Winter 2018

Identification And Characterization Of Tri-Colored Bat Hibernaculum In Russell County, Kansas

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IDENTIFICATION AND CHARACTERIZATION OF TRI-COLORED BAT

HIBERNACULUM IN RUSSELL COUNTY, KANSAS

being

A Thesis Presented to the Graduate Faculty

of Fort Hays State University

in Partial Fulfillment of Requirements for

the Degree of Master of Science

by

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This thesis for

The Master of Science Degree

By

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has been approved

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PREFACE

This thesis is written in the style of Journal of Mammalogy, to which a portion will be submitted for publication.

Key words: Cave macrohabitat, cave microhabitat, hibernacula, Hygrochron iButtons, *Myotis septentrionalis*, North-Central Kansas, *Perimyotis subflavus*, Russell County, threatened species, white-nose syndrome

ABSTRACT

Caves are often essential during hibernation, a sensitive stage in the life cycle of bats. Caves offer more stable conditions, such as temperature and relative humidity, during the changing seasons when food supply is limited, and weather is unstable. As caves in North-Central Kansas are sparse, the hibernating status of tri-colored bat (*Perimyotis subflavus*) is not well known. Bats are experiencing substantial population declines within the United States, caused primarily by white nose-syndrome, as well as habitat loss and degradation, and increased wind-power facilities.

In the winter seasons of 2015 and 2016, I studied tri-colored bat hibernacula. A man-made "cave" in Russell County, Kansas was the focal study area of my research. The tri-colored bat was the most common species found within the mixed-species hibernaculum. I described the cave by establishing microhabitat and macrohabitat parameters, which included at roost sites and overall characteristics within the cave. I placed Hygrochron iButtons at individual roost sites to automatically record environmental data. The hibernating tri-colored bat was more often located in areas that were closer to the ceiling than the ground and that were farther from the entrance. The cave provided unlimited access to water, subsequently this variable was not significant in roost site preference. At individual locations, the tri-colored bat was almost always roosting at sites that had constant temperatures and fluctuating relative humidity.

Continuing to study bat caves and roost site preference increases the understanding of bat natural history. Understanding bat behavior and how they are responding to environmental changes can be beneficial in determining potential protected habitat and to ensure the populations continued survival.

ACKNOWLEDGMENTS

I would never have had this amazing experience if my advisor, Dr. Elmer J. Finck had not agreed to accept me into his lab. It was a fateful, life-changing day and I will always be humbled and grateful for his acceptance. He guided me through my journey through graduate school and it is something I will never forget. I thank my graduate committee Dr. Rob Channell, Dr. Brian Maricle, and Mr. Curtis Schmidt. They were there to offer helpful advice, a good laugh, and an open ear. I give another thank you to Dr. Rob Channell, who not only helped me complete and understand the statistical analyses for my thesis, but also offered additional help with class projects, assignments, and general life obstacles. Thank you for always being so understanding to any question or problem I had.

I am thankful to Fort Hays State University including the Graduate School, Department of Biological Sciences, and the Kansas Wetlands Education Center for offering me wetlands and research assistantship positions, which allowed me the opportunity to grow and better myself as a person and as a biologist. My time at the Kansas Wetlands Education Center was a special one. I was new to the state and did not know anyone. Being at the wetlands center helped me learn so much history about the area, the people, and the culture. I loved the people that worked there and met so many new people, who came to visit the center. It helped me adjust to and appreciate the new place I called home. Thank you to Kansas Department of Wildlife, Parks, and Tourism as well as the many private landowners, who allowed us access to their land. They were all integral parts of the success of this project. Also, thank you to the Kansas Department of Wildlife, Parks, and Tourism for funding my research.

To all the amazing, and beautiful people I met during my time at FHSU, I could not have survived this process without you. To my partners-in-crime, my laughing buddies, and travel and eats companions – Mitchell Meyer and Holly Wilson. You will forever be truly special and dear to my heart. I am so lucky to have been in the right time and place in life to have met you both. Thank you to Elizabeth Bainbridge, Kasandra Brown, Diedre Kramer, Samantha Pounds, Taylor Rasmussen, Tej Man Tamang, Elizabeth Tharman, and fellow graduate and undergraduates for being the best colleagues, officemates, study buddies, advice givers, shoulders to cry on, storytellers, adventure and coffee dates, bad joke tellers, people to make me laugh, and so much more! You brought so much joy into my life and made this journey much more colorful. Thank you for letting me be myself and for your friendship, I will forever cherish it.

Thank you to my research team Geneva McKown, Mitchell Meyer, Adam Rusk, Elizabeth Schumann, and Holly Wilson for being the best bat research partners! Somehow through the scorching sun, insect bites, and long nights, you made field work the most amazing experience of graduate school. Thank you to the many people who came out to volunteer and spend their precious time catching bats with us, I hope you enjoyed it as much as I did. A special thank you to Taylor Rasmussen, who ended up slipping into the position of honorary team member as he spent many, many hours with us in the field, was always up for anything, and went along with our crazy ideas!

Thank you to my undergraduate university, California State University, Sacramento and the Department of Biological Sciences. It was there that I embraced my passion to study wildlife. This was fueled by the very charismatic and insightful lectures of Dr. William Avery and Dr. Winston Lancaster. I thank my undergraduate advisor, Dr.

Shannon Datwyler for always being so kind and understanding, and for taking the time out of her hectic schedule to encourage and guide me through my slightly rocky start into the field of Biology.

“Thank you” never seems to be enough when you want to recognize the support you received from your family and friends, but I will make a brief attempt. Thank you for believing in me, giving me the confidence to always be true to myself and to trust in life and the choices I make. Family is the backbone to anything successful in my life. The unconditional support I know I will always get from them means more to me than I could ever say. My mom, older brother and sister, dad, step-mom, baby sister, aunts, uncles and cousins are the reasons I wanted to do well and be a good person. To my oldest and dearest friends: Morgan Deane-Storelee, Stefanie Volturno, and Krystal Zamora, I would not be where I am today without you; you have been there through it all. Thank you for the late-night pep talks, the “you can do its!”, and for believing in me even if I was struggling. How amazing it is that our friendships have continued through distances, laughs and hard times, break ups, and marriages. I can always rely on you no matter where we are in our lives. That is the beauty of life, friends.

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INTRODUCTION

As climate change and temperatures continue to reach extreme levels, it is even more critical to understand the ecology of bats and how they affect our economy. Bats can be used as bioindicators, readily observable taxa that are sensitive to environmental changes (Jones et al. 2009). Their population status and function can indicate the condition of the environment because of their limited ability to cope with environmental stress or variability (Jones et al. 2009). One of the main stresses bats face is white-nose syndrome and its rapid anthropogenic spread. White-nose syndrome (hereafter WNS) is a recently described infectious disease that affects hibernating bats in the eastern United States. WNS is caused by a psychrophilic (cold-loving) fungus, *Pseudogymnoascus destructans,* which grows around the muzzle and wing membranes of bats (Reichard and Kunz 2009; Cryan et al. 2010; Wilder et al. 2011). The fungus erodes and invades the wing membranes of hibernating bats, disrupting homeostasis and causing altered torpor patterns; which can result in starvation and death (Cohn 2008; Britzke et al. 2010; Moosman et al. 2013). Other threats bats face includes habitat loss, fragmentation, and degradation, increased wind-power facilities (Kunz et al. 2007; Boyles et al. 2011), and predation by birds (Twente 1954), feral cats (*Felis catus*) (Ancillotto et al. 2013), and snakes (Barr and Norton 1965; Hopkins and Hopkins 1982).

Bats provide natural pest control, which affects our agricultural systems and our economy (Cleveland et al. 2006; Boyles et al. 2011). Bats decrease populations of some crop and forest pests. Bats eat approximately half their body mass in insects each night of foraging (Kunz et al. 1995; Cleveland et al. 2006). The over six million bats that have died from WNS are no longer eating pest insects. This means that, approximately 6000 metric tons of insects are no longer being consumed each year (Boyles et al. 2011). This leads to increased costs associated with the purchase and application of pesticides, which have subsequent harmful environmental effects as well as increased pesticide resistance. Pest control by bats is valued at 4 billion to 50 billion dollars per year (Boyles et al. 2011). The agricultural value of bats in the Midwest and Great Plains is substantial because crop production dominates the economies of those regions (Cleveland et al. 2006; Boyles et al. 2011). As bats have a positive impact on our environment and economy, it is imperative that we continue to further our understanding of bats, focusing on the sensitive stages in their life cycles, such as hibernation. Having this information can lead to stronger conservation and protection plans for bat populations and their future generations.

Hibernation is a state of inactivity, generally resulting in lower body temperatures, slower respiration, and slower metabolic rates (Hellgren 1998). Each year, hibernating bats undergo changes that allow them to survive in environments that experience seasonal changes and restrictions in food supply (Thomas 1995; Hellgren 1998). Vespertilionid bats accumulate body fat and mass in late summer or early autumn in preparation for migration during the fall and hibernation during the winter (Swanson and Evans 1936; Speakman and Racey 1989). They also lower their body temperatures in response to these seasonal changes, thus conserving water and energy reserves (Swanson and Evans 1936; Dietz and Kalko 2006).

Vespertilionid bats undergo daily torpor, which is a period of decreased body temperature that lasts less than 24 hours. Daily torpor allows bats to temporarily increase body temperature during the active portion of the day (Lyman et al. 1982; Britzke et al. 2010), while deep torpor maintains a constant reduction in body temperature for a longer period of time (Jonasson and Willis 2012). Longer periods of torpor will minimize energy use but can result in physiological stresses such as dehydration and the accumulation of metabolic waste. Daily torpor, although energetically costly, limits the physiological effects that would occur during deep torpor (Lyman et al. 1982; Jonasson and Willis 2012), while allowing them to have an active period at night.

Selecting an appropriate overwintering site is essential to the survival of hibernating bats. During the winter, bats can succumb to starvation and freezing (Raesly and Gates 1987; Speakman and Racey 1989; Neubaum et al. 2006). Bats choose hibernacula based on microhabitat: temperature, humidity, airflow, and physical structure, such as wall formations, crevices, and cavity volume (Willis and Brigham 2007). Physical structures could facilitate social thermoregulation and energy conservation (Willis and Brigham 2007). Bats also select hibernacula based on macrohabitat: cave and roost size, and relative exposure of the site (Raesly and Gates 1987).

The tri-colored bat (*Perimyotis subflavus*) is one of the most common bats in the United States. Although it is known that it hibernates in caves, buildings, and abandoned mines, little is known about how it selects its hibernacula and its habitat associations (Briggler and Prather 2003). As surveys can be time consuming, difficult and often hazardous, the documented use of caves is not always consistent. Sporadic surveys do not always yield strong or useful information about populations. Documentation of hibernacula locations, number of bats, use of resources and physical variables, most notably hibernacula temperature and humidity are needed to develop conservation plans for the species (Fenton 1997; Brack et al. 2003).

My initial research goal was to investigate the status of current northern myotis (*Myotis septentrionalis*) hibernacula in the state of Kansas based on historical localities. Although there are records of northern myotis throughout areas in Canada and the United States, the status in Kansas was not well known. The northern myotis was listed as federally threatened under the Endangered Species Act on April 2, 2015, because the species is experiencing substantial population declines throughout its range, primarily due to WNS (USFWS 2013). Research efforts resulted in one northern myotis capture during hibernation. As a result, my focus shifted to the tri-colored bat as it was the most common species found. The objectives of my tri-colored bat research were to 1) identify current tri-colored bat hibernacula in Kansas, 2) describe the microhabitat and macrohabitat characteristics of those hibernacula, and 3) assess Hygrochron iButton dataloggers in a cave environment. I hypothesized that 1) relative humidity would affect tri-colored bat roost selection and 2) temperature would affect tri-colored bat roost selection, as they are not known to roost in clusters.

Natural History of the Tri-colored Bat

The tri-colored bat can be distinguished from *Myotis* species, for which it is commonly misidentified, by its tri-colored hairs that are dark at the base, yellowishbrown in the middle, and dark at the tip (Barbour and Davis 1969). It is a small (4 to 8 g) insectivorous bat that belongs to the family Vespertilionidae. The tri-colored bat forages along water ways at forest edges and normally does not forage in regions of dense forest (Davis and Mumford 1962; Fujita and Kunz 1984). Its foraging flight pattern is relatively slow and erratic. It also is among the earliest species to emerge from evening roosts to forage (Fujita and Kunz 1984). The diet of tri-colored bat consists mostly of trichopterans (caddisflies), coleopterans (beetles), and hymenopterans (wasps, bees, and ants) (Fujita and Kunz 1984; Feldhamer et al. 2009).

Tri-colored bat occurs throughout eastern North and Central America (Fujita and Kunz 1984) (Fig. 1). Its most western extent reaches into Nebraska, Kansas, and the panhandle of Oklahoma and Texas. The northern portion extends into Nova Scotia, Quebec, and Ontario, Canada. Its southernmost extent reaches northeastern Honduras (Solari 2018). Recent evidence suggests the range of the tri-colored bat has expanded westward in the United States in the last two decades (White et al. 2006). Its previous western limit was Nebraska, Kansas, Oklahoma, and Texas, but records of tri-colored bat have been reported from South Dakota, Wyoming, Colorado, and New Mexico (White et al. 2006; Valdez et al. 2009). The tri-colored bat previously was known as the eastern pipistrelle and belonged to the genus *Pipistrellus* but has since been changed to the tri-colored bat belonging to the genus *Perimyotis* (Menu 1984; Hoofer and Van Den Bussche 2003). The tri-colored bat does not share a recent common ancestor with true *Pipistrellus* (Hoofer et al. 2006).

The tri-colored bat can hibernate between late September and early May (Briggler and Prather 2003; Damm and Geluso 2008). Before it enters hibernation, the tri-colored bats engage in swarming behavior between the ends of summer-beginning of autumn (August-September), where copulation occurs (Fraser et al. 2012). Hibernating females store sperm in their reproductive tract during the winter (Fujita and Kunz 1984). In the spring, the tri-colored bat leaves hibernaculum and migrates to maternity roost sites where ovulation and parturition occurs (Fujita and Kunz 1984). Tri-colored bat generally has two young between May and June. It raises its pups in foliage (Veilleux et al. 2003; Fraser et al. 2012), in piles of dead leaves (Veilleux et al. 2003), buildings such as barns (Fujita and Kunz 1984; Fraser et al. 2012), and trees, caves, or rock crevices (Fujita and Kunz 1984). Tri-colored bat females in Nova Scotia use a specific species of lichen, *Usnea trichodea*, on conifers as maternity colony roosts (Poissant et al. 2010). During the maternity period, females roost together while males roost singly (Fujita and Kunz 1984). The tri-colored bat is often the first bat species to enter hibernacula and last to emerge in spring (LaVal and LaVal 1980; Fujita and Kunz 1984). It also exhibits roost fidelity as banded individuals return to the same roost year after year (Allen 1921; Griffin 1934; LaVal and LaVal 1980; Fujita and Kunz 1984).

The tri-colored bat is classified as a short-distance regional migrant as hibernacula are usually within 100 km of its maternity roost sites (Vincent and Whitaker 2007). There is evidence for variation in migration distance between sexes, which has been seen with long-distance latitudinal migrants (Fraser et al. 2012). It has been noted to be one of the bat species most susceptible to wind-turbine deaths. Wind turbines might account for

25% of mortality in the tri-colored bat population (Fraser et al. 2012). The tri-colored bat has recently shown increases in latitudinal migration. Having knowledge of these behavioral changes can be useful in strategizing placement of future wind-turbines to help potentially avoid this high mortality rate (Fraser et al. 2012).

The tri-colored bat hibernates in caves and mines. It uses these structures for hibernation more than other cave-roosting bats in the eastern United States (Brack et al. 2003; Damm and Geluso 2008). It also has been found using a storm sewer (Goehring 1954) and other man-made structures, such as buildings, as hibernacula. Although males and females are found within the same hibernaculum, most tri-colored bats roost singly (Fujita and Kunz 1984; Vincent and Whitaker 2007). As the tri-colored bat does not form clusters to take advantage of social thermoregulation, it chooses deeper parts of the hibernaculum where ambient temperatures are relatively constant (Fujita and Kunz 1984; Vincent and Whitaker 2007). Tri-colored bat can be found in mixed-species hibernacula and depending on location can be seen amongst northern myotis, big brown bat (*Eptesicus fuscus*), and little brown bats (*Myotis lucifugus*) (Vincent and Whitaker 2007). The tri-colored bat remains near the same location within hibernaculum during much of the hibernation period (Vincent and Whitaker 2007). It remains in prolonged torpor longer than some of its hibernating neighbors of different species and does not often leave the hibernaculum (Vincent and Whitaker 2007).

METHODS

Study Areas

I conducted surveys in watersheds designated by the Kansas Department of Wildlife, Parks, and Tourism (KDWPT). The designated watersheds included Lower Arkansas, Lower Republican, Marais des Cygnes, Missouri, Neosho, Smoky Hill, Solomon, Verdigris, and Walnut Rivers (Fig. 2). Counties within these watersheds were then identified as having potential over-wintering habitat (Fig. 3).

Data collection occurred at the following region within the designated counties: North-Central; which included Ellis, Osborne, Phillips, Rooks, Russell and Trego counties (Fig. 4). Survey sites included both public and privately-owned land. The survey sites were dominated by elm (*Ulmus* sp.), willow (*Salix* sp.), honey locust (*Gleditsia triacanthos*), plains cottonwood (*Populus deltoides*), green ash (*Fraxinus pennsylvanica*), and hackberry (*Celtis occidentalis*).

The Russell County cave was approximately 2.5 km long and varied in length, width, and water level throughout the cave. There were two entrances, the open "main" entrance met at the end of a small ravine, which sloped downward and was full of vegetation and rocks. The second entrance fed into the Smoky Hill River and was not as easily accessible with sampling equipment. The main entrance was sunnier, warmer, and muddy, but with no standing water. The low sloped ceilings were rocky with crevices. Moving into the cave from the main entrance, the ceiling increased in height and the width of the cave decreased. This position of the cave had small pools of water and led to the middle portion of the cave. The middle section sloped downward and decreased in height and increased in width; standing water was approximately waist deep and the walls were damp and slippery. There was a closed surface entrance located in this section, where ceiling height and water level increased. In the last portion of the cave, the water level was between ankle to mid-thigh deep and the cave ceiling was 1.5 to 2 m high. The walls in the last portion were soft and sandy with pieces easily broken off.

Trapping

Methods were in accordance with the guidelines established by the American Society of Mammalogists (Sikes et al. 2016). Capture and handling procedures were approved by the Fort Hays State University Institutional Animal Care and Use Committee (protocol number 15-0002, Appendix 1).

Field Season 2015 – The first field season was from August 2015 to January 2016. Mist-nets were used to capture bats. They were placed in flyways between potential roost trees and water sources (i.e., creeks, streams, and ponds). Mist-net lengths used included 6 m, 9 m, and 12 m (Avinet Inc., Freeville, NY). These nets were mounted on net poles that were approximately 2 m high. Mist-nets were open from sunset until bat activity decreased substantially. If no bats were captured within 90 minutes from the last capture, nets were closed. Nets were closed when bat activity was too high to allow processing. When a bat was removed from a mist-net it was placed into a cloth bag until its data could be recorded.

For each bat, I recorded species, sex, age, reproductive condition, mass with a Pesola scale, ear length, tragus length, forearm length, hindfoot length, tail length and total body length with a field ruler (Reimer et al. 2014). Female reproductive condition included: 1) pregnant, identified by visual inspection and palpation of the abdomen; 2) lactating, identified by the presence of milk or bare patches around the nipples; 3) post-lactating, identified by bare patches around the nipples without expression of milk; or 4) non-reproductive, identified by the absence of milk and no bare patches around the nipples (Lacki and Schwierjohann 2001; Henderson and Broders 2008). Male reproductive condition included testes non-descended or descended, which were identified visually by scrotal development (Lacki and Schwierjohann 2001; Henderson and Broders 2008). The age of bats was classified as, juvenile or adult, and was determined by epiphyseal growth of the forearm and phalanges (Kunz and Anthony 1982; Reimer et al. 2014). This was done by back-illumination of the wing membrane (shining a flashlight on the bones on either side of the joint). If no space occurred between the diaphysis and epiphysis, the bat was classified as an adult. If there was space between the diaphysis and epiphysis, the bat was classified as a juvenile (Kunz and Anthony 1982; Reimer et al. 2014).

Field Season 2016 – The second field season was from August 2016 to January 2017. An acoustic system, Song Meter SM3BAT (Wildlife Acoustics, Inc., Maynard, MA), was used to determine bat presence in an area and discriminate between species (O'Farrell and Gannon 1999; Brabant et al. 2016). Acoustic software, Kaleidoscope Pro, was used to analyze collected acoustic data (Braun de Torrez et al. 2017). Acoustic data were used to assist with placement of nets to increase the likelihood of capture of northern myotis.

Mist-nets were placed in riparian corridors to capture flying bats. Mist-net sizes that were used included 6 m, 9 m, and 12 m. These nets were mounted on poles, which ranged from 2 m to 7 m high. The handling of bats changed with the addition of the U.S. Fish and Wildlife Service WNS decontamination protocol. In this protocol, captured bats were held in disposable wax cups to avoid cross contamination between bats. Latex gloves were used when handling bats. Between handling different bats, the gloves were either changed or wiped off with Clorox wipes. Clorox wipes were used to disinfect field equipment that came in contact with the bats. Trash was placed into biohazard bags. The biohazard bags and their contexts were autoclaved before disposal.

The data collected from bats were the same as during the 2015 field season. The right forearm of captured bats was banded by using aluminum alloy lipped bands (2.4 mm “narrow”, Porzana Limited, East Sussex, UK). Each band was marked with an identifier unique to the university and the Sternberg Museum of Natural History (FHSM0001-FHSM1000). Banding of bats helped me avoid mistaking a recaptured bat as a new capture. Banding also allowed for additional data to be collected over time for that individual (Kurta and Murray 2002; Krochmal and Sparks 2007; Reimer et al. 2014).

As part of a larger collaboration with the U.S. Fish and Wildlife Service to monitor the status of WNS in Kansas, I swabbed 15 tri-colored bats from the Russell County cave. On October 2, 2016, all 15 tri-colored bats captured from the Russell County cave were swabbed on the muzzle and wing membranes where WNS is most pervasive. Tri-colored bat are often known to enter hibernation the earliest, which was evident by the presence of the species within the cave (Vincent and Whitaker 2007). As swabbing occurred during hibernation, care was taken to minimize disturbance within the cave; limited field crew, red light headlamps, and only communicating when necessary. Bats were evaluated visually for additional signs of WNS; lesions and a wrinkling of the wing membrane (Blehert et al. 2009; Cryan et al. 2010). After bats were processed from the cave, swabs were submitted to the USGS National Wildlife Health Center in Madison, Wisconsin for surveillance of WNS.

Radiotelemetry

In 2016 and 2017, if a northern myotis was captured, a radio transmitter (Model: LB-2X, 0.31g, Holohil Systems Limited, Ontario, Canada) was attached to the interscapular region of the bat by using a non-toxic surgical adhesive (Osto-Bond Skin Bond Adhesive, Montreal Ostomy, Quebec, Canada) (Kurta and Murray 2002; Henderson and Broders 2008). The mass of the transmitter and adhesive was below 5% of body mass of the bat (Aldridge and Brigham 1988).

I released each bat near the site of capture and then radio tracked it to its roost site with the use of two TRX-2000S radio receivers and two 3-element yagi antennas (Wildlife Materials International, Inc., Murphysboro, IL) (Henderson and Broders 2008). I tracked and recorded locations of individual bats daily, as limited by weather and signal detection (Kurta and Murray 2002; Henderson and Broders 2008). I used a global positioning system (Garmin eTrex Vista Cx, Garmin Ltd., Olathe, KS) to mark the location of individuals. During hibernation, I attempted to track the northern myotis on a nightly basis, as the northern myotis will emerge periodically to drink, urinate, or defecate just after sunset.

In October 2016, I located one northern myotis individual in the Russell County cave. I attached a radio transmitter to the bat and tracked at its release location just prior to sunset. I also tracked one tri-colored bat from the Russell County cave. I fitted it with a radio transmitter and tracked from its release position.

Habitat Sampling

Once hibernacula and roost sites were identified within the cave, I measured microhabitat and macrohabitat characteristics, as these factors can be significant in roost selection and preference. I differentiated between microhabitat and macrohabitat classifications by establishing "macrohabitat" as the overall cave characteristics and "microhabitat" as where the bat chose to roost within the cave (Neubaum et al. 2006).

Characterize Microhabitat Parameters – I made more than 10 trips to the Russell County cave to locate roost sites. When a bat was found, I marked its location on the cave wall below or above the hibernating bat. I measured microhabitat variables, temperature (°C) and relative humidity (%RH), by using Hygrochron iButton data loggers (Model DS1923-F5, Maxim Integrated, San Jose, CA). I chose to use the Hygrochron iButton as it was small, lightweight, and durable. I used the marked locations of hibernating bats to determine placement of the iButton data logger by way of a random number generator. Once the initial "roost site" iButton was placed, a "random site" iButton was determined.

I chose the random site by using a quadrant system, which included quadrant number and distance (Fig. 5). A compass centered over the roost site iButton, was used to determine random quadrant location. This was done by using ranges between cardinal directions: between North and East, 0-89°, were quadrant 1, between East and South, 90-179°, were quadrant 2, between South and West, 180-269°, were quadrant 3, and between West and North, 270-359°, were quadrant 4. I determined the quadrant randomly by using these cardinal direction ranges. Once the quadrant was determined, the distance within that quadrant was chosen by setting a minimum and maximum range. The minimum distance was the tri-colored bats mean body lengths. The maximum distance was determined *a priori*, by using previous knowledge of the height and structure during initial exploratory assessment of cave habitat. I assigned Hygrochron iButton data loggers to the locations of 6 tri-colored bats, establishing the sample size as 6 roost locations and 6 random locations. I set the data loggers on 16 October 2016 and retrieved them 11 January 2017. However, only data from 17 October 2016 to 24 October 2016 was used in the statistical analyses, as restricted access to the cave began at the start of November until access was granted in January. There was also no guarantee that tri-colored bats remained at the same roost site the entire season. The iButton data loggers recorded temperature (°C) and relative humidity (%RH) every hour.

Characterize Macrohabitat Parameters – I focused on measuring characteristics within the hibernaculum site where the tri-colored bat was found, to quantify parameters of the location. These parameters included distance to entrance (m), distance to ground (cm), distance to ceiling (cm), and distance to the nearest water source (cm). I measured distance by using a 300-m fiberglass measuring tape (Keson Industries Inc., Aurora, IL). I recorded habitat parameters for every species found in the cave but omitted northern myotis and big brown bat as there was only one individual found for each species. Tri-colored bat was the most abundant species within the cave and was used in all statistical analyses. It was only found and captured within the Russell County cave; tri-colored bat was never captured in mist-nets.

Statistical Analyses

Microhabitat Parameters – To test if roost and random sites differed in temperature and relative humidity, I conducted an Analysis of Covariance (ANCOVA). I ran an individual ANOVA for relative humidity and temperature, with the covariate established as "Day". To determine if the data contained any outliers, I used Cook's distance to measure the influence individual datum had on the model. I then assessed the assumptions of normality by using a QQ plot and Shapiro-Wilks test, and homogeneity of variances by using Brown-Forsyth test. I conducted an ANOVA along with a graphical approach to test the assumption of equal slopes. I used a significance level of 0.05 for all statistical procedures.

Macrohabitat Parameters – To explore and visualize the variation in the dataset, I used a Principal Component Analysis (PCA) with variables "distance to nearest water source", "distance to ceiling", and "distance to entrance". I determined the number of eigenvectors, by using an *a priori* criterion. I used an outlier plot and matrix analysis to determine if outliers were present. After the outlier analysis, I tested for the assumptions of normality and multivariate normality by using a scatterplot matrix and a QQ plot to picture the Mahalanobis distance for the sample. Before performing the PCA, I applied a log transformation on variables distance to entrance and distance to ceiling as these variables did not meet assumptions for normality.

RESULTS

Trapping and Habitat Assessment

Field Season 2015 – The first season lasting from August 2015 to January 2016 consisted of 61.5 net nights. I captured a total of 4 northern myotis, 3 of those during hibernation season in September. I caught those 3 hibernating individuals between two different mist-nets sites at the same location on the same night (Fig. 6).

Field Season 2016 – The second season lasting from August 2016 to January 2017 consisted of 77 net nights. I used butterfly nets to capture bats in the Russell County cave on 7 nights. I captured a total of 4 northern myotis, one during hibernation season in October at the Russell County cave. I also captured 36 tri-colored bats in the Russell county cave between September and October. I used six of their roost site locations in statistical analyses (Fig. 6). The assessment for WNS in the Russell County cave tested negative for *Pseudogymnoascus destructans.* I also did not observe visual symptoms on any bat within the cave.

Radiotelemetry

In October 2016, I located a northern myotis individual in the Russell County cave and fitted it with a radio transmitter. I attempted to track this individual for a week after its release but did not locate it again. I also tracked a tri-colored bat individual from its release point at the cave in Russell County. I located this individual again in the cave a week later. Two months later, I saw the same tri-colored bat in the cave. I did not have sufficient data from radio tracking these individuals to support any inference or conclusion.

Statistical Analyses

Microhabitat Parameters – None of the data were outliers. My data were not normally distributed and did not have univariate normality. As the dataset was very small, no transformation allowed me to obtain normality; I continued using the original data. The Brown-Forsyth test for relative humidity (F = 1.558, df = 1 and 82, P-value = 0.215) and temperature (F = 1.152^{-29}, df = 1 and 82, P-value = 1) showed that there was equal variation between my roost and random sites. The equality of slopes was met as the ANOVA revealed that there was no difference between slopes for both relative humidity (F = 0.8905, df = 1, P-value = 0.348) and temperature (F = 0, df = 1, P-value =1).

The ANCOVA for relative humidity produced a statistically significant model (F = 9.368, df = 2 and 81, P-value = 0.0002), which showed that there was a significant difference of the covariate day (t = 4.223, df = 2 and 81, P-value < 0.001). Relative humidity levels varied over the 7-day recording period, but the percent differences between random and roost sites were not substantial (t = 0.949, df = 2 and 81, P-value = 0.345) (Table 1). The relative humidity of a random and roost site on day 1 differed by 3.23%. The same random and roost site on day 7 differed by 1.98%. The cave's relative humidity also varied throughout the entire recording period, from October to January with maximum humidity reaching 105% and minimum humidity reaching 52% for roost sites (Table 2).

The ANCOVA for temperature produced a statistically significant model (F = 24.21, df = 2 and 81, P-value < 0.001), which showed that there was a significant difference of the covariate day ($t = 6.958$, df = 2 and 81, P-value < 0.001). Temperature levels showed little variation over time but decreased slightly as the week progressed, with the range between 2.0°C and 3.0°C (Table 1). I recorded starting temperatures of 16.65°C and ending temperatures of 14.79°C, with the majority of the iButtons recorded around 15.0°C with little difference between random and roost sites ($t = 0$, df = 2 and 81, P-value = 1). Although bats did experience temperature stability, it also was relatively uniform throughout different areas of the cave. The range of temperature variability was limited.

Macrohabitat Parameters –The outlier analysis revealed that there was an outlier located under the variable distance to nearest water source. The outlier was removed. Univariate normality assessment showed that the variables distance to ceiling and distance to entrance did not have a normal distribution. I log transformed these variables, which resulted in a normal distribution. Multivariate normality assessment revealed the data were slightly platykurtic.

Using an *a priori* criterion, I chose two axes because it is easier to visualize two dimensions. Axis PC1 and PC2 represented the variation between treatments (roost and random sites) and macrohabitat variables. Axis PC1 with an eigenvalue of 1.9823, explained approximately 66% of the variation in the data set and axis PC2 with an eigenvalue of 0.7301, explained approximately 24% of the variation in the dataset.

Distance to nearest water source loaded mainly on principal component 1, distance to ceiling loaded about equally on the first and second principal components, and distance to entrance loaded mainly on the second principal axis. There were three separate groups in the PCA plot (Fig. 7). The largest group was closest to the variable distance to ceiling and far from distance to entrance and distance to nearest water source. The group consisted of 3 roost and 2 random sites. The second group consisted of an overlap of 1 roost and 1 random site, as well as a second roost site. The second group was farthest from distance to entrance and close to distance to ceiling. The mixture of random and roost sites could signify a possible effect. The third group was the smallest group and was closest to distance to nearest water source and far from distance to entrance. This group was represented by 1 roost and 1 random site. Variables distance to entrance and distance to ceiling separated the largest, most distinguishable groups.

DISCUSSION

Hibernation is a vulnerable and sensitive period in the life cycle of a bat. Bats rely on specific sites for protection and safety, and bats might select sites that provide them the highest chance of survival (Raesly and Gates 1987; Speakman and Racey 1989; Neubaum et al. 2006). Environmental conditions might affect the selection of an overwintering site. Choosing areas that minimize temperature and humidity fluctuations is vital when their physiological state is altered (Willis and Brigham 2007). I considered these variables when examining roost selection for the tri-colored bat. I hypothesized that temperature and humidity would be critical factors in selection of roost sites by the tri-colored bat. I also assessed macrohabitat characteristics within the cave as well as, the success of Hygrochron iButton dataloggers as an automatic recording device in a cave setting.

Bats were not choosing roost sites in the Russell County cave based on the microhabitat characteristics: temperature and relative humidity. I reject my hypotheses, acknowledging that my data violated the assumptions of normality. The dataset was very small (6 roost sites and 6 random sites) and might have experienced temporal and spatial autocorrelation as some roost and random sites were close to each other. This could have led to low power and failure to detect patterns. The only significant factor was the covariate day, which was the confounding variable. The confounding variable cannot be described in terms of correlations or associations because it can affect both the dependent and independent variables, potentially causing a false relationship. Relative humidity varied significantly with time, creating variability. Temperature levels showed little variation over time, creating stability.

Other studies find that the tri-colored bat hibernates in areas with warmer, stable temperatures and stable relative humidity (Brack 2007). It is often found in deeper parts of a cave or mine to roost in this specific environment. It conserves energy in these areas without clustering and remains in prolonged torpor (Vincent and Whitaker 2007). The tri-colored bat in a natural cave system is hibernating in longer caves that provide a wide range of temperature gradients and little temperature variation. It is hibernating more often at higher temperatures with a mean temperature of 11.4°C ± 0.33 during the winter (Briggler and Prather 2003). In a man-made abandoned clay, mine, tri-colored bat was roosting in warmer, deeper parts of the cave that provided stable temperatures. The hibernating population was in rooms with the most stable temperatures; but these rooms provided a high range of temperature regimes. Mean temperatures within the clay mine are 12.0°C ± 2 between October and May (Vincent and Whitaker 2007). The tri-colored bat in the man-made Russell County cave was not found exclusively in deeper sections of the cave, it was found equally throughout the cave. Although the cave provided temperature stability, it lacked a variety of temperature regimes. Temperatures within the Russell County cave ranged between 17.41°C and 14.55°C during the week in October (Table 1).

The difference in my outcome could be attributed to the man-made properties of the “cave”. Russell County cave could have lacked some of the natural structures found in caves that might support higher temperatures, little temperature variation, and steady relative humidity. Microhabitat characteristics might not have been critical factors in roost selection within the cave because of the uniformity of the cave. Essentially, bats could randomly choose any area in the cave and encounter the same conditions; the cave lacked a range in thermal gradients that tri-colored bat are often found in. The difference in outcome could also be attributed to the two entrances in the Russell County cave. Not having a closed system, open to fluctuations from each entrance could have influenced the lack of different temperature regimes within the cave.

I considered macrohabitat characteristics that could influence the selection of a particular cave and also specific sections within the cave. Areas closer to the entrance are exposed to more wind, light exposure, and predation. Another consideration is the location of the nearest water source; temperate bats often exhibit daily torpor and will awake to drink water during hibernation (Lyman et al. 1982; Britzke et al. 2010). Results of the macrohabitat analysis suggested that tri-colored bats formed groups according to distance to nearest water source, distance to entrance, and distance to ceiling. The most distinct groups formed closest to the ceiling and farthest from a water source and the entrance (Fig. 8). Groups consisted of both roost and random sites, suggesting a possible effect of the variables on tri-colored bat roost selection.

Almost the entire cave had water flow, there was a possibility that distinct groups did not occur near water sources as access to water was not limited. Bats choosing roost sites that were closest to the ceiling could be the avoidance of cooler temperatures closer to the ground and avoiding the water source. It also could be due to possible elevated temperatures closer to the ceiling. These conditions would be beneficial in conserving energy and decreasing moisture loss (Cryan et al. 2010). Most roost sites were far from the entrance where there was fluctuating air flow and temperatures; the cave had an open entrance with minimal vegetative obstructions. These groupings of bats within the cave potentially indicated preferences for specific chambers within the cave or be helpful in determining individual roost site preference.

I used an automatic data logger at each roost and random site. The iButton generally is known to be used in pharmaceutical, life science and fresh produce distribution as well as autoclave temperature data logging. It was not originally used in ecological or environmental research, although its use is becoming more common. I wanted to test the use of the Hygrochron model data logger with both temperature and relative humidity in cave systems. A study on the big brown bat successfully used the "Thermochron" iButton model to monitor temperature in rock crevices (Neubaum et al. 2006).

I originally glued the iButtons into individual slots cut out of an egg carton; I did this to protect the iButton from condensation on the device. The egg cartons were not successful in this particular cave as the cave walls were sandy and adhesive pads and glue did not adhere. I changed to mounting the iButtons with wall mounts that securely held the iButton in place; this method required the use of a drill and sturdy screws. My original plan did not include these as I feared the lack of a protective cover such that the egg carton would have provided, would lead to equipment failure. I can confirm the success and durability of the Hygrochron iButton in a cave environment. Each iButton successfully recorded temperature and relative humidity every hour over a 3-month winter period and did not experience malfunctions.

I analyzed chosen roost sites over a week-long period as I could not guarantee that the bats did not move to a new site. Although tri-colored bats are known to be one of the species that remains in the same spot within a cave for the longest period of time (Vincent and Whitaker 2007), I did not return weekly to confirm their roost site location. Confirming individual locations and possible movement throughout the hibernation period could be useful in further studies. For future studies, it would also be beneficial if additional hibernacula could be located, to create a more comprehensive dataset. Natural cave systems or man-made structures, such as buildings, could potentially add more information to the analysis and further the understanding of where and why bats choose over-wintering sites during the hibernation season. While additional hibernacula would strengthen our knowledge of hibernating bat behavior, tracking multiple bats within the same hibernaculum could provide more information about specific roost site preference.

I concentrated study efforts in locations with the highest number of recorded historical localities for the northern myotis. I surveyed and studied different sites within multiple counties. For future research, study efforts should include areas further from historical localities to include different habitats found in Kansas. Including different habitat conditions could help in further understanding the potential variety in bat hibernacula choice within the state. This can lead to a better understanding of the physiological and behavioral requirements and constraints of individuals and within the population of hibernating bats. As hibernation is a crucial but vulnerable time for bats, it is imperative that we continue to research and understand why bats are choosing roost locations. This can help guide future conservation efforts for the species by implementing potential land management policies in the areas they are found roosting.

Although WNS was not detected at the Russell County cave, factors such as disturbance, can potentially intensify the spread of WNS. Continuing to monitor these locations as well as additional locations where WNS sensitive species are found (which includes the northern myotis, tri-colored bat, and the little brown bat) is vital in understanding the transmission, and individual site factors that contribute to further WNS outbreak (Reichard and Kunz 2009; Cryan et al. 2010; Willis et al. 2011). Temperatures within the Russell County cave over the winter season were within the optimal growth range for WNS, approximately 3 to 15°, with an upper growth limit of 20° (Tables 1 and 2) (Blehert et al. 2009). Hibernacula with temperatures in the optimal growth range can be reservoirs for the psychrophilic fungus (Blehert et al. 2009).

While species have not gone extinct due to WNS, it continues to spread (Reichard and Kunz 2009; Willis et al. 2011). Tri-colored bats tested positive for WNS in Cherokee and Barber county caves in Kansas during spring 2018 (http://www.whitenosesyndrome.org). Educating the public about bats and bat conservation issues is a first step to control further WNS outbreaks. Controlling anthropogenic spread of *P. destructans* by committing to and strictly implementing WNS decontamination protocols in all areas, of field clothes and gear can help control rapid spread of WNS. Bats provide a natural, free pest control service to our forested areas and agricultural industries. Without this free service, an increased use of pesticides will continue to pollute our natural soil and water ways (Cleveland et al. 2006, Boyles et al. 2011).

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TABLES

Table 1. Maximum and minimum temperature and relative humidity for cave roost and random sites in Russell County, Kansas from 17 October 2016-24 October 2016.

Time Period	Maximum Temperature (°C)	Minimum Temperature (°C)	Temperature Range	Maximum Relative Humidity (%)	Minimum Relative Humidity (%)	Relative Humidity Range (%)
17 October 2016-24 October 2016						
Russell Co. Cave						
Roost Site	16.65	14.55	2.09	77.01	54.43	22.58
Random Site	17.41	14.59	2.82	81.03	54.38	26.65

Table 2. Maximum and minimum temperature and relative humidity for cave roost and random sites in Russell County, Kansas from 16 October 2016-11 January 2017.

Time Period	Maximum Temperature (°C)	Minimum Temperature (°C)	Temperature Range	Maximum Relative Humidity (%)	Minimum Relative Humidity (%)	Relative Humidity Range (%)
16 October 2016-11 January 2017						
Russell Co. Cave						
Roost Site	31.06	-2.99	34.06	104.67	52.36	52.03
Random Site	27.60	-2.49	30.09	107.61	50.36	57.26

FIGURES

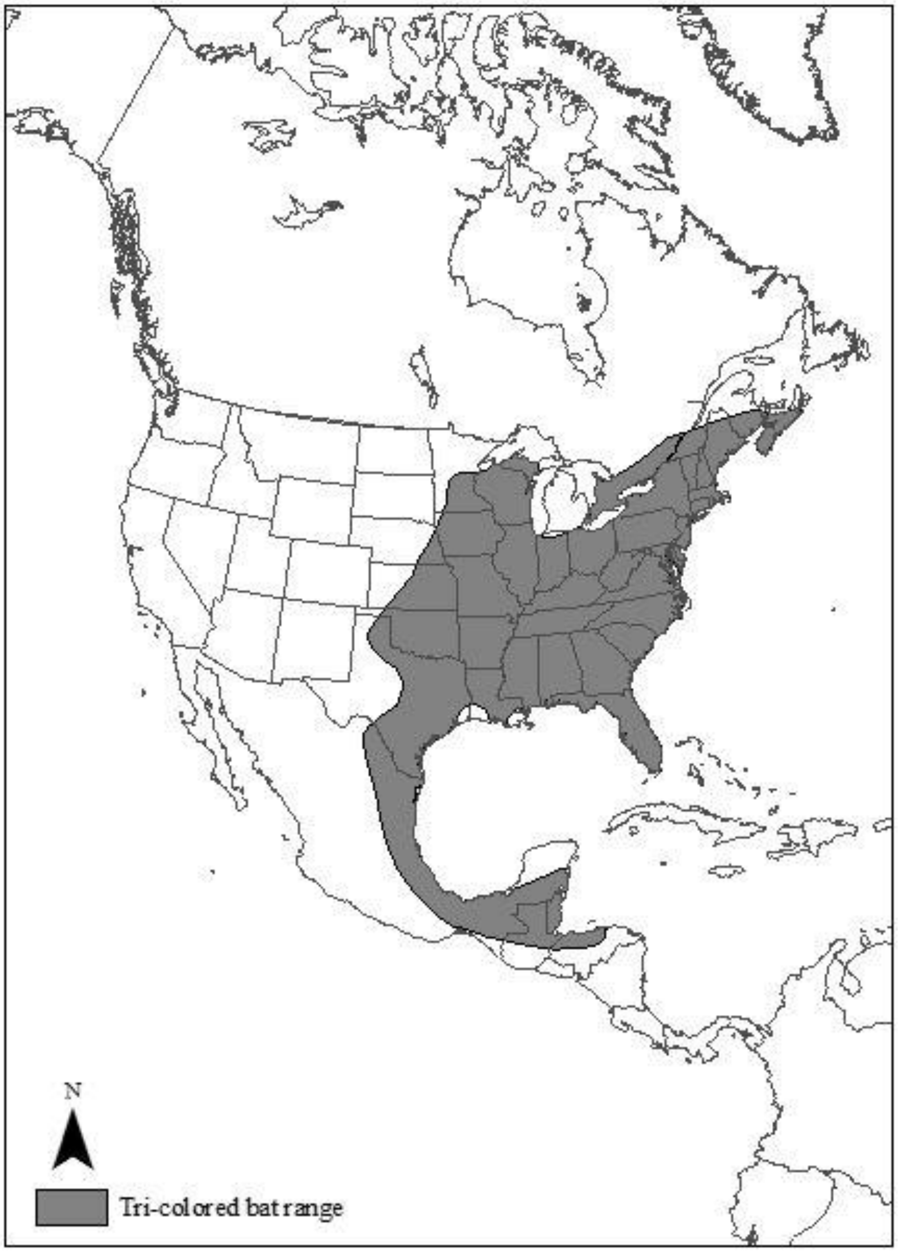


Fig. 1-- Range map of the tri-colored bat (*Perimyotis subflavus*) (Solari 2018).

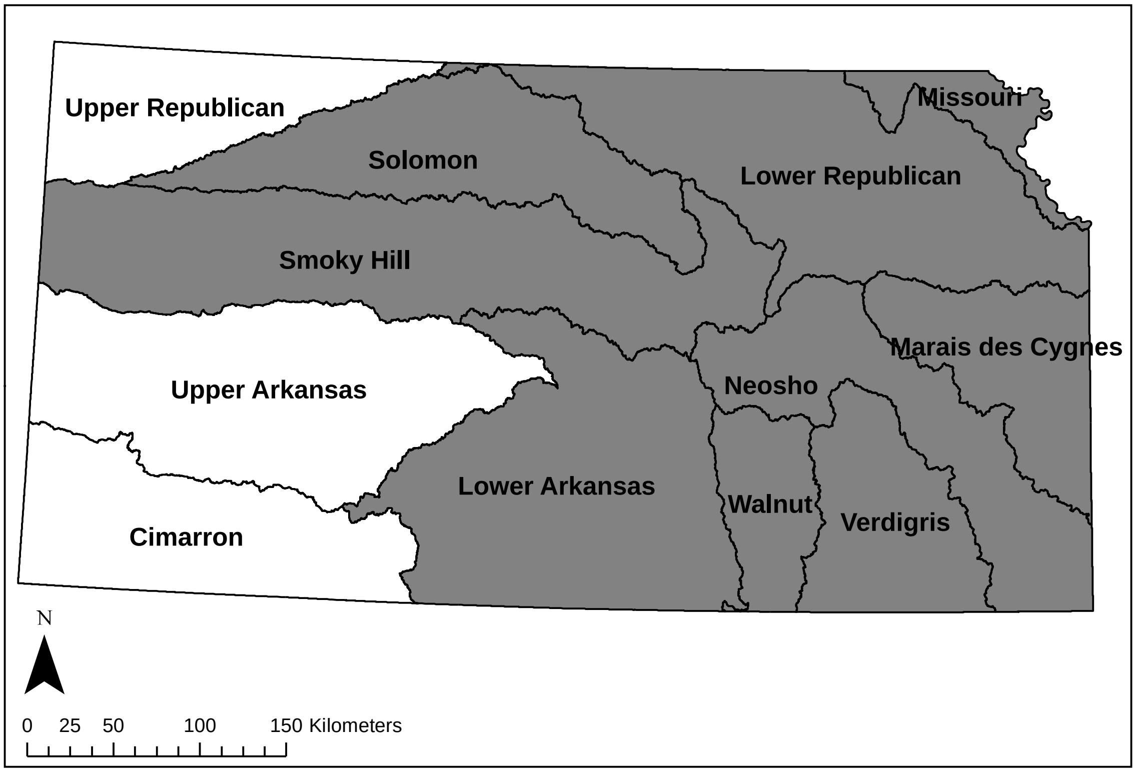


Fig. 2--Kansas watersheds designated by KDWPT that represent potential areas of northern myotis occurrence.

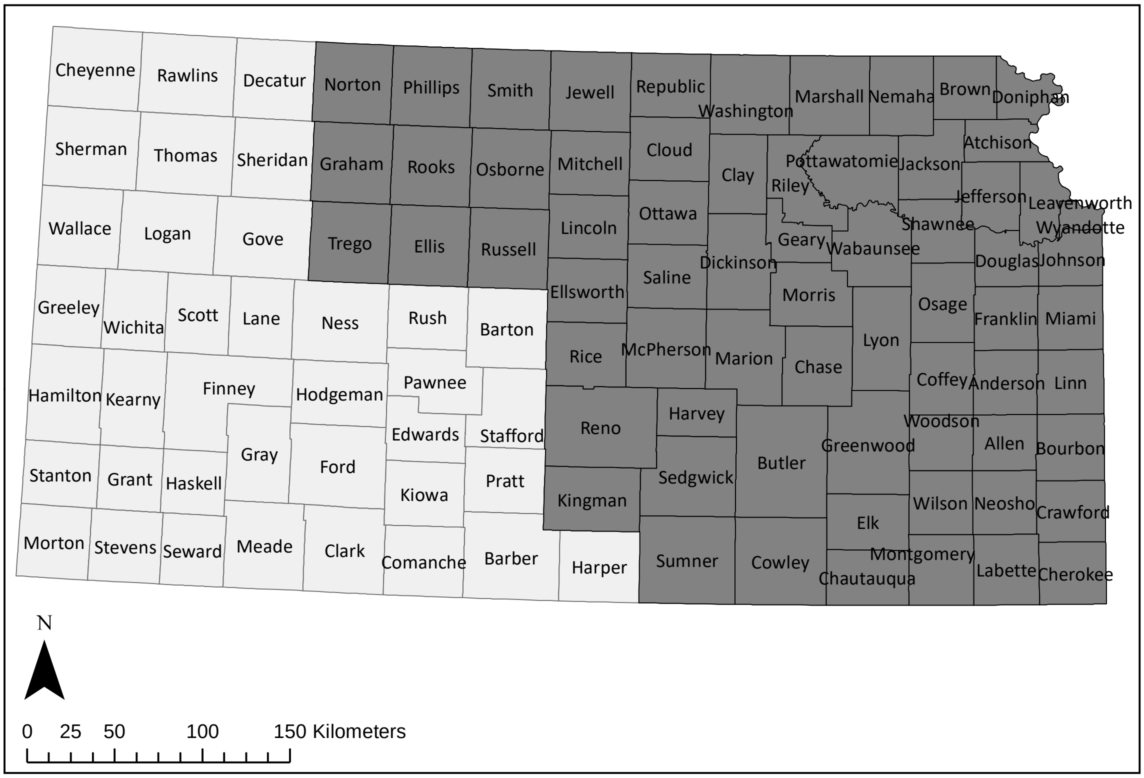


Fig. 3--The 67 counties in Kansas listed by KDWPT as possible occurrence for the northern myotis.

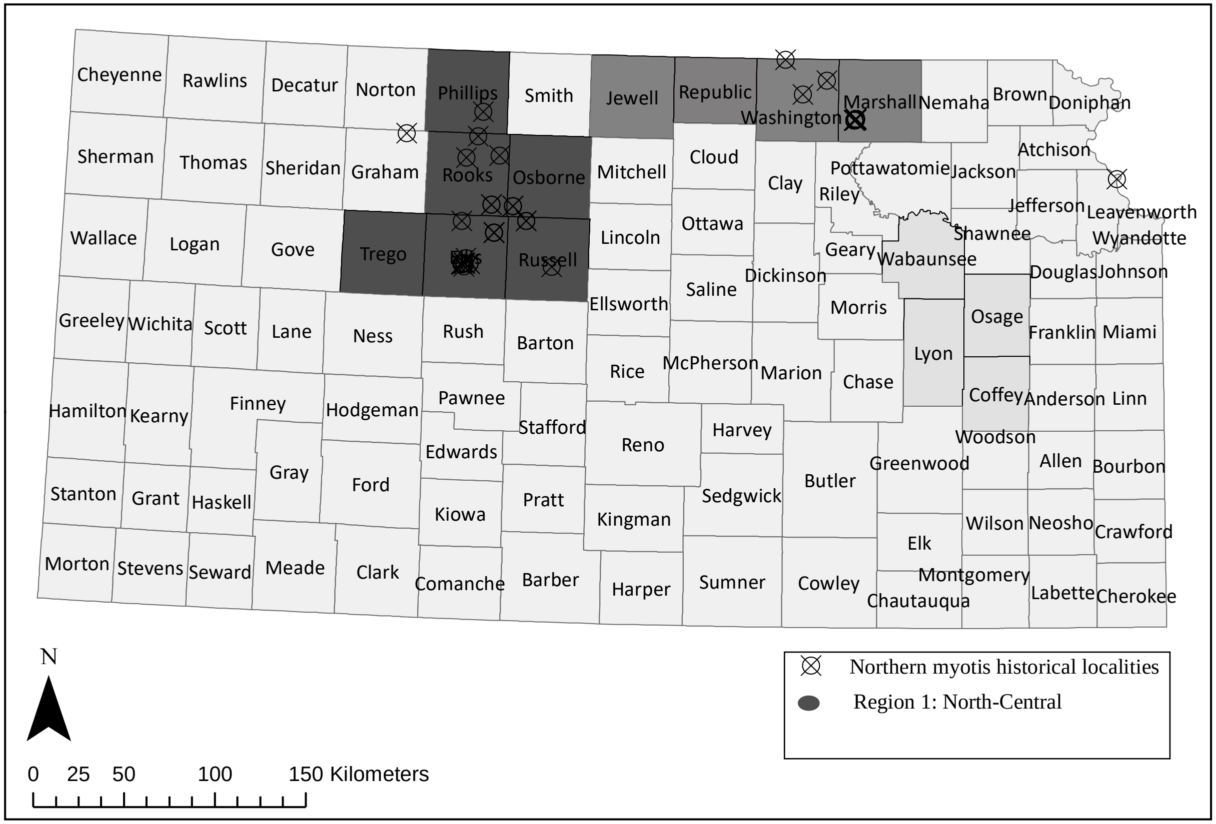


Fig. 4--Study areas from 2015 - 2017 field surveys were conducted in the following region. Region 1: North-Central included Ellis, Osborne, Phillips, Rooks, Russell, and Trego counties. Survey sites included both public and privately-owned land.

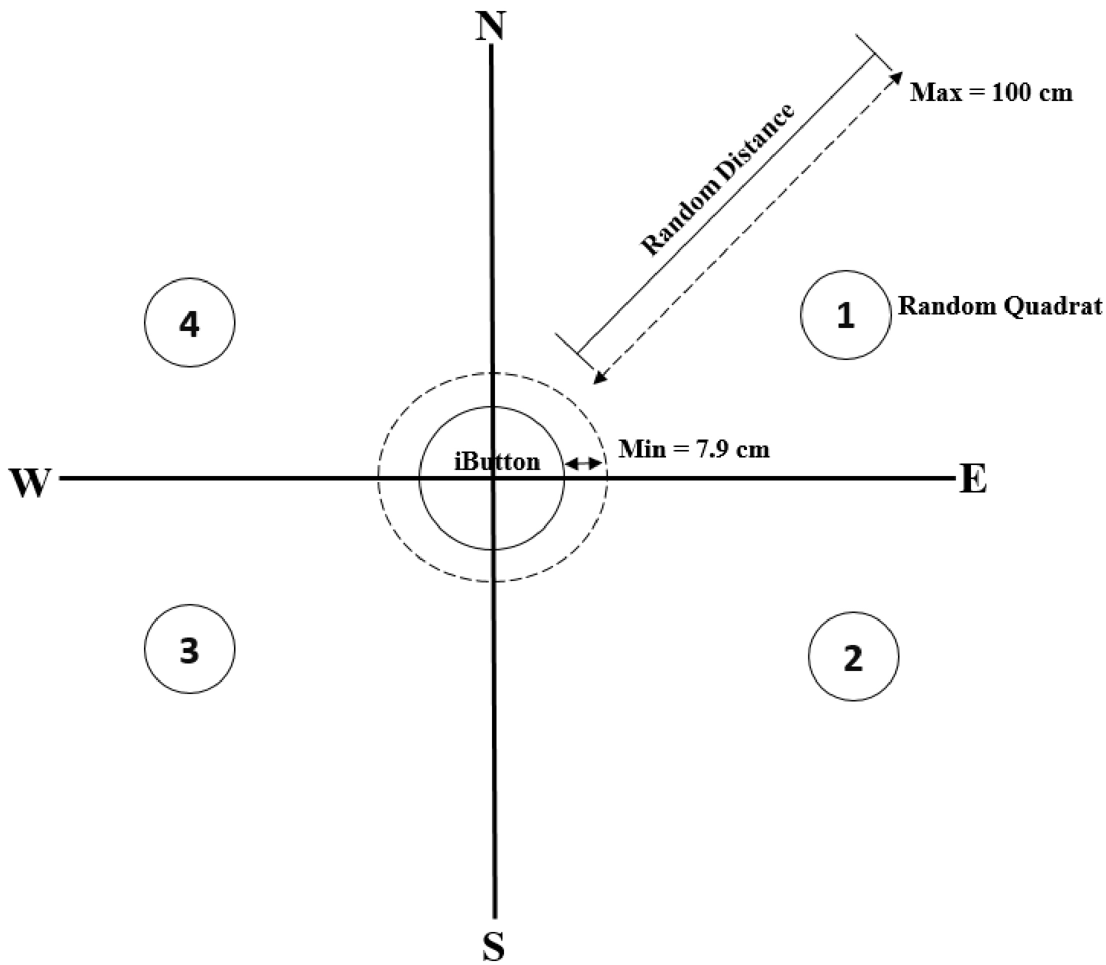


Fig. 5--Quadrant system to establish “roost” and “random” sites for Hygrochron iButton data loggers at Russell County cave, Kansas.

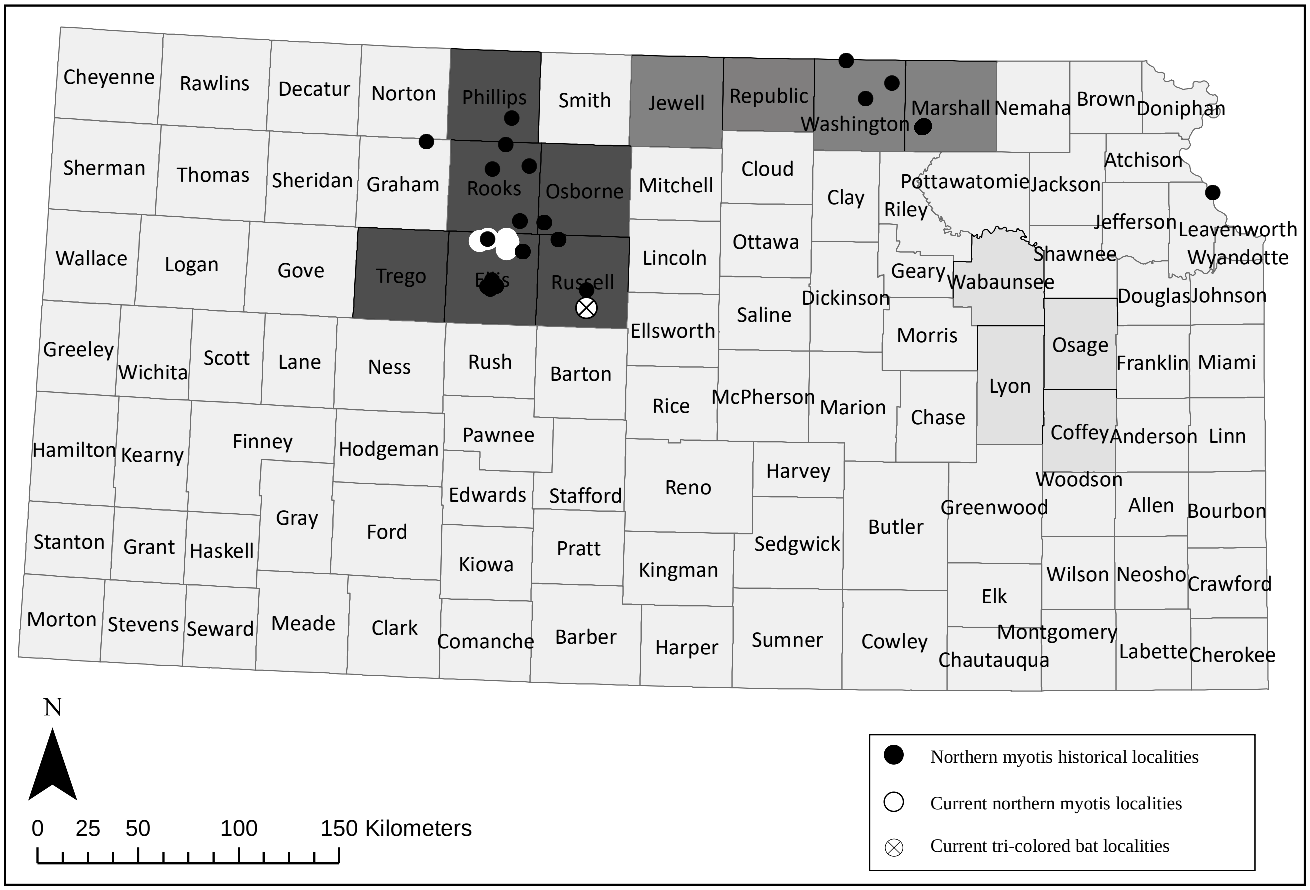


Fig. 6--Current northern myotis and tri-colored bat localities resulting from 2015 - 2017 survey efforts. These localities represent physical captures: 4 northern myotis in 2015, 4 northern myotis and 36 tri-colored bat in 2016.

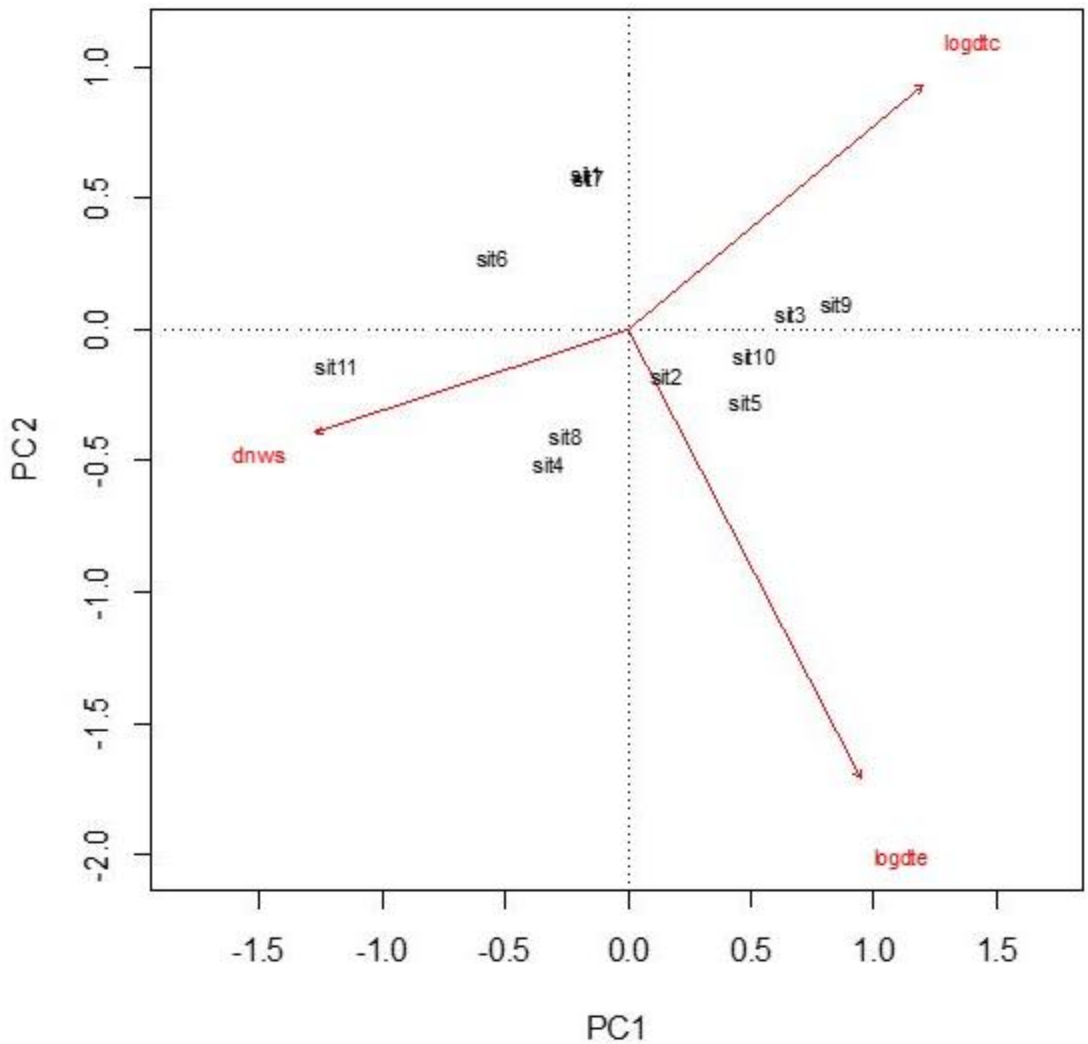
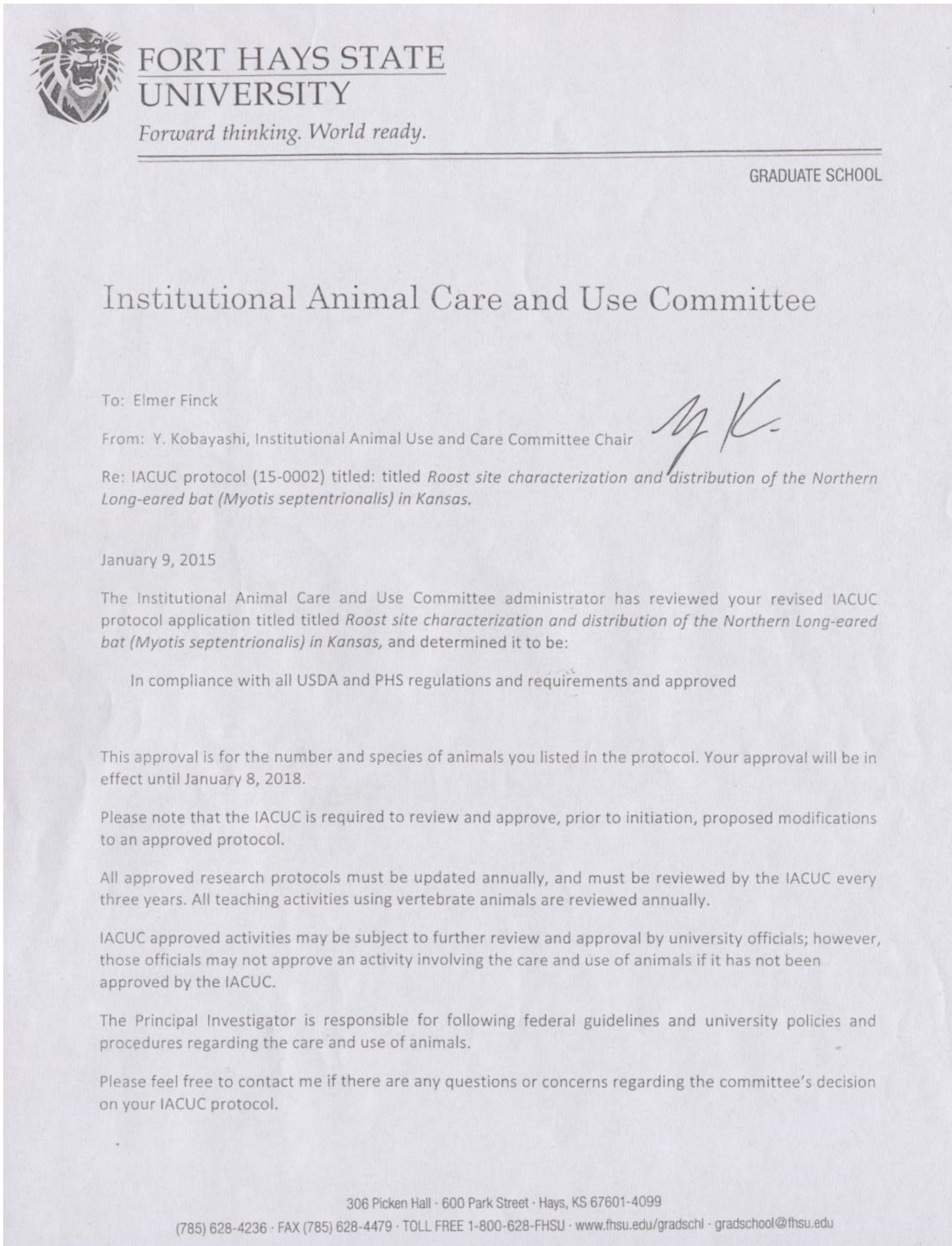


Fig. 7--Biplot of dimensions PC1 and PC2 resulting from the ordination technique, Principal Component Analysis. "Sit" or Sites represent random or roost sites and the arrows point to the variables "(log) distance to ceiling", "(log) distance to entrance", and "distance to nearest water source" within the cave. PC1 explains 66% of the variation within the dataset.

APPENDIX

Appendix 1. Project approval by the Fort Hays State University Institutional Animal Care and Use Committee (protocol number 15-0002).



FORT HAYS STATE UNIVERSITY

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Institutional Animal Care and Use Committee

To: Elmer Finck

From: Y. Kobayashi, Institutional Animal Use and Care Committee Chair

Re: IACUC protocol (15-0002) titled: titled *Roost site characterization and distribution of the Northern Long-eared bat (Myotis septentrionalis) in Kansas.*

January 9, 2015

The Institutional Animal Care and Use Committee administrator has reviewed your revised IACUC protocol application titled titled *Roost site characterization and distribution of the Northern Long-eared bat (Myotis septentrionalis) in Kansas,* and determined it to be:

In compliance with all USDA and PHS regulations and requirements and approved

This approval is for the number and species of animals you listed in the protocol. Your approval will be in effect until January 8, 2018.

Please note that the IACUC is required to review and approve, prior to initiation, proposed modifications to an approved protocol.

All approved research protocols must be updated annually, and must be reviewed by the IACUC every three years. All teaching activities using vertebrate animals are reviewed annually.

IACUC approved activities may be subject to further review and approval by university officials; however, those officials may not approve an activity involving the care and use of animals if it has not been approved by the IACUC.

The Principal Investigator is responsible for following federal guidelines and university policies and procedures regarding the care and use of animals.

Please feel free to contact me if there are any questions or concerns regarding the committee's decision on your IACUC protocol.

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