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WINTER TORPOR AND ROOSTING ECOLOGY OF TRI-COLORED BATS (*PERIMYOTIS
SUBFLAVUS*) IN TREES AND BRIDGES

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

by
Blaise Ashley Newman
May 2020

Accepted by:
David S. Jachowski, Committee Chair
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Patrick G. R. Jodice

ABSTRACT

Subterranean hibernating tri-colored bats (*Perimyotis subflavus*) have experienced precipitous declines from white-nose syndrome (WNS). However, tri-colored bats also use thermally unstable roosts like tree cavities, bridges, and foliage during winter. Our objective was to determine where tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts lie on the torpor continuum to understand their potential WNS susceptibility, as well as determine roost use and selection in an area devoid of subterranean roosts. From November to March 2017-2019, we used temperature-sensitive radio-transmitters to track bats to their day roosts and document their torpor and activity patterns on the Savannah River Site in south-central South Carolina. We measured habitat and tree characteristics of 24 used trees and 153 random trees and used discrete choice models to determine selection. Torpid bout duration (mean 2.7 ± 2.8 days SD) was negatively related to ambient temperature and positively related to precipitation. Bats maintained a non-random arousal pattern focused near dusk and were active on 33.6% of tracked days. Of arousals, 51% contained a passive rewarming component. Normothermic bout duration, general activity, and activity away from the roost were positively related to ambient temperature, and activity away from the roost was negatively related to barometric pressure. Days were cooler ($8.7^{\circ}\text{C} \pm 5.0$) when bats used bridges than on days that they used trees ($11.3^{\circ}\text{C} \pm 5.4$). Roost selection was negatively related to stream distance and

tree decay state and positively related to canopy closure and cavity abundance. Bats also appeared to favor hardwood forests and avoid pine forests. Tri-colored bats using thermally unstable roosts at SRS displayed winter torpor more reminiscent of daily torpor than classic hibernation. Our results suggest tri-colored bats in thermally unstable roosts may be less susceptible to white-nose syndrome than hibernating tri-colored bats in thermally stable roosts. Our results also suggest that access to multiple roost microclimates may be important for tri-colored bats during winter and forest management practices which retain live trees near streams with multiple roosting structures and foster cavity formation in hardwood forests will likely benefit this population. An understanding of tri-colored bat winter torpor and roosting ecology in areas devoid of subterranean roosts is increasingly important due to WNS-related declines of populations using subterranean hibernacula.

DEDICATION

Dedicated to my loving family and friends who are always there to help me find the fun in every challenge and keep a smile on my face. To paraphrase the song *Try Everything*, “Bats don’t just fly, they fall down and get up.”

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CHAPTER ONE

WINTER TORPOR OF TRI-COLORED BATS (*PERIMYOTIS SUBFLAVUS*) USING THERMALLY UNSTABLE ROOSTS

1. Introduction

Winter represents a time of negative energy balance for insectivorous temperate-zone bats due to decreased insect availability and increased thermoregulatory costs to maintain normothermic body temperatures (Turbill 2008; Jonasson and Willis 2012). Bats use torpor, or heterothermy, to overcome these challenging winter conditions. Torpor is an energy conservation strategy characterized by the controlled lowering of metabolic rate and, consequently, body temperature below normothermic levels (Geiser 2004). Torpid bouts in hibernators can last for days or weeks and result in energy savings as high as 97% of normothermic metabolic rates. However, all torpid bouts are interrupted by brief and energetically expensive arousals, during which a bat returns to normothermic body temperatures (Thomas et al. 1990; Geiser 2004). Normothermic periods are necessary for hibernators to recover from the physiological costs associated with metabolic depression and hypothermia including accumulated metabolic wastes (Humphries et al. 2003), reduced immunocompetence (Field et al. 2018), dehydration (Thomas and Cloutier 1992), and sleep deprivation (Daan et al. 1991). Despite representing a small fraction of a hibernator's time, arousals account for 83-90% of the energy expended during hibernation (Thomas et al. 1990). Therefore, hibernating bats must balance the energetic costs of normothermia with the

physiological and ecological costs (e.g., predation, missed opportunity) of being torpid (Humphries et al. 2003; Boyles et al. 2020).

Heterotherms employ a continuum of torpor between the two temporal extremes of daily torpor and hibernation to balance torpor costs and benefits (Canale et al. 2012; Boyles et al. 2013; Ruf and Geiser 2015; van Breukelen and Martin 2015; McNab and O'Donnell 2018). Hibernators maintain lower metabolic rates and body temperatures than daily heterotherms and are capable of multiday torpid bouts lasting more than 30 days (Geiser 2004; Ruf and Geiser 2015). In contrast, daily heterotherms generally remain torpid for only 3-12 hrs. In addition, hibernators have random patterns of arousal, while daily heterotherms remain entrained with the light-dark cycle and usually arouse prior to normal activity hours. In bats capable of hibernation, a species' position along the continuum reflects its specific behaviors, local climate and weather conditions, and roost microclimates (Ruf and Geiser 2015; McNab and O'Donnell 2018).

Identifying a species or population's position along the torpor continuum is increasingly important in North America because winter behaviors influence white-nose syndrome (WNS) susceptibility. WNS is an invasive fungal disease which has killed millions of North America's hibernating bats (Langwig et al. 2012; Frick et al. 2015; Powers et al. 2015) since its initial detection in 2006 (Blehert et al. 2009). Under amenable growth conditions (< 19.5°C; Verant et al. 2012) the causal fungal agent of WNS, *Pseudogymnoascus destructans* (Pd), invades and colonizes the cutaneous tissues

of the muzzle, ears, and wings of hibernating bats (Blehert et al. 2009). Affected bats experience a multi-stage disease progression with increased torpid metabolic rates and evaporative water loss resulting in more frequent arousals and premature fat depletion (Reeder et al. 2012; Warnecke et al. 2013; Verant et al. 2014; McGuire et al. 2017). Because bats near the hibernation end of the torpor continuum rely heavily on stored fat reserves to survive winter, disruption of natural hibernation patterns resulting in premature fat depletion can be fatal for these species. Hibernation and the thermally stable microclimates of subterranean hibernacula provide ideal conditions for Pd infection, WNS development, and subsequent disease related mortality.

Tri-colored bats (*Perimyotis subflavus*) are widely distributed across eastern North America (Geluso et al. 2005) and have experienced striking declines since the onset of WNS. Across five years of WNS spread (2007-2011), tri-colored bat populations in hibernacula in the northeastern U.S. declined between 16% in Virginia and 95% in New York (Turner et al. 2011). In the Southeast, tri-colored bat populations have declined > 91% in Georgia and South Carolina hibernacula (P. Pattavina, Southeast Regional White-nose Syndrome Coordinator, U.S. Fish and Wildlife Service, pers. comm.; S. Loeb, Research Ecologist, U.S. Forest Service Southern Research Station, unpub. data). The consistently high mortality rates of tri-colored bats in southeastern U.S. hibernacula, in contrast to the wide range observed in the Northeast, may be due to the region's relatively warm hibernacula temperatures (Sirajuddin 2018; Lutsch 2019) resulting in faster fungal growth and disease severity (Langwig et al. 2016). However, tri-

colored bats also use thermally unstable, non-cave roosts in regions devoid of subterranean hibernacula throughout the Southeast. Non-cave roosts used by tri-colored bats include bridges in Louisiana (Ferrara and Leberg 2005) and South Carolina (S. Loeb, Research Ecologist, U.S. Forest Service Southern Research Station, unpub. data), culverts in Texas (Sandel et al. 2001; Meierhofer et al. 2019a, 2019b) and Georgia (Lutsch 2019), and tree cavities in South Carolina (S. Loeb, Research Ecologist, U.S. Forest Service Southern Research Station, unpub. data).

Tri-colored bats using thermally unstable roosts are of interest because they may exhibit winter torpor patterns that offset the typical consequences of Pd infection similar to other species using thermally unstable roosts. For example, passive rewarming (exogenous heating prior to arousal) is rarely observed in subterranean hibernating species due to the thermally stable microclimates of most hibernacula, but tree-hibernating bats in the Southern Hemisphere (Turbill and Geiser 2008; Stawski et al. 2009; Czenze et al. 2017a) and non-subterranean hibernating bats of North America (Halsall et al. 2012) passively rewarm from torpor in thermally unstable roosts. Passive rewarming can result in energy savings of 20-47% per arousal compared to active rewarming and reduces cardiovascular demands and oxidative stress (Halsall et al. 2012; Currie et al. 2015). Additionally, bats in thermally unstable roosts frequently have short torpid bouts (i.e., < 5 days) and high winter activity levels associated with seasonally warm ambient weather conditions (Turbill and Geiser 2008; Stawski et al. 2009; Czenze et al. 2017b). These bats also often maintain non-random arousal patterns, similar to

daily torpor users, which allows them to exploit favorable foraging conditions and supplement winter fat reserves (Turbill 2006; Stawski and Geiser 2010; Johnson et al. 2012). Big brown bats (*Eptesicus fuscus*) and Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) with high winter activity levels (Johnson et al. 2012; Reynolds et al. 2017) and little brown bats (*Myotis lucifugus*) with increased body fat (Cheng et al. 2019) are less susceptible to WNS than bats with naturally low winter activity levels and minimal body fat. Therefore, bats in thermally unstable roosts with winter torpor patterns more reminiscent of daily torpor than hibernation may be less susceptible to WNS due to energetically conservative arousals, high winter activity levels, and winter fat supplementation.

Our objective was to determine where tri-colored bats using thermally unstable roosts lie on the torpor continuum. We hypothesized tri-colored bats using winter roosts with thermally unstable microclimates would exhibit torpor patterns closer to the daily torpor end of the continuum than the hibernation end. We predicted 1) tri-colored bats would use passive rewarming when possible, 2) that bat torpor and activity patterns would be related to ambient weather conditions because bats can track these conditions in thermally unstable roosts, and 3) that bats would exploit intermittent feeding opportunities by maintaining a non-random arousal pattern around dusk and increasing activity on nights with favorable foraging conditions. Our findings will add to

the growing knowledge base of torpor in thermally unstable roosts and help inform conservation actions related to WNS in the Southeastern US.

2. Methods

2.1 Study area

We conducted our study from November through March 2017-2018 and 2018-2019 on the Department of Energy's Savannah River Site (SRS) located in the Upper Coastal Plain of South Carolina (Figure 1). SRS is an 80,267 ha National Environmental Research Park ideal for studying non-subterranean hibernating tri-colored bats because it is devoid of caves, mines, and other subterranean hibernacula. Dominant vegetation types on SRS were loblolly pine (*Pinus taeda*), longleaf pine (*P. palustris*), and bottomland hardwood (e.g. *Nyssa* spp., *Quercus* spp.) forests (Imm and McLeod 2005). We focused our research efforts around three previously identified concrete I-beam bridges (Loeb and Zarnoch 2011). Tri-colored bats frequently hung from the ribbed metal decking under these bridges which provided easy walk-in access to hand-capture bats.

2.2 Field data collection

During daylight hours, we visually inspected the three study bridges for tri-colored bats and hand-captured available bats from the bridge ceiling. For each captured bat we determined sex, reproductive condition, and wing condition, and measured body mass and forearm length. Prior to transmitter attachment, we banded

each bat with a 2.4 mm aluminum lipped band (Porzana Ltd., East Sussex, UK). We trimmed hair between bats' scapulae and affixed a 0.42 g LB-2XT temperature-sensitive transmitter (Holohil Systems Ltd., Ontario, Canada) using surgical glue (Perma-Type Company, INC). After a minimum 15-minute holding period to ensure transmitter attachment, we placed bats back in their original roosting location. We also swabbed bats for the presence of Pd following protocols of the laboratory of Dr. Christopher Cornelison, Kennesaw State University, who analyzed these samples using qPCR. Swab samples from 2017-2018 were negative for the presence of Pd, but one of nine swabs from 2018-2019 had a presumptive positive detection of Pd. The real-time PCR assay for Pd provides a cycle threshold value inversely related to the amount of target DNA in the sample and the value generated for the presumptive positive detection was 37.8. Cycle threshold values > 37 are considered inconclusive since results from very small amounts of target DNA are less likely to be reproducible and laboratory contamination cannot be ruled out (C. Cornelison, Kennesaw State University, Georgia, pers. comm.). No visible signs of WNS were detected in either year. We followed the U.S. Fish and Wildlife Service Decontamination Protocol (whitenosesyndrome.org) when handling bats or visiting bridge roosts. All methods were approved by the Clemson University IACUC (#2017-057) and U.S. Forest Service IACUC (#2017-017).

We recorded skin temperatures (T_{sk}) at 15-minute intervals during a 20-second scan period for active transmitter frequencies using Lotek SRX-800D dataloggers. One or two 5-element Yagi antennas were attached to a datalogger placed under each study

bridge for the duration of the field season to ensure adequate roost coverage. We identified tree roost locations via daily radio-tracking and placed a 3- or 5-element Yagi antenna paired with a datalogger at these roosts. Dataloggers at tree roosts were maintained for the duration of the bat's transmitter life and if possible, longer to capture other transmitted bat visitations. We changed the external 12-volt battery powering each datalogger every 2-3 days or 3-4 days depending on battery size. Data were downloaded once a week or prior to moving the unit. We converted inter-pulse intervals recorded by the datalogger to Tsk using calibration curves supplied by Holohil Systems Ltd. We manually checked Tsk records for potentially inaccurate recordings (e.g., Tsk < 0°C or > 40°C) which were a result of low transmitter battery and/or poor signal strength.

We recorded roost temperature (Tr) and relative humidity (RHr) every 30 minutes using Hygrochron iButtons (Maxim Integrated, San Jose CA USA). We collected Tr and RHr from the study bridges during both years, Tr in accessible cavities (i.e., cavities with basal or mid-bole openings and an entrance width larger than 8 cm) during both years, and RHr in accessible cavities only during the second year. We acquired ambient temperature (Ta), relative humidity (RHa), barometric pressure (Pbar), and precipitation from an on-site climatology station through the Atmospheric Technologies Group of the Savannah River National Laboratory. In 2018-19, we suspended Hygrochron iButtons in inverted styrofoam cups from tree branches near used tree and bridge roosts for comparison with climatology station measurements.

To determine if bats were supplementing winter fat reserves with intermittent feeding, we placed 1.2 m² fabric and paper sheets under areas frequently used by bridge roosting tri-colored bats and inspected them weekly. If feces were present on the sheets, we collected the samples using tweezers. Additionally, we collected any feces expelled during bat handling. These samples were kept under refrigeration until ready to be examined for insect parts. We teased apart each sample in a petri dish containing a solution of 70% isopropyl alcohol while it was under a dissecting microscope and inspected the sample for insect parts at 10-40X magnification. Presence of insect parts would indicate bats were feeding during periods of activity and an absence of insect parts would indicate passage of metabolic wastes and no feeding.

2.3 Statistical analyses

We calculated vapor pressure deficit (VPD) and saturation vapor pressure (SVP) using simultaneous measurements of temperature (Ta or Tr) and relative humidity (RH_a or RH_r) and the following equation: $VPD \text{ (kilopascal [kPa])} = [(100-RH)/100 * SVP]/1000$ (Monteith and Unsworth 1990) where $SVP \text{ (kPa)} = [610.7 * 10^{7.5T/(237.3+T)}]/1000$ (Murray 1967). To ensure climatology tower data were comparable to Ta and ambient VPD (VPD_a) conditions of forests used by bats, we performed a one sample t-test on the daily mean difference between tower and forest measurements in 2018-19. We determined that daily tower measurements, although statistically different from forest measurements of Ta and VPD_a ($P < 0.001$), were not biologically different from forest

measurements based on estimated mean differences of only -0.26°C and -0.11 kPa , respectively. Prior to modeling, we assessed each response variable's distribution and if needed, transformed data using the best normalization function identified by the `bestNormalize` package (Peterson and Cavanaugh 2019). We tested for collinearity between our predictor variables using a variance inflation factor of five and found no models contained variables with high collinearity. All statistical analyses were performed in R version 3.5.2 (R Core Team 2019) and values are reported as the mean \pm *SD*. For models with transformed response variables, we present back-transformed data.

We defined *Tsk* below the maximal *Pd* growth threshold of 19.5°C (Verant et al. 2012) as amenable to fungal growth and infection (i.e., the *Pd*-zone). Additionally, we evaluated the length of time *Tsk* fell within the optimal temperature growth range of *Pd* ($12.5\text{-}15.8^{\circ}\text{C}$) and calculated the proportion of time within this optimum relative to the total time within the *Pd*-zone. We also identified *Pd* bout durations (*PdBD*) as the length of each continuous stretch of time that *Tsk* fell within the *Pd*-zone (note: multiple *Pd* bouts could occur within a single torpid bout).

We developed six *a priori* hypotheses including the null hypothesis and global model based on weather conditions, roost microclimate, and energetic status to predict *Pd* bout duration (Table 1). We tested our hypotheses using linear mixed effect models (LMMs) with a random effect of individual. We ranked competing models using Akaike's Information Criterion with correction for small sample sizes (AICc) and selected the most

parsimonious model(s) based on a $\Delta AICc < 2$. When multiple models were selected, we obtained model-averaged coefficients of the top models and used the conditional average to obtain parameter estimates. We determined the significance of model parameter estimates based on whether their upper and lower 95% confidence intervals overlapped 0.

While measures of metabolic rate are ideal for differentiating between thermoregulatory phases, we used Tsk which provides an indirect measure of body temperature to identify the thermoregulatory phases of torpor (Barclay et al. 2001; Willis and Brigham 2003). Following Jonasson and Willis (2012), we defined thermoregulatory phases for the analysis of torpor patterns as: (1) cooling, an abrupt decrease from normothermic Tsk for two consecutive readings ($> 5^{\circ}\text{C}$ in 30 min or less) with a slope < -2 ; (2) active warming, an abrupt increase in torpid Tsk for two consecutive readings ($> 5^{\circ}\text{C}$ in 30 min or less) that eventually stabilized at normothermic Tsk; (3) normothermic, period of raised Tsk between active warming and cooling phases; and (4) torpid, period of reduced Tsk between active warming and cooling phases (Figure 2). We also identified periods of passive rewarming during which Tsk increased in parallel with T_a . Due to complete passive rewarming by two bats in 2017-2018, we also categorized five periods of passive rewarming $> 28^{\circ}\text{C}$ as normothermic bouts. We defined a bat-day as starting at 08:00 and ending at 07:59 the next day. We excluded torpid bouts which occurred immediately following capture and did not proceed into the following bat-day of observation and normothermic bouts on the day of capture

from our analyses. We used the same six *a priori* hypotheses from the PdBD analysis to predict torpid bout duration (TBD) and normothermic bout duration (NBD) (Table 1). We tested our hypotheses using LMMs with a random effect of individual and used the same model selection procedures as outlined for the PdBD analysis.

We identified the hour of each arousal using Tsk records and the associated thermoregulatory phases of warming and normothermia. We analyzed hourly arousal data using package *circular* (Agostinelli and Lund 2017) and determined if arousal timing was non-random using a Rayleigh test. We then used a von Mises kernel density estimator with a cross-validators bandwidth selector minimizing Kullback-Leibler loss to identify the time of day with the highest probability of arousal (Agostinelli 2007; Oliveira-Santos et al. 2013).

We used three measures of activity: 1) general activity—bats warmed to normothermic Tsk in the late afternoon and evening; 2) activity away—bats left their day roost during a normothermic bout; and 3) a day-roost switch. We attributed nighttime gaps in the Tsk record that could not be explained by low transmitter strength or datalogger battery failure during a normothermic bout to activity away from the day roost. Roost switches were identified using telemetry logs from daily tracking efforts. For all activity analyses, we excluded events associated with the day of tagging.

We developed six *a priori* hypotheses based on weather conditions, roost microclimate, energetic status, warming costs, and seasonality to predict general

activity, nighttime activity away from the roost, and roost switching (Table 2). In addition to the above predictors, we also included a variable accounting for day roost structure in models for general activity and activity away from the roost since we found a significant difference based on roost structure in initial tests ($P < 0.001$ and $P < 0.001$, respectively). The roost structure variable was not added to roost switching models since we found no significant difference ($P = 0.073$). We tested our hypotheses using generalized linear models (GLMs) with a logit link. We used the same model selection procedures as outlined for the PdBD analysis.

3. Results

We recovered Tsk data from 12 of 19 transmittered bats (five in 2017-2018 and seven in 2018-2019; four females and eight males). We had one recapture in both years: a female in 2017-2018 that was originally banded in 2016-2017 during a pilot study (data not incorporated), and a male in 2018-2019 that was recaptured following initial capture in 2017-2018. The male recaptured in 2018-2019 was treated as a distinct individual and Tsk records were not pooled across years. The daily mean T_a during November through March was $10.7 \pm 6.1^\circ\text{C}$ in 2017-2018 and $10.5 \pm 4.8^\circ\text{C}$ in 2018-19 but the coldest daily mean T_a in 2017-18 (-4.5°C) was lower than the coldest daily mean in 2018-19 (1.2°C). Conditions were marginally more humid in 2018-2019 (0.31 ± 0.19 kPa) than 2017-2018 (0.47 ± 0.23 kPa) with a maximum daily mean VPDa of 1.185 kPa.

We recorded data during 304 bat-days with a mean tracking duration of 28 ± 10 days per bat. In addition to three previously identified I-beam bridge roosts, we identified 24 tree roosts. Tree roosts were in cavities with basal openings ($n = 5$), cavities with upper and mid-bole openings ($n = 6$), a hollow snag with a chimney and mid-bole opening, Spanish moss (*Tillandsia usneoides*) ($n = 2$), a cluster of dried sweetgum (*Liquidambar styraciflua*) leaves, and various unknown roost structures in the canopy ($n = 9$) such as potentially hidden cavities, dead limbs, bark, or foliage. The daily mean T_r was $12.9 \pm 4.9^\circ\text{C}$ in bridges and $11 \pm 4.6^\circ\text{C}$ in accessible cavities, while the daily mean roost VPD (VPD_r) was 0.42 ± 0.32 kPa in bridges and 0.04 ± 0.06 kPa in accessible cavities. The daily mean T_r fluctuation was $4.8 \pm 2^\circ\text{C}$ in bridges and $4.0 \pm 1.9^\circ\text{C}$ in accessible cavities with maximums of 13.8°C and 10.5°C , respectively. Bats usually roosted alone although we occasionally observed small clusters (2-3 individuals) in bridge roosts during January and February.

The proportion of time that bats' Tsk fell within the Pd-zone ranged from 0.6 to 0.96 (0.81 ± 0.11) (Appendix). While within the Pd-zone, the proportion of time Tsk was within the optimal growth range (12.5 to 15.8°C) varied from 0.24 to 0.66 (0.39 ± 0.13). We recorded 129 bouts within the Pd-zone ranging in duration from 15 minutes to 14.1 days with a median duration of 17.2 hours and a mean of 1.7 ± 2.4 days. The global model was the top model for predicting PdBD and carried 70% of the model weight (Table 3). Mean T_a and the interaction of mean VPD_a and roost structure were statistically significant in explaining PdBD (Table 4). PdBD decreased as mean T_a

increased and the median PdBD decreased to only 8 hours at $T_a > 10^\circ\text{C}$ (Figure 3a).

PdBD decreased in bridges and foliage as mean VPDa increased and we detected no relationship between PdBD and mean VPDa in cavities (Figure 4a).

All bats used torpor everyday of observation, and bats in all roost structures exhibited both short (<24 hr) and multi-day torpid bouts. During 94 recorded torpid bouts, the median torpid Tsk for each bat ranged from 12.3 to 18°C with a pooled mean Tsk across all bats of $15.4 \pm 4.3^\circ\text{C}$. The daily fluctuation in torpid Tsk averaged $4.8 \pm 3.7^\circ\text{C}$ with a maximum of 23.1°C. Torpid bouts ranged in duration from 3.5 hours to 15.5 days with a median of 1.6 days and mean of 2.7 ± 2.8 days. The simple weather, weather, and global models all fell within 2 ΔAICc units and combined carried 89% of the model weight (Table 3). Three predictors were significant: mean T_a , total precipitation, and the interaction between mean VPDa and roost structure (Table 4). TBD decreased as mean T_a increased and the median TBD decreased to only 21.7 hours at $T_a > 10^\circ\text{C}$ (Figure 3b). TBD was positively related to total precipitation and the longest TBD, 15.5 days, occurred during the second longest precipitation event which resulted in water covering the only known entrance to the bat's cavity for multiple days. TBD was negatively related to mean VPDa in bridges, positively related to mean VPDa in cavities, and we detected no relationship between TBD and mean VPDa in foliage roosts (Figure 4b).

We recorded 90 normothermic bouts ranging from 15 minutes to 10.1 hours with a median of 1.2 hours and a mean of 2.4 ± 2.6 hours. The longest observed NBD,

10.1 hours, was by a female roosting in foliage in late February; she maintained a $T_{sk} > 28^{\circ}\text{C}$ from 10:00 to 20:15. The top model for predicting NBD, simple weather, carried 89% of the model weight (Table 3) and contained the significant predictor mean T_a (Table 4). NBD increased with mean T_a , and we often observed multi-hour normothermic events at $T_a > 20^{\circ}\text{C}$ (Figure 3c).

Bats were active on 102 of 304 tracked days (33.55%) and 51% of arousals contained a passive rewarming component. For individual bats, days in which bats were active ranged from 14 to 64% of days followed and 97% of all activity events overlapped nighttime hours. Bats maintained a non-random arousal pattern throughout winter ($z = 0.5433$, $P < 0.001$, $n = 101$) with the highest probability of arousal near dusk (Figure 5). The top model for predicting general activity, energetic status, carried 60% of the model weight (Table 5) and contained the significant predictors mean daily T_a and roost structure (Table 6). The probability of general activity increased as mean T_a increased (Figure 6) and bats in foliage had a higher probability of general activity than those in bridges and cavities.

Of the nighttime arousal events, 71% involved activity away from the roost and 38% resulted in a roost switch. The weather and energetic status models were the best models predicting activity away from the roost and combined carried 97% of the model weight (Table 5) and contained the significant predictors mean nightly T_a and P_{bar} , and roost structure (Table 6). The probability of activity away from the roost increased with

increasing mean T_a and decreasing P_{bar} (Figure 7a and 7b), and bats in bridges and foliage had a higher probability of activity away than those in cavities. The seasonality, null, and warming cost models were the best models predicting a roost switch and combined carried 85 % of the model weight (Table 5). The quadratic predictor variable date (i.e., number of days from November 1) was significant (Table 6), with the probability of switching roosts being highest in fall and late winter-early spring and decreased towards mid-winter. We collected five guano samples, one in mid-January 2019, three in late February 2018, and one in early March 2018. Two of the five samples were collected during bat processing in the morning and the remaining three were collected from guano sheets. All five samples contained insect parts, a positive sign of winter feeding.

4. Discussion

Tri-colored bats using thermally unstable roosts at SRS displayed winter torpor more reminiscent of daily torpor than classic hibernation. In particular, tri-colored bats regularly used passive rewarming prior to arousals, maintained short TBD and relatively high winter activity levels related to ambient weather conditions, and exploited intermittent foraging opportunities during winter. These torpor and activity patterns are comparable to those recorded in other bat species in North America and Australia that use thermally unstable roosts (Stawski et al. 2009; Halsall et al. 2012; McNab and O'Donnell 2018).

We observed mean torpid Tsk and daily Tr similar to those observed in bats using cave and culvert systems of the southern US (Sirajuddin 2018; Meierhofer et al. 2019b) which fall within the optimal temperature growth range of Pd (Verant et al. 2012). However, while cave hibernating tri-colored bats use stable microclimates, bats in our study used roosts with thermally unstable microclimates. Tri-colored bats in both bridges and trees experienced daily Tsk and Tr fluctuations averaging 4-5°C with a maximum daily passive Tsk fluctuation of 23.1°C in a foliage roost. A change in Tr of 10-15°C (i.e., Tr fluctuation of 5°C) provokes energetically costly arousals in tri-colored bats accustomed to thermally stable microclimates (Davis and Reite 1967). In contrast, the ability to remain torpid during large passive fluctuations in Tsk appears relatively common for tree roosting species in Australia (Turbill and Geiser 2008; Stawski et al. 2009; Stawski and Currie 2016) and other non-subterranean hibernating species of North America (Davis and Reite 1967; Halsall et al. 2012). For example, *Nyctophilus bifax*, a subtropical vespertilionid bat, maintains multi-day torpid bouts even though daily Tsk fluctuates more than 10°C (Stawski et al. 2009). Our observation of tri-colored bats using torpor despite relatively large Tsk fluctuations adds to the growing body of literature describing torpor in thermally unstable roosts.

Thermoconforming at relatively high Tsk (> 20°C) as observed in tri-colored bats at SRS is of note for two reasons. First, few bat species have been recorded thermoconforming at Tsk > 20°C and examples of this behavior which do exist are from subtropical and tropical regions (Stawski and Geiser 2010; Liu and Karasov 2011). In

contrast, free-ranging bats in temperate regions that passively rewarm to approximately 20°C usually initiate active warming, ending the torpid bout (Turbill et al. 2008; Halsall et al. 2012). Multiple observations of tri-colored bats that thermoconformed at temperatures > 20°C expands the current knowledge of the physiological tolerances for tri-colored bats and highlights the need to explore critical arousal temperatures of bats using thermally unstable roosts. Second, Tsk > 20°C are not amenable to Pd growth, and changes in the structural morphology of Pd grown at elevated temperatures (12-19.5°C) suggest these conditions may be stressful for the fungus and limit its overall reproductive capacity (Verant et al. 2012). It is not known how frequent exposures to potentially stressful and unsuitable temperature conditions affect fungal load and viability. Studies that explore Pd's growth performance under environmental conditions which are representative of thermally unstable roosts will greatly increase our understanding of the bats' susceptibility to WNS in populations that use these types of roosts.

Passive rewarming prior to arousal appears to be a regular occurrence for tri-colored bats at SRS using thermally unstable roosts since 51% of arousals we recorded contained a passive component at the start of the warming period, and five arousals in foliage were performed exclusively via passive rewarming. Passive rewarming can result in energy savings of 20-47% per arousal when compared to complete active rewarming (Turbill et al. 2008; Halsall et al. 2012) and reduces cardiovascular demands and oxidative stress (Currie et al. 2015). Tri-colored bats in foliage likely experienced the

greatest opportunity for passive rewarming which may explain why bats in foliage had higher general activity than bats in bridges and cavities. The energetic benefits of passive rewarming for daily heterotherms increase exponentially over time since these species rewarm from torpor on a daily basis (Geiser 2004; Canale et al. 2012). Regular use of passive rewarming may also benefit tri-colored bats at SRS by reducing their need to save energy through hibernation and limiting the physiological demands of active arousals. However, the advantages of a passive rewarming strategy are constrained by microclimate and thermally unstable microclimates pose some disadvantages.

Fluctuations in T_r which allow passive rewarming also can expose bats to lethal freezing conditions during periods of extreme cold resulting in increased torpid metabolic rates and energy use during torpor (Boyles et al. 2020). Additionally, the exposure of some thermally unstable structures results in roost humidity conditions that likely increase evaporative water loss during torpor (Thomas and Cloutier 1992). For example, VPD_r in bridges (0.42 ± 0.32 kPa) often mirrored VPD_a whereas cavities remained consistently humid (0.04 ± 0.06 kPa) despite fluctuations in VPD_a. Furthermore, we found evidence that bats in bridges, but not cavities, decreased TBD as VPD_a increased potentially as a result of increased evaporative water loss and dehydration due to low humidity. Finally, tri-colored bats in bridges were more active away from the roost than bats in cavities which may also be related to dehydration. Therefore, the use and energetic advantages of thermally unstable roosts may be limited by local climate and weather conditions.

Our prediction that torpor and activity patterns would be related to ambient weather conditions was supported. As expected, TBD was negatively related to T_a and positively related to precipitation while NBD, general activity, and activity away from the roost were positively related to T_a . The relationship of torpor to ambient weather conditions may be in response to associated thermoregulatory costs of activity during certain conditions. As the differential between T_a and normothermic temperatures decreases so does the energetic cost of activity (Geiser 2004) and precipitation results in increased energetic costs on an actively flying bat (Voigt et al. 2011). Our results relating torpor and activity patterns to ambient weather conditions are similar to other bat species using thermally unstable roosts (Stawski and Geiser 2010; Stawski and Currie 2016; Czenze et al. 2017b). In contrast, TBD and NBD of cave hibernating populations of tri-colored bats in the southeast are not related to ambient weather conditions (Sirajuddin 2018). Differences in torpor strategy between tri-colored bats using thermally stable and unstable roosts may be related to their ability to monitor ambient weather conditions in thermally unstable roosts and subsequently arouse on nights with lower thermoregulatory costs and exploit intermittent feeding opportunities on nights with favorable foraging, as well as their ability to passively rewarm, further reducing energetic costs associated with activity.

We found multiple lines of evidence which suggest tri-colored bats at SRS exploit winter feeding opportunities. Tri-colored bats at SRS maintained a non-random arousal pattern throughout winter with peak activity near dusk. Bats active near dusk have

more foraging opportunities and many species suspected of feeding during winter concentrate their arousals near dusk (Turbill 2006; Stawski and Geiser 2010; Hope and Jones 2012; Johnson et al. 2012). In addition, we observed dramatic increases in NBD, general activity, and nighttime activity away from the roost on nights when temperatures were $> 10^{\circ}\text{C}$ which coincides with the lower critical flight temperature for most insects (Taylor 1963). Activity levels of bats in North Carolina's Coastal Plain during winter nights with $T_a > 10^{\circ}\text{C}$ are similar to activity levels during summer but, winter activity is much lower than summer activity levels at $T_a < 10^{\circ}\text{C}$ (Grider et al. 2016). We also observed a significant increase in nighttime activity away from the roost at lower Pbar. Multiple species appear to use changes in Pbar, which is associated with insect abundance, as a cue for emergence and activity (Turbill 2008; Czenze and Willis 2015) including tri-colored bats in Illinois (Paige 1995). Finally, though limited in number, the fecal samples we collected during our study all contained insect parts.

Increased activity levels and intermittent winter feeding on warm nights with low Pbar appears to be a readily employed strategy by bats in more mild winter climates (Park et al. 2000; Turbill 2008; Hope and Jones 2012; Czenze et al. 2017b) and a potential winter strategy of tri-colored bats at SRS. Our findings of activity during favorable foraging conditions and indications of winter feeding differ from tri-colored bats in Indiana which exhibit very little winter activity (Whitaker and Rissler 1992) and likely do not feed (Whitaker et al. 2004). These differences in intermittent feeding behavior may be a result of the mild winter climate found in the Upper Coastal Plain of

South Carolina compared to Indiana's much colder conditions. However, since evidence of common activity behaviors associated with foraging or activity away from the roosts were not observed by Sirajuddin (2018) in bats using thermally stable hibernacula under similar climatic conditions to our own, intermittent winter feeding in tri-colored bats at SRS may be a function of both a mild climate and use of thermally unstable roosts. Bats in thermally unstable roosts may respond to a critical threshold of passive T_{sk} fluctuation that signals reduced thermoregulatory costs and an opportunity to forage during winter (Dunbar et al. 2007; Turbill 2008) which is absent in cave hibernating bats.

Roost switching was related to season with a greater probability of roost switching in early and late winter. We have three potential explanations for this relationship. First, bats may switch roosts more often when foraging to reduce the energetic costs of commuting and early and late winter may have the most foraging opportunities due to warm conditions. Second, roost switching could reduce ectoparasite loads in early and later winter when parasites are more prevalent due to warm conditions. Female Bechstein's bats (*Myotis bechsteinii*) and pallid bats (*Antrozous pallidus*) switch roosts as a strategy of parasite avoidance (Lewis 1995, 1996; Reckardt and Kerth 2007) and tri-colored bats at SRS may as well. Third, increased roost switching in early and late winter may be in response to shifting microclimate suitability (Lewis 1995). Intermediate T_a , combined with thermally unstable roost structures, may result in a greater need to switch roosts to find a suitable roost microclimate.

Tri-colored bats' Tsk were often within the Pd-zone throughout winter at SRS. Consequently, susceptibility to WNS may be similar between tri-colored bats at SRS and subterranean hibernating populations. Alternatively, tri-colored bats at SRS may be less susceptible to WNS than subterranean populations for a combination of reasons related to their position nearer the daily torpor end of the continuum. First, PdBD was more responsive to Ta than TBD likely due to rising torpid Tsk (> 20°C) ending the Pd bout but not necessarily the torpid bout. Short PdBD may limit fungal growth and disease severity though susceptibility may vary across years depending on ambient weather conditions. Second, tri-colored bats at SRS regularly used passive rewarming. Increased arousal frequency to offset disease-related dehydration contributes to WNS-related mortality through premature fat depletion (Reeder et al. 2012; Warnecke et al. 2013; Verant et al. 2014; McGuire et al. 2017). Passive rewarming decreases the energetic costs of arousal (Halsall et al. 2012; Currie et al. 2015) and may reduce premature fat depletion in tri-colored bats at SRS. Third, tri-colored bats at SRS exhibit short TBD and relatively high winter activity levels. Big brown bats (*Eptesicus fuscus*) and Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) with high winter activity levels appear less susceptible to WNS than bats with naturally low winter activity levels (Johnson et al. 2012; Reynolds et al. 2017). During torpor bats experience reduced immunocompetence (Field et al. 2018) and long TBD may allow Pd to extensively colonize cutaneous tissues with little host resistance or immune response. However, bats with naturally high winter activity levels may be able to mount a more robust immune response throughout winter (van

Breukelen and Martin 2015; Humphries et al. 2003) potentially limiting WNS infection. Finally, we found evidence of intermittent winter feeding in tri-colored bats at SRS. Winter fat supplementation may enable bats to maintain winter activity and also result in decreased WNS susceptibility since higher fat reserves contribute to persistence in WNS affected populations (Cheng et al. 2019).

In summary, our results suggest winter torpor of bats using thermally unstable roosts lies nearer the daily torpor end of the continuum than the hibernation end. Therefore, tri-colored bats at SRS may be less susceptible to WNS due to short PdBD, regular use of passive rewarming, short TBD and relatively high winter activity levels, and winter fat supplementation from intermittent feeding opportunities. Understanding where bats lie along the torpor continuum when they use thermally unstable roosts is increasingly important as WNS advances into regions of North America with many non-cave roosting populations. Inherent differences in winter torpor strategy related to roost microclimate and weather conditions may result in varying degrees of WNS susceptibility among populations of the same species. Finally, these results highlight the need to explore the physiological flexibility of broadly distributed hibernators with consideration of climate change. Tri-colored bats using thermally unstable roosts appear well-adapted to climate change since, like some tropical bat species (Stawski and Geiser 2012), they continue to opportunistically use torpor at high T_a .

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Table 1 Hypotheses and variables for models predicting Pd bout duration, torpid bout duration, and normothermic bout duration for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Predictor abbreviations are as follows: Ta, ambient temperature; Precip, precipitation; VPDa, ambient vapor pressure deficit; and Pbar, barometric pressure.

Hypothesis	Predictors	Example Reference
Simple weather	Mean Ta	(Stawski et al. 2009)
Energetic status	Mean Ta	(Stawski et al. 2009)
	Mass	(Boyles et al. 2007)
Weather	Mean Ta	(Stawski et al. 2009)
	Mean Pbar	(Turbill 2008)
	Total Precip	(Voigt et al. 2011)
Roost microclimate	Mean VPDa*Roost	(Thomas and Cloutier 1992)
Global	Mean Ta	(Stawski et al. 2009)
	Mean Pbar	(Turbill 2008)
	Total Precip	(Voigt et al. 2011)
	Mass	(Boyles et al. 2007)
	Mean VPDa*Roost	(Thomas and Cloutier 1992)
Null	Null	

Table 2 Hypotheses and variables for models predicting general activity, nighttime activity away, and roost switching for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Predictor abbreviations are as follows: Ta, ambient temperature; Precip, precipitation; pVPDa, previous day's ambient vapor pressure deficit; Pbar, barometric pressure; and Continuous date2, a quadratic form of days from November 1st. Roost was only included in general activity and nighttime activity away models.

Hypothesis	Predictors	Example References
Weather	Mean Ta	(Stawski et al. 2009)
	Mean Pbar	(Turbill 2008)
	Precip	(Voigt et al. 2011)
	Roost	(Czenze et al. 2017a)
Energetic status	Mean Ta	(Stawski et al. 2009)
	Mass	(Boyles et al. 2007)
	Roost	(Czenze et al. 2017a)
Warming cost	Hr Max Ta	(Turbill et al. 2008)
	Max Ta	(Turbill et al. 2008)
	Roost	(Czenze et al. 2017a)
Past roost microclimate	Mean pVPDa*Roost	(Thomas and Cloutier 1992)
Seasonality	ConDate ²	(Czenze et al. 2017a)
	Roost	(Czenze et al. 2017a)
Null	Null	

Table 3 Top models for predicting Pd bout duration, torpid bout duration, and normothermic bout duration for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Abbreviations are as follows: model, refers to the hypothesis evaluated (Table 1); K, number of parameters; logLik, maximum log-likelihood; ΔAICc , difference of Akaike’s information criterion adjusted for small sample size (AICc) between a model and the model with the smallest AICc; weight, model weight.

	Model	K	logLik	ΔAICc	weight
Pd bout duration	Global	12	-262.01	0.00	0.70
	Simple weather	4	-272.47	2.55	0.20
Torpid bout duration	Weather	6	-107.17	0.00	0.34
	Simple weather	4	-109.47	0.09	0.33
	Global	12	-100.13	0.85	0.22
	Energetic status	5	-109.42	2.21	0.11
Normothermic bout duration	Simple weather	4	-43.65	0.00	0.89
	Energetic status	5	-44.69	4.32	0.10

Table 4 Coefficient estimates from top models of winter torpor patterns of tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Predictor abbreviations are as follows: Ta, ambient temperature; Precip, precipitation; MVPDa, mean ambient vapor pressure deficit; and Pbar, barometric pressure.

	Predictor	Estimate	SE	95% LCL	95% UCL
Pd bout duration	Mean Ta	-5.11	1.20	-6.69	-3.86
	Mean VPDa	-2.77	1.42	-4.89	-1.47
	Roost: Cavity	-1.85	1.60	-4.14	1.35
	Roost: Foliage	-4.16	1.57	-8.30	-1.91
	Total Precip	-1.06	1.18	-1.45	1.31
	Mean Pbar	1.05	1.22	-1.41	1.54
	Mass	-1.56	1.35	-2.65	1.15
	MVPDa*Roost: Cavity	3.18	1.61	1.35	6.71
	MVPDa*Roost: Foliage	1.56	1.51	-1.45	3.25
	Torpid bout duration	Mean Ta	-0.65	0.11	-0.92
Total Precip		0.25	0.08	0.09	0.42
Mean Pbar		0.15	0.11	-0.06	0.37
Mean VPDa		-0.06	0.13	-0.32	0.20
Roost: Cavity		-0.74	0.23	-1.41	-0.23
Roost: Foliage		-0.81	0.20	-1.40	-0.35
Mass		-0.32	0.16	-0.66	0.00
MVPDa*Roost: Cavity		0.42	0.20	0.02	0.90
MVPDa*Roost: Foliage		0.20	0.16	-0.12	0.54
Normothermic bout duration	Mean Ta	1.61	1.10	1.34	1.95

Table 5 Top activity models for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Abbreviations are as follows: model, refers to the hypothesis evaluated (Table 1); K, number of parameters; logLik, maximum log-likelihood; ΔAICc , difference of Akaike’s information criterion adjusted for small sample size (AICc) between a model and the model with the smallest AICc; weight, model weight.

	Model	K	logLik	ΔAICc	weight
General activity	Energetic status	6	-134.68	0.00	0.60
	Warming cost	6	-135.94	2.53	0.17
Activity away	Weather	7	-31.07	0.00	0.70
	Energetic status	6	-33.19	1.91	0.27
	Seasonality	6	-36.13	7.80	0.01
Roost switch	Seasonality	4	-62.49	0.00	0.44
	Null	2	-65.27	1.25	0.23
	Warming cost	4	-63.39	1.79	0.18
	Past roost microclimate	5	-63.08	3.40	0.08

Table 6 Coefficient estimates from top models of winter activity patterns of tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Predictor abbreviations are as follows: Ta, ambient temperature; Precip, precipitation; pVPDa, previous day's ambient vapor pressure deficit; Pbar, barometric pressure; and Continuous date², a quadratic form of days from November 1st.

	Predictor	Estimate	SE	95% LCL	95% UCL
General activity	Mass	0.32	0.23	-0.08	0.93
	Mean Ta	1.47	0.20	1.10	1.88
	Roost: Cavity	0.21	0.60	-0.71	1.51
	Roost: Foliage	1.80	0.55	0.79	2.95
Activity away	Mean Ta	1.00	0.47	0.07	1.94
	Mean Pbar	-1.03	0.48	-1.99	-0.08
	Total Precip	-0.46	0.51	-1.47	0.55
	Roost: Cavity	-3.26	1.09	-5.42	-1.10
	Roost: Foliage	1.47	1.66	-1.83	4.78
Roost switch	Mass	0.53	0.52	-0.50	1.56
	Continuous date ²	5.51	2.17	1.27	9.75
	Hr of Max Ta	0.47	0.27	-0.07	1.01
	Max Ta	-0.19	0.24	-0.67	0.29
	Mass	0.40	0.24	-0.07	0.86
	Mean Ta	-0.20	0.24	-0.68	0.28
	Mean Pbar	-0.21	0.26	-0.73	0.31
	Total Precip	0.08	0.27	-0.45	0.62
	Mean pVPDa	0.39	0.26	-0.12	0.90

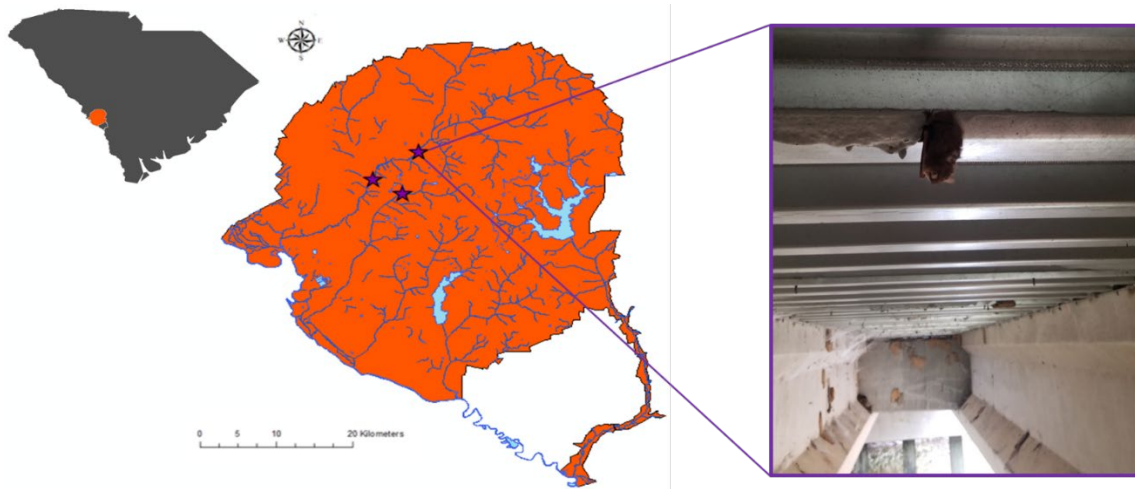


Fig. 1 Study area at the U.S. Department of Energy's Savannah River Site in the Upper Coastal Plain of South Carolina. Stars represent the three I-beam bridges where we captured tri-colored bats (*Perimyotis subflavus*). Inset photo: tri-colored bat roosting from the ribbed metal decking between two concrete I-beams.

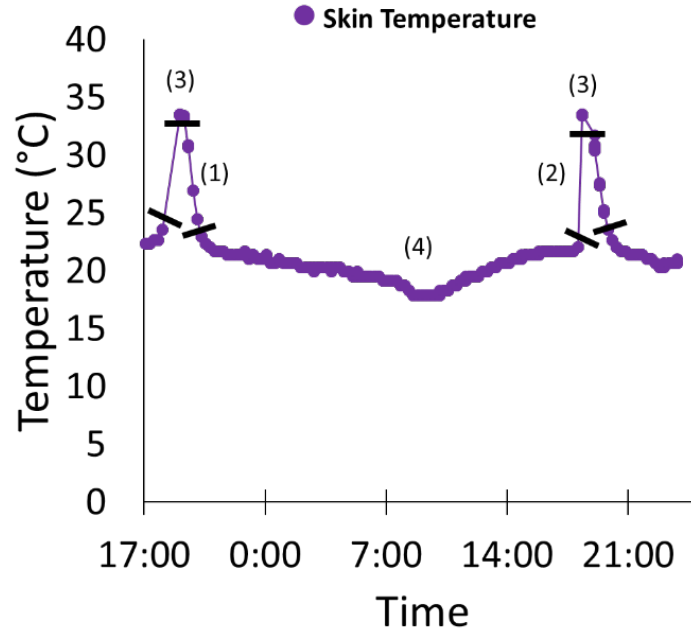


Fig. 2 Classification of thermoregulatory phases used in study of torpor patterns of tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019: (1) cooling, an abrupt decrease in skin temperature; (2) warming, an abrupt increase in skin temperature; (3) normothermic, relatively stable temperatures between warming and cooling phases; and (4) torpor, reduced skin temperatures between cooling and warming phases.

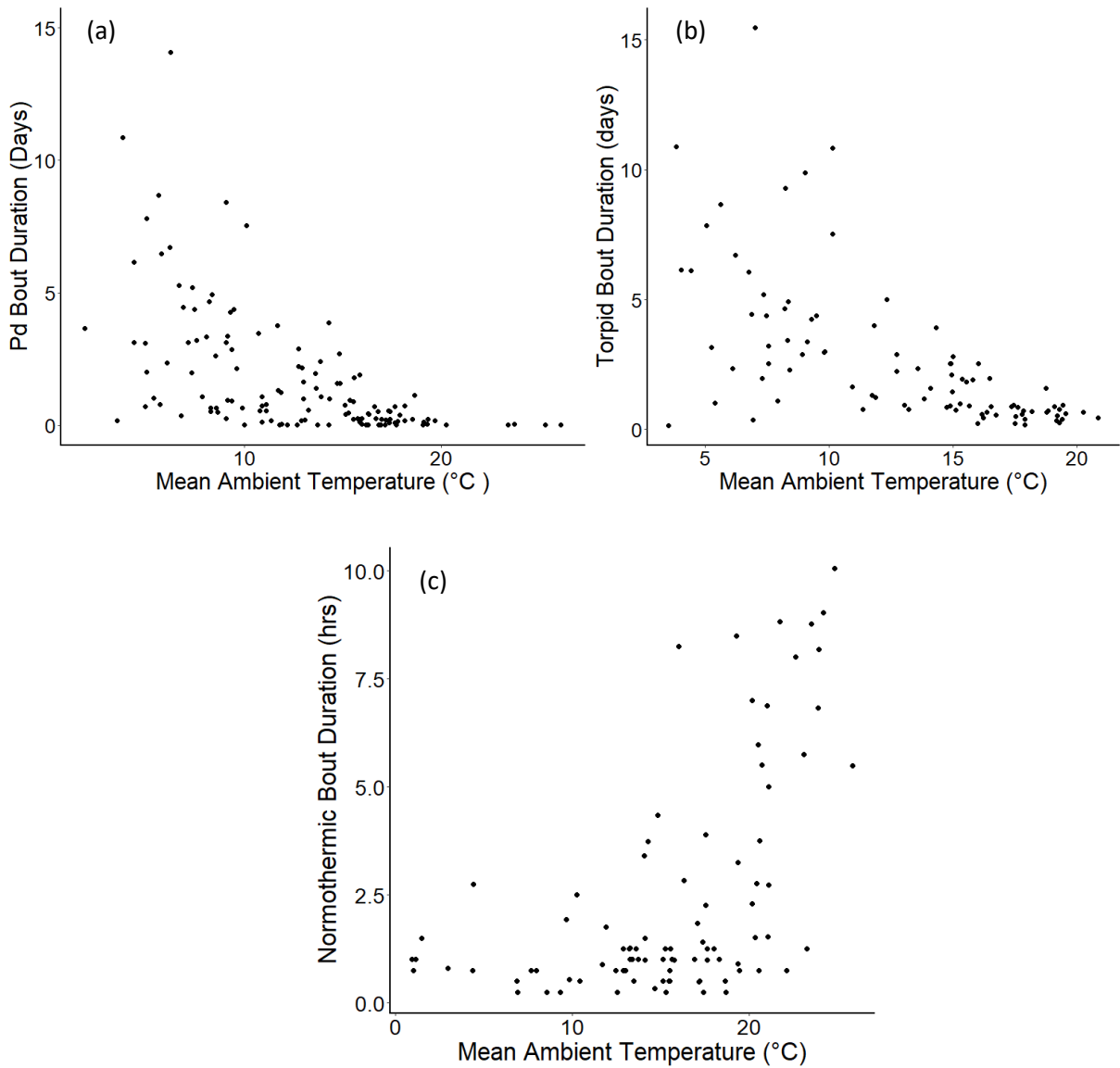


Fig. 3 (a) Pd bout duration, (b) torpid bout duration, and (c) normothermic bout duration as a function of mean ambient temperature for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019.

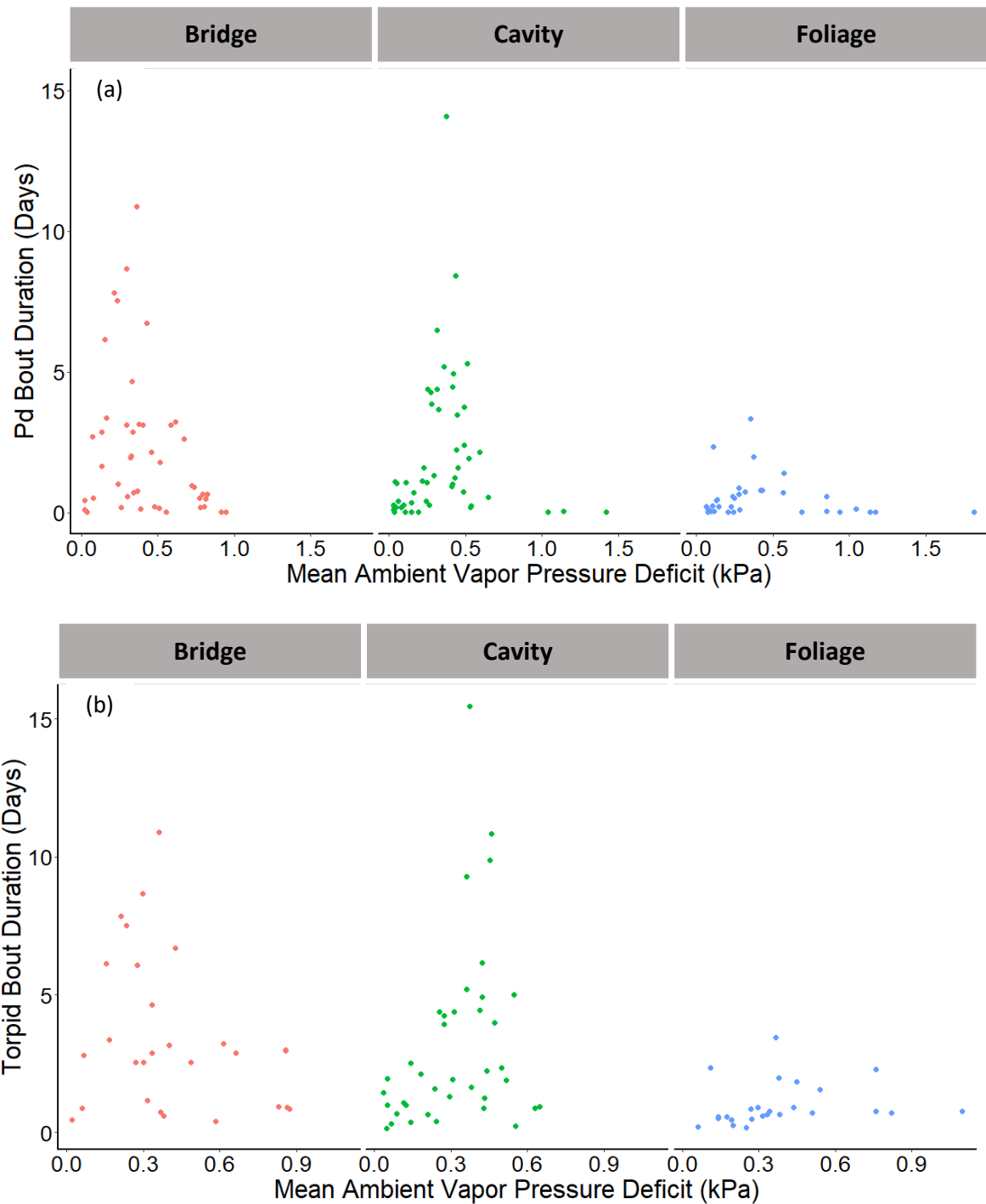


Fig. 4 (a) Pd bout duration and (b) torpid bout duration as a function of mean ambient vapor pressure deficit during bout in bridge, cavity, and foliage structures for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019.

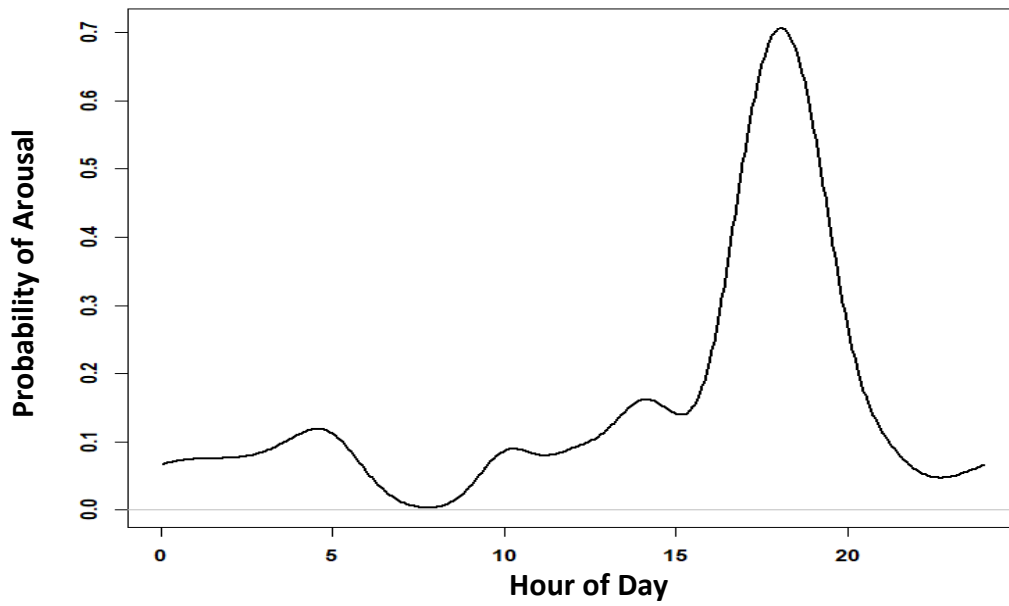


Fig. 5 Probability of arousal by hour for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019.

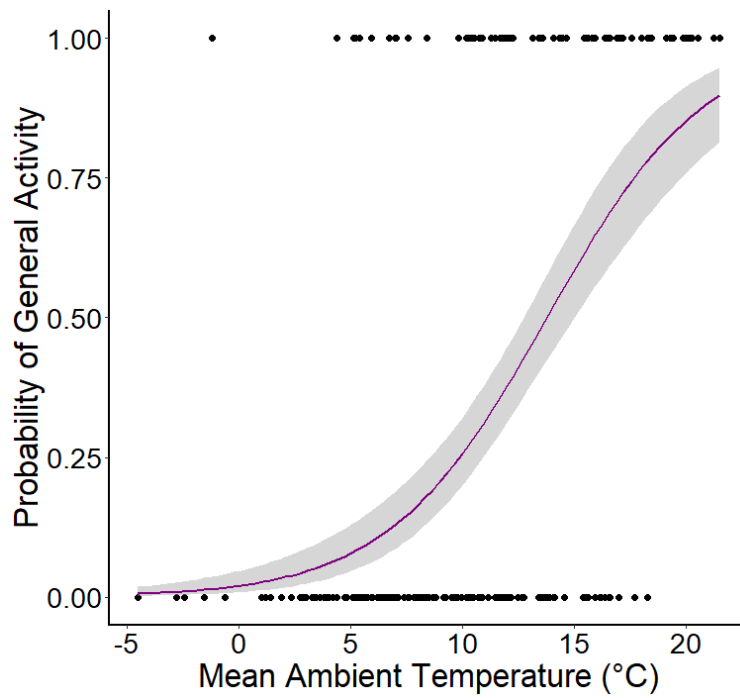


Fig. 6. Probability of general activity as a function of mean ambient temperature during the day for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019.

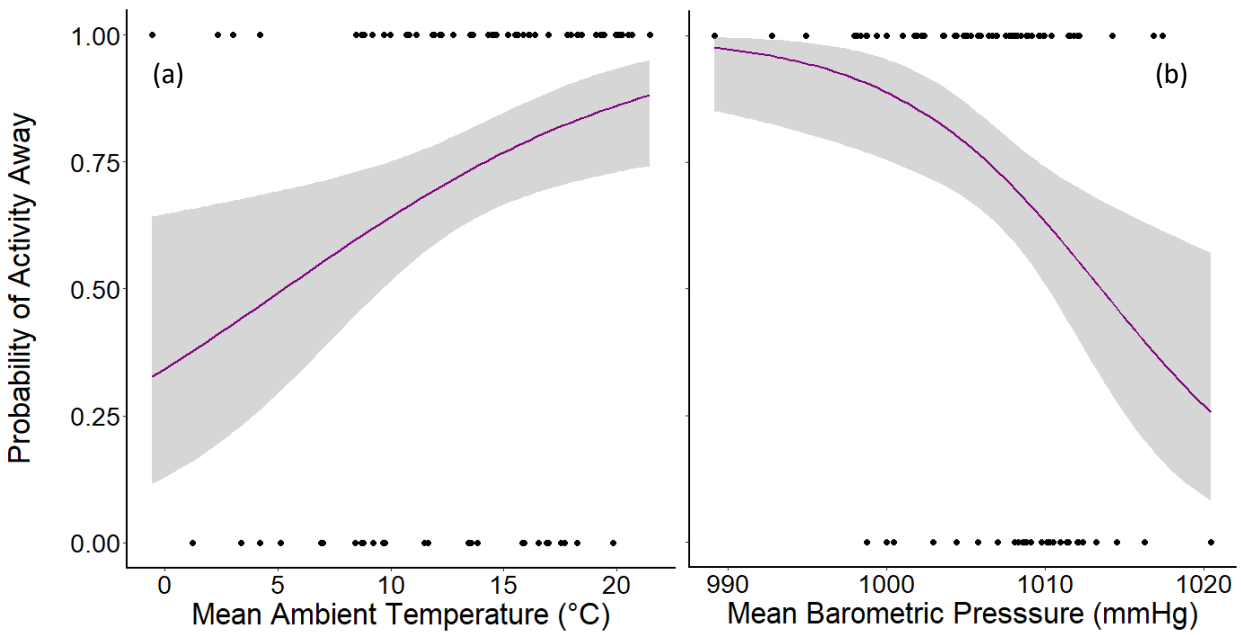


Fig. 7 (a) The probability of nighttime activity away as a function of mean nightly ambient temperature and (b) mean nightly barometric pressure for tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019.

Appendix—Capture and summary data from tri-colored bats (*Perimyotis subflavus*) using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017-2019. Predictor abbreviations are as follows: Recap, recapture; Wall Tr (°C), wall temperature in °C; Hours Logged, total time a bat’s skin temperature was recorded in hours; Pd-zone, proportion of time logged a bat’s skin temperature was amenable to *Pseudogymnoascus destructans* (Pd) growth (< 19.5°C); and Opt/Pd, proportion of time within the Pd-zone a bat’s skin temperature was within the optimal temperature growth range of Pd (12.5-15.8 °C).

Date of capture	Band	Bridge	Sex	Weight (g)	Recap	Wall T (°C)	Hours Logged	Pd-zone	Opt/Pd
30-Nov-17	SR0163	02G	M	6.25		17.3	472.02	0.96	0.24
05-Jan-18	SR0789	03G	F	6.25	Y	2.2	873.89	0.81	0.34
20-Jan-18	SR0165	01G	M	5.00		2.3	152.12	0.84	0.25
18-Feb-18	SR0167	02G	F	5.75		12.4	293.20	0.67	0.25
26-Feb-18	SR0168	02G	F	6.00		14.8	497.06	0.60	0.35
17-Nov-18	SR0163	02G	M	6.75	Y	10.6	352.43	0.87	0.34
29-Nov-18	SR0300	02G	M	7.50		7.6	922.14	0.87	0.48
13-Dec-18	SR0253	02G	M	5.75			601.98	0.76	0.29
19-Dec-18	SR0254	03G	M	5.25		7.9	791.28	0.67	0.53
31-Dec-18	SR0255	03G	M	6.00		16.2	484.56	0.89	0.43
08-Jan-19	SR0256	03G	M	6.00			467.96	0.92	0.66
12-Jan-19	SR0257	03G	F	4.75		8.4	482.71	0.88	0.51

CHAPTER TWO

WINTER ROOSTING ECOLOGY OF TRI-COLORED BATS (*PERIMYOTIS SUBFLAVUS*) IN TREES AND BRIDGES

1. Introduction

During winter, bats face a suite of challenges including disease, predation, and energetic stressors. Winter roost selection plays a critical role in mitigating these challenges and influences fitness and survival. Bats select roosts based on various ecological factors related to habitat characteristics available across multiple spatial scales such as tree characteristics, vegetation type, or landscape configuration (Fleming et al. 2013; Jachowski et al. 2016). Roosts may be selected for lowered predation risk (Ferrara and Leberg 2005), protection from adverse weather conditions, energetic benefits (Stawski et al. 2009; Stawski and Currie 2016; Ryan et al. 2019), access to foraging sites (Carter 2006; O’Keefe et al. 2009), and to facilitate social interactions and thermoregulation (Willis and Brigham 2007; Russo et al. 2017).

The tri-colored bat ranges from southeastern Canada to the Yucatan Peninsula of Mexico and west across the Great Plains into South Dakota and New Mexico (Geluso et al. 2005). During summer, tri-colored bats frequently roost in the foliage of trees (Veilleux et al. 2003; Perry and Thill 2007; O’Keefe et al. 2009) and select trees in mature hardwood forests and near riparian areas which may be related to their frequent use of riparian habitats for foraging (Ford et al. 2005; Menzel et al. 2005). During winter, tri-colored bats use caves and mines, as well as numerous non-subterranean structures

(Fujita and Kunz 1984). Relative to other species, tri-colored bats tend to select warmer, more humid environments within caves (Raesly and Gates 1987; Briggler and Prather 2003). Additionally, tri-colored bats prefer cave structures which provide a wide variety of thermally stable roost sites. Winter roost structures also include bridges in Louisiana (Ferrara and Leberg 2005) and South Carolina (S. Loeb, Research Ecologist, U.S. Forest Service Southern Research Station, unpub. data), culverts in Texas (Sandel et al. 2001; Meierhofer et al. 2019a, 2019b) and Georgia (Lutsch 2019), and tree cavities in South Carolina (S. Loeb, Research Ecologist, U.S. Forest Service Southern Research Station, unpub. data). Most accounts in these systems are descriptive and focus on anthropogenic structures, and few studies have explored drivers of use and selection in non-cave roosts. Nonetheless, tri-colored bats hibernating in Texas culverts select roosts based on microclimate (Meierhofer et al. 2019a) and potentially proximity to other roosts and suitable summer habitat (Sandel et al. 2001). Leivers et al. (2019) suggested ambient weather conditions, including humidity, may drive occupancy of culverts by tri-colored bats. However, no published information is available on microclimates of trees and bridges used by tri-colored bats or what influences winter tree roost selection or shifts between winter roost structures.

Hibernating tri-colored bats have experienced precipitous declines from white-nose syndrome (WNS) particularly in the Southeastern US; however, declines are so far limited to subterranean roosts. WNS is an invasive fungal disease which has killed millions of North America's subterranean hibernating bats (Langwig et al. 2012; Frick et

al. 2015; Powers et al. 2015) since its initial detection in 2006 (Blehert et al. 2009). *Pseudogymnoascus destructans* (Pd), the causal fungal agent of WNS, invades the cutaneous tissues of susceptible hibernating bats during amenable growth conditions (temperatures < 19.5°C; Verant et al. 2012). Affected bats experience a multi-stage disease progression with increased torpid metabolic rates and evaporative water loss resulting in more frequent arousals and premature fat depletion (Reeder et al. 2012; Warnecke et al. 2013; Verant et al. 2014; McGuire et al. 2017). Across five years of WNS spread (2007-2011), tri-colored bats in subterranean roosts in the northeastern U.S. declined between 16% in Virginia and 95% in New York (Turner et al. 2011). In the Southeast, tri-colored bat populations have declined > 91% in Georgia and South Carolina subterranean roosts (P. Pattavina, Southeast Regional White-nose Syndrome Coordinator, U.S. Fish and Wildlife Service, pers. comm.; S. Loeb, Research Ecologist, U.S. Forest Service Southern Research Station, unpub. data). The consistently high mortality rates of tri-colored bats in the southeastern U.S., in contrast to the wide range observed in the Northeast, may be due to the region's relatively warm hibernacula temperatures (Sirajuddin 2018; Lutsch 2019) resulting in faster fungal growth and increased disease severity (Langwig et al. 2016). Furthermore, the strict selection of humid regions by this species in subterranean roosts may mean tri-colored bats are especially susceptible to diseases which upset water balance like WNS (McGuire et al. 2017). However, while subterranean microclimates are ideal for Pd growth and WNS disease development (Langwig et al. 2012; Verant et al. 2012), other winter roost

structures like trees and bridges may have less suitable microclimates. Currently, there are no reported cases of WNS from bats hibernating in trees or bridges and this could be related to the microclimate conditions in these thermally unstable roost structures.

Understanding the tri-colored bat's winter roost ecology in areas devoid of subterranean roosts is essential for effective conservation of this species, especially if WNS susceptibility varies based on winter structure use and microclimate (see Chapter One). Our objectives were to: 1) determine winter roost use by tri-colored bats in an area devoid of caves or mines, 2) determine roost microclimates and relate them to Pd growth conditions, 3) determine if ambient weather conditions (temperature and humidity) influenced roost structure use, and 4) determine tree and habitat characteristics influencing tree roost selection. We hypothesized microclimates would differ between bridges and tree cavities, and predicted that cavities would be cooler and more humid than bridges. Additionally, we hypothesized use of bridges and trees would depend on ambient weather conditions and bats would shift among structures throughout winter to balance energetic costs and benefits related to roost microclimates. Specifically, we predicted bats would use thermally buffered tree cavities on the coldest days, bridges on days with intermediate temperatures, and foliage on the warmest days. We also predicted bats would limit use of bridges and foliage on days with low humidity and increase use of cavities. Finally, we hypothesized that tree and microhabitat characteristics influencing tree roost microclimate would be important for tri-colored bats since winter imposes many energetic constraints. We predicted bats

would select live, large diameter trees for increased thermal buffering and reduced freezing risks, and trees with high canopy closure resulting in low roost temperatures that are conducive for torpor. Our findings will expand the current understanding of tri-colored bat winter roosting ecology and help inform conservation action, particularly in response to WNS, for this species.

2. Methods

2.1 Study area

We conducted our study from November through March 2017-2018 and 2018-2019 on the Department of Energy's Savannah River Site (SRS) located in the Upper Coastal Plain of South Carolina. SRS is an 80,267 ha National Environmental Research Park ideal for studying non-subterranean hibernating tri-colored bats because it is devoid of caves, mines, and other subterranean hibernacula. Dominant vegetation types on SRS were loblolly pine (*Pinus taeda*), longleaf pine (*P. palustris*), and bottomland hardwood (e.g., *Nyssa* spp., *Quercus* spp.) forests (Imm and McLeod 2005). We focused our research efforts around three previously identified concrete I-beam bridges (Loeb and Zarnoch 2011). Tri-colored bats frequently hung from the ribbed metal decking under these bridges which provided easy walk-in access to hand-capture bats. The habitat surrounding the study bridges was a mix of mature and regenerating bottomland hardwood, upland hardwood, mixed pine-hardwood, and pine forests, as well as industrial facilities and waste remediation sites (large grass-covered clearings).

2.2 Field data collection

We visually inspected the three study bridges for tri-colored bats during daylight hours and hand-captured available bats from the bridge ceiling. For each captured bat we determined sex, reproductive condition, wing condition, and measured body mass and forearm length. Prior to transmitter attachment, we banded each bat with a 2.4 mm aluminum lipped band (Porzana Ltd., East Sussex, UK). We trimmed hair between bats' scapulae and affixed a 0.42 g LB-2XT temperature-sensitive transmitter (Holohil Systems Ltd., Ontario, Canada) using surgical glue (Perma-Type Company, Inc., Plainville, CT USA). After a minimum 15 minute holding period to ensure transmitter attachment, we placed bats back in their original roosting location. We followed the U.S. Fish and Wildlife Service Decontamination Protocol when handling bats or visiting bridge roosts. All methods were approved by the Clemson University IACUC (#2017-057) and U.S. Forest Service IACUC (#2017-017).

We recorded skin temperatures (Tsk) of bats in their roosts at 15 minute intervals during a 20 second scan period for active transmitter frequencies using SRX-800D dataloggers (Lotek Engineering Inc., Ontario, Canada) paired with a 3- or 5-element Yagi antenna. Dataloggers were maintained at bridges for the duration of the field season and at tree roosts for the duration of the bat's transmitter life. We changed the external 12-volt battery powering each datalogger every 2-3 days or 3-4 days depending on battery size. We manually checked Tsk records for potentially inaccurate recordings (e.g., Tsk < 0°C or > 40°C) which were a result of low transmitter battery

and/or poor signal strength. We classified thermoregulatory phases using abrupt increases and decreases in T_{sk} ($> 5^{\circ}\text{C}$ in ≤ 30 min). We also recorded roost temperature (T_r) and relative humidity (RHr) every 30 minutes using Hygrochron iButtons (Maxim Integrated, San Jose, CA USA). We collected T_r and RHr from the study bridges during both years, T_r in accessible cavities (i.e., cavities with basal or mid-bole openings and an entrance width larger than 8 cm) during both years, and roost RHr in accessible cavities only during the second year. We acquired ambient temperature (T_a) and relative humidity (RHa) from an on-site climatology station through the Atmospheric Technologies Group of the Savannah River National Laboratory. Climatology measurements of T_a and RHa were not biologically different from measurements of T_a and RHa collected using iButtons in forest habitat near used trees (see Chapter One).

We attempted to locate each bat's day roost every successive day following transmitter attachment using a 3-element Yagi antenna and a TR5 (Telonics, Inc., Mesa, AZ USA) or R1000 (Communication Specialist, Inc., Orange, CA USA) receiver. We tracked bats for the duration of their transmitter life or until they were undetectable for > 7 days. We located roosts by homing in on the signal and when we were within 20 m of the general location, we triangulated around the point until we identified an exact location. If we could not confirm the bat's location through visual sightings or emergence surveys, we used the suspected tree as the bat's location and noted its assumed position (e.g., canopy or trunk). We marked roost trees with a numbered aluminum tag and recorded the coordinate location using a Geo7X GPS (Trimble

Navigation, Ltd., Sunnyvale, CA USA). In order to minimize disturbance, the only tree characteristics collected while a bat was present were canopy closure, roost structure type, and tree species. Hemispherical photos for canopy closure estimates were taken 1.5 m from the base of the tree using a Coolpix digital camera (Nikon, Inc., USA) mounted with a fisheye lens and placed on a 1.5 m pole to stabilize shots and limit the amount of ground cover within the photo-capture area. At each location, we took three photos maintaining the same camera position, but with varying exposure settings (-1, 0, and +1).

After the bat switched roosts or a minimum of 10 days had passed, we returned to record diameter at breast height (DBH), tree height, cavity entrance width, cavity entrance height, number of potential roost structures, number of entrances on the tree, decay status, and distance to the nearest tree of equal or greater height. Decay status was assigned using a system modified from Boyles and Robbins (2006) to fit our study system with a scale from 1-4 as follows: 1) live, >75% of bark and canopy intact with the only obvious signs of decay being cavity formation; 2) declining live, 50-75% of bark and canopy intact with some obvious signs of decay including large dead and/or broken limbs; 3) severely declining live or dead, 25-50% of bark and canopy intact with multiple dead and/or broken limbs; and 4) dead, <25% of bark and canopy intact with extensive external and internal decay leaving mainly the trunk remaining. We also recorded DBH and tree species of all trees greater than 10 cm in DBH within a 0.1 ha plot centered on the roost tree, as well as the number of trees with cavities (i.e., plot cavity abundance).

We used these data to count the number of small trees (< 16 cm DBH) and large trees (≥ 25 cm DBH) within each plot.

Because we used discrete choice modeling, we needed to establish a choice set of used and available trees for selection analysis. The number of choice sets was determined by the number of roosting events, which we defined as the selection of a day roost following a period of normothermia by the individual. For example, if a torpid bat became normothermic but remained in its initial roost before reentering torpor, we considered this as an additional roosting event at the selected tree. The duration of time elapsed between repeated roosting events varied widely from 1 day to 15 days. We limited the number of roosting events per tree for each bat to three due to time and sampling constraints. For each roosting event, we buffered the used tree by 1.2 km and generated three random points within the buffer area to survey for available trees within the choice set. Due to limited knowledge of winter travel distances for tri-colored bats, our buffer was based on the maximum known distance travelled between two trees in a single night by a female tri-colored bat we tracked in 2017-2018. We defined a tree to be available at our random locations if it possessed at least one of the following characteristics: 1) one or more cavity structures with a minimum entrance width of 5 cm, 2) a decay class of 2 or greater, and 3) total of > 1 m² of dried foliage clumps. If we did not find a suitable tree within 100 m of a random point, we generated another

random point for survey. We collected the same tree and plot characteristics at random trees as used trees.

We accessed data on stand structure and vegetation type for SRS from the field sampled vegetation database within the Natural Resources Information System framework (USDA Forest Service 2018). We used a geographic information system (ArcGIS 10.5.1 Environmental Systems Research Institute, Redlands, CA USA) with the vegetation data and digital imagery to determine stand vegetation types and calculate distance to linear openings (e.g., roads, powerlines, and railroads), distance to nonlinear openings (e.g., stands <5 years in age, wildlife openings, and maintained clearings such as waste remediation sites), and distance to perennial streams for used and random tree locations. We reclassified the 13 vegetation types present within our study area into four broad vegetation types: bottomland hardwood forest, upland hardwood forest, mixed mesophytic pine-hardwood forest, and pine forest.

2.3 Statistical analyses

We calculated vapor pressure deficit (VPD) using simultaneous measurements of temperature (T_a or T_r) and relative humidity (RH_a or RH_r) and the following equation:

$VPD \text{ (kilopascal [kPa])} = [(100 - RH) / 100 * SVP] / 1000$ (Monteith and Unsworth 1990)

where $SVP \text{ (kPa)} = [610.7 * 10^{7.5T / (237.3 + T)}] / 1000$ (Murray 1967). We defined T_r below the maximal Pd growth threshold of 19.5°C (Verant et al. 2012) as amenable to fungal growth and infection (i.e., the Pd-zone).

We achieved a near continuous record of Tr and roost VPD (VPDr) under the bridges. In contrast, the accessible cavities were mostly monitored during use. The differences in these two sampling methods created an uneven number of observation days resulting in longer records from bridges than from cavities. Additionally, cavity records frequently represented only a small window of time at various stages throughout the winter. To address the first issue, we only used bridge records from days in which we also had a corresponding cavity measurement. For the second issue, we standardized measurements of mean daily Tr and VPDr by subtracting the corresponding mean ambient weather condition. The standardization created an adjusted daily mean Tr and VPDr representing the daily difference between roost and ambient weather conditions. We used a two-way analysis of variance (ANOVA) to analyze the effect of roost structure (bridge or cavity) and year (2017-18 or 2018-19) on the adjusted daily mean Tr. We used a one-way ANOVA to analyze the effect of roost structure on the adjusted daily mean VPDr. Both ANOVAs also included a random effect of roost to account for repeated measures. For post-hoc analyses of ANOVA results, we used Tukey's Honest Significant Difference to compare group levels of significance at a 95% confidence level. All statistical analyses were performed in R version 3.5.2 (R Core Team 2019) and values are reported as the mean \pm *SD*.

Our three roost structure categories were bridges, tree cavities, and foliage. Of the tree structures, cavities are thermally buffered from changes in Ta (Coombs et al. 2010), while foliage roosts are not thermally buffered. For tree roost structure use that

could not be visually verified (hidden cavity or foliage), we inferred the roost structure used based on Tsk fluctuations. For example, T_r in a cavity increases an hour or more after a corresponding increase in T_a as a result of thermal buffering. Since a bat can only be as cold as its roost, torpid Tsk which increase simultaneously with T_a are likely from bats in foliage or similarly thermally unbuffered structures. In contrast, those with a delayed increase in torpid Tsk following an increase in T_a are likely from bats in cavity roosts. We used this method of inference on visually confirmed cavity and foliage roosts as a test of its efficacy, and successfully classified 100% of these verified structures using Tsk fluctuations.

We developed nine *a priori* hypotheses to evaluate winter tree roost selection, including the global model and three sub-global models, based on roost availability, roost microclimate, foraging commute, ease of discovery, and general habitat (Table 1). We tested for collinearity among our predictor variables using a Pearson's correlation coefficient of 0.35. Only our global model contained variables that were highly correlated (cavity abundance with large tree density and distance to linear opening). Thus, we removed plot cavity abundance from the global model in order to retain the greatest number of predictors. We modeled tree roost selection with discrete choice models. The observational units were the choice sets (one used tree and three random available trees). We fit our discrete choice models using the package `mlogit` (Croissant 2015). This package is commonly used for discrete choice analysis and accounts for data obtained from multiple individuals. We ranked competing models using Akaike's

Information Criterion with correction for small sample sizes (AICc) and selected the most parsimonious model based on a $\Delta AICc < 2$. We determined the significance of model parameter estimates based on whether their upper and lower 95% confidence intervals overlapped 0. To assess the performance of our top model, we used 10-fold cross validation (Boyce et al. 2002). We randomly selected 80% of our data to function as training data (with 1:3 choice sets remaining intact) and allocated the remaining 20% of data as test sets for the newly trained model. We repeated this procedure nine times and used the trained model with our test sets to estimate the relative probability of selection of each used or available point in our choice sets. If the proportion of used sites correctly predicted from our pooled test sets was greater than 0.5, we determined that our model was a better fit than would be expected at random.

3. Results

We captured 19 tri-colored bats and tracked 15 bats for an average of 28 ± 10 days (eight in 2017-2018 and seven in 2018-2019; four females and eleven males). We had one recapture in both years: a female in 2017-2018 that was originally banded in 2016-2017 during a pilot study (data not incorporated), and a male in 2018-2019 that was recaptured following initial capture in 2017-2018. Tri-colored bats in bridges frequently hung from 5-cm lips created by the overlapping ribbed metal decking on the bridge ceiling or against the concrete I-beam itself. Bats usually roosted alone although we occasionally observed small clusters (2-3 individuals) in bridges.

We identified 18 tree roosts from November to March 2017-2018, and another six tree roosts from November to February 2018-2019. Bats used between 0 and 4 tree roosts while being tracked. Mean residence time was 8 ± 11 days (median = 3 days) in trees and 9 ± 8 days (median = 5 days) in bridges. The two longest residence times, 39 and 38 days, were by female bats in cavities with basal openings and occurred from mid-January to mid-February 2017-2018 and 2018-2019. The longest residence time in a bridge was 27 days by a male bat from early December to January. Tri-colored bats often returned to previously used bridges throughout winter and occasionally returned to a previously used tree after roosting in a bridge.

The most frequently used tree species were sweetgum (*Liquidambar styraciflua*) (n = 7) and oaks (*Quercus* spp.) (n = 6) including two roosts in swamp chestnut oaks *Q. michauxii*. Bats also used tupelo (*Nyssa* spp.) (n = 3), yellow poplar (*Liriodendron tulipifera*) (n = 3), sweetbay (*Magnolia virginiana*) (n = 2), loblolly pine (*Pinus taeda*) (n = 1), red maple (*Acer rubrum*) (n = 1), and American holly (*Ilex opaca*) (n = 1). Tree roost structures were in cavities with basal openings (n = 5), cavities with upper and mid-bole openings (n = 6), a hollow snag with a chimney and mid-bole opening (n = 1), Spanish moss (*Tillandsia usneoides*) (n = 2), a cluster of dried sweetgum leaves in an American holly (n = 1), and various unknown roost structures (n = 9) such as potentially hidden cavities, dead limbs, bark, or foliage. We tracked two individuals to the same sweetgum, one in 2017-2018 and the other in 2018-2019. We also found an unbanded tri-colored bat occupying a cavity with a basal opening in a sweetgum previously used by a tracked

individual while we were retrieving an iButton. Only a single individual occupied this cavity during each observation.

The daily mean T_a during November through March was $10.7 \pm 6.1^\circ\text{C}$ in 2017-2018 and $10.5 \pm 4.8^\circ\text{C}$ in 2018-19. The coldest daily mean T_a observed in 2017-18 (-4.5°C) was lower than the coldest daily mean in 2018-19 (1.2°C). Conditions were marginally more humid in 2018-2019 (0.31 ± 0.19 kPa) than 2017-2018 (0.47 ± 0.23 kPa) with a maximum daily mean VPDa of 1.185 kPa. The daily mean T_r was $12.9 \pm 4.9^\circ\text{C}$ in bridges and $11 \pm 4.6^\circ\text{C}$ in accessible cavities, while the daily mean VPDr was 0.42 ± 0.32 kPa in bridges and 0.04 ± 0.06 kPa in accessible cavities. The daily mean T_r fluctuation was $4.8 \pm 2.0^\circ\text{C}$ in bridges and $4.0 \pm 1.9^\circ\text{C}$ in accessible cavities with maximums of 13.8°C and 10.5°C , respectively. The proportion of time T_r fell within the Pd-zone was 0.88 ± 0.04 and 0.96 ± 0.04 for bridges and cavities, respectively. While within the Pd-zone, the proportion of time roost temperatures fell within the optimal growth range (12.5 to 15.8°C) was 0.28 ± 0.02 for bridges and 0.20 ± 0.07 for cavities. Finally, the proportion of time T_r within the Pd-zone were above 15.8°C was 0.22 ± 0.01 .

We found a significant interaction between roost structure and year on adjusted daily mean T_r ($F_{1,294} = 36.261$, $P < 0.001$). Post-hoc analyses showed significantly higher adjusted daily mean T_r in bridges than cavities in both 2017-18 ($P < 0.001$) and 2018-19 ($P = 0.012$), significantly higher adjusted daily mean T_r in 2018-19 than 2017-18 within cavity roosts ($P < 0.001$), and no significant difference between years within bridge

roosts ($P = 0.076$) (Figure 1a). We found a significant effect of roost structure on VPD_r, with higher adjusted daily mean VPD_r in bridges than cavities ($F_{1,5} = 36.261$, $P = 0.003$) (Figure 1b).

Daily mean T_a significantly differed on days that roost structures were used ($F_{2,48} = 10.282$, $P < 0.001$) (Figure 2a), but daily mean VPD_a did not ($F_{2,241} = 2.273$, $P = 0.105$) (Figure 2b). Mean T_a on days bats used bridges significantly differed from days bats used cavities and foliage ($P = 0.031$ and $P < 0.001$, respectively), while mean T_a on the days bats used the two tree structures did not significantly differ ($P = 0.268$). Days were cooler ($8.7^\circ\text{C} \pm 5.0$) when bats used bridges than on days that they used trees ($11.3^\circ\text{C} \pm 5.4$).

We modeled tree roost selection from 51 choice sets comprised of the habitat characteristics of 24 used trees and 153 random available trees (Table 2). The sub-global model containing predictors related to roost microclimate and availability was the top model for predicting tree roost selection, carrying 100% of the model weight (Table 3). Canopy closure, cavity abundance, stream distance, and decay status were significant predictors (Table 4). The relative probability of a bat selecting a tree was negatively related to stream distance (Figure 3a) and decay status and positively related to canopy closure (Figure 3b) and plot cavity abundance (Figure 3c). Although the 95% confidence intervals of stand vegetation type overlapped zero, we think it may be ecologically important. Based on availability, bats appeared to generally avoid roosting in pine

stands (Table 2). Additionally, the increase in the relative selection probability per unit increase in cavity abundance was lower in both pine forests and mixed hardwood-pine forests than in bottomland hardwood forests and upland hardwood forests (Figure 4). Based on the 10-fold cross validation, our top model accurately predicted tree roost selection by this group 89% of the time.

4. Discussion

Tri-colored bats at SRS used several different roost structures during winter. Cavity roosts were consistently cooler and more humid than bridge roosts. Both bridge and cavity T_r were amenable to Pd growth ($< 19.5^\circ\text{C}$) throughout most of the winter and approximately a quarter of the time they were within the optimal growth range of Pd ($12.5\text{-}15.8^\circ\text{C}$). These T_r are similar to temperatures in cave, tunnel, and culvert roosts of tri-colored bats during winter in the Southeast (Sirajuddin 2018; Lutsch 2019; Meierhofer et al. 2019b). Extended periods of time within the optimal growth range of Pd could contribute to increased fungal loads on torpid bats and disease severity (Langwig et al. 2016). However, differences in the adjusted daily mean T_r in cavities between years suggests Pd prevalence in these structures may vary depending on the ambient weather conditions. Further, frequent periods of low humidity in bridges may limit the growth of Pd in these structures (Marroquin et al. 2017) while potentially stressful high temperatures ($T_r > 15.8^\circ\text{C}$) may limit the overall reproductive capacity of Pd (Verant et al. 2012).

Contrary to our prediction, tri-colored bats did not use roost structures based on ambient humidity. Evaporative water loss in torpid bats increases at low humidity and bats must arouse more frequently and consequently, expend more energy to offset dehydration (Thomas and Cloutier 1992). Tri-colored bats are generally considered sensitive to evaporative water loss because they select very humid regions in caves (Raesly and Gates 1987; Briggler and Prather 2003). However, some bat species exhibit intraspecific variation in evaporative water loss. Big brown bats (*Eptesicus fuscus*) hibernating in arid microclimates have lower evaporative water loss during dry conditions than bats accustomed to hibernating in humid microclimates (Klüg-Baerwald and Brigham 2017). Our results suggest intraspecific variation may also occur in tri-colored bats. Tri-colored bats using trees and bridges may be less energetically constrained than subterranean hibernating populations during winter and able to tolerate more frequent arousals or are not as sensitive to evaporative water loss as subterranean populations. Our results highlight the need to explore physiological responses to environmental conditions across the entire suite of a species' habitat uses and range (Dunbar and Brigham 2010).

Tri-colored bats at SRS used bridges on cooler days ($8.7^{\circ}\text{C} \pm 5.0$) and trees on warmer days ($11.3^{\circ}\text{C} \pm 5.4$). Short-term shifts in structure use in response to T_a during winter appears to be a common strategy among tree roosting bats. Both eastern red bats (*Lasiurus borealis*) and Seminole bats (*L. seminolus*) roost in foliage during warm winter periods but shift to leaf litter during colder periods (Mormann and Robbins 2007;

Hein et al. 2008). Since cavities were consistently colder than bridges, and foliage provides poor insulation, trees may pose a greater freezing risk than bridges for tri-colored bats during cold periods. Bridges were likely warmer than cavities during cold periods as a result of solar radiation and concrete's high thermal mass. Therefore, bridges may provide predictable microclimates for tri-colored bats at SRS to use during periods of increased freezing risk.

As we predicted, bats selected trees based on decay status and canopy closure which may correspond to thermal properties of the roost. Tri-colored bats at SRS often selected live trees with minimal decay and closed canopy structures. Cavities in live trees buffer T_a better and thus respond more slowly to changes in T_a than dead trees resulting in increased thermal stability (Sedgeley 2001; Coombs et al. 2010). Additionally, a closed canopy reduces the amount of solar radiation and contributes to a cooler microclimate in cavity and foliage roosts. Colder temperatures allow for lower metabolic rates during torpor which reduces energy expenditure (Geiser 2004). Therefore, the relatively cool and stable microclimates of live trees with a closed canopy may be important for winter torpor in tri-colored bats.

Predicted selection of trees in areas with high cavity abundance may be related to access to multiple microclimates. No single microclimate provides 'optimal' roosting conditions for the entire winter period (Boyles et al. 2017). Cave hibernating bats shift locations within hibernacula based on microclimate throughout winter as needed

(Boyles et al. 2007, 2017; Ryan et al. 2019). Other tree roosting bats also vary their microclimate use in response to fat reserves and weather (Stawski et al. 2009; Stawski and Currie 2016). Tri-colored bats at SRS may prefer access to a wide range of microclimates which they can use depending on energetic needs associated with ambient weather conditions and food availability.

Our results suggest landscape-scale features are important for tri-colored bat tree roost selection during winter. Tri-colored bats at SRS appeared to avoid pine forests, favoring hardwood forests, and selected trees closer to streams. Of the three roosts we observed in pine forests, all were in foliage. Summer studies of tri-colored bat roost selection also recorded conifer use if there was dead vegetation to provide concealment (Perry and Thill 2007; O'Keefe et al. 2009). Selection of hardwood forests and trees near streams may be related to foraging and drinking resources, as well as roost suitability. During the summer, tri-colored bats favor riparian habitats for foraging (Ford et al. 2005; Menzel et al. 2005) and Sandel et al. (2001) suggested tri-colored bats in Texas may select culverts due to their proximity to other roosts and suitable summer habitat. Tri-colored bats at SRS may continue to use hardwood forests near streams because of their proximity to suitable summer habitat. Alternatively, tri-colored bats at SRS may return to summer roosting and foraging habitats during winter only if T_a are suitably mild ($> 10^\circ\text{C}$) for winter foraging opportunities.

In conclusion, cavity and bridge microclimates of roosts used by tri-colored bats at SRS were similar to microclimates used by tri-colored bats susceptible to WNS in subterranean roosts. However since Pd growth is temperature dependent (Verant et al. 2012) and fungal load is linked to disease severity (Langwig et al. 2016), tri-colored bats at SRS may experience varying degrees of Pd prevalence and WNS severity based on the influence of ambient weather conditions on TR and humidity conditions. Furthermore, roost structure use by tri-colored bats at SRS suggests these bats may either be less energetically constrained or less sensitive to evaporative water loss than subterranean populations which may alter host-pathogen dynamics and WNS-related disease effects. Therefore, tri-colored bats using bridge and tree roost structures may be less susceptible to WNS and forest habitats which support tri-colored bats during winter may represent important refugia from WNS. Our results suggest access to multiple roost microclimates may be important for forest dwelling tri-colored bats during winter and a diverse array of anthropogenic and tree roost structures may facilitate winter survival. Forest management practices which retain live trees near streams with multiple roosting structures and foster cavity formation in hardwood forests will likely benefit this population. Additionally, winter surveys of anthropogenic structures like bridges may help identify other tri-colored bat populations using non-subterranean roosts during winter. Given tri-colored bats at SRS used both tree and bridge structures, we encourage future studies to also explore the possibility of multiple roost structure use within a single winter season. More research is needed to determine the viability of

forest-dwelling tri-colored bat populations across the Southeastern US, as well as their broad conservation needs. Many parts of the Southeastern US are devoid of subterranean roosts and non-subterranean structures likely represent an important part of the tri-colored bat's winter roosting ecology in this region.

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Table 1 Hypotheses and variables for models predicting winter tree roost selection for tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina, 2017-2019.

Model	Predictor Variable	Prediction
Roost Microclimate (RM)	Decay	-
	DBH	+
	Canopy closure	+
Roost Availability (RA)	Cavity abundance	+
	Stand vegetation type	Bottomland hardwood
	Stream distance	-
Ease of Discovery (ED)	Tree height	+
	Distance to \geq height tree	+
	Canopy closure	-
	Small tree density	-
Foraging Commute (FC)	Linear opening distance	-
	Non-linear opening distance	-
	Stream distance	-
General Habitat	Small tree density	-
	Large tree density	+
	Tree species	<i>Nyssa</i> spp. & <i>Quercus</i> spp.
	Stand vegetation type	
FCED	Foraging commute	All of the above
	Ease of discovery	
FCRM	Foraging commute	All of the above
	Roost microclimate	
RMRA	Roost microclimate	All of the above
	Roost availability	
Global	All of the above excluding cavity abundance due to multicollinearity	

Table 2 Mean and standard deviations of predictor variables measured at tri-colored bat (*Perimyotis subflavus*) winter roost trees and available trees in the Upper Coastal Plain of South Carolina, 2017-2019.

Variable	Used Trees		Available Trees	
	Mean	SD	Mean	SD
Cavity abundance within 0.1 ha plot	8.1	2.5	3.8	2.8
Diameter at breast height (cm)	51.4	20.6	43.6	16.4
Distance to tree of \geq height (m)	4.10	2.4	4.3	3.3
Linear opening distance (m)	152.6	148.8	142.8	147.9
Non-linear opening distance (m)	424.9	290.6	440.4	415.1
Number large trees within 0.1 ha plot	23.1	5.6	18.3	8.6
Number small trees within 0.1 ha plot	18.9	8.4	18.6	13.1
Stream distance (m)	86.4	73.5	197.6	148.8
Tree height (m)	24.0	9.3	20.5	9.0
% canopy closure	78.2	7.4	74.6	12.4
% live	45.8		28.8	
% live declining	41.7		27.5	
% severely declining	4.2		17.6	
% dead	8.3		26.1	
% bottomland hardwood forest	62.5		41.2	
% upland hardwood forest	12.5		7.8	
% pine forest	12.5		34.6	
% pine-hardwood forest	12.5		16.3	

Table 3 Candidate set of winter tree roost selection models for tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina, 2017-2019. Abbreviations are as follows: model, refers to the hypothesis evaluated (Table 1); K, number of parameters; AICc, Akaike’s information criterion adjusted for small sample size; Δ AICc, difference of AICc between a model and the model with the smallest AICc; and weight, model weights.

Model	K	LogLik	AICc	ΔAICc	weight
RMRA	10	-9.2	43.8	0.0	1.0
Roost Availability (RA)	5	-22.3	55.8	12.0	0.0
FCRM	8	-38.6	96.7	52.8	0.0
Foraging Commute (FC)	3	-48.6	103.6	59.8	0.0
FCED	7	-43.9	104.3	60.5	0.0
Global	18	-26.8	111.1	67.2	0.0
Roost Microclimate (RM)	5	-52.9	117.2	73.4	0.0
General Habitat	8	-50.8	121.1	77.3	0.0
Ease of Discovery (ED)	4	-65.3	139.4	95.6	0.0

Table 4 Coefficient estimates from the top model, roost microclimate-roost availability, predicting tree roost selection of tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina, 2017-2019.

Covariate	Estimate	SE	LCL 95%	UCL 95%
Canopy Closure	5.1	2.4	0.4	9.9
Cavity Abundance	6.0	2.6	0.9	11.2
Diameter at Breast Height	-0.8	0.7	-2.2	0.7
Stream Distance	-7.0	3.0	-12.8	-1.1
Decay: Declining Live	0.7	1.2	-1.6	3.0
Decay: Severely Declining	-13.0	6.3	-25.4	-0.5
Decay: Dead	-2.0	2.3	-6.5	2.5
Stand: Upland Hardwood Forest	-1.0	1.8	-4.7	2.6
Stand: Pine Forest	-9.7	5.2	-19.9	0.5
Stand: Mixed Hardwood-Pine Forest	-4.2	12.8	-29.3	20.9

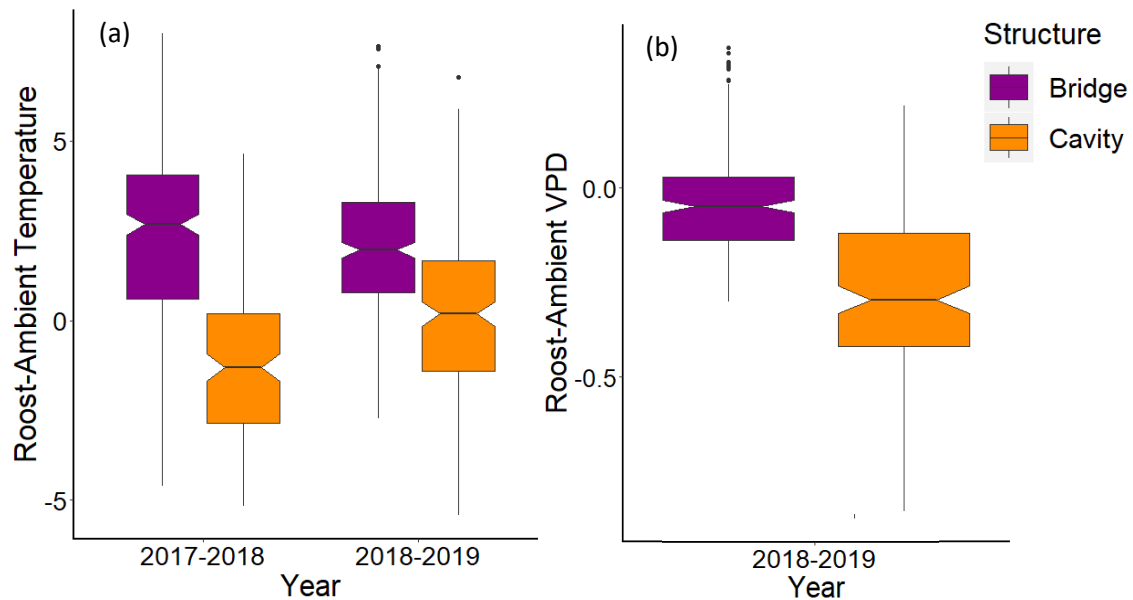


Fig. 1 We standardized measurement of roost conditions by subtracting the corresponding ambient weather conditions to create an adjusted daily mean representing the difference of roost and ambient conditions. (a) The adjusted daily mean temperature ($^{\circ}\text{C}$) and (b) vapor pressure deficit (kPa) of bridge and cavity roosts of tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina, 2017-2019. Vapor pressure deficit measurements are limited to one year of observation, 2018-2019.

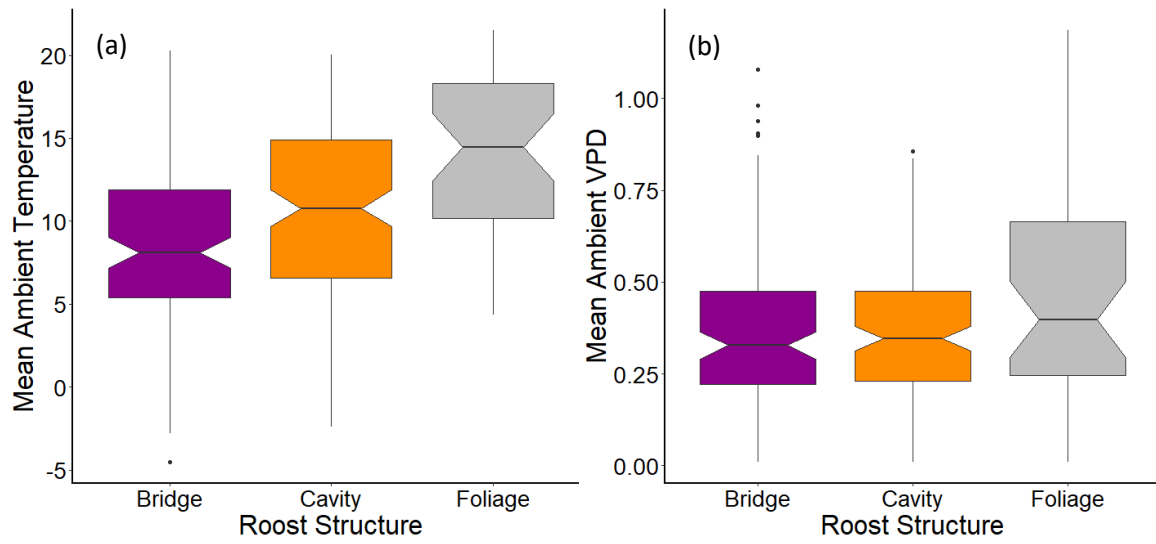


Fig. 2 (a) Ambient temperature (°C) and (b) vapor pressure deficit (VPD [kPa]) during structure use by tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina, 2017-2019.

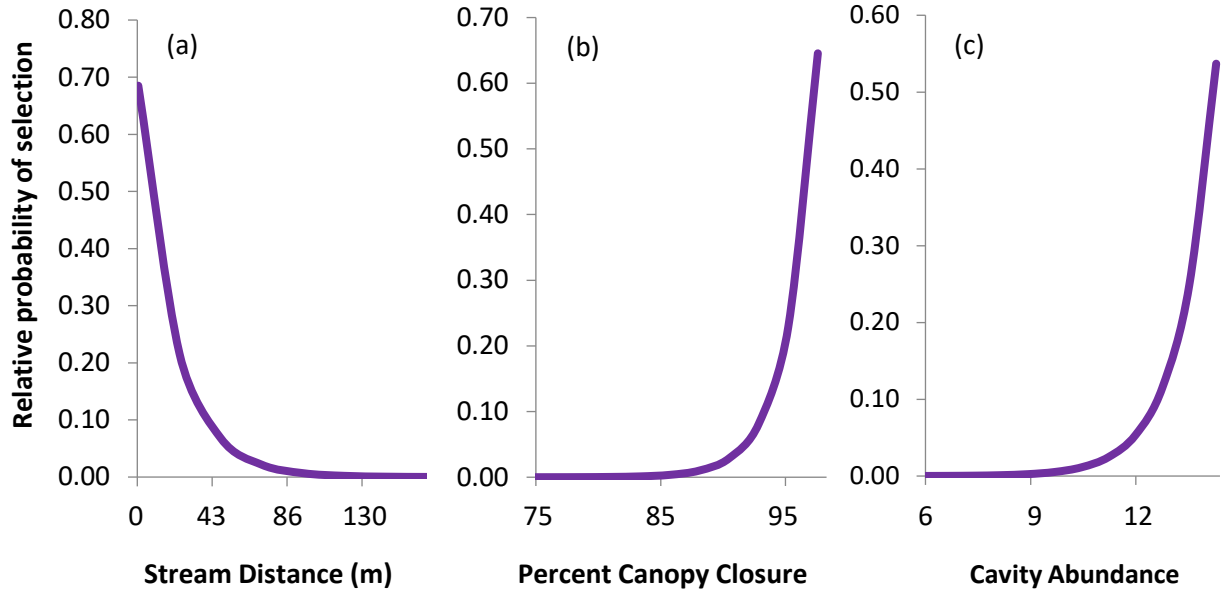


Fig. 3 (a) Relative probability of selection as a function of stream distance, (b) percent canopy closure, and (c) plot cavity abundance for tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina during winter, 2017-2019.

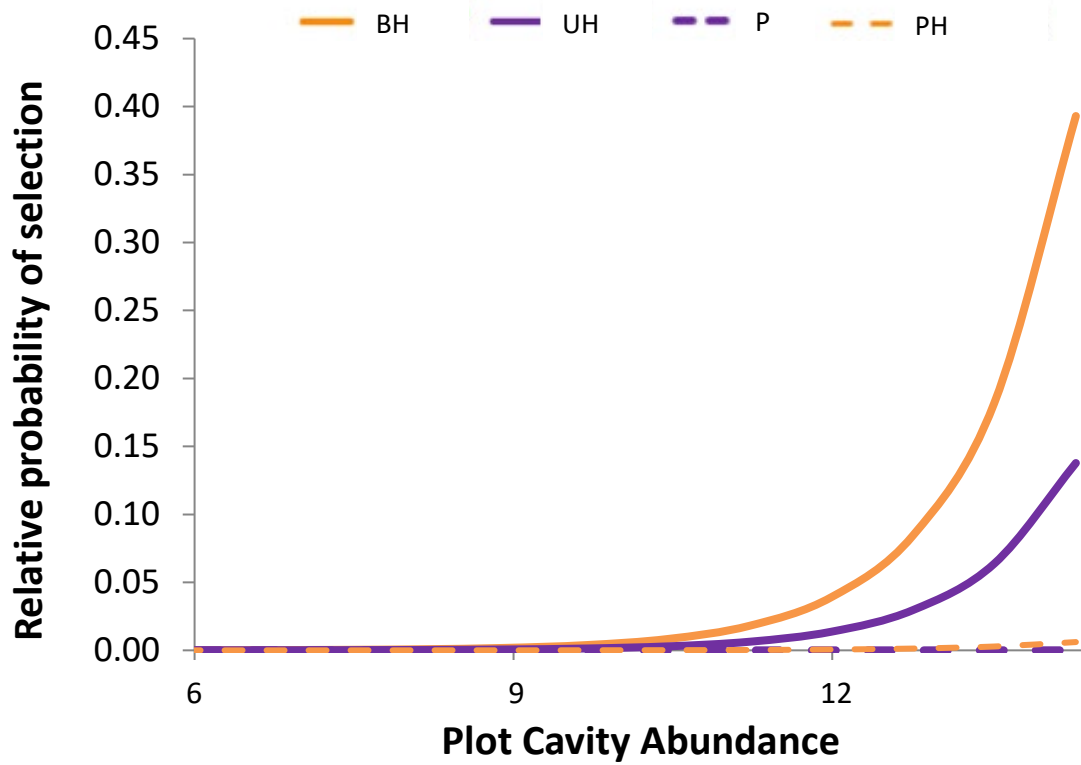


Fig. 4 Relative probability of selection as a function of plot cavity abundance within stand vegetation type for tri-colored bats (*Perimyotis subflavus*) in the Upper Coastal Plain of South Carolina during winter, 2017-2019. Stand vegetation type from left to right: bottomland hardwood forest (BH), upland hardwood forest (UH), pine forest (P), and mixed mesophytic pine-hardwood forest (PH).