

Thermally unstable roosts influence winter torpor patterns in a threatened bat species

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Many hibernating bats in thermally stable, subterranean roosts have experienced precipitous declines from white-nose syndrome (WNS). However, some WNS-affected species also use thermally unstable roosts during winter that may impact their torpor patterns and WNS susceptibility. From November to March 2017–19, we used temperature-sensitive transmitters to document winter torpor patterns of tricolored bats (*Perimyotis subflavus*) using thermally unstable roosts in the upper Coastal Plain of South Carolina. Daily mean roost temperature was $12.9 \pm 4.9^\circ\text{C}$ SD in bridges and $11.0 \pm 4.6^\circ\text{C}$ in accessible cavities with daily fluctuations of $4.8 \pm 2.0^\circ\text{C}$ in bridges and $4.0 \pm 1.9^\circ\text{C}$ in accessible cavities and maximum fluctuations of 13.8 and 10.5°C , respectively. Mean torpor bout duration was 2.7 ± 2.8 days and was negatively related to ambient temperature and positively related to precipitation. Bats maintained non-random arousal patterns focused near dusk and were active on 33.6% of tracked days. Fifty-one percent of arousals contained passive rewarming. 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. Our results suggest ambient weather conditions influence winter torpor patterns of tricolored bats using thermally unstable roosts. Short torpor bout durations and potential nighttime foraging during winter by tricolored bats in thermally unstable roosts contrasts with behaviors of tricolored bats in thermally stable roosts. Therefore, tricolored bat using thermally unstable roosts may be less susceptible to WNS. More broadly, these results highlight the importance of understanding the effect of roost thermal stability on winter torpor patterns and the physiological flexibility of broadly distributed hibernating species.

Key words: *Perimyotis subflavus*, roost microclimate, short-term torpor, thermally unstable roosts, white-nose syndrome

Editor: Rebecca Cramp

Received 21 March 2023; Revised 2 February 2024; Editorial Decision 24 February 2024; Accepted 14 March 2024

Cite as: Newman BA, Loeb SC, Jachowski DS (2024) Thermally unstable roosts influence winter torpor patterns in a threatened bat species. *Conserv Physiol* 12(1): coae014; doi:10.1093/conphys/coae014.

Introduction

Winter represents a time of negative energy balance for insectivorous temperate zone bats due to decreased insect availability and increased thermoregulatory costs (e.g. Turbill, 2008; Jonasson and Willis, 2011). 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). Torpor 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 (Geiser *et al.*, 1989), 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. missed opportunities, increased risk of predation) of being torpid (Humphries *et al.*, 2003; Boyles *et al.*, 2020).

Heterotherms generally fall into two physiologically and ecologically distinct categories: hibernation and daily heterothermy (Ruf and Geiser, 2015). Hibernators maintain lower metabolic rates and body temperatures than daily heterotherms and are capable of multiday torpor bouts lasting >30 days (Geiser, 2004; Ruf and Geiser, 2015). In contrast, daily heterotherms maintain higher metabolic rates and generally remain torpid for only 3–12 h with a maximum torpor bout duration of <2 days under all thermal, environmental and nutritional conditions (Geiser, 2020). Hibernators often have random patterns of arousal, while daily heterotherms usually arouse prior to normal activity hours (Ruf and Geiser, 2015). However, a hibernating species' temporal expression of torpor falls along a continuum between short-term torpor and hibernation that reflects its specific behaviors, local climate and weather conditions and roost microclimates (Turbill and Geiser, 2008; Ruf and Geiser, 2015; McNab and O'Donnell, 2018; McGuire *et al.*, 2021). The short-term torpor of hibernators shares many temporal characteristics with daily heterothermy, particularly in warmer climates, despite remaining metabolically distinct (Geiser, 2004, 2020; Ruf and Geiser, 2015). Populations of hibernators nearer the short-term torpor end of the continuum frequently have short torpor bouts (<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; Nowack *et al.*, 2020). Additionally, these populations often maintain non-random arousal patterns that allow them to exploit favorable foraging conditions and supplement winter fat reserves (Stawski and Geiser, 2010; Johnson *et al.*, 2012; Nowack *et al.*, 2020; Chenery *et al.*, 2022). Identifying a hibernating species or population's position along the torpor continuum is increasingly important in North America because winter behaviors have the potential to influence white-nose syndrome (WNS) susceptibility (Johnson *et al.*, 2012; Langwig *et al.*, 2012; Langwig *et al.*, 2016; Jackson *et al.*, 2022).

WNS is an invasive fungal disease that has killed millions of hibernating bats in North America (Langwig *et al.*, 2012; Frick *et al.*, 2015; Powers *et al.*, 2015) since its initial detection in 2006 (Blehert *et al.*, 2009). Under favorable 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 hibernating bats rely heavily on stored fat reserves to survive winter, disruption of natural hibernation patterns resulting in premature fat depletion can be fatal (Warnecke *et al.*, 2013; Verant *et al.*, 2014; McGuire *et al.*, 2017).

However, bats using thermally unstable roosts may exhibit winter torpor patterns nearer the short-term torpor end of the continuum that offset the typical consequences of Pd infection. For example, passive rewarming (use of 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 (Hallsall *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 complete active rewarming (Turbill, 2008; Hallsall *et al.*, 2012) and reduces cardiovascular demands and oxidative stress (Currie *et al.*, 2015). Additionally, bats in thermally unstable roosts frequently have short torpid bouts (<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 often maintain non-random arousal patterns, similar to daily heterotherms, that allow 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 indicative of short-term torpor than hibernation may be less susceptible to WNS due to energetically conservative arousals, high winter activity levels and winter fat supplementation.

Tricolored bats (*Perimyotis subflavus*) are widely distributed across eastern North America (Geluso *et al.*, 2005) and have experienced striking declines since the onset of WNS. Tricolored bat populations have declined by 90% over 59% of their range (Cheng *et al.*, 2021). In the southeastern USA, summer tricolored bat populations within the WNS zone in Georgia have declined by 50% (Perea *et al.*, 2022), whereas populations in an infected hibernaculum in South Carolina declined by >90% (Loeb and Winters, 2022) and in

Georgia by >95% (Gabriel *et al.*, 2022). The high mortality rates of tricolored bats in southeastern USA hibernacula despite shorter winters may be due to the region's relatively warm hibernacula temperatures (Sirajuddin, 2018; Lutsch *et al.*, 2022) resulting in faster fungal growth and disease severity (Langwig *et al.*, 2016). However, tricolored bats also use thermally unstable, aboveground roosts in regions devoid of subterranean hibernacula throughout the southeastern USA. Aboveground roosts used by tricolored bats include bridges in Louisiana (Ferrara and Leberg, 2005) and South Carolina (Newman *et al.*, 2021), culverts in Texas (Sandel *et al.*, 2001; Meierhofer *et al.*, 2019) and Georgia (Lutsch *et al.*, 2022) and tree cavities in South Carolina (Newman *et al.*, 2021).

Our objective was to determine where tricolored bats using thermally unstable bridge and tree roosts lie on the torpor continuum. We hypothesized tricolored bats using winter roosts with thermally unstable microclimates would exhibit skin temperatures (Tsk) and torpor patterns closer to the short-term torpor end of the continuum than the hibernation end. We predicted 1) that bats using thermally unstable roosts would experience Tsk unfavorable to Pd growth during torpor, 2) that bats would use passive rewarming to reduce arousal costs, 3) that bat torpor and activity patterns would be related to ambient weather conditions because bats can track these conditions in thermally unstable aboveground roosts and 4) 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.

Materials and Methods

Study area—We conducted our study from November to March 2017–19 on the Department of Energy's Savannah River Site (SRS), an 80,267-ha National Environmental Research Park. SRS is located in the Upper Coastal Plain of South Carolina, and the natural resources are managed by the United States Department of Agriculture Forest Service (Imm and McLeod, 2005). Dominant vegetation types on the SRS were loblolly pine (*Pinus taeda*), longleaf pine (*P. palustris*) and bottomland hardwood (e.g. *Nyssa* spp., *Quercus* spp.) forests (Imm and McLeod, 2005). Since SRS is devoid of subterranean hibernacula, we focused our research efforts around three concrete I-beam bridges (Loeb and Zarnoch, 2011) that provided easy walk-in access to hand-capture torpid tricolored bats.

Capture methodology and torpor measurements—During daylight hours we hand-captured tricolored bats from the ceilings of the bridges and recorded the sex, reproductive condition, wing condition (Reichard and Kunz, 2009), body mass and forearm length of each bat. We placed a 2.4-mm

aluminum lipped band on the forearm of each bat (Porzana Ltd, East Sussex, UK), trimmed the hair between the 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-min holding period to ensure transmitter attachment, we placed bats back in their original roosting location. We swabbed bats for the presence of Pd following protocols of the laboratory of Dr Christopher Cornelison, Kennesaw State University. All samples from 2017–18 were negative for the presence of Pd, but one of nine swabs from 2018–19 had an inconclusive detection of Pd (whitenosesyndrome.org). No visible signs of WNS were detected in either year at our site, despite the presence of WNS within ~100 km for 3 years prior. All applicable institutional and national guidelines for the care and use of animals were followed including the US 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 US Forest Service IACUC (#2017–017).

We recorded Tsk at roosts at 15-minute intervals during a 20-s scan period for active transmitter frequencies using Lotek SRX-800D data loggers. One or two 5-element Yagi antennas were attached to a data logger placed under each study bridge for the duration of the field season. We identified roost locations via daily radio tracking and placed a 3- or 5-element Yagi antenna paired with a data logger at newly identified roosts. Data loggers at day roosts in trees were maintained for the duration of the bat's transmitter life and if possible, longer, to capture other transmitted bat visitations. We converted inter-pulse intervals recorded by the data logger 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) that were a result of low transmitter batteries and/or poor signal strengths.

We considered Tsk below the maximal Pd growth threshold of 19.5°C (Verant *et al.*, 2012) as favorable to fungal growth and infection (i.e. the Pd zone). We defined Pd bout duration 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 torpor bout as defined below). We evaluated the length of time Tsk fell within the optimal temperature growth range of Pd (12.5–15.8°C) and calculated the proportion of time Tsk was within this optimum range relative to the total time within the Pd zone.

While measures of metabolic rate are ideal for differentiating between thermoregulatory phases of torpor, we used Tsk, which provides an indirect measure of body temperature to identify the thermoregulatory phases of torpor (Barclay *et al.*, 1996, 2001; Willis and Brigham, 2003). Although not exact, Tsk as measured by external temperature-sensitive transmitters is very close to body temperature, particularly during torpor (Willis and Brigham, 2003). Following

Jonasson and Willis (2012), we defined thermoregulatory phases for the analysis of torpor patterns as: 1) cooling bout, an abrupt decrease from normothermic Tsk for two consecutive readings ($>5^{\circ}\text{C}$ in ≤ 30 min) that ended when the slope of the cooling curve fell to $\leq 2^{\circ}\text{C}$ per 15 min; 2) active warming bout, an abrupt increase in torpid Tsk for two consecutive readings ($>5^{\circ}\text{C}$ in ≤ 30 min) that eventually stabilized at normothermic Tsk; 3) normothermic bout, period of raised Tsk between active warming and cooling phases; and 4) torpor bout, period of reduced Tsk between active warming and cooling phases. We also identified periods of passive rewarming during which Tsk increased in parallel with Ta. Due to complete passive rewarming by two bats in 2017–18, we also categorized certain periods of passive rewarming that reached Tsk $> 28^{\circ}\text{C}$ as normothermic bouts. We defined a bat-day as starting at 08:00 and ending at 07:59 the next day. We identified the hour of each arousal using Tsk records and the associated thermoregulatory phases of warming and normothermia.

Activity and roost switching—To determine if bats were feeding during the winter, we placed 1.2-m² fabric and paper sheets under areas frequently used by bridge-roosting tricolored bats, inspected them weekly, and collected fecal samples using tweezers. We also collected any feces expelled during bat handling. Fecal samples were refrigerated until we examined them 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 10–40 $\times$ magnification. Presence of insect parts indicates bats were feeding during periods of activity and an absence of insect parts indicates passage of metabolic wastes and no feeding (Whitaker and Rissler, 1993).

We used three measures of activity: 1) general activity—binary record for each bat representing whether a bat was normothermic (1) or torpid (0) in the late afternoon and evening for every day of observation; 2) activity away—binary record for each bat representing whether a bat left its day roost during a normothermic bout (1) or remained in the roost (0); and 3) a day-roost switch—binary record for each bat representing whether a bat switched roosts (1) or not (0) for each day of observation. The general activity record was based on torpor phases. For activity away, we attributed night-time gaps in the Tsk record that could not be explained by low transmitter strength or data logger battery failure during a normothermic bout to activity away from the day roost. Roost switches were identified using telemetry logs from daily radio tracking efforts.

Environmental covariates—We recorded roost temperature (Tr) and relative humidity every 30 min using Hygrochron iButtons (Maxim Integrated, San Jose, CA, USA). We collected Tr and relative humidity from the study bridges during both years, Tr in accessible cavities (tree cavities with basal or mid-bole openings and an entrance width > 8 cm) during both years, and relative humidity in accessible cavities only during the second year. We acquired

ambient temperature (Ta), relative humidity, 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. We calculated vapor pressure deficit (Kurta, 2014) and saturation vapor pressure using simultaneous measurements of temperature (Ta or Tr) and relative humidity (ambient or roost) using equations found in (Monteith and Unsworth, 1990) and (Murray, 1967). Vapor pressure deficit is an absolute measure of ambient moisture, unlike relative humidity, suitable for examining potential differences in evaporation (Kurta, 2014). To ensure climatology tower data were comparable to Ta and ambient vapor pressure deficit (VPDa) conditions of forests used by bats, we performed a paired *t*-test on the daily mean tower and forest measurements in 2018–19 with a null hypothesis of zero difference. We determined that daily tower measurements, although statistically different from forest measurements of Ta ($t = -4.28$, $df = 119$, $P < 0.001$) and VPDa ($t = -17.45$, $df = 119$, $P < 0.001$), were not biologically different from forest measurements based on estimated mean differences of only $-0.26 \pm 0.65^{\circ}\text{C}$ and -0.11 ± 0.07 kPa, respectively.

Statistical analyses—We excluded single-day torpor bouts and normothermic bouts that occurred on the day of capture from our analyses. Females and males were pooled for analyses due to limited sample size. We assessed model fit and suitability using QQ plots of our residuals. Due to non-normal model residuals in our linear response variables, we transformed these data using the best normalization function identified by the bestNormalize package (Peterson and Cavanaugh, 2019). We then reassessed and confirmed the normality of our model residuals. We tested for collinearity among our predictor variables using a variance inflation factor of 5 (Heiberger and Holland, 2015) 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 mean $\pm$ standard deviation.

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 $\Delta\text{AICc} < 2$. When multiple models were selected, we obtained model-averaged coefficients of the top models and used the conditional average to obtain parameter estimates (Burnham and Anderson, 2002). We determined the significance of model parameter estimates based on whether their upper and lower 95% confidence intervals overlapped 0 (Gardner and Altman, 1986). We used the same six *a priori* hypotheses and model selection procedures as outlined for the Pd bout duration

Table 1: Hypotheses and variables for models predicting Pd bout duration, torpid bout duration and normothermic bout duration for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. 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 <i>et al.</i> , 2009)
Energetic status	Mean Ta	(Stawski <i>et al.</i> , 2009)
	Mass	(Boyles <i>et al.</i> , 2007)
Weather	Mean Ta	(Stawski <i>et al.</i> , 2009)
	Mean Pbar	(Turbill, 2008)
	Total Precip	(Voigt <i>et al.</i> , 2011)
Roost microclimate	Mean VPDa	(Thomas and Cloutier, 1992)
	Roost Type	(Thomas and Cloutier, 1992)
	Mean VPDa*Roost Type	(Thomas and Cloutier, 1992)
Global	Mean Ta	(Stawski <i>et al.</i> , 2009)
	Mean Pbar	(Turbill, 2008)
	Total Precip	(Voigt <i>et al.</i> , 2011)
	Mass	(Boyles <i>et al.</i> , 2007)
	Mean VPDa	(Thomas and Cloutier, 1992)
	Roost Type	(Thomas and Cloutier, 1992)
	Mean VPDa*Roost Type	(Thomas and Cloutier, 1992)
Null	Null	

analysis to predict torpor bout duration and normothermic bout duration (Table 1).

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-validated bandwidth selector minimizing Kullback–Leibler loss to identify the hour of day with the highest probability of arousal (Oliveira-Santos *et al.*, 2013). 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 [activity away] and roost switching [day-roost switch] (Table 2). We also included a predictor variable accounting for day roost structure in models for general activity and activity away from the roost since we found a non-random association between our predictor and response based on Fisher's exact test ($P < 0.001$ and $P < 0.001$, respectively). The roost structure variable was not added to roost switching models since we found no significant difference ($P = 0.07$). We tested our hypotheses using generalized linear mixed effect models (GLMMs) with a logit link and a random effect of individual. We used the same model selection procedures as outlined for the Pd bout duration analysis to predict the probability of general activity, the probability of activity away and the probability of a roost switch.

Results

We recovered Tsk data from 11 of 18 transmittered bats (four females and seven males; Fig. 1a). We had one recapture in both years: a female in 2017–18 originally banded in 2016–17 during a pilot study (data not incorporated) and a male in 2018–19 recaptured following initial capture in 2017–18. We regarded the male recaptured in 2018–19 as a distinct individual, and Tsk records were not pooled across years; thus we assumed that we had readings from 12 unique individuals across 2 years. Daily mean Ta and VPDa during November through March 2017–19 were $10.9 \pm 5.5^\circ\text{C}$ (range = -4.5 – 22.1°C) and 0.42 ± 0.43 kPa (range = 0.01 – 1.185 kPa), respectively.

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 (five with basal openings, seven with mid-bole and chimney openings), Spanish moss (*Tillandsia usneoides*) or dead leaves ($n = 3$) and unknown structures in the canopy ($n = 9$). Daily mean Tr was $12.9 \pm 4.9^\circ\text{C}$ in bridges and $11.0 \pm 4.6^\circ\text{C}$ in accessible cavities, while daily mean roost vapor pressure deficit was 0.42 ± 0.32 kPa in bridges and 0.04 ± 0.06 kPa in accessible cavities. Daily mean Tr fluctuation was $4.8 \pm 2^\circ\text{C}$ in bridges and $4.0 \pm 1.9^\circ\text{C}$ in accessible cavities with maximum fluctu-

Table 2: Hypotheses and variables for models predicting general activity, night-time activity away, and roost switching for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. Predictor abbreviations are as follows: Ta, ambient temperature; pVPDa, previous day's ambient vapor pressure deficit; Pbar, barometric pressure; and Condate or Condate², linear or quadratic form of days from 1 November. Roost type was only included in the general activity and night-time activity away models

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

ations of 13.8 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) (Supplementary Table S1). While within the Pd zone, the proportion of time Tsk was within the optimal growth range (12.5–15.8°C) varied from 0.24 to 0.66 (0.39 ± 0.13). During 129 Pd bouts (Tsk < 19.5°C), Pd bout duration ranged from 15 min to 14.1 days with a median of 17.2 h and a mean of 1.7 ± 2.4 days. The global model was the top model predicting Pd bout duration and carried 70% of the model weight (Table 3). Mean Ta and the interaction of mean VPdA and roost structure were statistically significant in explaining Pd bout duration (Table 4). Pd bout duration decreased as mean Ta increased, and the median Pd bout duration decreased to only 8 h at Ta > 10°C (Fig. 2a). Pd bout duration decreased in bridges and foliage as mean VPdA increased, but we detected no relationship between Pd bout duration and mean VPdA in cavities (Supplementary Fig. S1a). However, plotted values suggest the relationship in cavities may be non-linear, with a peak at ~0.3–0.5 kPa, which was not detected by our model (Supplementary Fig. S1a).

All bats used torpor every day of observation, and bats in all roost structures exhibited both short (<24 h) and

multi-day torpor bouts (Fig. 1b). During 94 recorded torpor bouts, the median torpid Tsk for each bat ranged from 12.3 to 18.0°C with a pooled mean Tsk across all bats of $15.4 \pm 4.3^\circ\text{C}$ (Fig. 1a). The daily fluctuation in torpid Tsk averaged $4.8 \pm 3.7^\circ\text{C}$ with a maximum of 23.1°C. Torpor bouts ranged from 3.5 h to 15.5 days (Fig. 1b) 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 Ta, total precipitation and the interaction between mean VPdA and roost structure (Table 4). Torpor bout duration decreased as mean Ta increased, and the median torpor bout duration decreased to only 21.7 h at Ta > 10°C (Fig. 2b). Torpor bout duration was positively related to total precipitation, and the longest torpor bout duration, 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. Torpor bout duration was negatively related to mean VPdA in bridges, positively related to mean VPdA in cavities and we detected no relationship between torpor bout duration and mean VPdA in foliage roosts (Supplementary Fig. S1b).

We recorded 90 normothermic bouts ranging from 15 min to 10.1 h with a median of 1.2 h and a mean of 2.4 ± 2.6 h. The longest observed normothermic bout duration, 10.1 h, was by a female roosting in foliage in late February. The

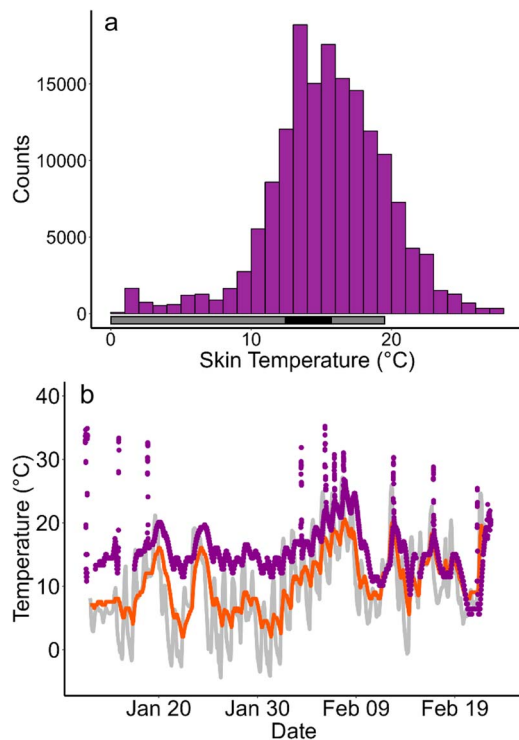


Figure 1: (a) Summary of torpid skin temperatures recorded every 15 min from tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. Skin temperatures favorable to Pd growth (i.e. $<19.5^{\circ}\text{C}$) occur above the grey shaded regions, while potential optimal growth conditions (i.e. $12.5\text{--}15.8^{\circ}\text{C}$) are represented by solid black shading. (b) Skin temperature trace (dotted purple) containing longest observed torpor bout duration of 15.5 days from a tricolored bat (SR0257) in 2019 using a tree cavity with roost temperature (solid orange) and ambient temperature (solid gray) for reference.

top model for predicting normothermic bout duration, simple weather, carried 89% of the model weight (Table 3) and contained the significant predictor mean T_a (Table 4). Normothermic bout duration increased with mean T_a , and we often observed multi-hour normothermic events at $T_a > 20^{\circ}\text{C}$ (Fig. 2c).

Bats were active on 102 of 304 tracked days (33.6%), and 51% of arousals contained a passive rewarming component. Days in which individual bats were active ranged from 14 to 64% of days followed, and 97% of all arousal events overlapped night-time hours. Bats maintained a non-random arousal pattern ($z = 0.5433$, $P < 0.001$, $n = 101$), with the highest probability of arousal near dusk (Fig. 3). 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 (Fig. 4a), and bats in foliage had a higher probability of general activity than those in bridges and cavities.

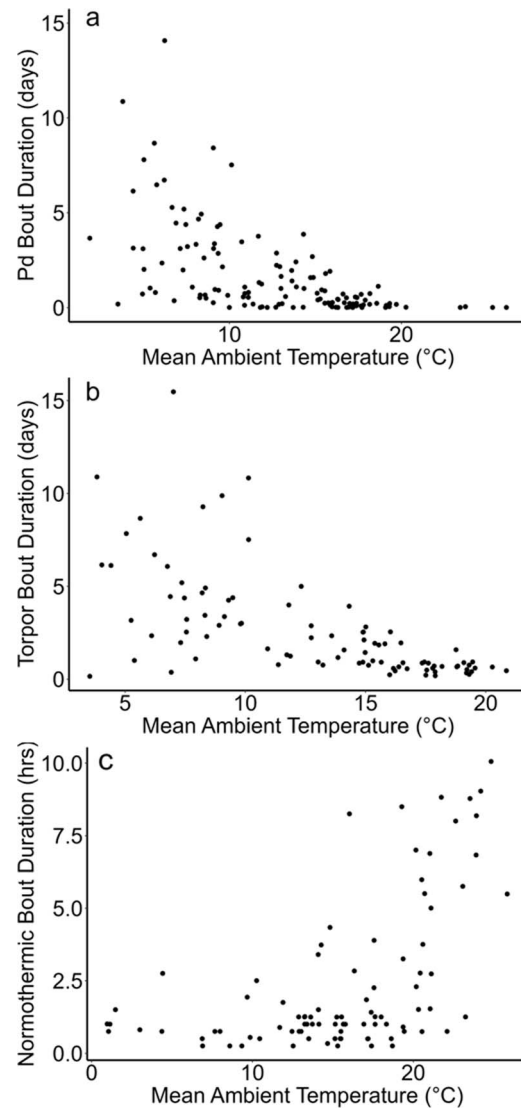


Figure 2: Observed (a) Pd bout duration, (b) torpor bout duration and (c) normothermic bout duration as a function of mean ambient temperature for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19.

Seventy-one percent of nighttime arousal events 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} (Fig. 4b and 4c), 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

Table 3: Top models predicting Pd bout duration, torpor bout duration and normothermic bout duration for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. Model refers to the hypothesis evaluated (Table 1); K, number of parameters; logLik, maximum log-likelihood; ΔAICc , difference of AICc between a model and the model with the smallest AICc; weight, model weight

	Model	K	logLik	AICc	ΔAICc	Weight
Pd bout duration	Global	12	−262.01	550.70	0.00	0.70
	Simple weather	4	−272.47	553.30	2.55	0.20
Torpor bout duration	Weather	6	−107.17	227.30	0.00	0.34
	Simple weather	4	−109.47	227.40	0.09	0.33
	Global	12	−100.13	228.20	0.85	0.22
Normothermic bout duration	Energetic status	5	−109.42	229.50	2.21	0.11
	Simple weather	4	−43.65	95.80	0.00	0.89
	Energetic status	5	−44.69	100.10	4.32	0.10

Table 4: Coefficient estimates from top models of winter torpor patterns of tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. Predictor abbreviations: Ta, ambient temperature; 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 precipitation	−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
Torpor bout duration	Mean Ta	−0.65	0.11	−0.92	−0.41
	Total precipitation	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

85% of the model weight (Table 5). The quadratic predictor variable date (number of days from 1 November) 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, three in late February 2018, one in early March 2018 and one in mid-

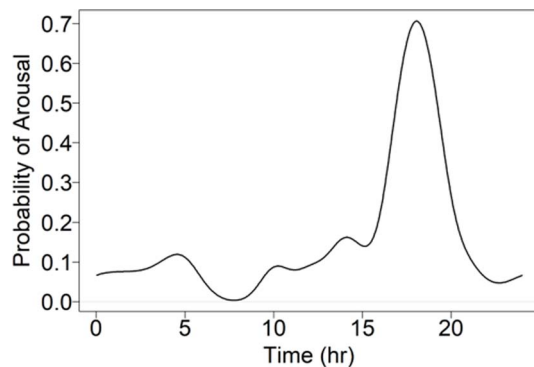


Figure 3: Probability of arousal by time of day (24-hour notation) for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19.

January 2019. 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, an indication of winter feeding.

Discussion

Tricolored bats at SRS exhibited winter torpor patterns nearer the short-term torpor end of the torpor continuum with characteristics reminiscent of daily heterotherms and, as a result, might be less susceptible to WNS than populations nearer the hibernation end. Although tricolored bats' Tsk often fell within the optimal temperature growth range of Pd (Verant *et al.*, 2012), similar to susceptible subterranean populations (Sirajuddin, 2018; Jackson *et al.*, 2022), Pd bout duration was responsive to Ta, and torpid Tsk often rose >20°C due to passive fluctuations. Bats in both bridges and trees experienced daily Tsk and Tr fluctuations averaging 4–5°C. In tricolored bats accustomed to thermally stable microclimates, Tr fluctuations of 5°C provoke energetically costly arousals (Davis and Reite, 1967), but bats at SRS frequently remained torpid. 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 above-ground hibernating species of North America (Davis and Reite, 1967; Halsall *et al.*, 2012). Few bat species are known to thermoconform at Tsk > 20°C, and examples of this behavior that exist are from subtropical and tropical regions (Stawski and Geiser, 2010; Liu and Karasov, 2011; Chenery *et al.*, 2022). Multiple observations of SRS bats thermoconforming at Tsk > 20°C highlight the need to explore critical arousal temperatures of bats using thermally unstable roosts. Of further importance, Tsk > 20°C are not favorable to Pd growth, and changes in the structural morphology of Pd grown at elevated temperatures (12–19.5°C) suggest these conditions are stressful for the fungus and limit its overall reproductive capacity (Verant *et al.*, 2012). Additionally, frequent temperature fluctuations

ranging from 8 to 25°C significantly reduce Pd growth *in vitro* (Forney *et al.*, 2022). Thus, short Pd bout duration due to passive Tsk fluctuations might limit fungal growth and disease severity, though severity might vary across years depending on ambient weather conditions.

Tricolored bats regularly used passive rewarming prior to arousals, maintained short torpor bout duration, had 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). Rarely observed in subterranean hibernating bats due to thermally stable microclimates, passive rewarming prior to arousal appears to be a regular occurrence for tricolored bats at SRS, and five arousals in foliage were performed exclusively via passive rewarming. Tricolored 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 daily (Geiser, 2004; Canale *et al.*, 2012). Regular use of passive rewarming by tricolored bats at SRS reduces the energetic and physiological demands of arousal and might limit premature fat depletion related to WNS. However, the advantages of a passive rewarming strategy are constrained by microclimate, and thermally unstable microclimates pose some disadvantages. Fluctuations in Tr that 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). Bats in bridges, but not cavities, decreased torpor bout duration as VPDA increased and were more active away from the roost than bats in cavities potentially because of increased evaporative water loss and dehydration. Therefore, the use and energetic advantages of thermally unstable roosts might be limited by local climate and weather conditions.

Tricolored bats in thermally unstable roosts exhibited clear differences in torpor expression when compared to climatically similar tricolored bat populations in subterranean hibernacula. Torpor patterns of bats at SRS were related to ambient weather conditions, similar to other bat species using thermally unstable roosts (Stawski and Geiser, 2010; Stawski and Currie, 2016; Czenze *et al.*, 2017a, 2017b), and unlike tricolored bats in subterranean hibernacula (Sirajuddin, 2018; Jackson *et al.*, 2022). The relationship of torpor to ambient weather conditions might be in response to associated thermoregulatory costs of activity during certain conditions. As the differential between Ta and normothermic temperatures decreases, so does the energetic cost of activity (Geiser, 2004), and precipitation results in increased energetic costs on

Table 5: Top activity models for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. Model refers to the hypothesis evaluated (Table 2); K, number of parameters; logLik, maximum log-likelihood; Δ AICc, difference of AICc between a model and the model with the smallest AICc; weight, model weight

	Model	K	logLik	AICc	Δ AICc	Weight
General activity	Energetic status	6	−134.68	281.60	0.00	0.60
	Warming cost	6	−135.94	284.20	2.53	0.17
Activity away	Weather	7	−31.07	77.40	0.00	0.70
	Energetic status	6	−33.19	79.30	1.91	0.27
	Seasonality	6	−36.13	85.20	7.80	0.01
Roost switch	Seasonality	4	−62.49	133.40	0.00	0.44
	Null	2	−65.27	134.70	1.25	0.23
	Warming cost	4	−63.39	135.20	1.79	0.18
	Past roost microclimate	5	−63.08	136.80	3.40	0.08

Table 6: Coefficient estimates from top models of winter activity patterns of tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19. Predictor abbreviations: Ta, ambient temperature; pVPDa, previous day's ambient vapor pressure deficit; Pbar, barometric pressure; and Condate or Condate², linear or quadratic form of days from 1 November

	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 precipitation	−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
	Condate	1.51	0.24	−0.90	0.06
	Condate ²	5.51	2.17	1.27	9.75
	Hour 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 precipitation	0.08	0.27	−0.45	0.62
	Mean pVPDa	0.39	0.26	−0.12	0.90

actively flying bats (Voigt *et al.*, 2011). The ability to closely monitor ambient weather conditions in thermally unstable roosts and subsequently arouse on nights with lower thermoregulatory costs to exploit intermittent feeding opportunities likely contributes to the expression of short-term torpor in

tricolored bats at SRS. Bats in thermally unstable roosts may respond to a critical threshold of passive Tsk fluctuation that signals reduced thermoregulatory costs and an opportunity to forage during winter (Dunbar *et al.*, 2007; Turbill, 2008), which is absent in subterranean hibernating bats. We found

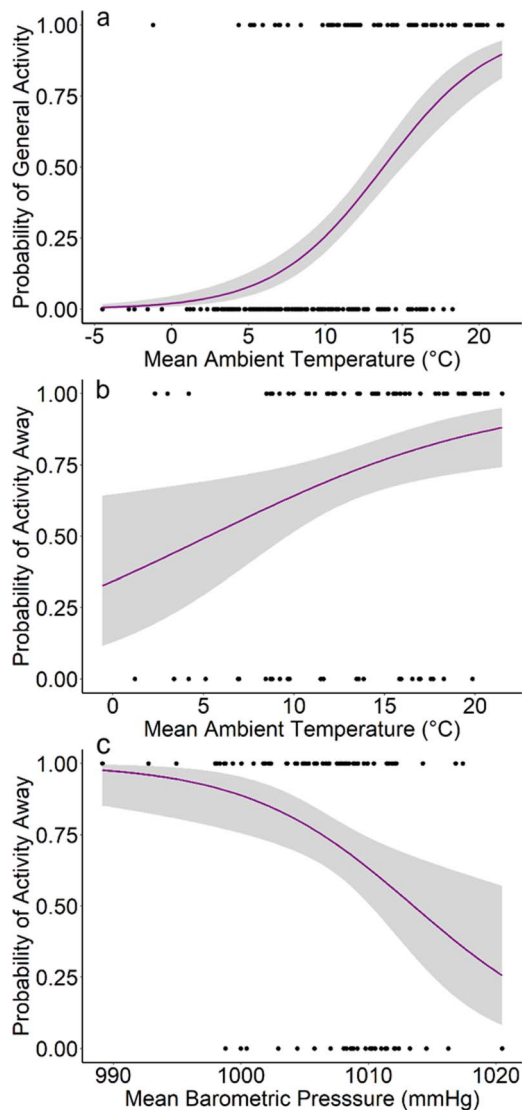


Figure 4: (a) Probability of general activity as a function of daily mean ambient temperature and the (b) probability of night-time activity away as a function of nightly mean ambient temperature and (c) mean nightly barometric pressure for tricolored bats using thermally unstable roosts in the Upper Coastal Plain of South Carolina, 2017–19.

multiple lines of evidence suggesting tricolored bats at SRS exploit winter feeding opportunities. Tricolored 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 increases in normothermic bout duration, 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). We also found a significant increase in night-time 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). Additionally, though limited in number, the fecal samples we collected during our study all contained insect parts. Since evidence of activity away from roosts was not observed by Sirajuddin (2018) in bats using thermally stable hibernacula under similar climatic conditions to our own, intermittent winter feeding by tricolored bats at SRS may be a function of both a mild climate and use of thermally unstable roosts. Big brown bats, Rafinesque's big-eared bats, and southeastern myotis (*Myotis austroriparius*) with high winter activity levels appear less susceptible to WNS than bats with naturally low winter activity levels (Johnson *et al.*, 2012, 2021; Reynolds *et al.*, 2017). During torpor, bats experience reduced immunocompetence (Field *et al.*, 2018), and long torpor bout duration 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 (Humphries *et al.*, 2003; van Breukelen and Martin, 2015) potentially limiting WNS infection. Finally, winter fat supplementation may enable bats to maintain high winter activity levels and also result in decreased WNS susceptibility since higher fat reserves contribute to persistence in WNS-affected populations (Cheng *et al.*, 2019).

Roost switching was related to season with a greater probability of roost switching in early and late winter. 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. Because roost switching is a strategy for parasite avoidance (Lewis, 1995; Reckardt and Kerth, 2007), tricolored bats may have switched roosts more often in early and later winter when parasites are more prevalent (Nickel and Hansen, 1967). Increased roost switching in early and late winter may have also been in response to shifting microclimates (Lewis, 1995). Intermediate Ta, combined with thermally unstable roost structures, may result in a greater need to switch roosts to find a suitable roost microclimate.

In summary, our results suggest winter torpor of bats using thermally unstable roosts lies nearer the short-term torpor end of the continuum than the hibernation end. Therefore, tricolored bats at SRS may be less susceptible to WNS due to short Pd bout duration, regular use of passive rewarming, short torpor bout duration 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 above ground 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. Further research to understand how short-term torpor and its associated physiological expression affect Pd growth and disease development is needed. Finally, these results highlight the need to explore the physiological flexibility of broadly distributed hibernators with consideration of climate change. Tricolored 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 Ta.

Acknowledgements

We thank Dr Patrick Jodice and the Clemson University Bat Lab for valuable feedback on an earlier draft. We thank Janette Perez-Jimenez and Laura Schenk for their hard work in the field and positive attitudes. We also thank the US Forest Service Savannah River funded under the Department of Energy's Interagency Agreement [grant number DE-EM0003622] for their support and help, especially Ed Olson, Dr Andy Horcher and Charles Davis. Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by B.A.N. The first draft of the manuscript was written by B.A.N. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Funding

This work was supported by the US Fish and Wildlife Service WNS fund [grant number F17PG00177] and the US Forest Service, Southern Research Station [grant number 18-JV11330134-001].

Data availability

Datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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