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Cover Photograph: Tricolored bats in a weep hole of a culvert in Putnam County. Photograph © Lisa Smith.

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Bats Roost in Culverts during Hibernation and the Maternity Season in North Florida

Lisa M. Smith[1*], Terry J. Doonan[2], and Jeffery A. Gore[3]

Abstract - Road infrastructure, such as bridges and culverts, can provide important habitat for bats that typically roost in caves, although culverts may facilitate disease transmission into Florida. To determine bat presence and abundance in culverts in north Florida, we surveyed 102 culverts during the primary winter torpor periods in 2018 and 2019, and in summer 2018. To determine temporal variation during winter, we surveyed 34 culverts multiple times between November 2019 and April 2020. Southeastern Myotis (*Myotis austroriparius*) was the most common species observed in both seasons. During winter 2019–2020, Southeastern Myotis was present in >50% of culverts during every survey. Average winter colonies of Tricolored Bats (*Perimyotis subflavus*) were small (≤3 bats). Tricolored Bats were first observed roosting in culverts in mid-November and increased in abundance until late January. Bats are susceptible to disturbance, human encroachment, and disease, and consequently, a better understanding of the role of culverts as roosts can improve conservation actions.

Introduction

Roads typically have a negative impact on wildlife populations, because they result in habitat loss, movement barriers, noise, and vehicle strikes (Jackson 2000, Moore et al. 2023, Rytwinski and Fahrig 2015, Shepard et al. 2008). These can impact populations directly through mortality or indirectly through habitat degradation and the fragmentation of populations, potentially increasing the susceptibility of wildlife to extirpation (Rytwinski and Fahrig 2015). However, culverts and bridges may help mitigate some of the challenges posed to wildlife by connecting subpopulations (Brunen et al. 2020, Glista et al. 2009) and there is approximately 1 culvert or bridge along every 0.25 mile (0.4 km) of paved road in North America (WNS CRWG 2018). Additionally, cave-roosting bats may derive benefits from structures associated with roads that provide suitable roosting sites (Detweiler and Bernard 2023, WNS CRWG 2018). For example, culverts of appropriate length, height, and material can provide roosting locations, with dark, humid, and stable microclimates (Leivers et al. 2019, Speakman and Thomas 2003). However, despite their potential as roosts, transportation structures may be ecological traps. Mortality may occur in transportation structures due to extreme cold events and flooding (Detweiler and Bernard 2023, Keely and Tuttle 1999). Nevertheless, at least 29 species of North American bats use culverts as night or day roosts, and even as hibernacula and maternity sites (WNS CRWG 2018).

Although culverts provide roosting sites for bats, use of culverts may entail a cost. Routine maintenance, such as repaving overlying roads, removing or replacing culverts, and applying concrete or epoxy patches can disturb, displace, or entomb roosting bats

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(WNS CRWG 2018). To minimize impacts to bats without impeding maintenance, transportation and wildlife managers need to understand how and when bats use culverts. Use of structures by temperate species is not constant throughout the year (Sandel et al. 2001), as bats may select different roosting locations and microclimates to meet changing physiological needs during hibernation, gestation, lactation, and fall swarming. For example, in summer, bats that are lactating and rearing pups require warmer temperatures (Kerth et al. 2001, Rodrigues and Palmeirim 2008), but in winter many species rely on cold, stable roost temperatures that allow individuals to enter torpor and minimize energy loss (Geiser 2004, Hock 1951).

Understanding the potential impacts of extreme weather and routine maintenance on culvert-roosting bats is of particular importance for *Perimyotis subflavus* (F. Cuvier) (Tricolored Bat), which is proposed for listing as endangered under the US Endangered Species Act (USFWS 2022) and is known to roost in culverts in the southeastern United States (Lutsch 2019, Meierhofer et al. 2019, Stevens et al. 2017). The Tricolored Bat has experienced significant declines across much of its range from white-nose syndrome (WNS). WNS, a disease caused by the fungus *Pseudogymnoascus destructans* (Gargas, Trest, Christensen, Volk, and Blehert) (*Pd*), has killed millions of cave-hibernating bats in North America (Blehert et al. 2009, Cheng et al. 2021). In addition to caves, the fungus has been detected in anthropogenic structures, including culverts and bridges (Blehert et al. 2009, Katzenmeyer 2016, Newman 2020). Because bats or humans moving between culverts may facilitate the spread of *Pd*, the broad distribution of culverts may allow *Pd* to develop in areas without caves or in karst landscapes where cave-roosting bats were not previously infected. Therefore, culverts may be important sites to monitor for *Pd* and WNS. Furthermore, culverts, like mines, may be ideal sites to administer or test potential WNS treatments, because collateral impacts to sensitive nontarget cave fauna could be avoided (Bernard et al. 2019, Meierhofer et al. 2019). For these reasons, a better understanding of use of culverts is important for developing comprehensive WNS management plans.

Most records and reports of bats roosting in culverts are anecdotal or limited in scope (Keeley and Tuttle 1999), and few studies have evaluated culvert use across a broad geographic area and multiple seasons (Meierhofer et al. 2019, Stevens et al. 2017). We investigated bat use of culverts in north Florida because it occurs within the same latitude and climate as Florida's cave hibernacula and within close proximity to *Pd*-positive areas in southern Georgia. Our objectives were to: 1) determine the species composition, occupancy, and abundance of bats in culverts, 2) assess temporal and spatial variation in bat presence and abundance to identify seasons and geographic regions where culverts are most important to bats, and 3) determine the minimum survey effort necessary to document bat presence in a culvert to inform survey recommendations. We hypothesized that culverts provide winter roosts for Tricolored Bats and *Myotis austroriparius* (Rhoads) (Southeastern Myotis) and summer roosts for Southeastern Myotis. We predicted that bat presence and abundance would be greater in culverts farther from caves.

Field-site description

North Florida is part of the subtropical southeastern Coastal Plain, and experiences mild winters (Bailey 1994). Bats in this region undergo a short, less intensive period of hibernation compared to bats in more temperate regions (Gore et al. 2012, McNab 1974). Results of annual surveillance at multiple hibernacula in Florida have been negative for *Pd* and WNS (L. Smith, Florida Fish and Wildlife Conservation Commission, Gainesville, FL, USA, 2024 unpubl. data), but the state is within 200 km of *Pd*-positive areas in Georgia (Muscogee County; whitenosesyndrome.org 2024). In winter 2018, summer 2018, and winter 2019, we assessed seasonal, interannual, and geographical variation from culverts in northwest (Jackson and Gadsden counties) and north-central (Alachua and Putnam counties; Fig. 1) Florida. These regions represent 2 different climatic conditions; average maximum winter temperature is 18.6 °C in the northwest region and 20.9 °C in the north-central region during winter (NOAA 2020, Smith et al. 2021). Additionally, Alachua and Jackson counties encompass areas of karst landscape with caves, but Gadsden, and Putnam counties have no caves. In winter 2019–2020, we assessed temporal variation in bat presence and abundance in culverts across the entire winter season. For practicality, we reduced the study area to culverts from the north-central region (Alachua and Putnam counties), as well as additional randomly selected culverts from adjacent Clay County to increase the sample size (L. Smith, Florida Fish and Wildlife Conservation Commission, Gainesville, FL, USA, 2024 unpubl. data).

Methods

Seasonal, interannual, and geographical variation

For culverts in Alachua, Gadsden, Jackson, and Putnam counties, we used Florida Department of Transportation (FDOT) maps known as "straight-line diagrams" (https://fdotewp1.dot.state.fl.us/slogis/) to identify all culverts under paved state or federal roads in both urban and rural areas. Because we selected culverts from straight-line diagrams that included only FDOT-maintained roads, smaller county or local roads were not surveyed. We considered all culverts ≥0.9 m tall for potential surveys, regardless of material type or shape (e.g., concrete box culverts, concrete pipe, and corrugated metal pipe), because smaller culverts are rarely used by bats (Boonman 2011, Meierhofer et al. 2019) and are difficult to sample. We randomly selected a similar number of culverts from each county to ensure an even sample size across each karst and geographic region, despite a varying number of culverts meeting the minimum height requirement in Alachua (n = 191), Gadsden (n = 78), Jackson (n = 202), and Putnam (n = 78) counties. For each county, we first used a random number generator to select a road from a list of state and federal roads in the county. Next, we used a random number generator to select the n^{th} culvert along that road starting at the beginning of the straight-line diagram. If the random number exceeded the number of culverts available along the road, we started the selection process over. We repeated the process until at least 25 culverts were selected per county.

At most sites, a single culvert was present, but at some locations, ≥2 adjoining, parallel culverts existed. At those sites, we combined counts of bats from adjacent culverts to create a single count for the site. Some culverts could not be surveyed because of high water, road-maintenance activities, or safety concerns or because the culvert could not be found. If we could not access a culvert in the initial survey period, we randomly selected a replacement to maintain a minimum sample of 25 culverts per county. However, if we could not survey a culvert in subsequent seasons, we did not add a replacement.

We surveyed all accessible culverts once each winter (1 January–15 March in both 2018 and 2019) and once in summer (15 June–15 August 2018). At each, we searched the full length of the culvert (range: 10.1–135.9 m; mean: 39.9 m) for bats and identified each to species. We counted individual bats in the culvert or, for clusters of >100 Southeastern Myotis, we estimated the number of bats by multiplying the observed area of roosting bats by 2000 bats/m^2 (Gore and Hovis 1998). To reduce disturbance, one biologist surveyed

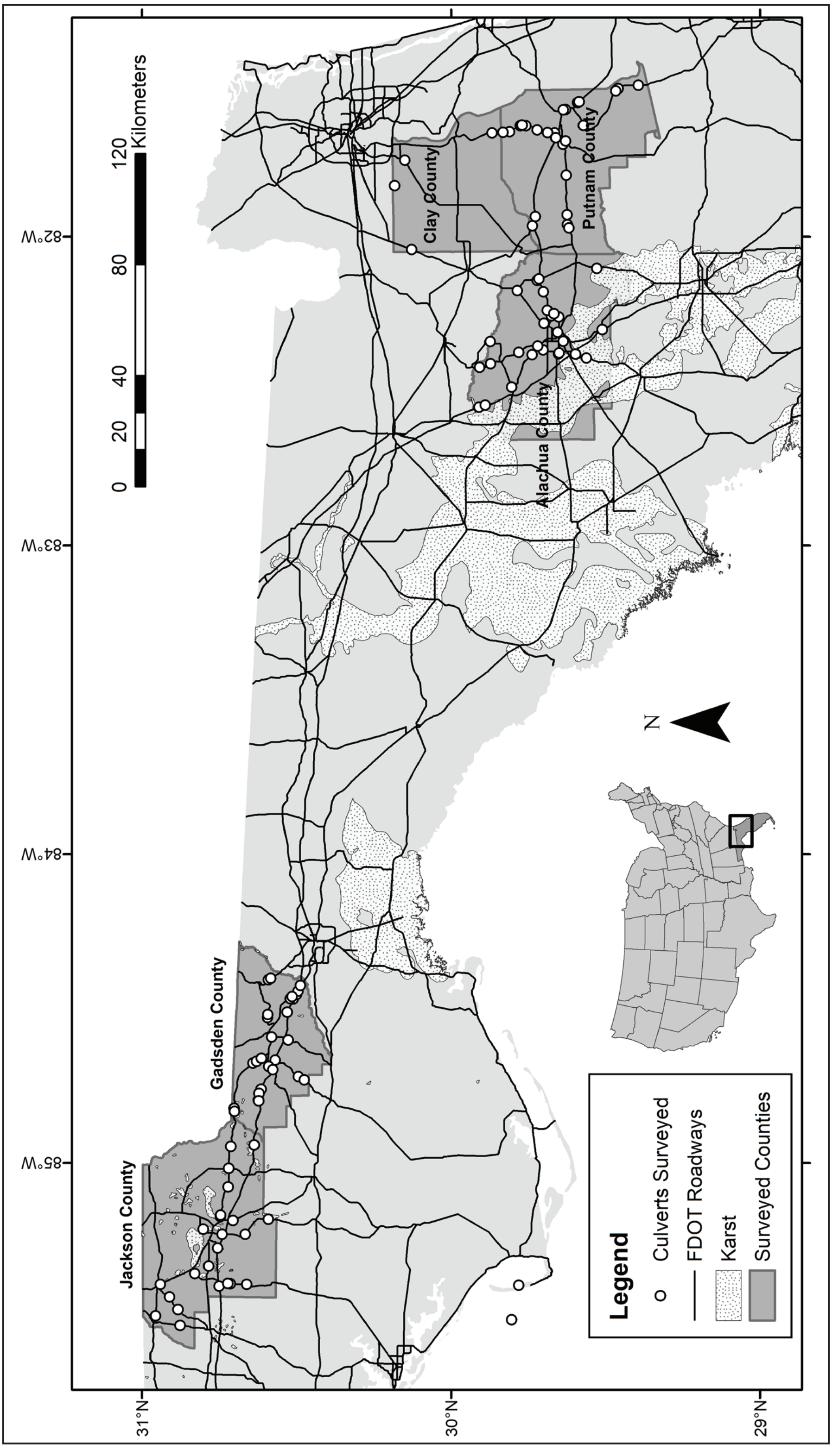

Figure 1. Locations of all culverts surveyed for bats along paved state or federal roads maintained by the Florida Department of Transportation (FDOT) in northwest (Jackson and Gadsden counties) and north-central (Alachua, Putnam, and Clay counties) Florida, in 2018–2020.

each structure and moved continuously through the culvert, only stopping to record data. Personnel used red lights, while minimizing noise in and around the culvert. We obtained the maximum ambient temperature recorded for the day of the survey from the nearest weather station (Florida Automated Weather Network; https://fawn.ifas.ufl.edu/), as an indication of conditions that may be suitable for arousal and foraging.

All analyses were conducted in R.3.6.0 (R Core Team 2020). We evaluated differences in bat presence for each species between seasons with a mixed-effects logistic regression model, using the glmer function (Bates et al. 2015). We evaluated differences in abundance for each species using a mixed-effects linear regression model with a negative binomial distribution, with function glm.nb (Venables and Ripley 2002). In each model, we included season, karst (presence or absence of caves in the county; Smith et al. 2021), and region (northwest and north-central) as covariates and a unique culvert identifier (culvert ID) as a random effect. We also evaluated interannual variation in bat presence in winter with a mixed-effects logistic regression model and differences in abundance using a linear regression model with a negative binomial distribution. In models evaluating annual variation in wintering bat presence, we included the year and maximum ambient temperature as covariates and culvert ID as a random effect. We considered a parameter significant if $P < 0.05$.

Within-winter variation

To assess variation in bat presence and abundance across winter, we selected a subset of 34 culverts in north-central Florida only. Culverts were selected from previously surveyed culverts in Alachua and Putnam counties, as well as adjacent Clay County (n = 8) to increase the sample size. This subset of culverts included all sites (n = 8) in these 3 counties previously known to support wintering Tricolored Bats, plus 26 culverts randomly selected from a list of the remaining culverts. One person surveyed each culvert every 2–3 weeks (12–21 days) starting on 6 November 2019, prior to the onset of sustained cool (average high temperature <25°C) weather and the presumed onset of hibernation, and continuing until 1 April 2020, when temperatures warmed (average high temperature >25°C) and no Tricolored Bats remained. Previous studies found no detectable impact of investigator disturbance to bats from repeated surveys of bridges and caves (Ferrara and Leberg 2005, Kilpatrick et al. 2020), but no studies report on the potential disturbance of surveys to bats at culverts.

To evaluate within-season variation, we used single-season occupancy modeling (Fiske and Chandler 2011) to estimate the probability of detecting bats (P) and site occupancy (Ψ). To account for the nonrandom selection of culverts, we included previous use by Tricolored Bats as a detection and occupancy covariate for all models for the species. We compared models using Akaike's Information Criterion (AICc), and we considered only models with a ΔAICc < 2 to have notable plausibility (Burnham and Anderson 2004). We then estimated the minimum number of surveys needed to detect bats in a culvert for each species using the equation $P_c = 1 - (1 - P)^{K}$, where P_c = the probability of detecting bats in a culvert at least once, P = the probability of detection, and K = the number of surveys (Bennett et al. 2008, Mackenzie and Royle 2005).

Results

We observed Southeastern Myotis, Tricolored Bats, and *Eptesicus fuscus* (Palisot de Beauvois) (Big Brown Bat) roosting in culverts. Southeastern Myotis was the most common species during every sampling period and in each county (≥50% of culverts in all winters, 29.6% of culverts in summer; Table 1). Tricolored Bats were less common and occurred in fewer culverts, albeit in every county (>10% of culverts in all winters and 1% of culverts in summer; Table 1). We only detected 2 Big Brown Bats in summer, 1 in Jackson County and the other in Putnam County. We encountered 3 types of culverts. Concrete box culverts comprised 58 (56.9%) of the total culverts surveyed, followed by 41 (40.2%) concrete pipe culverts, and 3 (2.9%) corrugated metal pipe culverts. Southeastern Myotis and Tricolored Bats roosted in all 3 types, but the 2 Big Brown Bats were only in concrete box culverts.

Seasonal, interannual, and geographical variation

Southeastern Myotis were common in culverts in both winter and summer and occupied ≥50% of culverts in both winters but fewer culverts in summer (Table 1). The number of individuals per occupied culvert ranged widely from 1–1002 (49.0 ± 150.8) in winter 2018, 1–1000 (64.7 ± 229.0) in summer 2018, and from 1–301 (22.0 ± 51.4) in winter 2019. The largest population of Southeastern Myotis in winter decreased from 1000 to 301 bats between the winters of 2018 and 2019. Although the range of bats in occupied culverts was similar between seasons, both presence and abundance of Southeastern Myotis were significantly lower in summer (Tables 2–3). We found no difference in presence or abundance of Southeastern Myotis between counties with or without karst or between north-central and northwest Florida (Tables 2–3). All means are ± *SD*.

Tricolored Bats were found in fewer culverts than Southeastern Myotis, especially in summer (Table 1). The number of Tricolored Bats ranged from 1 to 10 (2.0 ± 2.6) per occupied culvert in winter 2018 and from 1 to 7 (2.2 ± 2.0) in winter 2019. Only 2 Tricolored Bats were detected during summer, both in the same culvert, resulting in a significantly lower presence (Table 2) and abundance (Table 3) when compared to winter surveys. We did not detect a significant difference in presence or abundance of Tricolored Bats between counties with or without karst or between north-central and northwest Florida (Tables 2–3).

The number of culverts with Southeastern Myotis was significantly greater in winter 2019 (Table 4) compared to winter 2018. Despite the increase in occupied culverts in 2019, we observed fewer than half the number of Southeastern Myotis compared to 2018 (Table 1). However, the observed difference in abundance was not significant (Table 5). Maximum ambient external temperature did not explain differences in presence or abundance of Southeastern Myotis between winters. Tricolored Bat presence and abundance declined significantly from 2018 to 2019, but again, maximum ambient external temperature had no effect (Tables 4–5).

Within winter variation

The abundance of Southeastern Myotis across all sites was highest near the beginning of surveys in mid-November, then declined until the end of sampling on 1 April (Fig. 2a). Despite the decline in abundance, the percentage of culverts occupied by Southeastern Myotis remained between 60% and 80% throughout winter. During these repeat surveys of culverts, Southeastern Myotis had a probability of detection of 0.79 (*SE* = 0.02) and estimated occupancy of 0.88 (*SE* = 0.06). We estimated 2 surveys of a culvert would be needed to have a >0.9 probability of detecting a Southeastern Myotis during at least 1 survey (Fig. 3).

Tricolored Bats were detected during all survey periods, except the first survey in early November and the last survey in late March/early April. The abundance of Tricolored Bats and percentage of culverts occupied peaked during the 20–23 January sampling, then declined steadily until no bats remained (Fig. 2b). Previous use by Tricolored Bats was

Table 1. Total number of Southeastern Myotis and Tricolored Bats roosting in culverts, mean per culvert, and the number and percentage of culverts occupied by each species across 4 counties in north Florida during winter and summer 2018 and winter 2019.

	Winter 2018 (n = 102)			Summer 2018 = (n 98)*			Winter 2019 (n = 88)*		
Species	Total bats	Mean bats (± SD)	Occupied culverts	Total bats	Mean bats (± SD)	Occupied culverts	Total bats	Mean bats (± SD)	Occupied culverts
Southeastern Myotis	2,499	49.0 (± 158.8)	51 (50.0%)	1,813	65.0 (± 229.0)	29 (29.6%)	1,186	22.0 (± 51.4)	54 (61.4%)
Tricolored Bat	46	2.9 (± 2.6)	16 (15.7%)	2		1 (1.0%)	22	2.2 (± 1.9)	10 (11.4%)
Total	2,545		58 (56.9%)	1,815		32 (32.6%)	1,208		55 (62.5%)

Not all culverts from Winter 2018 were accessible for re-survey.

Table 2. Mixed-model estimates of logistic regression evaluating the effect of season, karst (yes or no), and region (northwest or north-central) on the presence of Southeastern Myotis and Tricolored Bats, roosting in culverts in north Florida in winter 2018, summer 2018, and winter 2019. Karst and region were coded as dummy variables, for which 0 = nonkarst, and 1 = karst; 0 = north-central, and 1 = northwest region of Florida.

	Estimate	Std Error	z value	P	SD
Southeastern Myotis					
Intercept	-1.36	0.68	-2.00	0.05*	
Season: Winter 2018	1.98	0.51	3.90	<0.001*	
Season: Winter 2019	2.69	0.57	4.68	<0.001*	
Karst	-0.89	0.71	-1.25	0.21	
Region	-0.23	0.70	-0.33	0.75	
Random effect					
Culvert	_	_	_	_	2.73
Tricolored Bat					
Intercept	-23.81	5.57	-4.28	<0.001*	
Season: Winter 2018	14.73	4.56	3.23	<0.005*	
Season: Winter 2019	9.96	3.53	2.82	<0.005*	
Karst	-0.79	2.08	-0.38	0.70	
Region	0.28	2.03	0.14	0.89	
Random effect					
Culvert	_	_	_	_	17.67

Asterisk (*) indicates $P \leq 0.05$.

Table 3. Mixed-model estimates of linear regression evaluating the effect of season, karst (yes or no), and region (northwest or north-central) on the abundance of Southeastern Myotis and Tricolored Bats, roosting in culverts in north Florida in winter 2018, summer 2018, and winter 2019. Karst and region were coded as dummy variables, for which 0 = nonkarst, and 1 = karst; 0 = north-central, and 1 = northwest region of Florida.

	Estimate	Std Error	z value	P	SD
Southeastern Myotis					
Intercept	-1.03	0.58	-1.79	0.07	
Season: Winter 2018	1.60	0.27	5.99	<0.001*	
Season: Winter 2019	1.73	0.27	6.39	<0.001*	
Karst	-1.16	0.61	-1.89	0.06	
Region	-0.36	0.61	-0.60	0.55	
Random effect					
Culvert	_	_	_	_	2.62
Tricolored Bat					
Intercept	-8.26	1.61	-5.13	<0.001*	
Season: Winter 2018	3.14	0.73	4.33	<0.001*	
Season: Winter 2019	2.40	0.74	3.24	<0.005*	
Karst	-0.58	1.04	-0.56	0.58	
Region	-0.10	1.03	-0.10	0.92	
Random effect					
Culvert	_	_	_	_	4.82

Asterisk (*) indicates $P \leq 0.05$.

Table 4. Mixed-model of logistic regression estimating the effects of survey year and maximum ambient temperature on presence of Southeastern Myotis and Tricolored Bats, roosting in culverts in north Florida during winter of 2018 and 2019.

	Estimate	Std Error	z Value	P	SD
Southeastern Myotis					
Intercept	-0.69	0.55	-1.25	0.21	
Max ambient	0.02	0.02	0.85	0.39	
Survey year: Winter 2019	0.20	0.19	1.09	0.28	
Random effect					
Culvert	—	—	—	—	2.62
Tricolored Bat					
Intercept	-4.37	1.41	-3.10	<0.005*	
Max ambient	-0.06	0.04	-1.66	0.10	
Survey year: Winter 2019	-0.90	0.29	-3.08	<0.005*	
Random effect					
Culvert	—	—	—	—	4.94

Asterisk (*) indicates $P \leq 0.05$.

Table 5. Mixed-model estimates of linear regression evaluating the effects of survey year and maximum ambient temperature on the abundance of Southeastern Myotis and Tricolored Bats, roosting in culverts during winter in north Florida in 2018 and 2019.

	Estimate	Std Error	z value	P	SD
Southeastern Myotis					
Intercept	-1.24	1.01	-1.22	0.22	
Max ambient	0.06	0.05	1.36	0.18	
Survey year: Winter 2019	1.12	0.51	2.18	0.03*	
Random effect					
Culvert	—	—	—	—	3.14
Tricolored Bat					
Intercept	-3.27	2.94	-1.11	0.27	
Max ambient	-0.44	0.25	-1.75	0.08	
Survey year: Winter 2019	-6.91	2.69	-2.56	0.01*	
Random effect					
Culvert	—	—	—	—	23.11

Asterisk (*) indicates $P \leq 0.05$.

included in the top model for detection and occupancy (see Supplemental File 1, available online at http://www.eaglehill.us/NABRonline/suppl-files/nabr-007-Smith-s1). Detection and occupancy were higher if the culvert was used by Tricolored Bats at least once in a previous winter (2018–2019). The probability of detecting Tricolored Bats was 0.15 (*SE* = 0.05), with an estimated occupancy of 0.32 (*SE* = 0.11). We estimated it would take 14 surveys of a culvert to have a >0.9 probability of detecting a Tricolored Bat during at least 1 survey (Fig. 3).

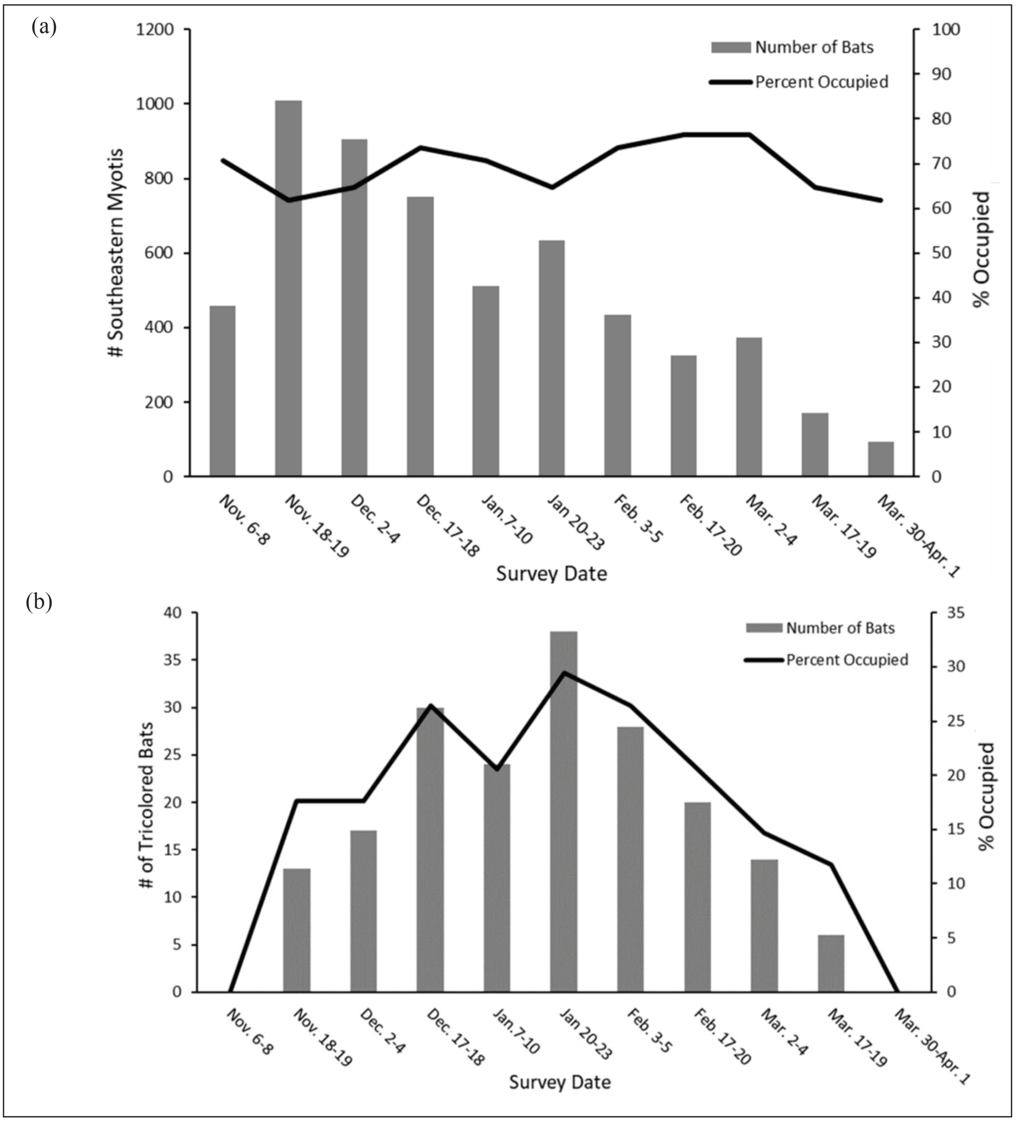

Figure 2. Number of bats observed in 34 culverts and percent of culverts occupied in north-central Florida recorded during 11 survey occasions from 6 November 2019–1 April 2020 for (a) Southeastern Myotis, and (b) Tricolored Bats.

Discussion

Our results demonstrate that culverts in north Florida provide roosting habitat for Southeastern Myotis and Tricolored Bats in summer, but more prominently in the winter. This is consistent with findings from other parts of the southeastern United States (Katzenmeyer 2016, Lutsch 2019, Stevens et al. 2017). In winter, we found no difference in bat use of culverts between areas with or without caves, suggesting that transportation structures may allow cave-roosting species to occur in areas where they would not naturally be expected in winter. Culverts may be particularly important as alternative roosting sites for the Tricolored Bat, which is proposed for federal listing as endangered due to recent population declines caused by WNS. Although bats may use culverts in summer and winter, we documented considerable seasonal and interannual variation in the presence and abundance of bats. This indicates that roost switching and abandonment not only complicate winter monitoring, but may also facilitate the transfer of *Pd* among sites.

Southeastern Myotis was the most commonly observed species in our study. This is consistent with other states in the Southeast (Lutsch 2019, Stevens et al. 2017). We found Southeastern Myotis occurred in larger colonies and occupied a higher percentage of culverts (≥50%) in Florida during winter than other states within their geographical range (Katzenmeyer 2016, Lutsch 2019, Meierhofer et al. 2019, Stevens et al. 2017). Tricolored Bat colonies were smaller than in other states and occupied between 11% and 16% of culverts in Florida, which is similar to neighboring Georgia (Lutsch 2019) but fewer than states elsewhere across their range (Katzenmeyer 2016, Meierhofer et al. 2019). We rarely

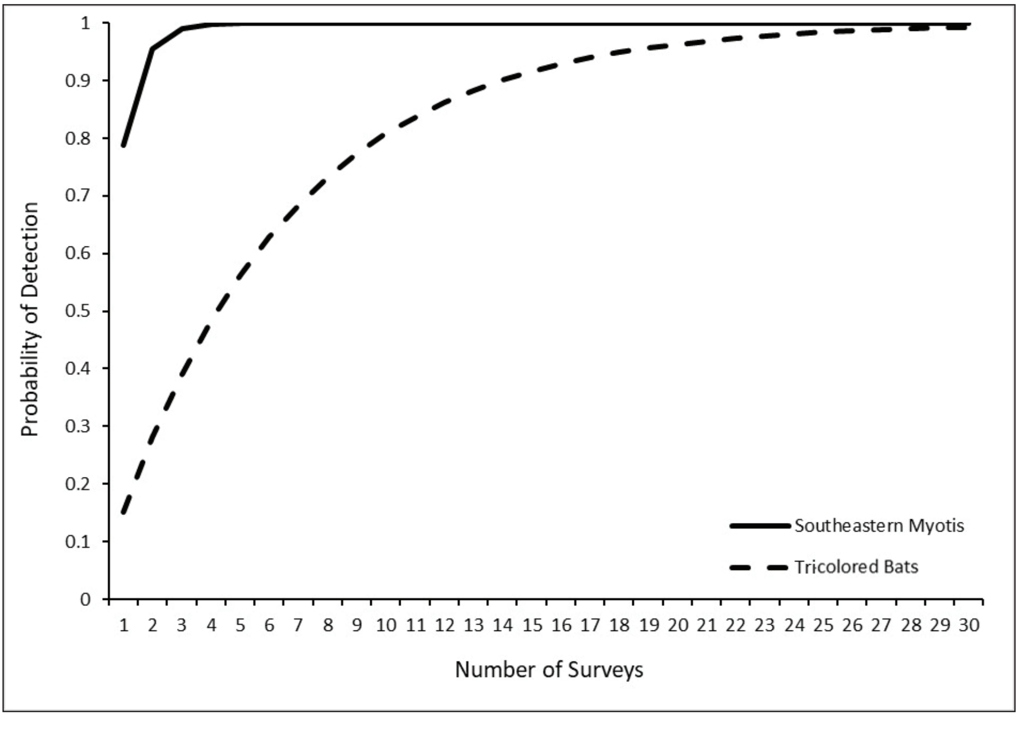

Figure 3. Estimated probability of bat detection by the number of surveys for Southeastern Myotis and Tricolored Bats in culverts in north Florida.

documented Big Brown Bats in culverts, and only in summer, which is consistent with other studies (Meierhofer et al. 2019, Stevens et al. 2017). However, in Mississippi, Big Brown Bats were present in almost a quarter of the culverts surveyed during winter, with up to 8 bats per site (Katzenmeyer 2016). We did not observe *Corynorhinus rafinesquii* (Lesson) (Rafinesque's Big-eared Bat), or *Tadarida brasiliensis* (I. Geoffery) (Brazilian Free-tailed Bat) in Florida culverts in any season, even though both species roost in culverts (Bender et al. 2010, Katzenmeyer 2016, Lewis 1995, Smith et al. 2020, Stevens et al. 2017, Walker et al. 1996); more extensive surveys may detect these species.

The reason bat presence and abundance in culverts varies so widely among southeastern states may result from differences in species distribution and abundance, geographic area, surrounding habitat, availability of other roost sites (natural and anthropogenic), climatic conditions, or flooding frequency. Unlike many other areas within the range of these species, Florida is unique in that it occurs in the subtropical coastal plain, where winters are shorter and warmer than more temperate regions, and insects are available prey on most nights (Frost 1962, McNab 1974, Myers and Ewel 1990). This may allow for increased flexibility in potential roosting locations and movement between roost types in accordance with temperatures (Newman et al. 2021). Additionally, Florida has a distinct wet and dry season with periods of heavy rainfall (Myers and Ewel 1990), which may increase the risk of flooding and reduce the suitability of some culverts as roosts. Differences in reported culvert use across the Southeast may also be related to differences in culvert selection between each study, in combination with ecological variation. We selected culverts randomly across each county, while other studies surveyed every culvert along a roadway (Stevens et al. 2017), in a GRTS cell (Meierhofer et al. 2019) or predominantly along interstates or other highways (Lutsch 2019, Stevens et al. 2017). Differences in methods of culvert selection may be necessary to accomplish a specific goal, but this complicates comparisons between studies by creating biases. For example, only surveying culverts along highways may not account for shorter culverts under smaller roads. Comprehensive and coordinated research across multiple states may help elucidate the factors responsible for the apparent interstate and intrastate differences in bat use of culverts, such as differences in latitude (e.g., Ferral 2022), climate, species abundance, and sampling design. Broad-scale studies or meta-analyses may provide more insights on variation and, therefore, better inform management practices for culvert maintenance and guide bat conservation and WNS-monitoring efforts.

We observed significant differences in the presence and abundance of both Southeastern Myotis and Tricolored Bats between seasons. In summer, Southeastern Myotis occupied fewer culverts and occurred in smaller numbers than in winter. We suspect that this is because, in summer, Southeastern Myotis typically form large maternity colonies in caves or other structures that have stable, warm temperatures and are, therefore, more suitable for raising young (Gore and Hovis 1998, Rice 1957). Although fewer Southeastern Myotis roosted in culverts in summer, maternity colonies with several hundred bats occupied 2 culverts. Lewis et al. (2022) also reported large maternity colonies of Southeastern Myotis in culverts during summer in Georgia. Maternity colonies are sensitive to disturbance, and road construction or maintenance near maternity sites could cause abandonment of the roost or entrapment and death of nonvolant young. Unlike Southeastern Myotis, Tricolored Bats typically roost in trees during the maternity season (Samoray et al. 2019, Veilleux et al. 2003) and that likely explains why we found only a single culvert with these bats in summer. Presence and abundance of bats in individual culverts in Florida varied between winters, but the variation was not explained by the maximum external ambient temperature. In contrast, in Mississippi and Texas, Tricolored Bats were found in larger numbers at sites with colder temperatures (Katzenmeyer 2016, Meierhofer et al. 2019). Warmer winters in Florida's subtropical climate may allow bats to use trees, buildings, bat houses, or bridges even during cold weather, potentially introducing additional variability in winter counts. Because culverts are an abundant resource across the landscape, the benefit of site fidelity may be reduced compared to caves (Lewis 1995). More roost switching may introduce variability into winter counts and increase the risk of *Pd* transfer between sites.

Bat abundance in culverts varied throughout the winter in north-central Florida. Southeastern Myotis were present in culverts for the duration of winter, but the number of individuals fluctuated. The number of Southeastern Myotis was highest in late November and then declined until late March, likely because bats began moving to maternity sites (Rice 1957). Variability in counts could also indicate roost switching or investigator disturbance. Southeastern Myotis are likely more susceptible to investigator disturbance because they are active most nights in winter and rarely rely on torpor in Florida's subtropical climate (McNab 1974). We made a concerted effort to reduce disturbance during surveys and note that bats rarely took flight. Although previous studies did not find an impact of investigator disturbance at bridges or caves during repeated surveys (Ferrara and Leberg 2005, Kilpatrick et al. 2020), culverts are often smaller and result in greater proximity of the surveyor and bats. Consistent with observations in Texas (Sandel et al. 2001), our highest counts of Tricolored Bats corresponded with the coldest part of winter, when bats are most likely to use deep and prolonged torpor (Barbour and Davis 1969, Laval and Laval 1980). To obtain the best estimates of the maximum number of Tricolored Bats using a site in winter in Florida, surveys should be conducted from mid-December to early February.

Developing a better understanding of culvert use by bats will allow transportation and wildlife managers to determine the best-management practices for highway infrastructure that conserves bat populations and allows for the necessary maintenance of culverts and safe roads. Bats may use culverts year-round in Florida, but bats are most sensitive to disturbance from routine maintenance operations in the summer maternity season and winter torpid period. In summer, maternity colonies containing nonvolant young should be identified so routine work may be postponed, if possible, to avoid harming young bats or causing adult bats to abandon the roost. Even without entering a culvert, loud noises and excessive vibrations near the culvert entrance may cause roost abandonment. During winter, bats may be torpid, even in a warm climate (McNab 1974), and disturbance can cause bats to arouse more frequently and deplete valuable energy stores. If torpid bats are unable to arouse quickly (e.g., Doty et al. 2018), they may be injured or killed during routine roadwork. If bats arouse from torpor and are forced to abandon a culvert due to maintenance, they may still be at risk of exposure to cold weather prior to finding a new suitable roost.

Although conducting a single survey prior to culvert maintenance would be ideal, we found that multiple surveys would be required to determine with 90% probability that a culvert would be used by Tricolored Bats at some point during winter. This is both impractical to execute and potentially disruptive. Therefore, when possible, it may be most efficient to schedule culvert maintenance for times when bats are less likely to be present. In north Florida, the ideal times are in spring (1 March–15 April) after the winter hibernation period but before the maternity season, or in fall (15 August–15 November) before hibernation. Regional assessments are needed to determine the ideal times for culvert maintenance.

There has been growing interest in culverts as sites that could both facilitate WNS transmission and serve as possible WNS treatment areas (Bernard et al. 2019, Lutsch et al. 2022, Meierhofer et al. 2019, WNS CRWG 2018). Ambient temperatures within many culverts in Florida (L. Smith, Florida Fish and Wildlife Conservation Commission, Gainesville, FL, USA, 2024 unpubl. data) are suitable for the growth of *Pd,* and the fungus has been found on bats roosting in culverts outside Florida (Bernard et al. 2019, Katzenmeyer 2016, Lutsch et al. 2022). A better understanding of why use of culverts varies would help inform monitoring programs by differentiating between natural fluctuations in bat populations and true declines caused by WNS. Additionally, the use of culverts as winter roost sites for bats raises concerns that a series of culverts along a roadway might facilitate the spread of *Pd,* extending its reach into karst areas that are currently devoid of *Pd* and WNS. Because we documented considerable variation in use between and within winters, additional study of roost switching among culverts by bats may provide more insight into the role of culverts in the transmission of *Pd*. Culverts with large, stable winter populations may provide preferred sites where treatments to inhibit or destroy *Pd* can be applied without the risk of negatively affecting sensitive cave fauna (Bernard et al. 2019). Therefore, WNS monitoring and surveillance programs that incorporate culvert roosts may better detect the spread of the disease and fully assess its impacts.

Our findings represent culvert use by bats in the subtropical, southeastern Coastal Plain. Bats in other regions may behave differently depending on the severity and duration of winter, and the variation in physical and internal microclimate characteristics of culverts. Our study highlights the use of culverts as alternative roosting sites for Southeastern Myotis and Tricolored Bats in Florida, and provides insights into annual and seasonal variation in culvert use. All bats are legally protected year-round from harm in Florida, and Tricolored Bats may soon be federally listed as endangered; knowing when bats are present in culverts is necessary to help avoid disturbance. However, additional information on the types and characteristics of culverts preferred by bats, and the relative importance of culverts compared to other types of roosts, is needed to guide surveys, develop conservation guidelines, inform potential WNS treatment locations, and minimize disturbance during road maintenance.

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