission occurs through direct bat-to-bat contact and exposure to fungus-infected substrates. This fungus may also be spread to new sites by human and animal vectors that have been in fungus-positive locations (Blehert et al. 2009, Lindner et al. 2010).

White-nose Syndrome infects the wing and skin tissues of hibernating bats (Blehert et al. 2009, Meteyer et al. 2009). Fungal hyphae spread across skin tissue of hibernating bats and create lesions and erode skin structures including sebaceous glands, hair follicles, and apocrine glands (Cryan et al. 2010). Bats with WNS may suffer from arousal from hibernation more frequently or for longer periods than average causing fat reserves to prematurely dissipate (Boyles and Willis 2010). Infection of the wings with *P. destructans* can cause direct mortality (Cryan et al. 2010). Abnormal behaviors associated with WNS have been reported in large numbers of bats including movement to roosting areas near cave entrances or other exposed sites and flying during the day from hibernacula in mid-winter. In spring, a few infected individuals may recover but will retain damage to the wing (Reichard and Kunz 2009).

Pseudogymnoascus destructans has been detected on 9 species of bats in North America including the endangered gray, Indiana, and northern long-eared bats *(Myotis grisescens, Myotis sodalis*, and *Myotis septentrionalis*), little brown bat (*Myotis lucifugus*), eastern small-footed bat (*Myotis leibii*), southeastern bat (*Myotis austroriparius*), cave bat (*Myotis velifer*), tricolored bat (*Perimyotis subflavus),* and big brown bat (*Eptesicus fuscus*; Foley et al. 2011). All species except for the eastern small-footed bat and cave bat are indigenous to Mississippi (Kennedy et al. 1974, Jones and Carter 1989).

Prior to initiation of this study, no testing for WNS had been accomplished in Mississippi. Due to the adaptations of the fungus to colder temperature regimes, biologist speculated that this pathogen would not be adapted to survive in Mississippi habitats. However, potential colder temperature regimes in caves and underground culvert systems caused state biologists to initiate testing to determine presence or absence of *P. destructans* on substrates of these potential roost sites for bats.

Objectives of this portion of the study were as follows:

1. Report results of testing for white-nose fungus (*Pseudogymnoascus destructans*)in surveyed culverts, box bridges, and caves and
2. Assess potential for negative impacts to bats based on recorded temperature regimes and numbers of roosting bats.

Methods

This study is a contribution to a nationwide "WNS/P.d. Continental Transmission Study" organized by Winifred Frick at University of California, Santa Cruz. Criteria of chosen sites were chosen based on three criteria as follows: a) previous knowledge of use by winter-roosting bat populations, b) accessibility to sites, and c) map of site depicting morphological characteristics of structure, such as shape and internal space dimensions.

Ten sites in Mississippi were selected for testing of *P. destructans* during January – April hibernacula surveys in 2014. In 2015 two additional sites were tested and seven sites from 2014 were re-tested. These sites included eight caves - Belding's Cave, Calcote Branch Cave, Eucutta Cave, Lamar Graham Cave, Nanih Waiya Cave, Triple H Cave, Waddell Cave, and William's (Pitt's) Cave; three culvert/ box-bridges, Louisville Culvert, Meridian NAS North Culvert, and Prison Culvert; and one abandoned mine, Tripoli Chalk Mine. Sample collection was conducted through use of a sampling kit that contained the following: nitrile gloves, Lysol spray, Ziploc bags, garbage bags, sterile swabs, 2 ml storage tubes with RNALater for storage, 2ml and 15 ml dipping vials filled with sterile water, boxes designed to hold collected storage tubes, ID tags each with a unique ID for bat and substrate swabs, and datasheets (Muller et al. 2013).

Mississippi Department of Wildlife, Fisheries and Parks biologist, Kathy Shelton, swabbed 10 to 20 bats per species in each of sampled location. No swabs were collected if < 5 bats were present at the time of culvert or cave inspection. At each location the following was recorded: location of bats within the structure, number of bats within 1 ft. of focal bat and approximate size of this cluster, and number of bats touching the swabbed bat. Any visible lesions, injuries, or fungal growth on the face or wings of swabbed bat were recorded. At each bat that was swabbed the following sample collection protocol was used: 1) dipped tip of swab in sterile water, 2) swabbed bat five times on both the wing and the muzzle, 3) stored swab in RNAlater vial, 4) application of unique identification tag, and 5) recorded unique identification and other data (ie. gender and body condition.) on the datasheet (Muller et al. 2013).

At each site 10 substrate samples were collected and a unique identification number was assigned to each. Swabs of substrate were taken directly under 10 different bats of the same species (Muller et al. 2013). Each substrate sample was collected using the following protocol: 1) dipped swab tip in sterile water, 2) swabbed substrate five times directly below the bat (not touching the bat with the swab), 3) swab was placed in RNAlater vial, 4) applied unique identification number, and 5) recorded unique identification and other data on datasheet. In 2015 surveys substrate sampling modifications were made to 2014 methodology that included the following: 1) five samples were collected in each of the 10 sites and 2) for each of the five focal bats chosen, a substrate sample was taken 10 cm (near) and 2 m (far) from the focal bat on substrate surfaces of similar type. The same above protocol of storage for samples was used in 2015. Following each survey, the National White-Nose Syndrome Decontamination Protocol Version 06.25.2012 was used for equipment and gear, footwear, and clothing (whitenosesyndrome.org).

All samples were placed in storages boxes that were maintained at temperatures recommended by Muller et al. (2013). Temporary storage prior to shipment and shipment of all samples to the Center for Microbian Genetics and Genomics at Northern Arizona University were accomplished following protocol recommended Muller et al. (2013). Presence of *P. destructans* DNA was determined by qPCR. Samples were typically analyzed > 4 times for detection of the pathogen's DNA and a single detection of pathogen's DNA was considered to be indicative of presence of *P. destructans.*

Results

In 2014, *P. destructans* was detected at four sites including Belding's Cave, Nanih Waiya Cave, Waddell Cave, and Louisville Culvert (Table 4.1). During 2014, fungal DNA was detected on fifteen individuals of two bat species (fourteen tricolor bats and one southeastern myotis) and eight substrate locations within three caves and one culvert (Table 4.1). In 2015, samples were collected from 90 bats from 9 locations and analyses yielded no presence of *P. destructans* DNA. (Table 4.2). No substrate samples were tested for fungal presence in 2015.

Discussion

Positive test results indicating fungal presence on cave and culvert substrates and bats in 2014 was the first sampling effort and first discovery of presence of *P. destructans* in bat roosting habitats of Mississippi. However, in 2015 samples there was no detection of this fungus. This finding may be due to the inability of the WNS fungus to thrive and infect bats in warmer temperature regimes of sampled sites in Mississippi. Repeated sampling of substrates and bats is necessary to provide a longer term record of fungal presence and infection of bats. Furthermore, continued monitoring of roosting bat numbers in sites with positive test results is needed to understand actual persistence of the fungus and potential impacts on bats. Negative test results from bats sampled in 2015 offer some hope that the fungus may not impact Mississippi bat populations as severely as bat populations in more northerly regions of the United States. However, conclusions concerning this concept are premature without more intensive study or temperature regimes in caves and culverts and continued monitoring of fungus presence and bat numbers over time. This type of monitoring could be extremely important for conservation of some species of bats, such as southeastern myotis and Rafinesque big-eared bats. Outbreak of WNS in Mississippi could have conservation implications for southeastern myotis, which is listed as protected, rare, and species of special concern in many southern states, including Mississippi (Foley et al. 2011, USFWS 2014). Tricolor bats although common in Mississippi could be negatively impacted over time with fungal outbreak. Although *P. destructans* has been found to affect hibernating populations of southeastern myotis in Virginia (Foley et al. 2011), populations of southeastern myotis in more southerly states may be less predisposed to WNS due to briefer hibernation periods in warmer climates and warmer temperature regimes in caves and other roosting sites which are not optimal for this fungus (Gore et al. 2012). Because of climatic conditions and winter dormancy behavior, the fungus may not decimate bat populations located in warmer regions of the U.S. (J. Gore, pers. comm.). I submit that these factors could explain the negative results of WNS testing in 2015. Negative test results found in 2015 were especially significant in caves and culverts that yielded positive test results in the previous year.

As discussed in previous chapters, tricolor and southeastern myotis bats have been found in multiple culverts and caves throughout Mississippi (Table 2.2; Figure 3.2). Based on results of previous chapters (Tables 3.2 and 3.5), temperatures were reported to be within temperature range of *P. destructans*. However, temperatures were not recorded over durations of time. To accomplish this longer term temperature sampling, I suggest placing data loggers in structures that are tested for WNS in the future to monitor temperature regimes and stability over night and day periods and throughout winter months.

I suggest continued investigation of this fungus throughout Mississippi using standardized protocols according to Muller et al. (2013). Also, data from this study and future monitoring should be used to supplement existing worldwide study on *P. destructans* and its impacts to bats. This study can teach us about temporal progression of transmission of *P. destructans* as well as provide early detection of low levels of infection at locations outside of known disease areas. A collaborative effort between biologists is needed to better understand the demographics of this disease as well as potential impacts.

Table 4.1 Results of samples collected for detection of *Pseudogymnoascus destructans* DNA during January- April 2014 in eight caves, one mine and one culvert in Mississippi.

Sampled Site	**Sampled Surface**	**Number of Positive Test Results of *P. destructans***	**Total Samples** [a]	***P. destructans* Percentage Prevalence**
Belding's Cave	Tricolor Bat[b]	2	10	0.2
	Substrate	1	9	0.11
Calcote Branch Cave	Tricolor Bat	0	10	0
	Substrate	0	5	0
Eucutta Cave	Tricolor Bat	0	10	0
Lamar Graham Cave	Tricolor Bat	0	10	0
	Substrate	0	10	0
Louisville Culvert	Tricolor Bat	6	15	0.4
	Substrate	2	8	0.25
Nanih Waiya Cave	Tricolor Bat	1	8	0.12
	Substrate	2	8	0.25
Triple H Cave	Tricolor Bat	0	13	0
	Substrate	0	10	0
Tripoli Chalk Mine	Tricolor Bat	0	4	0
	Substrate	0	4	0
Waddell Cave	Southeastern Myotis[b]	1	2	0.5
	Tricolor Bat	5	13	0.38
	Substrate	3	11	0.27
William's (Pitt's) Cave	Tricolor Bat	0	15	0
	Substrate	0	15	0

[a] Number of bats swabbed

[b] (tricolor bat- *Perimyotis subflavus*, southeastern myotis - *Myotis austroriparius*)

Table 4.2 Results of samples collected from bats for detection of *Pseudogymnoascus destructans* DNA during January- April 2015 in eight caves and one culvert in Mississippi.

Sampled Site	**Bat Species**[b]	**Number of Positive Test Results of *P. destructans***	**Total Samples**[a]	***P. destructans* Percentage Prevalence**
Belding's Cave	Tricolor Bat	0	10	0
Eucutta Cave	Tricolor Bat	0	10	0
Louisville Culvert	Tricolor Bat	0	10	0
Meridian NAS North Culvert	Southeastern Myotis	0	1	0
	Tricolor Bat	0	9	0
Nanih Waiya Cave	Tricolor Bat	0	10	0
Prison Culvert	Tricolor Bat	0	10	0
Triple H Cave	Tricolor Bat	0	10	0
Waddell Cave	Southeastern Myotis	0	3	0
	Tricolor Bat	0	7	0
William's (Pitt's) Cave	Southeastern Myotis	0	1	0
	Tricolor Bat	0	9	0

[a] Number of bats swabbed

[b] (tricolor bat- *Perimyotis subflavus*, southeastern myotis - *Myotis austroriparius*)

CHAPTER V

CONSERVATION AND RESEARCH IMPLICATIONS

Caves

Prior to this study, we lacked knowledge concerning use of caves by winter-roosting bats over Mississippi's landscape, this study provided baseline data that may help managers identify caves for future roosting surveys, monitoring of WNS incidence, and conservation efforts. If bat use of caves is to be compared between different regions of the state in the future, I recommend similar sampling size of caves targeted for survey within regions and similar sampling intensity in terms of repeated surveys within the same season and study year. For example, caves of central Mississippi exhibited the least number of roosting bats, and I submit that this finding was due, in part, to the number of caves surveyed in this region (n=2) (Table 2.3; Figure 2.2). In this study, comparisons of roosting bat numbers between caves of northern and southern Mississippi was most revealing due to similar sampling intensity with 7 caves being surveyed in each region.

I anticipate that this study will help state and federal agencies identify locations of conservation importance and educate landowners of caves and their importance. Because most caves in Mississippi are found on private land, educational outreach about conservation value of caves to bats, many of which are imperiled, could limit disturbance and damage to cave roosting bats. Caves are also of interest to other scientists, such as archaeologists and geologists, and with their help and participation, we potentially can strengthen efforts to protect these sites.

Culverts

Future research concerning bat use of culverts could be strengthened by increasing number of culverts sampled during a study year, repeating surveys at least 3 times per survey season, and conducting roosting surveys during at least three seasons to gain information on winter hibernacula and maternity colonies. Increases in sampling intensity could address seasonal changes in roosting bat numbers and provide stronger inferences concerning roosting bat numbers in culverts and box bridges throughout the state. For example, changes in numbers of bats detected in surveyed culverts in my study could be attributed to the random design of the study and the lack of repeated surveys during study years 2010 - 2014. Because this study was a pilot study intended to provide baseline information on bat use of culverts, culverts were surveyed during 2010 – 2014 as they were located in the field. Culverts that were "resurveyed" in 2015 (N=39) with at least two repeated surveys in November through March provided more reliable information on roosting bat numbers over the season and over study years.

I suggest a uniform protocol for all future monitoring of bat use in culverts and box bridges as follows: 1) all survey sites should meet size criteria for safety: < 0.5 m water depth, >1.7 m height, and > 1.5 m width, 2) surveys must be conducted between 11:00 A.M – 2:00 P.M. with no more than 3 observers, 3) using a Kestrel 3000 measure ambient air temperature, relative humidity and wind velocity at the entrance and center of surveyed culverts, 4) measure height and width of entrance and total length of surveyed culverts in meters, 5) record substrate or presence of water in surveyed culverts 6) place temperature loggers in the center of the surveyed culvert to measure temperature stability for duration of seasons of survey periods, 7) identify all bats detected to species, 8) record the position of detected bats (wall, ceiling, or crevice). Crevices should be defined as any crack or depression in the culvert where a bat could fit > 60% of its body, and 9) when present, record the number of available crevices to bats within surveyed culverts.

White Nose Syndrome

Loss of roosting sites, disturbance, and WNS are some of the most important known causes of bat decline. I submit that, as documented in this study, culverts could provide substitutional habitat where natural roosting habitat is not available or scarce. A greater understanding of use of human-made structures can enhance conservation efforts for bats that roost in these structures. Awareness of the presence of rare bats, such as southeastern myotis, can assist transportation departments and biologists in impact assessment and mitigation of potential negative impacts of culvert replacement and restoration projects. Also, enhanced knowledge of the role that culverts may play in roosting hibernacula for bats can improve conservation and monitoring approaches for Chiropterans. Knowledge of bat use rates and locations of used culverts can help focus conservation measures within and around these sites. These types of data are important in providing baseline information on roosting bat numbers and trends in bat populations over time. Baseline data on numbers of roosting bats is also very important due to the detection of WNS fungus on substrates of one study culvert and three caves in 2014. Greater information on fungal detection, numbers of roosting bats, and culverts most often used by greatest numbers of bats may assist biologist in developing plans to monitor and ameliorate impacts of WNS on bat species that use culverts and box bridges as winter roost sites.

REFERENCES

Adam, M. D., & Hayes, J. P. 2000. Use of bridges as night roosts by bats in the Oregon Coast Range. Journal of Mammalogy, 81(2), 402-407.

Bach, L., Burkhardt, P., & Limpens, H. J. 2004. Tunnels as a possibility to connect bat habitats. Mammalia mammals, 68(4), 411-420.

Barbour, R. W., & Davis, W. H. 1969. Bats of America (Vol. 7). Lexington: University Press of Kentucky.

Barclay, R.M.R., and Kurta, A. 2007. Ecology and behavior of bats roosting in tree cavities and under bark. Pages 17-59 *In*: M.J. Lacki, J.P. Hayes, and A. Kurta. (Eds). Bats in Forests: Conservation and Management. John Hopkins University Press, Baltimore, Maryland, USA.

Bender, M. J., Castleberry, S. B., Miller, D. A., and Wigley, T. B. 2010. Use of Culverts as Diurnal Roost by Bats in Butler Co., Alabama. Journal of the Alabama Academy of Science, 81 (3-4), 204-210.

Best, T. L., and Caesar, K. G. 2000. Distribution and abundance of bats in caves and mines of northeastern Mississippi. Occasional Papers of the North Carolina Museum of Natural Sciences and North Carolina Biological Survey, (12), 45-49.

Bigler, W. J., Hoff, G. L., and Buff, E. E. 1975. Chiropteran rabies in Florida: a twenty-year analysis, 1954 to 1973. The American journal of tropical medicine and hygiene, 24(2), 347-352.

Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., and Stone, W. B. 2009. Bat white-nose syndrome: an emerging fungal pathogen? Science, 323(5911), 227-227.

Boonman, M. 2011. Factors determining the use of culverts underneath highways and railway tracks by bats in lowland areas. Lutra, 54(1), 3-16.

Bouma, H. R., Carey, H. V., & Kroese, F. G. 2010. Hibernation: the immune system at rest? Journal of Leukocyte Biology, 88(4), 619-624.

Briggler, J. T., & Prather, J. W. 2003. Seasonal use and selection of caves by the eastern pipistrelle bat (*Pipistrellus subflavus*). The American midland naturalist, 149(2), 406-412.

Broders, H. G., Forbes, G. J., Woodley, S., and Thompson, I. D. 2006. Range extent and stand selection for roosting and foraging in forest-dwelling northern long-eared bats and little brown bats in the Greater Fundy Ecosystem, New Brunswick. Journal of Wildlife Management, 70(5), 1174-1184.

Cavallaro, L., K. Sanden, J. Schellhase, M. Tanaka. 2005. Designing Road Crossings for Safe Wildlife Passage: Ventura County Guidelines. Thesis. University of California, Santa Barbara, California.

Clark, D. R., Martin, C. O., and Swineford, D. M. 1975. Organochlorine insecticide residues in the free-tailed bat (*Tadarida brasiliensis*) at Bracken Cave, Texas. Journal of Mammalogy, 56(2), 429-443.

Clark, M. K. 1990. Roosting ecology of the eastern big-eared bat, *Plecotus rafinesquii*, in North Carolina. Thesis, North Carolina State University, Raleigh, USA.

Clark, M. K., E. Hajnos, and A. Black. 1997. Radio-tracking of *Corynorhinus rafinesquii* and *Myotis austroriparius* in South Carolina. Bat Research News 38:136–137.

Cochran, S. M. 1999. Roosting and habitat use by Rafinesque's big-eared bat and other species in a bottomland hardwood forest ecosystem. Thesis, Arkansas State University, Jonesboro. 50 p.

Collins, C. A., Wilkinson, D. W., and Evans, D. L. 2005. Multi-temporal analysis of Landsat data to determine forest age classes for the Mississippi statewide forest inventory–Preliminary results. In Proceedings of the Third International Workshop on the Analysis of Multi-Temporal Remote Sensing Images (pp. 16-18).

Cryan, P. M., Meteyer, C. U., Boyles, J. G., and Blehert, D. S. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC biology, 8(1), 135

Davidson-Watts, I., Walls, S., and Jones, G. 2006. Differential habitat selection by *Pipistrellus pipistrellus* and *Pipistrellus pygmaeus* identifies distinct conservation needs for cryptic species of echolocating bats. Biological Conservation, 133(1), 118-127.

Davis, W. H. 1959. Disproportionate sex ratios in hibernating bats. Journal of Mammalogy, 16-19.

Davis, W. H. 1966. Population dynamics of the bat *Pipistrellus subflavus*. Journal of Mammalogy, 47(3), 383-396.

Estók, P., Zsebők, S., & Siemers, B. M. 2009. Great tits search for, capture, kill and eat hibernating bats. Biology letters, rsbl20090611.

Fleming, T. H., & Estrada, A. (Eds.). 2012. Frugivory and seed dispersal: ecological and evolutionary aspects (Vol. 15). Springer Science & Business Media.

Foley, J., Clifford, D., Castle, K., Cryan, P., and Ostfeld, R. S. 2011. Investigating and Managing the Rapid Emergence of White-Nose Syndrome, a Novel, Fatal, Infectious Disease of Hibernating Bats. Conservation Biology, 25(2), 223-231.

Frost, C.C., J. Walker, and R.K. Peet. 1986. Fire-dependent savannas and prairies of the Southeast: original extent, preservation status, and management problems. Pages 348–357 *in* D.L. Kulhavy and R.N. Conner (Eds.). Wilderness and natural areas in the Eastern United States: a management challenge. Center for Allied Studies, School of Forestry, Stephen F. Austin St. Univ., Nacogdoches, TX.

Fujita, M. S., & Tuttle, M. D. 1991. Flying foxes (Chiroptera: Pteropodidae): threatened animals of key ecological and economic importance. Conservation Biology, 5(4), 455-463.

Golley, F.B. 1966. South Carolina Mammals. Contribution from the Charleston Museum. XV. E. Milby Burton (Ed.) Charleston, SC. 181 pp.

Gore, J. A., and Hovis, J. A. 1994. Southeastern myotis maternity cave survey. Final performance report, Nongame Wildlife Program, Game and Fresh Water Fish Commission, Tallahassee, FL.

Hall, J. S., and Wilson, N. 1966. Seasonal populations and movements of the gray bat in the Kentucky area. American Midland Naturalist, 317-324.

Hallam, T. G., and McCracken, G. F. 2011. Management of the Panzootic White-Nose Syndrome through Culling of Bats. Conservation Biology, 25(1), 189-194.

Harvey, C. A., Medina, A., Sánchez, D. M., Vílchez, S., Hernández, B., Saenz, J. C. and Sinclair, F. L. 2006. Patterns of animal diversity in different forms of tree cover in agricultural landscapes. Ecological applications, 16(5), 1986-1999.

Harvey, M. J. 1991. Status of endangered bats in the eastern United States. In Proceedings of the National Cave Management Symposium (pp. 351-355).

Hayes, J. P., Adam, M. D., Bateman, D., Dent, E., Emmingham, W. H., Maas, K. G., and Skaugset, A. E. 1996. Integrating research and forest management in riparian areas of the Oregon Coast Range. Western journal of applied forestry (USA).

Hefner, J. M., & Brown, J. D. 1984. Wetland trends in the southeastern United States. Wetlands, 4(1), 1-11.

Hoffmeister, D. F. 1989. Mammals of Illinois. University of Illinois Press, Urbana and Champaign, IL. 348pp.

Humphrey, S. R. 1978. Status, winter habitat, and management of the endangered Indiana bat, *Myotis sodalis*. Florida Scientist, 41(2), 65-76.

Humphrey, S. R., and Gore, J. A. 1992. Southeastern brown bat. Rare and endangered biota of Florida, 1, 335-342.

Hutson, A. M., and Mickleburgh, S. P. 2001. Microchiropteran bats: global status survey and conservation action plan (Vol. 56). IUCN.

Jackson, S.D. 1996. Underpass systems for amphibians. 4 pp. In G.L. Evink, P. Garrett, D. Zeigler and J. Berry (eds.) Trends in Addressing Transportation Related Wildlife Mortality, proceedings of the transportation related wildlife mortality seminar. State of Florida Department of Transportation, Tallahassee, FL. FL-ER-58-96.

Jones, C, and C. H. Carter. 1989. Annotated checklist of the recent mammals of Mississippi. Occasional Papers of the Museum of Texas Tech University, Lubbock, TX. 128:1-9.

Jones, C., and Manning, R. W. 1989. *Myotis austroriparius*. Mammalian species, 1-3.

Jones, C., and Suttkus, R. D. 1975. Notes on the natural history of *Plecotus rafinesquii*. Louisiana State University.

Keeley, B.W., and M.D. Tuttle. 1996. Texas bats and bridges project. Texas Department of Transportation, Austin, Texas, 16 pp.

Keeley, B.W. 1998. Bat use of bridges. Bureau of Land Management, Coos Bay District, and Oregon Department of Fish and Wildlife, Coos Bay, Oregon, 15 pp

Keeley, B. W., and Tuttle, M. D. 1999. Bats in American bridges. Resource publication, 4, 1-6.

Kennedy, M.L., K.N. Randolph, and T.L. Best. 1974. A review of Mississippi mammals. Natural Science Research Institute. Eastern New Mexico Univ., Portales, NM. 36 pp.

Knight, E. L., Irby, B. N., and Carey, S. D. 1974. Caves of Mississippi. University of Southern Mississippi.

Kunz, T. H. 1982. Roosting ecology of bats. In Ecology of bats (pp. 1-55). Springer US

Kunz, T. H., & Pierson, E. D. 1994. Bats of the world: an introduction. Walker's Bats of the World, 1-46.

Lance, R. F., Hardcastle, B. T., Talley, A., and Leberg, P. L. 2001. Day-roost selection by Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) in Louisiana forests. Journal of Mammalogy, 82(1), 166-172

LaVal, R. K. 1970. Infraspecific relationships of bats of the species *Myotis austroriparius.* Journal of Mammalogy, 51(3), 542-552.

Lindner, D. L., Gargas, A., Lorch, J. M., Banik, M. T., Glaeser, J., Kunz, T. H., and Blehert, D. S. 2011. DNA-based detection of the fungal pathogen *Geomyces destructans* in soils from bat hibernacula. Mycologia, 103(2), 241-246.

Ludlow, M., and Gore, J. 1997. Conservation of Cave Roosting Bats at a North Florida State Park. *In* 1997 Karst and Cave Management Symposium 13th National Cave Management Symposium.

McCoy, E. D., and Connor, E. F. 1980. Latitudinal gradients in the species diversity of North American mammals. Evolution, 193-203.

McCracken, G. F. 1986. Why are we losing our Mexican free-tailed bats? Bats, v. 3: 1-4

McNab, B. K. 1982. Evolutionary alternatives in the physiological ecology of bats. In Ecology of bats (pp. 151-200). Springer US.

Menzel, J. M., Menzel, M. A., Ford, W. M., Edwards, J. W., Sheffield, S. R., Kilgo, J. C., and Bunch, M. S. 2003. The distribution of the bats of South Carolina. Southeastern Naturalist, 2(1), 121-152.

Meteyer, C. U., Buckles, E. L., Blehert, D. S., Hicks, A. C., Green, D. E., Shearn-Bochsler, V., and Behr, M. J. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. Journal of Veterinary Diagnostic Investigation, 21(4), 411-414.

Middleton, A.L. 1976. A survey of cave-associated fauna in Mississippi. Master’s thesis. Univ. Southern Mississippi, Hattiesburg, MS. 92 pp.

Mirowsky, K. M., & Horner, P. A., R W. Maxey, and SA Smith. 2004. Distributional records and roosts of southeastern myotis and Rafinesque's big-eared bat in eastern Texas. Southwestern Nat, 49, 294-298.

Moore, C. M. 2006. Dissolution caves of Mississippi. ProQuest

Muller, L. K., Lorch, J. M., Lindner, D. L., O’Connor, M., Gargas, A., and Blehert, D. S. 2013. Bat white-nose syndrome: a real-time TaqMan polymerase chain reaction test targeting the intergenic spacer region of *Geomyces destructans*. Mycologia, 105(2), 253-259.

Neuendorf, K. K. 2005. Glossary of geology. Springer Science & Business Media.

Newling, C. J. 1990. Restoration of bottomland hardwood forests in the Lower Mississippi Valley. Ecological Restoration, 8(1), 23-28.

Perlmeter, S. I. 1996. Bats and bridges: patterns of night roost activity in the Willamette National Forest. In Bats and forests: proceedings of the Victoria Symposium. Ministry of Forests Research Program Working Paper (Vol. 23, No. 1996, pp. 132-150).

Raesly, R. L., and Gates, J. E. 1987. Winter habitat selection by north temperate cave bats. American Midland Naturalist, 15-31.

Reichard, J. D., and Kunz, T. H. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropterologica, 11(2), 457-464.

Rice, D. W. 1957. Life history and ecology of *Myotis austroriparius* in Florida. Journal of Mammalogy, 38(1), 15-32.

Roth, Z. U. 2014. A Phenological Study of Bat Communities in Southern Mississippi Caves.

Rudis, V. A. 1995. Regional forest fragmentation effects on bottomland hardwood community types and resource values. Landscape Ecology, 10(5), 291-307.

Ruediger, W. 2001. High, wide, and handsome: Designing more effective wildlife and fish crossings for roads and highways. International Conference on Ecology and Transportation 2001 Proceedings pp. 509-516.

Russ, J. M., and Montgomery, W. I. 2002. Habitat associations of bats in Northern Ireland: implications for conservation. Biological Conservation, 108(1), 49-58.

Sandel, J. K., Benatar, G. R., Burke, K. M., Walker, C. W., Lacher, Jr, T. E., and Honeycutt, R. L. 2001. Use and selection of winter hibernacula by the eastern pipistrelle (*Pipistrellus subflavus*) in Texas. Journal of Mammalogy, 82(1), 173-178.

Sealander, J. A., & Heidt, G. A. 1990. Arkansas mammals: their natural history, classification, and distribution. University of Arkansas Press.

Simmons, N. B. 2005. An Eocene big bang for bats. Science, 307(5709), 527-528.

Speakman, J. R., & Thomas, D. W. 2003. Physiological ecology and energetics of bats. Bat ecology, 430-490.

Stewart, R. A. 2003. Physiographic regions of Mississippi. Handout, Department of Biological Sciences, Delta State University, Cleveland, Mississippi.

Swanson, G., and Evans, C. 1936. The hibernation of certain bats in southern Minnesota. Journal of Mammalogy, 17(1), 39-43.

Tiner Jr, R. W. 1984. Wetlands of the United States: current status and recent trends. United States Fish and Wildlife Service.

Trousdale, A. W., and Beckett, D. C. 2002. Bats (Mammalia: Chiroptera) recorded from mist-net and bridge surveys in southern Mississippi. Officers of the Mississippi Academy of Sciences, 47(4), 185.

Trousdale, A. W., and Beckett, D. C. 2004. Seasonal use of bridges by Rafinesque's big-eared bat, *Corynorhinus rafinesquii*, in southern Mississippi. Southeastern Naturalist, 3(1), 103-112.

Trousdale, A. W., Beckett, D. C., and Hammond, S. L. 2008. Short-term roost fidelity of Rafinesque's big-eared bat (*Corynorhinus rafinesquii*) varies with habitat. Journal of Mammalogy, 89(2), 477-484.

Tuttle, M. D. 1979. Status, causes of decline, and management of endangered gray bats. The Journal of Wildlife Management, 1-17

Verboom, B., Boonman, A. M., and Limpens, H. J. 1999. Acoustic perception of landscape elements by the pond bat (*Myotis dasycneme*). Journal of Zoology, 248(01), 59-66.

Walker, C. W., Sandel, J. K., Honeycutt, R. L., and Adams, C. 1996. Winter utilization of box culverts by vespertilionid bats in southeast Texas. Texas Journal of Science, 48(2), 166-167.

Warren, R. D., Waters, D. A., Altringham, J. D., and Bullock, D. J. 2000. The distribution of Daubenton's bats (*Myotis daubentonii*) and pipistrelle bats (*Pipistrellus pipistrellus)(*Vespertilionidae) in relation to small-scale variation in riverine habitat. Biological Conservation, 92(1), 85-91.

Whitaker Jr, J. O. 1995. Food of the big brown bat *Eptesicus fuscus* from maternity colonies in Indiana and Illinois. American Midland Naturalist, 346-360.

White, J.S. 1960. Cave-inhabiting bats from an abandoned silica mine in Tishomingo County. J. Mississippi Acad. Sci. 6:198.

Wilkinson, G. S., and South, J. M. 2002. Life history, ecology and longevity in bats. Aging cell, 1(2), 124-131.

Wojciechowski, M. S., Jefimow, M., and Tęgowska, E. 2007. Environmental conditions, rather than season, determine torpor use and temperature selection in large mouse-eared bats (*Myotis myotis*). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 147(4), 828-840.

Zar, J. H. 1999. Biostatistical analysis. Pearson Education, India.