BAT COMMUNITY OF THE DUNBAR CAVE STATE NATURAL AREA, MONTGOMERY COUNTY, TENNSSEE

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Bat Community of the Dunbar Cave State Natural Area, Montgomery County, Tennessee

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ABSTRACT

LAUREN E. LEGGETT. Bat Community of the Dunbar Cave State Natural Area, Montgomery County, Tennessee (Under the direction of DR. DON DAILEY.)

Bats are an exceptional and diverse group of animals, occurring on all continents except Antarctica. As the only truly flying mammal, these creatures comprise approximately 20 percent of all mammalian species in the world. Being nocturnal and capable of flight, these animals are difficult to study. However, these obstacles can, in part, be overcome with the use of bioacoustic monitoring. The purpose of this study was to survey the bat community in the Dunbar Cave State Natural Area (DCSNA), survey for current presence of White Nose Syndrome (WNS), and assess the accuracy of acoustic monitoring used for identifying bat species by comparing acoustic data with physical capture data. Three species were found during cave surveys: Perimyotis subflavus, Eptesicus fuscus, and Myotis lucifugus. Two of these species and one additional species were collected using harp trapping: P. subflavus, E. fuscus, and Myotis septentrionalis. Between 81%-97% of individuals captured via harp trapping were male. Six additional species were detected in the DCSNA using acoustic recording methods: Tadarida brasiliensis, Lasiurus borealis, Corynorhinus townsendii, Lasiurus noctivagans, Nycticeius humeralis, and M. grisescens. Bats demonstrating lesions consistent with WNS were confirmed in 2013 and 2014. With the results of this study showing the continued presence of WNS and an overall decrease in total number of observed and captured bats at DCSNA underlie the importance of the continued need for more research on the bat communities of DCSNA.

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CHAPTER I

INTRODUCTION

Bats are an exceptional and diverse group of animals, occurring on all continents except Antarctica. As the only truly flying mammal, these creatures comprise approximately 20 percent of all mammalian species in the world (O'Shea et al. 2004). Bats are essential to world ecosystems, serving as seed dispersers, predators of insects, and pollinators (Boyles et al. 2011). Many insectivorous bats consume insects that act as vectors of human diseases (Hill & Smith 1984, Hutson et al. 2001). All temperate-climate North American bats, such as those native to the Southeastern United States, are insectivorous. Most of these bats belong to the family Vespertilionidae (Forsyth 1999). Of the bats in this family, there are two types: cave-dwelling and forest-dwelling.

Reproductively active adults of cave-dwelling species will congregate at the entrances of caves, their winter hibernacula, during autumn (Kerth 2008). This reproductive behavior, known as "swarming," is possibly explained by two hypotheses: 1) it functions to increase gene flow during mating and 2) it serves as a social learning opportunity for juvenile bats, where they are led to winter hibernacula by adults and allowed to familiarize themselves with these areas (Kerth et al. 2003, Parsons et al. 2003, Veith et al. 2004). This behavior is identified by increased flight activity in and around underground hibernacula in autumn (Fenton 1969).

As temperatures outside of a cave decrease, bats within the cave begin to enter torpor. Grouping behavior during torpor varies among species, but copulation has been observed during this time, as well as, during fall swarming (Cockrum 1955, Hill and Smith 1984).

Females store sperm until ovulation, which does not occur until they permanently awake from torpor in the spring (Barbour and Davis 1969, Hill and Smith 1984).

During the late spring and early summer, female and male bats of these cave-dwelling species will gather into species-segregated maternity and bachelor colonies. Within the maternity colonies, gestation and parturition take place. Newborn bats are then nursed until self-feeding behavior is learned, which typically takes from three to five weeks (Barbour and Davis 1969, Tuttle 2006).

Due to varying land management practices, deforestation, urbanization, and the wide use of pesticides, bat populations across the United States continue to decline. In addition, bat populations more recently have fallen under the threat of White Nose Syndrome. More than half of the endangered bat species are obligate cave-dwellers, depending on caves during a portion of their life cycle (Briggler & Prather, 2003). As a result, these species are of global conservation concern.

White Nose Syndrome (WNS) was first observed in a hibernating bat in New York (Blehart et al. 2008, Gargas et al. 2009). Since its discovery in 2006, WNS occurrence has spread as far west as Arkansas in the United States and into Canada (Boyles et al. 2011, Butchkoski 2012, Gargas et al. 2009). This condition has been linked to *Pseudogymnoascus destructans*, a psychrophilic fungus that grows at temperatures found in bat hibernacula.

During seasons when bats enter torpor, the fungus infests their epidermis and invades hair follicles and associated sweat and sebaceous glands, and then proceeds to invade membranes of the ears, muzzle, and wing (Blehart et al. 2008, Gargas, et al. 2009). Current studies indicate the most probable cause of death in individuals with WNS is the fungus's growth into tissues below the epidermis that produces irritation, which then would cause the

bats to wake on multiple occasions from torpor for repeated self-grooming. These multiple occasions of awakening could result in the depletion of fat reserves at a higher rate than is normal for bats, thus resulting in starvation or dehydration of infected individuals (Reichard and Kunz 2009). As of August 2012, WNS has caused the deaths of over 5.5 million bats, and has a fatality rate that ranges from 70% to almost 100% in infected areas (Boyles et al. 2011, Carr et al. 2011, Gargas et al. 2009, Smith 2012).

Being nocturnal and capable of flight, these animals are difficult to study (O'Shea and Bogan 2003, Ellison et al. 2003). Because of this difficulty, the current knowledge of bat behavior and population dynamics is limited (Fenton 2003). However, these obstacles can, in part, be overcome with the use of bioacoustic monitoring. This method provides a minimally invasive way of determining species presence in an area for organisms that use auditory signals (Adams 2013). In addition, many bat species fly above nets traditionally used for physical capture, but these species are detectable when conducting an acoustic survey (Adams 2013).

The purpose of this study was to survey the bat community in the Dunbar Cave State

Natural Area, survey for current presence of White Nose Syndrome, and assess the accuracy

of acoustic monitoring used for identifying bat species by comparing acoustic data with

physical capture data.

CHAPTER II

MATERIALS AND METHODS

Three methods were used to survey the bat community in Dunbar Cave State Natural Area: cave surveys, physical capture, and acoustic monitoring. Harp trapping was used for physical bat capture, and Avisoft 116Hme units were used to record bat vocalizations. Harp trapping was conducted during the same time frame as acoustic monitoring on July 17, July 31, and August 7, 2013. The dates of harp trapping, acoustic monitoring, and cave surveys are detailed in Appendix Table 1.

Cave Surveys

Cave surveys were conducted in accordance with previous surveys done by the

Center of Excellence for Field Biology at Austin Peay State University and the Tennessee

Department of Environment and Conservation, Tennessee Wildlife Resources Agency, and

U.S. Fish and Wildlife. All cave survey dates for this study were determined based on permit

restrictions designated by the Tennessee Department of Environment and Conservation and
the Tennessee Wildlife Resources Agency. These agencies require a decontamination

protocol to prevent the spread of WNS. Researchers wore Tyvek suits and protective gear

exclusively in and around Dunbar Cave. Quarantine containers, routinely decontaminated

with bleach wipes, were used to house and transport any items brought into the cave. Upon

return, these items were cleaned with bleach in the lab.

Fifteen cave chambers were surveyed during each trip into Dunbar Cave. During each survey, bats were counted, identified for species and sex if possible, and their location in the cave chamber recorded. Bats found during cave surveys that were conducted during fall, winter, and early spring were not handled for assessment of species and sex due to permit

restrictions, which only allowed for physical capture and handling during the summer season. Previous cave survey data were added to data collected during this study to more thoroughly assess the community of bats in Dunbar Cave.

Environmental characteristics of each bat location site were recorded. Temperatures at the entrance and middle portions of the cave route were recorded using standard mercury thermometers placed in the entrance and the "ballroom" chamber (Appendix Figure 1). In addition to temperature, any distinguishing characteristic of the location such as wetness or proximity to another bat was also recorded.

Harp-trapping

Harp trapping was conducted to capture bats entering and exiting the Dunbar Cave entrance during the spring and summer months. Each harp trap (Bat Conservation and Management, Inc., USA) was comprised of aluminum tubing forming a rectangular frame measuring approximately 2.4 m high by 1.8 m wide. Fishing line (3.6 kg test) was attached vertically to the frame of the harp trap approximately 2.5 cm apart. As they enter or exit the cave, bats fly into these strands and fall into a polyethylene bag that was fastened to the bottom of the trap. All harp traps were bleached and decontaminated upon return to the lab.

Captured bats were examined for species, sex, and vocalizations were recorded. All bats were handled carefully to ensure researcher and bat safety. In addition, all researchers designated to handle bats as a part of this permitted study were vaccinated against the rabies virus before handling any of the animals. These researchers also wore both leather and latex gloves during bat handling and switch them between bats. If more than one bat was captured at the same time, individuals waiting to be processed were placed in individual brown paper

bags until their characteristic field data was collected. All individuals captured were banded before released using the procedure described in Kurz (2011, unpublished M.S. thesis).

Acoustic Surveys

Acoustic surveys were used in conjunction with physical capture methods. Avisoft UltraSoundGate 116Hme units (Avisoft Bioacoustics, Germany), which are small, mobile recording devices used to capture ultrasound songs emitted by bats, were placed near harptrap sites during each capture event and used to acoustically survey the surrounding natural area. Six additional sites were selected to maximize coverage of the park (Appendix Figure 2). Every site was used for recording once per month June 2013 - November 2013. The order of site recordings within each month was determined randomly by drawing slips of paper labeled with site numbers out of a cup. Bat recordings were analyzed using Sonobat 3.20 software. Individuals that were both recorded and captured by harp trap were compared to examine software analysis accuracy in species identification.

CHAPTER III

RESULTS

Acoustic Surveys

A total of 13,312 sound files were analyzed using Sonobat 3.20. Of these, 99 files were identified as bat calls. Bat calls were detected at all 5 sites and for both seasons, but species detected varied among sites and season (Table 1).

Table 1. Summary of all species detected per recording season at each site after analyzing recorded files with Sonobat software.

Site	Summer	Fall
1	T. brasiliensis, P. subflavus	L. borealis, T. brasiliensis, P. subflavus,
		C. townsendii
3	T. brasiliensis	L. borealis, T. brasiliensis, P. subflavus
5	L. borealis, T. brasiliensis	L. borealis, T. brasiliensis, P. subflavus, C. townsendii
8	L. noctivagans	N/A
9	T. brasiliensis	L. borealis, T. brasiliensis
10	T. brasiliensis, N. humeralis, M. grisescens,	L. borealis, T. brasiliensis, C. townsendii
	L. noctivagans	

Harp Trapping

A total of 90 bats were captured in August 2009, 84 in 2011, 145 in 2012, and 29 in 2013 (Table 2). Of the total number of individuals caught each year in the harp trap, 6% (5/84) were recaptured in 2011, 12% (18/145) were recaptured in 2012, and 14% (4/29) were recaptured in 2013 (Figures 1 and 2). In 2011, 5.13% of captured *Perimyotis subflavus* bats were recaptured individuals; 12.23% were recaptured in 2012, and 14.29% were recaptured in 2013. Of the total number of *Eptesicus fuscus* individuals caught each year, 20% (1/5)

were recaptured in 2011 and 33% (1/3) in 2012. Of the total number of *Myotis* septentrionalis individuals caught, 33% (1/3) were recaptured in 2012. Females comprised 17% (15/90) of captured individuals in 2009, 19% (16/84) in 2011, 12% (18/145) in 2012, and 3% (1/29) in 2013 (Figures 1 and 3). Three species were captured using harp trapping: *P. subflavus*, *M. lucifugus*, and *E. fuscus*. All individuals captured in 2009 were identified as *P. subflavus* (Figure 4). *Eptesicus fuscus* comprised 6% (5/84) of the total number of bats captured in 2011, 2% (3/145) in 2012, and 3% (1/29) in 2013 (Figure 4). *M. septentrionalis* was only captured in 2012 and comprised 2% (3/145) of the total number of individuals (Figure 4).

All recaptured bats from 2011 - 2013 were identified as *P. subflavus* except one *E. fuscus* individual and one *M. septentrionalis* individual. From 2011-2013, only two females were recaptured, and both were identified as *P. subflavus*.

Table 2. The number of bats captured and recaptured of each sex and of each species during July and August harp trapping 2011-2013. Harp trapping data for 2009 only includes data from August.

Year	Recapture		Sex	Species				
		Male	Female	P. subflavus	E. fuscus	M. septentrionalis	Total	
August 2009	N/A	75	15	90	0	0	90	
2011	5	68	16	78	5	0	84	
2012	18	127	18	139	3	3	145	
2013	4	28	1	28	1	0	29	

Percentage of Harp Trap Recaptured Individuals Each Year

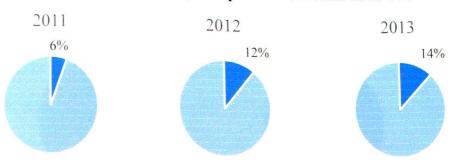


Figure 1. Percentage of recaptured individuals during harp trapping events during July and August 2011, 2012, and 2013.

Percentage of Males and Females Captured During July and August Harp Trapping by Year

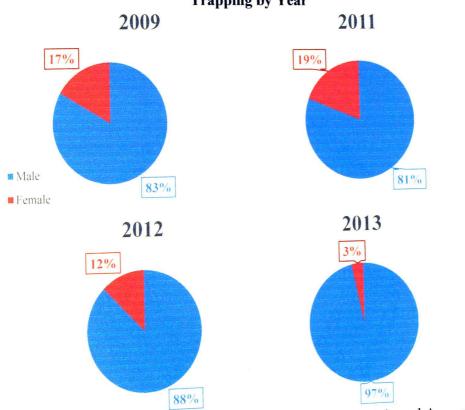


Figure 2. Percentage of males and females captured during July and August harp trapping in 2011-2013 and August 2009.

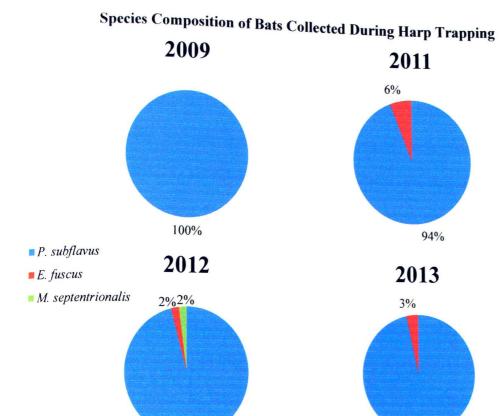


Figure 3. Species composition of bats captured during July and August harp trapping in 2011-2013 and August 2009.

96%

97%

Cave Surveys

Three species were found during cave surveys: P. subflavus, M. lucifugus, and E. fuscus. The minimum number of bats found during a cave survey season was 2 individuals in summer 2009, and the maximum number of bats found was 545 individuals in spring 2006 (Table 3). Eptesicus fuscus individuals were only found in the cave during spring and winter season cave surveys (Table 3, Figures 5, and 8). These individuals comprised 2.94% (16/545) of the total number of bats found in spring 2006, 4.21% (4/95) in winter 2006, 3.03% (7/231) in spring 2008, 0.47% (1/214) in spring 2009, 1.10% (2/181) in spring 2010, 1.11% (1/90) in winter 2011, and 2.44% (2/82) in winter 2013 (Table 3, Figures 5 and 8). Myotis lucifugus was found in the cave during all four seasons, but was only found during the summer in one year: 2007 (Table 3, Figure 6). When present, M. lucifugus comprised between 0.18% -35.14% of the individuals found each season, the maximum occurring in summer 2007, and the minimum occurring in spring 2006 (Table 3, Figure 5, Figure 6). Perimyotis subflavus comprised the majority of the bats found during all cave surveys, ranging from 64.86% -100% of the individuals identified (Figures 5, 6, 7, and 8).

Table 3. Number of bats found during cave surveys by season and species 2006-2013.

2006 P. subflavus M. lucifugus F fuscus Total

<u>2006</u> I	ber of bats four P. subflavus	M. luci	fugus	E. fus	cus	Total	_
Spring	528		1	1		545	
Summer	5				0	5	;
Fall	30		0		0	30	
Winter	91		0		4	9:	5
2007							
Spring	148		9		0	15	
Summer	2		13		0		7
Fall		8	3		0		1
Winter	3	5		5	0	- 4	40_
2008				_	-	2	21
Spring	21		1	0	7		31
Summer		3		0	0		13
Fall		19		0	(19
Winter		35		5	()	40
2009							21.4
Spring	2	06		7		-	214
Summer		2		0		0	2
Fall	7			1		0	8
Winter	118			33		0	151
<u>2010</u>		162		17		2	181
Spring		0		0		0	0
Summer		5		0		0	5
Fall		42		9		0	51
Winter		72					
<u>2011</u>		112		4		0	116
Spring		112		0		0	12
Summe	r	12		1		0	16
Fall		15		3		1	90
Winter		86					
2012				0		0	179
Spring		179		0			8
Summ		8		0			2
Fall		27	7 8			0	6
Winte	ľ	59		0			
2013				0		0	16
Spring		165				0	
Sumn		4		0		0	1
Fall	101	12		0		2	8
Winte		79		1			

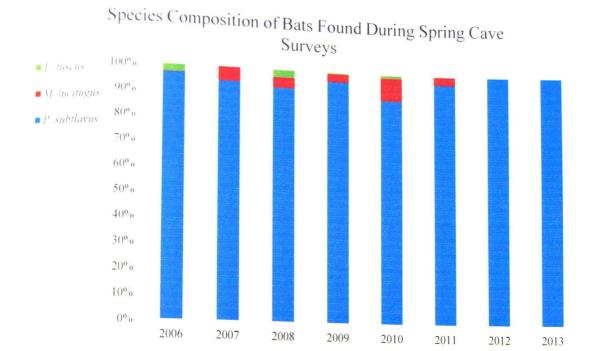


Figure 4. Species composition of bats found during spring cave surveys 2006-2013.

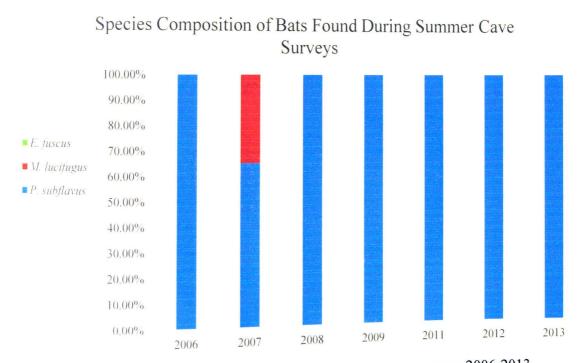


Figure 5. Species composition of bats found during summer cave surveys 2006-2013.

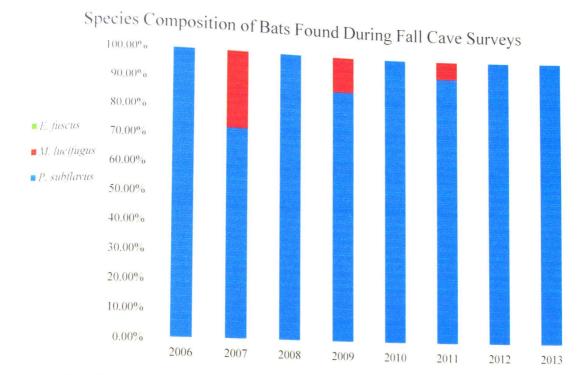


Figure 6. Species composition of bats found during fall cave surveys 2006-2013.

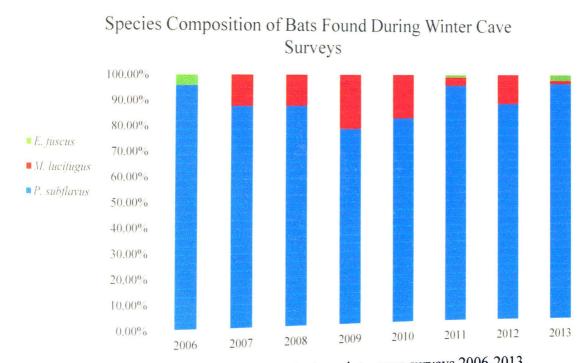


Figure 7. Species composition of bats found during winter cave surveys 2006-2013.

White Nose Syndrome

Five individuals were sent to Dr. Lisa Last at the College of Veterinary Medicine at the University of Georgia to determine if the bats were positive for White Nose Syndrome. One individual was found dead and sent in April 2013, and the other four were found dead or dying outside the cave and submitted in March 2014. All bats were identified as *P. subflavus* and were positive for White Nose Syndrome after histopathologic investigation.

CHAPTER IV

DISCUSSION

Bat Species of Dunbar Cave State Natural Area

Three species were found during cave surveys: *Perimyotis subflavus*, *Eptesicus fuscus*, and *Myotis lucifugus*. Three species were also collected using harp trapping for physical capture: *P. subflavus*, *E. fuscus*, and *Myotis septentrionalis*. Six additional species were detected in the state natural area using acoustic recording methods: *Tadarida brasiliensis*, *Lasiurus borealis*, *Corynorhinus townsendii*, *Lasiurus noctivagans*, *Nycticeius humeralis*, and *Myotis grisescens*.

In both cave surveys and harp trapping, *Perimyotis subflavus* comprised the majority of bats, ranging from 64.86%-100% of bats found or captured. *P. subflavus* was also detected at 1 acoustic site (site 1) during the summer and at 3 acoustic sites (site 1, 3, and 5) during the fall. The location of sites 3 and 5 indicate that *P. subflavus* is utilizing a large area of the park in addition to the cave that extends all the way to the northern border of the natural area (Appendix Figure 2). These findings are consistent with previous studies, as *P. subflavus* is known to roost in forested areas with an estimated minimum home range of 5km (Quinn and Broders 2007). All recaptured bats from 2011 – 2013 were identified as *P. subflavus* except one *E. fuscus* individual and one *M. septentrionalis* individual. The percentage of individuals recaptured comprised 6% of the total number of bats captured in 2011, 12% in 2012, and 14% in 2013. In 2011, 5.13% of captured *P. subflavus* bats were recaptured individuals; 12.23% were recaptured in 2012, and 14.29% were recaptured in 2013. This is consistent

with previously-measured percentages of recapture for this species (Whitaker and Rissler 1992).

Eptesicus fuscus was found during cave surveys and harp trapping but was not detected by acoustic monitoring. However, this species comprised a small portion of the bats obtained by these methods, ranging from 0.47%-6% of bats found or captured. This species was also only found in the cave during spring and winter cave surveys, which indicates that E. fuscus uses Dunbar Cave as a winter hibernacula, as this species is known to select large caves with relatively high airflow for use as winter hibernacula (Agosta 2002).

Myotis lucifugus was found only during cave surveys. This species appears to be utilizing the cave primarily for hibernacula during the winter and early spring, as it was found in the cave during fall and late spring every year and only once additionally in the summer during 2007. This species typically utilizes forest habitat near water during spring, summer, and fall months but use caves as hibernacula during the winter (Arroyo-Cabrales and Ticul Alvarez Castaneda 2008, Havens 2006).

Myotis septentrionalis was captured using harp trapping in 2012 only. Three individuals were captured, one of which was recaptured, having been banded in a separate study in February 2011. This species was not detected using acoustic monitoring nor observed during cave surveys, suggesting that this species is not utilizing the surrounding natural area and when in the cave utilizes a chamber that is not part of the survey area.

Because of the small number of this species captured at Dunbar Cave, it is unclear if there is a persistent population of M. septentrionalis in the cave that has not been observed or if this was a chance occurrence. Further surveys of additional cave chambers and openings would

be needed to determine if there is a population of *M. septentrionalis* that consistently inhabits Dunbar Cave.

Tadarida brasiliensis was detected using acoustic monitoring at every site during every season with the exception of the golf course site (site 8) (Appendix Figure 2). Individuals of this species primarily roost in manmade structures in the southeastern United States on their migratory flight to Mexico (Tennessee Bat Working Group 2013). The results from acoustic monitoring indicate that individuals of this species are utilizing the majority of Dunbar Cave State Natural Area with the exception of Dunbar Cave itself.

Lasiurus borealis was detected using acoustic monitoring at every site in the fall and one forested site (site 5) during the summer (Appendix Figure 2). This indicates that individuals of this species are utilizing the majority of the wooded parts of the state natural area. As these are forest-dwelling bats, they are most likely residing in the wooded areas of the natural area itself and emerging in the evening to feed (Arroyo-Cabrales et al. 2008).

Corynorhinus townsendii was detected using acoustic monitoring at two forest sites (site 1 and 5) and the cave entrance (site 10) (Appendix Figure 2), indicating that individuals of this species are utilizing the area near the cave entrance and at the northernmost part of the state natural area. However, these individuals have not been observed in the cave itself. This is relevant to note, as this is a cave-dwelling species (Arroyo-Cabrales and Ticul Alvarez Castaneda 2008). It is possible that members of this species are utilizing Dunbar Cave but, due to their sensitivity to disturbance, are roosting in inaccessible parts of the cave (Arroyo-Cabrales and Ticul Alvarez Castaneda 2008). However, this species has not been previously recorded in Tennessee nor the southeastern United States (Arroyo-Cabrales and Ticul Alvarez Castaneda 2008). It is also possible that the Sonobat identification was incorrect, as

this program is not 100% accurate at detecting and identifying bat calls (Tyburec-Tucson 2014). Because this species has never previously been recorded in the southeastern United States and is not known to utilize the type of forest habitat near and around sites 1, 5, and 10, it is more likely that Sonobat incorrectly identified the call (Arroyo-Cabrales and Ticul Alvarez Castaneda 2008).

Lasiurus noctivagans was detected using acoustic monitoring at the golf course (site 8) and the cave entrance (site 10) in the summer (Appendix Figure 2). Both of these sites are located in open areas in close proximity to wooded areas. Being forest-dwelling bats, members of this species are likely emerging from the nearby forest to feed in the evenings at these site locations (Arroyo-Cabrales et al. 2008).

Nycticeius humeralis was detected using acoustic monitoring at the cave entrance (site 10) in the summer (Appendix Figure 2). This species is a forest-dwelling bat, utilizing spaces beneath tree bark and in tree crevices throughout the year (Boyles and Robbins 2006, Arroyo-Cabrales and Ticul Alvarez Castaneda 2008). These data suggest that this species was present in the forest habitat surrounding site 10.

Myotis grisescens was detected using acoustic monitoring at the cave entrance (site 10) in the summer (Appendix Figure 2). This species has not been observed during a cave survey but is a cave-dwelling species (Arroyo-Cabrales and Timm 2008). Over 95% of the known population of this species resides in 8 or 9 caves, attributed to the fact that members of this species will only roost in caves with specific parameters such as domed halls, vertical shafts, and temperatures ranging from 6 °C -11°C in the winter and 14 °C -24 °C in the summer (Arroyo-Cabrales and Timm 2008). The temperature at Dunbar Cave measured close to 15°C in the entrance and 22°C in a central chamber during all seasons. Dunbar Cave may

not fit the habitat specifications required by *M. grisescens*, which could explain why no members of this species have been observed roosting in the cave. In addition, cave tours, which were held consistently from early 1980's through 2009, could have caused considerable disturbance to the population that may have utilized Dunbar Cave. These bats roost in large groups, averaging 10,000-25,000 and are sensitive to human disturbance (Arroyo-Cabrales and Timm 2008, Mullen 2012, unpublished M.S. thesis).

Bat Sex Ratios

Males make up the majority of bats utilizing Dunbar Cave. Females comprised a maximum of 19% of individuals captured using harp trapping and only 2 out of 27 recaptured individuals were female. Cave surveys could not be used to determine community sex ratios due to limitations in gathering the data. In many cases, bats were out of reach or present when permit restrictions did not allow handling of bats to determine sex. The results from harp trapping indicate that Dunbar Cave is primarily being used by a bachelor colony during July and August. In addition, no swarming behavior was observed during autumn, which further suggests that Dunbar Cave is not being used for reproduction and the raising of young by females but primarily to house a bachelor colony at this time. This is consistent with other studies, as harp trapping was conducted during the summer season when males and females will divide into maternity and bachelor colonies, with maternity colonies roosting in trees and/or other caves until winter when they use caves as winter hibernacula (Ollendorf 2002, Quinn and Broders 2007, Willis and Brigham 2003).

Harp Trapping and Acoustic Survey Comparison

Analysis of recordings taken during physical capture could not identify the species for any of the recorded bat vocalizations. This might be the result of the recording unit being

held too close to the bat while recording. However, previous studies have placed recording units as close as 15cm to an individual for call analysis (Kazial et al. 2001). For recordings of individuals, the microphone was held by a researcher, which would cause the microphone to move during the recording process. This accidental movement might have affected the sound quality and/or created additional noise in the sound file. The background noise could have interfered with the sound analysis program's ability to detect the bat call. It is also possible that the individuals were emitting stress calls, which may not be species-specific or be found in the database used for comparison in the Sonobat software. Future attempts to record individuals should standardize the distance between the bat and microphone as well as provide a stationary location for microphone placement so as to minimize background noise when recording.

White Nose Syndrome

In February-March 2014, a total of 30 bats were found dead or dying outside the cave entrance. A haphazardly selected sample of 4 bats taken from this group was tested for histopathologic evidence of White Nose Syndrome. All 4 bats were positive for WNS. This was the second occurrence of WNS among the bats in this study. The first occurred in April 2013 when a bat was observed with white fungal-like growth on its face. A second survey was conducted less than a week after this observation. The individual in question was not found roosting again, but a dead bat was collected from a ledge along the survey route. It is unknown if this was the same individual. This bat was submitted in April 2013 and tested positive for WNS. Because Dunbar Cave has been closed to the public, the spread of the fungus is most likely the result of physical contact between bats.

Conclusions

The results from this study provide more information about the bat species using Dunbar Cave State Natural Area. The data suggest the cave is used by cave-dwelling bats. Furthermore, this study has provided evidence that bioacoustic monitoring is a useful, minimally invasive method of detecting bat species in an area that would not have been detected using physical methods. This study also has confirmed the continued presence of WNS at Dunbar Cave in 2013 and 2014. With the results of this study showing the continued presence of WNS and overall decrease in total number of observed and captured bats at Dunbar Cave, understanding what species utilize the natural area and how it is being used by each species becomes more relevant. The knowledge of the species that are using the cave system and the natural area provides information for designing more effective bat conservation efforts. It is also hopeful this information can be used to develop policies allowing the public to enjoy the Dunbar Cave State Natural Area with minimal disturbance to the bat community.

Literature Cited

- Adams, A.M. 2013. Assessing and analyzing bat activity with acoustic monitoring: challenges and interpretations [dissertation]. University of Western Ontario. 2-3 p.
- Agosta, S.J. 2002. Habitat use, diet and roost selection by the Big Brown Bat (*Eptesicus fuscus*) in North America: a case for conserving an abundant species. Mammal Review. 32(2): 179-198.
- Arroyo-Cabrales, J. and Ticul Alvarez Castaneda, S. 2008. *Corynorhinus townsendii*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. http://www.iucnredlist.org/details/17598/0.
- Arroyo-Cabrales, J. and Ticul Alvarez Castaneda, S. 2008. *Myotis lucifugus*. The IUCN Red List of Threatened Species. Version 2014.2. http://www.iucnredlist.org/details/14176/0.
- Arroyo-Cabrales, J. and Ticul Alvarez Castaneda, S. 2008. *Nycticeius humeralis*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. http://www.iucnredlist.org/details/14944/0.
- Arroyo-Cabrales, J., Miller, B., Reid, F., Cuarón, A.D. & de Grammont, P.C. 2008.

 Lasionycteris noctivagans. In: IUCN 2014. IUCN Red List of Threatened Species.

 *Version 2014.1. http://www.iucnredlist.org/details/11339/0.
- Arroyo-Cabrales J., Miller B., Reid F., Cuarón A.D., de Grammont P.C. 2008. *Lasiurus borealis*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. http://www.iucnredlist.org/details/11347/0.

- Arroyo-Cabrales, J. and Timm, R. 2008. *Myotis grisescens*. In: IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1. http://www.iucnredlist.org/details/14132/0.
- Barbour R.W. and Davis W.H. 1969. *Bats of America*. Lexington, KY: University Press of Kentucky.
- Blehart D.S., Hicks A.C., Behr M., Meteyer C.U., Berlowski-Zier B.M., Buckles E.L., Coleman J.T.H., Darling S.R., Gargas A., Niver R., Okoniewski J.C., Rudd R.J., Stone W.B. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science*. 323, 227.
- Boyles J.G., Cryan P.M., McCracken G.F., Kunz T.H. 2011. Economic importance of bats in agriculture. *Science*. 332, 41-42.
- Boyles, J.G. and Robbins, L.W. 2006. Characteristics of summer and winter roost trees used by Evening Bats (*Nycticeius humeralis*) in Southwestern Missouri. The American Midland Naturalist Journal. 155:210–220
- Briggler J.T. and Prather J.W. 2003. Use and selection of caves by the Eastern Pipistrelle bat (*Pipistrellus subflavus*). *American Midland Naturalist*. 149(2): 406-412.
- Butchkoski, C. 2012. White Nose Syndrome (WNS) Occurrence by County/District. Image.
 PA Game Commission.
- Brunet-Rossini A.K. and Wilkinson G.S. 2009. Methods for Age Estimation and the Study of Senescence in Bats. *Age and Estimation of Senescence*. College Park, MD: University of Maryland.

- Carr S.L., Froschauer A., and MacKenzie T. 2011. White-Norse Syndrome Confirmed in Kentucky. Kentucky Department of Fish and Wildlife Resources.
- Cockrum E.L. 1955. Reproduction in North American bats. Transactions of the Kansas Academy of Science. 58 (4): 487-511.
- Ellison L.E., O'Shea T.J., Bogan M.A., Everette A.L., and Schneider D.M. 2003. Existing data on colonies of bats in the United States: summary and analysis of the U.S. Geological Survey's Bat Population Database. In: O'Shea TJ, and MA Bogan (Eds.). Monitoring trends in bat populations of the United States and territories: problems and prospects. Information and Technology Report 2003-0003.: U.S. Geological Survey. 127-237.
- Fenton M.B. 2003. Science and conservation of bats: Where to next? Wildlife Society Bulletin. 31 (1), 6-15.
- Forsyth A. 1999. Mammals of North America: Temperate and arctic regions. Buffalo, NY: Firefly Books (U.S.) Inc.
- Furmankiewicz J. 2008. Population size, catchment area, and sex-influenced differences in autumn and spring swarming of the brown long-eared bat (*Plecotus auritus*).

 Canadian Journal of Zoology. 86(3): 207-261.
- Gargas A., Trest M.T., Christensen M., Volk T.J., Blehart, D.S. 2009. *Geomyces destructans* sp. Nov. associated with bat white-nose syndrome. *Mycotaxon*. 108: 147-154.
- Havens, A. 2006. "Myotis lucifugus," Animal Diversity Web. University of Michigan Museum of Zoology.

- Hill J.E. and Smith J.D. 1984. *Bats: A Natural History*. Austin, TX: University of Texas Press.
- Hutson A.M., Mickleburgh S.P., Racey P.A. (Comp.) 2001. Microchiropteran Bats: Global Status Survey and Conservation Action Plan. Gland, Switzerland and Cambridge, UK; IUCN/SSC Chiroptera Specialist Group.
- Jones G., Jacobs D.S., Kunz T.H., Willig M.R., Racey P.A. 2009. Carpe noctum: The importance of bats as bioindicators. *Endangered Species Research*. 8: 93-115.
- Kazial K.A., Burnett S.C., Masters W.M. 2001. Individual and group variation in echolocation calls of Big Brown Bats, *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *Journal of Mammology*. 82(2): 339-351.
- Kerth G. 2008. Causes and Consequences of Sociality in Bats. BioScience. 58 (8), 737-746.
- Kerth G., Kiefer A., Trappmann C., Wewashaar M. 2003. High gene diversity at swarming sites suggest hot spots for gene flow in the endangered Bechstein's bat. *Conservation Genetics*. 4: 491-499.
- Kurz, M.E. 2011. Species richness, population genetics, reproductive behaviors, and sex ratios of the cave-dwelling bats at Dunbar Cave, Clarksville, Montgomery County, Tennessee. M.S. Thesis. Austin Peay State University: United States of America.
- Kunz, T.H. and Kurta, A. 1988. Capture methods and holding devices. In: Ecological and
 Behavioral Methods for the Study of Bats (Ed. By T.H. Kunz), pp. 1-30. Washington,

 D.C.: Smithsonian Institution Press.

- Mullen, V. 2012. "Bats of Dunbar Cave State Natural Area: An Update." Austin Peay State University. Tennessee Bat Working Group Conference. Fall Creek Falls State Park, Pikeville, Tennessee. 15 November 2012.
- Ollendorf, J. 2002. *Myotis septentrionalis*, Animal Diversity Web. University of Michigan Museum of Zoology.
- O'Shea, T.J and Bogan, M.A. (Eds.). Monitoring trends in bat populations of the United States and territories: problems and prospects. Information and Technology Report 2003-0003.U.S. Geological Survey.
- O'Shea T.J., Ellison L.E., Stanley T.R. 2004. Survival estimation in bats: Historical overview, critical appraisal, and suggestions for new approaches. In: Sampling Rare and Elusive Species: Concepts, Designs, and Techniques for Estimating Population Parameters. (Ed. By W.L. Thompson), pp. 297-336. Washington, D.C.: Wasland Press.
- Parsons K.N., Jones G., Davidson-Watts I., Greenaway F. 2003. Swarming of bats at underground sites in Britain-implications for conservation. *Biological Conservation*. 111: 63-70.
- Quinn, G.M. and Broders. 2007. Roosting and foraging ecology of eastern pipistrelle

 (Perimyotis subflavus) bats in SW Nova Scotia. A report prepared for: Nova Scotia

 Habitat Conservation Fund c/o NS Department of Natural Resources.

- Reichard J.D. and Kunz T.H. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). Acta Chiropterologica. 11(2): 457-464.
- Smith J. 2012. Forest Service Extends Closure of Abandoned Mines and Caves as New Studies Indicate 5.5 Million Bats Have Died. News Release. U.S. Forest Service.
- Tennessee Bat Working Group. 2013. Brazilian Free-tailed Bat. Tennessee Bat Working Group. http://www.tnbwg.org/TNBWG_TABR.html.
- Tuttle M.D. 2006. America's Neighborhood Bats: Understanding and Learning to Live in Harmony with Them. Austin, TX: University of Texas Press.
- Tyburec-Tucson, J. 2014. "Bat Echolocation Signal Analysis Software Calculating Accuracy Rates Among BCID, EchoClass, KaleidoscopePro and SonoBat." Bat Conservation and Management, Inc.
- Veith M., Beer N., Kiefer A., Johannesen J., Seitz A. 2004. The role of swarming sites for maintaining gene flow in the brown long-eared bat (*Plecotus auritus*). *Heredity*. 2004: 1-8.
- Whitaker, J.O. Jr. and Rissler, L.J. 1992. Seasonal activity of bats at Copperhead Cave.

 Proceedings of the Indiana Academy of Science. 1992(1):127-134
- Willis, C.K.R. and Brigham, R.M. 2003. Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, Eptesicus fuscus, conform to the fissionefusion model *Animal Behaviour*. 2004 (68):495-505.

Table 1. Dates of cave surveys, acoustic monitoring, and harp trapping for this

Date	Survey type Survey type
01/18/2013	Cave survey
03/26/2013	Cave survey
04/09/2013	Cave survey (not full survey- was sent in to find possible WNS bat)
04/19/2013	Cave survey
04/26/2013	Cave survey
05/20/2013	Acoustic (site 10) –trial run
05/22/2013	Acoustic (site 10) –trial run
05/30/2013	Acoustic (site 10) –trial run
06/04/2013	Acoustic (site 10)
06/11/2013	Acoustic (site 8)
06/12/2013	Cave survey & acoustic (site 10)
06/19/2013	Acoustic (sites 3 & 5)
06/25/2013	Acoustic (site 1 & 9)
07/09/2013	Acoustic (site 8)
07/10/2013	Cave survey & acoustic (sites 1 & 5)
07/17/2013	Harp trap & acoustic (site 10)
07/31/2013	Harp trap (site 10) & acoustic (site 10 & 3)
08/07/2013	Harp trap and acoustic (site 10)
08/21/2013	Cave survey
08/28/2013	Acoustic (sites 9 & 5)
08/30/2013	Acoustic (sites 1 & 3)
09/018/2013	Acoustic (sites 3 & 10)
09/25/2013	Acoustic (site 5)
09/27/2013	Acoustic (sites 9 & 1)
09/28/2013	Cave survey
10/18/2013	Cave survey & acoustic (site 9)
10/22/2013	Acoustic (site 10)
10/29/2013	Acoustic (sites 5, 3, & 1)
11/01/2013	Acoustic (sites 10 & 9)
11/02/2013	Cave survey
11/22/2013	Acoustic (sites 5, 3, 1)
12/05/2013	Acoustic (sites 3, 5, 9)
12/06/2013	Acoustic (sites 1 & 10)
12/07/2013	Cave survey

ArcGIS Template for Mapping of Bat Roost Sites within Dunbar Cave State Natural

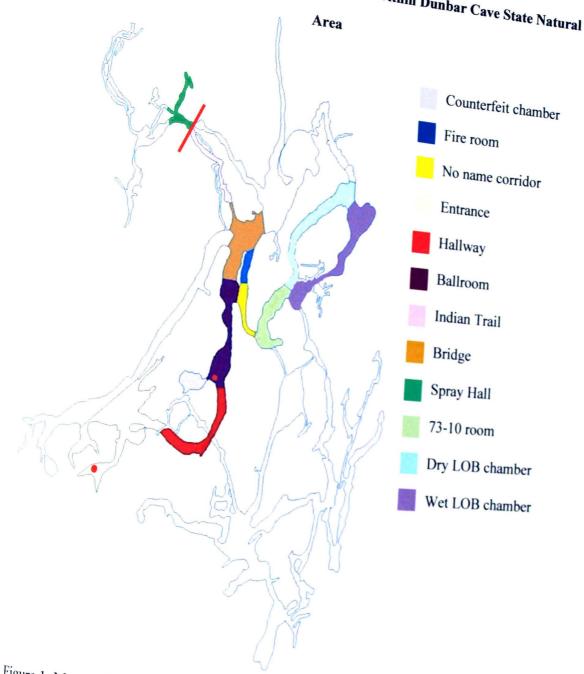


Figure 1. Map of Dunbar Cave, used as a template for mapping the roost sites of bats during cave surveys. The location of the thermometers used for temperature readings are marked with a red dot. The red line designates the area of the cave that was inaccessible after August 2013 due to the formation of a sinkhole in the path. The chambers surveyed are labeled with different colors

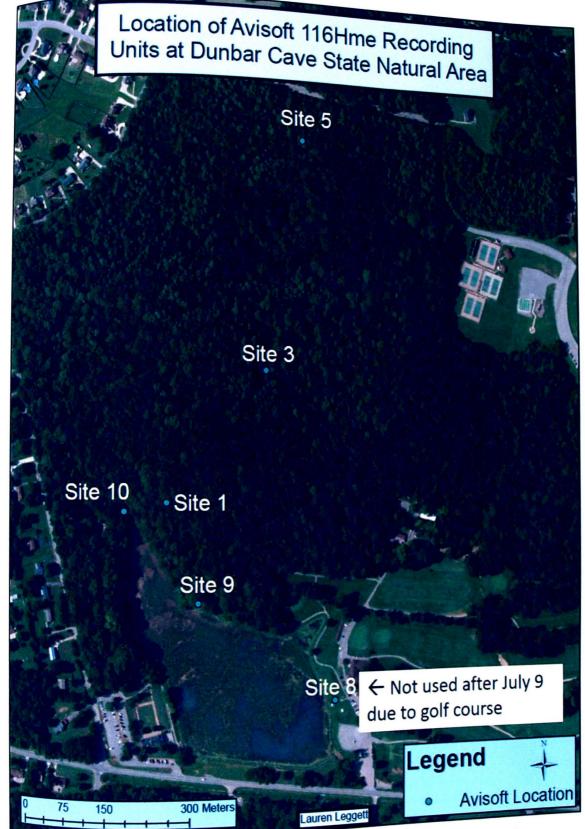


Figure 2. GIS map of unit recording sites used for acoustic data collection in this study.