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A Phenological Study of Bat Communities in Southern Mississippi Caves

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A PHENOLOGICAL STUDY OF BAT COMMUNITIES IN SOUTHERN MISSISSIPPI CAVES

by

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ABSTRACT

A PHENOLOGICAL STUDY OF BAT COMMUNITIES IN SOUTHERN MISSISSIPPI CAVES

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Mississippi is generally not known for its caves, and consequently its cave flora and fauna remain largely unstudied. From fall 2010 to winter 2013, we studied the bat populations in the three largest caves in Mississippi. The most common (and only) species found in these caves were *Myotis austroriparius* and *Perimyotis subflavus*. I collected monthly data on the number of bats per species, behaviors and locations of the bats within the caves, as well as atmospheric data at selected positions within each cave. All three caves were found to have significant temperature differences between seasons (winter<fall=spring<summer). Some of the caves also showed temperature differences between internal locations. *Perimyotis subflavus* was found in significantly higher numbers during winter, and individuals were usually in torpor. However, an experiment in winter with “marked” (by nearby strings) *P. subflavus* revealed that the majority of these bats did not remain in their original positions for more than two days. In contrast, *M. austroriparius* was found in significantly higher numbers in the summer than winters. Two of the caves were used as maternity roosts by *M. austroriparius*. The largest cave in Mississippi, which unfortunately was highly vandalized, usually contained ~8,000 *Myotis austroriparius* during the summer months.

ACKNOWLEDGMENTS

Special thanks to my committee director, Dr. David Beckett, and my other committee members, Dr. Frank Moore and Dr. Carl Quarls, for their advice and support throughout the duration of this research. Also, no research would have been possible without all of the dedicated cave explorers who accompanied me on my expeditions.

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CHAPTER I

DESCRIPTIONS OF THE THREE CAVES AND TWO BAT SPECIES IN THIS STUDY

Caves of Southern Mississippi

To most Mississippians, the word "cave" refers to something mystic, dangerous and out-of-state. In 1933, in a *Commercial Appeal* article, author Edwin Stainton indicated that not one person in a thousand in either Meridian or Laurel could remember having ever heard of Pitts Cave (Knight et al. 1974), the largest known cave in Mississippi.

There are approximately 47 documented caves in Mississippi (Knight et al. 1974; Moore 2006). The three caves I selected for this study are all found within a formation of limestone called the Vicksburg Group that stretches through southern Mississippi from Vicksburg, MS through Waynesboro, MS and slightly into western Alabama (Moore 2006). The caves I studied are Waddell Cave in Smith County, Triple H Cave in Wayne County, and Pitts Cave (also known as Williams Cave) also located in Wayne County (Figure 1). These particular caves were selected due to their size and previous observations of their occupation by relatively large numbers of cave dwelling bats. All three caves are among the largest in Mississippi (Knight et al. 1974; Moore 2006) and bats were observed in surveys of all three caves in the year 2000 (Beckett and Trousdale 2001).

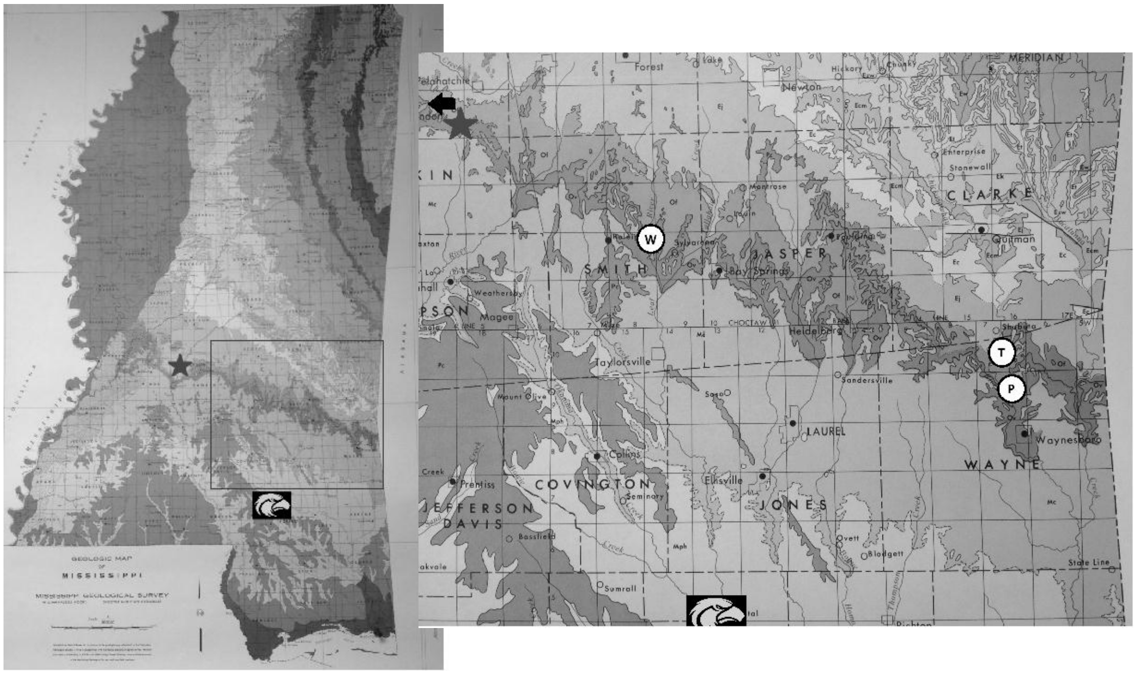

Figure 1. State and County location of Caves in southern Mississippi. This is a geological map of Mississippi, courtesy of the USM Department of Geography and Geology. The area in the box is enlarged on the right and shows the Vicksburg Group limestone formation (dark area across Rankin, Smith, Jasper, Clarke and Wayne counties) and the approximate locations of the caves in this study. W = Waddell Cave, T = Triple H Cave, and P = Pitts Cave. The University of Southern Mississippi is also marked by the Eagle emblem.

Physical Description of Waddell, Triple H and Pitts Cave

Waddell Cave

Waddell Cave is the second longest cave in Mississippi with a total of ca. 235 meters of passageway. The entrance to the cave is 1.5m in width and height and is located at the base of a large, steeply sloped hill. A stream of water flows through the length of the cave year round, beginning from the back of the cave and exiting out of the mouth of the cave, then flowing down to the Leaf River. The cave floor consists of mostly sand and small pebbles with an occasional large chunk of limestone. The ceiling and walls are limestone with seashells embedded in the stone.

The cave meanders back into the hillside through a series of crawlways with no major branching (Figure 1.1). These crawlways connect a few larger domed areas that are located near the entrance and near the back of the cave, with the largest dome being less than 2m in height. It was in these front and back domed areas that I found colonies of *Myotis austroriparius* (southeastern myotis) in the summer. *Perimyotis subflavus* (tricolored bat) individuals were found in these areas, as well, but only during the cooler months. Just past the entrance of the cave, beyond these first few domes, there is a very small, tight crawlway that is about 1m wide, 0.5m in height, and about 3m long (see "Very Low Crawl" in Figure 1.1). Passing this small crawl is difficult and likely acts as a natural barrier to most human visitors. Four areas of the cave, including three domed areas and one crawlway, were given names by Dr. David Beckett and me and were used as points to collect atmospheric data (only temperature was used) for the study. They include the Antechamber near the entrance, the Chamber of Secrets just after the very low crawl, a crawlway approximately halfway to the back called the Shelves, and the last dome called the Back of the Cave (Figure 2).

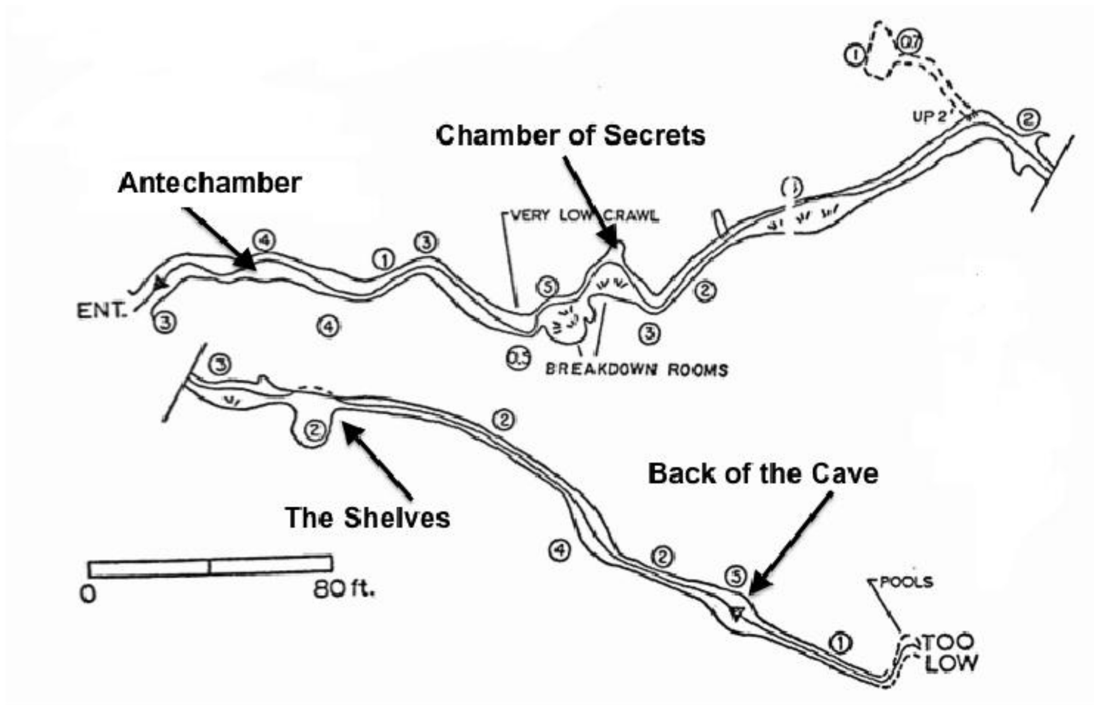

Figure 2. Map of Waddell Cave. This map shows the general layout of the cave. The names and areas of interest for this study are marked with arrows. Adapted from Knight et al. (1974).

Triple H Cave

Triple H Cave is the one of the longer caves in Mississippi with a total of ca. 177m of walkable/crawable passageway. Its mouth is located at the base of a drainage area. During downpours, the cave can largely fill with water, and I have observed post-rain debris stuck to the cave wall at a height of approximately 1.5m above the cave floor. The cave mouth is low to the ground, 2m wide and 1m high. Once inside the mouth, the cave opens up into a fairly large dome. This Front Dome, as I called it, contained a large portion of the *P. subflavus* that were found in the cave during the winter months. Beyond the Front Dome, a crawlway continues for 20m before finally becoming high enough to stand and walk (Figure 3). The floor of the cave is littered with limestone rubble and organic debris from the surrounding forest.

Just after 40m, there is a small crawlway that branches off to the left and dead ends after a few meters. I describe this point as the Fork in the Road. The linear passageway continues off to the right where, after another 10m, it meets a unique geographical feature. It was a waist high wall that was topped with a large stalagmite and a large stalactite directly overhead. This feature was given (by our laboratory) the name The Stalagmite. Prior to and immediately after The Stalagmite is a small pool of water no more than a few centimeters deep.

From here the passageway continues for another 30m, finally opening up into another domed chamber called the Back Dome. This area is fairly large with higher ceilings and a lower floor. There are three passages that radiate from the Back Dome. The first is the lowest part of the floor where the water flows past the Back Dome and very quickly drops into an area too small to explore. The second is a crawlway that continues off the left of the Back Dome and dead ends after a few meters. The third is to the right of the Back Dome. It is a smaller, very tight crawlway resembling a gopher tortoise burrow. This crawlway curves upwards and bifurcates with one passage quickly ending in a wall with the other passageway extending to the east through “Keyholes” (Figure 3).

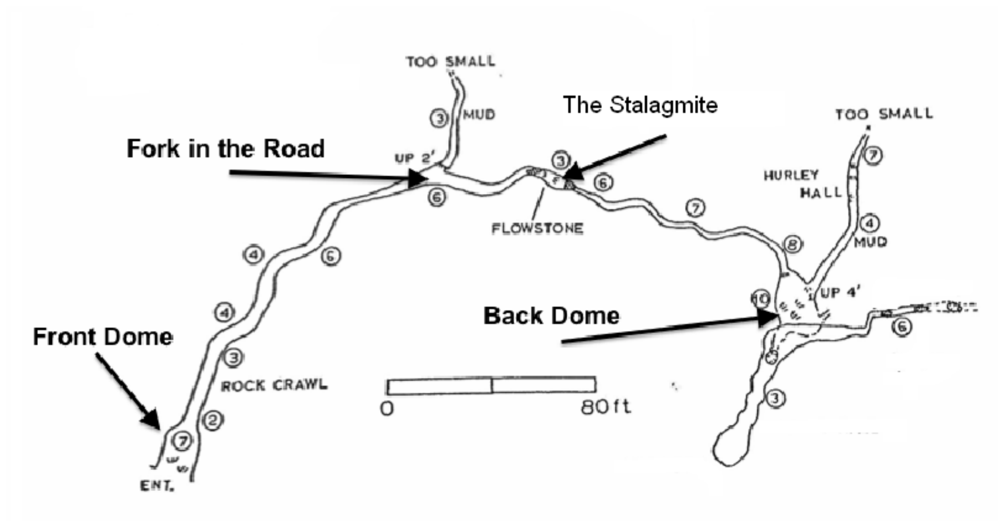

Figure 3. Map of Triple H Cave. This map shows the general layout of the cave. The names and areas of interest for this study are marked with arrows. Adapted from Knight et al. (1974).

Pitts Cave

Pitts Cave is the largest cave in Mississippi and thus the largest cave in my study with over 420m of passageway. Its entrances are located at the bottom of a small drainage area, though other parts of this area are much lower than the cave mouths. It has two large openings, one on the north side of the cave system and another on the southern side (Figure 1.3). The northern entrance is 2m wide by 2m high and is very spacious with a flat ceiling. After several meters, this passageway funnels down to a tight but walkable passage. After another few meters, this passageway drops down, and the ceiling raises up over a large chamber named the Rain Room, so named because the constant dripping of water falling from the ceiling into the cave stream after outside rains resembles the sound of rain. This upper portion of the room is called Above the Rain Room, and the lower portion of this chamber is called Bottom of the Rain Room.

To reach the rest of this cave, it is easier to go through the southern entrance. This entrance is 2 to 3m wide by 2m high and is also very spacious with a flat ceiling. After a few meters the cave opens up into a very large domed area with a 6m ceiling. There are three passages that diverge from this area. The first is the path of the natural course of water called The Chute (our laboratory's description). It is a low crawl about 1m by 1m and continues on until it forms a T-intersection with the back passage of the cave (the End of the Chute). The second passageway goes up to the right shortly after entering the cave via the south entrance. An area along the second passageway is called The Peanut Butter Room (see Moore 2006) because of the deep thick mud floor that has the consistency of peanut butter. This passageway is very difficult to move through and was not used in my study; however, it has been mapped in the past.

The third passageway is to the left and goes up to a large open passageway that contained many small stalactites. On the right hand side of this passageway there is a large hole in the floor that is over 9 feet deep, earning it the name The Bottomless Hole/Pit. This open passageway continues on for a distance until it forms a T-intersection. The passageway to the left comes to a dead end after a few meters. The passageway to the right slopes down through a tight area where explorers might find it difficult to squeeze through before it joins the Bottom of the Rain Room. From this point, the passageway has a stream of water that flows year round with occasional debris on the walls showing that floodwaters can reach 1.5m-2m above the cave floor.

From the Bottom of the Rain Room, the passageway follows the water through a 0.5m wide by 4 to 5m high tunnel-like ravine area for ca. 10m or so. A low crawlway tunnel branches off to the right; this is the End of the Chute (the tunnel that goes straight up to the cave's southern entrance). Continuing past the End of the Chute, the passageway becomes smaller but still large enough to walk through. After 10m, it curves to the right and enters a long area called The Black Top due to the dark ceiling stains that run the length of this area, (ea. 10m). The passageway following the Black Top is a long, low, and wet crawl; therefore, throughout most of my studies I would stop checking for bats at the end of the Black Top.

Over the last several months of my study I pushed forward past the end of the Black Top; the passageway at this point becomes very small indeed (see "low, narrow and wet" in Figure 4). The floor drops so that the water level is 0.5m deep, and the overall height and width of the passageway is <1m. At times it is necessary to lie down in the water and crawl on hands and knees in order to pass. This continues for 10 to 15m, and then the passageway begins to open up into a series of smaller domed areas that are 2 to 3m in height with soda straw stalactites overhead. After a few domes, a dry passageway branches off to the right, and the water passageway continues to the left. This area is called the Roost (in my description), simply because of the large number of bats we later found in this area. The right dry passageway continues into the Peanut Butter Rooms and is still difficult to enter. The left passageway continues for 10 to 15m and gets smaller in height and width. This low area also has dark ceiling stains running its length and is home to a colony of thousands of *M. austroriparius* during the warmer months (Figure 4).

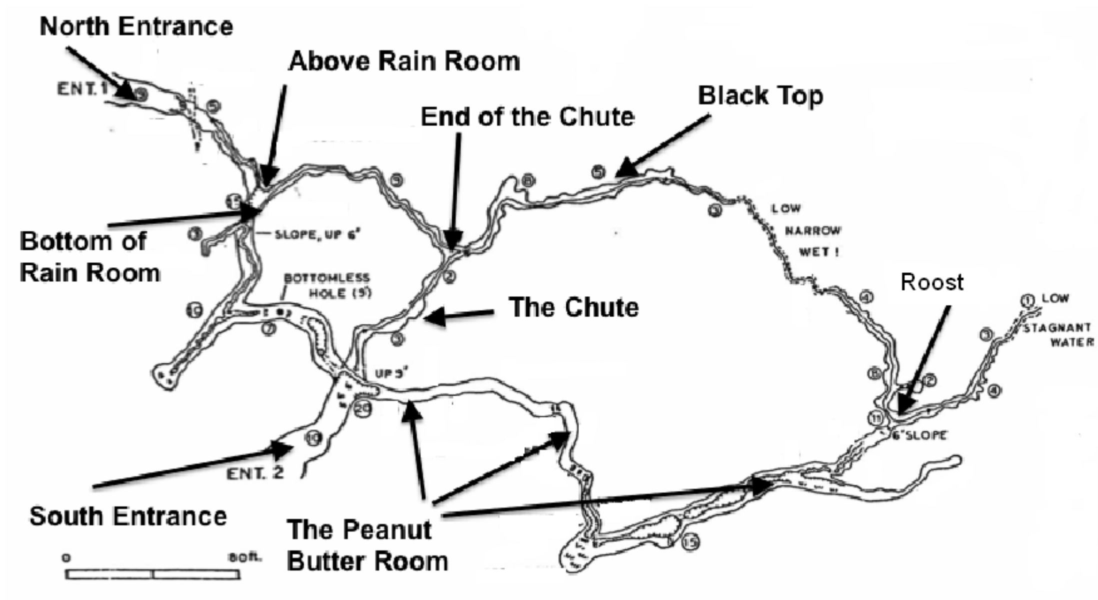

Figure 4. Map of Pitts Cave. The names and areas of interest for this study are marked with arrows. Adapted from Knight et al. (1974).

Vandalism

It is worth mentioning that all three caves show some type of vandalism. This is worse in Waddell and Pitts caves, though Triple H Cave does have some writing in the mud on its walls. Waddell cave has spray-painted signatures and arrows used for directions on its walls. Also, the area where water first enters Waddell caves is literally a trash dump. During times of high rain, trash such as beer cans and plastic cups are washed into the cave and can be found scattered throughout its length.

Pitts Cave has seen the worst of the vandalism over its years. Its location is very well known to local citizens, and it sits on land that is both rural and easy to reach. During its earlier days, the owner had wooden walkways and electricity wired through portions of the cave and visitors could take a tour for only a small fee. Though the tours have stopped, many of the boards, though degraded to some degree, remain. At both of the entrances to the cave, campfires have been burned, and graffiti from scratching one's name and the date covers the walls. Beer cans are commonly found in the entrance and within the cave. Clothing, shoes, and boots can be found discarded in these areas, as well. Worse still, vandals have removed all of the larger stalactites from the passable portions of the entire cave, presumably for souvenirs (Figure 5).

Figure 5. Vandalism in Pitts Cave. This picture shows trash and graffiti that are common to the entrance and tunnels of Mississippi's largest cave, Pitts Cave. The beer can in the picture is sitting exactly as it was found: upright on a limestone outcropping.

Bats in the Caves of Southern Mississippi

Biological surveys and research of southern Mississippi caves started in the early 1970's with Steven D. Carey's (a USM graduate student) thesis work in which he investigated cave populations of three-lined salamanders in Mississippi caves (Carey 1982). Around the same time, Arthur L. Middleton Jr., another USM graduate student, began his thesis work surveying the general fauna of caves in Mississippi, including the phyla Annelida, Mollusca, Arthropoda, and Chordata (Middleton 1976). Middleton (1976) observed tricolored bats (then called eastern pipestrelles) in six caves in Mississippi, including the three caves I studied. In the late 1990's and early 2000's, Dr. David Beckett and Austin Trousdale III conducted further surveys of Mississippi caves with a focus on bats. They surveyed a total of 11 caves in south-central Mississippi. The most common (and only) bat species found during their surveys were *M. austroriparius* (found in four of the 11 caves) and *P. subflavus* (found in five of the 11 caves). The surveys of Beckett and Trousdale (2000) also showed that Pitts, Triple H, and Waddell Caves contained both *P. subflavus* and *M. austroriparius*. Also, they identified all three caves as active or possible maternity sites for *M. austroriparius.*

Description of Perimyotis subflavus (Tricolored Bat)

The tricolored bat (*Perimyotis subflavus*), formally known as the eastern pipestrelle (*Pipestrellus subflavus*), is a small insectivorous bat weighing 4 to 8g with a forearm length of 31 to 35mm. Its fur is tri-colored: black at the base, light brown in the middle, and darker at the tips, thus the name tricolored bats (Figure 6).

This species inhabits eastern North America from southern Canada south to Honduras in Central America. Generally a solitary species, *P. subflavus* roosts mostly in caves and mines during the winter but will also inhabit buildings, tree cavities, and rock crevices.

Emerging at night earlier than most other bat species, *P. subflavus* generally has two daily foraging bouts: one starting immediately after sunset with another near midnight where they fly in an erratic and fluttery pattern around forest edges, ponds, and waterways. Their diets include smaller insects such as leafhoppers, flies, flying ants, small moths, and ground beetles. Catching up to one insect every two seconds, an individual can increase its body mass by 25% in just 30 minutes (Gould 1955). Foraging continues throughout the summer and the fall. Copulation begins in the autumn before hibernation and sometimes again in the spring. Sperm is stored in the females over the winter months. Fertilization occurs with ovulation in late April and early May when these bats become more active (Nowak 1991).

The sexes live separately during the summer months. Males live solitarily in buildings and tree cavities. Females form small maternity colonies of less than 35 individuals in buildings and tree cavities (Harvey et al. 1999; Fujita and Kunz 1984). After a 44 day gestation period, twin pups are born blind and hairless in late May to mid-July, and are flight capable in three weeks (Nowak 1991). The young are weaned after the fourth week (Whitaker 1998). In the wild, they live four to eight years with the highest mortality occurring during the first two hibernations, usually from failure to store enough fat reserves for winter. The known record for oldest *P. subflavus* is a 14.8 year-old male (Nowak 1991; Wally and Jarvis 1972; Whitaker and Hamilton 1998).

P. subflavus is one of the first bats to enter hibernation and one of the last to emerge, sometimes spending 6 to 9 months in hibernation (Davis 1964). It generally roosts singly on cave ceilings or walls where there are stable conditions, minimal airflow, and an ambient temperature of 6.8 to 18 °C, depending on the latitude, with cooler temperatures observed in more northern latitudes and warmer temperatures in more southern latitudes (Davis 1964; McNab 1974; Rice 1957; Swanson and Evans 1936). This allows the bats to arouse infrequently. *P. subflavus* is loyal to its hibernation site, often returning to the same cave throughout its life (Damm and Geluso 2008; Fujita and Kunz 1984; Sandel et al. 2001). This species is known to perform seasonal migrations up to 100km between its winter hibernation sites and its summer nursery sites (Vincent and Whitaker 2007).

Like most cave dwelling species, disturbance of hibernation sites is a leading cause of population declines in *P. subflavus.* The effects of forest management techniques on these bats are not known. However, bat friendly gates and better knowledge of its summer roosting and feeding requirements could help reduce this threat. With the onset of *Pseudogymnoascus destructans* (White Nose Syndrome, WNS) affecting several bat species (including tricolored bats), *P. subflavus* is being monitored more closely so that the impact of WNS on this species may be judged more accurately. Currently, the U.S. Fish and Wildlife service does not list *P. subflavus* as either threatened or endangered.

On August 11th, 2012, the Game Commission of Pennsylvania released a notice requesting actions for protection of the remaining populations of northern long-eared bats, tricolored bats, and little brown bats. Along with precipitous declines in the other two species, this notice stated that comparative pre- and post-WNS hibernacula surveys show a 98% decline in tricolored bats in Pennsylvania since 2008 (http://www.pabulletin.com/secure/data/vol42/42-32/1555.html).

Figure 6. Picture of *P. subflavus*. This picture shows the typical characteristics of *P. subfalvus*. The fur has light tips with darker bases. The nose and muzzle are pink. The forearms are light tan or pink and contrast greatly against the dark black or brown wing membrane. Photograph by Zac Roth, 2011.

Description of Myotis austroriparius (Southeastern Myotis)

The southeastern myotis is a small insectivorous bat weighing 5 to 9g with a forearm length of 35 to 42mm. Its short, wooly fur is bi-colored, dark gray on top with lighter gray to white underneath (Figure 1.6). The species also has a russet color variation. Inhabiting the southeastern United States, *M. austroriparius* is a colonial species roosting in a variety of shelters including caves, mines, bridges, culverts, and the tree hollows of hardwoods (Rice 1957).

Emerging after dark, *M. austroriparius* flies in a steady pattern around streams and ponds where it feeds. Its diet includes many freshwater invertebrates such as adult chironomids, mosquitoes, craneflies, small moths, and small beetles. Foraging continues through the summer and fall. Breeding takes place from October through December with sperm stored in the females over the winter months. Ovulation and fertilization takes place in early spring. In some southern populations, such as the ones in Florida, foraging continues throughout the winter months, and breeding occurs in early spring (Rice 1957).

In the early spring through the summer, females gather in large nursery colonies that are warm (capable of trapping the bats' body heat) and free of predators such as corn snakes, opossums, and rat snakes. In late April and early May females give birth, usually to twin pups, making them the only species of *Myotis* to give birth to more than one pup each season. The pups are born hairless with ears and eyes closed and stay behind in clusters when the mothers leave to feed. The young are weaned at an age of 5 to 6 weeks. During this time, males often cluster in bachelor colonies or roost individually (Rice 1957).

Loss of habitat is an important reason for a decline in numbers of *M. austroriparius*. As their highland roosts are lost, they are forced to lowland areas where they often roost in the low hollows of trees and are therefore vulnerable to the threat of drowning during floods (Arroyo-Cabrales and Castaneda 2008). Management practices that affect water quality and thus the abundance of aquatic insects, which is this species' major food source, are also likely to affect *M. austroriparius*. However, flooding of lowland roosts and caves, vandalism, and the smoke from campfires (made inside the entrances of caves by visitors) are a primary cause of the decline for *M. austroriparius* (Gore and Hovis 1992). Gore and Hovis (1992) argued for the enforcement of cave protection, as maternity caves urgently needed protection, especially in northern Florida.

Best et al. (1992) stated that a colony of 8,000 *M. austroriparius* was found in one of the coastal plain caves in Alabama, and Alabama had only this one known maternity roost of M. austroriparius in a cave (out of 3000 caves in the state). The cave containing this roost was highly visited by people, extremely susceptible to destruction, and *M. austroriparius* had at least once before been extirpated from this cave by vandals and careless cave explorers. This led to the listing of *M. austroriparius* as a species in need of special attention in coastal plain caves.

Due to this reduction in numbers throughout its range, the U.S. Fish and Wildlife service has listed *M. austroriparius* as a species of special concern (Texas Parks and Wildlife). Now with the looming threat of *P. destructans*, it is very important to determine the population numbers of *M. austroriparius* in our area prior to the onset of WNS. By doing so, we may accurately judge its impact if *P. destructans* is manifested in Mississippi bat populations.

Figure 7. Picture of *M. austroriparius*. This picture shows the typical characteristics of *M. austroriparius*. The muzzle is pink with a slightly darker nose. The fur is dark gray on the back and light gray on the belly. The rust color variation can be seen at the bottom. Photograph by Zac Roth, 2011.

CHAPTER II

SEASONAL AND LOCATION TEMPERATURE DIFFERENCES IN TRIPLE H, WADDELL, AND PITTS CAVE

Introduction

I tested the hypothesis that the temperatures of the three studied caves are not homogeneous throughout their passageways or from season to season. I wanted to first determine if the caves themselves had 1) differences in temperatures among the different seasons and 2) if there were differences from the fronts of the caves to the backs of the caves within each season. This would help provide an explanation if I found bats in larger numbers at certain areas within the caves and suggest how thermal differences might affect their distribution throughout the seasons.

Methods

Over a 27 month period starting in October, 2010, and ending in February, 2013, I made monthly visits to the all three caves and took temperature readings using a Kestrel 3000 Pocket Weather Meter, Model # 0830, at set locations (usually at the set geographical features within each cave, see Chapter I of this thesis) as well as a few locations outside the caves.

For all of my analyses, temperatures were grouped into seasons in the following manner: March, April, and May were included in spring; June, July, and August were included in summer; September, October, and November were included in fall; and December, January, and February were considered as winter. This gave me five spring surveys, six summer surveys, seven fall surveys, and nine winter surveys over a total of 27 months. For the analysis of temperature differences by season and location, only the locations inside the caves were used. These readings were divided into the four seasons and compared to each other to determine if there were any statistical differences in temperatures among seasons. I also divided the temperature data into the set locations within each cave (see Chapter I of this thesis) to see if there were any differences among the locations within each cave. I also tested for interaction between season and location. A mixed design ANOVA with one between factor (season) and one repeated factor (location) was used to test for significance and a Tukey's LSD pairwise comparison test was used to rank the differences.

Results

Seasonal and location temperature differences in the Triple H, Waddell and Pitts Cave

During the winter and the summer seasons, it is easy to notice a change in temperature when entering the caves. As a rule of thumb, in the summer the caves feel much cooler than the outside temperature. However, in the winter, the caves feel much warmer than the outside temperature. The deeper into the cave I ventured, the more of a temperature difference I would notice from the outside conditions during the winter and the summer seasons. However, this was less noticeable during the fall and spring months as the temperatures outside and within the cave were often very close to one another.

In Triple H Cave, the mean temperature for all the seasons was 19.7 °C. Over the springs' investigations, the lowest mean temperature among the four cave locations was 18.1 °C in the Front Dome with the highest mean of 20.7 °C in the Back Dome. Over the summer's investigations, the lowest mean temperature was 20.6 °C at the Stalagmite with a high mean of 24.8 °C in the Front Dome. The lowest mean temperature determined over the observations in the fall was 19.1 °C in the Front Dome with a high mean of 20.4

°C in the Back Dome. The lowest mean temperature over the winter's investigations was 13.9 °C in the Front Dome with a high mean of 19.4 °C in the Back Dome (Figure 8). The temperature data from Triple H Cave clearly manifests the rather wide temperature variations at the beginning of the cave (the Front Dome) relative to the remainder of the cave. Mean temperatures over the four seasons at the Front Dome varied by almost 11 °C; by the next site in the cave (Fork in the Road) the variation in the mean temperatures over the four seasons had deminished to less than 3 °C. The variability in mean temperatures over the seasons was less than 2 °C at the Back of the Cave. Consequently, the front area of this cave presents a wide range of temperatures over a year while the remainder of the cave presents a stable environment, temperature-wise, of 18 to 21 °C year around.

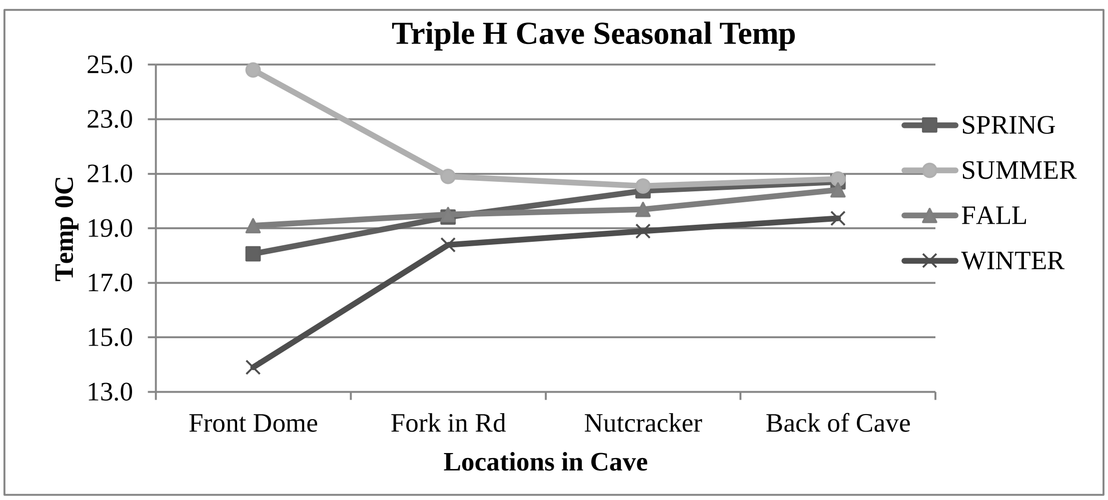

Figure 8. Seasonal Temperatures in Triple H Cave. This figure shows the mean temperature for each season in Triple H Cave. The mean temperatures for the seasons are shown for four locations inside the cave proceeding from the front of the cave to the back.

In Waddell Cave, the mean temperature for the four locations over the four seasons was 19.7 °C, the same as Triple H Cave. The lowest mean temperature for the spring observations was 18.5 °C in the Antechamber with a high mean of 19.8 °C in the Back of the Cave. The lowest mean temperature over the summer observations was 20.7 °C at the Shelves with a high mean of 23.3 °C in the Antechamber. The lowest mean temperature over the fall observations was 19.8 °C at the Shelves with a high mean of 20.7 °C in the Chamber of Secrets. The lowest mean temperature over the winter for the four locations was 16.7 °C in the Antechamber with a high mean of 18.4 °C in both the Chamber of Secrets and the Back of the Cave (see Figure 9). The temperature pattern in Waddell Cave was therefore similar to that of Triple H Cave, with the greatest variability over the year near the cave mouth, and differences of only 2 to 3 °C further back in the cave.

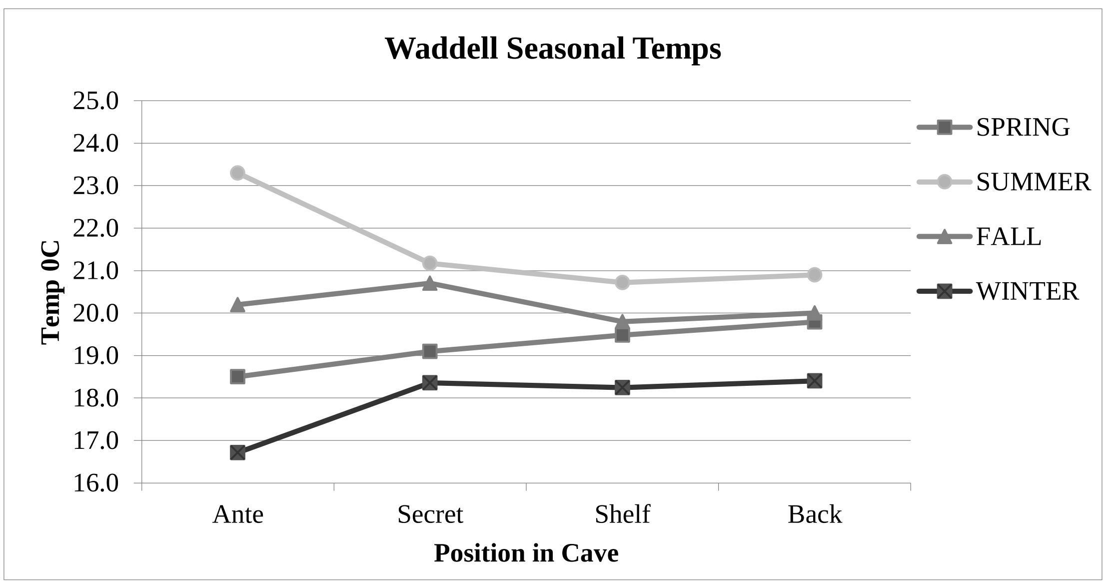

Figure 9. Seasonal Temperatures in Waddell Cave. This figure shows the mean temperature for each season in Waddell Cave. The mean temperatures for the four seasons are shown for four locations inside the cave. Ante = Antechamber; Secret = Chamber of Secrets; Shelf = Shelves; Back = Back of the Cave.

In Pitts Cave, the mean temperature for all measured locations over all the seasons was 18.3 °C. The lowest mean temperature over the spring measurements was 18.6 °C at the End of the Chute with a high mean of 20.1 °C in the Above the Rain Room. The lowest mean temperature for the summer observations was 19.9 °C at the Black Top with a high mean of 24.0 °C in the mouth of the North entrance to Pitts Cave. The lowest mean temperature for the fall observations was 17.2 °C at the mouth of the South entrance to Pitts Cave with a high mean of 19.0 °C in the Black Top. The lowest mean temperature for the winter observation was 13.4 °C in the mouth of the South entrance to Pitts Cave with a high mean of 16.2 °C in the Black Top area (Figure 10).

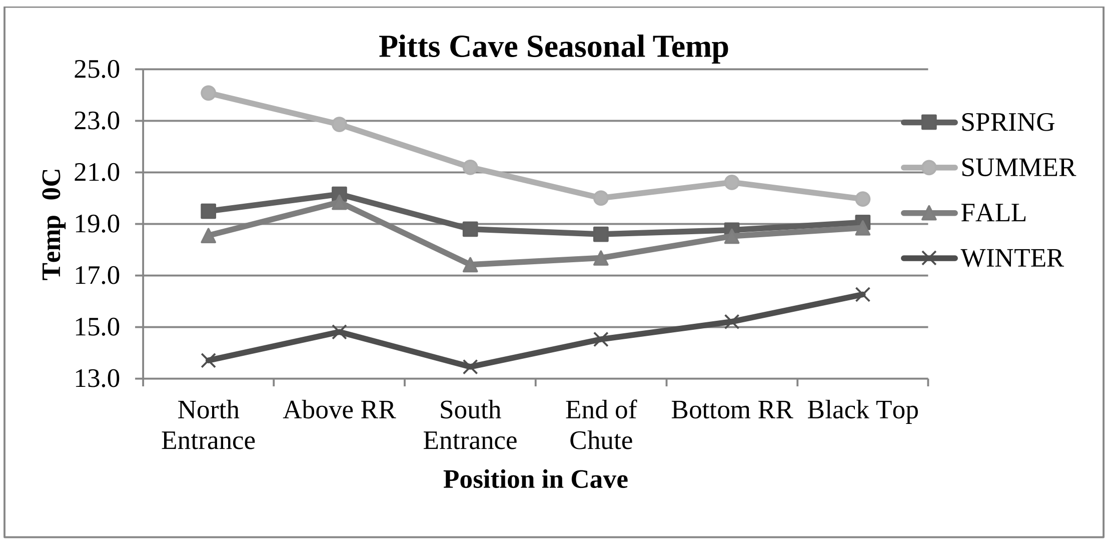

Figure 10. Seasonal Temperatures in Pitts Cave. This figure shows the mean temperature for each season in Pitts Cave. The mean temperatures for the four seasons are shown for six locations inside the cave. Above RR = Above the Rain Room; Bottom RR = Bottom of the Rain Room.

Discussion

The mixed design ANOVA with one between factor (season) and one repeated factor (location) revealed an interaction of location and season in both Triple H Cave ($F_{9, 78}$=3.449, p=0.001) and Waddell Cave ($F_{9, 45}$=2.332, p=0.03). This interaction is clearly caused by the wide variations in temperature at the front of the caves over the four seasons versus the rather uniform temperatures throughout the remainder of each cave (Figures 2.1 and 2.2). Temperatures in Triple H Cave do not vary much beyond the Fork in the Road, even across seasons. Similarly, temperatures in Waddell Cave exhibited little variation beyond the Chamber of Secrets across all seasons. These two caves are long, relatively flat, narrow tubes with only one major opening, and airflow throughout the cave is very restricted. The back of each of these caves is far enough from the major entrance that external air does not effectively circulate very far back in the caves. Thus, they have very little variation in temperature throughout the seasons. This kind of relationship would be expected in long caves that have one opening. However, this relationship was not seen in Pitts Cave. The layout of its tunnels form a (albeit, roughly) a figure-eight with a large opening at each end which allows air to flow in and out of the caves, thereby reaching the most inner parts with relative ease.

The mixed design ANOVA with one between factor (season) and one repeated factor (location) of temperatures across locations in Pitts Cave, (N=6, Mean=18.37°C, SD=0.385°C, $F_{5, 16}=13.556$, $p<0.001$) led me to reject the null hypothesis and conclude that there was a significant variation in temperature among locations. The analysis did not yield a significant interaction between location and season ($F_{15, 54}=1.569$, $p=0.114$). This provided two conclusions: 1) some areas of Pitts Cave exhibit significantly different temperatures from other areas and 2) these differences are consistent across all seasons. A pairwise comparison using Tukey's LSD showed that the South Entrance, Bottom of the Rain Room, End of the Chute, and Black Top were not significantly different from each other in regard to temperature, and it is in these areas during the winter that I found the largest number of *P. subflavus.* The South Entrance had significantly different temperatures from the North Entrance and Above the Rain Room. Temperatures in the North Entrance and Above the Rain Room were not significantly different from each other. The analysis, therefore, distinguished two somewhat dissimilar thermal regimes within the cave, the North Entrance, which is directly (and shortly) connected with the upper portions of the Rain Room, and the remainder of my temperature observations points, which are all situated at a lower level within the cave than the North Entrance and Above the Rain Room.

CHAPTER III

BATS IN SOUTHERN MISSISSIPPI CAVES: AN OVERVIEW

Only *P. subflavus* and *M. austroriparius* were found to use the three caves during this study, and they varied greatly on how each species used the caves and what season they were present. *P. subflavus* individuals were found in all three caves and were at their highest numbers during the winter months. A high of 194 individuals were found in Triple H Cave, a high of 53 individuals were present in Waddell Cave, and a high of 244 individuals were found in Pitts Cave during winter surveys. During this time, most individuals were found in torpor and were located in the cooler (entrances) parts of the caves. A few individuals were found to be awake and were generally found in the warmer (deeper) parts of the caves.

Conversely, during the summer surveys, *P. subflavus* were found infrequently in the three caves. Per single visit over the summer visits, a high of five individuals were found in Triple H Cave, zero individuals were found in Waddell Cave, and a high of four individuals were present in Pitts Cave. During the summer, these few bats used the caves as day roosts, as they were easily awakened and often were quick to fly when disturbed (Figure 11).

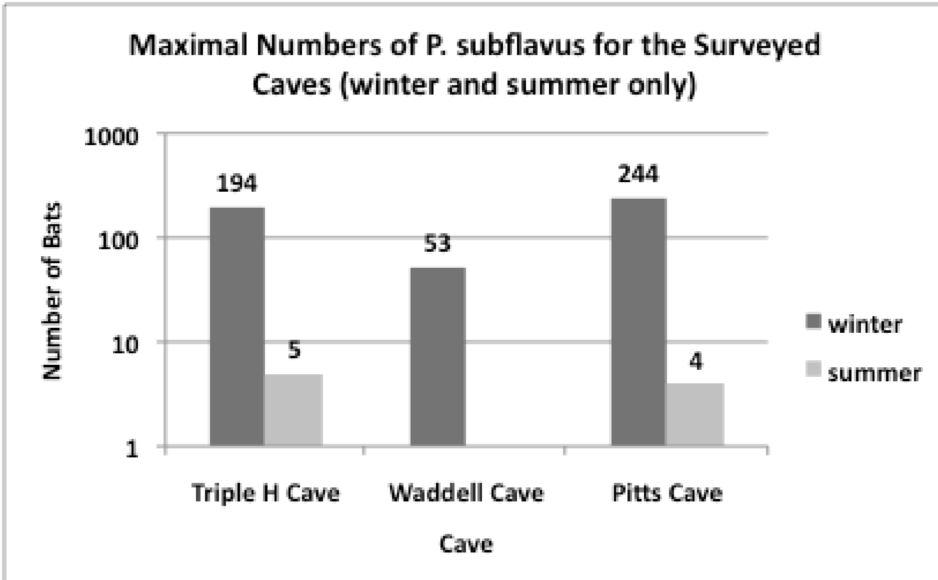

Figure 11. Max numbers of *P. subflavus*. This figure shows the maximum number of *P. subflavus* counted during the winter and summer surveys of Triple H, Waddell, and Pitts Caves. Note that the scale is logarithmic.

During this study *M. austroriparius* was found in Waddell and Pitts Cave, but by comparison this species was virtually non-existent in Triple H Cave. During the winter surveys, a high of only three individuals was found in Triple H Cave, whereas a high of 225 individuals was found in Waddell Cave, and a high of 200 individuals was found in Pitts Cave. During this time, most individuals were found in the deeper (warmer) areas of the caves, were awake, and immediately flew when approached.

However, during the summer surveys, *M. austroriparius* were found in large colonies. A high of 2,700 individuals was encountered during a visit to Waddell Cave, and a high of 10,000 was found in Pitts Cave during summer surveys (Figure 12). Only one *M. austroriparius* was found in Triple H Cave during the summer. I can confirm that Waddell Cave is used as a maternity colony by *M. austroriparius* as I observed pups present in late May. I was quite cautious not to disturb the colony as that might cause pups to fall from their mothers and become stranded in the stream that flowed beneath them. This may have resulted in a lower estimate of the number of bats present during summer month surveys, as I did not proceed past the initially observed colony when pups were present.

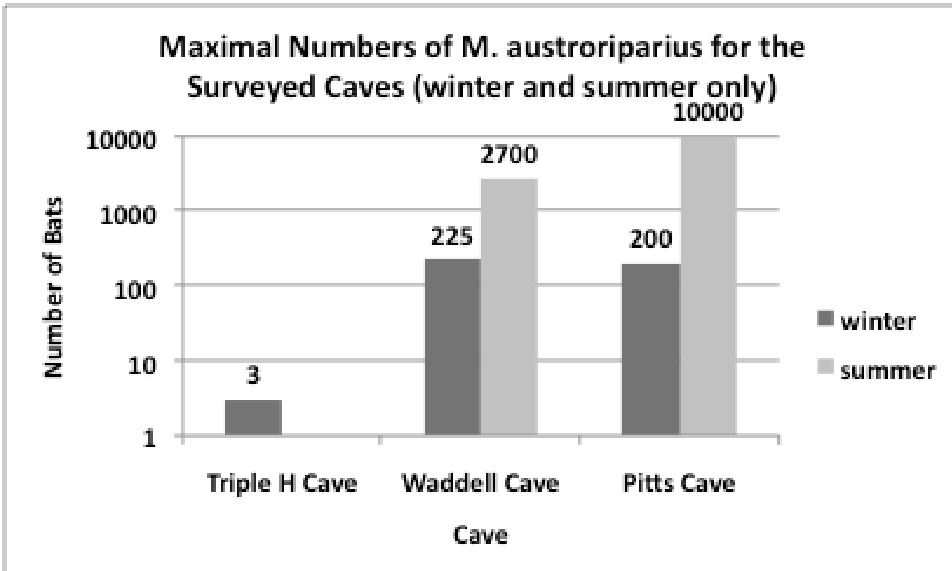

Figure 12. Max numbers of *M. austroriparius.* This figure shows the maximum number of *M. austroriparius* counted during the winter and summer surveys of Triple H, Waddell, and Pitts Caves. Note that the scale is logarithmic.

CHAPTER IV

BATS IN WADDELL CAVE

Introduction

Prior to March of 2012, Waddell Cave was thought to hold the largest known maternity colony of *M. austroriparius* in southern Mississippi caves (Beckett and Trousdale 2000). The colony was located near the entrance, which made surveying both quick and easy. However, surveying after this part of the cave was much more physically demanding than in other caves. Its low ceiling and long passageway made for a long uncomfortable and wet crawl. Despite the difficult traverses, the presence of both *P. subflavus* and *M. austroriparius* made Waddell Cave an ideal choice for studying the differences in seasonal abundance between the two species.

Methods

I surveyed the bat communities of Waddell Cave from October 2010 through February 2013. Crawling from the entrance towards the Back of the Cave, the species and numbers of bats were identified and counted. General information on the location of the bats (i.e., number of *P. subflavus* at the entrance to the cave and a total throughout the cave) was noted for this cave, but was studied in much greater detail with Triple H Cave.

For the analysis of seasonal differences in abundance between the two species, the data from both Triple H Cave and Pitts Cave were not suitable for use due to 1) the virtual non-existence of *M. austroriparius* in Triple H Cave and 2) a low sample size of usable data in Pitts Cave. Although the data from Waddell Cave did not have these constraints, the data did not meet the requirement of being normally distributed. Consequently, I used a non-parametric Kruskal-Wallis Ranked Sum Test (K-W) to compare seasonal differences in abundances between the two species in their use of Waddell Cave.

For each of the two species, count data was divided into four seasons. I then compared the seasons to each other using the Kruskal-Wallis test, assigning a rank for the number of bats present per seasonal count. The ranks for each season were then compared and checked for significance.

Results

A high of 53 individual *P. subflavus* were found in Waddell Cave during the winter, and zero were found during summer surveys. A high of 225 individual *M. austroriparius* were found in Waddell Cave during the winter, and a high of 2,700 were found in a maternity colony during summer surveys. It was obvious from my observations and from my counts that though both species use the cave, they do not use it during the same season nor for the same reasons.

Although visits to Waddell Cave were made on somewhat different dates over the three year sampling span, numbers of *P. subflavus* were always highest in the winter and were either very low or at zero in the remaining seasons each year. In 2010 and 2011, *P. subflavus* were fairly abundant in the cave during the visits from mid-December through early March, from early November through late January in 2011-2012, and from mid-December to late February in 2012-2013 (Figure 4.1). No *P. subflavus* were observed within Waddell Cave in the surveys from late May through mid-September of 2011 or 2012 (Figure 4.1). *P. subflavus* first appear in Waddell Cave in mid-September to early November and leave the cave by April.

Conversly, numbers of *M. austroriparius* exceeded 1,000 in each visit to the cave from March 31, 2011, to October 27, 2011, a span of seven months. A similar pattern was evident in 2012 when numbers of this species exceeded 1,000 from April 19 through September 13 (Figure 4.1). It was also evident that the use of the cave by *M. austroriparius* was minimal in the cold weather months of December, January, and February.

Observations of their behavior confirmed that *P. subflavus* used Waddell Cave as a hibernacula. Conversly, observation also confirmed that *M. austroriparius* used Waddell Cave as a maternity colony as pups were observed in Waddell Cave in May, 2011. The count data revealed a pattern of cave usage for both bat species. *Perimyotis subflavus* was much more abundant in Waddell Cave during the winter, and *M. austroriparius* was much more common in the Cave during the summer (Figure 13).

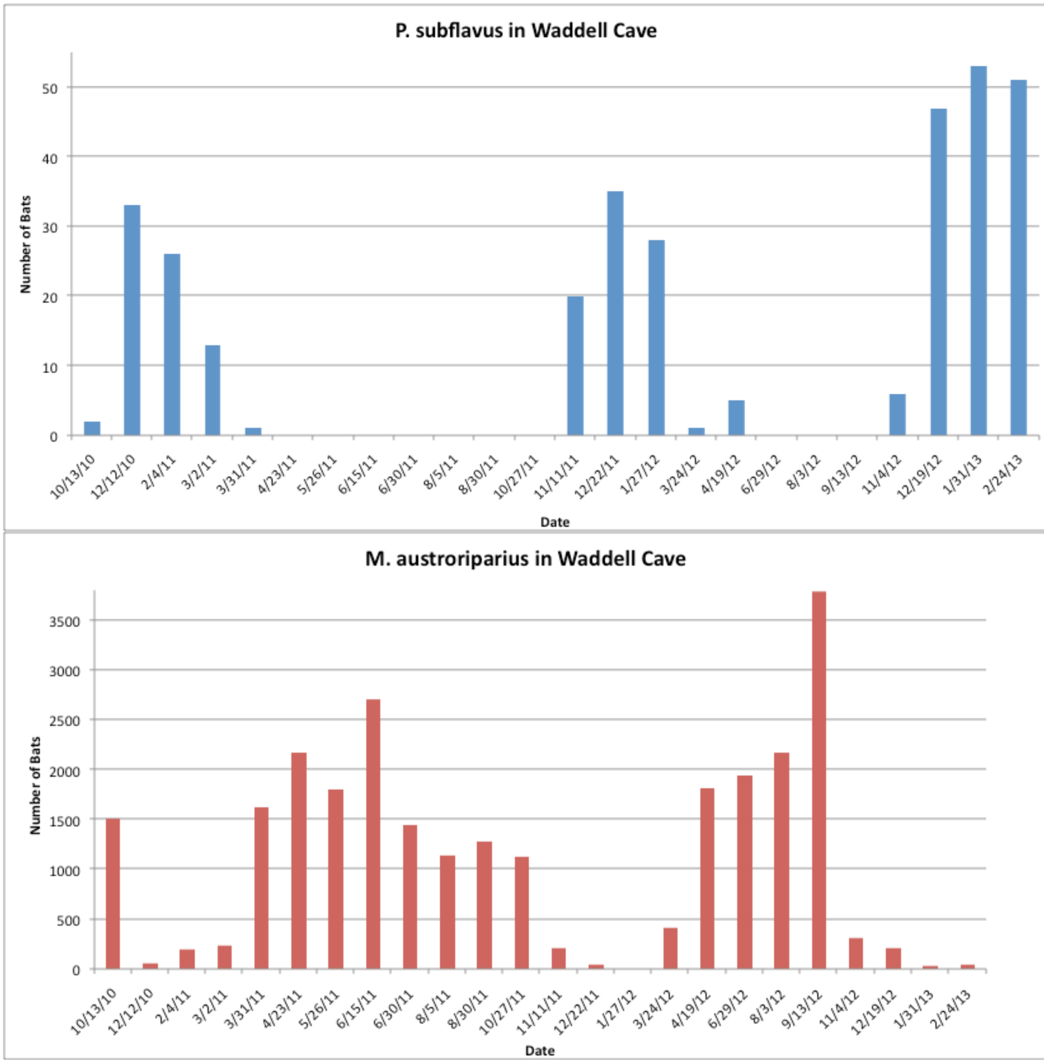

Figure 13. Numbers of *P. subflavus* and *M. austroriparius* in Waddell Cave. These figures show the number of *P. subflavus* and *M. austroriparius* surveyed in Waddell Cave. Note the difference in scale between the two graphs. *P. subflavus* is more abundant during the winter, and *M. austroriparius* is more abundant during the summer.

My observations were confirmed when a Kruskal-Wallis test for numbers of *P. subflavus* per season in Waddell Cave showed statistically significant differences among some of the seasons (χ^2=17.856, DF=3, p<0.001) with a mean rank of 21.00 for winter, 10.67 for spring, 5.50 for summer, and 11.20 for fall. Winter counts were significantly higher than all other seasons. Fall and spring counts were not significantly different from each other but were significantly higher than summer counts.

A Kruskal-Wallis test for numbers of *M. austroriparius* per season in Waddell Cave showed statistically significant differences among some of the seasons (χ^2=14.895, DF=3, p=0.002) with a mean rank of 4.07 for winter, 15.83 for spring, 17.83 for summer, and 13.90 for fall. Winter counts were significantly lower than all other seasons whereas fall, spring, and summer counts were not significantly different from one another.

Discussion

From this analysis it is apparent that *P. subflavus* and *M. austroriparius* use Waddell Cave differently from one another. *P. subflavus* uses the cave during the cooler months as a hibernaculum while *M. austroriparius* uses the cave during the warmer months as a maternity roost. This behavior of *P. subflavus* as a winter hibernator is consistent with other studies for this species in multiple sites throughout its range: Florida (McNab 1974), Indiana (Vincent and Whitaker 2007), Tennessee (Rabinowitz 1981), and Nebraska (Damn and Geluso 2008) just to name a few.

My findings are also consistent with Rice's (1957) observations on the winter habitats of *M. austroriparius* in peninsular Florida. Rice found that *M. austroriparius* left the cave during the winter and roosted in small colonies in non-cave locations. However, his observations of *M. austroriparius* in western (panhandle) Florida and observations from the upper Mississippi valley (Illinois and Indiana) showed that this species stays in caves for prolonged periods of time over the winter months in these areas. In Illinois *M. austroriparius* was found hibernating in caves for seven months spanning from September to March and were found hibernating in the caves of western Florida for four or five months spanning from late October to early March (Rice 1957). Rice (1957) explained that the difference between the western Florida and peninsular Florida populations of *M. austroriparius* was due to climatic differences. According to Rice (1957), the continental climate of western Florida provided habitat conditions that were similar to the conditions in the temperate zone of the upper Mississippi valley. The subtropical climate of peninsular Florida provided warmer temperatures and thus a nearly yearlong supply of food. Thus, the bats there were not dependent on torpor to survive.

In southern Mississippi, Waddell, Triple H, and Pitts Caves are all at more northern latitudes than the caves studied by Rice (1957) and are more influenced by the continental climate than the caves in the panhandle Florida. Yet, the results from my study show that the winter and summer habitat selections of the populations of *M. austroriparius* in southern Mississippi are comparable to that of the subtropical populations in peninsular Florida rather than the geographically closer temperate zone populations of western Florida. A study by Best et al. (1992) in Sanders Cave in the coastal plains of Alabama (again a slightly more northern latitude than western Florida) also showed a pattern of winter and summer cave usage by *M. austroriparius* that was comparable to the peninsular Florida populations and not to the western Florida populations, despite the geographical closeness of coastal Alabama and the panhandle of Florida. Perhaps there are other conditions and variables influencing the western Florida population of *M. austroriparius* that cause them to behave differently from the southern Mississippi, coastal plain of Alabama, and peninsular Florida populations.

CHAPTER V

BATS IN PITTS CAVE

Introduction

Before March of 2012, I did not observe large numbers of *M. austroriparius* in the areas of Pitts Cave that I surveyed. This was always puzzling as some areas of the cave's ceiling were covered in stains, indicating usage by this species. Also, fresh guano on the floor and outcroppings, the distant sound of flapping wings, and the smell of boiled peanuts (this is what the smell of a large colony of *M. austroriparius* reminds me of) were evident in The Black Top area of Pitts Cave. In addition, Beckett and Trousdale (2002) reported dense clusters of *M. austroriparius* on two visits to Pitts Cave in July 2000, and interpreted the cluster as evidence of a maternity colony within the cave. They also collected juvenile *M. austroriparius* at the entrance to the cave. Yet, before April of 2012, no *M. austroriparius* were found in Pitts Cave during the summer months of my survey.

Methods

Beginning with North Pitts, the total number of bats was carefully counted throughout most of Pitts Cave. The cave's high ceilings with many folds and crevices, combined with 420m of passageway made surveying go more slowly than in the other two caves. Exiting out of the North Pitts entrance area and circling around outside to the South Pitts entrance, surveys continued throughout the remainder of Pitts Cave with the exception of the Peanut Butter Rooms (See Chapter I of this thesis). During the surveys, I made observations on the behavior of the bats and kept very generalized notes on the number of bats that were found in specific areas (i.e., number of bats in the entrances, number of bats between the Rain Room and the End of the Chute, etc.). Surveys beyond the Black Top area and into the Roost area did not begin until March of 2012. No statistical tests were run on this data set due to a low sample size of usable data.

Results

The numbers of *P. subflavus* were greatest in Pitts Cave in the winter months with a high of 244 individuals found on Feb. 22, 2011 (Figure 5.1). Only a few individual *P. subflavus* were found in the cave during the summer months. The abundance of *M. austroriparius* was the greatest during the summer months with a high of approximately 10,000 bats found in the Roost area. During the winter a high of 200 individual *M. austroriparius* were found in Pitts Cave (on Dec. 20, 2012) (Figure 5.1).

In all three years of the surveys, numbers of *P. subflavus* first reached appreciable numbers (>50 bats) in Pitts Cave in late October to early November, and remained at abundances greater than 150 bats over December, January, and February (Figure 5.1). By late March to early April, the number of *P. subflavus* in the cave had appreciably decreased. The number of *P. subflavus* in Pitts Cave was always very low from late April to early October (Figure 5.1).

In March of 2012, after venturing into a portion of Pitts Cave that is very difficult to reach, a large colony of an estimated 8,000 to 10,000 *M. austroriparius* was found roosting. The characterization of the use of Pitts Cave by *M. austroriparius* was obviously influenced by this discovery of the large colony in the previously unsurveyed portion of the cave (Figure 14). Numbers of individuals were difficult to estimate precisely due to the dense packing of large numbers of bats into small areas. However, approximately 8,000 to 10,000 bats were consistently present on each of the six survey dates from March 28, 2012, to September 15, 2012. A few thousand *M. austroriparius* were still present in October 27, 2012 (Figure 14). Given the dense packing of *M. austroriparius* in Pitts Cave, the high numbers of bats present, and the use of the cave in the warm weather months, it is apparent that this cave is used as a maternity site by this species.

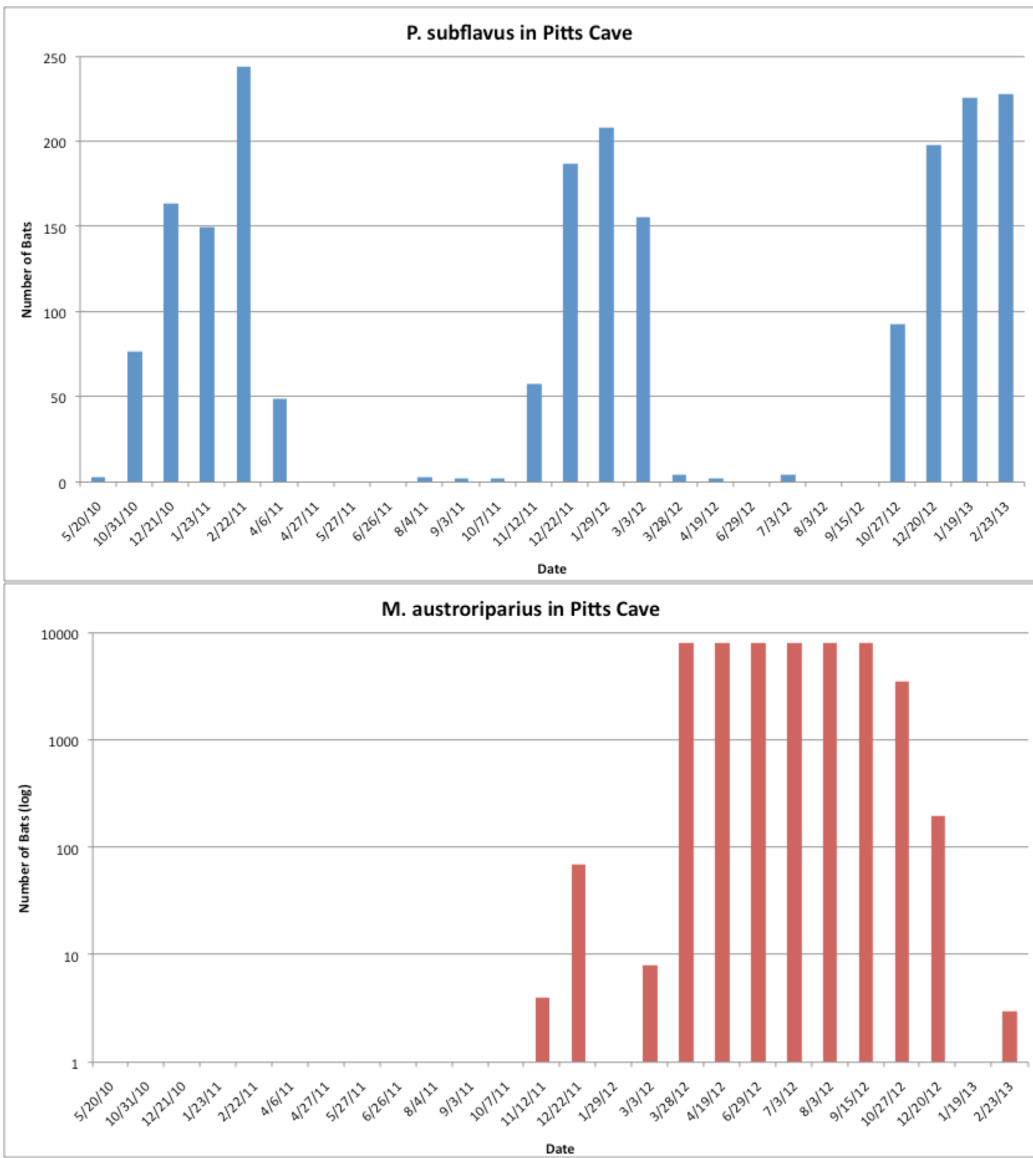

Figure 14. Numbers of *P. subflavus* and *M. austroriparius* in Pitts Cave. This figure shows the number of *P. subflavus* and *M. austroriparius* found in Pitts Cave during surveys. Notice that the number of *M. austroriparius* is shown on a logarithmic scale. Numbers include surveys from the North and South portions of Pitts Cave.

Discussion

Although Beckett and Trousdale (2000) observed a thousand to a few thousand *M. austroriparius* in Pitts Cave in July 2000, the discovery of the colony of 8,000 to 10,000 *M. austroriparius* in my study, and their consistent presence throughout spring, summer and fall of 2012 (Figure 5.1), revealed the importance of Pitts cave for this species. Before my study, the largest colony of *M. austroriparius* in a Mississippi cave or at any location in Mississippi was thought to be Waddell Cave. This discovery is important, as Pitts Cave is not only the largest cave in Mississippi, it is now known to hold what is probably the largest colony of *M. austroriparius* in Mississippi.

Best et al. (1992) stated that a maternity colony of *M. austroriparius* (consisting of an estimated 8,000 individuals) was found in only one cave (Sanders Cave) in Alabama, although there are 3,000 caves throughout the state. According to Best et al. (1992), a report from 1986 states that *M. austroriparius* temporarily abandoned its use of Sanders Cave as a maternity site due to vandals and careless cave explorers and shows how sensitive these colonies are to disturbance. Although the colony of *M. austroriparius* had returned by 1992, Sanders Cave is still highly visited by people and remains extremely susceptible to destruction. Both Pitts and Waddell Caves share these features as they are frequently visited by people and show signs of vandalism. Given that *M. austroriparius* is currently listed as a species of special concern (United States Fish and Wildlife Service), this makes Pitts Cave and Waddell Cave very important caves and makes them prime candidates for management practices such as gating.

Bats, especially *M. austroriparius,* are often loyal to their roost (Humphrey 1975). Their loyalty is even stronger to those caves, hollow trees, and manmade structures that are used as maternity sites because very few are known to have the conditions (i.e., flowing water, high humidity, large flat ceilings) that make them suitable for maternity sites. Thus, maternity sites are a limiting factor for *M. austroriparius* (Rice 1957; Tuttle 1975). I would suggest Waddell and Pitts Caves would therefore make excellent sites to study year-to-year cave loyalty in *M. austroriparius*. Also, as very few cave maternity sites are known in southern Mississippi, locating and protecting such sites is crucial for the continued existence of the species in this portion of their range.

CHAPTER VI

BATS IN TRIPLE H CAVE

Introduction

I wanted to determine whether the two bat species, *P. subflavus* and *M. austroriparius*, were found evenly throughout the cave and whether there were changes in their abundance throughout the different seasons. Past studies have shown that bats might be seeking out specific temperature profiles in caves (Briggler and Prather 2003; Rabinowitz 1981; Rice 1957). If the temperatures are not homogenous throughout the cave, then the bats might not be evenly distributed throughout the cave and might also change according to the seasons.

The literature states that *M. austroriparius* uses caves in the summer as sites for their maternity colonies. Conversely, *P. subflavus* use caves in the winter, hibernating in the deepest part of caves where temperatures are most stable. This feature of stable temperatures is thought to be the driving force for these two species in their selection of locations within caves (Briggler and Prather 2003; Damm and Geluso 2008; McNab 1974; Rabinowitz 1981; Rice 1957; Schmidly 1991; Vincent and Whitaker 2007). Preliminary surveys of Beckett and Trousdale (2000) showed that *M. austroriparius* and *P. subflavus* use caves in southern Mississippi, but their pattern of distribution either statistically (from front to back of the caves) or seasonally has not been studied.

When observing *P. subflavus* in Triple H Cave, I wanted to determine: 1) how the abundance of *P. subflavus* differs among the seasons; 2) if there is a spatial pattern of distribution within the cave for *P. subflavus*; 3) if such a pattern exists, does it differ among seasons; 4) why *P. subflavus* might be demonstrating such a pattern; 5) does it exhibit a preferred temperature especially for hibernation; and if so 6) compare this temperature to other areas and caves within in the range of this species. Triple H Cave was chosen for this portion of the study as observations by Beckett and Trousdale (2000) indicated its possible usage as a hibernaculum by wintering *P. subflavus*. Also, ease of accessibility to the *P. subflavus* which hang on the walls and ceilings of the tunnel-like Triple H Cave, with few side branches, made surveying relatively easy.

Methods

Over a 27 month period starting in Oct. 2010 and ending in February 2013, I made monthly visits to Triple H Cave and counted the number of both *P. subflavus* and *M. austroriparius* occupying the cave. The date, number and species of the bats, and their behavior (i.e., use of the caves as a day roost, hibernaculum or maternity roost) were recorded for any bats that were found.

To answer the questions regarding the distribution of *P. subflavus* within Triple H Cave, I marked off the length of the cave in two (2) meter intervals with a meter tape, starting immediately behind the Front Dome and running along the length of the cave to the beginning of the Back Dome. This gave me a total linear cave length of 86 meters. Thin metal tent spikes were then pushed into the softer parts of the cave wall at every even numbered meter. Masking tape was then attached to the spike and a meter number was written in permanent marker on the tape. One (1) meter intervals were then estimated based off the two (2) meter location markers. The number of *P. subflavus* in the Front and Back Domes and the number of bats per meter of cave were recorded during every cave survey.

For my analysis of number of *P. subflavus* per season in Triple H Cave, abundances of bats for each month were grouped into seasons in the following manner: March, April, and May were included in spring; June, July, and August were included in summer; September, October, and November were included in fall and December, January and February were considered as winter. This gave me five spring surveys, six summer surveys, seven fall surveys and nine winter surveys over a total of 27 months.

For the analysis of numbers and locations of *P. subflavus* in Triple H, I grouped all of the bats in the cave into 10-meter intervals, giving me 11 separate areas including the Front Dome and Back Dome. This was done for each season. For the analysis of seasonal differences in abundance between the two species, the virtually non-existent numbers of *M. austroriparius* in Triple H Cave made this test impossible to run. However, Waddell Cave did not have this constraint and is discussed in Chapter IV of this thesis.

Results

During my study of Triple H Cave, a mean number of 29.8 *P. subflavus* were counted in the spring surveys with a low of one and high of 103 individuals per visit. A mean of 3.3 were counted in the summer surveys with a low of two and high of five individuals per survey. The fall surveys produced a mean of 45.8 bats with a low of zero and a high of 124 individuals, whereas the winter surveys yielded a mean of 153.6 with a low of 106 and a high of 194 individuals.

During the winter, the Front Dome always contained a large gathering of *P. subflavus*. On average this area contained 65.8 individuals for the days surveyed in the winter, which made up roughly 43% of the bats in the cave on those days. The lowest number of *P. subflavus* recorded at this location in the winter was 50, and the high was 91, which made up 36% and 49% of the *P. subflavus* in the cave, respectively on those survey dates. On average, the Front Dome, combined with meters zero to 49, contained 89.5% of the *P. subflavus* in the winter on the days surveyed. The Back Dome contained a mean of 5.3 *P. subflavus* with a low of one and a high of 13 individuals on winter days.

In sharp contrast, the summer months contained almost no *P. subflavus* in the Front Dome on surveyed days. Only one *P. subflavus* was found in that location over all the summer surveys. A mean of two *P. subflavus* were found between meters 20 and 60 in the summer. The Back Dome contained a mean of 1.5 *P. subflavus* found during the summer with a low of zero and a high of three *P. subflavus* (Figure 6.1).

In the winter months I would sometimes find 2 to 3 *M. austroriparius* around meter 56 just past the Stalagmite, beyond a small pool of still water. As indicated by a dark ceiling stain on the limestone rim, they seemed to have a preference for a small hole in the wall that allowed them to climb up into a hollow area. They were generally in the same spot each time they were observed and were easily detectable from their loud calls. Other than this, *M. austroriparius* were not found in Triple H Cave during the summer.

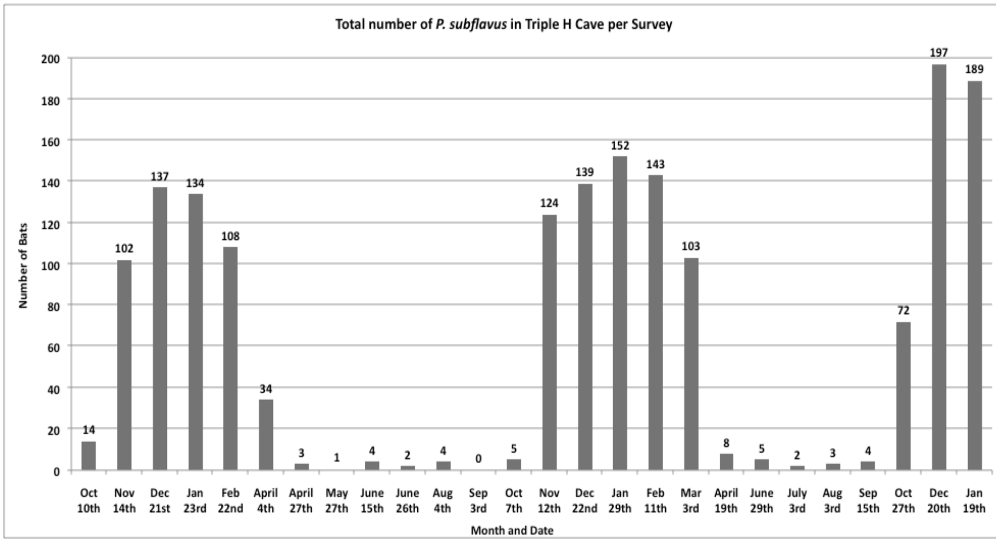

Figure 15. Number of *P. subflavus* in Triple H Cave per survey. This figure shows the total number of *P. subflavus* found in Triple H Cave per survey from October, 2010 to February, 2013. Bats were more abundant in the late fall and winter and were nearly absent during the spring and summer.

Analysis of Numbers of P. subflavus by Season in Triple H Cave

I analyzed the numbers of *P. subflavus* in Triple H cave across seasons using a mixed design ANOVA with one between factor (season) and one repeated factor (location). There was no interaction between location and season ($F_{30, 48}$ = 1.397, p=0.148). The result for season ($F_{3, 23}$=25.168, p<0.001) led me to reject the null hypothesis and conclude that the number of *P. subflavus* varied among the seasons. A post-hoc Tukey's LSD pairwise comparison revealed that winter (mean rank of 13.97) was significantly higher than all the other seasons. Fall (mean rank of 4.17) was not significantly different from spring (mean rank of 2.71), but was significantly higher than summer (mean rank of 0.3). Spring was not significantly higher than summer, and summer was significantly lower than the winter and fall (Figure 16).

Location of P. subflavus per Season in Triple H Cave

The results of the same mixed design ANOVA for locations of *P. subflavus* in Triple H Cave by season ($F_{10, 14}$=13.332, p<0.001) led me to reject the null hypothesis and conclude that there is significant difference (or differences) among the locations. I therefore concluded that 1) some difference (or differences) exists among locations in Triple H Cave in terms of numbers of *P. subflavus,* and 2) these differences were similar for each season (except summer, which always had low numbers of *P. subflavus*).

In terms of mean number of *P. subflavus* over all seasons, Tukey's LSD pairwise comparisons showed that the Front Dome numbers of *P. subflavus* were significantly higher than at the rest of the locations (Figure 17). Numbers of bats in intervals 0m, 20m, 30m, 40m were not significantly different from each other and were significantly higher than the Back Dome which is significantly higher than bat numbers in intervals 10m, 50m, 60m, and 70m. Meters 10, 50, 60, and 70 were not significantly different from each other. *P. subflavus* numbers in interval 80m were significantly lower than all other locations. Therefore, numbers in the FD > 0m, 20m, 30m, 40m > BD > 10m, 50m, 60m, 70m > 80m (Figure 17).

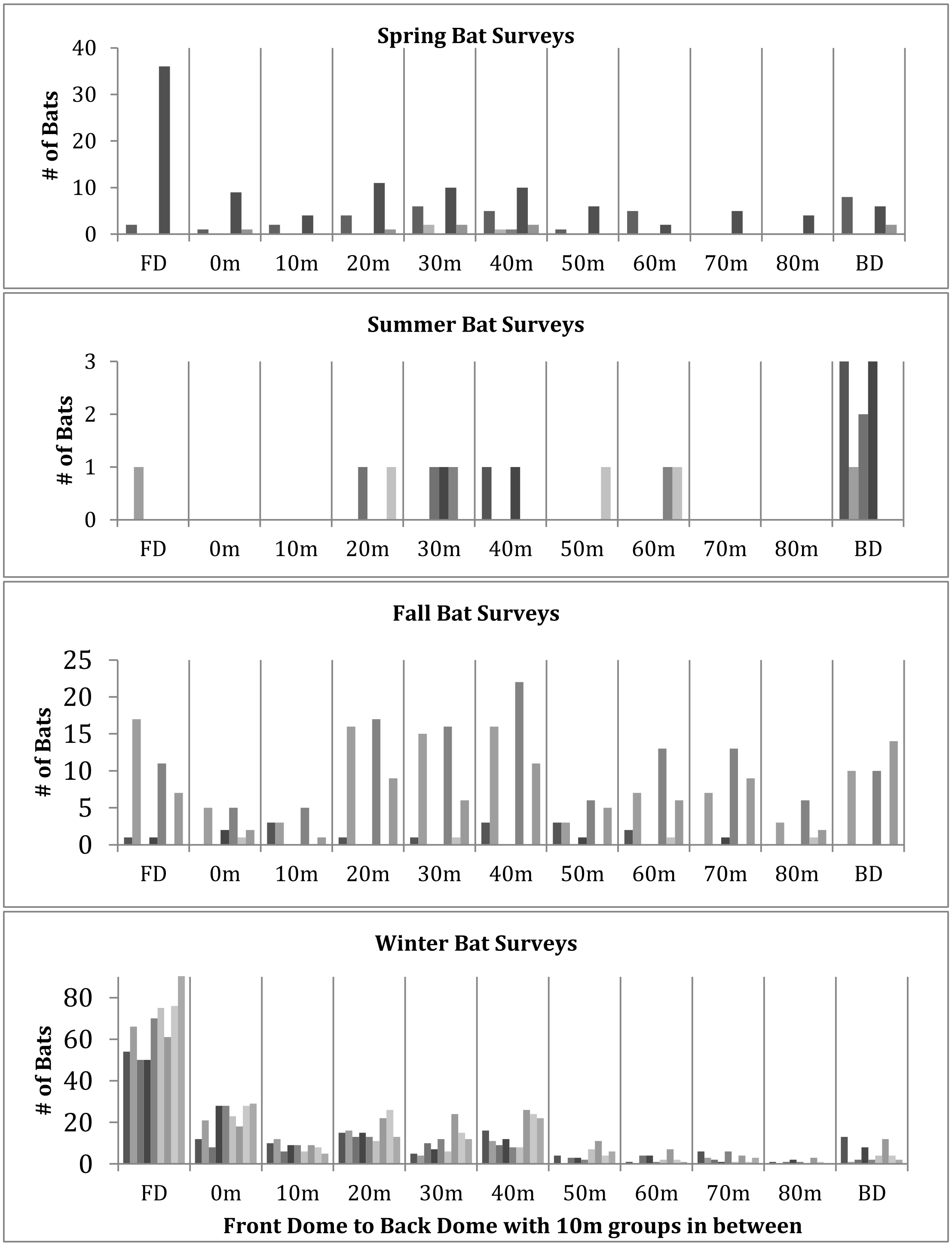

Figure 16. Seasonal distribution of *P. subflavus* in Triple H Cave. This graph shows the number of *P. subflavus* counted in Triple H Cave for all four seasons. The results for each season are split into locations, generally of 10m in length, and the locations are split showing the results of each individual survey. 0m = the interval between 0m and 9m, 10m = the 10m-19m interval, etc. The winter had a significantly higher number of bats

than all other seasons. Note the scale on the y-axis varies according to the numbers of bats present. FD = Front Dome, BD = Back Dome.

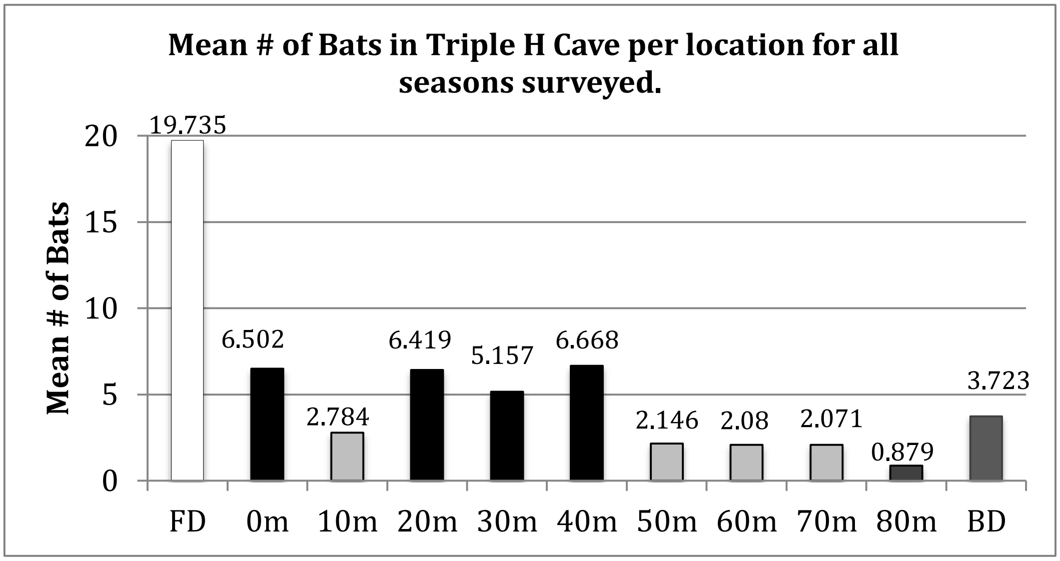

Figure 17. Distribution Pattern of *P. subflavus* in Triple H Cave. This graph shows the differences in the distribution of *P. subflavus* in Triple H cave for all the seasons surveyed. The values shown are the means for that area of the cave. Statistically similar groups are the same color. In general, the front dome and locations in the front of Triple H Cave had a statistically higher number of *P. subflavus* than locations near the back of the cave. FD = Front Dome, BD = Back Dome.

Discussion

Location and temperature preference of P. subflavus

Data regarding location and temperature was kept for all three caves in this study, though it was kept in much greater detail for Triple H Cave. Observations show that the entrance to each cave was very heavily used by *P. subflavus* (Table 1) and, during the winter, were the coolest locations in each of the caves (Figure 18). The temperatures of these areas suggest a preferred temperature for *P. subflavus* while in hibernation in the southern United States. In Triple H Cave, the Front Dome was the heaviest area used by *P. subflavus* with a mean winter temperature of 13.9 °C. The entrance to Waddell Cave was also heavily used by this species with a mean winter temperature of 16.7 °C.

Similarly, the South Entrance of Pitts Cave exhibited heavy use by *P. subflavus* and a mean winter temperature of 13.4 °C. This suggests the mean preferred temperature for *P. subflavus* wintering in southern Mississippi caves is between 13 and 17 °C.

Table 1

Comparison of P. subflavus distribution in cave entrances.

Cave	*Number of P. subflavus in Entrances / Total Number of P. subflavus in Caves*							
Triple H	54/137	66/134	50/106	50/139	70/151	75/141	61/194	76/186
Waddell	10/33	13/26	13/13	30/35	19/28	37/47	45/53	47/51
Pitts	46/164	52/150	79/244	43/187	48/208	47/156	31/198	55/226

This table shows the number of *P. subflavus* in the entrance of the each cave compared to the total number in the cave. Each box is one winter survey for that cave. Numbers in Pitts Cave are a combination of both the North and South entrances.

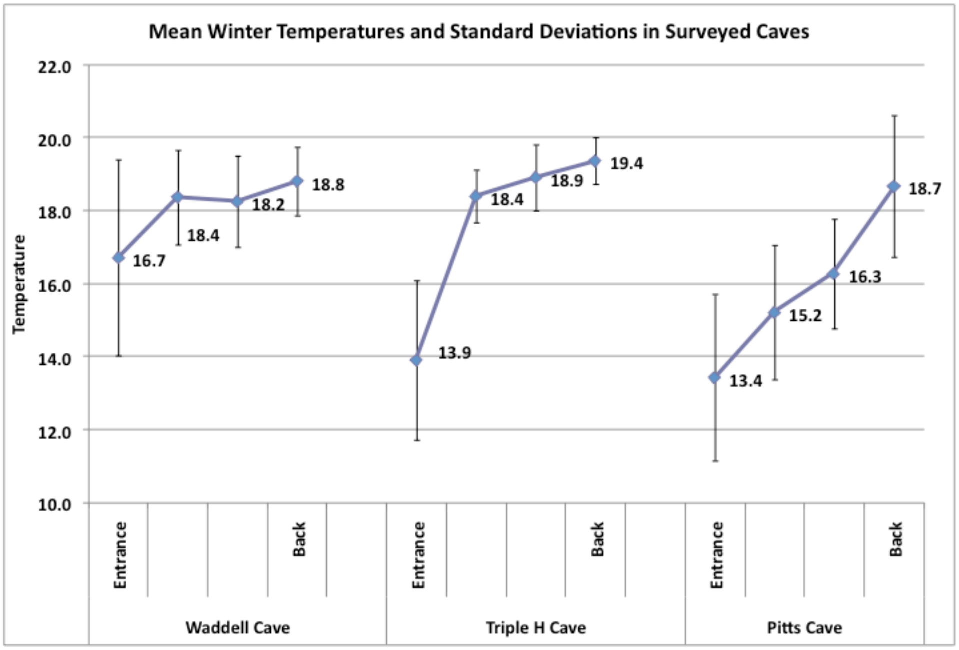

Figure 18. Mean Winter Temperature of Waddell, Triple H, and Pitts Caves. This figure shows the mean winter temperatures °C and their standard deviation for locations in Waddell, Triple H, and Pitts Cave. The entrances to each cave contained a large abundance of *P. subflavus* during the winter and were also the coolest area in each cave with the least amount of thermal stability.

Comparison of the preferred temperature of P. subflavus *to other areas in its range*

For bats to hibernate they must choose an environment where temperatures will aid them in both entering and prolonging torpor. In his seminal work describing the behavior of temperate bats in a subtropical environment, McNab (1974) describes *P. subflavus* as an obligate hibernator. However, contrary to my findings, he also stated that this species, which is the smallest bat in eastern North America, “selects the warmest innermost part of a cave” for hibernation (pg. 949). However, a later study of hibernation by *P. subflavus* in Tennessee caves over a range of possible temperatures showed that the majority of the individuals selected temperatures of 8 to 11 °C even when cooler or warmer temperatures were available (Rabinowitz 1981) (Table 2). Extreme temperatures such as near freezing or temperatures too high to induce torpor were avoided in Tennessee caves.

Table 2

Temperature effect on Distribution of P. subflavus

Cave Name	*Number of Bats*	*Temperature Range °C*	
		Cave	Hibernation Site
Gregory	391	8.4 to 12.1	8.5 to 10.7
Saltpeter	108	2.0 to 8.5	8.0 to 8.5
Scott Gap	65	5.1 to 10.7	8.3 to 9.0
Blow Hole	1305	5.0 to 14.3	8.2 to 10.8
Tory Shields	9	2.3 to 10.5	10.0 to 10.5
Hatcher	315	8.5 to 13.5	8.5 to 10.5
Bull	300	8.5 to 11.4	9.3 to 9.8

This table shows the range of cave and hibernation site temperatures and the numbers of *P. subflavus* found in seven eastern Tennessee Caves. Hibernation site temperatures represent where 75% or more of the bats were found. Note that despite how warm or cold the caves were *P. subflavus* preferred to hibernate in areas that were between 8 and 11 °C.

Given the wide geographical distribution of *P. subflavus* (from Canada to Central America), it is evident that there is no one specific hibernation temperature that is shared by all populations throughout the species range, as populations in the most northern and southern parts of the range may not be able to find that specific temperature. However, McNab (1974) argued that bats have a *preferred* temperature and that this preferred temperature is dependent on the geographical location, especially latitude. During the winter months, populations in the north might prefer the warmest temperatures available in order to keep from freezing, while populations in the south would prefer the coolest temperatures available to help them enter torpor.

Davis and Reite (1967) showed experimentally that a drop in air temperature from 5 °C to -5 °C produces arousal in *Myotis lucifugus* (little brown bat), *Myotis sodalis* (Indiana bat) and *Eptesicus fuscus* (big brown bat). However, no individuals of *P. subflavus* aroused when the temperature was dropped from 5 °C to -5° C and *P. subflavus* kept at -5 °C for three to four hours died. Since the other bat species responded to below 0 °C temperature (-5 °C) by arousal and *P. subflavus* did not and died shortly thereafter, *P. subflavus* clearly needs to avoid colder temperatures. However, at least in the southern United States (e.g., Florida and Mississippi), they must also find temperatures cool enough to produce at least short durations of torpor. Since the geographical range of *P. subflavus* extends into Guatemala and Honduras (Fajita and Kunz 1984), it would be interesting to determine if they are capable of hibernation/going into torpor in tropical areas.

In Minnesota, *P. subflavus* was reported to hibernate at 6.8 °C (some of the warmest temperatures available) (Swanson and Evans 1936). In Indiana they hibernate at 11 to 13 °C (Vincent and Whitaker 2007), while in Florida they hibernate at 14 to 18 °C (some of the coldest temperatures available) (Rice 1957). Rice (1957) also suspected that *P. subflavus* may not be able to maintain torpor at temperatures above 18 °C. Similarly, in a cave in Florida, McNab (1974) observed that *P. subflavus* in a cooler passageway were in deep torpor, while bats in a warmer portion of the same cave were active. McNab identified an air temperature of 18 °C as the line separating torpor from activity.

As seen from the examples above, latitude does seem to have an effect on the preferred temperature for hibernation of *P. subflavus*, and the caves of southern Mississippi are no exception. Their preferred temperature was found to be 13 to 17 °C in southern Mississippi (the coldest temperatures available). This preferred temperature is very close to the preferred temperature range in Florida (14 to 18 °C) (McNab 1974 and Rice 1957) but is warmer than the caves in Indiana and Minnesota (Swanson and Evans 1936; Vincent and Whitaker 2007). This information adds strength to the argument that *P. subflavus'* preferred temperature range is dependant on latitude, and when faced with warmer environments, it will generally seek out the coldest temperature available and vice versa in cooler environments.

Pattern of dispersal for P. subflavus in southern Mississippi caves differs from patterns of dispersal in more northern latitudes.

The pattern of distribution of *P. subflavus* in Triple H Cave is driven almost entirely by the winter season due to *P. subflavus* being most abundant during that season (Figure 6.2). The winter distribution pattern shows that ~ 81% of the *P. subflavus* surveyed were located within the first 50m of the cave, and ~ 34% of all *P. subflavus* surveyed were located in the Front Dome (Table 1). This area of Triple H Cave is prone to the largest variation in temperature during the winter months (see Figure 18) due to its proximity to the cave mouth but is also statistically significantly cooler than all other locations in Triple H Cave.

A repeated measures ANOVA comparing the winter months' temperatures among the Front Dome, (mean=13.9 °C, SD=2.178 °C), the Fork in the Road, (mean=18.4 °C, SD=0.723 °C), Stalagmite (mean=18.8 °C, SD=0.934 °C), and the Back Dome (mean=19.4 °C, SD=0.639 °C) ($F_{(3,7)}$=15.209, p=0.002) showed that there was a difference in the temperatures among the locations. A Bonferroni pairwise comparison showed that the Front Dome was significantly cooler than all other areas in the cave during the winter. Temperatures at the Fork in the Road and the Stalagmite were significantly cooler than the temperatures at the Back of the Cave. These results support the idea that *P. subflavus* chooses specific temperatures rather than thermal stability (Table 2).

Even though no data was kept on specific locations (to the meter) of *P. subflavus* in Waddell or Pitts Cave, most of the *P. subflavus* that I counted were also in the coolest and most thermally unstable areas of their caves, and their patterns of dispersal were roughly the same as that of Triple H Cave.

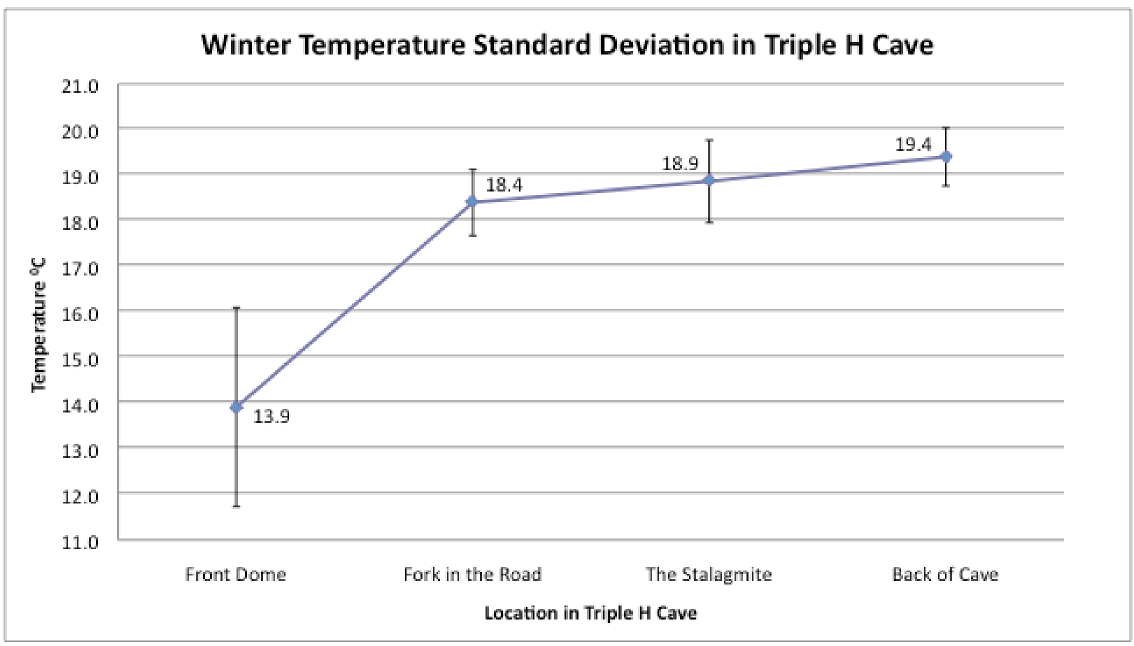

Figure 19. Triple H Cave Distribution of Winter Temps. This figure shows the winter temperature °C variations at each location within Triple H Cave. Not only is the Front Dome significantly cooler than the rest of the cave, it also has the greatest variation in temperature during the winter months.

McNab (1974) stated that *P. subflavus* in Illinois and Kentucky caves would choose to hibernate in the deeper recesses of the cave, emphasizing that this species would choose locations where temperatures for hibernation were the warmest and the most stable. Many authors, including Fujita and Kunz in their 1984 paper "Pipestrellus subflavus" in the journal *Mammalian Species,* and Schmidly in his 1991 book *The Bats of Texas* reused this idea of choosing both warmer and more stable temperatures for hibernation. Despite finding evidence to the contrary (90% of winter roosting sites being along the outer passageways of a mine in Nebraska), Damm and Geluso (2008) also repeat the idea that *P. subflavus* commonly roost in deep recesses of caves where warmer and more stable temperatures exist. A study in Tennessee caves by Rabinowitz (1981) argued that warmer temperatures would be avoided by *P. subflavus*. Later, Briggler and Prather's (2003) research in Arkansas caves combined McNab's and Rabinowitz's ideas and suggested that *P. subflavus* could handle a wide range of winter temperatures as long as the temperatures were stable and further suggested that a specific temperature was being sought. Vincent and Whitaker's (2007) research continued this idea and suggested that thermal stability was the driving factor in selecting winter hibernation site for *P. subflavus* in Indiana caves, whereas the range of available temperatures was of secondary importance.

While clearly important, these combined studies are biased in that all took place in more northern areas of *P. subflavus'* range (other than Mississippi and Florida), such as Kentucky, Tennessee, Arkansas, Missouri, Nebraska, Minnesota, Illinois, Indiana, and Iowa; thus, they do not represent the entire range of this species (Figure 20). Consequently, the idea that *P. subflavus* seeks out thermally stable hibernation sites has nonetheless been used as a general rule of thumb for the species as whole. However, the data collected in my study does not show this pattern in southern Mississippi caves. I found that *P. subflavus* collected around the coolest portions of the cave, despite greater thermal instability in that area. Rabinowitz (1981) suggested that in caves with available warmer temperatures, there might be cooler temperatures which *P. subflavus* would select, thereby enabling the bats to enter torpor. Based on my data, I think that searching for a cooler temperature within the caves during winter is the driving factor for the distribution of *P. subflavus* within southern Mississippi caves.

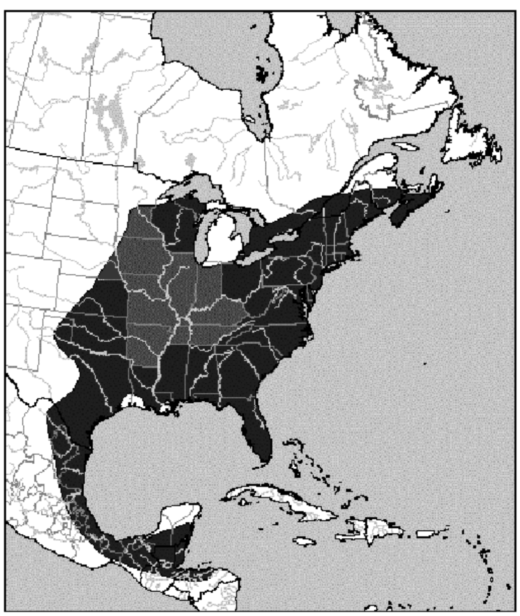

Figure 20. Range Versus Study Areas. This figure shows the range of *P. subflavus*. The states that are lightly shaded represent the areas where many of the studies of temperature preferences of this species have taken place. Note that no previous studies have been done in the northeastern U.S., Canada, or in the more southern parts of this species range.

Though temperature stability and temperature optimum might both be preferred, it would make sense that getting into torpor would take precedence. If *P. subflavus* were simply choosing the most stable area of Triple H Cave then I would see a pattern of dispersal with larger numbers of *P. subflavus* in the back of the cave. Even a preference for a blend of thermal stability and preferred temperatures would lend a more bell-shaped pattern of dispersal through Triple H Cave. However, such dispersal patterns were not seen in any of the caves surveyed.

This pattern of dispersal toward the coolest locations might suggest that optimal temperatures in the southern areas of its range could be a limiting resource for entry into hibernation for *P. subflavus* and might be worth future study, especially in tropical areas. It is obvious from my study that *P. subflavus* in southern Mississippi caves have a strong preference for the cooler available temperatures during the winter months.

M. austroriparius in Triple H Cave

The low number of *M. austroriparius* in Triple H Cave was interesting considering evidence from its past. The ceiling of the Back Dome and a few meters of passageway leading into the Back Dome, are stained black from the large colony of *M. austroriparius* that had once inhabited the cave. There is evidence that a stream flowed through Triple H cave in the mid 1970's since Middleton (1976) reported two species of aquatic crustaceans from the cave. It is possible that the water table had lowered (a stream did not regularly flow through the cave during my study), and the *M. austroriparius* abandoned the cave, as it no longer met the requirements for this species (a stream below the colony). This reason for abandonment of caves by *M. austroriparius* has been observed in this species in Florida caves (Rice 1957), but not by Gore and Hovis (1994).

CHAPTER VII

SUMMARY OF CAVES' TEMPERATURES AND BAT USAGE THROUGHOUT THE SEASONS

Within Waddell, Triple H, and Pitts Caves, there was a significant difference in temperature among the seasons. In general, the greatest difference was between the summer and winter. Some of the different locations within Triple H and Pitts Caves showed significant temperature differences, with the greatest differences existing between the parts of the caves near the entrances and areas near the back of the caves. Waddell Cave did not show significant temperature differences among its locations (see Chapter II of this thesis). The winter months have warmer temperatures than outside ambient temperatures and vice versa for the summer months. These thermally dynamic caves give options to bats that are seeking certain condition in certain seasons.

The bats will use the cave when their requirements match up with what the cave currently has to offer in both location and temperature. My data showed that *P. subflavus* were significantly more numerous in these caves during the winter. My observations also showed that this species uses the caves as hibernacula (see Chapter IV of this thesis). In the winter season, *P. subflavus* is hibernating with statistically higher numbers in areas that are cooler than the warmer, deeper areas within the cave (see Chapter VI of this thesis). I also showed that *M. austroriparius* is more numerous in Waddell and Pitts Caves during the summer, and my observations also show that they use the caves as day and maternity roosts (see Chapters IV and V of this thesis). They are found in the areas of the caves that are both relatively warm and have flowing water.

The winter pattern of cave usage by *P. subflavus* is seen in all of the caves that were surveyed while the pattern of warm-weather usage of the caves as maternity sites by *M. austroriparius* was observed in only Waddell and Pitts Caves. These data correspond with earlier studies throughout the United States which state that *P. subflavus* hibernates in caves and mines during the winter and roosts in buildings, trees, and man-made structures during the summer (Damm and Geluso 2008). It also agrees with Rice's (1957) assessment that *M. austroriparius* uses caves during the summer months as day roosts and maternity colonies in some parts of its range.

Clumping Numbers and Mixed Species Clumps

Although *P. subflavus* are known to generally hibernate as individuals, they have also been known to form small clusters. Previous investigators have found clusters mostly in pairs with an occasional cluster of three bats and rarely of four (Vincent and Whitaker 2007). McNab (1974) described *P. subflavus* as bats that do not cluster and, rely instead, on thermally stable environments to mitigate the impact of temperature on their small body size. During the winter month surveys, most of the bats were found as singles. Yet, groupings of two to three were rather common, especially in the in the Front Dome and between meters zero and 40. Clusters of four to five were rare but did occur. On more than one occasion, a grouping of *P. subflavus* in the Front Dome of Triple H Cave numbered between seven and 10 individuals (Figure 21). Groupings of this size were not common (only found in the Front Dome of Triple H Cave) but were noted in the winters of 2011, 2012, and 2013. Clusters of *P. subflavus* were also noted in Waddell and Pitts Cave but not in high numbers.

It is also worth noting that on a more than one occasion I observed a single *P. subflavus* and *M. austroriparius* clustered together in both Triple H and Pitts Caves. The pairs were awake and watched me as I walked by (Figure 22).

Figure 21. Clustering in *P. subflavus*. The top clump has 10 individuals, the smaller clump at the bottom middle has eight individuals, and the clump to the bottom left has four individuals. This picture was taken in the Front Dome of Triple H Cave in winter 2013. Photographs by Zac Roth, 2013.

Figure 22. Interspecies Clustering. This picture shows a *P. subflavus* clumped with a *M. austroriparius* in Triple H Cave during the winter of 2013. Photographs by Zac Roth, 2013.

CHAPTER VIII

THERMAL RELATIONSHIPS OF *P. SUBFLAVUS* WITHIN TRIPLE H CAVE

Introduction

On many surveys during the winter months, I noticed that *P. subflavus* showed certain characteristics when in torpor, such as no visible chest expansion during breathing, resistance to waking up when touched, eyes remaining closed when disturbed, and also the collection of dew on their fur. Stones and Wiebers (1967) noticed similar behaviors with little brown bats. However, it still seemed possible to me that some of these bats could simply be sleeping and not necessarily in torpor. Initially, I wanted a more quantifiable way to distinguish between torpor and non-torpor (sleeping) bats.

Since hibernating (torpid) bats allow their body temperature to approximate ambient temperature, i.e., that of the surrounding environment (Brack 2004), I thought it might be possible to distinguish between the two thermally, as the sleeping bats would (in theory) be warmer than torpid bats. Taking internal body temperatures was ruled out as too disruptive to the bats. Instead, I used a handheld laser thermometer to measure the surface temperature of the bats. A similar technique using thermal infrared imaging cameras has been used in tracking bats during flight and has also been used in monitoring temperature regulation of large colonies of Brazilian Free-Tailed bats in Carlsbad Cavern (Hirstov et al. 2008).

As I measured the surface temperature of individual *P. subflavus* (both in torpor and awake) down the length of the Triple H Cave. I also measured the surface temperature of the cave wall immediately next to the bat for comparison. After doing this throughout Triple H Cave, two patterns emerged from these initial observations: 1) during the winter months the surface temperature of the cave was warmer the further back I went and 2) the surface temperature of the bats (at least of the ones in torpor) seemed to depend on the surface temperature of the cave; the cooler the cave surface, the cooler the bat and vice versa. In addition to the idea of distinguishing sleeping bats from bats in torpor, I decided to also determine if there was a dependence of bat surface body temperature on cave surface temperature down the length of Triple H Cave.

Methods

I used a Ryobi Tek4 Professional 4-Volt Infrared Thermometer, Model # RP4030 purchased at Home Depot for ~$70 to measure bat body and cave surface temperatures. This would later be known as the "Ray Gun" (Figure 23). The ray gun can measure temperatures from -20 to 310 °C with an accuracy of +/-0.1 °C. It is primarily used for detecting pipes and wires in floors and walls and also for checking mechanical equipment or electrical circuit breaker boxes or outlets for hot spots. In addition it is also used for checking for cold air drafts around doors and windows, heaters, air conditioning units, and oven and freezer temperatures. Its construction makes it impact-, dust- and water-resistant, and it can withstand tough job site conditions, which makes it ideal for caves. A 10:1 distance-to-spot ratio makes it more accurate when compared to the standard 8:1 used by most other infrared thermometers. Its red laser pinpoint and backlit LED screen with continuous readout made collecting measurements in the dark both quick and easy.

Only *P. subflavus* was used in this experiment, as the behavior of the few *M. austroriparius* present in Triple H Cave did not lend itself to this type of testing. Surface body temperatures of fifteen seemingly torpid bats were determined from different locations in Triple H Cave over three visits (five per visit) during late January and early

February 2012. This winter time frame was chosen because the bats were in deep torpor with predicted minimal differences between the bats' body and cave surface temperatures. On October 27, 2012, 40 bat samples comprised of both awake and seemingly torpid bats were once again collected from different locations in Triple H Cave. This fall time frame was chosen because it had a large number of both seemingly torpid and awake bats available to sample.

The surface body temperatures of the *P. subflavus* determined in January, February, and October, 2012, were all taken by centering the ray gun directly over the dorsal surface and then pulling the trigger of the ray gun. While holding the trigger the ray gun was adjusted so that the laser pointer's dot was on the center of the bats' backs. While still holding the trigger of the ray gun, I moved the laser pointer off the bat a few inches onto the cave surface. This was done at a distance of less than half a meter. The temperature of each bat's surface and cave surface was recorded as well as the location of the bat within the cave. This process was repeated on *P. subflavus* throughout the cave.

Figure 23. Surface Temperature Measuring. This picture shows the “Ray Gun” being used in measuring the surface temperature of the bat and the adjacent cave surface. Photographs by Zac Roth, 2012.

For the fifteen bats collected in January and February of 2012, a simple linear regression analysis was used to check for dependency. I tested the null hypothesis that the slope was equal to zero. For the 40 bats collected in October, 2012, I calculated the difference between the bat’s surface temperature and the cave surface temperature for each bat, both those awake and those seemingly in torpor. A Student’s t-test was then used to test the null hypothesis that the temperature differences of the awake *P. subflavus* were equal to the temperature differences for the seemingly torpid *P. subflavus*. Alpha was set at 0.05.

Results

A test for dependency (linear regression) showed that there was a significant positive relationship between the surface temperature of the bat and its distance within the cave. The bats’ body temperature was (n=15, mean=12.8 °C, SD=2.4 °C) and distance from the Front Dome was (n=15, mean=23.7m, SD=24.8m). After meeting the assumptions for all three tests (slope of the residuals over the predicted y are equal, the variances were equal, and the number of points above and below the line were equal), I found that the relationship between distance and bat body temperature was significant (y-intercept=10.75, slope=0.085, $F_{1,13}$=40.56, R^2=0.755, p<0.001) which led me to reject the null that slope=0 and conclude that the body temperature of the bat was dependent on distance in the cave from the Front Dome (Figure 24).

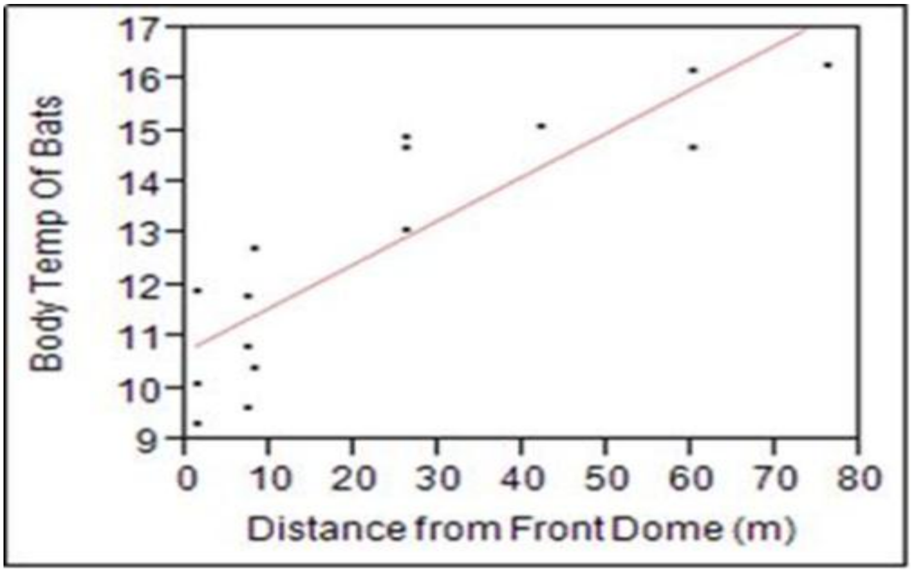

Figure 24. Dependence Relationship of Bats and Cave Surface. This figure shows the dependence relationship between the surface body temperature of *P. subflavus* and the cave surface temperature. The further back in the cave the warmer the cave surface, and thus the warmer the bat. Each dot represents one bat surveyed in January or February 2012.

During the process of measuring the temperature of bats in torpor in October of 2012, I found that the differences between the bats' surface temperature and the temperatures of the cave surfaces next to the bats were generally very small. As I ventured further back into the cave, the cave surfaces had slight variations in temperature and so did the surface temperatures of the bats in torpor. In most cases they were less than 1 °C apart. In contrast, when I measured the temperatures of bats that were awake (actively looking around, cleaning their wings, etc.), the difference in the bats' surface temperatures was, in most cases, greater than 2 °C above the cave's surface temperature (Figure 25).

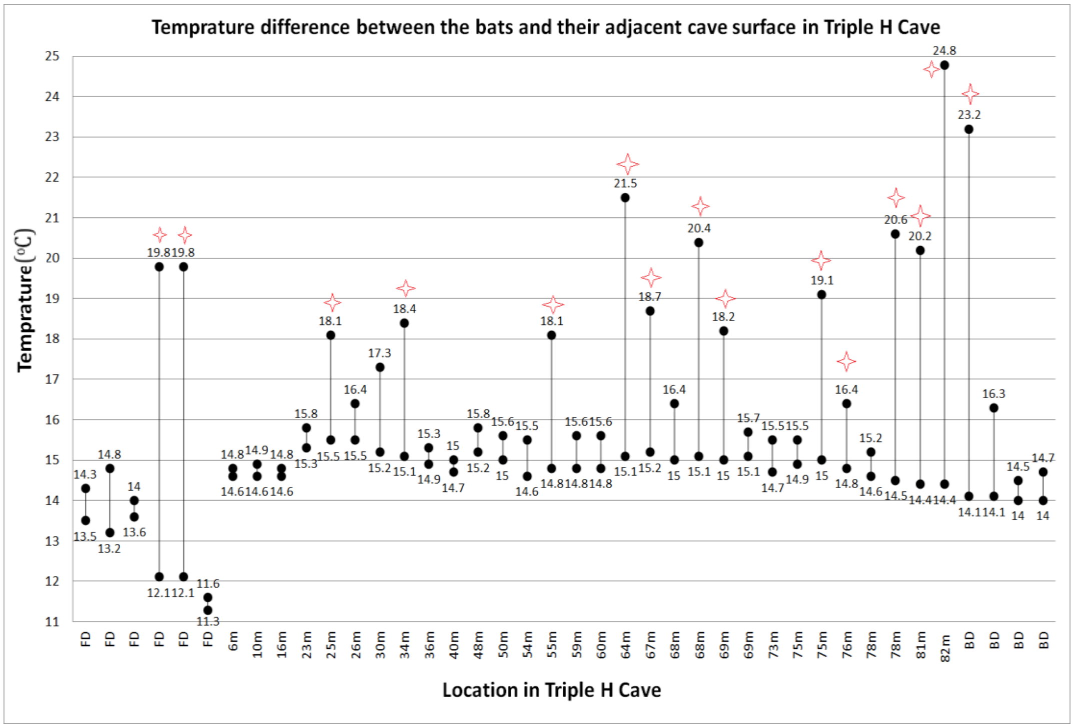

Figure 25. Temperature Difference between bats and the cave surface. This figure shows the surface body temperature (higher dot) and the adjacent cave surface temperature (lower dot) for each of 40 bats sampled in the Triple H Cave. Bats whose body temperatures were close to the adjacent cave surface temperature (generally < 1 degrees difference) were thought to be in torpor. Bats that were found to be awake (moving their heads or wings) were found to be much warmer (generally > 2 degrees difference) than the adjacent cave surface temperature and are marked with a star. FD = Front Dome. BD = Back Dome.

The differences in the awake bats' body surfaces and the cave's surface temperatures (n=15, mean=5.3 °C, SD=2.6 °C) and the differences in the bats' body surfaces and cave surface temperatures of the seemingly torpid bats (n=25, mean=0.76 °C, SD=0.53 °C) were compared using an unpooled two-tailed, two sample *t* test. The results (t=6.84, DF=28, p<0.001) led me to reject the null hypothesis and conclude that a significant differences existed between the awake versus the seemingly in torpid bats in relation to their nearby cave surface temperature.

Discussion

From the experiments conducted in this study it is apparent that 1) the surface body temperature of individuals of *P. subflavus* in torpor depend on their distance within Triple H Cave; and 2) the difference between the bats' body surface temperatures and cave surface temperatures is a helpful way to determine if the bats are indeed in torpor. The closer the two temperatures (generally <1 °C) the more likely the bat is in torpor. The more distant the two temperatures (generally >2 °C and often > 5 °C) the more likely the bat is awake or simply asleep. The difference between a bat's surface body temperature and the adjacent cave surface temperature is an interesting aspect of hibernation that deserves more study not only during the winter but during other seasons of the year as well.

In the winter months in Triple H Cave, the surface temperatures of the cave's walls are increasingly warmer toward its back. Thus, bats in torpor in the back of the cave will be warmer than the bats in the front of the cave. In theory, if a bat wished to regulate its temperature, it only needed to fly further into Triple H Cave to be warmer and fly toward the entrance (and thus a state of torpor) if it wished to be cooler.

Although this experiment was conducted only in Triple H Cave, (a long, linear tube with one major opening), I suspect that Waddell Cave (also a long, linear tube with one major opening) would have a similar relationship between *P. subflavus* in torpor and their temperature choices as a function of distance within the cave. This relationship in Pitts Cave is more complex as its features two major openings that allow winds to mix the air, very tall ceilings that allow air to thermally stratify when wind is not present, and multiple passageways that circle back and connect to one another, allowing external temperatures to more easily reach the innermost parts of the cave. Nonetheless, different locations in Pitts Cave have different temperatures during the winter months, especially among the South Entrance, End of the Chute (cooler), and the Black Top (warmer). A comparison between Waddell Cave/Triple H Cave and Pitts Cave would be worthwhile to determine if there are any differences in how *P. subflavus* uses a linear cave versus a non-linear cave for winter hibernation.

Due to its physical conditions and layout, Triple H Cave offers a unique opportunity to study how a bat's body temperature is related to cave surface temperature along the length of a cave. When trying to determine if an individual *P. subflavus* is in torpor, my data suggests that the temperature difference between the bat and the surrounding cave wall is a useful and salient indicator. Bats in torpor will be very close in temperature to the adjacent cave surface, and bats not in torpor will be at least a few degrees warmer than the adjacent cave surface.

CHAPTER IX

DURATION OF TORPOR IN *P. SUBFLAVUS* IN TRIPLE H CAVE

Introduction

P. subflavus uses more small caves and mines for hibernacula than do other species of bats (Brack et al. 2003, 2004). During hibernation, all mammalian hibernators awaken periodically during the season of hibernation (Lyman et al. 1982), and bats are no exception. A study by Twente (1955) in the caves of Kansas and Oklahoma concluded that the biggest problem faced by bats is the conservation of energy. His observations showed that cave dwelling bats in unfavorable (warmer) places would become irritated, awake from torpor, and move after a short period of time (presumably to a cooler place), and bats in cooler, more favorable places would stay in torpor for a longer period, thus helping them to conserve energy.

During the winter, bats will periodically arouse between torpor bouts (Daan 1973; Folk 1940; Krzanowski 1959) and will often fly within and/or leave their hibernacula (Daan 1972; Guthrie 1993; Hooper and Hooper 1956; Mumford 1958; Tinkle and Patterson 1965). Twente (1955) suggested that the length of the hibernation period in bats was related to the temperature during that period and that the lower the temperature the longer the hibernation. This idea was revisited later by Ransom in 1971 and Twente et al. in 1977 where their studies on bats and other small mammals confirmed that frequency of arousal is, in fact, regulated by temperature with warmer temperatures causing more frequent arousals from hibernation.

Other species of *Perimyotis* bats are known to move within their hibernacula and will exit their hibernacula and feed on warm days during all months of the winter (Avery

1985). Vincent and Whitaker (2007) noted that *P*. *subflavus* in Indiana caves and mines were not remaining in one location during the length of winter. During the winter of 2012, I noticed a similar pattern while conducting surveys of *P*. *subflavus* in Triple H Cave. The bats in the warmer areas of the caves seemed more awake that the bats in the cooler areas of the cave. Based on these observations I wanted to 1) see how long *P*. *subflavus* in Triple H Cave were staying in torpor; 2) if this differs between different areas of Triple H Cave; and 3) how these results would compare to other studies of wintering activity of *P*. *subflavus.*

Methods

During the winter of 2013, I marked the positions of *P. subflavus* in Triple H Cave using white string as position indicators. The strings were labeled with duct tape and used staples at either end to anchor the string to the cave surface (Figure 26). These were placed under 20 inactive (presumably torpid) *P. subflavus*; 10 of which were in the front portion of Triple H Cave from the Front Dome to the 10m mark. The remaining 10 strings were placed further back under inactive *P. subflavus* just past the Fork in the Road (42m mark) up to the Stalagmite (52m mark). Few usable *P. subflavus* were located past the Stalagmite during this time, so that area of the cave was not used in this experiment.

I checked to see if each bat was still in its original position on days one, seven, 14 and 28, thereafter. This was done twice during the winter of 2013. This first trial ran from January 12th to January 26th and the second February 1st to March 2nd. On each day a picture was taken of the marked bats. This picture was then viewed on the next visit to see if the bat had moved. I was therefore able to determine if a bat remained in one spot over the observation period. I also compared the data from the front of the cave to the data from the back of the cave to see if position (and therefore temperature) in the cave played a role in the length of the torpor bout. For this experiment, movement was defined as a detectable, physical shift in position (Figure 27).

Figure 26. String Experiment Setup. This figures shows the general makeup of the strings used to mark the bat’s position during this experiment. Photographs by Zac Roth, 2013.

Figure 27. String Experiment Demonstration. This figure shows how the strings were used to mark the bats position. The top picture was taken on day zero (the day we first marked the bats). The bottom picture was taken after one day. The position of the bat, variations in the pelage of the bat and the mottled pattern on the cave surfaces were used to determine if the bats had moved during the observational period. This particular pair had not moved after one day. Photographs by Zac Roth, 2013.

Results

For the January trial, 18 bats were still present in their original locations after day one (nine in the front and nine in the back). However, after seven days, only five bats were still present in their original location (three in the front and two bats in the back). By day 14 all of the bats had moved from their original locations (Figures 4.3 and 4.4).

For the February to March experiment, 17 bats were still present in their original locations (nine in the front and eight in the back) after one day. After seven days, nine bats were still present in their original locations (four in the front and five in the back). After 14 days, six bats were still present in their original location (three in the front and three in the back). After four weeks, two bats were still present in their original locations (both in the front) (Figures 28 and 29).

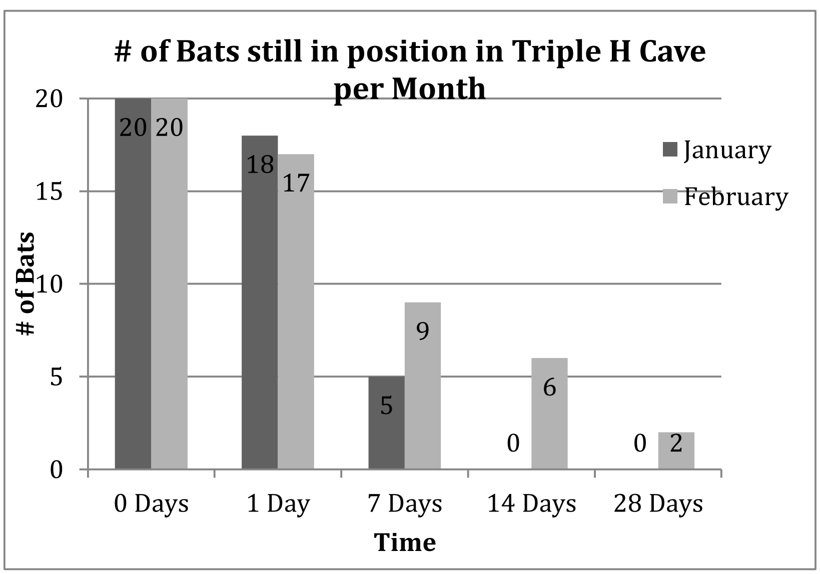

Figure 28. Monthly Comparison of Torpor length. This figure shows the number of bats that were still in their original positions after a certain number of days for observations begun in January and February 2013.

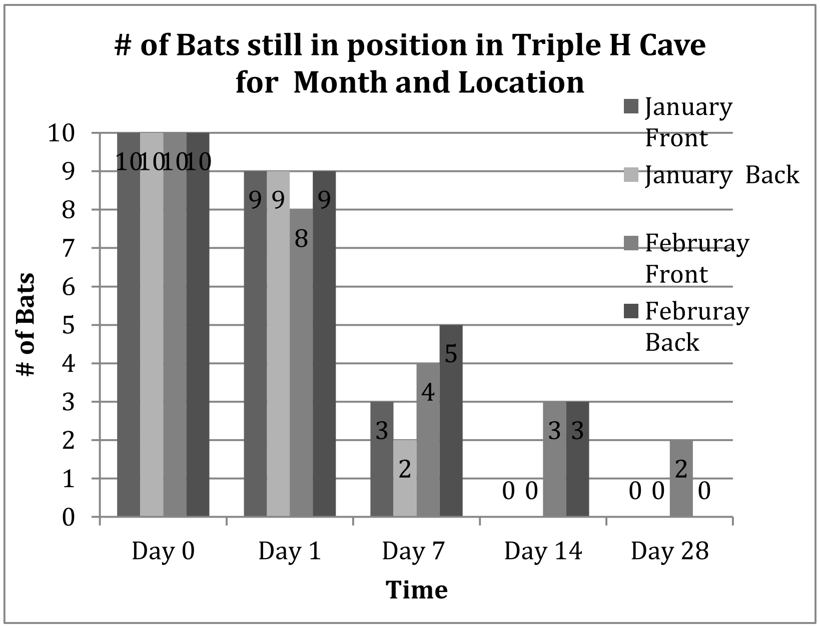

Figure 29. Distribution Comparison of Torpor length. This figure shows the number of "front" and "back" bats (see text) that were still in their original positions after a certain number of days for observations begun in January and February 2013.

Discussion

These results show that individuals of *P. subflavus*, for the most part, do not stay in one place over the winter in Triple H Cave. In January, all 20 of the observed bats had moved by 14 days with 11 of the 20 observed bats moving by day 14 in the February to March experiment. A similar study by Hassell (1967) showed the mean hibernation duration of *P. subflavus* in Kentucky Caves to be 14 to 17 days. This pattern is very close to what I observed in Triple H Cave despite differences in latitude between our study areas. In contrast, other studies have reported much longer periods of hibernation. Brach and Twente's (1985) research on *P. subflavus* in Missouri Caves showed 20 instances of bats staying in one spot longer than 50 days. Davis (1964) marked and monitored 20 *P. subflavus* in natural hibernacula in Bat Cave, Kentucky, and after two weeks 17 of the 20 were still present.

Differences in torpor length between these two studies and my study could be attributed to the differences in the cave's winter temperatures. The caves in Missouri and Kentucky are exposed to longer periods of cold weather with less daily temperature variations during the winter than caves in southern Mississippi. This prolonged and relatively stable cold weather in more northern latitudes should lengthen the torpor bouts of *P. subflavus* (provided it does not get too cold), keeping them inactive and in one spot for a longer period of time. This would be beneficial to *P. subflavus* in helping them to conserve energy by lowering the number of arousals over the winter months (Table 3).

Table 3

Comparison of Torpor Bouts

	Day 0	*Day 1*	*Day 7*	*Day 14*	*Day 21*	*Day 28*	*Day 56*	*Day 70*	*Day 77*	*Day 91*
Bat Cave	100		95	85	55	55	50	35	25	10
Triple H Jan.	100	90	25	0						
Triple H Feb.	100	85	45	30		10				

This table shows the percent of *P. subflavus* remaining in the original marked locations in two caves, Bat Cave, Kentucky (see Davis 1964) and Triple H Cave, Mississippi (present study). The Kentucky cave has a larger percentage of *P. subflavus* remaining in their original positions longer than the southern Mississippi cave. Blank boxes represent days not checked.

Since temperature does have an effect on the duration of hibernation, I was surprised that there was not a larger difference in the movement from the original position of *P. subflavus* between the front (Front Dome) and farther back (Fork in the Road) areas within Triple H Cave, given the significantly different temperatures in the two areas. Brack and Twente's (1985) field experiments showed a large change in hibernation length from 15.2 days to 25.3 days when the temperature was lowered from

12 to 10 °C, a difference of only 2 °C. A repeated measures ANOVA showed that during the winter, the mean temperature in the Front Dome (13.9 °C) and the mean temperature in the Fork in the Road (18.4 °C) differed significantly ($F_{(3,7)}$=15.209, p=0.002) (see Figure 18 of this thesis) with a difference of 4.5 °C and together contained 82% of the bats in the caves. Yet, I did not see any large differences in the movements of the bats between these two areas within the cave. Differences between my results and Brack and Twente's (1985) field experiments could be attributed to the differences in cave locations and the exposure to prolonged colder air temperatures in their Missouri caves.

For Triple H Cave, the location of the most highly used area during hibernation is just a few meters from the entrance and is prone to relatively quick changes in temperature. Often in southern Mississippi the weather swings from cold to warm and back to cold all within just a few days. If the bats are attempting to maintain a certain temperature, these fluctuations might cause some of the bats to wake up and move to more thermally suitable portions of the cave, move to a different cave, or even venture outside to feed, especially if the weather is warmer. This may help to explain why we see equal movement between the front and back groups of bats in Triple H Cave (Figure 30).

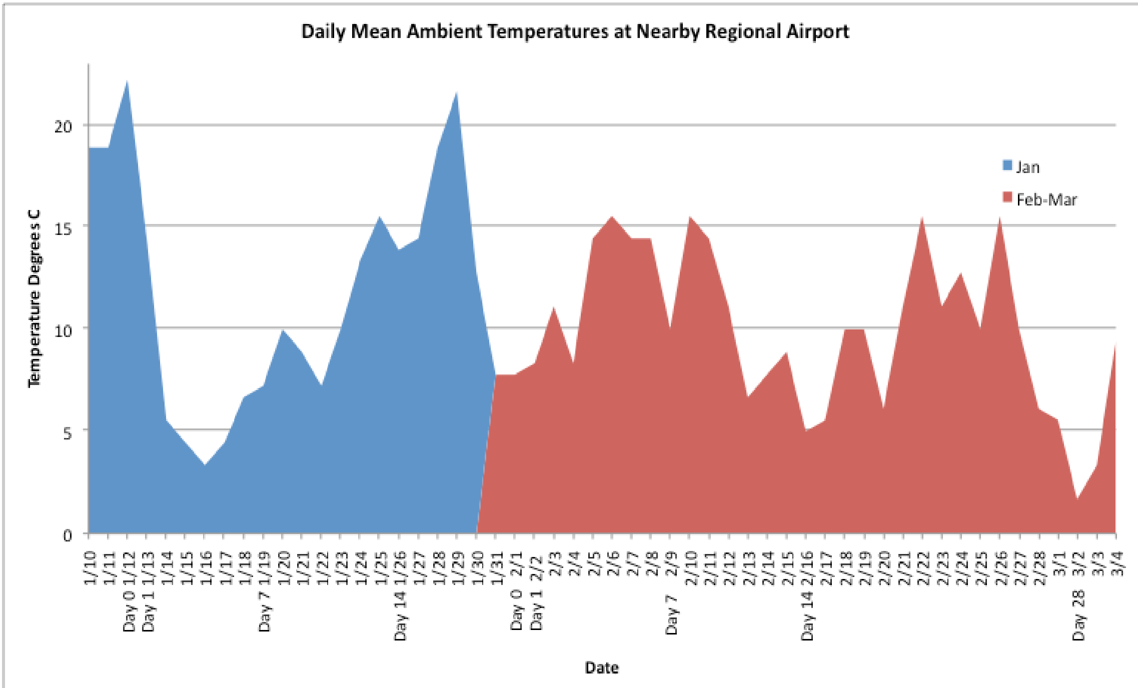

Figure 30. Daily Mean Temperature Variation. This figure shows the daily mean temperature variations during the two trials in movements of *P. subflavus* in the Triple H Cave in January, February, and early March 2013. These inconstant temperatures might have an effect on the duration of torpor length of *P. subflavus*. Temperature data is from the Hattiesburg/Laurel airport.

This pattern of winter arousal in *P. subflavus* is also seen in other vespertilionid bat species in temperate locations. Of these, most cave-hibernating species are less likely to arouse than tree-hibernating species, as they are not as exposed to external ambient temperature (Boyles et al. 2006). When flights outside of the hibernacula do occur, they tend to be more common when the ambient temperature rises above 15 °C (Avery 1985; Fenton 1970; Twente 1955a). Many investigators have concluded that this is due to an increase in prey availability. Yet, many of the bat species that are active at temperatures above 15 °C are not known to feed. Therefore, these bats may change locations within or between caves to thermoregulate or to drink (Boyles et al. 2006). However, Avery (1985) has concluded that *Periomytis pipestrellus*, a sister species of *P. subflavus*, feeds on warm nights in the winter in England.

Feeding, drinking, and copulation have been noted as the main reasons for winter arousal (Boyles et al. 2006). However, winter feeding has only been directly observed in a few species (Avery 1985; Whitaker et al. 1997). Bats hibernating in wet caves with fresh water supplies are commonly known to leave the hibernaculum (Whitaker and Rissler 1992), and reports of winter copulation, while known to happen in some cave-dwelling species, are rare. Predator avoidance may be an important part of winter movements by bats (Twente 1955).

The timing of arousal during the winter appears to be an extension of circadian cycles and was assumed to lengthen with a drop in temperature (Heller et al. 1989). A study by Parks et al. (2000) suggest that this is not the case, and that bat's circadian rhythms continue through hibernation. The arousal of most hibernating bats appears to coincide with dusk (Hays et al. 1992) with few individuals arousing randomly throughout the day (Thomas 1993). This dusk centered arousal is thought to allow the bats a way to continually reset their biological clock (DeCoursey and DeCoursey 1964; Kortner et al. 1998; and Parks et al. 1999). Arousing at dusk also suggests that feeding is an important part of arousal since any other reason for arousal could happen at any other time of the day (Parks et al. 2006).

Clearly, there is no one set length of hibernation duration for *P. subflavus* and variables that are specific to each region, area or cave whether it is latitude, temperature variability, disturbance, or cave morphology have an effect on the torpor length of *P.*

subflavus. However, my study shows that in the southern United States, *P. subflavus* is generally not in hibernation continuously over the duration of the winter months.

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