

BAT POPULATION STATUS AND ROOST SELECTION OF TRI-COLORED BATS IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK IN THE ERA OF WHITE-NOSE SYNDROME

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DEDICATION

To my nieces and nephews. May you always be awed by the weird and beautiful creatures around you.

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ABSTRACT

The ongoing spread of white-nose syndrome is causing devastating declines range-wide for certain North American bat species. Baseline population data that would help managers monitor bat populations in the face of WNS is lacking. Likewise, knowledge of summer roosts, a limiting resource for tri-colored bats (*Perimyotis subflavus*), a species threatened by WNS, is lacking in the southern portion of their range. In our study, we investigated the effect that WNS has had on a population of tri-colored bats in the Great Smoky Mountains National Park, TN-NC. We also characterized summer roosts for the species at the microhabitat and landscape levels. Summer capture rates declined significantly for tri-colored (-76%), little brown (-98%), northern long-eared (-99%), and Indiana bats (-69%) following the arrival of WNS, and winter cave counts also declined significantly for tri-colored (-94%), little brown (-98%), and Indiana bats (-87%). Male tri-colored bats selected for roosts in forest stands with a lower density of stems and fewer conifers in the overstory, as well as taller and larger trees than were generally available. They also selected roosts that were closer to water and foraging resources, and were generally located at lower elevations.

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LIST OF ABBREVIATIONS

GSMNP: Great Smoky Mountains National Park

NPS: National Park Service

USFWS: United States Fish and Wildlife Service

WNS: White-nose syndrome

1. GENERAL INTRODUCTION

BACKGROUND

Bats in North America face numerous threats caused by habitat loss and wind energy, but currently, the most urgent threat is posed by the disease white-nose syndrome (WNS). The fungal pathogen *Pseudogymnoascus destructans*, the cause of the disease (Gargas *et al.* 2009; Lorch *et al.* 2011; Minnis and Linder 2013), was first discovered in a cave in Albany, NY in winter 2006–07 (Blehert *et al.* 2009). Spread through direct contact with infected individuals or with contaminated environments (Lorch *et al.* 2011; Lorch *et al.* 2013), WNS has reached 31 states and five Canadian provinces, killing an estimated 6 million bats (USFWS 2012, USFWS 2016;). Nine species of bats in North America are known to develop symptoms of WNS, including the big brown bat (*Eptesicus fuscus*), little brown bat (*Myotis lucifugus*), northern long-eared bat, eastern small-footed bat (*M. leibii*), Indiana bat (*M. sodalis*), gray bat (*M. grisescens*), southeastern bat (*M. austroriparius*), Yuma bat (*M. yumanensis*), and tri-colored bat (*Perimyotis subflavus*), and an additional six species have been documented carrying *P. destructans* with no diagnostic signs of WNS, including the Eastern red bat (*Lasiurus borealis*), cave bat (*M. velifer*), silver-haired bat (*Lasionycterus noctivagans*), Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), and Virginia big-eared bat (*C. townsendii virginianus*; Turner *et al.* 2011, Bernard *et al.* 2015). The disease poses a significant threat of regional extirpation to certain species of bats. To date it has resulted in the federal listing of the northern long-eared bat as threatened, and led to the tri-colored bat being considered for federal listing (Frick *et al.* 2010; Langwig *et al.* 2012; Center for Biological Diversity and Defenders of Wildlife 2016; USFWS, 2016).

The cause of mortality from WNS is apparently a change in metabolism, resulting from epidermal invasion by *P. destructans* and consequential depletion of fat stores that leads to starvation (Meteyer 2009; Cryan *et al.* 2010; Warnecke *et al.*, 2012). While the physiological cause of mortality is not fully understood, there is compelling evidence that the presence of ulcerations in the bats' tissues induces hypotonic dehydration, loss of electrolytes, and, even in early stages of disease manifestation, development of severe, chronic acidosis, and hyperkalemia (Cryan *et al.* 2013; Verant *et al.* 2014). In some species, this results in infected individuals losing body fat at twice the rate of healthy individuals (Verant *et al.* 2014). During hibernation, healthy bats will periodically arouse to drink water, forage, or relocate to more suitable locations within the hibernacula (Jonasson and Willis 2012). While Verant *et al.* (2014) did not notice a significant increase in number or duration of arousals, there have been numerous other such observations of increased activity during harsh winter months, potentially exacerbating energy depletion and further increasing the likelihood of mortality (Reeder *et al.* 2012; Warnecke *et al.*, 2013; Carr *et al.* 2014). Growth of fungal bodies within epidermal tissues on the wings, muzzle, and ears of bats is most prolific between the range of 12.5 and 15.8°C (Verant *et al.* 2012), within the temperature range of many winter cave hibernacula and maintained by bats during winter torpor (Briggler and Prather 2003; Verant *et al.* 2012; Langwig *et al.* 2012). Fungal loads tend to sharply increase between late fall and early winter, then build throughout the season, resulting in mortality in late winter and early spring (Langwig *et al.* 2015).

Big brown bats appear to have a strong ability to recover from WNS, and, coupled with their ubiquity across North America, populations have a strong likelihood of

surviving through the plight with minimal management (Frank *et al.* 2014). Eastern small-footed bats also appear resistant to the disease, carrying smaller loads of *P. destructans*, and fewer individuals of that species showing signs of infection within contaminated caves (Bernard *et al.* 2016, unpublished data). While transmission models for WNS predict population growth rates for big brown and eastern small-footed bat will eventually stabilize, Indiana, little brown, northern long-eared, and tri-colored bats are at considerable risk of regional extirpation if their annual declines do not drop below 5% population loss (Frick *et al.* 2010; Langwig *et al.* 2012; Thogmartin 2013; Alves *et al.* 2014; Frick *et al.* 2015). Despite predictions, some remnant populations of little brown bats have shown resistance to the infection by hibernating at cooler temperatures within caves (Lilley *et al.* 2016), and some individuals have recovered to produce offspring the following summer (Dobony *et al.* 2011). While these populations represent hope for little brown bats, the grim predictions for the remainder of susceptible species have proven true across affected areas, with significant declines being widely reported in northern long-eared, Indiana, and tri-colored bats (Franci *et al.* 2012; Holliday 2012).

Of the species predicted to experience regional extirpation, the tri-colored bat is one of the least studied. Before the arrival of WNS, 87% of bat species found in the Southeast carried special conservation designations somewhere within the region (Laerm *et al.* 2000). Tri-colored bat populations, however, were stable and had no major threats (Arroyo-Cabrales *et al.* 2008). In studies conducted in Virginia and South Carolina, tri-colored bats were the fourth most commonly observed species, captured at about half the rate of the most commonly seen species, the big brown bat

(Menzel *et al.* 2003; Timpone *et al.* 2011). Because their populations have not been threatened in recent history, very few studies exist that can directly inform their management. Knowing that large declines are imminent in novel areas, management strategies must be developed to support WNS survivors.

Monitoring range-wide populations is one way to determine when and where management actions are needed to slow the spread of WNS and aid in recovery of tri-colored bats (Foley *et al.* 2011). These data are often collected through mist-netting at caves and on the landscape, passive and active acoustic surveys, and annual or biennial hibernacula surveys. The effect of WNS on populations in the northeastern U.S. has been well-monitored, and some species now have regional management strategies that may aid in population stabilization (Szymanski *et al.* 2009; Langwig *et al.* 2012). However, tri-colored bats have large distributions (Barbour and Davis 1969) and, as energy use during hibernation in bats varies along a latitudinal gradient (Dunbar and Brigham 2010), populations in more southern regions of North America may be affected differently by WNS. Therefore, tri-colored bats in the southeastern U.S. may require different management strategies than populations in the northeastern U.S. Located within a temperate deciduous biome, the Great Smoky Mountains National Park (GSMNP) contains a highly diverse suite of climates and forest types throughout (Simon *et al.* 2005), and represents one such area where differential responses to WNS may exist. There is little knowledge to inform the management efforts in this region, therefore implementation of optimized mist net and cave surveys could be used to describe trends in population size, sex ratios, and body condition of tri-colored bats in this region for future population monitoring.

Assemblages of bats can change seasonally as many species are migratory, while others overwinter in hibernacula that are often geographically separated from summer home ranges (Fraser *et al.* 2012). Therefore, it is important to monitor both summer and winter populations of bats within a region for changes in size, potentially resulting from WNS. During the summer, mist-netting provides a means of obtaining monitoring data (Weller 2007). Mist-netting involves capturing bats in fine nets placed in areas where bats tend to aggregate. Data from these surveys can be used to create indices of abundance and demographic parameters such as age and sex ratios.

Winter monitoring is achieved directly through counting hibernating individuals for cave-dwelling species. Although, locating bats during surveys can sometimes be inefficient in tall caves where there is little visibility towards the cave ceiling. Identifying hibernacula that are used by tri-colored bats allows for managers to better protect the species during the winter months. Caves that are known to harbor sensitive species, and the areas surrounding them can be blocked off to disturbances caused by human entry, as is seen at numerous caves in GSMNP (NPS 2015). Documentation of winter population trends and of basic cave use by tri-colored bats that would allow for implementation of management strategies is severely lacking in the Southern Appalachian region.

There is also a deficit in knowledge of basic habitat requirements for the species across their range. To cultivate conditions in which populations of bats can flourish, environmental features important to a species survival and population growth must be identified. Summer roosts are a defining feature of bat habitat, as safety from adverse weather conditions and predators allows for increased survival of adults and recruitment

of juveniles (Barclay and Kurta, 2007). Searching for roosts can be energetically costly (Ruczynski *et al.* 2007), so in areas with increased roosting options, bats may be able to spend less time searching, and allocate those metabolic resources to recovery from WNS and reproductive efforts. When survival and fecundity are optimized through summer months and the maximum number of individuals enter hibernation, the number of individuals to survive the hibernation season may be maximized. The varying life history strategies of bats leads to different requirements for roosts, and for species with wide geographic distributions, differences in roost preference can exist across the range (Humphrey 1975). Therefore, conservation needs for bats can be as varied as the geographic locations they inhabit, and managing for a species within a region requires knowledge of that species' habits that are unique to that landscape. There are only six studies investigating the roost requirements of tri-colored bats; two that took place in the Midwest (Veilleux *et al.* 2003; Veilleux *et al.* 2004; IN), two in the Ouachita Mountains (Perry *et al.* 2007; Perry and Thill 2007; AR), and two small pilot studies; one in South Carolina and one in the Nantahala National Forest (Leput 2004; O'Keefe *et al.* 2009; NC). These studies show that tri-colored bats roost in many types of structures that vary range-wide, including living and dead *Acer*, *Quercus*, and *Celtis* spp., buildings and rock crevices (Winchell and Kunz 1996; Whitaker 1998), and lichen or Spanish moss (Carter *et al.* 1999; Poissant *et al.* 2010). The summer roosting habits of this species throughout the Southern Appalachian range is poorly documented, and will be needed to aid in the recovery of the species within the region.

There is a great need for knowledge of the summer and winter status of GSMNP's bats, and habitat requirements of the tri-colored bat, a species greatly

threatened by WNS. Our study will be the first to examine tri-colored bat populations in the Southern Appalachian region post-WNS. It will provide managers with information needed to manage habitat for these sensitive species, and will help to fill in knowledge gaps on important life-history characteristics of these animals, ultimately enhancing stewardship and conservation.

STUDY OBJECTIVES

The objectives of my study were to:

1. Determine distribution, relative abundance, age, and sex ratios of bat species across GSMNP, and compare to pre-WNS populations.
2. Identify characteristics of roost trees used by tri-colored bats and determine roost selection on two levels:
 - a. Microhabitat
 - b. Landscape

I address these objectives in the subsequent two chapters. Chapter 2 examines the changes in population following the arrival of white-nose syndrome, and Chapter 3 examines the summer roost tree selection of tri-colored bats at the microhabitat and landscape levels.

STUDY AREA

We conducted our research in the Great Smoky Mountains National Park, located at the southern end of the Appalachian Mountain range (Figure 1.1). The park encompasses 211,183 ha in Blount, Sevier, and Cocke Counties, TN, and Swain and

Haywood Counties, NC. It is approximately 80% forested and comprised of five main forest types; cove hardwood forest, spruce-fir forest, northern hardwood forest, hemlock forest, and pine-and-oak forest. Unforested portions of the park contain grassy balds, open fields, and roadways. Midstory throughout the park closely resembles surrounding forest type, and shrub and groundcover vary widely. Elevations throughout the study area range from 267 to 2025 m, and slope angles range from 0 to 28°. The average annual rainfall ranges from 165 to 248 cm. There are numerous karst regions throughout the park, many containing caves and crevices known to be used by bats for roosting and hibernation.

LITERATURE CITED

- Alves, D.M.C.C., C.T. Levi, and D. Brito. 2014. The potential impact of white-nose syndrome on the conservation status of North American bats. *PloS one* e107395.
- Arroyo-Cabrales, J., B. Miller, F. Reid, A.D. Cuarón, and P.D. de Grammonst. 2008. *Pipistrellus subflavus*. The IUCN Red List of Threatened Species 2008: e.T17366A7011135. <http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T17366A701135.en>. [accessed 4/22/2016]
- Barbour, R.W.D., and W.H. Davis. 1969. Bats of America. *University Press*, Lexington, KY, USA.
- Barclay, R.M.R., and A. Kurta. 2007. Ecology and behavior of bats roosting in tree cavities and under bark. In: Lacki MJ, Hayes JP, Kurta A (Eds.), Bats in Forests: Conservation and Management. *John Hopkins University Press*, Baltimore, Maryland, USA. Pp. 17-59.
- Bernard, R.F., J.T. Foster, E.V. Willcox, K.L. Parise, G.F. McCracken. 2015. Molecular detection of the causative agent of white-nose syndrome on Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) and two species of migratory bats in southeastern USA. *Journal of Wildlife Diseases* 51:519-522.
- Bleher, D.S., A.C. Hicks, M. Behr, C.U. Meteyer, B.M. Berlowski-Zier, E.L. Buckles, J.T.H. Coleman, S.R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227.
- Briggler, J.T., and J.W. Prather. 2003. Seasonal use and selection of caves by the eastern pipistrelle bat (*Pipistrellus subflavus*). *Am. Mid. Nat.* 149:406-412.
- Carr, J.A., R.F. Bernard, and W.H. Stiver. 2014. Unusual bat behavior during winter in Great Smoky Mountains National Park. *Southeastern Naturalist* 13:18-21.
- Carter, T., M. Menzel, B. Chapman, and K. Miller. 1999. Summer foraging and roosting behaviour of an eastern pipistrelle, *Pipistrellus subflavus*. *Bat Research News* 40: 5-6.
- Center for Biological Diversity, Defenders of Wildlife. 2016. Petition to list the tri-colored bat (*Perimyotis subflavus*) as threatened or endangered under the Endangered Species Act.

- Cryan, P.M., C.U. Meteyer, J.G. Boyles, and D.S. Blehert. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biology* 8:135.
- Cryan, P.M., C.U. Meteyer, D.S. Blehert, J.M. Lorch, D.M. Reeder, G.G. Turner, J. Webb, M. Behr, M. Verant, R.E. Russell, and K.T. Castle. 2013. Electrolyte depletion in white-nose syndrome bats. *Journal of Wildlife Diseases* 49:398-402.
- Dobony, C., A. Hicks, K. Langwig, R.I. von Linden, J.C. Okoniewski, R.E. Rainbolt. 2011. Little brown *Myotis* persist despite exposure to white-nose syndrome. *Journal of Fish and Wildlife Management* 2 (2):190-195 e1944-687X.
- Dunbar, M., and R. Brigham. 2010. Thermoregulatory variation among populations of bats along a latitudinal gradient. *Journal of Comparative Physiology* 180:885-893.
- Foley, J., D. Clifford, K. Castle, P. Cryan, and R.S. Ostfeld. 2011. Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. *Conservation Biology* 25:223-231.
- Francl, K.E., W.M. Ford, D.W. Sparks, and V. Brack. 2012. Capture and reproductive trends in summer bat communities in West Virginia: assessing the impact of white-nose syndrome. *Journal of Fish and Wildlife Management* 3(1):33-42.
- Frank, C.L., A. Michalski, A.A. McDonough, M. Rahimian, R.J. Rudd, and C. Herzog. 2014. The resistance of a North American bat species (*Eptesicus fuscus*) to White-Nose Syndrome (WNS). *PLoS ONE* 9:1-14
<http://doi.org/10.1371/journal.pone.0113958>
- Fraser, E.E., L.P. McGuire, J.L. Eger, F.J. Longstaffe, and M.B. Fenton. 2012. Evidence of latitudinal migration in Tri-colored Bats, *Perimyotis subflavus*. *PLoS ONE* 7(2): e31419.
- Frick, W.F., J.F. Pollock, A.C. Hicks, K.E. Langwig, D.S. Reynolds, G.G. Turner, C.M. Butchkoski, and T.H. Kunz. 2010. An emerging disease causes regional population collapse of a common North American bat species. *Science* 329:679-682.

- Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B.A. Nickel, K.E. Langwig, J.T. Foster, and A.M. Kilpatrick. 2015. Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography* 24(7): 741–749.
- Gargas, A., M.T. Trest, M. Christensen, T.J. Volk, and D.S. Blehert. 2009. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon* 108:147-154.
- Holliday, C. 2012. White-nose syndrome disease surveillance and bat population monitoring report. Tennessee WNS Response cooperators. July.
- Humphrey, S.R. 1975. Nursery roosts and community diversity of Nearctic bats. *American Society of Mammalogists* 2:321-346.
- Jonasson, K, and C.K.R. Willis. 2012. Hibernation energetics of free-ranging little brown bats. *Journal of Experimental Biology* 215:2141-2149.
- Laerm, J., W.M. Ford, B.R. Chapman. 2000. Conservation status of terrestrial mammals of the southeastern United States. Pp. 4–16, *In* Chapman BR (Ed.). Fourth Colloquium of Conservation on Mammals in the Southeastern United States. Occasional Papers of the North Carolina Museum of Natural Sciences. No. 12. 92 pp.
- Langwig, K.E., W.F. Frick, J.T. Bried, A.C. Hicks, T.H. Kunz, and A.M. Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 15:1050-1057.
- Langwig KE, Frick WF, Reynolds R, Parise KL, Drees KP *et al.* 2015. Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proceedings of the Royal Society B* 282: 20142335.
- Leput, D. W. 2004. Eastern red bat (*Lasiurus borealis*) and eastern pipistrelle (*Pipistrellus subflavus*) maternal roost selection: Implications for forest management. Clemson University. Masters Thesis.
- Lilley, T.M., J.S. Johnson, L. Ruokolainen, E.J. Rogers, C.A. Wilson, S.M. Schell, K.A. Field, and D.M. Reeder. 2016. White-nose syndrome survivors frequent arousals associated with *Pseudogymnoascus* infection. *Frontiers in Zoology* 13:12.

- Lorch J.M., C.U. Meteyer, M.J. Behr, J.G. Boyles, P.M. Cryan, et al. 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 480: doi:10.1038/nature10590.
- Lorch J.M., L.K. Muller, R.E. Russel, M. O'Connor, D.L. Linder, and D.S. Blehert. 2013. Distribution and Environmental Persistence of the Causative Agent of White-Nose Syndrome, *Geomyces desctructans*, in Bat Hibernacula of the Eastern United States. *Applied Environmental Microbiology* 79:1293-1301.
- Menzel J.M., M.A. Menzel, W.M.Ford, J.W. Edwards, S.R. Sheffield, J.C. Kilgo, and M.S. Bunch. 2003. The distribution of the bats of South Carolina. *Southeastern Naturalist* 2:121-152.
- Meteyer C.U., L.E. Buckles, D.S. Blehert, A.C. Hicks, D.E. Green, V. Shearn-Bochsler, N.J. Thomas, A. Gargas, and M.J. Behr. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 21:411-414.
- Minnis A.M., and D.L. Linder. 2013. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology* 117:638-649.
- National Park Service (NPS). 2015. Park Closes White Oak Sink area to protect declining bat populations. Press Release, <https://www.nps.gov/grsm/learn/news/wns-whiteoak-sink-closure.htm> [accessed 4/22/2016]
- O'Keefe, J.M., S.C. Loeb, J.D. Lanham, and H.S. Hill. 2009. Macrohabitat factors affect day roost selection by eastern red bats and eastern pipistrelles in the southern Appalachian Mountains. *Forest Ecology and Management* 257:1757-1763.
- Perry, R.W., and R.E. Thill. 2007. Tree roosting by male and female Eastern pipistrelles in a forested landscape. *Journal of Mammalogy* 88:974-981.
- Perry, R.W., R. Thill, and D. Leslie. 2007. Selection of roosting habitat by forest bats in a diverse forested landscape. *Forest Ecology and Management* 238:156-166.
- Poissant, J.A., H.G. Broders, and G.M. Quinn. 2010. Use of lichen as a roosting substrate by *Perimyotis subflavus*, the tri-colored bat, in Nova Scotia. *Ecoscience* 17:372-378.

- Reeder, D.M., C.L. Frank, G.G. Turner, C.U. Meteyer, A. Kurta, E.R. Britzke, M.E. Vodzak, S.R. Darling, C.W. Stihler, A.C. Hicks, R. Jacob, L.E. Grieneisen, S.A. Brownlee, L.K. Muller, and David S. Blehert. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. PLoS ONE 7:e38920.
- Reichard, J.D., and T.H. Kunz. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown Myotis (*Myotis lucifugus*). Acta Chiropterologica 11:457-464.
- Ruczynski, I., E.K.V. Kalko, B.M. Siemers. 2007. The sensory basis of roost finding in a forest bat, *Nyctalus noctula*. Journal of Experimental Biology 210:3607-3615.
- Simon, S.A., T.K. Collins, G.L. Kauffman, W.H. McNab, and C.J. Ulrey. 2005. Ecological zones in the Southern Appalachians: First Approximation. Southern Research Station SRS-41.
- Szymanski, J.A., M.C. Runge, M.J. Parkin, and M. Armstrong. 2009. White-nose syndrome management: Report on structured decision making initiative. Department of Interior, U.S. Fish and Wildlife Service, Fort Snelling, MN, USA.
- Thogmartin, W.E., C.A. Sanders-Reed, J.A. Szymanski, P.C. McKann, L. Pruitt, R.A. King, M.C. Runge, and R.E. Russell. 2013. White-nose syndrome is likely to extirpate the endangered Indiana bat over large parts of its range. Biological Conservation 160:162-172.
- Timpone, J., K.E. Francl, D. Sparks, V. Brack, and J. Beverly. 2011. Bats of the Cumberland Plateau and Ridge and Valley Provinces, Virginia. Southeastern Naturalist 10:515-528.
- Turner, G.G., D.M. Reeder, and J.T.H. Coleman. 2011. A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future. Bat Res News 52:13-27.
- USFWS (US Fish and Wildlife Service). 2012. North American bat death toll exceeds 5.5 million from white-nose syndrome. USFWF Press Release., https://www.whitenosesyndrome.org/sites/default/files/files/wns_mortality_2012_nr_final.pdf [accessed 19 April 2016].
- USFWS (US Fish and Wildlife Service). 2016. White-nose syndrome: Where is it now? www.whitenosesyndrome.org. [accessed 19 April 2016].

- Veilleux, J.P., J.O. Whitaker, and S.L. Veilleux. 2003. Tree-roosting ecology of reproductive female Eastern pipistrelles, *Pipistrellus subflavus*, in Indiana. American Society of Mammalogists 84:1068-1075.
- Veilleux, J.P., S.L. Veilleux. 2004. Colonies and reproductive patterns of tree-roosting female eastern pipistrelle bats in Indiana. Proceedings of the Indiana Academy of Science 113:60-65.
- Verant, M.L., J.G. Boyles, W. Waldrep, G. Wibbelt, and D.S. Blehert. 2012. Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. PLoS ONE 7(9): e46280.
- Verant, M.L., C.U. Meteyer, J.R. Speakman, J.M. Lorch, and D.S. Blehert. 2014. White-nose syndrome initiates a cascade of physiological disturbances in the hibernating at host. BMC Physiology 14:10.
- Warnecke, L., J.M. Turner, T.K. Bollinger, J.M. Lorch, V. Misra, and P.M. Cryan. 2012. Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. PNAS 109:6999-7003.
- Warnecke, L., J.M. Turner, T.K. Bollinger, V. Misra, P.M. Cryan, D.S. Blehert, G. Wibbelt, and C.K.R. Willis. 2013. Pathophysiology of white-nose syndrome in bats: A mechanistic model linking wing damage to mortality. Biology Letters 9:20130177.
- Weller, T.J., and C.J. Zabel. 2002. Variation in bat detections due to detector orientation in a forest. Wildlife Society Bulletin 30(3):922–930.
- Weller, T.J. 2007. Assessing Population Status of Bats in Forests: Challenges and Opportunities. In: Lacki M.J., J.P. Hayes, and A. Kurta (Eds.), Bats in Forests: Conservation and Management. John Hopkins University Press, Baltimore, Maryland, USA. Pp. 17-59.
- Whitaker, J. 1998. Life history and roost switching in six summer colonies of Eastern Pipistrelles in Buildings. American Society of Mammalogists 79:651-659.
- Winchell, J.M., and T.H. Kunz. 1996. Day-roosting activity budgets of the eastern pipistrelle bat, (Ciroptera: Vespertilionidae). Canadian Journal of Mammalogy 79:651-659.

APPENDIX 1

Figures



Figure 1.1 Location of the Great Smoky Mountains National Park, USA.

2. CHANGE IN STATUS OF BATS POPULATIONS AFTER THE ARRIVAL OF WHITE- NOSE SYNDROME IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

ABSTRACT

Since the arrival of white-nose syndrome (WNS) in the northeastern U.S. in 2006, populations of cave-hibernating bats have declined across the disease's spreading range. To determine whether bat populations in the Great Smoky Mountains National Park (GSMNP) experienced WNS-related declines at rates similar to northern populations, we investigated the change in relative abundance, age and sex ratios of several species known to be susceptible to WNS using summer capture surveys, and winter hibernacula count data in GSMNP. We compared capture rates to a study carried out in 1999–2004 (pre-WNS), and inspected numbers reported during cave counts from 2009–2016. We captured a significantly lower ratio of adult to juvenile big brown bats, and significantly lower ratios of male to female eastern red and tri-colored bats in post-WNS years than captured in pre-WNS years. Summer capture rates declined significantly for tri-colored (-76%), little brown (-98%), northern long-eared (-99%), and Indiana bats (-69%) following the arrival of WNS, and winter cave counts also declined significantly for tri-colored (-94%), little brown (-98%), and Indiana bats (-87%). These results indicate that WNS has had a dramatic impact on southern populations of tri-colored bats, similar to that seen in the northeast.

INTRODUCTION

Bat populations across the globe are in decline due to myriad anthropogenic factors, including changes in land cover and general habitat disturbance (Voigt & Kingston, 2016; IUCN 2017; Johnson et al. 1998; Jones et al. 2009; Gorreson and Willig 2004). Certain species with flexibility in their habitat usage have demonstrated positive reactions to these changes. However, while some edge-adapted species have seen sustained population numbers, other clutter-adapted species have shown declines in regions with reduced forest cover (Estrada-Villegas et al. 2010; Ethier & Fahrig, 2011; Luck et al. 2013). Across the Eastern U.S., the downward trend for these and other species has been exacerbated by the development of wind energy (Arnett and Baerwald 2013), and most recently, the arrival of white-nose syndrome (WNS) (USFWS 2012).

Caused by the fungus *Pseudogymnoascus destructans*, WNS causes skin lesions and changes in behavior during hibernation that lead to premature usage of fat stores, and ultimately death by starvation for many afflicted individuals (Lorch et al., 2011; Minnis & Lindner, 2013; Verant et al., 2014). Of the nine species found with symptoms of the disease, the endangered Indiana bat (*Myotis sodalis*), threatened northern long-eared bat (*M. septentrionalis*), little brown bat (*M. lucifugus*), and tri-colored bat (*Perimyotis subflavus*) have shown disproportionately negative reactions, resulting in collapse of populations across the northeastern US (Frick et al. 2010). If amelioration of less than 5% population loss per year is not reached, it has been suggested that these four species will be at considerable risk of regional extirpation

(Frick et al., 2010; Langwig et al., 2012; Thogmartin et al., 2013; Alves et al., 2014; Frick et al. 2015).

Among these species affected by WNS, baseline demographic data that would aid in monitoring of local populations are lacking range-wide (Foley et al. 2011). Additionally, much of the existing baseline population data for these species originates from winter hibernacula count data. As some of these species are migratory, winter hibernation residents may not represent the summer reproductive population (Fraser et al. 2012), therefore it is important to monitor changes in both summer and winter populations of bats within a region.

In addition to an understanding of population baselines, information on species' reactions to WNS range-wide may increase the accuracy of predictions of species status. Current prediction models for several microchiropteran species, assume that since southern latitudes experience shorter and milder winters, the resulting change in energy usage by individuals at these extents (Dunbar & Brigham, 2010) would slow WNS-related population decline (Maher et al., 2012). However, few studies testing this prediction exist (Bernard & McCracken, 2017).

Pseudogymnoascus destructans arrived in the Great Smoky Mountains National Park (GSMNP) in winter 2009/2010 and around this time anecdotal evidence of change in behaviors to bat species associated with WNS was seen (Carr et al. 2014). To investigate whether these changes were effecting the bat populations in GSMNP, our objectives were to 1) compare summer capture data collected prior to and following the arrival of WNS and 2) assess changes to winter population sizes of bats in cave hibernacula before and after the arrival of WNS using count data.

We hypothesized that, in spite of warmer temperatures in southern climates, the summer capture rate of WNS affected species such as the Indiana, little brown, northern long-eared, and tri-colored bats would have declined significantly from pre- to post-WNS years. We predicted that there would be no change in age or sex ratios as WNS has not been seen to effect sexes differently, and the number of juveniles captured should correlate to the abundance of females. Lastly, we hypothesized that counts of Indiana, little brown, northern long-eared, and tri-colored bats in winter hibernacula would be significantly smaller in post-WNS years than in pre-WNS years.

METHODS

Study Area

Our study took place within the GSMNP, a 2,100 km² area within South Carolina's Haywood and Swain Counties, and Tennessee's Blount, Cocke, and Sevier Counties. Located within the Southern Appalachians, GSMNP is comprised of several ecological zones (i.e. spruce-fir forest, northern hardwood forest, acidic cove) representative of the Southeastern U.S. (Simon et al. 2005), and has records of little brown, northern long-eared, Indiana, and tri-colored bat populations prior to the arrival of WNS. This park is composed of 80% forest, with oak forests primarily found in the lowlands, and spruce-fir forests found at the higher elevations. There are numerous land cover types seen in the Park, including some areas of high intensity visitor traffic and building complexes, as well as agricultural and pastoral fields and grassy balds. The lowland areas of the park have a high density of perennial and ephemeral streams,

receiving an estimated 1.4–2.2 m of rain annually. Temperatures range from 16–32° C in summer and -2.8–12° C in the winter.

Summer Capture Rates

From May – August 2015 and 2016, we sampled bats using mist net surveys at 24 sites that were surveyed in a pre-WNS (1999–2004) study (Britzke et al. 2003; Britzke 2005, unpublished data). The majority of these net sites were along or near streams, consistent with traditional methods for capturing Myotis bats (Kunz and Kurta 1988). By sampling in the same locations that were surveyed pre-WNS, we were able to compare capture rates, age and sex ratios, and body condition of bats post-WNS outbreak. To reduce sampling bias, we placed nets in the same locations used in the 1999 – 2004 study.

To capture bats across GSMNP, we used 6, 9, or 12 m long mist nets, in various single, double, and triple high arrangements (75 denier, 2-ply; Avinet, Dryden, New York; Appendix 2). We opened nets 30 minutes before sunset and kept them open for a total of five hours. While open, we checked nets every ten minutes and closed them during rain or strong winds. For captured individuals, we determined species, age, sex, and reproductive class following protocols used in Kunz (1988), and measured forearm length, mass, and wing damage due to WNS (indexed on a scale of 0–3; Reichard & Kunz, 2009). We collected epidermal swab samples from each individual to test for presence of *P. destructans*, checked all bats captured for presence of WNS lesions (orange fluorescence under black light; Turner et al., 2014), and banded them with lipped aluminum alloy forearm rings (2.4mm or 2.9mm, Porzana Ltd., UK).

To assess changes in the summer distribution and relative abundance of bats in GSMNP, we calculated captures/ net area (m²)/ hour. We reported number of males, females, and juveniles captured by level of effort (e.g. individuals/ net m²/ hour). This facilitated comparisons over multiple years of data collection. Effort in net hours was not available for the 1999–2004 study, hence we assumed that two nets combining to an area of 293.1 m² were used per site per night, and that nets were open for five hours beginning at sunset, per US Fish and Wildlife Indiana bat survey protocols in place at the time (USFWS 1999).

Cave Survey

To investigate the change in winter populations of bats, we used hibernacula survey data collected for 10 caves within GSMNP, primarily located in the Cade's Cove region, including popular tourist sites such as Gregory's Cave, Bull Cave, and White Oak Blowhole. Since the mid 1970's, many of these caves have been surveyed for the endangered Indiana bat, and Rafinesque's big-eared bat, a species of concern in North Carolina. Though anecdotal data appear for other species, earnest hibernacula counts did not begin for most until after the WNS threat was identified in 2009. The 10 caves mentioned in this study are those for which surveys were conducted both prior to and following the arrival of *P. destructans* in the Park in the winter of 2009/2010, and did not show zero counts for both pre- and post-WNS years.

Cave surveys typically took place on a biannual basis to avoid excessive disturbance to hibernating bats, and similar routes, protocols (USFWS 2016), and technicians were used in pre- and post-WNS years to reduce sampling error. Technicians searched caves thoroughly for bats as far into the cave as they were able

to traverse. Bats were counted individually, and number of bats within large clusters was estimated by counting number of individuals within a square meter and multiplying by the total area of the cluster.

Data Analysis

To determine statistical differences in summer capture rates (captures/ net m²/ hour) between pre- and post-WNS years, we used Mann-Whitney U-tests (Francl et al. 2012). To assess potential differences in summer age and sex ratios between pre- and post-WNS years, we used a Fisher's exact test.

Historical counts of certain species exist for some but not all of the 10 caves examined, so we did not average counts across years. Instead, to investigate changes in winter relative abundance in cave hibernacula, we compared the last count preceding the detection of WNS in GSMNP, which for most caves was 2009 (Nolfi 2011), and the latest count following the arrival of the disease following the methods of Turner et al. (2011). This resulted in a dataset of 20 surveys from 10 caves. We did not control for survey date, as it was seen by Ingersoll et al. (2013) that survey date did not affect relative abundance of tri-colored bats in winter hibernacula surveys, though it may have affected abundance estimates for other species. All statistical analyses were run in RStudio 1.0.136 (R Core Team 2017).

RESULTS

Summer Captures

During the summers of 2015–2016, we netted for 48,818 net*hours over 85 nights compared to 37,382 net*hours over 60 nights surveyed in 1999–2004, and

captured 333 bats compared to 628. We encountered 12 species post-WNS compared to 10 pre-WNS; big brown bat (33%; *Eptesicus fuscus*), red bat (30%; *Lasiurus borealis*), silver-haired bat (9.1%; *Lasionycterus noctivagans*), tri-colored bat (8.6%; *Perimyotis subflavus*), Rafinesque's big-eared bat (4.7%; *Corynorhinus rafinesquii*), evening bat (3.5%; *Nycticeus humeralis*), hoary bat (2.7%; *Lasiurus cinereus*), Indiana bat (2.4%; *M. sodalis*), little brown bat (1.5%; *M. lucifugus*), and northern long-eared bat (0.3%; *M. septentrionalis*; Table 2.1), with eastern small-footed (2.9%; *Myotis Leibeii*) and gray bats (0.9%; *M. grisescens*) appearing in the post-WNS years (Table 2.2). While we saw an increase in relative abundance for the big brown bat (24.3%) and eastern red bat (17.2%), we saw notable decreases for the little brown bat (-38.7%), northern long-eared bat (-14.0%), and tri-colored bat (-5.1%; Figure 1).

The summer capture rate for all species combined in 1999–2004 was 0.0168 captures/ net m²/ hour, with the highest capture rates seen for little brown bats (0.0067 captures/ net m²/ hour), and the lowest capture rate for evening bats (8.03E-05 captures/ net m²/ hour). The mean capture rate for all bat species in 2015–2016 was 0.00057 captures/ net m²/ hour, a -66.2% decrease from pre-WNS years (Table 2.3). Capture rates for several species were significantly reduced in 2015–2016, including the little brown bat, northern long-eared bat, Indiana bat, and tri-colored bat. In contrast, we saw a significant increase in the capture rate of eastern small-footed bats, as well as dramatic increases in capture rates of Rafinesque's big-eared bats, evening bats, and big brown bats (Table 2.3). While we saw few changes to age and sex ratios of bat populations, we saw a significant difference in ratios of male to female tri-colored bats, with an increase from 1:84 to 1:8 (Table 2.4). We also saw a greater proportion of

females to males in the post-WNS years for eastern red bats. There was no significant difference in the ratios of adult to juvenile bats for species affected by WNS, however, we did see an increase in the ratio of juveniles to adults for big brown bats (Table 2.4).

Cave Survey

We found that relative abundance of little brown (-98.2%; Table 2.7), Indiana (-86.6%; Table 2.8), and tri-colored bats (-94.3%) in the 10 cave hibernacula surveyed had dropped dramatically (Table 2.5). We also saw declines in Rafinesque's big-eared bats (Table 2.6). Large decreases of tri-colored bats were seen at most hibernacula, including Gregory's Cave where historical records of tri-colored bats show that the cave consistently maintained an average population of 577 individuals. Likewise, decreases in Indiana bats at White Oak Blowhole cave represented a 62.7% decrease in the known winter population of that species within GSMNP.

DISCUSSION

We found that summer capture rates for little brown, northern long-eared, Indiana and tri-colored bats were significantly reduced from pre-WNS years. We expected to see this trend because similar WNS-associated declines in summer capture rates have been observed for these species across their ranges, even at more southern extents (Francl et al. 2012; O'Keefe et al. 2016, abstract). These declines confirm that southern bat populations are likely just as vulnerable to WNS as those in the northeast.

We found no difference in the ratio of adults to juveniles for little brown, northern long-eared, Indiana, or tri-colored bats between pre- and post-WNS years, meaning that the females present appear to still be able to produce young in similar ratios to pre-WNS

populations. However, we did see an increase in the number of females to males for tri-colored bats. This is a positive sign because, as tri-colored bats have low annual fecundity, a disproportionate reduction in females during the winter would mean a disproportionate reduction in the number of offspring available to help rebuild the population (Hoying & Kunz, 1998). It has been suggested that some female bats may be able to partition energy for recovery from WNS-related wounds while still maintaining reproductive success (Dobony et al. 2011), and our finding of similar adult to juvenile ratios before and after the arrival of WNS supports this idea. The increase in age ratio within big brown bats may be due to a reduction in competition from species affected by WNS (Ford et al. 2011). While the pre-WNS study spans five summers, ours only encompasses two. Additional years of survey would help to ensure that our findings reflect long-term trends, and were not due to a population eruption.

A potential source of error in the summer portion of our study was that we were unable to quantify the effort put forth in the pre-WNS years, as exact hours of survey and size of nets were not available. If we underestimated the number of hours surveyed or the size of nets used, then capture rates for all species in pre-WNS years would be less than we estimated, and not as different from post-WNS years as we calculated. This is unlikely as we estimated that effort was standardized with the protocols followed during the survey, and capture rates of species unaffected by WNS were similar between studies.

Similar to trends seen for summer populations, we found a drastic reduction in the number of tri-colored bats hibernating in caves between pre- and post-WNS years within GSMNP. The finding mirrors the 90–100% declines seen in caves across

northeastern populations of tri-colored bats (Frick et al. 2010; Turner et al. 2011; Ingersoll et al. 2013; Frick et al., 2015). We expected to see this result, even at a more southern extant of the WNS zone, as reduction in capture rates and acoustic activity were observed at several caves across East TN following the arrival of the disease (Bernard and McCracken 2017). This decline seen in our direct counts of hibernating individuals is a more accurate representation of the decline in abundance of these species within these hibernacula. While we saw 100% declines at caves containing small numbers of tri-colored bats pre-WNS, a decline of 97.6% at Gregory Cave is particularly alarming as it contained 53.5% of the known winter metapopulation prior to the discovery of WNS in GSMNP. Hence, the decline at this cave alone represents a 52.2% loss of GSMNP's total known winter population. Though no tri-colored bats were seen at White Oak Blowhole in 2009, the cave harbored significant numbers historically, and many were seen in the 2011 and 2013 surveys. Prior to WNS concern, Indiana bats were the only species recorded at this cave, with others noted only when researchers noticed abnormalities. As tri-colored bats prefer to hibernate in the deepest regions of caves, it is possible that their eruptive numbers were due to perturbations from WNS drawing them closer to the cave entrance, as has been seen in other regions (Langwig et al. 2012).

Since historical counts for tri-colored bats are lacking in most of the caves used in our study, it is difficult to determine if trends seen between 2009 and 2016 reflect recent WNS-related declines, or if they are normal within a long term fluctuating population cycle. However, historical records suggest that caves such as Gregory's did at least incidentally contain numbers of tri-colored bats similar to the 2009 pre-WNS figure

we used for our analyses. We take this to mean that the decline is due to WNS, and does not reflect a natural fluctuation in the population.

We saw declines in both summer and winter populations of little brown, northern long-eared, Indiana, and tri-colored bats in GSMNP. This finding was expected because, while the winter hibernation season is shorter and typically milder than experienced by populations in northern latitudes, evidence suggests that individuals in southern latitudes maintain higher body temperatures, and begin hibernation with fewer fat reserves than their northern counterparts (Dunbar and Brigham 2010; Bernard and McCracken 2017). This suggests that predictions of population decline, which assume reduced WNS-related mortality in southern latitudes are likely underestimating the number of individuals that will die from disease. It is possible that in latitudes where these species hibernate for under the amount of time it takes WNS to cause mortality, populations may not see much change. Moreover, it is possible that beyond a latitudinal threshold, populations of microchiropteran bats may not require the energetic savings of hibernation, hence would be safe from the effects of WNS. Studies looking at the hibernation behaviors of bats in southern edges of their ranges would help determine whether these latitudes can function as safe harbors from WNS. Otherwise, the disease is likely just as destructive across all populations, due to the energy trade-offs seen along a latitudinal gradient.

We have shown that in the presence of milder hibernation seasons, certain species of bats are still badly affected by WNS, and this trend may exist range-wide. Whether due to metabolic optimization or other factors, managers may not be able to rely on mildness of winters to sustain their hibernating bat populations. Continued study

of the effect of WNS on the physiology of bats at varying latitudinal gradients will help managers in regions as of yet affected by WNS know what to expect upon arrival of the disease, and help them to determine which habitats and hibernacula will need protection or improvement, such as restriction of access to caves harboring sensitive species. Deeper understanding of the effects on differing latitudinal scales would also help project the future status of these species and possibly help in prioritizing the use of funding sources. Early establishment of summer and winter baseline population data will be key in helping managers understand long-term trends and help to differentiate WNS-related declines from normal fluctuations within populations. This will also set an abundance goal to aim for as populations recover and gain resistance to WNS. As the disease continues to spread south, updating prediction models to fit the observed effects of WNS on populations across latitudes will help managers know what changes to expect within populations.

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LITERATURE CITED

- Alves, D.M.C.C., C.T. Levi, and D. Brito. 2014. The potential impact of white-nose syndrome on the conservation status of North American bats. *PloS One* 9(9): e107395.
- Arnett, E., and E. Baerwald. 2013. Impacts of wind energy development on bats: implications for conservation. *In* R. Adams and S. Peterson (Eds.), *Bat evolution, ecology, and conservation* (pp. 435–456). Springer, New York, USA.
- Bernard, R. F., and G.F. McCracken. 2017. Winter behavior of bats and the progression of white-nose syndrome in the southeastern United States. *Ecology and Evolution*, November 2016: 1–10.
- Britzke, E. R., M.J. Harvey, and S.C. Loeb. 2003. Indiana bat, *Myotis sodalis*, maternity roosts in the Southern United States. *Southeastern Naturalist*, 2(2): 235–242.
- Carr, J.A., R.F. Bernard, and W.H. Stiver. 2014. Unusual bat behavior during winter in Great Smoky Mountains National Park. *Southeastern Naturalist*, 13(2): N18–N21.
- Carroll, S. K., T.C. Carter, and G.A. Feldhamer. 2002. Placement of nets for bats : Effects on perceived fauna. *Southeastern Naturalist*, 1(2): 193–198.
- Dobony, C., A. Hicks, K. Langwig, R.I. von Linden, J.C. Okoniewski, and R.E. Rainbolt. 2011. Little brown myotis Persist Despite exposure to white-nose syndrome. *Journal of Fish and Wildlife Management* 2(2): 190–195.
- Dunbar, M.B., and R.M. Brigham. 2010. Thermoregulatory variation among populations of bats along a latitudinal gradient. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology* 180(6): 885–893.
- Estrada-Villegas, S., C. Meyer, and E. Kalko. 2010. Effects of tropical forest fragmentation on aerial insectivorous bats in a land-bridge island system. *Conservation Biology* 143: 597–608.
- Ethier, K., and L. Fahrig. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. *Landscape Ecology* 26(6): 865–876.
- Foley, J., D. Clifford, K. Castle, P. Cryan, and R.S. Ostfeld. 2011. Investigating and managing the rapid emergence of white-nose syndrome, a novel, fatal, infectious disease of hibernating bats. *Conservation Biology* 25(2):223–231.

- Ford, W.M., E.R. Britzke, C.A. Dobony, J.L. Rodrigue, and J.B. Johnson. 2011. Patterns of acoustical activity of bats prior to and following white-nose syndrome occurrence. *Journal of Fish and Wildlife Management* 2:125–134.
- Francl, K.E., W.M. Ford, D.W. Sparks, and V. Brack. 2012. Capture and reproductive trends in summer bat communities in West Virginia: Assessing the impact of white-nose syndrome. *Journal of Fish and Wildlife Management* 3(1): 33–42.
- Fraser, E.E., L.P. McGuire, J.L. Eger, F.J. Longstaffe, and M.B. Fenton. 2012. Evidence of latitudinal migration in tri-colored bats, *Perimyotis subflavus*. *PLoS ONE* 7(2).
- Frick, W.F., J.F. Pollock, A.C. Hicks, K.E. Langwig, D.S. Reynolds, G.G. Turner, C.M. Butchkoski, and T.H. Kunz. 2010. An emerging disease causes regional population collapse of a common North American bat species. *Science* 329:679–682.
- Frick, W.F., S.J. Puechmaille, J.R. Hoyt, B.A. Nickel, K.E. Langwig, J.T. Foster, and A.M. Kilpatrick. 2015. Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography* 24(7):741–749.
- Gorresen, P., and M. Willig. 2004. Landscape responses of bats to habitat fragmentation in Atlantic forest of Paraguay. *Journal of Mammalogy* 85(4):688–697.
- Hoying, K., and T.H. Kunz. 1998. Variation in size at birth and post-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). *Journal of Zoology* 245:15–27.
- Ingersoll, T.E., B.J. Sewall, and S.K. Amelon. 2013. Improved analysis of long-term monitoring data demonstrates marked regional declines of bat populations in the eastern United States. *PLoS ONE*, 8(6).
- IUCN 2017. The IUCN Red List of Threatened Species. Version 2017-1. <<http://www.iucnredlist.org>>. Downloaded on 12 May 2017.
- Johnson, S.A., V. Brack Jr., and R.E. Rolley. 1998. Overwinter weight loss of Indiana bats (*Myotis sodalis*) from hibernacula subject to human visitation. *American Midland Naturalist* 139: 255–261.
- Jones, G., D.S. Jacobs, T. H. Kunz, M.R. Willig, and P.A. Racey. 2009. Carpe noctem: the importance of bats as bioindicators. *Endangered Species Res.* 8: 93–115.

- Kunz, T., and A. Kurta. 1988. Capture methods and holding devices. *In* T. Kunz (Ed.), *Ecological and behavioral methods for the study of bats* (pp. 1–29). Smithsonian Inst. Press, Washington, D.C., USA.
- Langwig, K.E., W.F. Frick, J.T. Bried, A.C. Hicks, T.H. Kunz, and A.M. Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 15:1050-1057.
- Lorch J.M., C.U. Meteyer, M.J. Behr, J.G. Boyles, P.M. Cryan, A.C. Hicks, A.E. Ballmann, J.T.H. Coleman, D.N. Redell, D.M. Reeder, and D.S. Blehert. 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 480(7377): 376–378.
- Luck, G., L. Smallbone, C. Threlfall, and B. Law. 2013. Patterns in bat functional guilds across multiple urban centers in south-eastern Australia. *Landscape Ecology* 28: 455–469.
- Maher, S. P., A.M. Kramer, J.T. Pulliam, M.A. Zokan, S.E. Bowden, H.D. Barton, and J.M. Drake. 2012. Spread of white-nose syndrome on a network regulated by geography and climate. *Nature Communications* 3(1306): 1–8.
- Minnis, A. M., and D.L Lindner. (2013). Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology* 117(9): 638–649.
- US Fish and Wildlife Service (USFWS). 2012. North American bat death toll exceeds 5.5 million from white-nose syndrome. Retrieved from http://www.fws.gov/whitenosesyndrome/pdf/WNS_Mortality_2012_NR_FINAL.pdf
- O’Keefe, J. M., J.L. Pettit, and S.C. Loeb. 2016. White-nose syndrome extirpated one-quarter of the bat species in Great Smoky Mountains National Park. Abstract. *In* 46th Annual Symposium: North American Society for Bat Research (p. 56).
- Reichard, J. D., and T.H. Kunz. 2009. White-Nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). *Acta Chiropterologica*, 11(2): 457–464.

- Simon, S.A., T.K. Collins, G.L. Kauffman, W.H. McNab, and C.J. Ulrey. 2005. Ecological zones in the Southern Appalachians : First Approximation.
- Thogmartin, W.E., C.A. Sanders-Reed, J.A. Szymanski, P.C. McKann, L. Pruitt, R.A. King, M.C. Runge, and R.E. Russell. 2013. White-nose syndrome is likely to extirpate the endangered Indiana bat over large parts of its range. *Biological Conservation* 160:162-172.
- Turner, G. G., D.M. Reeder, and J.T.H. Coleman. 2011. A five-year assessment of mortality and geographic spread of White-nose Syndrome in North American bats and a look to the future. *Bat Research News* 52(2): 13–27.
- Turner, G. G., C.U. Meteyer, H. Barton, J.F. Gumbs, D.M. Reeder, B. Overton, and D.S. Blehert. 2014. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases* 50(3): 566–573.
- US Fish and Wildlife Service (USFWS). 1999. Agency Draft: Indiana bat (*Myotis sodalis*) revised recovery Plan. USFWS, Ft. Snelling, Minnesota, (March), 53. Retrieved from [http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:AGENCY+DRAFT+INDIANA+BAT+\(+Myotis+sodalis+\)+REVISED+Recovery+Plan#0](http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:AGENCY+DRAFT+INDIANA+BAT+(+Myotis+sodalis+)+REVISED+Recovery+Plan#0)
- US Fish and Wildlife Service (USFWS). 2012. North American bat death toll exceeds 5.5 million from white-nose syndrome. News Release, U.S. Fish and Wildlife Service, Arlington, Virginia.
- USFWS. 2016. Indiana Bat (*Myotis sodalis*): Bat Survey Protocol for Assessing Use of Potential Hibernacula. https://www.fws.gov/midwest/endangered/mammals/inba/inba_srvyprtcl.html
- Verant, M.L., C.U. Meteyer, J.R. Speakman, J.M. Lorch, D.S. Blehert. 2014. White-nose syndrome initiates a cascade of physiological disturbances in the hibernating at host. *BMC Physiology* 14:10.
- Nolfi, D.C. 2011. National Park Service cave and karst resources management case study: Great Smoky Mountains National Park. Masters Theses & Specialist Projects. Paper 1053. <http://digitalcommons.wku.edu/theses/1053>

Voigt, C.C., and T. Kingston. 2016. Bats in the Anthropocene: conservation of bats in a changing world. *Bats in the anthropocene: Conservation of bats in a changing world*.

APPENDIX 2

Tables

Table 2.1 Number of bats captured by age and sex, over 48,818 net*hours in 85 nights during summer 2015–2016, in Great Smoky Mountains National Park, USA.

	Female		Male		Unknown	Total
Species	Adult	Juvenile	Adult	Juvenile		
<i>Eptesicus fuscus</i>	32	8	62	9	2	113
<i>Lasiurus borealis</i>	13	2	83	2	2	102
<i>Lasionycterus noctivagans</i>	0	0	31	0	0	31
<i>Perimyotis subflavus</i>	2	1	24	0	2	27
<i>Corynorhinus rafinesquii</i>	8	1	6	1	0	16
<i>Nycticeus humeralis</i>	0	0	12	0	0	12
<i>Myotis leibei</i>	2	1	6	1	0	10
<i>Lasiurus cinereus</i>	2	0	7	0	0	9
<i>Myotis sodalis</i>	1	0	7	0	0	8
<i>Myotis lucifugus</i>	2	0	3	0	0	5
<i>Myotis grisescens</i>	0	0	2	1	0	3
<i>Myotis septentrionalis</i>	1	0	0	0	0	1
Total	63	13	243	14	6	339

Table 2.2 Number of bats captured by age and sex, over 37,382 net*hours in 60 nights during summer 1999–2004, in Great Smoky Mountains National Park, USA.

	Female		Male		Unknown	Total
Species	Adult	Juvenile	Adult	Juvenile		
<i>Eptesicus fuscus</i>	23	2	30	0	2	57
<i>Lasiurus borealis</i>	3	0	73	5	0	81
<i>Lasionycterus noctivagans</i>	0	0	24	0	0	24
<i>Perimyotis subflavus</i>	1	0	82	2	1	86
<i>Corynorhinus rafinesquii</i>	2	0	0	0	0	2
<i>Nycticeus humeralis</i>	0	0	3	0	0	3
<i>Myotis leibei</i>	0	0	0	0	0	0
<i>Lasiurus cinereus</i>	0	0	13	0	0	13
<i>Myotis sodalis</i>	9	2	6	3	0	20
<i>Myotis lucifugus</i>	148	31	46	26	1	252
<i>Myotis grisescens</i>	0	0	0	0	0	0
<i>Myotis septentrionalis</i>	37	0	52	1	0	90
Total	223	35	329	37	4	628

Table 2.3 Summer capture rates of bat species in number of captures/ net m²/ hour between pre- and post-WNS years (1999–2004 and 2015–2016, respectively), and comparison with Mann-Whitney U test, in the Great Smoky Mountains National Park, USA.

Species	1999–2004	2015–2016	P	% Change
<i>Myotis lucifugus</i>	0.006741	0.000102	9.76E-10	-98.5
<i>Myotis septentrionalis</i>	0.002408	2.05E-05	2.30E-06	-99.2
<i>Perimyotis subflavus</i>	0.002301	0.000553	1.86E-07	-76.0
<i>Lasiurus borealis</i>	0.002167	0.002048	0.450	-5.5
<i>Eptesicus fuscus</i>	0.001525	0.002274	0.265	49.1
<i>Myotis sodalis</i>	0.000535	0.000164	0.001	-69.4
<i>Lasionycterus noctivagans</i>	0.000642	0.000635	0.656	-1.1
<i>Lasiurus cinereus</i>	0.000348	0.000184	0.073	-47.0
<i>Corynorhinus rafinesquii</i>	5.35E-05	0.000328	0.069	512.6
<i>Nycticeus humeralis</i>	8.03E-05	0.000246	0.238	206.3
<i>Myotis leibei</i>	0	0.000205	0.010	-
<i>Myotis grisescens</i>	0	6.15E-05	0.237	-
Effort hours	37,382	48,818		
No. nights	60	85		

Table 2.4 Fisher's exact test showing the change in age and sex ratios of bat species between pre- and post-WNS years (1999-2004 and 2015-2016, respectively), in Great Smoky Mountains National Park, USA.

Species	Sex	Age
<i>Eptesicus fuscus</i>	0.3108	0.03584
<i>Lasiurus borealis</i>	0.01242	0.5165
<i>Lasionycterus noctivagans</i>	1	1
<i>Perimyotis subflavus</i>	0.04286	0.5666
<i>Corynorhinus rafinesquii</i>	0.4967	1
<i>Nycticeus humeralis</i>	1	1
<i>Myotis leibei</i>	1	1
<i>Lasiurus cinereus</i>	0.1558	1
<i>Myotis sodalis</i>	0.08822	0.2808
<i>Myotis lucifugus</i>	0.1518	0.5898
<i>Myotis grisescens</i>	1	1
<i>Myotis septentrionalis</i>	0.4176	1

Table 2.5 Number of tri-colored bats in cave hibernacula counted during pre- and post-WNS winters (2009 and 2015–2016, respectively) in the Great Smoky Mountains National Park, USA.

Site Name	Pre-/Post-WNS Count Year	Pre-WNS Count	Post-WNS Count	% Change
Gregory's Cave	(2009/2015)	1365	33	-97.5
Scott's Gap Cave	(2009/2015)	384	18	-95.3
Rainbow Cave	(2009/2016)	350	21	-94.0
Saltpeper Cave	(2009/2015)	216	6	-97.2
Kelly Ridge Cave	(2009/2015)	149	17	-88.6
Snake Dance	(2009/2016)	50	14	-72.0
Bull Cave	(2009/2016)	25	17	-32.0
Rich Mountain Blowhole	(2009/2016)	12	1	-91.7
Hazel Creek	(2009/2015)	2	0	-100.0
White Oak Blowhole	(2009/2015)	0	18	-

Table 2.6 Number of Rafinesque's big-eared bats in cave hibernacula counted during pre- and post-WNS winters (2009 and 2015–2016, respectively) in the Great Smoky Mountains National Park, USA.

Site Name	Pre-/Post-WNS Count Year	Pre-WNS Count	Post-WNS Count	% Change
Eagle Creek	(2009/2015)	845	312	-63.1
Hazel Creek	(2009/2015)	440	248	-43.6
Kelly Ridge Cave	(2009/2015)	350	67	-80.9

Table 2.7 Number of little brown bats in cave hibernacula counted during pre- and post-WNS winters (2009 and 2015–2016, respectively) in the Great Smoky Mountains National Park, USA.

Site Name	Pre-/Post-WNS Count Year	Pre-WNS Count	Post-WNS Count	% Change
Kelly Ridge Cave	(2009/2015)	996	16	-98.4
Bull Cave	(2009/2016)	236	3	-98.7
Rainbow Cave	(2009/2016)	127	3	-97.6
Scott's Gap Cave	(2009/2015)	165	3	-98.2
White Oak Blowhole	(2009/2015)	1038	20	-98.1

Table 2.8 Number of Indiana bats in cave hibernacula counted during pre- and post-WNS winters (2009 and 2015–2016, respectively) in the Great Smoky Mountains National Park, USA.

Site Name	Pre-/Post-WNS Count Year	Pre-WNS Count	Post-WNS Count	% Change
Kelly Ridge Cave	(2009/2015)	904	188	-79.2
Bull Cave	(2009/2016)	2097	140	-93.3
Scott's Gap Cave	(2009/2014)	40	36	-10.0
White Oak Blowhole	(2009/2015)	7983	1117	-86.0

Figures

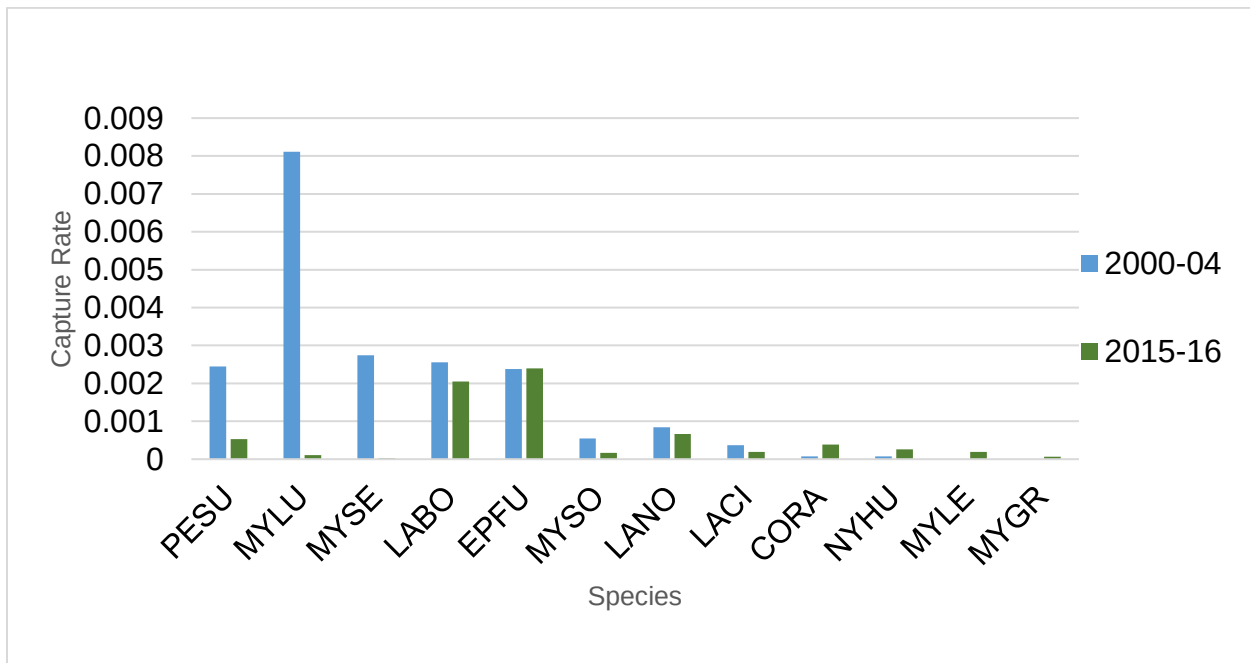


Figure 2.1 Percent change in summer capture rate of bats pre- and post-WNS (1999–2004 and 2015–2016 respectively) in Great Smoky Mountains National Park, USA.

Netting Protocol

Procedure:

Netting will be conducted at each site twice throughout the season

- We will use a combination of mist nets (single, double, and triple-high) and harp traps at each site
 - Nets will be checked every 10–15 minutes; harp traps will be checked every 5–10 minutes
- Captured bats will be placed individually in cloth or paper bags and held for 30–60 minutes to ensure defecation
 - Note: Pregnant females will be processed immediately and released to minimize disturbance
 - Data collection may include the following: forearm and weight measurements; fecal, tissue, hair or swap sample collection; reproductive condition (pregnant, lactating, post-lactating, scrotal), age, parasite load, WDI, and overall body condition
 - Bats will be banded with UTK# 2.9 narrow Porzana bands and released at the point of capture
 - Bats with forearm <30mm (PESU & MYLE) will be banded with 2.4 narrow Porzana bands or marked with permanent marker on the elbow.
 - LABO and LACI have a tendency to break forearms easily, thus banding them is not suggested
 - In the event of injury to a bat while in captivity, Isoflurane or cervical dislocation will be used by individuals listed on the federal permit

**3. ROOST SELECTION BY TRI-COLORED BATS (*PERIMYOTIS SUBFLAVUS*) IN
THE GREAT SMOKY MOUNTAINS NATIONAL PARK**

ABSTRACT

The tri-colored (*Perimyotis subflavus*) bat was once common across its range but due to the arrival of white-nose syndrome populations have declined significantly, such that tri-colored bats have been recommended for federal listing under the Endangered Species Act. A key component of tri-colored bat habitat is summer roosts, however, not much is known about roost requirements for the species in the southeastern region. We investigated roost selection of tri-colored bats at the micro- and landscape levels within the Great Smoky Mountains National Park to characterize roost selection for the species within the region. On a microhabitat level, we found that male tri-colored bats selected for taller trees with greater canopy volumes than were proportionately available, and for forest stands that contained fewer overstory trees and fewer overstory conifers, than the surrounding forest. On a landscape level, we saw selection for roost locations that were closer to roads; had lower elevations, shallower slopes, and more north-facing aspects than other areas of GSMNP. As GSMNP contains a uniquely diverse suite of forest types and microclimates, the characteristics identified in our study likely represent true preference for tri-colored bats in the Southern Appalachian region.

INTRODUCTION

The discovery of white-nose syndrome (WNS) in North America in 2006 (Blehert et al., 2009) marked the beginning of significant declines in the populations of numerous cave-hibernating bat species. These declines have led to the federal listing of the northern long-eared bat (*Myotis septentrionalis*) (USFWS, 2016) and a petition for listing of the tri-colored bat (*Perimyotis subflavus*; Center for Biological Diversity and Defenders of Wildlife 2016). The disease, caused by the fungus *Pseudogymnoascus destructans*, disrupts physiology during winter torpor, leading to morbidity and death (Verant et al., 2014). Having spread unchecked across the eastern U.S. and into the midwestern and western states, WNS is associated with hibernacula mortality rates as high as 98%. The disease has proved particularly deadly to little brown (*M. lucifugus*), Indiana (*M. sodalis*), northern long-eared, and tri-colored bats (Turner et al 2011) and threatens all four species with regional extirpation (Frick et al. 2010; Langwig et al. 2012; Thogmartin et al. 2013; Alves et al 2014; Frick et al. 2015).

With little advance in treatments for WNS, and logistical constraints on administration of control compounds, protection of summer forest habitats needed for successful recruitment is likely to be critical to species conservation. Supporting spring survival and summer reproduction by WNS infected bats through habitat protection and enhancement may promote species recovery and support or accelerate the evolution of resistance to *P. destructans* in remnant populations (Willis et al., 2016, abstract). Roosts are a key component in spring survival and the summer recruitment process, as optimal selection of roost site affords protection from weather, safety from predators for both adults and young, and energetic benefits such as thermoregulatory support and

reduced commuting costs to resources (Barclay and Kurta, 2007). Additionally, increased density of roosting locations may lead to an increase in bat abundance, where carrying capacity is only reached at very high roost densities (Hayes and Loeb 2007), hence, modification of the landscape to include increased numbers of suitable roosting structures may be an effective method of increasing a population's size.

Many studies on roost requirements for North American bat species focus on endangered species and their congeners, but less is known about species with historically stable populations, such as the tri-colored bat. Though populations of this species are rapidly declining and it has been petitioned for federal listing, very little is known about its roost selection. However, such information may be important in population recovery. The majority of published accounts of tri-colored bat summer roost selection are anecdotal in nature (*lichen*, Poissant et al. 2010; *basal cavity of sweet gum tree*, Menzel 1996; *Spanish moss*, Davis & Mumford, 1962; Menzel et al. 1999; *cavity roosts in SC*, Carter et al. 1999), and report of maternity colonies in man-made structures and caves (*house* Allen, 1921; *barns* Lane 1946, Poole 1938; *attic in garage* Golley 1966; Cope et al. 1961; Jones & Pagels, 1968; Jones & Suttikus, 1973; Whitaker Jr., 1998; Winchell & Kunz, 1996; Humphrey 1975). Studies focusing on their selection of forest roosts are limited to sites in Indiana (Veilleux et al. 2003; Veilleux & Veilleux, 2004) and the Ouachita Mountains of Arkansas (Perry et al. 2007; Perry & Thill, 2007; Perry et al. 2008), with two studies of limited sample size conducted in North Carolina (O'Keefe et al. 2009; n = 7) and South Carolina (Leput 2004; n = 4). These studies offer insight to the usage of local flora, but availability of roosting structures varies across the species' range, hence, roost selection likely varies as well. As conservation of the

species will likely be of interest range-wide, identification of these locally preferred habitat features will be important.

Our objective was to characterize roost selection by tri-colored bats at the microhabitat and landscape levels in the Great Smoky Mountains National Park, a portion of their range that remains largely uninvestigated. As studies of other microchiropteran bats have seen selection for warmer locations was important, we hypothesized that tri-colored bats would select roosts at the microhabitat level with greater thermoregulatory potential (McNab 1969). As more energy is required to maneuver through dense vegetation (Menzel et al. 2005), we also hypothesized that individuals would select roosts that require less effort to access, i.e., contain less structural complexity in adjacent midstory and canopy, than what is proportionally available. Similarly, bats may chose roosts across the landscape that receive greater amounts of solar radiation (Willis & Brigham, 2005) and are closer in proximity to limiting resources, such as water and linear corridors that may serve as foraging areas, than random sites (Kalcounis-Ruppell et al 2005; Carter & Feldhamer, 2005; Broders et al. 2006; Timpone et al. 2010). Studies looking at similarly-sized bats demonstrated that proximity to viable drinking water sources, and density of such hydrologic features may influence roost selection (Lacki & Schwierjohann, 2001; O'Keefe et al. 2009; Pauli et al. 2015). Hence, we hypothesized that bats would select roosts in locations on the landscape with increased thermoregulatory benefits and located close to important resources.

METHODS

Study Site

We conducted our study in Great Smoky Mountains National Park (GSMNP) a 1300 km² area encompassing portions of Cocke, Sevier, and Blount counties in Tennessee, and Swain and Haywood counties in North Carolina. The National Park ranges in elevation from 267–2025 m, and is 80% forested with two primary forest types; spruce-fir above 1,370 m, and oak at lower elevations. Many areas of the park have man-made structures and high visitor traffic. Other portions of the park include horse pasture, fields, and grassy balds, with numerous rivers and streams throughout. The park receives between 1.4–2.2 m of rain per year and low elevation summer temperatures normally range from 16–32° C.

Radiotelemetry

From mid-May to early August 2015 and 2016, we determined the roosting locations of tri-colored bats using radio telemetry. To capture bats to which we could attach radio transmitters, we conducted mist netting at 24 sites across GSMNP. We netted bats using 6, 9, or 12 m long mist nets in various single, double, and triple high arrangements (75 denier, 2-ply; Avinet, Dryden, New York), for five hours beginning 30 minutes before sunset, and discontinuing for rain or strong winds. We primarily placed nets over water or in forest corridors, and checked for captures every ten minutes. We recorded species, sex, and age for each bat captured using protocols outlined in Kunz (1988). We also recorded reproductive status, mass, forearm length, and wing damage index (Reichard and Kunz 2009). We fitted all tri-colored bats with a transmitter (BD-2X, Holohil Systems, Ltd.; Ontario, Canada) weighing 0.26 – 0.35 g, not exceeding 5% body

weight (Kurta & Murray, 2002), by trimming a small amount of fur from between the scapulae, applying directly to the skin a small amount of surgical glue (Perma-Type, Plainville, CT), and holding for 5 –10 minutes to ensure glue had dried (Appendix 3). We used handling and transmitter application procedures consistent with guidelines of the American Society of Mammalogists (Sikes *et al.* 2016). Our methods were approved by the University of Tennessee Animal Care and Use Committee (IACUC 2253) and research was conducted under state and federal scientific collection and recovery permits (US Fish and Wildlife Service, TE353135-3; National Park Service, GRSM-01228; Tennessee Wildlife Resources Agency, 3742).

After release, we tracked all bats for the duration of the transmitter battery life (14–21 days), or until transmitters fell off. We used 3- and 5-element Yagi antennae and Telonics TR-5 receivers (Telonics, Inc., Mesa, AZ) to track bats to their roosts during the day by vehicle and on foot. Once a roost tree was found, we determined roost location within the canopy using telemetry signals to approximate the bat's position, and confirmed the position using visual detection whenever possible.

Microhabitat characterization

To determine roost site preference of tri-colored bats at the microhabitat level, we compared characteristics of selected roosts and surrounding 0.1 ha plots to those of a random, available but unused tree from within the same forest stand (James and Shugart 1970). We selected random trees for microhabitat-level comparison by pairing a random distance 40–100 m away from the roost tree with a random azimuth 0–360°, and locating the nearest stem ≥ 10 cm diameter at breast height (dbh) to this point. The minimum distance of 40 m ensured that measurements within 0.1 ha circular roost and

random plots did not overlap, and the maximum distance of 100 m ensured that roost and random trees were located within the same forest stand.

For each roost tree located, we recorded tree species, the substrate the bat was found roosting on (e.g. bark, live leaves, dead leaves), roost height (m), and roost aspect (compass bearing [°] in the direction of the roost extending away from the base of the tree). Additionally, we recorded qualitative data, including ground substrate and dominant groundcover plant species, to aid in characterization of habitat. We measured 12 total characteristics of roosts and corresponding random trees suggested to be important for tri-colored bat selection (Table 4.1; Veilleux et al. 2003; Yates & Muzika 2006; Perry and Thill 2007; O’Keefe et al. 2009).

It has been suggested that the size of a tree and those around it may affect the ability of a bat to successfully thermoregulate within its roost (Perry and Thill 2007; Menzel et al. 2002; Yates and Muzika 2006). Hence, we measured six variables that may provide thermoregulatory benefits, including: tree dbh (cm); tree height (m); crown volume index (m^3), estimated as the width of the roost tree crown in two dimensions taken 90° apart and multiplied by the total canopy height; percent canopy closure, measured as the mean of 16 spherical densiometer (Model-C, Forest Densiometers, Rapid City, SD) readings taken at sampling locations 0, 5, 10, and 15 m away from the center point of the roost tree in each of the cardinal directions; percent canopy closure in the 90° quadrant (e.g. NE, SE, SW, NW) radiating away from the trunk of the tree that contained the roost; and average height of the forest stand (m), recorded as the mean height of four trees visually representative of the 0.1 ha plot. All height measurements were taken using a Suunto PM5 clinometer (Suunto, Helsinki, Finland).

Complexity of canopy structure and surrounding forest may create obstacles, and affect the ease of access to roosts or foraging areas (Yates and Muzika 2006). Hence we measured six variables that reflected the complexity of the forest structure, including: height to the base of the roost tree's crown (m); distance to the nearest overstory tree with dbh ≥ 10 cm (m); basal area, derived from stand dbh measurements; number of midstory trees (clutter), saplings, shrubs and woody vines taller than 1.4 m and ≥ 5 cm dbh; number of overstory trees ≥ 10 cm dbh; and number of coniferous softwood trees in the overstory, as many species of conifer have dense growth from the ground up, and may create more midstory clutter than other species.

Landscape Characterization

We also measured landscape variables to determine importance of various features to tri-colored bat roost tree selection. We used a handheld GPS (Garmin International, Olathe, KS, USA) to determine locations of roost trees, and ArcMap 10.3 (ESRI, Inc., Redlands, CA), along with shapefiles from the United States Geological Survey National Hydrography Dataset (nhd.usgs.gov), National Land Cover Dataset (Homer et al. 2015) and the National Parks Service (nps.gov) to create maps of potential selection variables, including hydrological features, roads, forest, and other land cover types. To determine roost preference of tri-colored bats at the landscape level, we compared characteristics of each selected landscape location to those of a random, unused location within GSMNP. We selected random landscape coordinates for comparison from a matrix of the forest types within GSMNP (nps.gov) that tri-colored bats used during our study (Miles et al. 2006). To ensure that random landscape locations were available to bats, points were located within 1 km of roosts. This roughly

represents the distance that a tricolored bat may fly in a given night, as calculated from the average maximum distances flown between roosts or sites of capture by bats in our study (880 m), and those of another study (1,137 m; Krishon et al. 1997). We used a presence-only modeling technique to investigate the selection of landscape characteristics. Roost searching requires considerable man hours and while we could confirm that random landscape points were not used by tagged bats, we could not determine if they were used by untagged bats (Foster & Kurta, 1999; Menzel et al., 2002; Bellamy et al. 2013).

As landscape position can affect a bat's need for thermoregulation through varying amounts of solar radiation, we measured three landscape variables that may affect temperature within a roost plot, including: elevation, slope and aspect of the hillside containing the roost, all determined using ArcGIS (Table 4.2). We transformed aspect degrees to continuous linear measurements using a cosine transformation. As distance to resources has been seen to be important in roost selection, we measured groups of variables that may be important in reducing commuting costs, including distances from roosts and random points to nearest water feature (stream polyline or water body) and nearest roads (Carter et al. 2002). Within a 1 km buffer we calculated the total area of forest, length of forest edge, total area of water bodies and total length of streams. We designated forest edges as any locations where forest pixels touched non-forest pixels, and defined water sources for bats as any stream center lines or water bodies greater than 3 m in width.

Data Analysis

For each radio-tagged bat, we detailed the number of days tracked, number of trees used, and, for trees to which bats returned repeatedly, number of days used. As tri-colored bats are known to switch day roosts often, we assumed that all day roosts selected by individuals were independent of each other, hence we included all observations as experimental units (Lewis 1995). We used a Fisher's exact test to examine selection of roost tree species compared to available overstory trees, and a Chi-square goodness of fit test to determine if bats over- or underused any polar quadrant of a tree (NE, SE, NW, SW), assuming equal usage of each direction. We calculated mean characteristics of all identified roost trees, and used an information theoretic approach to determine characteristics of roost trees that best predicted bat presence.

Step-wise regression and best subsets have been used in previous studies to determine habitat features that best predict roost selection of tri-colored bats (Perry and Thill 2007, O'Keefe et al. 2009). These types of null hypothesis model selection methods are dubious because the arbitrary alpha levels (e.g., $P = 0.01$ or $P = 0.05$) used to add or remove variables from models could preclude habitat features that are biologically significant to roost selection. Since biologically important variables could be removed from models with even a high alpha level (e.g., $P = 0.15$ or 0.20), an information-theoretic approach using Akaike information criterion (AIC) has been recommended instead of null hypothesis testing (Anderson et al. 2000; Burnham and Anderson 2002). Following with this method, we developed *a priori* linear regression models for the microhabitat and landscape-levels, which we used to investigate the

differences between selected and available microhabitat and landscape-level variables (e.g., tree species, dbh, height, basal area etc.). We tested these models using AIC_c, a variation of AIC used to assess small sample sizes (Anderson et al. 2002). We created models using measured microhabitat variables and landscape variables (Table 3.1; Table 3.2). We tested variables for correlation, and none were highly correlated (Pearson $r > 0.70$) except for percent canopy closure within the 5 m roost quadrant and total canopy closure of the plot. Hence, we dropped roost quadrant closure in our models, as closure around the plot has been seen to be an important roost selection factor in studies of similar bat species (Willis and Brigham 2005). We included variables in our models that had a very high alpha value ($P > 0.35$) to ensure that biologically significant variables were not removed. This resulted in the removal of the stand basal area, distance to nearest water, length of streams and forest edge, and area of forest and water variables. After we removed the one highly correlated variable and insignificant variables, we were left with 10 variables in our microhabitat global model: dbh, tree height, distance to nearest overstory tree, height to the base of crown, crown volumes, average height of plot, number of stems in midstory, percent canopy closure, number of overstory trees, and number of softwoods in the overstory (Table 3.1). There were four variables in our landscape global model: elevation, slope, aspect, and nearest road (Table 3.2).

We created groupings of models to explain microhabitat and landscape-level selection based on our hypotheses (Appendix 3). The microhabitat model suite was broken into two groups of 18 models, one group containing five variables we hypothesized to be important in thermoregulation, and the other group including 5

variables we hypothesized were important for ease of access to roosts and foraging areas. The landscape-level selection suite contained twenty two models, including the four variables we hypothesized to important for selection of a position on the landscape. Using AICc scores and model weights (w_i ; Burnham and Anderson 2002), we examined differences between candidate models, with best models represented by lowest scores and highest weights. AIC weight can be interpreted as the probability that a candidate model is the best among potential models. Parameter estimates of variables from the top model, and models within 2 Delta AIC units, were averaged using the full model averaging approach (Burnham and Anderson 2002). We carried out all statistical tests using RStudio 1.0.136 (R Core Team 2017).

RESULTS

Radiotracking

Over the two summers, we captured 26 tri-colored bats (7.83% of all bat captures) during 85 net-nights. Eighty-eight percent of tri-colored bat captures were males ($n = 23$) and 12% were females ($n = 3$). We placed transmitters on all individuals, plus an additional three adult males captured by another research team within the study area, totaling 29 tri-colored bats: 25 adult males, one juvenile male, two post-lactating females, and one juvenile female. Eleven males and two females were not detected following release, including both juvenile bats. Among the remaining 16 bats (15 adult male, 5 in 2015 and 9 in 2016, and 1 adult female in 2016), we located 67 total roost trees, 66 for male bats and one for a single female bat. Due to small sample size, we only analyzed roost tree data for male bats. Radiotelemetry signals indicated all bats

roosted in the foliage of live trees (i.e., no evidence to suggest bats were roosting in cavities of dead or damaged trees), which we confirmed visually for five roosts. Number of roosts per bat was 1 – 9 (4.2 ± 2.2). Of the bats we tracked on multiple days, 5 individuals used a single roost tree twice, one individual used two different roost trees twice each, one individual used a single roost tree 4 times, and another used a single roost tree 5 times. Bats flew an average of 885 m to roosts from sites of capture with a maximum of 20 km and a minimum of 57 m.

Microhabitat Characterization

We found all bats roosting in live trees. We found 65.2% of roosts in live foliage, and only 27.3% in dead foliage. For the remaining 7.5 of roosts, we were unable to determine whether roosts were in live or dead vegetation. We found bats tended to overuse roosts on NE- and SE-facing branches and underuse NW- and SW-facing branches ($\chi^2 = 11.459$, $df = 3$, $p = 0.009$), using east-facing branches 70.5% of the time. A majority of roosts were located in live oaks (28.4%; *Quercus*), yellow-poplars (28.4%; *Liriodendron tulipifera*), and maples (17.9%; *Acer spp.*). Usage of these tree species was proportionally greater than other available tree types: softwoods, other hardwoods, and snags ($P < 0.001$; Table 3.3).

Male tri-colored bats tended to use trees that were taller, larger in diameter and with greater crown volume than random, and they roosted on average 16.7 m from the ground. They roosted in plots that tended to have fewer trees than random plots, though basal area did not differ between roost and random plots ($P = 0.859$, Table 3.4).

Of the 36 total microhabitat candidate models tested, three were within 2 AIC_c units of the top model, indicating that these models were the best at differentiating roost

trees from random trees. The top model was from the clutter group of variables and contained total density, clutter, density of soft woods, distance to nearest overstory tree, and height to base of crown, while the second two most important models were from the thermoregulation group and contained the variables canopy volume, and tree height. The sum of the AIC_c weights for these three models was 0.544, meaning that there is a 54.4% chance that they are the true predicting models of the response variable (Table 3.5).

Within top models for the clutter group, the total number of overstory trees, total number of overstory softwoods, distance to nearest overstory tree, and height to base of canopy were the most informative variables, with confidence intervals not crossing 0. Within the thermoregulation group, both roost tree height and canopy volume were informative. Estimated odds ratios showed that for every one unit increase, odds of bat presence in a roost tree would decrease by 5.8% for each additional overstory tree, 7.7% for each additional overstory softwood tree, 36.2% for each additional meter to nearest overstory tree, and would increase by 15% for each meter increase in roost tree height, 9.4% for each increase in height to base of canopy, 0.01% for each increase in canopy volume. Selected plots had 12.1% fewer overstory trees and 46.2% fewer overstory softwoods than random, and roost trees were 19.7% closer to nearby overstory trees, and 24.2% taller than random (Table 3.6).

Landscape Characterization

We frequently found roosts in oak-hickory, successional hardwood, cove, yellow-pine, or floodplain forests with streams or wetlands located within or adjacent to plots. Groundcover in all plots contained 10–30% herbaceous plants and small saplings that

generally reflected overstory species, with remaining cover comprising leaf litter and bare ground. Several roosts were located near roads with heavy traffic during daylight hours. Distances from roosts to water sources were not significantly different from random landscape location, and there was no difference in forest edge, total length of streams, area of water, or area of forest within 1 km buffers than random landscape locations. Roost plots were located on gentler grades than random, were more north-facing, and were significantly lower in elevation. Additionally, a majority of roosts were located on west-facing aspects, while random plots showed no pattern (Table 3.7).

Of the nine landscape level variables we measured, we used four in candidate models investigating selection at the landscape scale (Table 3.8). Of the 22 total candidate models tested, one model was within 2 AIC units of the top model, and another four were within 4 AICc units of the top model, indicating that these may help to differentiate roost sites from random points on the landscape. The top model for the landscape-level selection group (land10) contained the distance to road and cosine aspect variables, of which distance to road was the only informative variable. The average distance from road to roost was 185.9 m, and 95% of the roosts within the sample were located within 590.6 m of a road. Estimated odds ratios showed that for every one meter distance increase from a road, odds of bat presence in a roost location would decrease by 0.4% (Table 3.9). Other models within four AIC units of the top model included elevation and slope variables, and all contained distance to road.

DISCUSSION

Reflecting the extreme ratio of males to females and adults to juveniles captured during our study, all but one of the roosts located belonged to male tri-colored bats, with only one located for a female and none located for juveniles. We found that male tri-colored bats generally roosted in oak-hickory, successional hardwood, cove, yellow-pine, or floodplain forests within or adjacent to riparian zones, often characterized by 10–30% groundcover, though this was generally uninformative as roost and random plots had similar groundcover composition. Roosts occurred at varying heights throughout roost tree crowns, with a majority found near the center and top. Fidelity to a specific tree was low, but each individuals' roosts were primarily located in one forest stand, which is a trend that has been commonly observed in tri-colored bats (Fujita and Kunz 1984, Veilleux et al. 2003).

Microhabitat characterization

Most roosts were primarily in live vegetation of live trees, similar to findings of more northern and western populations of tri-colored bats (Perry et al 2008; Perry et al 2007; Perry & Thill 2007; Veilleux & Veilleux 2004; Veilleux et al 2003). We found tri-colored bats using east-facing branches for roosts more often than west-facing branches, possibly to maximize collection of solar radiation, as was seen in other foliage roosting species in Saskatchewan, Canada (Wills and Brigham 2005).

A preference for *Quercus* species appeared similar to findings of previous studies where male tri-colored bats used them 27–87% of the time, while the group made up only 3.1–8% of available stems (Perry and Thill 2007; Veilleux et al. 2003). We saw tri-colored bats use *Acer* species more often than was expected, similar to one study in

southwestern Indiana (Veilleux et al. 2003). Other studies saw under-utilization of *Liriodendron tulipifera* (O'Keefe et al. 2009; Perry and Thill 2007; Veilleux et al. 2003) and *Magnolia spp* (O'Keefe et al. 2009) compared to available proportions, however male tri-colored bats in our study used both species in greater proportions than available, suggesting selection for these tree species.

A reduction in the density of overstory trees, overstory conifers, increased height to the base of the canopy, and a reduced distance to nearest overstory tree characterized roost selection of tri-colored bats at the microhabitat scale. Previous studies of the species have shown disparity in preference for density of overstory hardwoods and softwoods, with some individuals appearing to select for roosts in stands of greater mid- and overstory density than the surrounding forest (Perry and Thill 2007), and others selecting for low density (Yates and Muzika 2006). The species has been seen to inhabit dense forest (Menzel et al. 2005), though testing at finer scales suggests that they may be trending towards staying within forest gaps (Loeb and O'Keefe 2006). While individuals in our study selected for fewer overstory trees than available patches, the number of trees within selected plots was similar to numbers found in other studies. While tri-colored bats have shown adaptability to dense forests, our roost and random plots were generally more densely forested than those of other studies. Additionally, many of the conifers within random plots in our study were Eastern hemlocks (*Tsuga canadensis*), a species with very dense branching from the ground up. This may have additionally pushed the clutter density beyond the species' tolerance.

While clutter-related variables populated the strongest model predicting male tri-colored bat presence, variables hypothesized to enhance thermoregulation budgets appeared in strong models as well, tree height and canopy volume being the most informative among them. Male tri-colored bats used taller trees with larger canopies than were available proportionally, which is consistent with findings for the species in other studies (Veilleux et al 2003; Veilleux & Veilleux 2004; Perry & Thill 2007; Perry et al. 2007; Perry et al 2008), and has been generally seen for most species of bats (Kalcounis-Ruppell et al. 2005). Roosts in tall trees with large canopies are advantageous for thermoregulation as they receive more solar radiation while allowing some protection from wind, and may additionally afford protection from terrestrial predators (Vonhof and Barclay 1996; Ormsbee and McComb 1998; Vonhof and Barclay 1997; Elmore et al. 2004).

Landscape characterization

Roost selection at the landscape scale was characterized by proximity to roads. Our findings are similar to those seen in studies where selected roosts of tri-colored and other species of bats were located in close proximity to linear corridors such as roads or trails that may serve as foraging areas or commuting paths (Elmore et al. 2005; Menzel et al. 2005; O'Keefe et al. 2009; Pauli et al. 2015). Other studies have shown avoidance of roads by bats, suggesting that noise disturbance can work as a deterrent (Zurcher et al. 2010; Bennett and Zurcher 2013; Bennett et al. 2013). However, as roads within GSMNP have generally low levels of traffic during night hours or are closed to traffic, they likely do not present such cause for avoidance. While we found that selection for sites on the landscape that decrease commuting costs for bats were the

most important in predicting presence, we found that elevation, aspect of the hillside containing the roost plot, and slope of the roost plot were likely informative as well. Lower elevations and shallower slopes have been seen to afford bats with greater protection from winds (Willis and Brigham 2005; Yates and Muzika 2006), which supports our findings. Other studies have shown that microchiropteran bats, such as hoary bats in Saskatchewan, often select positions that receive increased amounts of sunlight, such as south-facing aspects (Willis and Brigham 2005). This contradicts our finding, as male tri-colored bats in GSMNP tended to select for north-facing slopes. Because GSMNP is located in a more southern latitude, aspect may be more important for protection from south and westerly winds, rather than selection for increased solar radiation.

Successful management of wildlife requires knowledge of preferred habitat, and looking at selection of factors on multiple scales can help managers to protect and enhance habitat for sensitive species, such as the tri-colored bat, more effectively. Based on our findings, habitat for male tri-colored bats within GSMNP includes oak-hickory, successional hardwood, cove, yellow-pine, or floodplain forest patches near roads. Within these patches, mature deciduous trees in low-density stands appear to be preferred microhabitat. As GSMNP has a variety of forest types, topographies, and microclimates available, tri-colored bat selection at this site is likely an accurate representation of habitat preference for the species throughout the Southern Appalachian region.

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LITERATURE CITED

- Allen, A. A. 1921. Banding Bats. *Journal of Mammalogy* 2(2): 53–57.
- Alves, D.M.C.C., L.C. Terribile, and D. Brito. 2014. The potential impact of white-nose syndrome on the conservation status of North American bats. *PloS One*, 9(9): e107395.
- Anderson, D.R., K.P. Burnham, and W.L. Thompson. 2000. Null hypothesis testing: problems, prevalence and an alternative. *Journal of Wildlife Management* 64:912–923.
- Barclay, R.M.R., and A. Kurta. 2007. Ecology and behavior of bats roosting in tree cavities and under bark. *In*: Lacki M.J., J.P. Hayes, and A. Kurta (Eds.), *Bats in Forests: Conservation and Management*. John Hopkins University Press, Baltimore, Maryland, USA. Pp. 17-59.
- Bellamy, C., C. Scott, and J. Altringham. 2013. Multiscale, presence-only habitat suitability models: fine-resolution maps for eight bat species. *Journal of Applied Ecology* 50:892–901.
- Bennett, V.J., and A.A. Zurcher. 2013. When corridors collide: road-related disturbance in commuting bats. *Journal of Wildlife Management* 77:93–101.
- Bennett, V.J., D.W. Sparks, and P.A. Zollner .2013. Modeling the indirect effects of road networks on the foraging activities of bats. *Landscape Ecology* 28:1–13.
- Blehert, D.S., A.C. Hicks, M. Behr, C.U. Meteyer, B.M. Berlowski-Zier, E.L. Buckles, J.T.H. Coleman, S.R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome : An emerging fungal pathogen? *Science* 323(Jan): 227.
- Broders, H.G., G.J. Forbes, S. Woodley, and I.D. Thompson. 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* 70:1174–1184.
- Burnham, K.P., and D.R. Anderson. 2002. *Model selection and multimodel inference: a practical information-theoretic approach*. Springer, New York, USA.

- Carter, T.C., M.A. Menzel, B.R. Chapman, and K.V. Miller. 1999. Summer foraging and roosting behavior of an eastern pipistrelle, *Pipistrellus subflavus*. *Bat Research News* 40:5–6.
- Carter T.C., S.K. Carroll, J.E. Hofmann, J.E. Gardner JE, and G.A. Feldhamer. 2002. Landscape analysis of roosting habitat in Illinois. *In*: Kurta A, Kennedy J (eds) *The Indiana bat, biology and management of an endangered species*. Bat Conservation International, Austin, pp 160–164.
- Carter, T. C., and G.A. Feldhamer. 2005. Roost tree use by maternity colonies of Indiana bats and northern long-eared bats in southern Illinois. *Forest Ecology and Management* 219(2–3): 259–268.
- Center for Biological Diversity, Defenders of Wildlife. 2016. Petition to list the tri-colored bat (*Perimyotis subflavus*) as threatened or endangered under the Endangered Species Act.
- Cope, J. B., W. Baker, J. Confer, and E. College. 1961. Breeding colonies of four species of bats. *Proceedings of the Indiana Academy of Science* 70: 262–266.
- Davis, W. H., and R.E. Mumford. 1962. Ecological notes on the bat *Pipistrellus subflavus*. *The American Midland Naturalist* 68(2): 394–398.
- Elmore, L. W., D. A. Miller, and F. J. Vilella. 2004. Selection of diurnal roosts by red bats in an intensively managed pine forest in Mississippi. *Forest Ecology and Management* 199:11–20.
- Foster, R. W., and A. Kurta. 1999. Roosting ecology of the northern bat (*Myotis septentrionalis*) and comparisons with the endangered Indiana bat (*Myotis sodalis*). *Journal of Mammalogy* 80(2): 659–672.
- Frick, W.F., J.F. Pollock, A.C. Hicks, K.E. Langwig, D.S. Reynolds, G.G. Turner, C.M. Butchkoski, and T.H. Kunz. 2010. An emerging disease causes regional population collapse of a common North American bat species. *Science* 329:679–682.
- Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B.A. Nickel, K.E. Langwig, J.T. Foster, and A.M. Kilpatrick. 2015. Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography* 24(7): 741–749.

- Golley, F.B. 1966. South Carolina Mammals. Contrib. from the Charleston Museum. XV. E. Milby Burton (Ed.). Charleston, SC. 181 pp.
- Hayes, J.P., and S.C. Loeb. 2007. The influences of forest management on bats in North America. *In*: Lacki MJ, JP Hayes, and A Kurta (Eds.), Bats in Forests: Conservation and Management. John Hopkins University Press, Baltimore, Maryland, USA. Pp. 17–59.
- Homer, C.G., J.A. Dewitz, L. Yang, S. Jin, P. Danielson, G. Xian, J. Coulston, N.D. Herold, J.D. Wickham, and K. Megown. 2015. Completion of the 2011 National Land Cover Database for the conterminous United States-representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing* 81(5): 345-354.
- Hoying, K. M., and T.H. Kunz. 1998. Variation in size at birth and post-natal growth in the insectivorous bat *Pipistrellus subflavus* (Chiroptera: Vespertilionidae). *Journal of Zoology* 245(1): 15–27.
- Humphrey, S. R. 1975. Nursery populations of *Pipistrellus subflavus* (Chiroptera, Vespertilionidae) in Missouri. *Transactions of the Illinois State Academy of Science* 69:367.
- Jones, C., and J. Pagels. 1968. Notes on a population of *Pipistrellus subflavus* in southern Louisiana. *Journal of Mammalogy* 49(1): 134–139.
- Jones, C., and R.D. Suttus. 1973. Structure and organization of *Pipistrellus subflavus* in southern Louisiana. *Journal of Mammalogy* 54(4): 962–968.
- Kalcounis-Ruppell, M. C., J. M. Psyllakis, and R. M. Brigham. 2005. Tree roost selection by bats: an empirical synthesis using meta-analysis. *Wildlife Society Bulletin* 33:1123–1132.
- Krishon, D. M., M. A. Menzel, T. C. Carter, and J. Laerm. 1997. Notes on the home range of four species of vespertilionid bats (Chiroptera) on Sapelo Island, Georgia. *Georgia Journal of Science* 55:215–223.
- Kunz, T.H. (Ed.), 1988. *Ecological and Behavioral Methods for the Study of Bats*. Smithsonian Institution Press, Washington, DC, USA.

- Kurta, A., and S.W. Murray. 2002. Philopatry and migration of banded Indiana bats (*Myotis Sodalis*) and effects of radio transmitters. *Journal of Mammalogy* 83(2): 585–589.
- Lacki, M. J., and J.H. Schwierjohann. 2001. Day-roost characteristics of northern bats in mixed mesophytic forest. *Journal of Wildlife Management* 65(3): 482–488.
- Lane, H.K. 1946. Notes on *Pipistrellus subflavus subflavus*. *Pennsylvania Academy of Science* 20:57–61.
- Langwig, K.E., W.F. Frick, J.T. Bried, A.C. Hicks, T.H. Kunz, and A.M. Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 15(9):1050–1057.
- Leput, D. W. (2004). Eastern red bat (*Lasiurus borealis*) and eastern pipistrelle (*Pipistrellus subflavus*) maternal roost selection: Implications for forest management. Thesis, Clemson University, Clemson, USA.
- Lewis, S.E. 1995. Roost fidelity of bats: a review. *Journal of Mammalogy* 76:481–496.
- Menzel, M. A. 1996. Possible use of a tree cavity as a maternity roost by the eastern pipistrelle bat *Pipistrellus subflavus*. *Bat Research News* 37:115–116.
- Menzel, M. A., D.M. Krishon, T.C. Carter, and J. Laerm. 1999. Notes on tree roost characteristics of the northern yellow bat (*Lasiurus intermedius*), the Seminole bat (*Lasiurus seminolus*), the evening bat (*Nycticeius humeralis*), and the eastern pipistrelle (*Pipistrellus subflavus*). *Florida Scientist* 62(3/4):185–193.
- Menzel, M. A., S.F. Owen, W.M. Ford., J.W. Edwards, P.B., Chapman, B.R., and K.V. Miller. 2002. Roost tree selection by northern long-eared bat (*Myotis septentrionalis*) maternity colonies in an industrial forest of the central Appalachian mountains. *Forest Ecology and Management* 155(1–3): 107–114.
- Menzel, J.M., M.A. Menzel, J.C. Kilgo, W.M. Ford, J.W. Edwards, and G.F. McCracken. 2005. Effect of habitat and foraging height on bat activity in the coastal plain of South Carolina. *Journal of Wildlife Management* 69:235-245.
- Miles, A. C., S.B. Castleberry, D.A. Miller, and L.M. Conner. 2006. Multi-scale roost-site selection by evening bats on pine-dominated landscapes in southwest Georgia. *Journal of Wildlife Management* 70(5): 1191–1199.

- O'Keefe, J. M., S.C. Loeb, J.D. Lanham, and H.S. Hill. 2009. Macrohabitat factors affect day roost selection by eastern red bats and eastern pipistrelles in the southern Appalachian Mountains, USA. *Forest Ecology and Management* 257(8): 1757–1763.
- Ormsbee, P. C., and W. C. McComb. 1998. Selection of day roosts by female long-legged *Myotis* in the central Oregon Cascade Range. *Journal of Wildlife Management* 62:596–603.
- Pauli, B. P., H.A. Badin, G.S. Haulton, P.A. Zollner, and T.C. Carter. 2015. Landscape features associated with the roosting habitat of Indiana bats and northern long-eared bats. *Landscape Ecology* 30(10): 2015–2029.
- Perry, R. W., and R.E. Thill. 2007. Tree roosting by male and female eastern pipistrelles in a forested landscape. *Journal of Mammalogy* 88(4): 974–981.
- Perry, R. W., R.E. Thill, and D.M. Leslie Jr. 2007. Selection of roosting habitat by forest bats in a diverse forested landscape. *Forest Ecology and Management* 238: 156–166.
- Perry, R. W., R.E. Thill, and D.M. Leslie Jr. 2008. Scale-Dependent Effects of Landscape Structure and Composition on Diurnal Roost Selection by Forest Bats. *Journal of Wildlife Management* 72(4): 913–925.
- Poissant, J. A., H.G. Broders, and G.M. Quinn. 2010. Use of lichen as a roosting substrate by *Perimyotis subflavus*, the tricolored bat, in Nova Scotia. *Ecoscience* 17(4): 372–378.
- Poole, E.L. 1938. Notes on the breeding of *Lasiurus* and *Pipistrellus* in Pennsylvania. *Journal of Mammalogy* 19:249.
- Reichard, J. D., and T.H. Kunz. 2009. White-Nose syndrome inflicts lasting injuries to the wings of little brown *Myotis* (*Myotis lucifugus*). *Acta Chiropterologica* 11(2): 457–464.
- Sikes, R. S., and Animal Care and Use Committee. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* 97: 663–688.
- Thogmartin, W.E., C.A. Sanders-Reed, J.A. Szymanski, P.C. McKann, L. Pruitt, R.A. King, M.C. Runge, and R.E. Russell. 2013. White-nose syndrome is likely to

- extirpate the endangered Indiana bat over large parts of its range. *Biological Conservation* 160:162-172.
- Timpone J.C., J.G. Boyles K.L. Murray, D.P. Aubrey, and L.W. Robbins. 2010. Overlap in roosting habits of Indiana bats (*Myotis sodalis*) and northern bats (*Myotis septentrionalis*). *American Midland Naturalist* 163:115–123.
- Turner, G. G., D.M. Reeder, and J.T.H. Coleman. 2011. A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future. *Bat Research News* 52(2): 13–27.
- US Fish and Wildlife Service (USFWS). 2016. Endangered and Threatened Wildlife and Plants; 4(d) Rule for the Northern Long-Eared Bat. *Federal Register*, 81(9), 1900–1922.
- Veilleux, J. P., and S.L. Veilleux. 2004. Colonies and reproductive patterns of tree-roosting female eastern pipistrelle bats in Indiana. *Proceedings of the Indiana Academy of Science* 113(1): 60–65.
- Veilleux, J. P., J.O. Whitaker Jr., and S.L. Veilleux. 2003. Tree-roosting ecology of reproductive female eastern pipistrelles, *Pipistrellus subflavus*, in Indiana. *Journal of Mammalogy* 84(3): 1068–1075.
- Verant, M.L., C.U. Meteyer, J.R. Speakman, J.M. Lorch, and D.S. Blehert. 2014. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. *BMC Physiology* 14(1): 10.
- Vonhof, M. J., and R. M. R. Barclay. 1996. Roost-site selection and roosting ecology of forest-dwelling bats in southern British Columbia. *Canadian Journal of Zoology* 74:1797–1805.
- Vonhof, M. J., and R.M.R. Barclay. 1997. Use of tree stumps as roosts by the western long-eared bat. *Journal of Wildlife Management* 61:674–684.
- Whitaker Jr., J. O. 1998. Life history and roost switching in six summer colonies of eastern pipistrelles in buildings. *American Society of Mammalogists* 79(2): 651–659.
- Willis, C. K. R., and R.M. Brigham. 2005. Physiological and ecological aspects of roost selection by reproductive female hoary bats (*Lasiurus cinereus*). *Journal of Mammalogy* 86(1): 85–94.

- Willis, C. K. R., Q.M.R. Webber, Q.E. Fletcher, K.J.O. Norquay, A.M. Briet, and C.M. Davy. 2016. To Spray or Not to Spray : Evaluating Alternative Management Approaches for White-nose Syndrome. Abstract. 46th Annual Symposium: North American Society for Bat Research, 85.
- Winchell, J. M., and T.H. Kunz. 1996. Day-roosting activity budgets of the eastern pipistrelle bat, *Pipistrellus subflavus* (Chiroptera : Vespertilionidae). Canadian Journal of Zoology 74: 431–441.
- Yates, M., and R. Muzika. 2006. Effect of forest structure and fragmentation on site occupancy of bat species in Missouri Ozark forests. Journal of Wildlife Management 70(5): 1238–1248.
- Zurcher, A.A., D.W. Sparks, and V.J. Bennett. 2010. Why the bat did not cross the road? Acta Chiropterologica 12:337–340.

APPENDIX 3

Tables

Table 3.1 Microhabitat variables used in candidate models to determine roost selection by male tri-colored bats (*Perimyotis subflavus*) in Great Smoky Mountains National Park, USA, 2015–2016.

Variable	Definition
DBH	Roost tree diameter at breast height (m)
T_Ht	Roost tree height (m)
C_Vol	Crown volume index (m ²)
Can_Cover	Percent canopy density (%)
Can_5	Percent canopy density in 90° roost quadrant (%)
Overst_Ht	Average overstory height (m)
BC_Ht	Height to base of tree crown (m)
D_10	Distance to nearest overstory tree (m)
BA	Basal area (m ² /ha)
Clutter	Number of midstory stems
Total_den	Number of overstory stems
Soft_den	Number of conifers in overstory

Table 3.2 Landscape variables used in candidate models to determine roost selection by male tri-colored bats (*Perimyotis subflavus*) in Great Smoky Mountains National Park, USA, 2015–2016.

Variable	Definition
elevation	Elevation (m)
aspect	Hillside aspect of roost location (rad)
slope	Percent slope of plot (%)
dist_road	Distance to nearest road (m)
dist_water	Distance to nearest stream, river or pond (m)
area_water	Area of water features in 1 km buffer (m ²)
area_forest	Area of forest within 1 km buffer (m ²)
len_edge	Total length of forest edge within 1 km buffer (m ²)
len_stream	Total length of stream within 1 km buffer (m ²)

Table 3.3 Number of roosts of tri-colored males and female in live or dead vegetation, by tree species, and composition of tree species ≥ 5.0 cm diameter at breast height (dbh) in random plots in the Great Smoky Mountains National Park, 2015–2016.

Species	Males			Females		Tree composition in random plots (%) ^a
	Live	Dead	Unknown	Live	Dead	
<i>Quercus</i> spp.	10	7	2	0	0	8.3
<i>Quercus alba</i>	5	1	2	0	0	4.3
<i>Q. rubra</i>	0	3	0	0	0	1.4
<i>Q. montana</i>	5	3	0	0	0	2.6
<i>Acer</i> spp.	9	0	3	0	0	10.2
<i>Liriodendron tulipifera</i>	15	3	0	0	1	18.9
<i>Carya</i> spp.	1	0	0	0	0	3.8
<i>Tilia americana</i>	1	0	0	0	0	0.5
<i>Magnolia macrophylla</i>	0	8	0	0	0	0.1
<i>Liquidambar styraciflua</i>	2	0	0	0	0	4.1
<i>Betula lenta</i>	1	0	0	0	0	3.2
<i>Aesculus flava</i>	4	0	0	0	0	0.4
^a Composition of available tree groups (random plots) was 8.2% oaks, 10.2% maples, 24.7% conifers, 11.5% snags, 3.8% hickories, 18.9% yellow poplars, 22.7% other hardwoods						

Table 3.4 Characteristics of roost trees selected by male tri-colored bats compared with unselected available random trees in the Great Smoky Mountains National Park, USA, 2015–2016. P-values are the result of qualifying logistic regression test. Asterisk denotes variables used in thermoregulation models, double asterisk denotes variables used in clutter models.

Variable		Roost		Random		P
		Mean	S.E.	Mean	S.E.	
Tree Characteristics						
Roost tree diameter (cm)		40.05	2.07	28.39	2.23	0.0005*
Roost tree height (m)		22.38	0.75	16.96	0.7	0.0000*
Nearest overstory tree (m)		2.4	0.22	2.99	0.19	0.0515**
Base canopy height (m)		12.37	0.56	10.15	0.55	0.0071**
Crown volume (m ³)		725.3	93.82	316.25	51.1	0.0016*
Plot Characteristics						
Average height of overstory (m)		21.99	0.59	20.52	0.67	0.1050*
Number of stems in midstory		21.24	1.42	24.78	1.79	0.1320**
Percent canopy density		97.28	0.32	95.68	0.64	0.0413*
Number of overstory trees		33.97	1.06	38.63	1.4	0.0117**
Number of softwoods in overstory		5.13	0.67	9.54	1.09	0.0016**
Overstory basal area (m ² /ha)		26.44	1.04	26.18	0.98	0.8590

Table 3.5 Best candidate models predicting selection of roost microhabitat by male tri-colored bats in the Great Smoky Mountains National Park, USA, 2015–2016. We present the number of parameters in the model (K), the Akaike's Information Criterion score (AIC_c), difference in AIC from top model score (Δ AIC), and relative weight for each model (w). Clutter1 is the global model for the clutter group.

Model	Variables	K	AIC _c	Δ AIC	W
clutter1	total_den+Clutter+soft_den+D_10+BC_Ht	6	149.62	0.00	0.260
therm12	T_Ht+C_Vol	3	150.47	0.86	0.170
therm7	T_Ht	2	151.27	1.65	0.114
therm3	overst_Ht+T_Ht	3	151.81	2.19	0.087
clutter18	total_den+soft_den+Clutter+C_Vol	5	152.25	2.63	0.070
therm5	T_Ht+Can_Cover	3	152.37	2.75	0.066
therm18	overst_Ht+T_Ht+Can_Cover+C_Vol	5	152.78	3.16	0.054
therm15	T_Ht+DBH	3	152.85	3.23	0.052
therm2	overst_Ht+T_Ht+Can_Cover	4	153.10	3.48	0.046
Null	.	1	171.87	22.25	0.000

Table 3.6 Model-averaged parameter estimates, unconditional SE, 95% confidence intervals, and odds ratios for variables of models within 2 IAC units of the top model explaining microhabitat selection in male tri-colored bats in Great Smoky Mountains National Park, USA, 2015–2016.

Variable	Parameter estimate	SE	Lower CI	Upper CI	Odds ratio
Micro: Clutter					
total_den	-0.064	0.024	-0.111	-0.017	0.942
Clutter	-0.031	0.018	-0.067	0.005	0.970
soft_den	-0.080	0.030	-0.139	-0.021	0.923
D_10	-0.446	0.148	-0.736	-0.157	0.638
BC_Ht	0.092	0.047	3.57E-04	0.183	1.094
Micro: Thermoregulation					
T_Ht	0.145	0.042	0.063	0.227	1.150
C_Vol	7.34e-04	4.66E-04	-1.80E-04	1.65E-3	1.001

Table 3.7 Characteristics of roost plots selected by male tri-colored bats compared with random plots in the Great Smoky Mountains National Park, USA, 2015–2016. P-values are the result of qualifying logistic regression test. Asterisk denotes variables used in selection models.

Landscape variable	Roost		Random		P
	Mean	S.E.	Mean	S.E.	
Elevation (m)	530.12	12.75	575.84	13.17	0.016*
Slope (%)	37.96	2.60	41.66	2.47	0.000*
aspect (rad)	0.11	0.09	-0.25	0.09	0.000*
near_road (m)	185.89	24.91	427.13	39.26	0.000*
near_water (m)	98.84	9.99	101.85	9.71	0.828
len_edge (m)	6576.40	345.25	7058.95	494.62	0.422
len_stream (m)	9668.10	189.96	9602.10	256.05	0.835
forest_area (m ²)	2.9151	0.04	2.91	0.042	0.926
water_area (m ²)	0.0176	0.0023	0.0198	0.003	0.570

Table 3.8 Best candidate models predicting landscape-level roost selection by male tri-colored bats in the Great Smoky Mountains National Park, USA, 2015–2016. We present the number of parameters in the model (K), the Aikake’s Information Criterion score (AICc), difference in AIC from top model score (ΔAIC), and relative weight for each model (w).

Model	Variables	K	AIC_c	ΔAIC	W
land10	near_road +CosAsp	3	159.8	0	0.333
land5	near_road	2	161.4	1.57	0.152
land13	near_road+CosAsp+elevation	4	161.9	2.08	0.117
land15	near_road+CosAsp+Slope	4	161.9	2.11	0.116
land8	near_road+elevation	3	163.5	3.64	0.054
land11	near_road+Slope	3	163.5	3.66	0.053
Null	.	1	185	25.2	0

Table 3.9 Parameter estimates, unconditional SE, 95% confidence intervals, and odds ratios for variables of models within 2 IAC units of the top model explaining landscape-level roost selection in male tri-colored bats in Great Smoky Mountains National Park, USA, 2015–2016.

Variable	Parameter estimate	SE	Lower CI	Upper CI	Odds ratio
near_road	-3.54E-03	8.75E-04	-5.25E-03	-0.82E-03	0.996
CosAsp	0.509	0.268	-0.017	1.035	1.664

Transmitter Application Protocol

Procedure:

1. Prepare transmitter for application.
 - a. Record the serial number and frequency on the transmitter to be applied and remove the tape over the wires.
 - b. Twist wires gently, use a small amount of solder to join wires.
 - c. Fold wires in to wax on side of transmitter.
 - d. Adjust receiver to transmitter frequency and test transmitter output.
2. Prepare bat for attachment
 - a. Place a glob (~1/2 tsp) of glue on a piece of paper. Use paint brush to apply glue to the transmitter.
 - b. Part the fur on between the bat's shoulders, trimming some fur, and brush additional glue to parted/trimmed fur.
 - c. Place transmitter flat-side down to the bat, add additional glue to the top of the transmitter and fold hair from either side over the top to encase.
 - d. For the glue to dry allow 5–10 minutes/or until tacky.
 - e. When the glue is dry (somewhat tacky, but no longer runny), it is safe to release the bat.

Selection Models

Appendix 3.1. Candidate models used in model selection for male tri-colored bat summer microhabitat in 2015–2016, in the Great Smoky Mountains National Park, USA.

Model	Variables	Df	AIC _c
clutter1	total_den+Clutter+soft_den+D_10_m+BC_Ht_m	6	149.6195
clutter2	total_den+Clutter+soft_den	4	160.1608
clutter3	total_den+Clutter	3	168.4975
clutter4	total_den+soft_den	3	159.8192
clutter5	Clutter+soft_den	3	163.8681
clutter6	total_den	2	168.2798
clutter7	soft_den	2	163.9767
clutter8	Clutter	2	173.6294
clutter9	D_10_m	2	170.9478
clutter10	BC_Ht_m	2	169.0468
clutter11	total_den+D_10_m	3	159.1407
clutter12	Clutter+D_10_m	3	169.1308
clutter13	soft_den+D_10_m	3	162.405
clutter14	total_den+BC_Ht_m	3	166.2373
clutter15	soft_den+BC_Ht_m	3	159.1509
clutter16	Clutter+BC_Ht_m	3	169.7275
clutter17	total_den+soft_den+Clutter+DBH	5	153.5807
clutter18	total_den+soft_den+Clutter+C_Vol	5	152.2472
therm1	overst_ht_m+T_Ht_m+Can_Cover+DBH+C_Vol	6	154.8943
therm2	overst_ht_m+T_Ht_m+Can_Cover	4	153.1033
therm3	overst_ht_m+T_Ht_m	3	151.8136
therm4	overst_ht_m+Can_Cover	3	173.2271
therm5	T_Ht_m+Can_Cover	3	152.3699
therm6	overst_ht_m	2	174.0537
therm7	T_Ht_m	2	151.2743
therm8	Can_Cover	2	172.9258
therm9	DBH	2	163.3407
therm10	C_Vol	2	160.4497
therm11	overst_ht_m+C_Vol	3	161.7586
therm12	T_Ht_m+C_Vol	3	150.4747
therm13	Can_Cover+C_Vol	3	160.232
therm14	overst_ht_m+DBH	3	163.5801
therm15	T_Ht_m+DBH	3	152.853
therm16	Can_Cover+DBH	3	164.4472
therm17	overst_ht_m+T_Ht_m+Can_Cover+DBH	5	155.275
therm18	overst_ht_m+T_Ht_m+Can_Cover+C_Vol	5	152.7787
Null	.	1	171.87

Appendix 3.2. Candidate models used in model selection for male tri-colored bat landscape-level habitat variables in the Great Smoky Mountains National Park, USA, 2015–2016.

Model	Variables	Df	AICc
land1	elevation+CosAsp+Slope+near_road	5	164
land2	elevation	2	180.9
land3	CosAsp	2	179.2
land4	Slope	2	186
land5	near_road	2	161.4
land6	elevation+CosAsp	3	175.7
land7	elevation+Slope	3	181.7
land8	elevation+near_road	3	163.5
land9	CosAsp+Slope	3	180
land10	CosAsp+near_road	3	159.8
land11	Slope+near_road	3	163.5
land12	elevation+CosAsp+Slope	4	176.4
land13	elevation+CosAsp+near_road	4	161.9
land14	elevation+Slope+near_road	4	165.6
land15	CosAsp+Slope+near_road	4	161.9
land16	CosAsp*Slope+near_road+elevation	6	166.2
land17	CosAsp*Slope+near_road	5	164.1
land18	CosAsp*Slope+elevation	5	178.5
land19	elevation*CosAsp*Slope*near_road	16	175.6
land20	elevation*CosAsp	4	171
land21	CosAsp*Slope	4	182.2
land22	near_road+elevation*Slope*CosAsp	9	167.3
Null	.	1	185

4. CONCLUSION

In this thesis, I investigated the change in the populations of bats species found in Great Smoky Mountains National Park (GSMNP), and roost selection in one species for which little was known about roost selection in the southeastern U.S.

I found that at least four species of bats in GSMNP have declined significantly following the arrival of white-nose syndrome, a disease caused by a fungus that infects bats during hibernation. I saw significant declines in the summer capture rates for tri-colored (-76%), little brown (-98%), northern long-eared (-99%), and Indiana bats (-69%), well as winter hibernacula counts for tri-colored (-94%), little brown (-98%), and Indiana bats (-87%) demonstrating that declines are effecting both the summer and winter residents of the national park.

To provide information that may help wildlife managers conserve the species should they receive federal protection under the Endangered Species Act, I characterized the summer roost selection of male tri-colored bats in GSMNP. Previously, little was known about their summer habitat needs in the southeastern US, but through the course of this research, I found that they select locations on the landscape that are near roads. I also found that “northness,” slope grade, and elevation may also be important for the species when selecting a roost site. At the tree and forest stand level, I found that male tri-colored bats select trees that are generally taller and have larger canopies than those around them, and that they select forest stands that have fewer overstory trees and fewer overstory conifers.

Managers may be able to use this knowledge to protect habitat that tri-colored bats need to recover their populations. Future monitoring of bat populations within GSMNP may increase the accuracy of demographic data, such as age and sex ratios.

Additionally, study of the roost selection in female tri-colored bats would offer further information to aid in development of management strategies.

VITA

Grace Carpenter was born and raised in Ann Arbor, MI where she was the youngest of her four siblings: Emily, Peter, Tim, and Ben. She attended Pioneer High School in Ann Arbor for two years before transferring to Washtenaw Technical Middle College where she received her associate degree in Photographic Imaging. She went on to receive her BS in Biology from Eastern Michigan University in 2012, where she began her first foray into fieldwork with bats with Dr. Allen Kurta.

Before starting her master's, she worked with the Bird Conservancy of the Rockies, Hawk Watch International, and Round River Conservation Studies on various projects investigating anthropogenic effects on migratory birds and wolverines. She also worked for Sanctuary Mountain in Pukeatua New Zealand helping with native songbird reintroductions.

She accepted a master's research assistantship with Dr. Emma Willcox at the University of Tennessee, and now focuses her research on the habitat needs of bats affected by White-nose syndrome in the Great Smoky Mountains National Park.