

SUMMER FORAGING RANGE AND DIURNAL ROOST
SELECTION OF TRI-COLORED BATS, *PERIMYOTIS*
SUBFLAVUS.

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Dustin Bradley Thames

December 2020

Copyright © 2020 by Dustin Bradley Thames

All rights reserved.

DEDICATION

For Addy and Arlo.

ACKNOWLEDGEMENTS

I thank the Tennessee Wildlife Resources Agency for funding my project and the State of Tennessee for providing a tuition waiver as a benefit of full-time employment that made this possible. I thank all my technicians, volunteers, and co-workers that helped with field work, particularly Mallory Tate. This project would not have been possible without her expertise and dedication. I thank my advisor Dr. Emma Willcox for her support, encouragement, friendship, and technical acumen. Also, I thank my committee members, Josh Campbell, Dr. Mona Papes, and Dr. John Zobel, for their input and support.

ABSTRACT

Tri-colored bat populations are declining in eastern North America where the fungal pathogen *Pseudogymnoascus destructans* has been introduced. The pathogen causes disease and mortality in cave hibernating bats. Once considered a common species in Tennessee, tri-colored bats are now being considered for protection under the Endangered Species Act. There is a paucity of research examining the basic ecology of tri-colored bats. Research to fill these knowledge gaps is needed to inform conservation plans and to define critical habitat. The first objective of my research was to characterize the summer diurnal roosts of tri-colored bats and to examine roost selection at the forest stand and landscape scale. The second objective was to determine the size of tri-colored bat foraging ranges and determine land cover selection of foraging tri-colored bats. During the summers of 2016/17, I radio-tagged 7 male tri-colored bats. I visually confirmed 15 roosts of male tri-colored bats. The roosts were primarily in clumps of dead oak (*Quercus* spp.) and hickory (*Carya* spp.) leaves. Forest stand characteristics were not good predictors of male tri-colored bat roost selection likely due to homogeneity of forests within my study area. At the landscape scale, tri-colored bats selected roosts near the intersect of the Eastern Highland Rim and Cumberland Escarpment physiographic subregions. Roosts were in large tracts of mature forest near more open land covers of the Eastern Highland Rim. Additionally, tri-colored bats selected roosts further from streams, yet with a higher density of streams around the roost tree. Male tri-colored bats traveled a long nightly mean maximum distance ($11.4 \text{ km} \pm 7.1 \text{ SD}$) from their roost area while foraging. They also had relatively large 95% minimum convex polygon foraging ranges ($2350 \text{ ha} \pm 3303 \text{ SD}$). Male tri-colored bats selected fixed kernel density foraging ranges with less forest cover than available ($\Delta = 0.085$, $P = 0.014$) and core foraging ranges with more open water and wetlands than available ($\Delta = 0.11$, $P =$

0.03). Conservation plans for tri-colored bats should protect large tracts of mature oak-hickory forests near waterbodies where tri-colored bats forage.

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION	1
WINTER ECOLOGY	5
ROOSTING ECOLOGY.....	6
FORAGING ECOLOGY	8
MANAGEMENT IMPLICATIONS.....	10
LITERATURE CITED	12
CHAPTER 2: SUMMER DIURNAL ROOST SELECTION OF MALE TRI-COLORED BATS, <i>PERIMYOTIS SUBFLAVUS</i> , ALONG THE CUMBERLAND PLATEAU IN MIDDLE TENNESSEE.....	22
ABSTRACT	23
INTRODUCTION.....	24
METHODS AND MATERIALS.....	26
RESULTS.....	32
DISCUSSION	34
LITERATURE CITED	39
APPENDIX 1	45
CHAPTER 3: SUMMER FORAGING RANGE SELECTION OF TRI-COLORED BATS, <i>PERIMYOTIS SUBFLAVUS</i> , ALONG THE CUMBERLAND PLATEAU IN MIDDLE TENNESSEE.....	51
ABSTRACT	52
INTRODUCTION.....	53
METHODS AND MATERIALS	55
RESULTS.....	60
DISCUSSION	63
LITERATURE CITED	67
APPENDIX 2	75
VITA.....	87

LIST OF TABLES

Table 1. Stand scale covariates used in logistic regression models to generate candidate models describing male tri-colored bat, <i>Perimyotis subflavus</i> , roost selection in Franklin, County, Tennessee, USA, 2016/17.....	46
Table 2. Landscape scale covariates ^a used in logistic regression models to generate candidate models describing male tri-colored bat, <i>Perimyotis subflavus</i> , roost selection in Franklin, County, Tennessee, USA, 2016/17.....	47
Table 3. Mean ($\bar{x}$) and standard error (SE) of selected roosts of tri-colored bats, <i>Perimyotis subflavus</i> , and random sites, results of paired sample t-tests for stand level variables, and t-tests or Wilcoxon rank-sum test for landscape level variables in Franklin County, Tennessee, USA.....	48
Table 4. Values of Akaike's Information Criterion adjusted for small sample sizes, difference in AICc when compared to the model with the lowest AICc ($\Delta AICc$), Akaike weight (ω_i), and generalized R^2 values of models with $\Delta AICc < 2$ for models of roost selection of tri-colored bats, <i>Perimyotis subflavus</i> , in Franklin County, Tennessee, USA.....	49
Table 5. Model averaged parameter estimates, 95% confidence interval of parameter estimates, adjusted standard errors (S.E.), odds ratios, and sum of Akaike weights of covariates describing roost selection of male tri-colored bats, <i>Perimyotis subflavus</i> , in Franklin County, Tennessee, USA. Only one landscape model had substantial support, so estimates are from that one model and sum of Akaike weights is excluded.	50
Table 6. Land cover categories used for compositional analysis of male tri-colored bat, <i>Perimyotis subflavus</i> , foraging ranges in Franklin County, TN, USA, and the 2016 National Land Cover Database categories that were merged for the analyses.	76
Table 7. Transmitter to body mass ratio, number of nights tracked, number of foraging locations, size of the foraging range, and maximum distance traveled from the center of the roost area for tri-colored bats, <i>Perimyotis subflavus</i> , tracked in Franklin County, Tennessee in 2016/17.	77
Table 8. Ranking matrices from compositional analyses of tri-colored bat, <i>Perimyotis subflavus</i> , foraging range selection in Franklin County, Tennessee, USA, 2016/17.....	78

LIST OF FIGURES

Figure 1. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> , (ID = 063) in Franklin County, Tennessee, USA.	79
Figure 2. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a female tri-colored bat, <i>Perimyotis subflavus</i> , (ID =124) in Franklin County, Tennessee, USA.	80
Figure 3. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> , (ID = 182) in Franklin County, Tennessee, USA.	81
Figure 4. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> (ID = 483) in Franklin County, Tennessee, USA.	82
Figure 5. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> , (ID = 663) in Franklin County, Tennessee, USA.	83
Figure 6. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> (ID = 752) in Franklin County, Tennessee, USA.	84
Figure 7. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> , (ID = 785) in Franklin County, Tennessee, USA.	85
Figure 8. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, <i>Perimyotis subflavus</i> (ID = 903) in Franklin County, Tennessee, USA.	86

CHAPTER 1: GENERAL INTRODUCTION

Bats provide a variety of ecosystem, provisioning, and cultural services that improve human well-being (Kunz et al. 2011). Insectivorous bats suppress populations of nocturnal insects and have profound benefits to agriculture (Kalka et al. 2008). Declines in bat abundance and diversity could impact natural ecosystem function and lead to agricultural losses of over 3.7 billion dollars (USD) annually in North America (Pierson 1998, Boyles et al. 2011). Unfortunately, North American bats are currently experiencing dramatic population declines caused by the cumulative effects of several threats, including habitat loss, disturbance of hibernacula and maternity roosts, and pesticide use in agriculture (Harvey 2011). However, emerging threats, such as wind energy development and disease, currently pose the greatest risk to bats in North America. Interactions with wind turbines are estimated to have killed 600,000 bats in 2012 alone (Hayes 2013). With wind energy being one of the fastest growing energy development sectors in the United States, wind facilities are a growing danger to bat populations, including those of tri-colored bats, *Perimyotis subflavus* (Horn et al. 2008, Cryan and Barclay 2009). The emerging fungal pathogen, *Pseudogymnascus destructans* (Pd), the causal agent of white-nose syndrome (WNS) is the leading threat to hibernating bats in the eastern United States and Canada. First discovered in a cave in Schoharie County, New York in the winter of 2006/07, WNS has spread from the epicenter to 35 states and seven Canadian provinces. In addition, four states have confirmed the presence of Pd, but have yet to confirm the presence of the disease, WNS (USFWS 2019).

Pseudogymnascus destructans previously described as *Geomyces destructans* (Minnis and Lindner 2013), invades and colonizes the epidermal and dermal tissue of torpid bats causing erosion and ulceration. In addition to tissue damage, infected bats increase their metabolism resulting in an accelerated depletion of fat reserves. As the disease progresses, arousal periods

increase in frequency and, consequently, further accelerate the depletion of fat reserves. In laboratory experiments involving *Myotis lucifugus*, fat energy utilization in WNS positive bats was twice the rate of WNS negative bats (Verant et al. 2014). Furthermore, lab experiments have shown that WNS causes dehydration and electrolyte imbalance in torpid bats (Warnecke et al. 2013). White-nose syndrome often causes mortality to infected bats and is recognized as the first sustained epizootic ever documented in the taxon (Cryan et al. 2010).

Tri-colored bats (*Perimyotis subflavus* Menu, 1984) belong to the class Mammalia, order Chiroptera, and family Vespertilionidae. The species was historically placed in the genus *Pipistrellus*. However, anatomical and phylogenetic evidence has resulted in tri-colored bats being re-classified in the monotypic genus *Perimyotis* (Hoofer and Van Den Bussche 2003, Menu 1984). In literature published before 2003, tri-colored bats are referred to as eastern pipistrelles and in vernacular tri-colored bats are still often referred to as “pips”.

Tri-colored bats occur from southeastern Canada through the eastern United States into eastern Mexico and Central America (Harvey 2011, NatureServe 2016). The species has historically been considered common throughout much of its range and range expansion has been documented in the western United States and central Great Lakes region (Geluso et al. 2005, Kurta et al. 2007). In Tennessee, tri-colored bats have been documented in 84 of Tennessee’s 95 counties (Tennessee Bat Working Group 2020).

Tri-colored bats are one of the smallest species of bat in Tennessee and across the eastern and midwestern United States (Amelon 2006). Females weigh more than males on average ranging from 5.8–7.9 g, and males weigh between 4.6–7.5 g. Forearm length measures 31.4–36 mm and total wingspan 200–206 mm. Tri-colored bats are easily distinguished from superficially

similar species by their fur, which is dark at the base, yellowish-brown in the middle, and darker at the tips, hence the common name tri-colored bat (Fujita and Kunz 1984). Also, tri-colored bats have reddish colored forearms that contrast with their black colored wing membranes.

Tri-colored bats appear to be particularly susceptible to WNS. Bernard et al. (2017) found tri-colored bats have a greater fungal load of *P. destructans* on average through the winter than other cave hibernating bats sampled including northern long-eared bats (*Myotis septentrionalis*), which were federally listed as threatened in April 2015 due to declines caused by WNS (USFWS 2015). In Virginia, tri-colored bats at 18 hibernacula declined by 89.5% from 2009 to 2013 (Powers et al. 2015). In Tennessee, similar patterns of decline have been documented in many hibernacula within the state (Campbell 2017). Tri-colored bats have a NatureServe (2016) global conservation status rank of G2-imperiled. NatureServe's conservation status ranks species on a scale from G5-secure to G1-critically imperiled. A G2-imperiled status indicates that tri-colored bats have a high risk of extinction due, primarily, to recent population declines. In Canada, the species was designated endangered in February 2012 during an emergency assessment. While population trends in Canada were generally unknown for the tri-colored bat prior to the arrival of WNS, >75% declines are expected both in hibernacula and on the landscape, as have been documented in the northeastern United States (Turner et al. 2011, Forbes 2012). In Tennessee's 2015 State Wildlife Action Plan, tri-colored bats along with other WNS susceptible bats, were designated as a species of greatest conservation need (Tennessee State Wildlife Action Plan Team 2015). In June of 2016, the United States Fish and Wildlife Service (USFWS) was petitioned by The Center for Biological Diversity and Defenders of Wildlife to protect tri-colored bats under the endangered species act (Center for Biological Diversity and Defenders of Wildlife 2016).

Bats are long lived (~5-15 years or more) relative to other small mammals and have low reproductive potential. Bats have the longest lifespan of all mammals when the effects of body size are considered, and tri-colored bats have a maximum reported lifespan of 14.8 years after initial capture for a male tri-colored bat in Illinois (Walley and Jarvis 1971, Salmon et al. 2009). Since bats typically only have one or two pups per year, high rates of adult survival are critical for population growth and population declines from WNS will take a long time to recover (Coleman 2011).

Effective conservation strategies for tri-colored bats should consider both seasonal roosting sites and foraging areas (Pierson 1998). Limiting disturbance in WNS infected caves could be critical for successful hibernation and survival, as increased disturbance could increase arousal periods, further compounding the effects of WNS. Summer is also a critical period in the life history of tri-colored bats. Following emergence from winter torpor, bats are recovering from the effects of WNS and building fat reserves for reproduction in females and preparing for the fall swarming or mating season for males. Reproductive success is vital to the persistence of the species and developing strategies to identify, create, and manage summer habitat could help ensure the lasting survival of the species in the presence of WNS.

WINTER ECOLOGY

Winter hibernacula and summer roost sites for tri-colored bats are typically in different locations. Tri-colored bats are considered regional migrants and have been documented traveling up to 243 km from their winter hibernacula to their summer maternity area (Samoray 2019). In winter, tri-colored bats inhabit more caves in eastern North America than any other species of bat and will also utilize mines and rock crevices as hibernacula (Harvey 2011). The species most often roosts either singly or in small clusters (McNab 1974). Since copulation primarily occurs

in the fall and winter, the need to delay pregnancy until spring makes the tri-colored bat an obligate hibernator, even in central Florida. McNab (1974) hypothesized that hibernation is maintained in Florida due to reproductive requirements and the long period required for sperm storage. Tri-colored bats often roost in warmer, more humid areas of caves than other sympatric species (Ploskey and Sealer 1979, Harvey et al. 2011). Disproportionate sex-ratios have been reported in many hibernacula and Davis (1959) attributed this to high survival rates of males and probable selection of hibernacula outside of caves by females. Tri-colored bats appear to have high fidelity to both winter hibernacula and summer roost sites (Whitaker and Hamilton 1998).

Tri-colored bats copulate in the fall before hibernation and again in the spring during ovulation (Guthrie 1933). While the species typically gives birth to two pups, between two and seven ova are implanted during ovulation with the excess being reabsorbed in the uterus (Wimsatt 1975). Gestation lasts approximately 44 days. Timing of parturition largely depends on latitude and has been reported from late May to the first week in July (Fujita and Kunz 1984). Pups are born hairless and pink in the breech position. The young are capable of flight in 3 weeks and assume adult like foraging capabilities in 4 weeks. The fourth metacarpal-phalangeal epiphyseal gap which is used to differentiate adults from juveniles in the field is typically completely closed 45 days after birth (Hoying and Kunz 1998).

ROOSTING ECOLOGY

Relatively few studies have been conducted on foliage roosting bats, including the tri-colored bat, when compared to studies of cavity roosting bats (Carter and Menzel 2007). Most research on tri-colored bat summer roosting ecology has focused on colonies in man-made structures (Allen 1921, Jones and Pagels 1968, Jones and Suttkus 1973, Winchell and Kunz 1996, Whitaker 1998). Summer roosts of tri-colored bats have been documented in numerous

other structures including spanish moss (Davis and Mumford 1962), rock shelters in cliffs (Lacki and Hutchinson 1999), tree hollows (Whitaker 1998) and lichen (*Usnea spp.*; Quinn and Broders 2007). However, in forests tri-colored bats likely most often roost in foliage. In the first comprehensive study of reproductive female tri-colored bat roosting ecology conducted in Indiana, Veilleux et al. (2003) found that tri-colored bats roosted in dead foliage (65%), live foliage (30%), and squirrel nests (5%). In Arkansas Perry and Thill (2007) documented roosts of 7 female tri-colored bats, where 3 (43%) were in dead needles of live pine trees (*Pinus spp.*) and 4 (57%) were in dead leaves of live deciduous trees. In addition, they documented 33 roosts of male tri-colored bats of which 87% were located in dead leaves of live oak trees (*Quercus spp.*).

Oak trees as roosts appear to be an important habitat component in forests for tri-colored bats in the eastern United States (Perry and Thill 2007). In Indiana, Veilleux et al. (2003) found that reproductive female tri-colored bats roosted in oak trees more than expected. Perry and Thill (2007) also found that, in a forested landscape in Arkansas, male tri-colored bats roosted in oaks more than expected, but females did not significantly select certain species of trees more than expected. Veilleux (2003) hypothesizes that because dead oak leaves often form an umbrella shape when hanging downward and are relatively stable compared to other tree genera, they provide protection from wind and rain and moderate temperature extremes, as well as concealment from predators.

Male tri-colored bats typically roost alone in foliage. Females of the species often form small maternity colonies. Perry and Thill (2007) reported a mean colony size of 6.9 individuals from exit counts. Both sexes of tri-colored bats have been shown to have roost fidelity. Veilleux (2003) reported the mean use of a single roost at 6.0 consecutive days for 18 female tri-colored

bats. Bats used on average 2.8 roosts during a mean of 9.1 days monitored. Perry and Thill (2007) documented a single male tri-colored bat in a foliage roost for 33 consecutive days.

To date there is no published research that models roost selection for female tri-colored bats in the eastern United States and only a couple for males. Perry and Thill (2007) modeled roost selection for male tri-colored bats in Arkansas and discovered that selected sites contained more large pines, more mid-story hardwoods, lower canopy cover and were farther from the nearest overstory tree than random locations. Macrohabitat features were not considered in their models but are likely significant. O’Keefe et al. (2009) found that distance to non-linear openings and distance to streams were important variables in roost selection for male tri-colored bats in the Southern Appalachians. Furthermore, in Nova Scotia, Quinn and Broders (2007) found that distance to water was an important variable driving roost selection of tri-colored bats.

FORAGING ECOLOGY

Wing morphology determines speed and maneuverability of flying bats and morphological metrics can indicate the degree of clutter bats are adapted to forage in (Lacki et al. 2007). Clutter refers to the amount of foliage and woody debris that can interfere with flight and echolocation transmission. Wing loading describes the size of the wing and is calculated as the bat’s mass divided by the total wing area and is often expressed as g/cm^2 (Menzel et al. 2005). Bats with relatively low wing loading (i.e. large wings relative to body size) generally exhibit greater maneuverability than bats with a high wing loading (Lacki et al. 2007). Low wing loading allows bats to forage in more cluttered areas. Aspect ratio describes wing shape and is calculated by the wingspan squared divided by the wing area (Norberg and Rayner 1987). Bats with high aspect ratio (e.g. *Lasiurus cinereus*) have long, pointed wings and fly at high rates of speed. Conversely, bats with short, blunt wings have low aspect ratio and are generally slow

fliers (e.g. *Myotis sodalis*). Menzel et al. (2005) report the wing loading and aspect ratio of 20 tri-colored bats as 0.10 g/cm² and 2.15 (wing length/wing width), respectively. These values represent relatively low wing loading and moderate aspect ratio indicating that tri-colored bats generally have good maneuverability and are capable of moderate flight speed. Therefore, the species is morphologically adapted to forage in relatively high clutter areas such as forest gaps and riparian forests (Menzel et al. 2005, Lacki et al 2007).

The structure of search-phase echolocation calls is correlated with foraging land cover types and strategy. Tri-colored bats have 5 millisecond-long, broadband search phase echolocation calls that typically consist of two harmonics. A harmonic in a broadband call is two separate ranges of frequencies emitted simultaneously. The first harmonic is the lowest in intensity and sweeps from 18 – 35 kHz, while the second most intense harmonic sweeps from 70–36 kHz (MacDonald et al. 1994). Tri-colored bats are on the high end of the frequency spectrum and often grouped with sympatric species that have characteristic frequencies around 40 kHz (Szewczak et al. 2011). Characteristic frequency is defined as the point near the end of a broadband call with the lowest slope. Bats with high frequency broadband calls generally perform well in cluttered environments, whereas bats with low frequency narrowband calls perform well in open areas (Fenton 1990). However, tri-colored bats have a relatively high intensity call with a relatively long narrowband portion near the characteristic frequency, which indicates a physiological adaption to forage in more open areas (MacDonald et al. 1994, Quinn and Broders 2007).

Tri-colored bats have an aerial hawking foraging strategy (Barclay and Bringham 1991). The species' flight pattern is described as slow and erratic (Fujita and Kunz 1984, Harvey et al. 2011) and they are reported to have consumed 1.4–1.7 g of prey in one 30-minute foraging bout

(Gould 1955). Foraging tri-colored bats are most often associated with riparian areas and forest edges (Fujita and Kunz 1984). In South Carolina using acoustic detectors, Menzel et al. (2003) found that tri-colored bat activity was highest in hydric communities and clearcuts. Furthermore, Schirmacher et al. (2007) found that open or less structurally cluttered riparian areas were important foraging areas for tri-colored bats in Virginia. Only anecdotal accounts of the foraging range size of tri-colored bats are currently available in the literature. Carter et al. (1999) documented a 395.5 ha foraging range for one male tri-colored bat in South Carolina. The foraging range was concentrated in bottomland hardwood and upland pine stands.

Tri-colored bats are strictly insectivorous and are sometimes considered foraging opportunist taking advantage of whatever prey items are available on the landscape (Lacki et al. 2007). Prey items are usually between 4-10 mm in length and consist of a variety of orders including coleopterans, homopterans, dipterans, hymenopterans, and lepidopterans (Amelon 2006). However, on Sapelo Island, Georgia tri-colored bats consumed lepidopterans more than available and coleoptera and homoptera less than available (Carter et al. 1998).

MANAGEMENT IMPLICATIONS

With the current rate of decline and petition for federal protection under the Endangered Species Act, furthering our understanding of the ecology of tri-colored bats is imperative to making informed forest management and regulatory decisions. The summer maternity period is an exceptionally important time in the life-history of bats because of the energetic requirements of reproduction and recovery from WNS, and the energetic demands of hibernation. For most species of bats, basic information about roosting and foraging ecology is lacking (Fenton 2003). Roosts are critical to the survival and reproduction of bats and few reports have focused on foliage roosting species like the tri-colored bat (Carter and Menzel 2007). More research is

needed to determine if geographic variation exists in summer roost selection (Veilleux et al 2003). If drastic declines continue, regulations will only be successful if critical roosting areas can be identified and protected. Except for a couple anecdotal accounts with small sample sizes, size of the tri-colored bat foraging range is unknown. Furthermore, selection of land covers for foraging within the foraging range is unknown. Several studies have employed acoustic detectors to determine foraging land cover associations. This method fails to provide details on the degree to which different land cover types are utilized by individual bats and cannot determine if males and females select resources in different ways. Telemetry studies are needed to determine which land cover types are selected in relation to what land covers are available (Miller et al. 2003, Loeb and O’Keefe 2006). O’Keefe et al. (2009) recommends that future studies evaluate the relative importance of small openings to the roosting and foraging ecology of tri-colored bats in hardwood dominated forests. Our proposed methods will allow us to determine the relative importance of small openings and other land cover features and how roost selection is driven by proximity to these features.

Improving our knowledge of the variables driving roost and foraging range selection of tri-colored bats will enable land managers to more effectively identify, create, and maintain high quality summer habitat. Also, land managers will be better equipped to analyze the effectiveness of current management regimes at providing summer habitat for the species. The continued persistence of tri-colored bats may depend on manager’s ability to provide quality summer habitat, especially in areas affected by WNS. If the species is federally listed for protection under the Endangered Species Act, accurately identifying critical habitats will be important to the success of regulations and ultimately, the recovery of the species.

LITERATURE CITED

- Allen, A.A. 1921. Banding bats. *Journal of Mammalogy* 2:53–57.
- Amelon, S. 2006. Conservation assessment: *Pipistrellus subflavus* (Eastern Pipistrelle) in the Eastern United States. Pages 11-30 in F.R. Thompson, editor. Conservation assessments for five forest bat species in the eastern United States. Gen. Tech. Rep. NC- 260. U.S. Forest Service, North Central Research Station, St. Paul, MN, USA.
- Bernard, R.F., E.V. Willcox, K.L. Parise, J.T. Foster, and G.F. McCracken. 2017. White-nose syndrome fungus, *Pseudogymnoascus destructans*, on bats captured emerging from caves during winter in the southeastern United States. *BMC Zoology* 2:12. DOI 10.1186/s40850-017-0021-2.
- Boyles J.G., P.M. Cryan, G.F. McCracken, and T.H. Kunz. 2011. Economic importance of bats in agriculture. *Science* 332:41–42.
- Campbell, J. 2017. Tennessee Winter Bat Population and White-nose Syndrome Monitoring Report for 2016-2017.
<<http://www.tnbwg.org/2017%20Annual%20Monitoring%20Report.pdf>>. Accessed 26 Oct 2017.
- Carter T.C., and J.M. Menzel. 2007. Behavior and day-roosting ecology of North American foliage-roosting bats. Pages 60–81 in M. J. Lacki, J. P. Hayes, and A. Kurta, editors. *Bats in forests: conservation and management*. Johns Hopkins University Press, Baltimore, Maryland, 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., M.A. Menzel, D.M. Krishon, and D.B. Warnell. 1998. Prey selection of five species of vespertilionid bats on Sapelo Island, Georgia. *Brimleyana* 25:158–170.
- Center for Biological Diversity and Defenders of Wildlife. 2016. Petition to list the tricolored bat *Perimyotis subflavus* as threatened or endangered under the Endangered Species Act. < <https://ecos.fws.gov/docs/petitions/92000/885.pdf>>. Accessed 26 Aug 2016.
- Coleman, J., A. Ballmann, L. Benedict, E. Britzke, K. Castle, W. Cottrell, P. Cryan, T. Deliberto, A. Elliot, R. Ewing, A. Hicks, R. Reynolds, J. Rubado, B. Slack, and L. Williams. 2011. A national plan for assisting states, federal agencies, and tribes in managing white-nose syndrome in bats. U.S. Fish and Wildlife Publications. 454, Washington, D.C., USA.
- 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., and R.M. Barclay. 2009. Causes of bat fatalities at wind turbines: hypotheses and prediction. *Journal of Mammalogy* 90:1330–1340.
- Davis, W.H. 1959. Disproportionate sex ratios in hibernating bats. *Journal of Mammalogy* 40:16–19.
- Davis, W.H., and R.E. Mumford. 1962. Ecological notes on the bat *Pipistrellus subflavus*. *American Midland Naturalist* 68:394–398.
- Fenton, M.B. 1990. The foraging behavior and ecology of animal-eating bats. *Canadian Journal of Zoology* 68:411–422.

- Forbes, G. 2012. Technical summary and supporting information for an emergency assessment of the tri-colored bat *Perimyotis subflavus*. Committee on the status of endangered wildlife in Canada.
- <https://www.registrelepsararegistry.gc.ca/virtual_sara/files/cosewic/ca_pipistrelle_tricolored_bat_0212_e.pdf>. Accessed 22 Dec 2015.
- Fujita, M.S., and T.H. Kunz. 1984. *Pipistrellus subflavus*. Mammalian species. 228:1–6.
- Geluso, K., T.R. Mollhagen, J.T. Tigner, and M.A. Bogan. 2005. Westward expansion of the eastern pipistrelle (*Pipistrellus subflavus*) in the United States, including new records from New Mexico, South Dakota, and Texas. Western North American Naturalist 65:405–409.
- Gould, E. 1955. The feeding efficiency of insectivorous bats. Journal of Mammalogy 36:399–407.
- Guthrie, M.J. 1933. The reproductive cycle of some cave bats. Journal of Mammalogy 14:199–216.
- Harvey, M.J., J.S. Altenbach, and T.L. Best. 2011. Bats of the United States and Canada. John Hopkins University Press. Baltimore, Maryland, USA.
- Hayes, M.A. 2013. Bats killed in large numbers at United States wind energy facilities. Bioscience 63:975–979.
- Hoofer, S.R., and R.A. Van Den Bussche. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. Acta Chiropterologica, 5 supplemental:1–63.

- Horn, J.W., E.B. Arnett, and T.H. Kunz. 2008. Behavioral responses of bats to operating wind turbines. *Journal of Wildlife Management* 72:123–132.
- 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* 245:15–27.
- Jones, C., and J. Pagels. 1968. Notes on a population of *Pipistrellus subflavus* in southern Louisiana. *Journal of Mammalogy* 49:134–139.
- Jones, C., and R. D. Suttkus. 1973. Colony structure and organization of *Pipistrellus subflavus* in southern Louisiana. *Journal of Mammalogy* 54:962–968.
- Kalka M.B., A.R. Smith, and E.K.V. Kalka. 2008. Bats limit arthropods and herbivory in a tropical forest. *Science* 320:71.
- Kunz, T.H., E. Braun de Torrez, D. Bauer, T. Lobova, and T.H. Fleming. 2011. Ecosystem services provided by bats. *Annals of the New York Academy of Sciences* 123:1–38.
- Kurta, A., L. Winhold, J.O. Whitaker, Jr., and R. Foster. 2007. Range expansion and changing abundance of the Eastern Pipistrelle (Chiroptera: Vespertilionidae) in the central Great Lakes region. *American Midland Naturalist* 157:404–411.
- Lacki, M.J., S.K. Amelon, and M.D. Baker. 2007. Foraging ecology of bats in forests. Pages 82–127 in M.J. Lacki, J.P. Hayes, and A. Kurta, editors. *Bats in forests: conservation and management*. Johns Hopkins University Press, Baltimore, Maryland, USA.

- Lacki, M.J., and J.T. Hutchinson. 1999. Communities of bats (Chiroptera) in the Grayson Lake Region of northeastern Kentucky. *Journal of the Kentucky Academy of Science* 60:38–44.
- Loeb, S.C., and J.M. O’Keefe. 2006. Habitat use by forest bats in South Carolina in relation to local, stand, and landscape characteristics. *Journal of Wildlife Management* 70:1210–1218.
- MacDonald, K., E. Matsui, R. Stevens, and M.B. Fenton. 1993. Echolocation calls and field identification of the eastern pipistrelle (*Pipistrellus subflavus* Chiroptera: Vespertilionidae), using ultrasonic bat detectors. *Journal of Mammalogy* 75:462–465.
- McNab, B.K. 1974. The behavior of temperate cave bats in a subtropical environment. *Ecology* 55:943–958.
- Miller, D.A., E.B. Arnett, and M.J. Lacki. 2003. Habitat management for forest-roosting bats of North America: a critical review of habitat studies. *Wildlife Society Bulletin* 31:30–44.
- Minnis, A.M., and D.L. Lindner. 2013. Phylogenetic evaluation of *Geomyces* and allies reveals no close relatives of *Pseudogymnoascus destructans*, comb. nov., in bat hibernacula of eastern North America. *Fungal Biology* 117:638–649.
- Menu, H. 1984. Revision of the status of *Pipistrellus subflavus* (F. Cuvier, 1832). A new generic name is proposed: *Perimyotis* new genus. *Mammalia* 43:409–416.
- 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.

- Menzel, M.A., J.M. Menzel, J.C. Kilgo, W.M. Ford, T.C. Carter, and J.W. Edwards. 2003. Bats of the Savannah River Site and vicinity. U.S. Forest Service, Southern Research Station. Gen. Tech. Rep. SRS-68. Asheville, North Carolina, USA.
- NatureServe. 2016. NatureServe Explorer: An online encyclopedia of life [web application]. Version 7.1. NatureServe, Arlington, Virginia. <<http://explorer.natureserve.org>>. Accessed 17 Aug 2016.
- Norberg, U.M., and J.M.V. Rayner. 1987. Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Philosophical Transactions of the Royal Society of London B 316:335–427.
- O’Keefe, J.M., S.C. Loeb, J.D. Lanham, and H.S. Hill Jr. 2009. Macrohabitat factors affect day roost selection by eastern red bats and eastern pipistrelles in the southern Appalachian Mountain, USA. 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.
- Pierson, E.D. 1998. Tall trees, deep holes, and scarred landscapes. Pages 309-325 in T.H. Kunz and P.A. Racey, editors. Bat biology and conservation. Smithsonian Institution Press. Washington D.C., USA.
- Ploskey, G.R., and J.A. Sealer. 1979. Lipid deposition and withdrawal before and during hibernation in *Pipistrellus subflavus*. Southwestern Naturalist 24:71–78.

- Powers, K.E., R.J. Reynolds, W. Orndorff, and C.S. Hobson. 2015. Post-White-nose syndrome trends in Virginia's cave bats, 2008-2013. *Journal of Ecology and the Natural Environment* 7:123–130.
- Quinn, G.M., and H.G. Broders. 2007. Roosting and foraging ecology of eastern pipistrelle (*Perimyotis subflavus*) in SW Nova Scotia. Report prepared for Nova Scotia Department of Natural Resources. Halifax, Nova Scotia, Canada.
- Salmon, A.B., S. Leonard, V. Masamsetti, A. Pierce, A.J. Podlutsky, N. Podlutska, A. Richardson, S.N. Austad, and A.R. Chaudhuri. 2009. The long lifespan of two bat species is correlated with resistance to protein oxidation and enhanced protein homeostasis. *Journal of the Federation of American Societies for Experimental Biology* 23:2317–2326.
- Samoray, S.T., S.N. Cotham, and M.W. Gumbert. 2019. Spring migration behavior of *Perimyotis subflavus* (Tri-colored bat) from Tennessee. *Southeastern Naturalist* 18: doi.org/10.1656/058.018.0302.
- Schirmacher, M.R., S.B. Castleberry, W.M. Ford, and K.V. Miller. 2007. Habitat associations of bats in south-central Virginia. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies* 61:46–52.
- Szewczak, J.M., S. Parsons, and M.L. Morrison. 2011. Automated acoustic identification of bats and birds. Final Report prepared for Department of Defense. SERDP Project RC-1394.
- Tennessee Bat Working Group 2020. Tri-colored bat.
<http://www.tnbwg.org/TNBWG_PESU.html>. Accessed 10 Aug 2020.

- Tennessee State Wildlife Action Plan Team. 2015. Tennessee State Wildlife Action Plan. Tennessee Wildlife Resources Agency. Nashville, Tennessee, USA.
- Turner, G., D. Reeder, and J. 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* 52:13–27.
- U.S. Fish and Wildlife Service. 2019. Current WNS occurrence map (updated 30 August 2019). <<https://www.whitenosesyndrome.org/about/where-is-it-now>>. Accessed 18 Aug 2019.
- U.S. Fish and Wildlife Service. 2015. Endangered and threatened wildlife and plants; threatened species status for the northern long-eared bat with 4(d) Rule. U.S. Federal Register 80:17974–18033.
- Verant, M.L., C.U. Meteyer, J.R. Speakman, P.M. Cryan, J.M. Lorch and D.S. Blehert. 2014. White-nose syndrome initiates a cascade of physiological disturbances in the hibernating bat host. *BMC Physiology* 14:10.
- Veilleux, J.P., J.O. Whitaker, and S.L. Veilleux. 2003. Tree-roosting ecology of reproductive female eastern pipistrelles, *Pipistrelles subflavus*, in Indiana. *Journal of Mammalogy* 84: 1068–1075.
- Walley, H.D. and W.L. Jarvis. 1971. Longevity record for *Pipistrellus subflavus*. *Illinois Academy of Science* 64:305.
- Whitaker, J.O. 1998. Life history and roost switching in six summer colonies of eastern pipistrelles in buildings. *Journal of Mammalogy* 79:651–659.

Whitaker, J.O., and W.J. Hamilton. 1998. Mammals of the Eastern United States. Cornell University Press. Ithaca, New York.

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.

Wimsatt, W.A. 1945. Notes on breeding behavior, pregnancy, and parturition in some vespertilionid bats of the eastern United States. Journal of Mammalogy 26:23–33.

CHAPTER 2: SUMMER DIURNAL ROOST SELECTION OF MALE TRI-COLORED BATS, *PERIMYOTIS SUBFLAVUS*, ALONG THE CUMBERLAND PLATEAU IN MIDDLE TENNESSEE.

This chapter is modified from a paper in preparation:

Thames, D.B., M.E. Tate, J.R. Campbell, and E.V. Willcox. *In prep.* Summer diurnal roost selection of male tri-colored bats, *Perimyotis subflavus*, along the Cumberland Plateau in middle Tennessee. Planned submission to Southeastern Naturalist.

In this chapter I use the term “we” in reference to my co-authors and myself. The chapter is formatted for publication in the Southeastern Naturalist. I am responsible for all the following: (1) project design and data collection, (2) literature review, (3) statistical analyses, and (4) all writing. My co-authors advised on the design of the project, assisted with field work, and/or editing of the manuscript.

ABSTRACT

Tri-colored bat populations are declining across eastern North America, primarily due to impacts of the fungal disease white-nose syndrome. As regulators and lands managers begin developing and implementing conservation strategies for the species, more information is needed to determine if there is geographic variation in roost selection across its range. The objectives of this research were to characterize the diurnal roosts of tri-colored bats and model roost selection at the forest stand and landscape scale to determine the features that best predict roost selection. In general, tri-colored bats selected roosts in clumps of dead oak and hickory leaves of similar shape as has been reported in other areas. However, males in our study selected roosts lower in the canopy than female tri-colored bats from a previous study. Stand level features were found to be less important to roost selection than landscape level features, likely due to the uniformity of forests in our study area. At the landscape scale, tri-colored bats selected roosts near the intersect of the Eastern Highland Rim and Cumberland Escarpment physiographic subregions. Roosts were located in large tracts of mature forest on the escarpment in close proximity to more open foraging areas on the Eastern Highland Rim. Additionally, tri-colored bats selected roosts further from streams, yet with a higher density of streams in a buffer around the roost tree. Tri-colored

bats may be utilizing streams as corridors for nightly commutes, but actually moving away from streams to roost.

INTRODUCTION

Tri-colored bats, *Perimyotis subflavus*, were considered common throughout eastern North America prior to the introduction of the fungal pathogen *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome (WNS; Fujita and Kunz 1984, Blehert et al. 2009, Harvey et al. 2011). Tri-colored bat populations have declined dramatically in areas where WNS occurs and the United States Fish and Wildlife Service (USFWS) has been petitioned to protect them under the Endangered Species Act (Center for Biological Diversity and Defenders of Wildlife 2016). During winter, tri-colored bats use more caves in eastern Northern America than other sympatric species (Harvey et al. 2011). Therefore, conservation and management efforts should not be limited to winter hibernacula, as these efforts may be inefficient. The summer habitat components of tri-colored bats should also be identified and considered in conservation strategies, especially considering that the species was gradually declining before the introduction of *P. destructans*, indicating WNS is not the only threat to their populations (Ingersoll et al. 2013).

Diurnal roosts are critical to the survival of bats (Carter and Menzel 2007). Summer diurnal roosts are especially important for reproduction, concealment from predators, and protection from adverse weather conditions. Diurnal roosts of tri-colored bats have been documented in man-made structures and many early studies focused on these structures (Allen 1921, Jones and Pagels 1968, Jones and Suttikus 1973, Winchell and Kunz 1996, Whitaker 1998). Diurnal roosts have additionally been documented in Spanish moss (*Tillandsia usneoides*; Davis and Mumford 1962), rock shelters in cliffs (Lacki and Hutchinson 1999), tree hollows

(Whitaker 1998), and lichen (*Usnea spp.*; Quinn and Broders 2007). In forests, tri-colored bats often roost in clumps of dead leaves and oak leaves (*Quercus spp.*) were selected significantly more than other available tree genera by male tri-colored bats in Arkansas and by females in Indiana (Veilleux et al. 2003, Perry and Thill 2007). The species' unique propensity to roost in dead leaf clumps rather than tree cavities and sloughing bark may free tri-colored bats from competition with other sympatric species while still providing concealment from predators and protection from the weather (Veilleux et al. 2003).

Forest stand characteristics and proximity to landscape features (streams, roads, etc.) can influence selection of summer diurnal roosts by bats (Carter and Menzel 2007). In Arkansas, tri-colored bats selected forest stands with more large pines, more mid-story hardwoods, lower canopy cover, and trees further from the nearest over story tree. These characteristics suggest that tri-colored bats favor forests with mature pines, yet structurally complex mid-story hardwoods, with relatively open canopies (Perry and Thill 2007). In Nova Scotia, stand level features were also important to roost selection. Selected forest stands had more softwood trees and fewer trees as tall as the roost tree within 5 m (Quinn and Broders 2007). In South Carolina, proximity of features on the landscape was determined to have more influence on roost selection than forest stand characteristics. Here, tri-colored bats favored roost sites in close proximity to non-linear openings and streams (O'Keefe et al. 2008). Roosting in close proximity to optimal foraging areas, such as forest openings and streams, may decrease the cost of nightly commutes.

More studies are needed to determine if geographic variation in roost selection exists for tri-colored bats (Veilleux et al. 2003). Conservation efforts may need to be regionally specific to protect or augment roosting areas across the species' range. The identification and protection of these areas may be necessary for the development of efficient and effective conservation

strategies. This may be particularly important along the hibernacula and cave rich Cumberland Escarpment physiographic subregion in Tennessee (Tennessee Wildlife Resources Agency 2005). This area has a high density of winter tri-colored bat hibernacula and may represent an important area on the landscape during the summer. Therefore, the objectives of our research were to characterize summer diurnal roosts of tri-colored bats in this region and to determine the stand and landscape characteristics that best predict roost selection.

METHODS AND MATERIALS

Study Area.—We primarily conducted our study on Bear Hollow Mountain Wildlife Management Area in Franklin County, Tennessee. The management area is owned and managed by the Tennessee Wildlife Resources Agency and encompasses approximately 6,880 ha in the Cumberland Plateau and Cumberland Escarpment physiographic subregions of Tennessee (Tennessee Wildlife Resources Agency 2005). The heavily dissected and steep Cumberland Escarpment is primarily composed of mature forests and is rich in karst features. Above the escarpment is the Cumberland Plateau subregion, which is devoid of karst features due to sandstone bedrock that is not water soluble like the predominant limestone of the escarpment. The plateau is also primarily forested; however, a slight proportion of the flatter areas have been cleared for early successional land covers and wildlife openings. During the second year of our study, we focused our capture efforts on private land in the Eastern Highland Rim physiographic subregion to increase our captures of tri-colored bats. This region is primarily composed of more open agricultural type land covers and more development relative to the escarpment and plateau subregions. Average rainfall for the area is 134 cm with an average temperature of 14.6°C (NOAA 2016).

Capture and Telemetry. — Between 26 April and 29 July of 2016/17, we attempted to capture tri-colored bats for a total of 45 nights. We set polyester mist nets (38mm mesh size, Avinet, Inc. Dryden, New York) of varying lengths between 6 m and 12 m and varying heights between 2.6 m and 7.8 m in areas where a high level of bat activity was anticipated. For each bat captured, we recorded species, sex, age, right forearm length, body mass, and reproductive condition. We differentiated juvenile and adult bats by the degree of ossification of the metacarpal epiphyseal gaps (Anthony 1988) and determined reproductive condition of female bats by abdominal palpation, degree of abdominal distention, and examination of the mammae (Racey 1988). We banded tri-colored bats with 2.4mm aluminum alloy forearm bands (Porzana Ltd. United Kingdom) with a unique identification number.

To track tri-colored bats to their diurnal roosts, we used 0.27 g radio transmitters (Holohil Systems Ltd., Ontario Canada) with a battery life of 14 days. We attached transmitters by trimming a small amount of fur from between the scapulae, applying surgical glue (Perma-Type, Plainville, CT, USA) to the exposed skin, and holding the transmitter in the glue for 5–10 minutes until dry. Transmitter mass was on average $4.75\% \pm 0.0036$ ($\bar{x} + SD$) of the bats mass with a range from 4.32% to 5.4%. It is recommended that transmitter load should not exceed 5% of the bats mass (Aldridge and Brigham 1988) and only one individual's transmitter exceeded 5% in our sample. Other studies of tri-colored bats have exceeded this recommendation with average transmitter loads being greater than 5% (Veilleux et al. 2003, Perry and Thill 2007, O'Keefe et al. 2008).

We used 3 or 5 element Yagi-Uda antennas and Wildlife Materials TRX 1000s telemetry receivers (Wildlife Materials, Inc., Murphysboro, Illinois) to track bats to their diurnal roosts. We determined the roost location of each bat daily for the life of the transmitter by physically

walking to the tree and observing the bat with binoculars or using triangulation. Additionally, we conducted emergence counts in an attempt to determine the exact roost location of each individual.

Collection of Roost and Stand Data. — To characterize the roosts of tri-colored bats, we recorded the species of roost tree and the genus of leaf clump for each visually confirmed roost. Two observers independently estimated the percent of vegetation within 2 m above and below the roost and the height of each roost was measured with a clinometer (Suunto, Vantaa, Finland; Veilleux et al. 2003). At visually confirmed roost trees, we established a 0.1-ha plot centered on the roost tree in which to collect forest stand characteristics that may be important to roost selection for tri-colored bats (Table 1; All tables for Chapter 2 are located in Appendix 1). For each tree in the plot with a diameter at breast height (dbh) ≥ 10 cm (O’Keefe et al. 2009), species was recorded and dbh measured (cm) with a dbh tape (Forestry Suppliers, Inc., Jackson, Mississippi). Using a clinometer, we measured the height of the roost tree (m), height of all other trees ≥ 10 cm dbh in the plot, and height to the base of the canopy (m) of the roost tree. We measured the width of the roost tree at two points 90° apart and multiplied the widths by the canopy height (tree height-height to base of canopy) to obtain an index of canopy volume (m^3 ; Perry and Thill 2007). Additionally, two independent observers visually estimated the percent of canopy closure in quadrants located 10 m from the roost tree to the nearest 10%. We averaged estimates for each observer and across the quadrants to get an overall estimate of canopy closure in the plot. To obtain a stem count for the plot, we counted all trees < 10 cm dbh and > 1 m in height. All trees < 10 cm dbh and > 1 m in height were counted to get a stem count for the plot. We collected the same stand level information at random sites that were determined by walking

at a random azimuth a random distance between 50–100 m to the closest canopy tree >10 cm dbh.

At each roost tree, whether visually confirmed or not, a geographic coordinate was recorded with a handheld Garmin GPSmap 64 (Garmin International, Inc., Olathe, Kansas) global positioning system (GPS). We then entered each coordinate into Arcmap 10.3 (ESRI, Redlands, CA) to analyze characteristics outside of the stand at the landscape level (Table 2). Elevation for each roost was also recorded from the GPS. We used the near analysis tool in Arcmap 10.3 to measure the closest distance (m) to features that may influence roost selection of tri-colored bats (i.e., water body, open land cover, streams, non-primary or secondary road, and the intersect of the Eastern Highland Rim and the Cumberland Plateau Escarpment physiographic subregions). The density of features within a 452 m buffer of each roost tree was also determined (i.e., forested land cover, streams, non-primary or secondary roads). We chose a 452 m buffer because this is the furthest distance between any two roosts of a typical bat in our sample. One bat had a much larger maximum distance between two roosts at 4,512 m. However, this individual's roosting behavior was non-typical and a 452 m maximum distance between roosts is similar to what other researchers have reported for male tri-colored bats (O'Keefe et al. 2009). For random points at the landscape scale, a minimum convex polygon was drawn around the roosts of all bats in our sample and the center of the polygon was determined. We buffered the center point by 24.4 km, which represents the furthest distance any one bat was documented foraging from the center of its roosting area (Chapter 3) and drew random points in forested areas within the buffer. We measured elevation for each random point in Delorme Topo North America 10.0 (Delorme, Yarmouth, Maine) and the other variables the same way as for selected roosts.

Data Analysis. — Our tri-colored bat captures were lower than initially expected, so we used roosts as the experimental unit rather than individual bats with the assumption that the observations were independent (Perry and Thill 2007). Additionally, due to our small sample size, we did not attempt to test for temporal variation in roost selection across years. All statistical analyses were conducted in R (R Version 3.3.1, www.r-project.org, accessed 6 Oct 2016).

To determine if male tri-colored bats select certain roost trees more than random, we used a Fisher's exact test. Also, because 4 of the 15 visually confirmed roosts were in leaves that had fallen from a canopy tree and became lodged in a different tree lower in the canopy, we used a Fisher's exact test to determine if genus of leaf clump was selected more than random. Due to the relatively small sample size for these tests, only 3 categories were used: oak, hickory (*Carya spp.*), or other. Since foliage roosting bats are thought to select roosts with little clutter blocking exit and entry below and more clutter above for concealment, we conducted a Welch's *t*-test to determine if clutter or foliage was on average lower below selected roosts than above (Carter and Menzel 2007).

To elucidate which forest stand and landscape characteristics best predicted roost selection, we took an information theoretical approach rather than hypothesis testing (Anderson et al. 2000), as variables that are not necessarily statistically significant may be biologically important. We used logistic regression with tree use as the binomial dependent variable at both spatial scales. Of the 11 potential stand level predictor variables, 3 were removed due to multicollinearity (Table 1). We examined Person correlation coefficients of the independent variables. The variable *rth* had a strong positive correlation ($r \geq 0.74$) with *cnpy_vol* and *m_hght*. The three variables relate to the height or size of trees in the forest stand. Additionally, the

variable *m_dbh* had a strong positive correlation ($r = 0.74$) with *dbhlow*, as both variables relate to the size of trees in the forest stand. We removed the variables *rth*, *m_dbh*, and *m_hght* to eliminate multicollinearity among the independent variables. With these removed, all variance inflation factors (vifs) for the remaining variables in the stand level model were less than 2 (Hair et al. 2004). One confirmed roost tree and random tree observation was removed from the model at the stand scale. This roost tree was in a fence row with open fields on either side, whereas, all other selected roosts were within forest interiors. The random observation had only one tree in the plot, which was in the lawn of a residence. We considered these observations to be too influential in the models. Pearson correlation coefficients amongst independent variables at the landscape scale were acceptable ($r < 0.64$). However, we removed the variable *dist_wtr* because it was redundant with the variable *dist_strms* and inflated the vifs. In the final landscape global model, all variance inflation factors were ≤ 7.8 , so we assume that multicollinearity was not an issue at either spatial scale (Hair et al. 2004). Summary statistics for both stand and landscape variables are provided in Table 3.

For landscape and stand variables, we constructed a global generalized linear model with all potential predictor variables and then the best subset of candidate models was selected using Akaike's Information Criterion for small sample sizes (AICc; Burnham and Anderson 2002). Due to the relatively small sample size, only models with up to 3 explanatory variables were considered (Psyllakis and Anderson 2006, O'Keefe et al. 2009). In total we examined 93 candidate models at each spatial scale because little is known about tri-colored bat roost selection especially in our study area. The model with the lowest AICc value was considered the most likely model; however, candidate models within 2 AICc values of the model with the lowest AICc were considered to have substantial support (Burnham and Anderson 2002). Akaike's

weights were computed to examine the likelihood of the competing models and, additionally, the generalized R^2 was examined for each candidate model to evaluate model fit (Nagelkerke 1991). We averaged the parameter estimates and standard errors for models with a $\Delta AICc < 2$ and calculated the odds ratios from the averaged parameters.

RESULTS

During the summer of 2016, we captured 389 individual bats during 27 nights of netting with tri-colored bats representing 1.8% of our captures. In 2018, during 18 nights of netting we captured 126 individual bats with tri-colored bats representing 4.8% of our captures. In total we captured 12 individual male tri-colored bats and applied transmitters to 8 during the two summers. Additionally, we captured and applied a transmitter to one female. Because male and female tri-colored bats may select roosts differently, we excluded the female from our analyses and one male was not located after being radio-tagged.

We tracked each tri-colored bat daily for 5–15 days (10.57 ± 3.4 ; $\bar{x} + SD$) until the transmitter failed or detached from the bat. Male tri-colored bats used 1–7 diurnal roosts (2.86 ± 2.27) and all 15 of the confirmed roosts were in clumps of dead oak leaves (10) and dead hickory leaves (4) with the exception of one in a live eastern red cedar (*Juniperus virginiana*). To our knowledge, this is the first time a diurnal roost of a tri-colored bat has been documented in an eastern red cedar. While it is speculation, we suspect that at least 2 of the unconfirmed roosts were in live foliage of grapevines (*Vitis sp.*) based on triangulation and signal strength.

Considering only confirmed roosts, male tri-colored bats used a total of 5 genera of tree: oak (7), hickory (4), sugar maple (*Acer saccharum*; 2), eastern red cedar (*Juniperus virginiana*; 1), and winged elm (*Ulmus alata*; 1). However, the actual roosts in dead leaf clumps were only

in oak and hickory leaves, several of which were attached to limbs that had broken and fallen into other trees lower in the forest canopy. Tri-colored bats did not select certain genera of roost tree more than available ($P=1$) or certain clumps of leaves more than available ($P=0.4646$) based on Fisher's exact tests.

On average roost tree height was 18 ± 7.1 m and the actual roosts were on average 9.1 ± 4.5 m from the ground and 7.5 ± 5.1 m below the top of the canopy of the roost tree. Roost height was $52.6 \pm 0.24\%$ of tree height. Roost tree height was on average shorter than random trees ($t = -2.25$, d.f. = 13, $P=0.04$). However, we only selected random trees with a dbh ≥ 10 cm and two of the documented roosts had a dbh < 10 cm, so we cannot be certain that tri-colored bats were actually selecting shorter roost trees than available. We can infer though, that male tri-colored bats were not selecting the tallest available trees. On average, each dead leaf clump selected as a roost had significantly less clutter or vegetation below than above ($t = -4.348$, d.f. = 13, $P < 0.001$).

At the stand scale, a total of 6 models had substantial support with $\Delta AIC_c < 2$. The intercept only null model was the top ranked model and, overall, the remaining models were a poor fit with generalized R^2 values all ≤ 0.15 (Tables 4 and 5). Basal area was present in three of the supported models, followed by stem count in two of the models. Canopy closure and count of trees > 25 cm dbh each appear in one supported model. However, the 95% confidence interval of the averaged parameter estimates for each variable contains 0 and, overall, it appears that stand level variables are not good predictors of roost selection in our study area.

At the landscape scale, only one model had substantial support, with all other models having $\Delta AIC_c > 2$. This model contained three explanatory variables: distance to the intersect of

the Eastern Highland Rim and Cumberland Escarpment physiographic subregions (negative relationship), density of streams, and distance to the nearest stream (positive relationships). The 95% confidence interval of the parameter estimates did not contain 0 for the variables in this model. For every 1000 m increase away from the intersect of the two physiographic subregions, the odds of roost selection is reduced by approximately half (odds ratio: 0.55). For every 100 m increase in distance from the nearest stream, the odds a tree in the area will be selected as a roost increases by a factor of 5.5. Tri-colored bats are roosting in areas further from the nearest stream. For every 100 m increase in the density of streams, the odds of a tree in the area being selected as a roost increases by a factor of 1.197. Even though roosts are further from streams, there is a slightly higher density of streams in the buffer around the roost tree.

Overall, the landscape roost selection model was a good fit to our dataset. It explained almost 80% of the variation in roost selection of male tri-colored bats in our sample. However, due to our relatively small sample size, the model may not transfer to other study areas or datasets and may be overfit. Additionally, some of our random observations took extreme values due to our methods of determining random sites and the heterogeneity of the landscape. While the model is useful in our study area, we urge extreme caution in drawing absolute conclusions and projecting the model to other areas.

DISCUSSION

While tri-colored bats have been found to use various substrates as diurnal roosts across their range, all confirmed roosts in our study were in dead clumps of oak and hickory leaves, with the exception of one that was in the foliage of a live eastern red cedar. All dead leaf clumps were shaped like an umbrella as described in Indiana (Veilleux et al. 2003). Ten of the 15 confirmed roosts were in clumps of dead oak leaves and researchers in Arkansas and Indiana

found that tri-colored bats selected oaks significantly more than available (Veilleux et al. 2003, Perry and Thill 2007). Oaks were not selected more than available in our study potentially because oaks made up 40% of the trees over 10 cm dbh in our plots. We would need a larger sample size to detect selection of oaks as roost. However, this research supplies more evidence that oak trees are an important component of forests as roosts for tri-colored bats.

In Indiana, female tri-colored bat roost tree height averaged 20.8 ± 7.1 m, which is only slightly taller than the roost trees of males in our study 18 ± 7.1 m. However, actual roost height for the males in our study (7.5 ± 5.1 m) was half that of females in Indiana (15.7 ± 6.8 m; Veilleux et al. 2003). Furthermore, roost height relative to roost tree height was on average greater for females in Indiana ($74.7 \pm 17.6\%$) than males in our sample ($56.6 \pm 16.9\%$). In general, air temperature and wind speed decreases lower in the forest canopy and roosts lower in the canopy are more buffered from extremes in the weather (Veilleux et al. 2016). Since males are free from the energetic requirements of pregnancy and lactation, they can withstand lower temperatures and may select sites that are more buffered from the weather than reproductive females. Females may roost higher in the canopy during the maternity season when solar exposure is critical to avoid torpor, which can slow the reproductive process (Racey 1973). Interestingly though, non-reproductive female tri-colored bats roosted higher in the canopy than reproductive female tri-colored bats in Indiana (Veilleux et al. 2016). The authors do concede that time of the year may have influenced the differences between the reproductive classes as selective pressures change through the reproductive cycle.

Stand level characteristics have been shown to influence roost selection for tri-colored bats (Perry and Thill 2003, Quinn and Broders 2007). In our study area, however, stand level variables described only 15% of the variation in roost selection and these variables appeared less

important than landscape level variables. We found that proximity to and densities of features on the landscape are more important to roost selection than stand level features. O’Keefe et al. (2003) had a similar result in South Carolina and speculated that the relatively homogenous forest type in their study may have influenced the result. Similarly, our study area is relatively uniform, especially forests along the Cumberland Escarpment and Plateau subregions where male tri-colored bats roosted. In Arkansas, where stand level characteristics were important, Perry and Thill (2003) describe a study area with a diversity of forest types available for tri-colored bats to utilize.

Forested corridors are often utilized by bats for foraging and commuting (Verboom and Huitema 1997) and riparian areas are particularly important as foraging areas (Grindal et al. 1999). Several researchers have found that foraging tri-colored bats are often recorded with acoustic devices near riparian areas and early successional land covers (Davis and Mumford 1962, Menzel et al. 2003, Loeb and O’Keefe 2006). Additionally, tri-colored bats in our study area selected open water and wetlands within the foraging range significantly more than other land cover types (Chapter 3). In South Carolina, areas closer to the nearest intermittent or perennial stream and with a greater density of intermittent streams were selected as roosts by male tri-colored bats (O’Keefe et al. 2003). The density of intermittent streams also had a slight, yet important, positive relationship with roost selection in our study. We were unable to empirically differentiate intermittent and perennial streams. The Cumberland Escarpment physiographic subregion, where the bats in our sample primarily roosted, is characterized by high gradient, intermittent streams (Hack 1966), so we suspect that streams near the roosts were intermittent. Roosting near areas with a high density of intermittent streams may provide tri-colored bats with corridors leading from roosting areas to their core foraging areas over bodies of

water and more open land covers. In contrast, roosts were selected further from streams in our study area. More research is needed to elucidate how distance to and densities of streams impact roost selection across the species geographic range.

Additionally, roosting near the intersect of the Eastern Highland Rim and Cumberland Escarpment may provide male tri-colored bats with abundant roosting resources in mature forests on the escarpment, yet still in close proximity to more open foraging land covers on the Highland Rim. Every bat in our sample commuted to and foraged on the Eastern Highland Rim. Density of forests within a 452 m buffer of the roost tree was significantly greater than random ($W = 135$, d.f. = 38, $P = 0.0448$). However, the variable was not included as a predictor variable in models with substantial support due to the extremely non-normal distribution of values of random and selected roosts. Regardless, it demonstrates the importance of large tracts of forests for diurnal roosts of male tri-colored bats. In South Carolina, distance to non-linear forest opening had a strong positive relationship with roost selection (O’Keefe et al. 2003). However, their study area was primarily forested with only approximately 5.4% of the area in early successional land cover, so open land covers for foraging may have been a limiting resource. Distance to the nearest open land cover was not an important predictor of roost selection in our study, but in contrast, the Eastern Highland Rim is a largely open landscape and roosts were relatively close to this subregion (Tennessee Wildlife Resources Agency 2005).

Male Indiana bats, *Myotis sodalis*, typically roost near their winter hibernacula (Whitaker and Brack 2002) and our results suggest that male tri-colored bats may exhibit the same behavior. While we can’t be certain that males in our sample were actually roosting near their winter hibernacula, the Cumberland Escarpment is rich in karst features and known tri-colored bat hibernacula (Tennessee Wildlife Resources Agency 2005) and distance to the intersect of this

subregion and the Eastern Highland Rim was an important predictor of roost selection for tri-colored bats. Research is needed on the spring migration patterns of male tri-colored bats to test this hypothesis.

Conservation strategies to protect tri-colored bat roosting areas should protect mature forests where oak and hickory trees are abundant, especially in close proximity to bodies of water where tri-colored bats forage (Menzel et al. 2003, Ford et al. 2005, Chapter 2). While it is unclear if forests in the Cumberland Escarpment physiographic subregion are important due to the presence of hibernacula or if they are simply the largest continuous tract of mature oak hickory forest available, protection of these forest will preserve male tri-colored bat summer roosts.

LITERATURE CITED

- Aldridge, H.D. and R.M. Bringham. 1988. Load carrying and maneuverability in an insectivorous bat: a test of the 5% rule of radiotelemetry. *Journal of Mammalogy* 69:379–382.
- Anderson, D.R., K.P. Burnham, and W.L. Thomson. 2000. Null hypothesis testing: Problems, prevalence and an alternative. *The Journal of Wildlife Management* 64:912–923
- Allen, A. A. 1921. Banding bats. *Journal of Mammalogy* 2:53–57.
- Burnham, K.P., and D.R. Anderson. 2002. Model selection and multimodel inference: a practical information- theoretic approach. 2nd ed. Springer- Verlag, New York.
- 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. Okonieski, R.J.Rudd, and W.B. Stone. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227.
- Carter T.C., and J.M. Menzel. 2007. Behavior and day-roosting ecology of North American foliage-roosting bats. Pages 60-81 in M. J. Lacki, J. P. Hayes, and A. Kurta, editors. *Bats in forests: conservation and management*. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Center for Biological Diversity and Defenders of Wildlife. 2016. Petition to list the tricolored bat *Perimyotis subflavus* as threatened or endangered under the Endangered Species Act. < <https://ecos.fws.gov/docs/petitions/92000/885.pdf>>. Accessed 26 Aug 2016.
- Davis, W.H., and Mumford, R.E. 1962. Ecological notes on the bat *Pipistrellus subflavus*. *American Midland Naturalist* 68:394–398.

- Ford, W.M., M.A. Menzel, J.L. Rodrigue, J.M. Menzel, and J.B. Johnson. 2005. Relating bat species presence to simple habitat measures in a central Appalachian forest. *Biological Conservation* 126:528–539.
- Fujita, M.S., and T.H. Kunz. 1984. *Pipistrellus subflavus*. *Mammalian species*. 228:1–6
- Grindal, S.D., J.L. Morissette, and R.M. Brigham 1999. Concentration of bat activity in riparian habitats over an elevational gradient. *Canadian Journal of Zoology* 77:972–979.
- Hack, J.T. 1966. Interpretation of the Cumberland Escarpment and Highland Rim, south-central Tennessee and northeast Alabama. US Department of the Interior, Geological Professional Paper 524-C.
- Hair Jr., J.F., W.C. Black, B.J. Babin, R.L. Tatham, and R.E. Anderson. 1998. Multivariate data analysis. 5th ed. Prentice-Hall, Upper Saddle River, New Jersey, USA.
- Harvey, M.J., J.S. Altenbach, and T.L. Best. 2011. Bats of the United States and Canada. John Hopkins University Press. Baltimore, Maryland, USA.
- Ingersoll, T.E., B.J. Sewall, and S.K. Amelon. 2013. Improved analysis of long-term monitoring data demonstrates marked regional declines of bat populations in the eastern United States. *Plos One* 8:e65907.
- Jones, C., and J. Pagels. 1968. Notes on a population of *Pipistrellus subflavus* in southern Louisiana. *Journal of Mammalogy* 49:134–139.
- Jones, C., and R.D. Suttus. 1973. Colony structure and organization of *Pipistrellus subflavus* in southern Louisiana. *Journal of Mammalogy* 54:962–968.

- Lacki, M.J., and J.T. Hutchinson. 1999. Communities of bats (Chiroptera) in the Grayson Lake Region of northeastern Kentucky. *Journal of the Kentucky Academy of Science* 60:38–44.
- Loeb, S.C., and J.M. O’Keefe. 2006. Habitat use by forest bats in South Carolina in relation to local, stand, and landscape characteristics. *Journal of Wildlife Management* 70:1210–1218.
- Menzel, M.A., J.M. Menzel, J.C. Kilgo, W.M. Ford, T.C. Carter, and J.W. Edwards. 2003. Bats of the Savannah River site and vicinity. U.S. Forest Service, Southern Research Station. Gen. Tech. Rep. SRS-68. Asheville, North Carolina, USA.
- Nagelkerke, N.J.D. 1991. A note on a general definition of the coefficient of determination. *Biometrika* 78:691–692.
- National Oceanic and Atmospheric Administration. 2016. National Oceanic and Atmospheric Administration National Climate Data Center. Local climatological data. <<http://www.ncdc.noaa.gov/most-popular-data#loc-clim>>. Accessed 13 Jan 2016.
- O’Keefe, J.M., S.C. Loeb, J.D. Lanham, and H.S. Hill Jr. 2009. Macrohabitat factors affect day roost selection by eastern red bats and eastern pipistrelles in the southern Appalachian Mountain, USA. *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.
- Psyllakis, M.J., and R.M. Bigham. 2006. Characteristics of diurnal roosts used by female *Myotis* bats in sub-boreal forests. *Forest Ecology and Management* 223:93–102.

- Quinn, G.M., and H.G. Broders. 2007. Roosting and foraging ecology of eastern pipistrelle (*Perimyotis subflavus*) in SW Nova Scotia. Report prepared for Nova Scotia Department of Natural Resources. Halifax, Nova Scotia, Canada.
- Racey, P.A. 1973. Environmental factors affect the length of gestation in heterothermic bats. *Journal of Reproduction and Fertility*. 19:175–189.
- Tennessee Wildlife Resources Agency. 2005. Tennessee's comprehensive wildlife conservation strategy. Tennessee Wildlife Resources Agency. Nashville, Tennessee, USA.
- Veilleux, J.P., J.O. Whitaker, and S.L. Veilleux. 2003. Tree-roosting ecology of reproductive female eastern pipistrelles, *Pipistrelles subflavus*, in Indiana. *Journal of Mammalogy* 84: 1068–1075.
- Veilleux J.P., J.O. Whitaker, and S.L. Veilleux. 2016. Reproductive stage influences roost use by tree roosting female eastern pipistrelles, *Pipistrellus subflavus*. *Ecoscience*, 11:249–256.
- Venables, W.N., and B.D. Ripley. 2002. Modern applied statistics with S. 4th ed. Springer-Verlag, New York.
- Verboom, B. and H. Huitema. 1997. The importance of linear landscape elements for the pipistrelle, *Pipistrellus pipistrellus*, and the serotinus bat, *Eptesicus serotinus*. *Landscape Ecology* 12:117–125.
- Walters B.L., C.M. Ritzi C.M., D.W. Sparks, and J.O. Whitaker. 2007. Foraging Behavior of Eastern Red Bats (*Lasiurus borealis*) at an urban-rural interface. *American Midland Naturalist* 157:365–373.

- Whitaker Jr., J.O., and V. Brack Jr. 2002. Distribution and summer ecology in the state of Indiana, Pages 48–54, in A. Kurta and J. Kennedy, editors. The Indiana bat: biology and management of an endangered species. Bat Conservation International, Austin, Texas, USA.
- 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.
- Whitaker, J.O. 1998. Life history and roost switching in six summer colonies of eastern pipistrelles in buildings. Journal of Mammalogy 79:651–659.

APPENDIX 1

Table 1. Stand scale covariates used in logistic regression models to generate candidate models describing male tri-colored bat, *Perimyotis subflavus*, roost selection in Franklin, County, Tennessee, USA, 2016/17.

Variable Abbreviation	Units	Description
rth ^a	m	Height of roost or random tree
cnpy_clsr	%	Estimate of overstory canopy closure
dist_cntr	m	Distance from roost tree to the nearest canopy tree
cnpy_vol	m ³	Index of roost tree canopy volume
stm_cnt	n	Number of trees <10 cm dbh and > 1 m tall in a 0.1 ha plot
dbhlow	n	Number of trees 10- 24.9 cm dbh in a 0.1 ha plot
dbhhgh	n	Number of trees > 25 cm dbh in a 0.1 ha plot
ba	m ² /ha	Basal area of the stand
oakhck	%	Percent of <i>Quercus spp.</i> and <i>Carya spp.</i> in a 0.1 ha plot
m_dbh ^a	cm	Mean dbh of trees ≥ 10 cm dbh in a 0.1 ha plot
m_hght ^a	m	Mean height of trees in a 0.1 ha plot with a dbh ≥ 10 cm

^a Variables removed from logistic regression models due to multicollinearity.

Table 2. Landscape scale covariates ^a used in logistic regression models to generate candidate models describing male tri-colored bat, *Perimyotis subflavus*, roost selection in Franklin, County, Tennessee, USA, 2016/17.

Variable Abbreviation	Description
ele	Roost tree elevation
dist_wtr ^b	Distance to nearest water body
dist_open	Distance to nearest open land cover
dist_intrsct	Distance to intersection of the E. Highland Rim and the Cumberland Escarpment physiographic regions
dens_frst	Density of forests in a 452 m buffer
dist_strm	Distance to nearest stream
dens_strms	Density of streams in a 452 m buffer
dist_rd	Distance to nearest road
dens_rd	Density of roads in a 452 m buffer

^a All units are in meters.

^b Variable removed from logistic regression models.

Table 3. Mean ($\bar{x}$) and standard error (SE) of selected roosts of tri-colored bats, *Perimyotis subflavus*, and random sites, results of paired sample *t*-tests for stand level variables, and *t*-tests or Wilcoxon rank-sum test for landscape level variables in Franklin County, Tennessee, USA.

Stand Variable ^a	Selected Roosts (n=14)		Random Trees (n=14)		Test	P value	d.f.
	$\bar{x}$	SE	$\bar{x}$	SE			
rth ^b	17.1	2.2	21	1.4	$t = -2.255$	0.042 ^c	13
cnpy_clsr	0.682	0.032	0.733	0.024	$t = -1.230$	0.240	13
dist_cntr	2.98	0.239	3.29	0.551	$t = -0.352$	0.730	13
cnpy_vol	859	265	921	200	$t = -0.251$	0.806	13
stm_cnt	142	16	112	13.1	$t = 1.730$	0.107	13
dbhlow	27.9	3.53	29.3	4.26	$t = -0.320$	0.754	13
dbhhgh	12.2	1.74	11.6	0.96	$t = 0.382$	0.710	13
ba	22.3	0.97	24.9	1.54	$t = -1.864$	0.085	13
oakhck	0.521	0.062	0.491	0.063	$t = 0.666$	0.517	13
m_dbh	24.4	0.886	26	1.712	$t = -0.960$	0.355	13
m_hght	15.62	0.95	16.32	0.98	$t = -1.232$	0.240	13
Landscape Variable ^a	Selected Roosts (n=20)		Random Points (n=20)		Test	P value	d.f.
	$\bar{x}$	SE	$\bar{x}$	SE			
ele	1412	58	1353	87	$t = -0.564$	0.577	33
dist_wtr	1173	194	1430	257	$W = 216$	0.675	38
dist_open	610	70	575	99	$t = -0.285$	0.777	34
dist_intrsct	1112	266	10030	1768	$t = 6.475$	<0.001 ^c	38
dens_frst	0.917	0.056	0.898	0.038	$W = 135$	0.045 ^c	38
dist_strm	169	34	169	28	$W = 212$	0.766	38
dens_strms	4014	622	1358	145	$W = 113$	0.019 ^c	38
dist_rd	502	53	638	122	$W = 208$	0.839	38
dens_rd	484	180	615	211	$W = 231$	0.352	38

^a Refer to Tables 1 and 2 for a description of each covariate.

^b Two roost trees had a dbh <10 cm and only trees >10 cm dbh were chosen as random trees.

^c Significant at the 0.05 level.

Table 4. Values of Akaike's Information Criterion adjusted for small sample sizes, difference in AICc when compared to the model with the lowest AICc (ΔAICc), Akaike weight (ω_i), and generalized R^2 values of models with $\Delta\text{AICc} < 2$ for models of roost selection of tri-colored bats, *Perimyotis subflavus*, in Franklin County, Tennessee, USA.

Candidate Model ^a	AICc	Δ_i	ω_i	R^2
Stand Scale				
Intercept only	41	0	0.224	0
stm_cnt	41.1	0.09	0.215	0.1023
-ba	41.2	0.18	0.205	0.0983
-cnpy_clsr	41.6	0.61	0.165	0.0792
-ba + dbhhgh	42.6	1.62	0.100	0.1453
-ba + stm_cnt	42.8	1.81	0.091	0.1371
Landscape Scale				
dens_strms - dst_intrsct + dist_strms	28.2	0	1	0.7960

^a The + symbol indicates a positive relationship and - indicates a negative relationship.

Table 5. Model averaged parameter estimates, 95% confidence interval of parameter estimates, adjusted standard errors (S.E.), odds ratios, and sum of Akaike weights of covariates describing roost selection of male tri-colored bats, *Perimyotis subflavus*, in Franklin County, Tennessee, USA. Only one landscape model had substantial support, so estimates are from that one model and sum of Akaike weights is excluded.

Variable	Parameter estimate	95% CI	S.E.	Odds Ratio	$\Sigma\omega_i$
Stand Scale					
ba	-0.129	-0.343–0.085	0.109	0.879	0.40
stm_cnt	0.010	-0.007–0.027	0.009	1.010	0.31
cnpy_clsr	-4.961	-13.10–3.181	4.154	0.007	0.17
dbhgh	0.102	-0.130–0.334	0.118	1.108	0.10
Landscape Scale					
dist_intrsct	-0.001	-1.517– -0.0001	0.0003	0.999	
dens_strms	0.002	4.347–0.005	0.001	1.002	
dist_strm	0.016	1.377–0.040	0.009	1.016	

CHAPTER 3: SUMMER FORAGING RANGE SELECTION OF TRI-COLORED
BATS, *PERIMYOTIS SUBFLAVUS*, ALONG THE CUMBERLAND PLATEAU
IN MIDDLE TENNESSEE.

This chapter is modified from a paper in preparation:

Thames, D.B., M.E. Tate, J.R. Campbell, and E.V. Willcox. *In prep.* Summer foraging range selection of tri-colored bats, *Perimyotis subflavus*, along the Cumberland Plateau in middle Tennessee. Planned submission to the Journal of Wildlife Management.

In this chapter I use the term “we” in reference to my co-authors and myself. The chapter is formatted for publication in the Journal of Wildlife Management. I am responsible for all the following: (1) project design and data collection, (2) literature review, (3) statistical analyses, and (4) all writing. My co-authors advised on the design of the project, assisted with field work, and/or editing of the manuscript.

ABSTRACT

Tri-colored bat populations are declining in Tennessee and throughout the temperate portions of their range, primarily from the impacts of white-nose syndrome. The United States Fish and Wildlife Service has been petitioned to protect the species under the Endangered Species Act. However, limited understanding of the foraging ecology of the species precludes the ability of managers and regulators to effectively identify, manage, protect, and enhance critical summer foraging areas required for listing and designating critical habitat. The objectives of this research were to determine the size of tri-colored bat foraging ranges and to determine if foraging tri-colored bats select certain land cover types significantly more than available. During the summers of 2016 and 2017, we radio-tagged 7 male tri-colored bats and biangulated an average of 102 (range 49–177) foraging locations for each bat. The mean maximum distance traveled from the roost area in a night for male tri-colored bats was 11.4 ± 7.1 km ($\bar{x} + \text{SD}$) and the mean 95% minimum convex polygon foraging range was $2,350 \pm 3,303$ ha. To determine land cover selection of foraging tri-colored bats, we created a fixed kernel density estimate of each individual bat's foraging range and generated a 95% and 50% percentage volume contour (PVC). We compared used versus available land cover proportions at two spatial scales using a MANVO randomization test. Selection of foraging range (95% PVC) within the study area was nonrandom ($\lambda = 0.085$, P

= 0.014). Forests were the least selected land cover and were selected significantly less than other land covers in the study area. Selection of core foraging area (50% PVC) within the foraging range (95% PVC) was also nonrandom ($A = 0.11$, $P = 0.03$). Male tri-colored bats selected core foraging areas over open water and wetlands significantly more than other available land covers.

INTRODUCTION

In eastern North America tri-colored bats, *Peryimyotis subflavus*, are declining rapidly due to the impacts of the introduced fungal pathogen *Pseudogymnoascus destructans* (Pd), the causative agent of the disease white-nose syndrome (WNS; Blehert et al. 2009). White-nose syndrome impacts cave-hibernating bats and is the first sustained epizootic ever documented in bats (Cryan et al. 2010). Tri-colored bats appear to be particularly susceptible to WNS relative to sympatric heterospecifics (Bernard et al. 2017). In Virginia, tri-colored bats at 18 hibernacula declined by 89.5% from 2009 to 2013 (Powers et al. 2015). In Tennessee, similar patterns of decline have been documented in many hibernacula within the state (Campbell 2017). These declines could lead to local extirpations (Frick et al. 2015) and the United States Fish and Wildlife Service (USFWS) has been petitioned to protect tri-colored bats under the Endangered Species Act (Center for Biological Diversity and Defenders of Wildlife 2016). With regulatory decision imminent, it is imperative that decision makers and managers have the best available information to drive effective and efficient conservation and management plans. Foraging areas are an important habitat component for temperate bat species and should be considered in conservation plans (Pierson 1998). Identifying, enhancing, managing, and protecting important foraging sites in spring/summer could accelerate tri-colored bat's recovery from the effects of WNS in winter hibernacula and could ultimately help remnant populations develop resistance to Pd.

Animals utilize resources on the landscape at multiple spatial scales. Johnson (1980) describes four broad orders of resource selection. First order selection refers to an animal's selection of geographic range. Second order selection refers to an animal's home range selection on the landscape and third order selection is the utilization of resources or land covers within the home range. Fourth order selection is the actual food resources consumed by the animal. Many studies have utilized bioacoustic devices to record the echolocation calls of tri-colored bats to analyze land cover associations at both the second and third order of selection. At the landscape scale or the second order of selection, tri-colored bat land cover associations are unclear (Silvis et al. 2016). In Nova Scotia, research with bioacoustics suggest that tri-colored bats are less active in areas where forests are cleared for agriculture or development (Farrow and Broders 2011). However, in Ontario as the amount of forest cover increased the relative abundance of tri-colored bats recorded decreased suggesting that tri-colored bats avoid forests (Ethier et al. 2011).

At the stand scale or third order of selection, there is more agreement across bioacoustic studies and the relationship between forest structure and tri-colored bat activity is better understood (Silvis et al. 2016). In general tri-colored bats appear to gravitate to more open landscapes especially near waterbodies. In West Virginia, tri-colored bats were mostly recorded in riparian areas along watercourse, especially more open stream and river corridors where canopy heights were relatively high (Ford et al. 2005). Along the Coastal Plain of South Carolina, recordings of tri-colored bats were predominantly from lakes, pond, bottomland hardwood forests, and grass-brush land covers (Menzel et al. 2005). In the Appalachian Mountains of South Carolina, tri-colored bats were most often recorded in areas with sparse vegetation and early successional forest stands such as small gaps in intact forests and larger forest openings (Loeb and O'Keefe 2006).

The shortcoming of acoustic studies is that they do not provide information on the availability of land covers and cannot determine the geographic scale at which individual bats are selecting foraging ranges. Radio-telemetry studies are needed to determine the size of a species' foraging range to define availability. Researches have speculated that the foraging range of tri-colored bats is relatively small (Harvey et al. 2011). In Indiana, female tri-colored bats traveled a maximum distance of 4.4 km from roost tree to foraging areas. However, this study did not assess the nightly foraging patterns and the distance represents the maximum distance from capture site to roost tree (Veilleux et al. 2003). In South Carolina, a single male tri-colored bat had a 395-ha foraging range with activity predominantly concentrated in bottomland hardwood forests (Carter et al. 1999).

Radio-telemetry studies are needed to determine foraging range selection in relation to the availability of land covers. There are no published studies to our knowledge of the foraging patterns of tri-colored bats using radio-telemetry (Silvis et al. 2016). The objectives of our research were, therefore, to utilize telemetry equipment to determine land cover selection of tri-colored bats at two spatial scales and to elucidate the size of tri-colored bat foraging ranges. The results of these objectives will give regulators and managers more information on which foraging land covers should be incorporated into conservation strategies and the spatial scale at which to identify foraging areas when roosting areas are identified.

METHODS AND MATERIALS

Study Area. — Our research was conducted in Franklin County, Tennessee. The county has three distinct physiographic subregions. To the northwest half of the county lies the Eastern Highland Rim physiographic subregion. This area is a fragmented landscape with small patches of deciduous forest interspersed among open agricultural fields and development. The Eastern

Highland averages 300 m elevation. To the southeast lies the Cumberland Plateau physiographic subregion. This area is primarily intact mature forest with small patches of early successional areas and development. The Cumberland Plateau averages between 457 m and 549 m in elevation and is considered a true plateau. Between the Eastern Highland Rim and the Cumberland Plateau lies the Cumberland Escarpment. This physiographic subregion is steep and highly dissected by low order ephemeral streams. Caves and karst features are dense in the subregion and due to the rugged steep terrain, it is composed almost entirely of mature forest (Tennessee Wildlife Resources Agency 2005).

Bat Capture and Handling.— Our research was conducted between 26 April 2016 and 29 July of 2017. We used polyester mist nets with 38 mm mesh size (Avinet, Inc. Dryden, New York) of varying lengths between 6 m and 12 m and of varying heights between 2.6 m and 7.8 m to capture flying bats. For each bat captured we recorded species, sex, age, right forearm length, body mass and reproductive condition. We differentiated juvenile and adult bats by the degree of ossification of the metacarpal epiphyseal gaps (Anthony 1988) and reproductive condition of female bats by abdominal palpation, degree of abdominal distention, and examination of the mammae (Racey 1988).

We banded tri-colored bats with 2.4 mm aluminum alloy bands (Porzana Ltd. United Kingdom) with a unique identification number. We radio-tagged male tri-colored bats with 0.27 g VHF radio transmitters (Holohil Systems Ltd., Ontario Canada) with a 14-day battery life. One female tri-colored bat was radio-tagged with a 0.23 g VHF transmitter with a battery life of 7 days. To attach the transmitter, we trimmed a small patch of fur from the mid-scapular region of the bat and used non-toxic surgical cement (The Perma-Type Company Inc., Plainville, Connecticut) to adhere the transmitter to the bat.

Radio-tracking. — On the night of capture, we did not collect foraging locations to reduce the chance of recording non-normal behavior. We tracked each radio-tagged bat from the time it emerged from its diurnal roost through the first or second foraging bout. There was individual variation in the length of the first foraging bout, so for most individuals we recorded foraging locations through the second foraging bout to ensure enough locations for statistical analyses. Two teams used 5 element Yagi-Uda antennas mounted on trucks and Wildlife Materials TRX 1000 telemetry receivers (Wildlife Materials, Inc., Murphysboro, Illinois) to take azimuths to the strongest signal while each bat foraged. Azimuths were taken using a Suunto MCA-D mirror compass (Suunto, Vantaa, Finland). If the bat stopped foraging, we would cease taking azimuths until the bat began foraging again. We used two-way radios for communication to take simultaneous azimuths at ≥ 5 -minute intervals and recorded the time. This interval is commonly used in bat telemetry studies (e.g., Bergeson et al. 2013) and minimizes the risk of autocorrelation, as independence of observations is an implicit assumption of our statistical analyses (Swihart and Slade 1985). We recorded the geographic coordinate from which the azimuth was taken with a Garmin GPSMAP 64 (Garmin International, Inc., Olathe, Kansas). Each corresponding azimuth and telemetry station coordinate was entered into DeLorme Topo USA software (Delorme, Yarmouth, Maine). Using the software, we drew a straight line from the telemetry station coordinate in the azimuth of the strongest signal. The coordinate where corresponding azimuths crossed was recorded as an estimate of the bat's foraging location at a point in time.

We estimated the error of biangulation by activating a transmitter and placing it on the tip of a 3 m pole. We placed the transmitter at 29 random spots on the landscape and a geographic coordinate was recorded. Without knowing the position of the transmitter, two teams then took

azimuths in the direction of the strongest signal from truck mounted Yagi-Uda antennas and telemetry receivers in the same way that azimuths were taken for foraging tri-colored bats. We recorded the coordinates of telemetry stations, known transmitter locations, and azimuths. We then calculated the difference from where each azimuth crossed and the known location of the transmitter using DeLorme Topo USA. Additionally, we calculated the difference in the estimated azimuth and true azimuth for each known transmitter location.

Estimation of Foraging Range.— We entered each foraging location into Geospatial Modeling Environment (Spatial Ecology LLC, www.spataleecology.com, accessed 18 August 2020) to estimate a 95% minimum convex polygon (MCP) foraging range for each individual radio-tagged tri-colored bat. This parametric technique assumes bivariate normality and is a polygon with the least possible area with internal angles <180 degrees drawn around 95% of the foraging locations (Worton 1987). While studies caution against the use of MCPs for intraspecific or interspecific comparison (Nilsen et al. 2007), we report MCP foraging ranges due to the precedent set by other bat foraging studies (e.g., Sparks et al. 2005, Walters et al. 2007, Istvanko et al. 2016, Moore et al. 2017). We use extreme caution in interpreting comparisons of home range size across studies because much of the variation can be explained by study design, study area, and sample size (Nilsen et al. 2007).

Another metric used to examine the size of the foraging range is the mean maximum distance traveled from the roosting area. We determined the center of each bat's roosting area in ArcMap 10.3 (ESRI, Redlands, CA, USA). If the bat used two roost trees, we used the center point between the two trees as the roosting area. If the bat used three or more roost trees, we drew a polygon around the roosts and used the center point of the polygon to define roosting

area. We measured the distance between the roosting area and the furthest foraging point and averaged across individuals.

To examine home range selection of individual tri-colored bats, we examined the utilization distribution using fixed kernel density estimates (KDE). An animal's utilization distribution (UD) is a 3-dimensional estimate of the animal's home range with 2-dimensions being the animal's position on a plane and the third dimension being time. The UD can be interpreted as an estimate of the probability of an animal occurring in an area at a specified time (Worton 1987). The KDE method is a nonparametric technique and, therefore, does not make assumptions about the distribution of the data. The technique has been demonstrated to result in more accurate home range estimates than parametric techniques such as MCP (Worton 1995). The technique works by placing a kernel, whose size is specified by the smoothing parameter, over each observation or foraging location. At any point on the plane the probability density is estimated by adding the contribution from each kernel. (Horne and Garton 2006). A smoothing parameter or kernel size must be specified for a KDE and can impact the accuracy of the estimate (Seaman 1999). We used least-squares cross validation (LSCV) as the smoothing parameter. The LSCV algorithm minimizes the mean integrated square error between the estimated UD and the true UD and is recommended in animal movement studies with sample sizes >30 (Seaman 1999).

From each KDE, which is a raster, we generated a 95% and 50% percent volume contour (PVC) as polygon shapefiles. The interpretation is that there is a 95% or 50% probability that during the bat's first or second foraging bout the bat's location on the plane is within the respective contour. We considered 95% PVC to be the overall foraging range and the 50% PVC to be the core foraging range of each bat.

Compositional Analysis.— For compositional analysis, we evaluated foraging range selection in a hierarchy at two spatial scales to determine the relative importance of used versus available land cover types. Each radio-tagged bat was the sample unit (Aebischer 1993). We extracted land cover categories from the 2016 National Land Cover Database (Jin et al. 2019). Because of our relatively small sample size, we merged land cover categories into four broad categories: forest, open water and wetlands, agricultural areas and grasslands, and developed areas (Table 6; All tables and figures from this chapter are located in Appendix 2). At the second order of selection or selection of foraging range within the study area, we compared proportions of used land covers in the 95% PVC to those in the study area for each individual. We define study area as a buffer of each bats roosting area, the furthest distance any individual travels while foraging. The assumption is that all bats in the sample are capable of flying the furthest distance any one bat flies. At the third order of selection, we compare used land covers in the core foraging range (50% PVC) to those available in the overall foraging range (95% PVC).

We conducted all statistical analyses in R (R Version 3.3.1, www.r-project.org, accessed 6 Oct 2016). The program adehabitatHS in R was used to conduct compositional analyses (Calenge 2006). This method uses a MANOVA randomization test to compare used versus available land cover proportions to determine if selection significantly deviates from random. Where selection is non-random, the relative importance of each land cover category is ranked. We ran the randomization test with 500 iterations and where a used or available land cover proportion was absent, we inserted a small value of 0.001 (Aebischer 1993).

RESULTS

During the summer of 2016, we captured 389 individual bats during 27 nights of netting with tri-colored bats representing 1.8% of our captures. We used the foraging patterns of male

tri-colored bats from 2016 to increase our efficiency the following field season. In 2017, during 18 nights of netting we captured 126 individual bats with tri-colored bats representing 4.8% of our captures. In total we captured 12 individual male tri-colored bats and applied transmitters to eight during the two summers. Additionally, we captured and applied a transmitter to one female tri-colored bat. One radio-tagged male tri-colored bat could not be located after transmitter application.

We tracked each radio-tagged male tri-colored bat for six nights on average and collected an average of 102 ± 41 ($\bar{x} \pm SD$) foraging locations for each individual (Table 7). Generally, it is recommended that transmitter to body mass ratio for bat research should be less than 0.05 (Aldridge and Bingham 1988). Transmitter to body mass ratio in our sample averaged 0.047 for male tri-colored bats with only one individual slightly exceeding the 0.05 rule with a ratio of 0.054. The female tri-colored bat was tracked for 4 nights and we obtained a total of 82 foraging locations. The transmitter to body mass ratio was 0.04 for the female. We estimated the mean error of biangulation to be $7.8 \pm 6.5^\circ$. On average each estimated location was 121 ± 100 m from known locations.

There was variation in the maximum distance traveled amongst individual male tri-colored bats from their roost area and the size of the foraging range (Table 7 and Figures 1-8). The maximum distance male tri-colored bats traveled from the center of their roosting area was 11.4 ± 7.1 km (Table 7). One individual, bat 483, traveled a maximum distance of 24.4 km from its roosting area on the Cumberland Plateau to its core foraging area over Woods Reservoir each night (Figure 4). The mean 95% MCP foraging range for male tri-colored bats was $2,350 \pm 3,303$ ha with a range from 234–9655 ha. The single non-reproductive female tracked had a 95% MCP foraging range of 364 ha.

Several male tri-colored bats in our study demonstrated nightly fidelity to their foraging area with two exceptions. Every night following a rapid commute from its roosting area bat 063 would forage over a large farm pond, bat 483 over Woods Reservoir, bat 663 over a large limestone quarry pond, bat 752 over Cowan Swamp, and bat 785 over Tim's Ford Reservoir (Figures 1, 4, 5, 6, and 7, respectively). Bat 182 foraged over Tim's Ford Reservoir, Cowan Swamp, and farm ponds (Figure 3). It appeared to select either Tim's Ford Reservoir or Cowan Swamp each night, but never both on the same night. Bat 903 foraged over several farm ponds, but only foraged over one pond on separate nights (Figure 8).

Male and female tri-colored bats in our study foraged intermittently. With telemetry equipment it was challenging to pinpoint the exact time, but after emergence bats would generally forage for one to two hours. Then, depending on the individual, they would either stop at a night roost or return presumably to their diurnal roost. Time spent in the night roosts between the first and second foraging bout varied greatly between individuals. For instance, male tri-colored bat 483 only stopped foraging for brief periods of approximately 15 minutes periodically throughout the night. Other individuals would stay in the night roosts for up to two hours before resuming to forage. To the other extreme, male tri-colored bat 903 roosted in a night roost for at least 3 hours after a one-hour foraging bout on two separate nights.

We excluded the female tri-colored from compositional analysis because males and females may select foraging ranges differently (Istvanko et al. 2016). The female tri-colored bat we tracked roosted adjacent to a small tributary of Woods Reservoir on the boarder of Franklin and Coffee Counties. The female's foraging area was concentrated along the tributary and Woods Reservoir (Table 6 and Figure 2).

Second order selection of foraging range (95% PVC) within the study area was significantly non-random ($\lambda = 0.085$, $P = 0.014$) for male tri-colored bats. The longest distance traveled by an individual tri-colored bat in our sample was 24.4 km, so we defined each study area as a buffer with a 24.4 km radius of each bat's roosting area. Forests were used significantly less than other available land covers (Table 9). While we observed individual bats foraging around their roost trees in mature forests along the Cumberland Escarpment and Cumberland Plateau physiographic subregions, each bat commuted away from these forests nightly to forage over more open landscapes of the Eastern Highland Rim.

Third order selection of core foraging range (50% PVC) within the foraging range (95% PVC) was also significantly non-random ($\lambda = 0.11$, $P = 0.03$) for male tri-colored bats. Water/wetlands were selected significantly more than other land cover categories for core foraging areas. Open bodies of water were the destination for each male tri-colored bat's nightly commute from their roosting area. Three of the individuals in our sample had core foraging ranges over Cowan Swamp (Figures 3, 4, and 6). The margins of the swamp are forested, and it contains approximately 14 ha of shallow, open water. However, approximately 2/3 of the open water is covered in emergent vegetation. Three individuals had foraging ranges over either Tim's Ford Reservoir or Woods Reservoir (Figures 3, 4, and 7). Other bodies of water used as core foraging areas were farm ponds (Figures 1 and 8) and one individual's core foraging areas was over a pond created by a limestone quarry (Figure 5).

DISCUSSION

Our research demonstrates that tri-colored bats are capable of long distance flights despite having a flight pattern that is described as weak, slow, and erratic (Fujita and Kunz 1984, Harvey et al. 2011), and a wing morphology more adapted for maneuverability than speed

(Menzel et al. 2005, Lacki et al. 2007). Female tri-colored bats have been documented flying 243 km during spring migration over two days (Samoray et al. 2019) and our study demonstrates that male tri-colored bats fly long distances outside of migration to forage during the summer.

Researchers have speculated that tri-colored bats have small foraging ranges (Harvey et al. 2011). However, our research indicates that male tri-colored bats have larger foraging ranges than many sympatric species. Comparisons of foraging range size using MCP foraging ranges is prone to error (Nilsen et al. 2007), so for discussion we examine the maximum distance traveled while foraging. The mean maximum distance traveled for male tri-colored bats in our study was $11.4 \text{ km} \pm 7.1 \text{ km}$, a longer distance than reported for male *Myotis septentrionalis* ($0.158 \pm 0.127 \text{ km}$; Broders et al. 2006), male *Myotis lucifugus* ($0.275 \pm 0.406 \text{ km}$; Broders et al. 2006), and male *Lasiurus borealis* ($1.4 \pm 7.4 \text{ km}$; Hutchinson and Lacki 1999). However, *Myotis grisescens* were documented to have a greater mean maximum distance traveled ($21 \pm 2.9 \text{ km}$; Moore et al. 2017) than tri-colored bats in our study. While tri-colored bats likely do not have the largest foraging range of bats in the eastern United States, our research suggests that male tri-colored bat foraging ranges are relatively large.

At the landscape scale, or second order of selection, male tri-colored bats in our study tended to avoid forests, corroborating a study using bioacoustics in Ontario (Ethier et al. 2011). At the third order of selection, our study suggests that tri-colored bat core foraging areas tend to be over water bodies. In Nova Scotia, where forest cover and tri-colored bat activity had a positive correlation based on bioacoustic monitoring, all monitoring sites were placed along river corridors. Therefore, tri-colored bats may have been selecting more optimal foraging areas along forested river corridors rather than avoiding areas where forests have been cleared as suggested by the authors (Farrow and Broders 2011). While our study did not seek to assess the relative

importance of forested riparian areas along water bodies or forested edges, previous research has suggested forested edges, whether field edges or riparian areas, are where foraging tri-colored bats are most often encountered (Davis and Mumford 1962). Future research with radio telemetry should examine the relative importance of forest edges for foraging tri-colored bats.

Several studies have found that tri-colored bats are often recorded with bioacoustic devices near riparian areas or watercourses (Menzel et al. 2003, Ford et al. 2005, Schirmacher et al. 2007). Generally, waterbodies and riparian zones are important foraging areas for insectivorous bats relative to upland areas due the availability of emergent insects (Grindal et al. 1999). Our research indicates that tri-colored bats are no exception and select core foraging area over bodies of water. Nightly commutes from roost to foraging area have been documented in other species of bats (e.g., *Myotis lucifugus*; Bergeson et al. 2013), but this is the first time it has been documented in tri-colored bats. Like *M. lucifugus*, male tri-colored bats in our study would rapidly commute from their roost tree to their core foraging areas near water.

Sexual segregation has been document in forest dwelling bats in eastern North America (Istvanko et al. 2016). We actively tracked one female tri-colored bat that roosted adjacent to, and foraged over, Woods Reservoir. Other research using aerial telemetry found similar foraging and roosting patterns with female tri-colored bats at Arnold Air Force Base around the reservoir (J. Lamb unpublished data). It appears that female tri-colored bats may have smaller foraging ranges than males and may select roosts nearer to core foraging areas over bodies of water to avoid the energetical cost of nightly commutes. Additionally, while nursing, female bats return to the maternity roost between foraging bouts to nurse and provide maternal care to pups (Henry et al. 2002). This need to provide maternal care to pups may preclude female tri-colored bats from roosting far from their core foraging areas which our research suggests is over bodies of water.

Additionally, tri-colored bats are considered regional migrants and the need to be spatially near core foraging areas for reproduction may explain why females migrate and males, we speculate, remain near hibernacula in the reproductive season (Samoray et al. 2019). More research is needed on foraging range selection of female tri-colored bats to provide more evidence for this hypothesis.

Conservation and management plans for tri-colored bats should consider foraging areas at broad spatial scales across the landscape due to the large foraging ranges of male tri-colored bats. Waterbodies appear to be particularly important features on the landscape for foraging tri-colored bats. Creating and managing wetlands and ponds will potentially augment and improve tri-colored bat foraging areas. Our research did not directly assess the importance of forested edges and forested riparian areas for tri-colored bats. However, based on anecdotal observations from our research and previous research (Davis and Mumford 1962), we speculate that both features are important and maintaining forested corridors for commuting and forested riparian areas for foraging may be critical for tri-colored bat conservation.

LITERATURE CITED

- Aebischer, N.J., P.A. Robertson, and R.E. Kenward. 1993. Compositional analysis of habitat use from radio-tracking data. *Ecology* 74:1313–1325.
- Aldridge, H.D., and R.M. Bringham. 1988. Load carrying and maneuverability in an insectivorous bat: a test of the 5% rule of radiotelemetry. *Journal of Mammalogy* 69:379–382.
- Anthony, E.L.P. 1988. Age determination in bats. Pages 47–57 in T.H. Kunz, editor. *Ecological and behavioral methods for the study of bats*. Smithsonian Institution Press, Washington, D.C., USA.
- Bergeson, S.M., T.C. Carter, and M.D. Whitby. 2013. Partitioning of foraging resources between sympatric Indiana and little brown bats. *Journal of Mammalogy* 94:1311–1320.
- Bernard, R.F., E.V. Willcox, K.L. Parise, J.T. Foster, and G.F. McCracken. 2017. White-nose syndrome fungus, *Pseudogymnoascus destructans*, on bats captured emerging from caves during winter in the southeastern United States. *BMC Zoology* 2:12. DOI 10.1186/s40850-017-0021-2.
- 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. Okonieski, R.J. Rudd, and W.B. Stone. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227.
- 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.

- Calenge, C. 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. *Ecological Modeling* 197:516–519.
- Campbell, J. 2017. Tennessee Winter Bat Population and White-nose Syndrome Monitoring Report for 2016–2017.
<<http://www.tnbwg.org/2017%20Annual%20Monitoring%20Report.pdf>>. Accessed 17 Aug 2020.
- 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.
- Center for Biological Diversity and Defenders of Wildlife. 2016. Petition to list the tricolored bat *Perimyotis subflavus* as threatened or endangered under the Endangered Species Act.
<<https://ecos.fws.gov/docs/petitions/92000/885.pdf>>. Accessed 26 Aug 2016.
- 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.
- Davis, W.H., and R.E. Mumford. 1962. Ecological notes on the bat *Pipistrellus subflavus*. *American Midland Naturalist*. 68:394–398.
- Ethier, K., and L. Fahrig. 2011. Positive effects of forest fragmentation, independent of forest amount, on bat abundance in eastern Ontario, Canada. *Landscape Ecology* 26:865–876.
- Farrow, L.J., and H.G. Broders. 2011. Loss of forest cover impacts the distribution of the forest dwelling tri-colored bat (*Perimyotis subflavus*). *Mammalian Biology* 76:172–179.

- Ford, W.M., M.A. Menzel, J.L. Rodrigue, J.M. Menzel, and J.B. Johnson. 2005. Relating bat species presence to simple habitat measures in a central Appalachian forest. *Biological Conservation* 126:528–539.
- Frick, W.F., S.J. Puechmaille, J.R. Hoyt, B.A. Nickel, K.E. Langwig, J.T. Foster, K.E. Barlow, T. Bartonička, D. Feller, A. Haarsma, C. Herzog, I. Horáček, J. van der Kooij, B. Mulkens, B. Petrov, R. Reynolds, L. Rodrigues, C.W. Stihler, G.G. Turner, and M.A. Kilpatrick. 2015. Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography* 24:741–749.
- Fujita, M.S. and T.H. Kunz. 1984. *Pipistrellus subflavus*. *Mammalian species*. 228:1–6
- Grindal, S.D., J.L. Morissette, and R.M. Brigham. 1999. Concentration of bat activity in riparian habitats over an elevational gradient. *Canadian Journal of Zoology* 77:972–977.
- Harvey, M.J., J.S. Altenbach, and T.L. Best. 2011. *Bats of the United States and Canada*. John Hopkins University Press. Baltimore, Maryland, USA.
- Henry, M., D.W. Thomas, R. Vaundry, and M. Carrier. 2002. Foraging distances and home range of pregnant and lactating little brown bats (*Myotis lucifugus*). *Journal of Mammalogy* 83:767–774.
- Horne J.S., and E.O. Garton. 2006. Likelihood cross-validation versus least squares cross-validation for choosing the smoothing parameter in kernel home-range analysis. *Journal of Wildlife Management* 70:641–648.
- Hutchinson J.T., and M.J. Lacki. 1999. Foraging behavior and habitat use of red bats in mixed mesophytic forests of the Cumberland Plateau, Kentucky. Pages 171–177, *in* J.W.

- Stringer and D.L. Lofis, editors. Proceedings, 12th central hardwood forest conference. USDA Forest Service, Southern Research Station, General Technical Report SRS-24. Asheville, North Carolina, USA.
- Istvanko, D.R., T.S. Risch, and V. Rolland. 2016. Sex-specific foraging habits and roost characteristics of *Nycticeius humeralis* in north-central Arkansas. *Journal of Mammalogy* 97:1336–1344.
- Jin, S., C.G. Homer, L. Yang, P. Danielson, J. Dewitz, C. Li, Z. Zhu, G. Xian, and D. Howard. 2019. Overall methodology design for the United States National Land Cover Database 2016 products. *Remote Sensing* 11:19.
- Johnson, D.H. 1980. The comparison of usage and availability measurements for evaluating resource preference. *Ecology* 61:65–71.
- Lacki, M.J., S.K. Amelon and M.D. Baker. 2007. Foraging ecology of bats in forests. Pages 82–127 in M. J. Lacki, J. P. Hayes, and A. Kurta, editors. *Bats in forests: conservation and management*. Johns Hopkins University Press, Baltimore, Maryland, USA.
- Loeb, S.C., and J.M. O’Keefe. 2006. Habitat use by forest bats in South Carolina in relation to local, stand, and landscape characteristics. *Journal of Wildlife Management* 70:1210–1218.
- Menzel J.M., M.A. Menzel, J.C. Kilgo, 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.

- Menzel, M.A., J.M. Menzel, J.C. Kilgo, W.M. Ford, T.C. Carter and J.W. Edwards. 2003. Bats of the Savannah River Site and vicinity. U.S. Forest Service, Southern Research Station. Gen. Tech. Rep. SRS-68. Asheville, North Carolina, USA.
- Moore, P.A., T.S. Risch, D.K. Morris, and V. Rolland. 2017. Habitat use of female gray bats assessed using aerial telemetry. *Journal of Wildlife Management* 81:1242–1253.
- Nilsen, E.B., S. Pedersen, and J.D.C. Linnell. 2007. Can minimum convex polygon home ranges be used to draw biologically meaningful conclusions? *Ecological Research* 23:635–639.
- Pierson, E.D. 1998. Tall trees, deep holes, and scarred landscapes. Pages 309–325 in T.H. Kunz and P.A. Racey, editors. *Bat biology and conservation*. Smithsonian Institution Press. Washington D.C., USA.
- Powers, K.E., R.J. Reynolds, W. Orndorff, and C.S. Hobson. 2015. Post-white-nose syndrome trends in Virginia's cave bats, 2008-2013. *Journal of Ecology and the Natural Environment* 7:123–130.
- Racey, P.A. 1988. Reproductive assessment in bats. Pages 31–43 in T.H. Kunz, editor. *Ecological and behavioral methods for the study of bats*. Smithsonian Institution Press, Washington, D.C., USA.
- Samoray, S.T., S.N. Cotham, and M.W. Gumbert. 2019. Spring migration behavior of *Perimyotis subflavus* (Tri-colored bat) from Tennessee. *Southeastern Naturalist* 18: doi.org/10.1656/058.018.0302.

- Schirmacher, M.R., S.B. Castleberry, W.M. Ford, and K.V. Miller. 2007. Habitat associations of bats in south-central Virginia. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies* 61:46–52.
- Seaman, D.E., J.J. Millsaugh, B.J. Kernohan, G.C. Brundige, K.J. Raedeke, and R.A. Gitzen. 1999. Effects of sample size on kernel home range estimates. *Journal of Wildlife Management* 63:739–747.
- Silvis, A., R.W. Perry, and W.M. Ford. 2016. Relationships of three species of bats impacted by white-nose syndrome to forest condition and management. Gen. Tech. Rep. SRS–214. Asheville, NC: U.S. Department of Agriculture Forest Service, Southern Research Station. 48 p.
- Sparks, D.W., C.M. Ritzi, J.E. Duchamp, and J.O. Whitaker. 2005. Foraging habitat of the Indiana bat (*Myotis sodalis*) at an urban-rural interface. *Journal of Mammalogy* 86:713–718.
- Swihart, R.K., and S.A. Slade. 1985. Testing for independence of observations in animal movements. *Ecology* 66:1176–1184.
- Tennessee Wildlife Resources Agency. 2005. Tennessee’s comprehensive wildlife conservation strategy. Tennessee Wildlife Resources Agency. Nashville, Tennessee, USA.
- Veilleux, J.P., J.O. Whitaker, and S.L. Veilleux. 2003. Tree-roosting ecology of reproductive female eastern pipistrelles, *Pipistrelles subflavus*, in Indiana. *Journal of Mammalogy* 84: 1068–1075.

Worton, B.J. 1987. A review of models of home range for animal movement. *Ecological Modelling* 38:277–298.

Worton B.J. 1995. Using Monte Carlo simulations to evaluate kernel-based home range estimators. *Journal of Wildlife Management* 59:794–800.

APPENDIX 2

Table 6. Land cover categories used for compositional analysis of male tri-colored bat, *Perimyotis subflavus*, foraging ranges in Franklin County, TN, USA, and the 2016 National Land Cover Database categories that were merged for the analyses.

Land cover categories used for Compositional Analyses	2016 National Land Cover Database ^a categories merged for compositional analyses
Forests	Deciduous Forest Evergreen Forest Mixed Forest Shrub/Scrub
Water/ Wetlands	Open Water Barren Land Woody Wetlands Emergent Herbaceous Wetlands
Development	Developed Open Space Development- Low Intensity Development- Medium Intensity Development- High Intensity
Open Ag./ Grasslands	Grassland/Herbaceous Pasture/Hay Cultivated Crops

^a Jin et al. 2019

Table 7. Transmitter to body mass ratio, number of nights tracked, number of foraging locations, size of the foraging range, and maximum distance traveled from the center of the roost area for tri-colored bats, *Perimyotis subflavus*, tracked in Franklin County, Tennessee in 2016/17.

Bat ID	Transmitter to body mass ratio	Nights Tracked	Number of Foraging locations	Foraging Range Size 95% MCP (ha)	Foraging Range Size 95% FK (ha)	Foraging Range Size 50% FK (ha)	Maximum Distance from roost area (km)
Male Tri-colored bats							
63	0.049	6	111	1117	242	22	8.3
182	0.043	6	114	2306	490	73	17.2
483	0.047	7	177	9655	1617	275	24.4
663	0.049	5	89	991	348	56	7.9
752	0.054	6	110	366	60	11	6.6
785	0.045	6	67	1783	1245	214	11.6
903	0.045	5	49	234	224	40	3.9
Average	0.047	5.9	102	2350	604	99	11.4
Female Tri-colored bat							
124	0.040	4	82	364	316	77	3.2

Table 8. Ranking matrices from compositional analyses of tri-colored bat, *Perimyotis subflavus*, foraging range selection in Franklin County, Tennessee, USA, 2016/17.

	Water/ Wetlands	Developed	Open Ag./ Grasslands	Forests	Rank
Second Order Selection ^{a,b}					
Water/ Wetlands		+	+	+++	3
Developed	-		-	+++	1
Open Ag./ Grasslands	-	+		+++	2
Forests	---	---	---		0
Third Order Selection ^{a,c}					
Water/ Wetlands		+++	+++	+++	3
Developed	---		-	-	0
Open Ag./ Grasslands	---	+		+	2
Forests	---	+	-		1

^a The symbol "+++" indicates that the land cover is selected significantly more than the corresponding land cover at $P = 0.05$. The "---" symbol indicates that the land cover is selected significantly less than the corresponding land cover at $P = 0.05$.

^b Second order selection compares used proportions of land covers in the foraging range to those available in the study area.

^c Third order selection compares the proportion of land covers in the core foraging area to those in the foraging range.

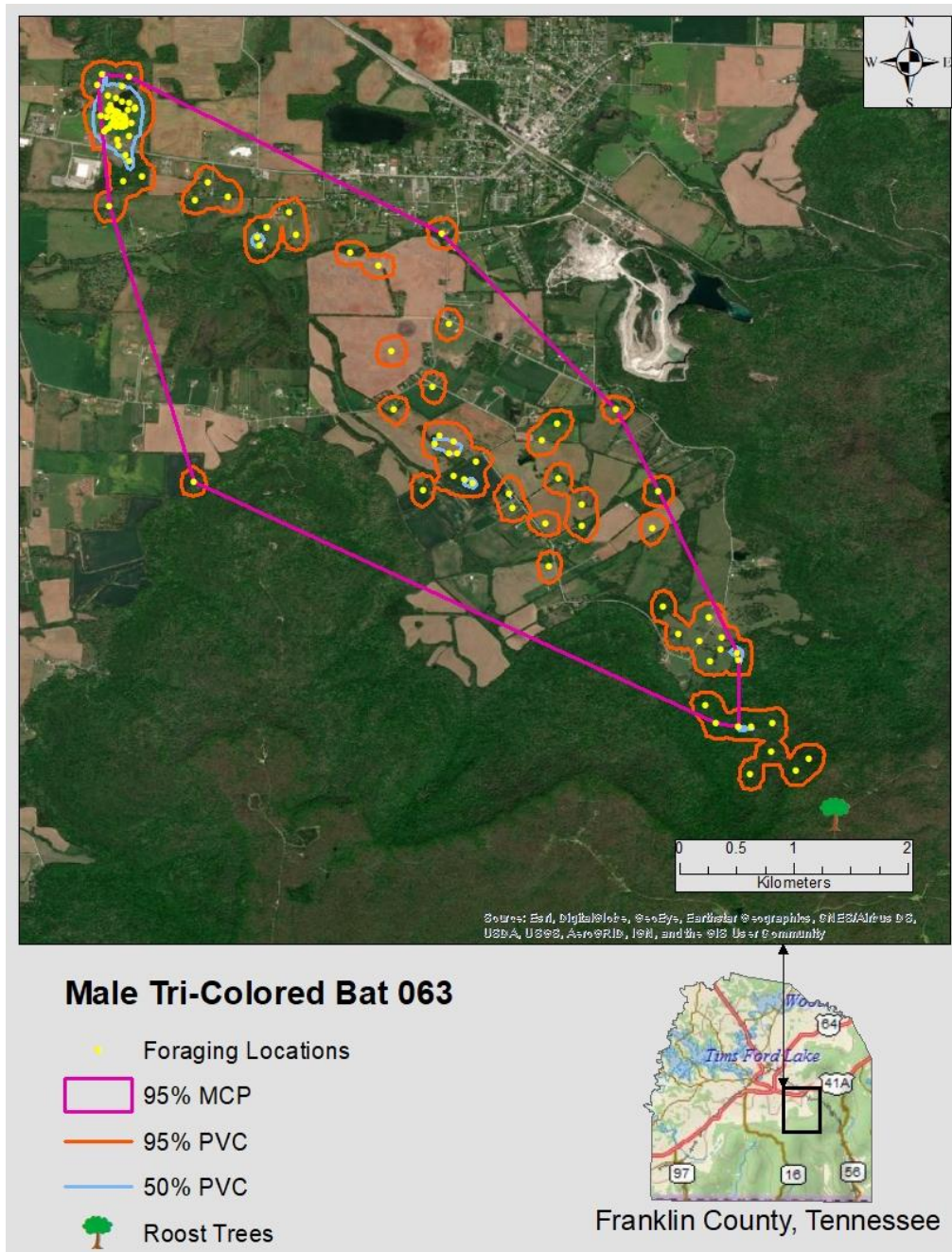


Figure 1. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus*, (ID = 063) in Franklin County, Tennessee, USA.

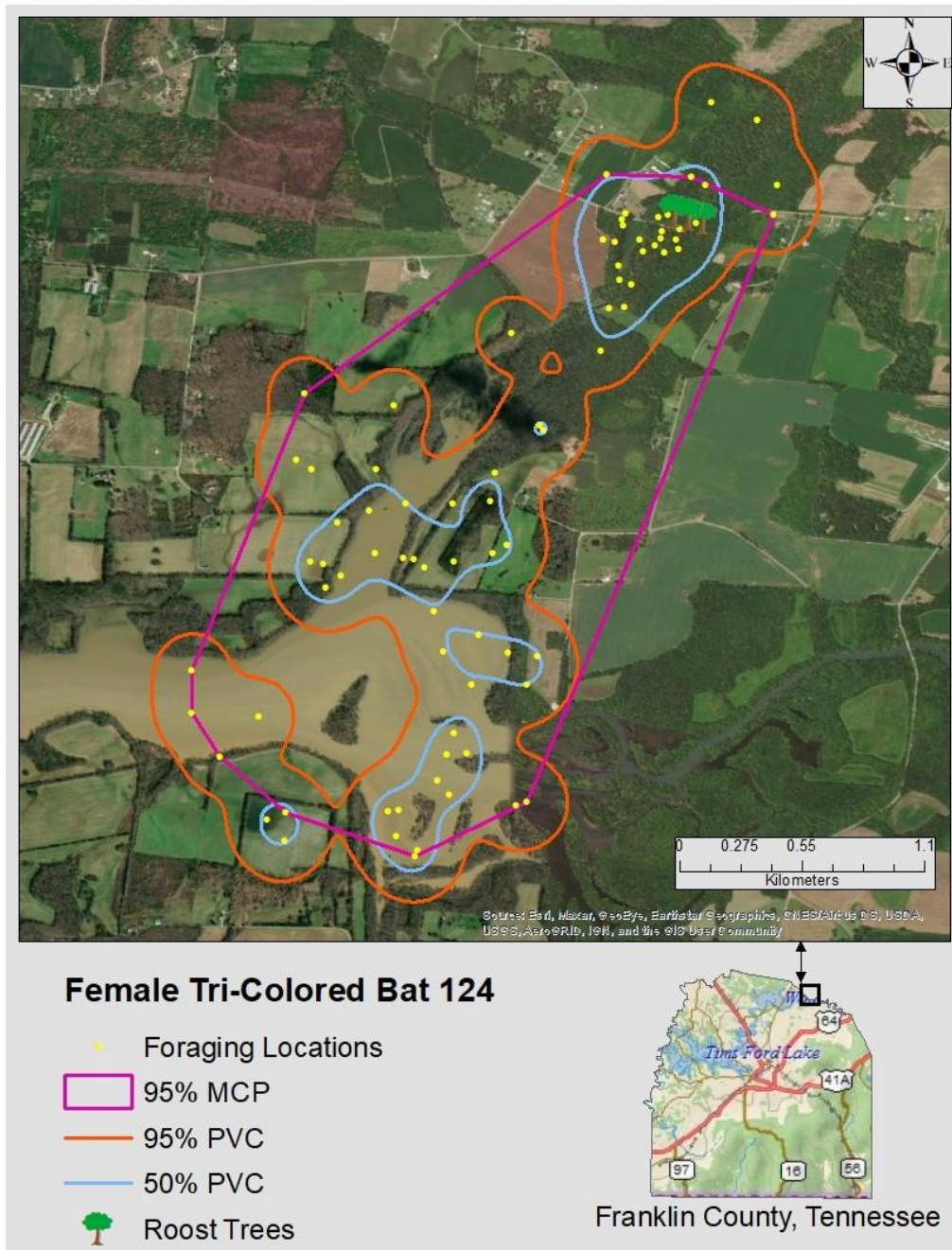


Figure 2. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a female tri-colored bat, *Perimyotis subflavus*, (ID =124) in Franklin County, Tennessee, USA.

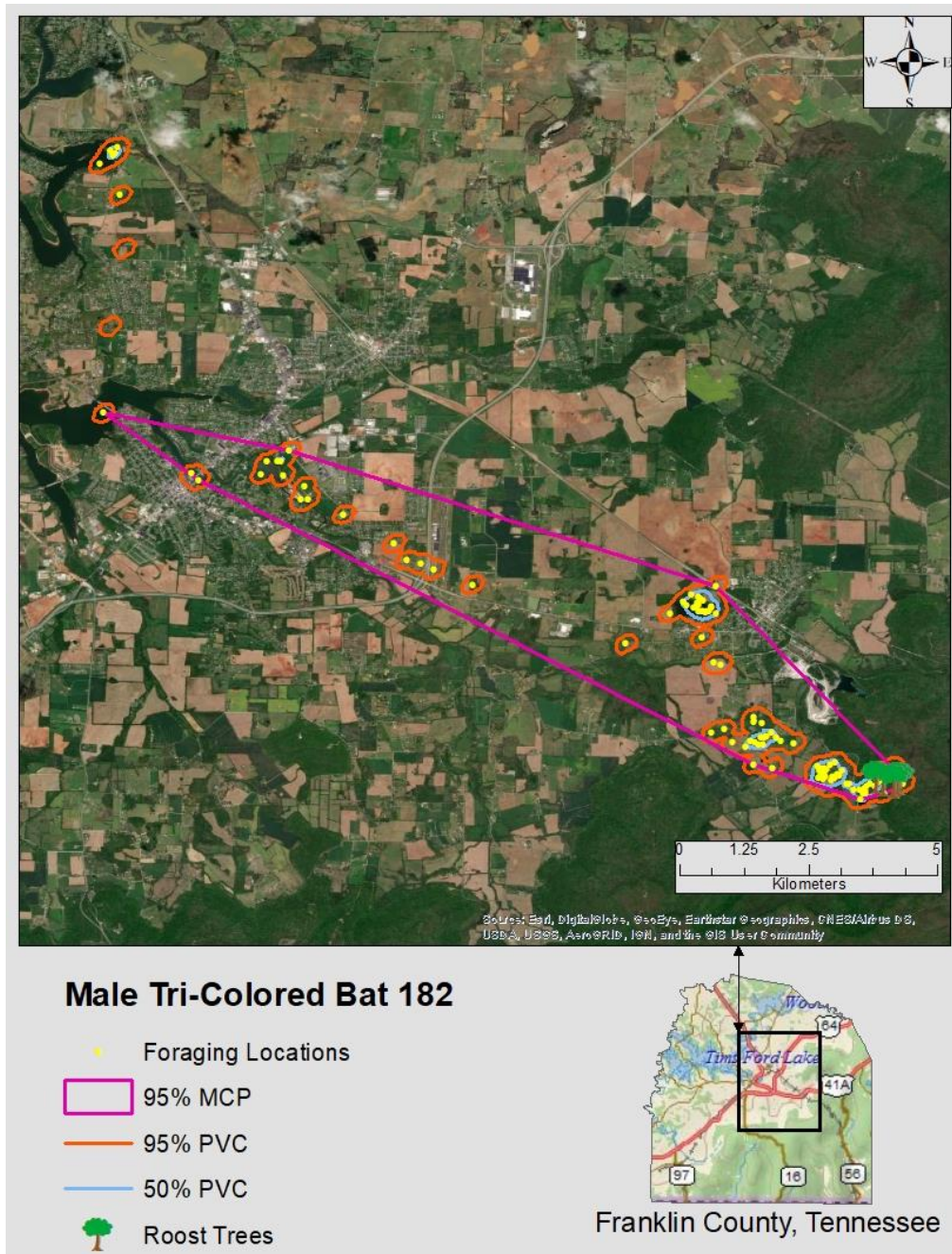


Figure 3. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus*, (ID = 182) in Franklin County, Tennessee, USA.

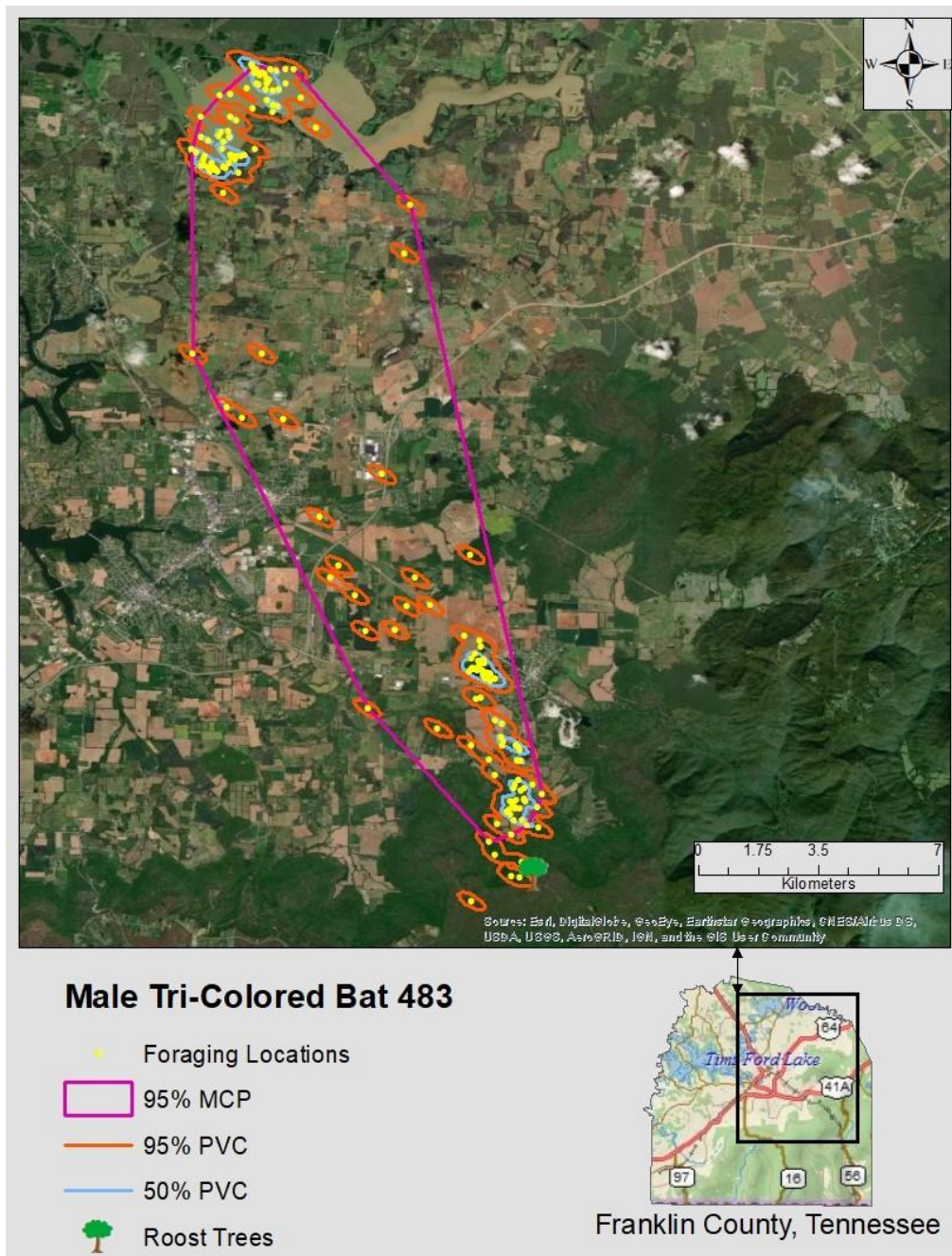


Figure 4. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus* (ID = 483) in Franklin County, Tennessee, USA.

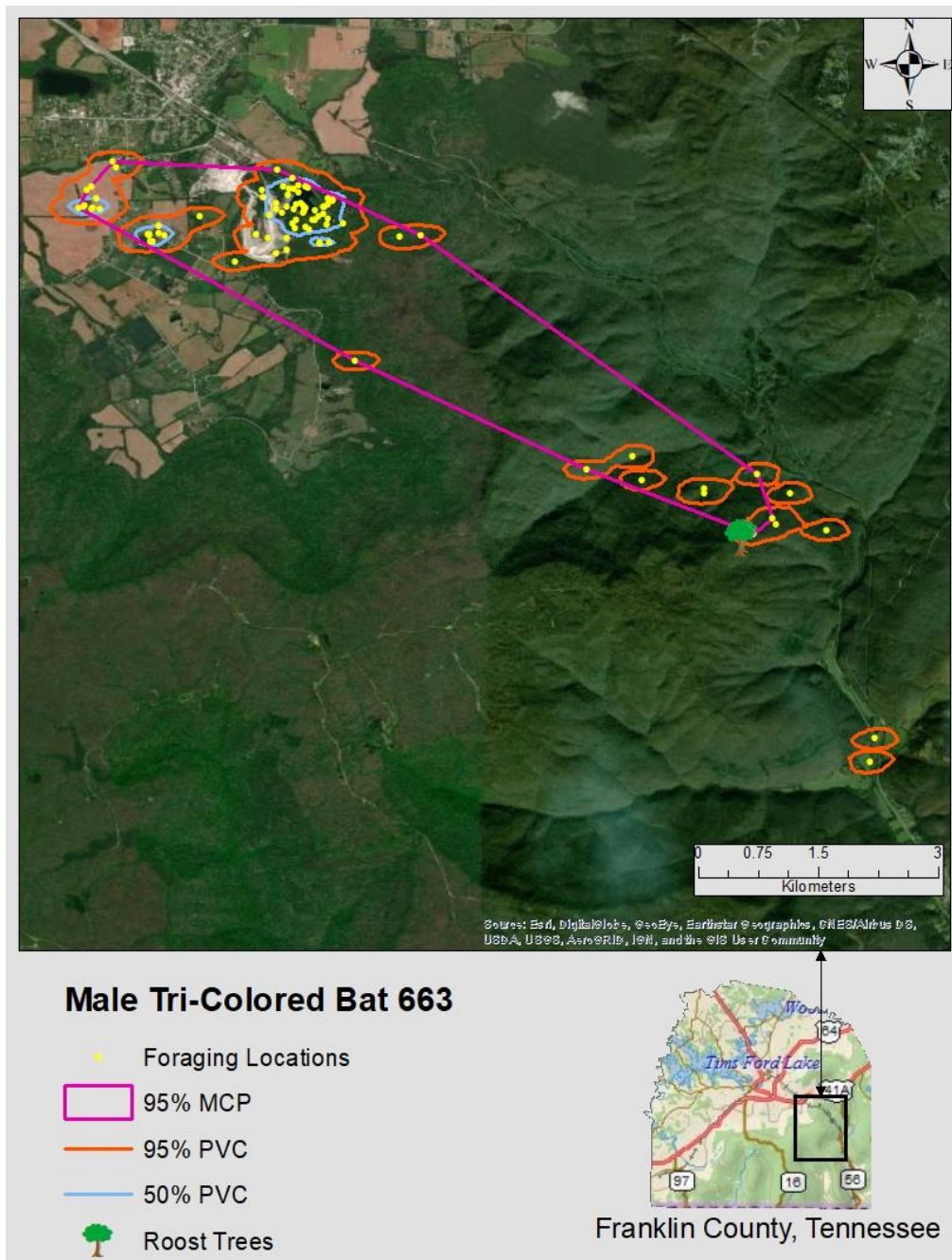


Figure 5. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus*, (ID = 663) in Franklin County, Tennessee, USA.

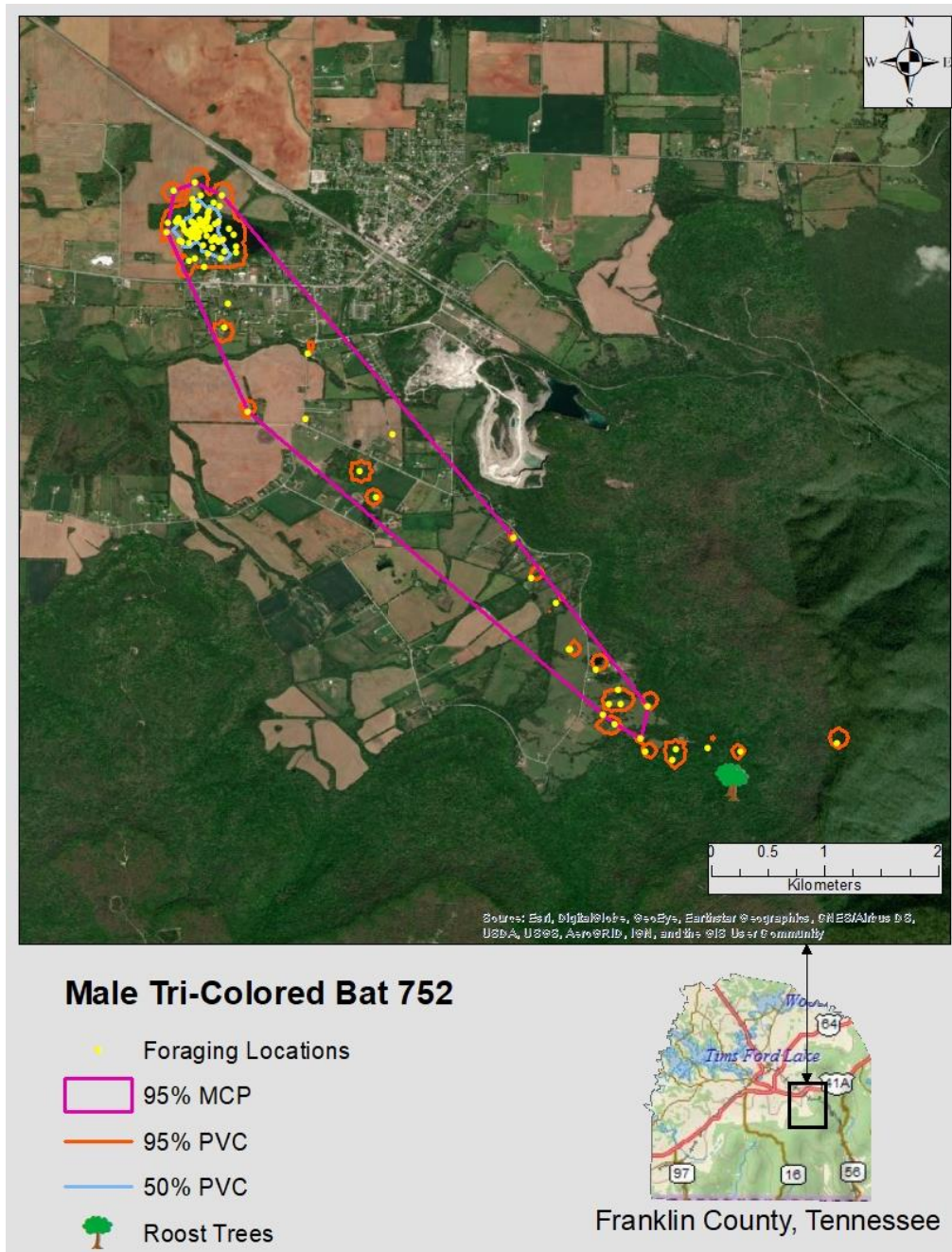


Figure 6. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus* (ID = 752) in Franklin County, Tennessee, USA.

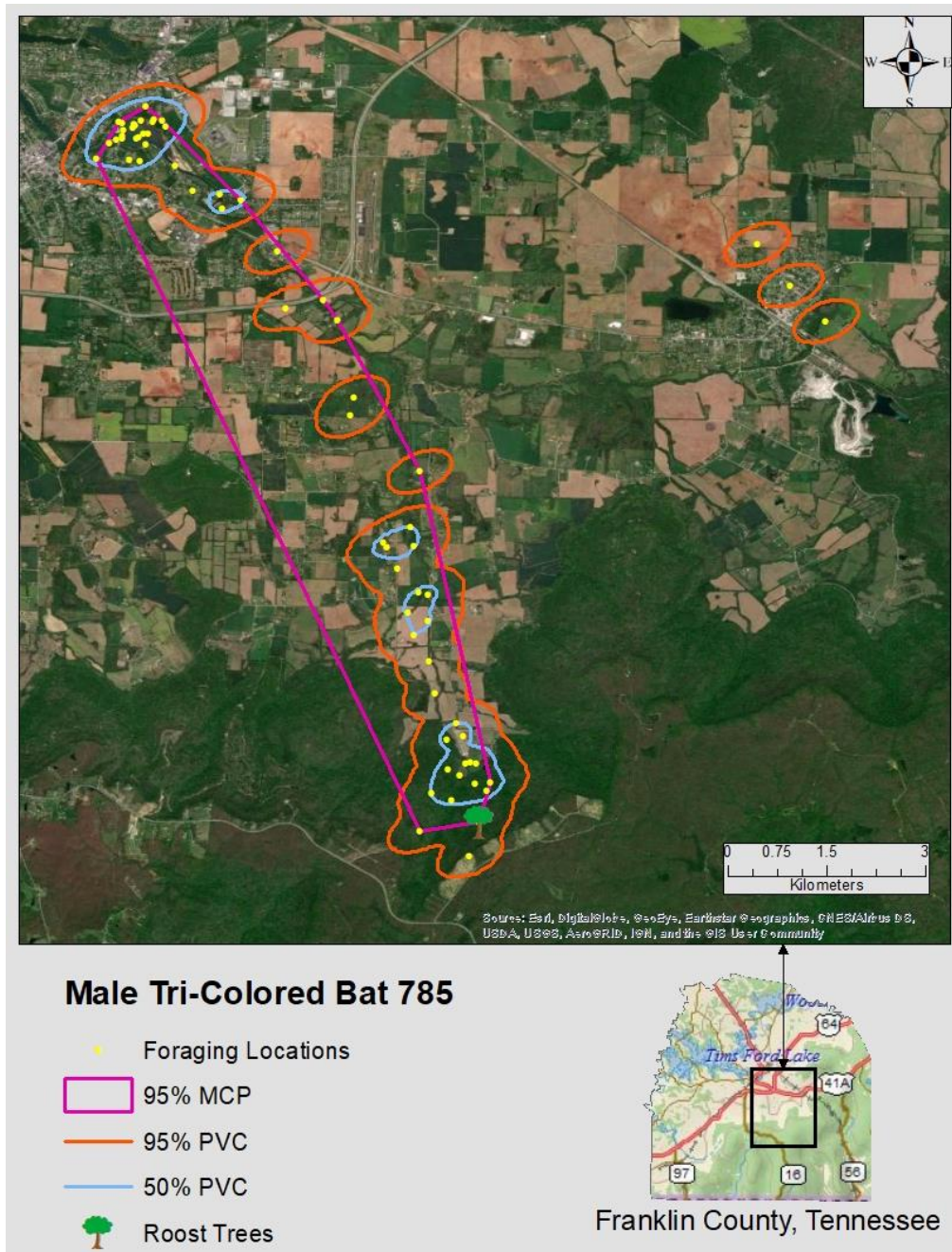


Figure 7. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus*, (ID = 785) in Franklin County, Tennessee, USA.

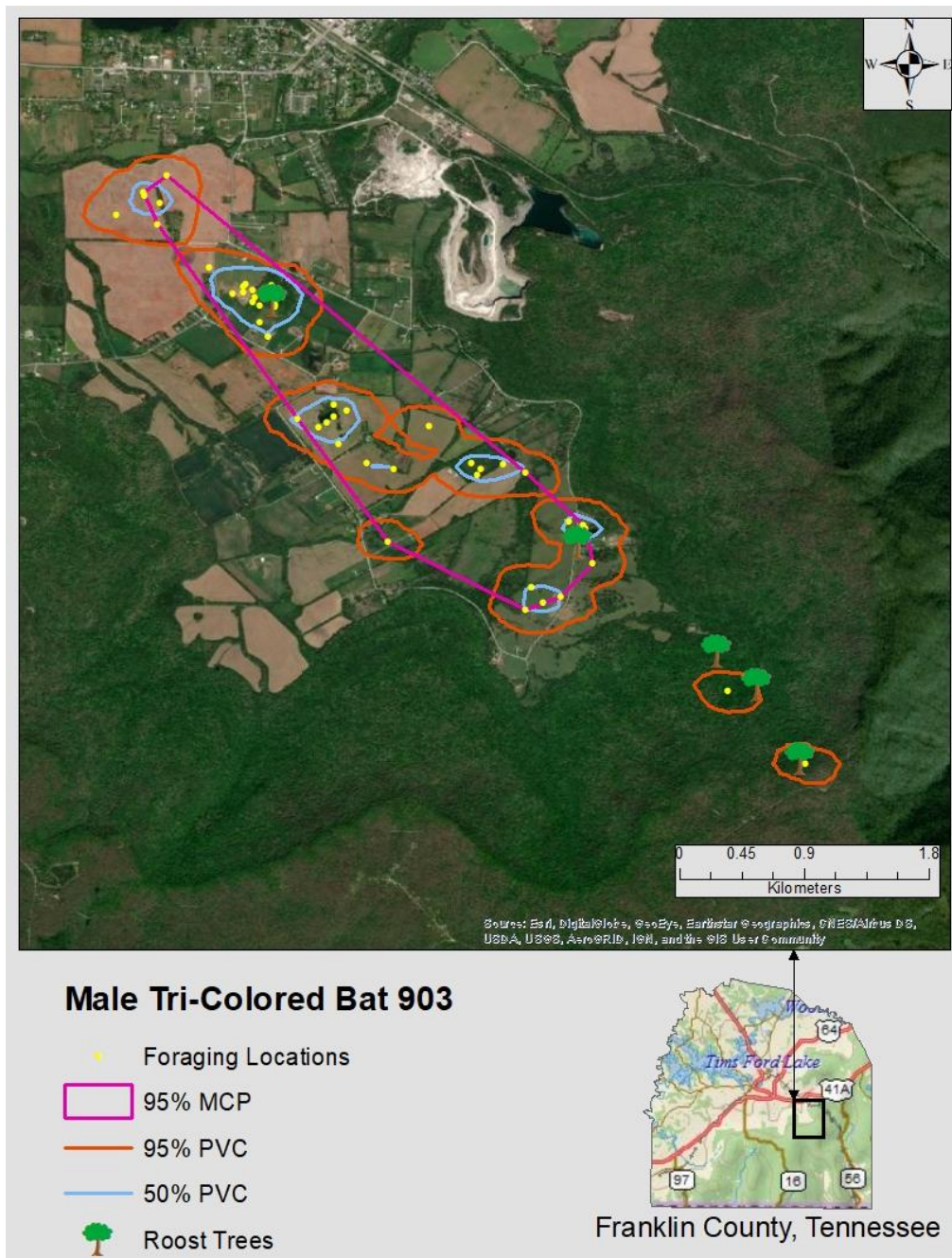


Figure 8. Estimated foraging locations, 95% minimum convex polygon (MCP), 95% and 50% percent volume contours derived from fixed-kernel density home range estimation and roost trees of a male tri-colored bat, *Perimyotis subflavus* (ID = 903) in Franklin County, Tennessee, USA.

VITA

Dustin Thames was born in Winchester, Tennessee. He graduated from Tullahoma High School in 2000 and attended Motlow State Community for 2 years before transferring to Tennessee Technological University in Cookeville, Tennessee. Dustin graduated with a Bachelor of Science degree in Wildlife and Fisheries Science in 2005. After receiving his undergraduate degree, Dustin completed a summer internship with the National Forest Service conducting rare plant surveys, and then worked miscellaneous landscaping and maintenance jobs before getting hired on with the Tennessee Wildlife Resources Agency (TWRA) in 2007 as a Fisheries Management Technician. Dustin has been fortunate to receive several promotions with TWRA and currently serves as a regional Biodiversity Survey Coordinator. He joined the Willcox lab in 2015 to research tri-colored bats while working full time.