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Survival and persistence of tricolored bats hibernating in Arkansas mines

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White-nose syndrome (WNS) has caused large declines in bat populations across eastern North America, making information on demographics of affected species critical to determining their risk for extinction. We used Cormack–Jolly–Seber models to estimate apparent survival rates of hibernating tricolored bats (*Perimyotis subflavus*) for 5 years in four small abandoned mines in the Ouachita Mountains of Arkansas, located within the WNS endemic area of the United States. Populations in individual mines varied greatly in survival rates, with one mine displaying annual survival rates as high as 0.706 and another as low as 0.101. Differences in survival among bats in different mines could not definitively be attributed to WNS, but may have varied based on a combination of WNS, disturbance, mine climate, and other unknown factors. Further, some hibernacula may have served as temporary winter shelter for young transient males. Sites housing small colonies of hibernating bats may result in high survival rates despite WNS, and protecting these smaller sites may be important for overall species perseverance.

Key words: caves, disturbance, fat stores, mass, *Perimyotis subflavus*, white-nose syndrome

The disease, white-nose syndrome (WNS), has been spreading across North America since 2006, causing widespread declines in certain species of cave-hibernating bats, including little the brown bat (*Myotis lucifugus*), northern long-eared bat (*M. septentrionalis*), and tricolored bat (*Perimyotis subflavus*—Frick et al. 2010; Langwig et al. 2012). This disease is caused by the cold-adapted (3–15°C) fungus, *Pseudogymnoascus destructans* (*Pd*), which invades the skin of bats while they hibernate (Meteyer et al. 2009; Verant et al. 2012). Dehydration caused by evaporative water loss and irritation associated with this fungal infection jointly contribute to frequent arousals of bats during hibernation (Cryan et al. 2010; Willis et al. 2011; Reeder et al. 2012; Lilley et al. 2016). These arousals prematurely exhaust the accumulated fat reserves required to survive winter while insect foods are not available, which reduces overwinter survival of bats.

After *Pd* invades a region, some bats persist, but in greatly reduced numbers (Langwig et al. 2012; Maslo et al. 2015; Cheng et al. 2018). It is unclear what percentage of these remaining bats are survivors or immigrants at a specific site; however, Maslo et al. (2015) found that populations of little brown bats (*M. lucifugus*) persisted and had increasing annual survival after initial invasion of *Pd* in the northeastern United States. It was suggested that individuals persisted due to resistance of the host (behavioral, physiological, or microbial factors), which resulted in reduced fungal growth on individuals (Langwig et al. 2016). Factors that may influence survival by WNS-infected bats include hibernaculum temperature, prehibernation body condition, and initial *Pd* loads (Langwig et al. 2012; Johnson et al. 2014).

Growth of *Pd* varies based on temperature, with temperatures of 12.5–15.8°C resulting in optimal growth (Verant et al. 2012). Hibernacula temperatures vary according to latitude and, in southern portions of the United States, temperatures often exceed 16°C in hibernacula, with little air flow (Perry 2013). However, it is unknown how bats in these more southerly latitudes respond to WNS at the higher end of the *Pd*-temperature spectrum: research is needed on potential survival and persistence of bats affected by *Pd* in these warmer climates. These more southerly latitudes may also offer opportunities for bats to forage during warmer winter periods, thereby increasing chances of overwinter survival.

With the recent invasion of *Pd* and its detrimental effects on bat survival, robust estimates of population parameters for North American bats are needed, including birth rates, population growth rates, and survival rates. Historically, large-scale

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banding efforts in the 1950s to 1970s resulted in numerous studies on bat survival, but most were based on life tables for bats of unknown age (Keen 1988; O'Shea et al. 2004). Only a handful of more recent studies have used the rigorous methods of maximum likelihood to model parameters such as survival rates (O'Shea et al. 2004, 2011). Modern methods of survival analysis using maximum likelihood, such as open-population, Cormack–Jolly–Seber (CJS) models (Lebreton et al. 1992) estimate apparent survival (ϕ) and probability of reencountering an individual (*p*; recapture probability). CJS models do not estimate true probability of survival, but instead estimate "apparent survival" because they do not distinguish mortality from emigration. Similarly, older studies on bat survival using life tables and other methods assumed that permanent disappearance of banded individuals was due to death rather than emigration (e.g., Davis 1966; Keen and Hitchcock 1980). CJS models increasingly are being used to estimate survival of bats (e.g., Sendor and Simon 2003; Frick et al. 2007; O'Shea et al. 2011).

The tricolored bat (*P. subflavus*) is historically one of the most common bat species across eastern North America, but has suffered substantial declines due to WNS (e.g., Francl et al. 2012; Ingersoll et al. 2013). Because of these declines, the U.S. Fish and Wildlife Service was petitioned to review the tricolored bat in 2017 for potential listing. Although a number of previous studies existed on survival rates for other cave-hibernating bats in North America, only one study (Davis 1966) estimated survival rates of tricolored bats. The tricolored bat differs from many other species of cave-hibernating bats in eastern North America. Members of the species usually hibernate alone instead of in dense clusters like many other cave-hibernating bats and can produce multiple offspring each year, instead of a single pup (Barbour and Davis 1969; Sealander and Heidt 1990; Hoying and Kunz 1998). Tricolored bats typically do not remain in caves year-round and may hibernate in road culverts in more southern portions of their range (Sandel et al. 2001). Because of these differences, demographic parameters for other North American bat species may not be applicable to the tricolored bat.

Our goal was to compare survival of tricolored bats hibernating in different abandoned mines in Arkansas to determine how survival in a population may differ among sites. These data are important for developing conservation strategies for this species with diminishing population numbers. We conducted winter banding of tricolored bats over 5 years (2014–2019) in small abandoned mines to determine survival rates in a southern portion of the WNS endemic region where *Pd* first was detected in February 2015.

MATERIALS AND METHODS

Study sites.—We conducted the study in Garland County, located in the Ouachita Mountains of west-central Arkansas, United States, on the Caddo-Womble Ranger District of the Ouachita National Forest (approximate coordinates: latitude = 34.5°, longitude = −93.3°). The Ouachita Mountains extend from central Arkansas into east-central Oklahoma. Elevations in the region range from 100 to 800 m. Mean annual precipitation ranges from 112 to 142 cm and the growing season is 200–240 days (McNab and Avers 1994). The dominant forest type in the region is mixed shortleaf pine (*Pinus echinata*)–hardwood forests, but also includes diverse hardwood forests (*Quercus* spp. and *Carya* spp.). Caves are generally absent from the Ouachita region and numerous abandoned mines provide most of the usable hibernacula for cave bats in the region. The Ouachita region of Arkansas is within the WNS endemic area of the United States (U.S. Fish and Wildlife Service 2018).

Based on data from a weather station centrally located to our study sites, mean daily winter (December–February) temperatures during 2014–2019 were 0.3°C (min) to 11.7°C (max), and mean annual surface temperature (MAST) of our study area was 15.5°C (National Centers for Environmental Information 2019). Because underground temperatures are typically close to MAST of an area, we took this datum as the approximate temperature in the mines in our study area (Perry 2013). Mines had little air flow and periodic temperature measures taken during winter in our mines ranged from 14.5°C to 17.0°C.

Mines.—Among abandoned mines available throughout western Garland County, we selected four that housed small numbers (< 30) of hibernating tricolored bats during winter. All mines consisted of one or two horizontal adits that ranged in length from approximately 12 m (Monte Cristo mine) to approximately 100 m (Spillway mine; Table 1). Three of the mines were gated, only allowing access to U.S. Forest Service personnel; one mine was not gated and was commonly visited by the public despite warning signs forbidding entrance. Three of the mines also were considered wet, having standing water on the floor and abundant water seepage on ceiling and walls; one mine was dry, with a dry floor, ceiling, and walls, and little water seepage. All mines had passageways < 2.1 m tall, and had few crevices; bats were clearly visible and accessible.

Mines were located within a 6.5 × 15 km area. The greatest distance between individual mines was 18 km (Monte Cristo and Spillway), and the closest distance was 2 km (Monte Cristo and Charlton). Spillway mine was located in mature forest on the edge of a 26,840 ha reservoir, Charlton mine was located in

Table 1.—Characteristics of four small abandoned mines where survival rates of tricolored bats were estimated in the Ouachita Mountains of Arkansas, 2015–2019.

Mine	Approximate length (m)	Climate	Aspect of entrance	Public access	Approximate mine age
Monte Cristo	12	Wet	North	Yes	Late 1800s
Sleeping Child	18	Dry	East	No	Late 1800s
Charlton	30	Wet	West	No	Late 1800s
Spillway	100	Wet	East	No	1940s

mature forest along a creek and adjacent to a recreation area, and Monte Cristo mine was located along a creek in mature forest. Sleeping Child mine was located in the center in a large open area with no standing mature trees (approximately 50 ha) that resulted from an EF3 tornado in April 2011.

Bat surveys.—We entered mines at the beginning of hibernation (late November to early December) and again near the end of hibernation (late February to early March), starting in November 2014 until March 2019 (5 winters of surveys). Mines were entered during the day and each mine visit generally lasted < 1 h. During each entry, tricolored bats were removed individually from the wall and weighed to the nearest 0.25 g with a 30-g spring scale. We determined sex, measured forearm length, and affixed a single, uniquely numbered 2.4-mm pinch band (Porzana, Icklesham, United Kingdom) to the forearm. Bats were then reattached to the wall. During each reentry, previously banded bats were recorded and newly encountered individuals were banded. We adhered to guidelines of the American Society of Mammalogists for the capture, handling, and care of wild mammals (Sikes et al. 2016); permits were granted by the Arkansas Game and Fish Commission.

We collected swab samples from random bats to determine the presence of *Pd* in the four mines. For Sleeping Child and Spillway mines, we collected swabs from 10 bats at each site, and 20 swabs from mine walls or ceiling near where bats were located, in February 2015, 2016, and 2017, as part of a larger study on WNS (Frick et al. 2017). Presence of *Pd* was determined in these two mines with qPCR using the Muller assay (Muller et al. 2013). For Charlton and Monte Cristo mines, five bats from each mine were swabbed in late February 2017; these swabs were tested for presence of *Pd* using PCR by the Southeast Cooperative Wildlife Disease Study (SCWDS). We followed strict WNS decontamination protocols to avoid spreading *Pd* among the mines (U.S. Fish and Wildlife Service 2018).

Analysis.—To determine whether bat numbers in each mine had an increasing or decreasing trend during the study, we tested for a linear relationship between bat numbers (total individuals encountered during late winter surveys) and time using linear regression (PROC REG—SAS Institute, Inc. 2011). We used late winter surveys because hibernacula surveys are typically conducted in late winter, when symptoms of WNS are most visible. We tested for differences in mean mass between males and females and among mines using mixed model analysis of variance (Littell et al. 1996). We included individuals encountered over multiple years, and included individual bats as a random effect. We used Kenwood–Roger degree of freedom adjustments if variances were unequal (Littell et al. 1996). For multiple comparisons after analysis of variance, we used the Benjamini–Hochberg method to control the false-positive discovery rate (FDR) for the overall experiment at 0.10 (Benjamini and Hochberg 1995; Waite and Campbell 2006). For mean mass loss, we only included bats that were present at both the beginning and end of hibernation such that loss per individual was our sample unit.

We used open-population, CJS models (Lebreton et al. 1992) in program MARK (Cooch and White 2019) to determine apparent survival (ϕ; hereafter survival) and probability of reencountering an individual (p; recapture probability). Sample occasions were entered into MARK based on number of months between each sample (three for winter and nine for summer); estimates of survival therefore were monthly survival rates (ϕ). We also determined annual survival rates for comparison with previous studies of bat survival. For each mine, we compared nine competing a priori models where survival and recapture probability were: 1) held constant (.) over time and did not vary between sexes; 2) varied among sample periods (t); or 3) varied based on sex (s; Table 2). We differentiated sex in our models because it has a strong effect on survival in microbats (Lentini et al. 2015), including the tricolored bat (Davis 1966). We also included time, to determine if differences existed between summer and hibernation survival, and whether survival decreased as WNS spread though the region (Maslo et al. 2015). For reencounter probability, we considered sex in the models because of differences in removal migration and dispersal between the sexes (Baker 1978; Fraser et al. 2012), and we included time because we believed effects of WNS invasion could have changed site fidelity over time. We did not include interactions (e.g., $t * s$) in our model comparisons because initial testing resulted in confounded models with unreliable *SE*s (e.g., ± infinity—Cooch and White 2019). We also determined survival and recapture probability for all mines combined to estimate overall population survival of the tricolored bat.

Table 2.—Mass (± *SE*) of bats (g) and sample size (in parentheses) in four mines during early winter hibernation and late hibernation in the Ouachita Mountains, 2014–2019. Mass loss and percent loss was collected only for individual bats that were present at both the beginning and end of hibernation.

Mine	Sex	Early	Late	Mass loss (g)	% loss
Sleeping Child	F	7.3 ± 0.1 (49)	6.1 ± 0.1 (42)	1.3 ± 0.1 (34)	16.4 ± 0.1 (34)
Spillway	F	7.0 ± 0.1 (55)	5.7 ± 0.1 (55)	1.5 ± 0.1 (35)	20.6 ± 0.1 (35)
Monte Cristo	F	6.5 ± 0.1 (7)	5.9 ± 0.1 (10)	0.8 ± 0.1 (3)[a]	11.1 ± 0.3 (3)[a]
Charlton	F	6.8 ± 0.2 (22)	5.6 ± 0.2 (8)	1.4 ± 0.3 (7)	19.7 ± 0.0 (7)
All mines	F	7.1 ± 0.1 (133)	5.9 ± 0.1 (115)	1.3 ± 0.1 (79)	18.4 ± 0.8 (79)
Sleeping Child	M	6.3 ± 0.1 (48)	5.1 ± 0.1 (42)	1.3 ± 0.1 (31)	20.0 ± 0.1 (31)
Spillway	M	6.3 ± 0.1 (68)	4.9 ± 0.1 (54)	1.4 ± 0.1 (40)	21.3 ± 0.1 (40)
Monte Cristo	M	6.0 ± 0.1 (44)	5.3 ± 0.1 (45)	0.9 ± 0.1 (12)	14.4 ± 0.2 (12)
Charlton	M	6.1 ± 0.1 (67)	5.0 ± 0.1 (20)	1.2 ± 0.1 (11)	19.0 ± 0.2 (11)
All mines	M	6.2 ± 0.1 (227)	5.1 ± 0.1 (161)	1.3 ± 0.1 (93)	19.7 ± 0.7 (94)

[a] Sample size too small for reliable estimates and was not included in analyses.

We compared competing models using Akaike's Information Criterion for small samples (AICc—Burnham and Anderson 2002). We considered models within 2 AICc units of the best model to be in the plausible model set (Burnham and Anderson 2002). Models that contained unreliable *SE*s for ϕ or *p* were discarded and AICc was compared for remaining models. We averaged models for each mine to determine ϕ and *p*, using the weighted averages for the plausible model set, and we calculated unconditional *SE*s for ϕ and *p* (Burnham and Anderson 2002).

RESULTS

Bat numbers.—Over the 5 winters, we banded 325 tricolored bats hibernating in the four mines (Fig. 1). Total number of different individuals encountered in each mine was 94 in Spillway, 52 in Sleeping Child, 83 in Monte Cristo, and 96 in Charlton. The mean number of bats found in each mine during a survey was 24.3 ± 2.7 (*SE*) in Spillway, 17.8 ± 0.9 in Sleeping Child, 12.0 ± 2.1 in Monte Cristo, 12.2 ± 3.6 in Charlton, and 66.3 ± 7.2 in all mines combined. Number of bats in Sleeping Child was most stable (smallest variance), whereas numbers in Charlton were most variable. Over the 5 years, there was no significant increase or decrease in total number of bats encountered during late winter surveys in Spillway ($F_{1,3} = 0.09$, $P = 0.780$), Sleeping Child ($F_{1,3} = 3.42$, $P = 0.162$), Monte Cristo ($F_{1,3} = 0.27$, $P = 0.641$), Charlton ($F_{1,3} = 3.11$, $P = 0.176$), or all mines combined ($F_{1,3} = 0.09$, $P = 0.779$).

The percentage of bats captured more than once was 85% in Sleeping Child and 65% in Spillway. Charlton and Monte Cristo had lower reencounters (25% and 27%, respectively). Over the 10 total encounter periods during the 5 years of study, mean number of times an individual bat was encountered was 3.52 ± 0.28 in Sleeping Child, 2.51 ± 0.18 in Spillway, 1.34 ± 0.07 in Monte Cristo, and 1.25 ± 0.04 in Charlton. The greatest number of encounters (nine times) was by two bats in Sleeping Child, followed by two bats encountered eight times in Spillway. New individuals frequently entered mines during mid-winter while others left; this turnover occurred more often in Charlton and Monte Cristo than the other two mines. For example, at the beginning of hibernation in December 2018, there were 14 bats in Monte Cristo, but by the following February, only six of those bats remained and 13 additional bats had arrived, one of which was banded 3 years prior. Therefore, additional, unidentified hibernacula were present in the area.

Two incidences of banded bats moving between mines occurred. A female bat was banded in early hibernation in 2014 in Charlton, but was found at the beginning of hibernation in 2016 in Sleeping Child, 12 km away. A male bat, also originally banded in Charlton (February 2017), was found in Monte Cristo in February 2018, approximately 2 km away.

Among all mines combined, 109 bats were female (33.5%) and 216 were male (66.5%). We found equal sex ratios in Sleeping Child (51% M, 49% F) and in Spillway (49% M, 51% F), but sex ratios were skewed heavily toward males in Charlton (77% M, 23% F) and Monte Cristo (83% M, 17% F).

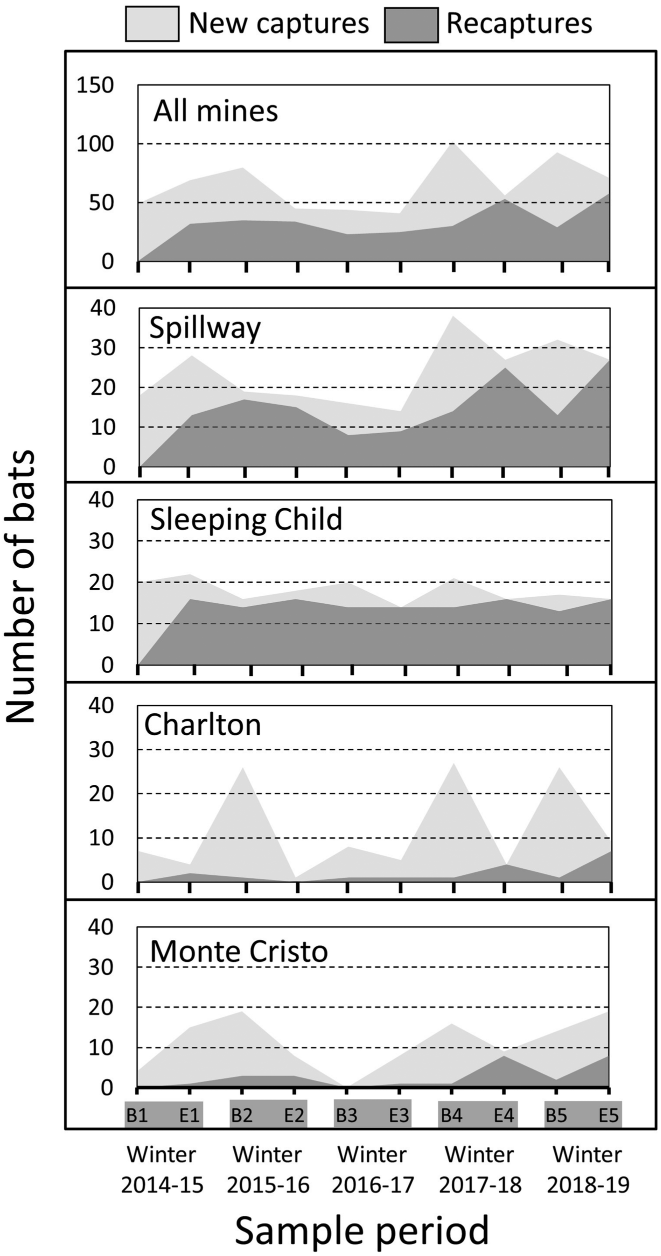

Fig. 1.—Numbers of new and recaptured tricolored bats encountered at the beginning (B) and end (E) of each hibernation period over 5 winters (1–5 on x-axis) in the Ouachita Mountains of Arkansas, 2014–2019.

Bat mass.—Females weighed more than males at both the beginning of hibernation ($F_{1,358} = 146.57$, $P < 0.001$) and at the end of hibernation ($F_{1,274} = 136.88$, $P < 0.001$; Table 2). At the beginning of hibernation, females in Sleeping Child weighed the most among all mines and sexes. Females in Sleeping Child at the beginning of hibernation weighed 11% more than females in Monte Cristo and 7% more than females in Charlton ($F_{3,128} = 3.81$, $P = 0.012$), but were not significantly different from females in Spillway. In late hibernation, females in Sleeping Child weighed significantly more than females in Spillway or Charlton ($F_{3,111} = 4.29$, $P = 0.007$). Mass of males did not differ among mines at the beginning of hibernation ($F_{3,97.6} = 2.09$, $P =$

0.107), but males in Monte Cristo weighed significantly more than males in Spillway at the end of hibernation ($F_{3,158}$ = 3.57, P = 0.016).

On average, both sexes lost around 19% of their body mass over winter, with males in Spillway losing the greatest percentage of mass overwinter (21.3%). For bats present at both the beginning and end of hibernation, mean mass loss was 1.3 ± 0.1 g for both females and males, which did not differ significantly ($F_{1,171}$ = 1.82, P = 0.180). Males and females in Spillway lost the greatest percentage of mass among all mines, and males in Monte Cristo lost significantly less mass than in Spillway or Sleeping Child ($F_{3,90}$ = 3.09, P = 0.031). Females lost significantly less mass in Sleeping Child than in Spillway ($F_{2,72.6}$ = 3.24, P = 0.0448), but females in Monte Cristo were not included in this analysis due to low sample size (n = 3).

Bat survival rates.—Three models were in the best model set for Sleeping Child, Spillway, Charlton, and all mines combined, whereas only one model was in the best model set for Monte Cristo (Table 3). For Monte Cristo, only three of the nine initial models had reliable parameter estimates; all other models contained implausible *SE* estimates and were discarded. For Spillway, Sleeping Child, and Charlton, the best model set included effects of sex (s) on both survival and recapture probability. For Monte Cristo, only constant survival and recapture (.) were included in the best model set. Effects of time (t; season) on survival was included only in the model for all mines combined, along with the effects of sex. Recapture probability was affected by sex in all mines except Monte Cristo.

Based on model-averaged estimates, survival probability varied greatly among the 4 mines with annual survival for both sexes greatest in Sleeping Child (0.7058) and lowest in Charlton (0.1097; Table 4; Appendix I). Monthly probability of survival differed between sexes in all mines except Monte Cristo, with males having slightly higher survival than females in Sleeping Child and Spillway, but females had slightly higher survival than males in Charlton. For all mines combined, monthly survival probability ranged from a low of 0.9368 for males during winter to a high of 0.9455 for females during summer, and annual survival probability was 0.4925 for both sexes. Monthly survival rates were greater during the active season (summer) than during hibernation (winter) in both males and females in the population.

Trends in probability of recapture (p) were similar to survival, with Sleeping Child and Spillway having the greatest probability of recapture and Charlton and Monte Cristo having the lowest (Table 4). There was only a 0.255 probability of reencountering an individual bat in Monte Cristo mine, whereas there was a 0.782 probability of reencountering an individual female in Sleeping Child.

We observed only one mortality event during the study. A bat in Spillway, which was present in winter 2014–2015 and again at the December 2016, was found dead at the entrance in February 2017. Only skeletal remains with an attached band were found and decomposition did not allow us to identify the cause of mortality or test for *Pd*.

Pd presence.—Pd was not detected on bats in Monte Cristo or Charlton in 2017 (the only year those mines were tested). In Spillway, 20% of sampled bats tested positive for *Pd* in February 2015. In February 2016, no samples tested positive for *Pd*, but in February 2017, 10% of bats and 10% of substrate samples tested positive for *Pd* in Spillway. In Sleeping Child, all bat and substrate samples tested negative for *Pd* in February 2015 and 2016. However, in February 2017, 10% of bats and 10% of substrate samples tested positive. Anecdotally, one bat from Sleeping Child that tested positive for *Pd* in February 2017 was encountered throughout the winters of 2017–2018 and 2018–2019 with no apparent adverse effects of WNS. However, three other bats in Spillway that tested positive for *Pd* in late winter were never encountered again. Despite these positive *Pd* detections, no outward symptoms of WNS (e.g., fungal growth or lesions) were observed on any tricolored bats.

Table 3.—Plausible set (< 2.0 from AIC_{min}) of models for monthly survival (ϕ) and encounter probability (p) for four abandoned mines and all mines combined in the Ouachita Mountains of Arkansas, 2014–2019. Models delineate probability of survival and reencounter based on differences in time periods (seasons; t), difference between sexes (s), or no effects of time or sex (.), including difference between each model and the model with lowest AICc (ΔAICc), model weights (ω_i), model likelihood, number of parameters in each model, and model deviance. Models with unreliable *SE*s were discarded from the model set and AICc was recalculated.

Mine	Model	ΔAICc	Weight (ω_i)	Model likelihood	No. of parameters (k)	Deviance
Sleeping Child	$\phi(.)\ p(s)$	0	0.366	1	3	199.32
	$\phi(.)\ p(.)$	0.54	0.278	0.760	2	201.94
	$\phi(s)\ p(s)$	1.48	0.174	0.477	4	198.71
Spillway	$\phi(.)\ p(.)$	0	0.447	1	2	208.06
	$\phi(.)\ p(s)$	1.36	0.227	0.508	3	207.36
	$\phi(s)\ p(.)$	1.83	0.179	0.401	3	207.83
Monte Cristo	$\phi(.)\ p(.)$	0	0.624	1	2	76.37
Charlton	$\phi(.)\ p(s)$	0	0.333	1	3	30.17
	$\phi(.)\ p(.)$	0.07	0.321	0.964	2	32.36
	$\phi(s)\ p(.)$	1.02	0.200	0.601	3	31.19
All mines	$\phi(.)\ p(s)$	0	0.370	1	3	414.41
	$\phi(t)\ p(s)$	0.10	0.352	0.953	11	398.08
	$\phi(s)\ p(s)$	0.85	0.241	0.653	4	413.23

Table 4.—Model-averaged estimates of monthly survival probability (ϕ), recapture probability (*p*), unconditional *SE*s for ϕ and *p*, number of models averaged for each mine (and all mines combined), and overall annual survival rates for tricolored bats in four abandoned mines of the Ouachita Mountains, 2014–2019. Annual survival is over a 12-month period and incorporates differences in seasonal survival when time (*t*) was included in the best model set.

Mine	Sex (season)	ϕ	*SE*	*P*	*SE*	No. of models	Annual
Sleeping Child	Females (all seasons)	0.9705	0.0063	0.7816	0.0559	3	0.6981
	Males (all seasons)	0.9723	0.0060	0.7004	0.0560		0.7137
	Both sexes (all seasons)	0.9714	0.0061	0.7410	0.0556		0.7058
Spillway	Females (all seasons)	0.9586	0.0069	0.6541	0.0474	3	0.6023
	Males (all seasons)	0.9598	0.0068	0.6713	0.0460		0.6115
	Both sexes (all seasons)	0.9592	0.0068	0.6627	0.0467		0.6069
Monte Cristo	Both sexes (all seasons)	0.9301	0.0161	0.2554	0.0601	1	0.4191
Charlton	Females (all seasons)	0.8389	0.0406	0.4511	0.1601	3	0.1215
	Males (all seasons)	0.8247	0.0397	0.3539	0.1031		0.0990
	Both sexes (all seasons)	0.8318	0.0402	0.4025	0.1317		0.1097
All mines	Females (all seasons)	0.9421	0.0189	0.6942	0.0401	3	0.5005
	Females (summer)	0.9455	0.0138	0.6942	0.0401		
	Females (winter)	0.9393	0.0230	0.6942	0.0401		
	Males (all seasons)	0.9395	0.0189	0.5117	0.0363		0.4845
	Males (summer)	0.9430	0.0142	0.5117	0.0363		
	Males (winter)	0.9368	0.0227	0.5117	0.0363		
	Both sexes (all seasons)	0.9408	0.0189	0.6030	0.0382		0.4925
	Both sexes (summer)	0.9442	0.0140	0.6030	0.0382		
	Both sexes (winter)	0.9380	0.0228	0.6030	0.0382		

Discussion

Massive die-offs of bats have been reported across North America due to WNS, with bats in some hibernacula declining by > 90% (Maslo et al. 2015). The extent to which the Ouachita Mountains have been affected by WNS is unclear. Arkansas has seen sizable declines in some species, particularly the northern long-eared bat (*Myotis septentrionalis*) in the Ozark region. We saw no decline in number of bats using our mines during the 5 years of study, and tricolored bats persisted in relatively low numbers in our study mines despite possible *Pd* infections. Further, survival rates in some mines were similar to survival rates prior to WNS (Davis 1966).

Persistence of bats at sites affected by WNS may be attributable to changes in hibernating behavior, selection for genetically resistant individuals, or enhanced immune responses (Maslo et al. 2015), although immune response may be potentially costly yet ineffective (Johnson et al. 2015; Lilley et al. 2017). In the northeastern United States, smaller colonies may be at greater risk of extinction than larger colonies (Frick et al. 2015), but we hypothesize that small colonies in southern regions hibernating in sites with winter temperatures exceeding 15°C also may persist, depending on the site.

Age-dependent survival rates for tricolored bats were provided by Davis (1966); these varied greatly between sexes and among age cohorts in a pre-WNS population, but direct comparisons with this study are problematic because that author did not present overall survival rates. Nevertheless, we used his age-specific survival rates for both sexes weighted by his theoretical age distribution to estimate his annual survival rate at 0.687 for males, 0.490 for females, and 0.641 for combined sexes in his population. Comparatively, annual survival rates for combined sexes in Sleeping Child (0.706) were above his estimates, and survival in Spillway (0.607) were slightly below his estimates. Annual survival rates in male-dominated Monte Cristo (0.419) were below survival rates for males presented by Davis (1966), and annual survival in Charlton (0.110) was substantially less than his estimates. Thus, survival rates in two of our mines were comparable to those in a pre-WNS population, whereas one mine was less and one mine was substantially less. However, we caution that it may be problematic to compare studies that used different methods to estimate survival, particularly to compare older studies that used life tables with newer studies that use maximum likelihood methods (O'Shea et al. 2004). We believe that banding injury did not affect our overall survival estimates because tricolored bats are not subject to band injury, like many other species (Davis 1966), and we saw little evidence of injury in our banded bats.

Bat species that produce a single offspring, such as the Indiana bat (*Myotis sodalis*) and the little brown bat, have annual survival rates (pre-WNS) around 0.70–0.86 (e.g., Humphrey and Cope 1977; Keen and Hitchcock 1980; O'Shea et al. 2004). Annual survival rates > 0.70 may be considered typical for growing populations of temperate-zone bats (Frick et al. 2007; O'Shea et al. 2011). However, bat species that produce multiple offspring per year may have lower survival rates than those that produce a single pup each year (Lentini et al. 2015). The tricolored bat is one of the few cave-hibernating species in North America that produce two, and sometimes even three, offspring (Barbour and Davis 1969; Sealander and Heidt 1990; Hoying and Kunz 1998). Both our study and that of Davis (1966) suggest tricolored bat survival rates are generally lower than many other cave-hibernating species, and their higher reproductive rate may compensate for this lower survival.

Bats in Monte Carlo and Charlton had high turnover rates, low probability of recapture, lower survival, and were male-dominated. Comparatively, survival was relatively high, turnover was low, sex ratios were equal, and bats weighed more at the beginning of hibernation in Spillway and Sleeping Child.

Although total number of bats occurring in each mine was similar each winter, total numbers in two mines were maintained by immigrants, whereas bat numbers in the other two mines were maintained by survivors. The exact cause of these demographic differences among mines is unknown, but potential explanations include differences in *Pd* loads, length of time since *Pd* infected a site, microclimate of mines, disturbance patterns, and composition of the individuals using a particular mine, including age structure, sex ratio, and prehibernation fat levels.

Differences in disturbance also may have affected our results. Monte Carlo mine was ungated and visible from a state highway (approximately 50 m from the mine) during winter. Evidence indicated human visitors and vandalism occurred during the winter in this mine, and overwinter disturbance in Monte Cristo may have caused the high turnover and perceived lower survival. The reasons for the low survival, male dominance, and high turnover in Charlton are equivocal, but this mine may be more of a transitional site (Barbour and Davis 1969; Perry 2013), and bats may have moved to other sites later in the winter. Alternatively, lower survival in Monte Cristo and Charlton may have been a relic of bats abandoning those mines due to unsuitable microclimate, or undetected *Pd* infection.

Differences in sex ratios and age also could have influenced survival among mines. Apparent survival, ϕ, likely underestimates true survival because some bats will permanently emigrate (O'Donnell 2009), and lower apparent survival may be a relic of differing emigration and dispersal patterns of young males (O'Shea et al. 2004). Removal migration (dispersal) in the tricolored bat occurs mostly during the first 2 years of age (Baker 1978), and male tricolored bats tend to undergo longer-range migrations during spring and fall than females (Fraser et al. 2012). We found a relatively equal proportion of males and females that were present in early hibernation remained until late hibernation in Monte Cristo (38% of females and 35% of males), suggesting disturbance may have caused an equal number of each sex to exit. However, in Charlton, 45% of females remained overwinter, whereas only 15% of males remained, suggesting Charlton was more influenced by males leaving, and that these males could have been transient male juveniles.

Finally, differences in body condition of bats in early hibernation might have affected survival, and heavier bats in Sleeping Child may have contributed to the greater survival rates in that mine. Bats with greater fat stores in early hibernation may be capable of tolerating *Pd*, resulting in greater survival than bats with less fat (Lilley et al. 2016; Cheng et al. 2018). Why bats in Sleeping Child were heavier is unknown, but they may have consisted of fewer juveniles than those in other mines, or the landscape surrounding that mine may have been more optimal for gaining fat reserves during the critical prehibernation period.

Our findings underscore the importance of conserving small colonies to the overall survival of species facing WNS. Solitary bats in small colonies have lower initial declines than those in large colonies (Langwig et al. 2012). Numerous small hibernacula (as opposed to a few large sites) that provide adequate survival could potentially maintain a population, although likely at lower overall numbers compared to pre-WNS populations. Our results showed that hibernacula that appear similar and result in similar numbers of bats counted during annual surveys may have substantial differences in survival and site fidelity. This highlights the importance of individual sites that may differ in a number of ways, including differences in microclimate (Langwig et al. 2012) and disturbance (natural or anthropogenic). These hibernation sites likely also differ in ways that we do not currently understand, such as juxtaposition of hibernation sites to needed resources during the prehibernation period when building critical fat reserves for hibernation is important. Different hibernation sites appear to support similar numbers of bats, but without knowing which sites provide greater survival over others, protecting numerous smaller sites can improve the likelihood that sites with a high potential for survival are maintained.

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Appendix I

Estimates of monthly survival (ϕ) and recapture probability (*p*) for each model in the best model set prior to model averaging, including 95% confidence intervals (95% *CI*s) for each estimate for tricolored bats in four abandoned mines of the Ouachita Mountains, 2014–2019. Models included constant survival and/or recapture (.), survival and/or recapture differing by sex (*s*), and survival and/or detection differing over time (*t*).

					95% CI	
Mine	Model	Parameter	Estimate	*SE*	Lower	Upper
Sleeping Child	ϕ(.) *p*(*s*)	ϕ	0.9713	0.0053	0.9588	0.9802
		p (females)	0.8030	0.0504	0.6859	0.8838
		p (males)	0.6826	0.0538	0.5693	0.7777
	ϕ(.) *p*(.)	ϕ	0.9714	0.0053	0.9589	0.9802
		p	0.7379	0.0376	0.6579	0.8047
	ϕ(*s*) *p*(*s*)	ϕ (females)	0.9672	0.0081	0.9471	0.9798
		ϕ (males)	0.9756	0.0071	0.9572	0.9862
		p (females)	0.8063	0.0499	0.6899	0.8862
		p (males)	0.6780	0.0547	0.5631	0.7748
Spillway	ϕ(.) *p*(.)	ϕ	0.9593	0.0062	0.9453	0.9698
		p	0.6632	0.0397	0.5816	0.7361
	ϕ(.) *p*(*s*)	ϕ	0.9591	0.0062	0.9452	0.9696
		p (females)	0.6298	0.0572	0.5125	0.7335
		p (males)	0.6944	0.0535	0.5811	0.7883
	ϕ(*s*) *p*(.)	ϕ (females)	0.9564	0.0089	0.9352	0.9709
		ϕ (males)	0.9621	0.0083	0.9421	0.9754
		p	0.6622	0.0398	0.5803	0.7354
Monte Cristo	ϕ(.) *p*(.)	ϕ	0.9301	0.0161	0.8913	0.9557
		p	0.2554	0.0601	0.1558	0.3893
Charlton	ϕ(.) *p*(*s*)	ϕ	0.8319	0.0352	0.7514	0.8902
		p (females)	0.5588	0.1776	0.2358	0.8387
		p (males)	0.3093	0.0940	0.1590	0.5146
	ϕ(.) *p*(.)	ϕ	0.8295	0.0360	0.7471	0.8890
		p	0.3773	0.0966	0.2129	0.5757
	ϕ(*s*) *p*(.)	ϕ (female)	0.8657	0.0442	0.7535	0.9314
		ϕ (male)	0.8051	0.0457	0.7002	0.8796
		p	0.3905	0.1012	0.2178	0.5958
All Mines	ϕ(.) *p*(*s*)	ϕ	0.9416	0.0047	0.9317	0.9502
		p (females)	0.6942	0.0389	0.6131	0.7648
		p (males)	0.5047	0.0345	0.4375	0.5718
	ϕ(*t*) *p*(*s*)	ϕ (winter 1)	0.9460	0.0297	0.8486	0.9821
		ϕ (summer 1)	0.9636	0.0116	0.9328	0.9806
		ϕ (winter 2)	0.9021	0.0311	0.8220	0.9484
		ϕ (summer 2)	0.9139	0.0154	0.8784	0.9397
		ϕ (winter 3)	0.9613	0.0298	0.8379	0.9917
		ϕ (summer 3)	0.9681	0.0122	0.9333	0.9850
		ϕ (winter 4)	0.9018	0.0278	0.8322	0.9445
		ϕ (summer 4)	0.9479	0.0134	0.9146	0.9686
		ϕ (winter 5)	0.9458	0.0340	0.8262	0.9846
		p (females)	0.6994	0.0406	0.6145	0.7726
		p (males)	0.5170	0.0375	0.4436	0.5896
	ϕ(*s*) *p*(*s*)	ϕ (females)	0.9473	0.0068	0.9324	0.9591
		ϕ (males)	0.9372	0.0064	0.9233	0.9486
		p (females)	0.6868	0.0400	0.6035	0.7595
		p (males)	0.5148	0.0356	0.4452	0.5837