SPECIES RICHNESS, POPULATION DENSITIES, REPRODUCTIVE BEHAVIORS, AND SEX RATIOS OF THE CAVE-DWELLING BATS AT DUNBAR CAVE, CLARKSVILLE, MONTGOMERY COUNTY, TENNESSEE

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Morgan Eve Kurz

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Of the Requirements for a

Master of Science Degree in Biology

Morgan Eve Kurz

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DEDICATION

This thesis is dedicated to my mother, Kathy L. Watts, my father Arnold W. Kurz, and my grandparents, Estelle P. Grambihler and Kenneth L. Grambihler. Their love, guidance, and unconditional support throughout my graduate career made my dreams of becoming a field biologist a reality. Thank you all.

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Without the support of many state and federal agencies, this research would not have been possible. I am very grateful that I was able to continue and finish my field work despite the detection of an individual bat that tested positive for *Geomyces destructans*, a fungal pathogen associated with White-Nose Syndrome in some North American bat species. Special thanks to Tennessee Wildlife Resource Agency, Dunbar Cave State Natural Area, U.S. Fish and Wildlife Service, and U.S. Forest Service.

I am greatly indebted to the Center of Excellence for Field Biology at Austin Peay State University for funding my research. It was through the Center that I was allowed to join the bat research team as an undergraduate, and also received funding to continue my research at the graduate level. I would also like to thank Dr. Steven Hamilton for his continued support of the project. I also owe thanks to the College of Graduate Studies at Austin Peay State University for their financial support of the study.

ABSTRACT

MORGAN EVE KURZ. The Species Richness, Population Densities, Sex Ratios, and Reproductive Behaviors of the Cave-dwelling Bats at Dunbar Cave, Clarksville, Montgomery County, TN (under the direction of ANDREW N. BARRASS).

Eastern Pipistrelles (*Perimyotis subflavus*) also known as tri-colored bats, and Little Brown bats (*Myotis lucifugus*) are two of the most prevalent and common species of bats found in eastern North America. They are known to hibernate during winter months and demonstrate tree roosting or day roosting during spring, summer, and early autumn. The primary objective of this study was to create a detailed evaluation and data base of the dominate species and other species of cave-dwelling bat at Dunbar Cave State Natural Area. Many public recreational activities take place at Dunbar Cave, such as nature awareness programs, cave tours, fishing, and bird watching. Over the last five years, research assistants and project directors at Austin Peay State University Center of Excellence for Field Biology, with the help of Dunbar Cave staff, have discovered a fragile bat population that hibernates in specific chambers of the cave during the late fall and winter months. Over the last four years, the Eastern Pipistrelle population has been the largest, followed in decreasing numbers by Little Brown bats and Big Brown (*Eptesicus fuscus)* bats. The population sizes were based on the observable number of individuals roosting within the chambers of Dunbar Cave that were surveyed. This study was designed to determine the numbers of each species present in the cave system during hibernation, emergence, and swarming seasons. Other focal points of the project were to assess the sex ratios of sampled bats per survey or banding date, site fidelity of any recaptured individuals, and the usage of space within each individual cave chamber.

Data were recorded for every individual that was captured. Bats were captured using three trapping techniques, due to the change in protocol during mid-study mandidated by federal and state agencies after detection of a diseased bat that was infected with *Geomyces destructans. Geomyces destructans* is the fungal agent associated with the bat-killing disease White-Nose Syndrome. Individuals were initially trapped using the harp-nets. During the winter hibernation in 2010, individuals were removed by hand and banded. During the swarming period in the late summer of 2010, bats were sampled using mist-nets method of sampling. All emerging and swarming bats were banded on the forearm, and released at the entrance to the cave during the summer and early fall months of 2009 and 2010. There was a total of 205 bats banded throughout the duration of the study, the majority of which were male *P. subflavus*. There were four species of bats trapped; *Perimyotis subflavus*, *Myotis lucifugus*, *Eptesicus fuscus*, and *Lasiurus borealis*. The sex ratio of the total sampled *P. subflavus* was disproportionate and more males than females were sampled collectively, with each survey type. Mating pairs were observed on five occasions. There were several reproductive female and juvenile bats sampled during the study. Cave surveys indicate a disproportionate use of cave chambers by the various species surveyed. This indicates that Dunbar Cave is an important swarming and mating site in addition to the winter hibernaculum for bat populations.

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CHAPTER I

INTRODUCTION

Introduction and General Information

The purpose of the project was to create a data base for the cave-dwelling bat populations at Dunbar Cave State Natural Area (DCSNA), in Clarksville, Tennessee. As bat populations across the northeastern United States have been affected by the bat-killing pathogen *Geomyces destructans*, which is responsible for the disease known as White-Nose Syndrome (WNS), bat-population monitoring has become a major conservation effort across the country. Researchers must regularly survey bat populations in order to effectively monitor the status of WNS at DCSNA and other known bat hibernacula in the state of Tennessee. This includes determining the species and the numbers of individuals of each species present in the cave system and in each individual chamber; these data can then be used to determine the species richness and densities of the bats present in the cave during hibernation. The sex ratio for the cave-dwelling bats at Dunbar Cave has never before been assessed. Determining sex ratios for the species present in the cave is an important management strategy, especially if this location is a summer maternity roost or fall swarming location. In many species, female bats form maternity colonies in late spring to increase the survival of their offspring. It is possible that Dunbar Cave is a maternity colony site for emerging female bats in late spring. Males will often isolate themselves from the female maternity colony and forage independently during spring and summer before returning to the swarming site, often located near the winter hibernacula, in late summer and early fall (Parsons et al., 2003).

It has been documented that males will sometimes utilize a separate summer roost location, forming bachelor colonies (Martin, et al., 2003). It is possible that Dunbar Cave is a summer bachelor colony for independently foraging male vespertilionid bats. Determining the sex ratio of the species of bats that roost, swarm, and forage in or near Dunbar Cave may allow researchers to better understand the reason for the seasonally differing sex ratio. During late summer and early fall, males and females will exhibit a swarming behavior at specific resource-rich locations (Parsons, et al., 2002). Determining the sex ratio of the bat species present at Dunbar Cave will help to determine if this is a potential or developing maternity colony, bachelor colony, or swarming location. Another main goal for the project was to capture, band, and release individuals to determine seasonal sex ratio in the cave and within individual chambers during hibernation. Both Eastern pipistrelles (*Perimyotis subflavus*) and Little Brown bats (*Myotis lucifugus*) are species of vespertilionid bats that have previously hibernated in the chambers of Dunbar Cave (Matthews, 2005). Researchers from Austin Peay State University's Center of Excellence for Field Biology have been monitoring the bat populations at DCSNA for the past five years, with efforts directed at investigating cave tour impacts on the roosting bats and monitoring the return of these bats to the cave as a useful winter hibernacula or as a resource-rich swarming site during the fall months. Gathering this information will benefit future conservation efforts and research on the cave-dwelling bats at this site.

These are the main predictions made using the data from previous studies, field surveys, and literature reviews:

1. The population of *P. subflavus* will be larger than *M. lucifugus* and other bat populations hibernating in the cave throughout the study;
2. The cave chambers with the highest mean number of individuals will be chambers that are not on the path of the cave tours directed by Dunbar Cave park staff;
3. The sex ratio of males to females will be equal in both Eastern pipistrelles' and Little Brown bats' populations;
4. Bats exhibit some degree of site fidelity to Dunbar Cave as a winter hibernacula; and
5. Dunbar Cave is not only a hibernaculum, but also an important site for mating activities.

Background Information of Dunbar Cave

Dunbar Cave (Figures 1 and 2) is a state natural area located in Clarksville, Montgomery County, Tennessee. Many public recreational activities take place at Dunbar Cave, such as nature awareness programs, cave tours, fishing, and bird watching. According to Dunbar Cave staff, prior to the 1970's, thousands of bats inhabited the chambers of Dunbar Cave (Matthews, 2005).

Concerts and numerous other public events were commonly held at the entrance to the cave. The cave itself was accessible to the public, but bat populations still thrived. Unfortunately, in the late 1970's vandals set fire to numerous nuclear fallout shelter supplies stored in the cave, creating toxic smoke that filled the chambers of the cave system (Figure 3). After the fire, Dunbar Cave staff and members of the National Speleological Society found thousands of bat skeletons in a major exit/entrance passage, now known as Bat Bone Passage. To this day, bats have seldom been observed roosting in the chamber where the fire originated. Almost all of the known bat populations were eradicated by the fire, and for over fifteen years bats were not known to inhabit the cave (Amy Wallace, park staff personal communication). DCSNA recently installed a "bat-friendly" cave gate to protect the cave and bat populations from human disturbances and vandalism. Such gates protect bat populations, but must be constructed to fit emerging bats with a suggested width between horizontal bars that is greater than 150 mm (Pugh, 2005). Over the last three years, various researchers at Austin Peay State University's Center of Excellence for Field Biology, with the help of Dunbar Cave staff, have discovered a fragile bat population that hibernates in specific chambers of the cave during the late fall and winter months.

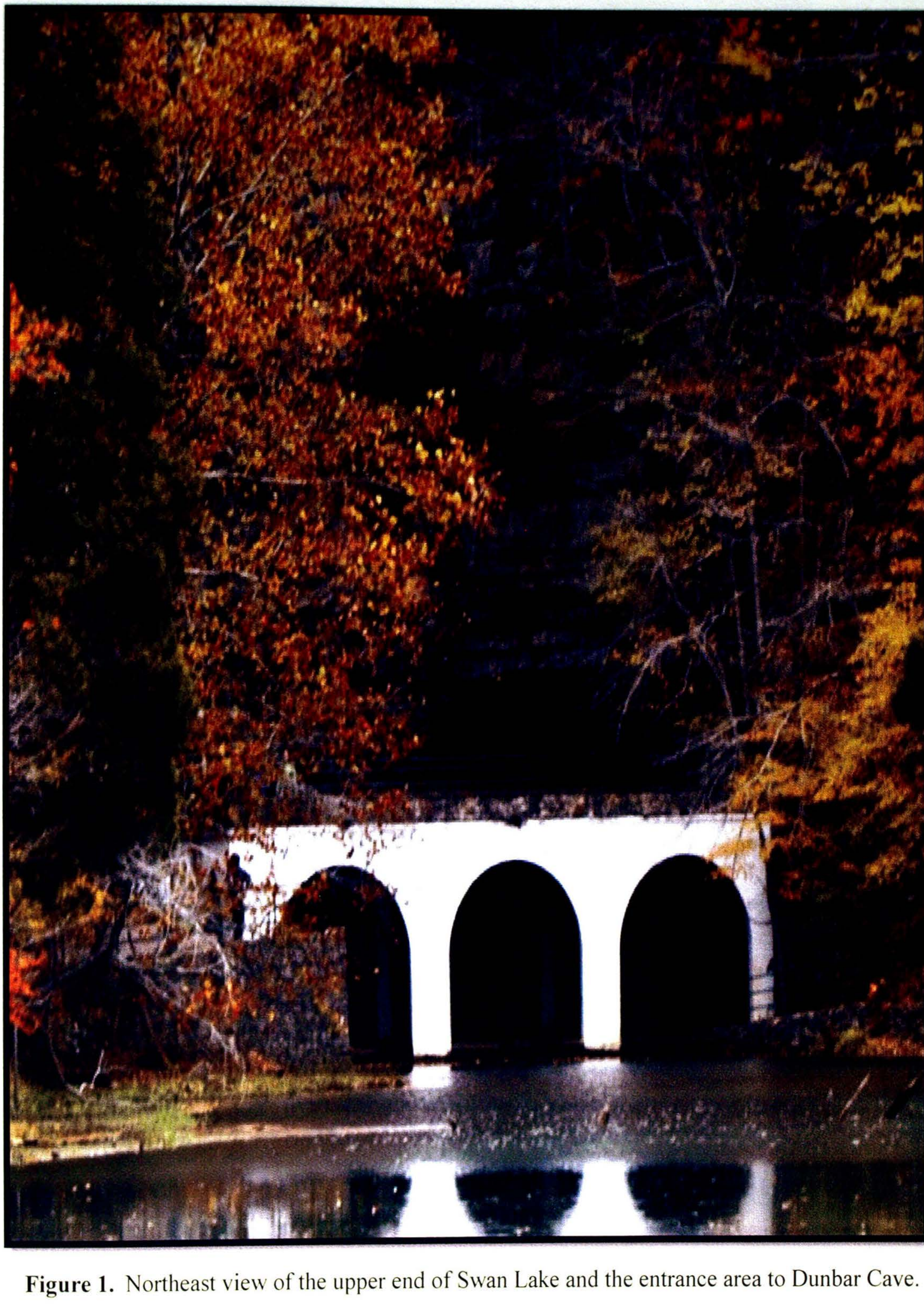

Figure 1. Northeast view of the upper end of Swan Lake and the entrance area to Dunbar Cave.

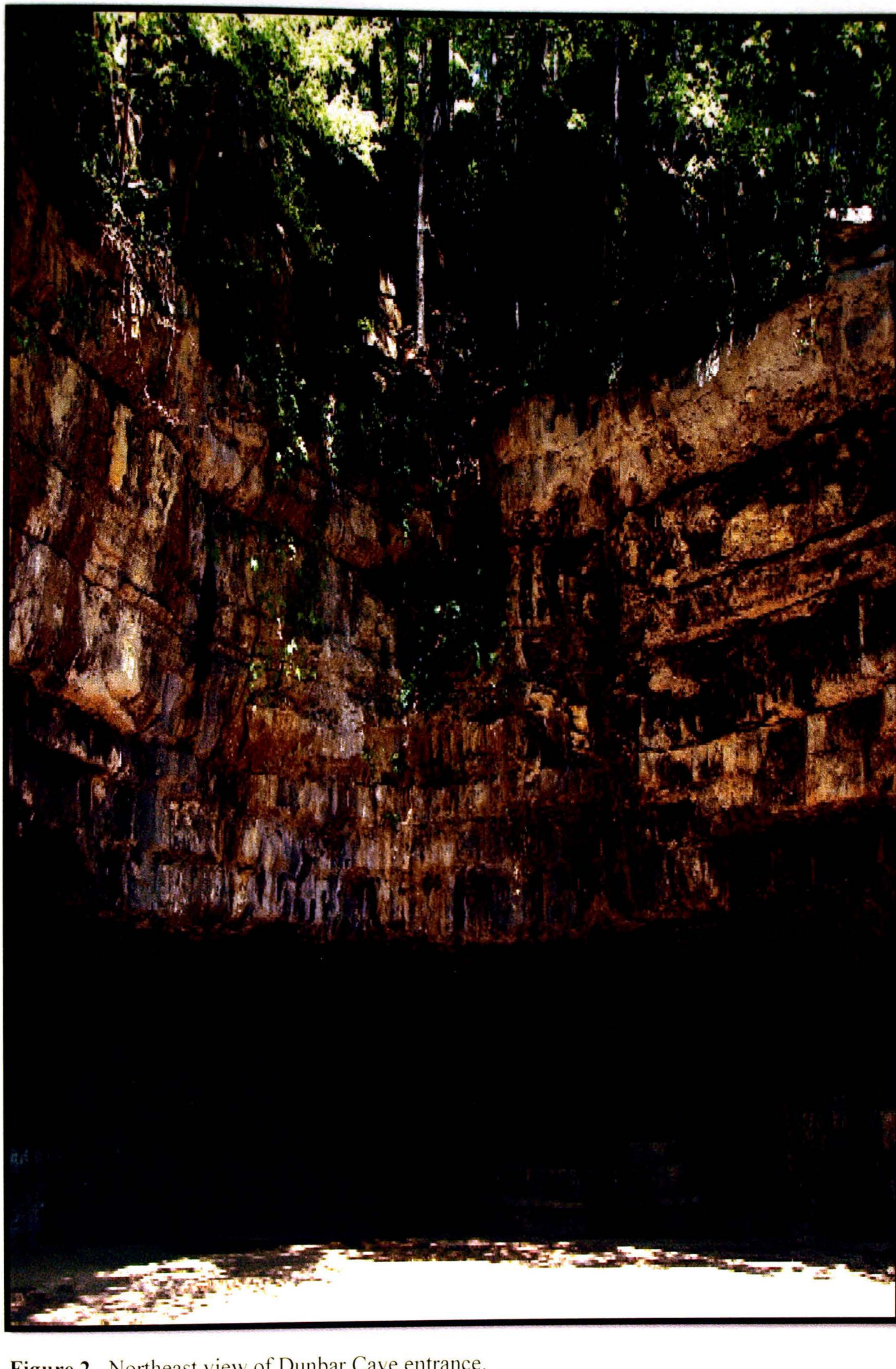

Figure 2. Northeast view of Dunbar Cave entrance.

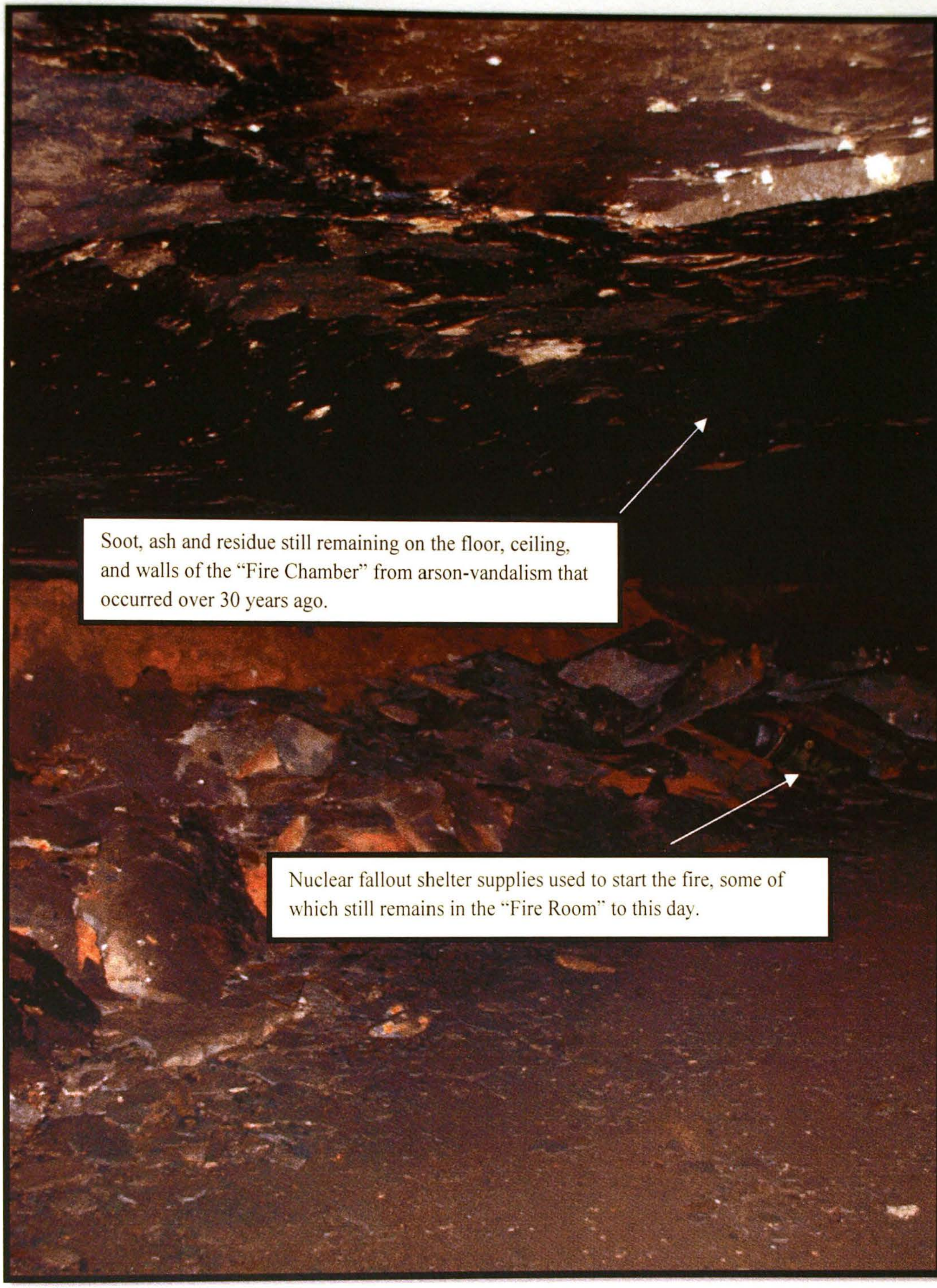

Figure 3. Fire Room chamber, over thirty years after the fire was set by vandals.

Eastern pipistrelle (*P. subflavus*) populations have been the largest, Little Brown bats (*M. lucifigus*) the next largest, and Big Brown bats, (*E. lucifugus*) the smallest. There were less than 90 bats observed roosting in the cave system during hibernation, over a three-year observation period. In late August 2007, however, a single feral cat eradicated over half of the known bat population over an eight-day period. The cat was eventually trapped and retrieved by wildlife officials. It was important to the cave recovery project to determine the number of bats and species that are present after the bat massacre of 2007.

Cave tours can negatively affect cave-dwelling bats that are roosting in chambers, which are exposed to frequent human-related disturbances. Factors such as light-exposure, noise levels, and the approximate size of cave tour groups have been documented as disturbances associated with public cave tours. Some studies show that cave-dwelling bat populations will increase when recreational cave touring or human access has been restricted (Stihler and Hall, 1993). The individual energy reserve or conservation of fat stores of hibernating bats is vital for survival throughout winter months. When cave tours persist, however, bats will use this energy for movement away from sound and light, and will also make audible distress and aggression calls in response to the disturbances (Speakman et al., 1991; Thomas, 1995). Bats require almost twice the metabolic energy for survival than other land mammals (Thomas, 1975). The intensity of light exposure and frequency of cave tours along with increased noise levels from tour groups have negative impacts on the roosting and hibernating bats (Mann et al., 2002). Previous studies also indicate that cave tours should be prohibited during critical roosting periods, such as hibernation in winter months and maternity roost colonization from early June to late July (Mann et al., 2002).

General Information on the Cave-dwelling Bats in Dunbar Cave

Bats are unique to other mammals in many ways, but the most exceptional of these traits is the fact that bats are the world's only true flying mammal. The vespertilionid bats are commonly known as the Old World bats and belong to the suborder Microchiroptera. They are generally small to medium sized bats, and use echolocation to hunt and communicate. There are over 267 species of these bats, and they are distributed worldwide (Simmons, 2005). Eastern pipistrelles and Little Brown bats are some of the most prevalent and common species of vespertilionid bats found in the eastern parts of North America (Briggler and Prather, 2003). They are known to hibernate during late fall and winter months and demonstrate tree roosting in spring, summer, and early autumn (Sandel, 2001). Mating behaviors and copulation take place in the fall before hibernation and parturition occurs in spring (Wimsatt, 1945). During winter months, cave-dwelling bats such as the Eastern Pipistrelle and Little Brown bat, often hibernate in old buildings, houses, rock crevices, and caves (Barbour and Davis, 1969). Temperature stability is an important factor in determining hibernacula roost site location (Briggler and Prather, 2003). Caves often offer stable temperatures throughout the year.

Regardless of common perception, bats are not blind. Rather they have generally good eyesight, but since most species are nocturnal, hunting mainly at night, eyesight is a poor tool for hunting prey effectively. Instead vespertilionid bats use echolocation, also referred to as biosonar. Bats will emit pulses and calls in the form of high frequency wavelengths. These emissions will literally echo off of objects in the environment. The echo or bounce back of the acoustic pulses creates a 3-dimensional image or map of the environment as well as indicates the presence of predators and prey. The initial acoustic pulses come from the larynx through the mouth and nose. Most of these calls are beyond human audibility. Prey location requires the use of lower pulse rates, while distress and aggression calls are emitted at a much higher rate (Speakman and Racey, 1991). Distress, mating, and aggression calls are audible to the human ear.

Bat species in general have a long lifespan and high survivorship rates when compared to other terrestrial mammals. This may be attributed to the fact that temperate region bats hibernate for more than 50% of each year. Essentially, a hibernating bat that has lived for ten years has only utilized metabolic activity necessary for five years of active life. It should be noted that tropical and Neotropical bat species that do not hibernate also have remarkably long life spans. This may be accredited to the lower predation risk during bat nocturnal activity peaks while foraging (Simmons, 2005). Bats do not have primary predators with the exception of some owls, hawks, snake species, and both feral and domestic cats that are localized near bat populations and hibernacula sites (Winkler and Adams, 1972).

Eastern pipistrelles (*P. subflavus*), also known as the Tri-colored bat (Figure 4), is the chiropteran species that is most numerous in Dunbar Cave during winter hibernation. *P. subflavus* are widespread throughout the Eastern United States, Mexico, and Canada as are Little Brown bats (Figure 5), which are also known to hibernate in Dunbar Cave (Figures 6 and 7).

Figure 4. Eastern pipistrelle, *P. subflavus,* roosting in the Hallway chamber of Dunbar Cave.

Figure 5. The Little Brown bat, *M. lucifugus*, is a common species of bat found roosting in Dunbar Cave.

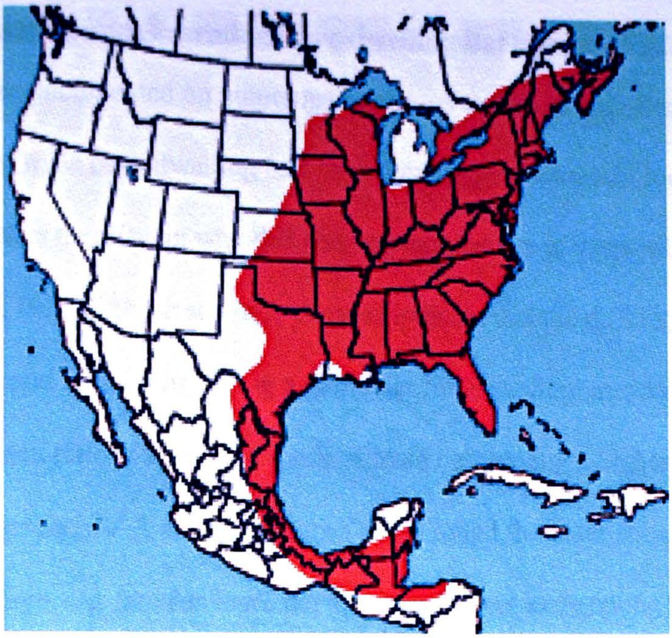

Figure 6. Range Map of *Perimyotis subflavus* in United States. "North American Mammals: *Myotis lucifugus*." *Smithsonian Institution National Museum of Natural History NMNH.* Web. 01 May 2011. (http://www.mnh.si.edu/mna/image_info.cfm?species_id=283).

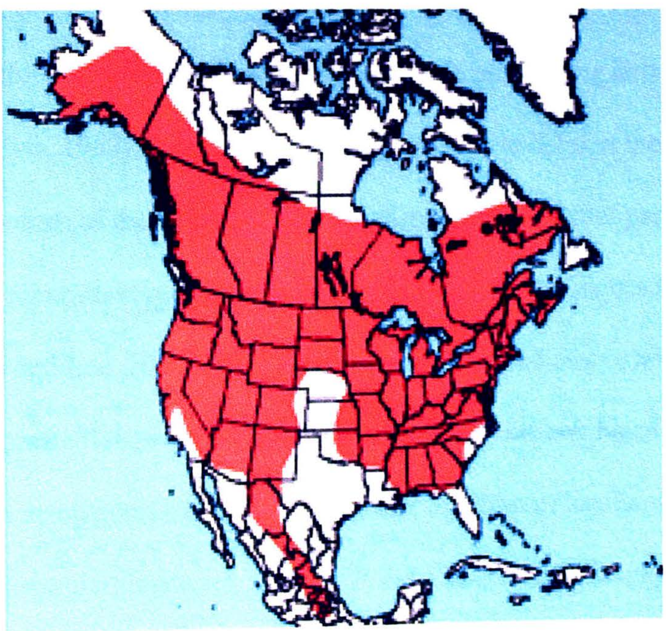

Figure 7. Range Map of *Myotis lucifugus* in United States. "North American Mammals: *Myotis lucifugus*." *Smithsonian Institution National Museum of Natural History NMNH.* Web. 01 May 2011. (http://www.mnh.si.edu/mna/image_info.cfm?species_id=199).

Sex Ratio of Roosting and Swarming Cave-dwelling Bat Species

It has been documented on numerous occasions that there are often a substantially greater number of male cave-dwelling bats than females at hibernacula locations. There have been numerous theories on why this phenomenon occurs in vespertilionid bat species (Barclay, 1991). These sex ratio theories indicate that the difference in the number of males and females present at a particular hibernaculum may be the result of a disproportionate sex ratio at birth, or a result of male bats having a higher survival rate in the cave-dwelling species. A study by Davis (1959) found the latter reason as a cause for the unequal sex ratio. As females leave the hibernaculum in early spring to establish maternity colonies, males will often remain in torpor, the inactive state of hibernation for a longer period of time, well into the summer months. This means that the males are not faced with foraging obstacles and predation threats to the extent that the females have to overcome. Therefore, the average survival rate for male hibernating bats is higher than it is for females (Davis, 1959). Females must forage enough to support their metabolism as well as the metabolism of the unborn offspring when pregnant. After parturition, females will nurse young for up to seven weeks until proper foraging techniques have been established (Shen and Lee, 2000). This is a constant metabolic cost, which also may contribute to the greater longevity of male bats' lifespan. This combined with the probability that in many populations, females winter at different locations than do males, may explain the disproportionate sex ratios of *P. subflavus* and *M. lucifugus* that have been observed in roosting bat populations in past studies (Davis, 1959; Barclay, 1991).

During late summer and early fall, temperate-zone vespertilionid bat species often exhibit a behavior in which multiple species will utilize the same resource-rich site, known as swarming (Parsons, et al., 2003). This behavior occurs in middle to late August through October for the bat populations at Dunbar Cave. While swarming has been observed by researchers and documented on numerous occasions, the reason for this behavior has yet to be confirmed (Parsons et al., 2003). There are many hypotheses regarding swarming behaviors and the sex ratio of these swarming bats. Most all species that exhibit the swarming behavior are cave-dwelling bat species that winter in stable hibernacula such as Dunbar Cave. The swarming of bats takes place at or near the cave or winter hibernation location. Swarming often encompasses multiple species, most of which spend the winter at that site (Davis, 1959). There are usually many more males compared to females during this swarming behavior and at the site of the swarm. It would be beneficial to males, which have undergone spermatogenesis and are ready to reproduce as this is optimal copulatory and mating season (Fujita and Kunz, 1984). Dunbar Cave is a unique swarm site because it is extremely rich in resources needed by reproductively active and soon-to-be hibernating bats. Dunbar Cave is located directly behind a small impoundment called Swan Lake. The cave is surrounded by the forested and protected state-park grounds. This is an optimal protected foraging site with an ample water supply directly in front of the hibernaculum. Swarming male bats at this site would have a high chance of mating, foraging, and having access to water and a stable hibernaculum (Fenton and Barclay, 1980). This makes Dunbar Cave a favorable swarming site for individuals. While Dunbar Cave offers a moist and stable roost site, it has been noted in previous studies that there is low site fidelity of swarming bats to the hibernaculum. This means that there is a mixing of migratory bat species and individuals, many of which do not hibernate at the swarming location. It has been hypothesized that this promotes mixing of the gene pools and increases outbreeding, which is highly beneficial to the population (Parsons et al., 2003).

Reproductive Biology of Vespertilionid Bat Species Present in Dunbar Cave

The reproductive cycle of the vespertilionid bat is complex. Most temperate species mate in the fall months, from September to November (Guthrie, 1933; Wimsatt, 1945). While copulation and insemination occur before hibernation, female bats demonstrate delayed ovulation, and do not release the ova until spring (Pearson and Koford, 1952). The sperm are stored in the uterus and kept viable until emergence from the hibernaculum when the female will undergo ovulation, gestation, and parturition. During the hibernation period, females are said to be in a state of sub-estrus with the Graffian follicle continuously growing in preparation for ovulation in spring (Krishna and Abhilasha, 2000). Male bats will experience withering of the genitals during hibernation, and spermatogenesis ceases until spring (Fenton and Barclay, 1980). The purpose of the review is to examine the general reproductive cycle, mating behaviors, copulation, gestation, and parturition of the temperate vespertilionid bats.

General Reproductive Cycle

Female microchiropteran bats are usually larger than the males (Myers, 1978). Some studies correlate this with the information that the female lactates and increases foraging activities with food intake to make and produce milk (O'Donnell, 2002). The energy loss of a lactating female is less harmful if the female is larger and has stored more fat during the foraging period prior to late fall hibernation (Myers, 1978; Koehler and Barclay, 2000). Females also carry the offspring on their backs or under their wings until they can fly on their own, which also requires more food intake (Myers, 1978). According to Sendor and Simon (2003), females often exhibit a higher seasonal survival rate than males. There are some details of the reproductive cycles among species of vespertilionid bats that vary to a certain degree, but the temperate species of these bats do share most traits.

Microchiropteran bats are polygamous. Males and females will mate opportunistically and frequently during the mating season. Females are mounted and inseminated in the fall, prior to the onset of hibernation (Fujita and Kunz, 1984). During this time, Graffian follicles are formed in the ovary and the follicle will slowly begin to enlarge and develop (Guthrie, 1933). This will ensue through hibernation but the follicle will not become terminally mature until spring emergence (Krishna and Abhilasha, 2000). The male will undergo specific changes during hibernacula as well. Male bats will experience withering of the genitals. Unlike tropical bats that are able to mate during the other seasons of the year, temperate bats will undergo a significant weight loss percentage of their fall testes mass during torpor (Tamsitt and Valdevieso, 1965). The male accessory glands are still functional. The caudal epididymis is filled with mature sperm as winter begins and stores the semen there until spring emergence. However, the testes and the seminiferous tubules are inactive through winter and shrink in size (Avery, 1985). Male bats do not produce any new spermatozoa during hibernacula.

Courtship and Copulation Behavior

The courtship behaviors of the vespertilionid bats are rather simple and without intense displays or visual cues. It is not energy efficient to present an elaborate dance or song to a female when the males will mate so frequently. Male and females will swarm, often near prospective hibernacula during or prior to the mating season (Schowalter, 1980). Immediately after the mating season ends in late fall, the bats will enter a state of torpor until spring. Mates are located by a specific call, and the female will emit a series of click-like sounds while the male faces her and paces briefly before assuming the copulatory position. The female bat will remain in a passive state, showing no aggression or resistance while roosting. This is how she behaviorally indicates reception to the male's actions prior to copulation. Like many other mammals, bats mate in the position referred to as *coitus a posteriori,* with the male mounted behind the female (Wimsatt, 1945). Copulation can last for several minutes, and in some species, longer. Wimsatt (1945) reported, "*The male grasped with his teeth the hair of the female at the base of the skull and pulled her head far back, usually at a right angle to her body. The male strengthened his grip by using his thumbs to hold her body in place, and then pushed his hindquarters backward and downward around the rump of the female, bringing his protruded penis beneath her interfemoral membrane.*" Numerous sources (e. g. Cockrum, 1955) have witnessed incidents of late and early mating during the hibernation season, and copulating pairs have even been found sleeping while still in *coitus a posteriori*. Males will also often mount other males as the mating season comes to an end. Most active sperm is released earlier in the mating season, and females will often enter hibernacula after copulation and when food sources become less available, leaving males attempting to mate with other males (Barclay, 1989). It is a last attempt of males to copulate and pass on their genetic information to the future generation. Determining the reproductive behaviors and gestational period for a group of animals, such as the bats in this study, is a useful way of studying and understanding population characteristics such as the disproportionate sex ratio, which is exhibited by many vespertilionid bat species.

Ovulation, Gestation, and Parturition Behaviors

As the temperature drops and winter settles in, bats will re-locate towards a hibernation roost location. Previous studies have shown that both temperature and rainfall influence reproductive activities and the timing of torpor (Grindal et al., 1992). The female will have obtained sperm during the fall mating, and will store it in the uterus until emergence the next spring. The Graffian follicle will grow and enlarge until it reaches maturity, at which point it will be ovulated (Krishna and Abhilasha, 2000). Some species such as those that belong to the genus *Myotis*, will release one ovum. Other species, such as the Eastern pipistrelles, give birth to twins, and can release two to seven ova at one time. The twin offspring can represent anywhere from one third to one half of the mother's maternal body weight by the time they are born (Fujita and Kunz, 1984). The gestation period can vary slightly from species to species, but generally the gestation period lasts 40-50 days after implantation (Wimsatt, 1945). Gestation usually occurs in spring and early summer. Physiological fetal development of bats basically follows the stages of fetal development in other mammals. Parturition occurs in early to late summer (Kunz, 1974). Females will emerge from hibernation and form maternal colonies with other females in foliage such as oak trees (Veilleux and Whitaker, 2003). Parturition in the Old World bats is a fairly short process. When giving birth, the female will orient herself opposite to the normal roosting position, with her head facing up. The interfemoral membrane is positioned to cup the emerging offspring (Wimsatt, 1945). Most female vespertilionid bats give birth within 15-30 minutes after the first contractions begin. There are accounts of longer birthing times, but these incidents almost always produced stillborn offspring. The young are born without fur, and depend on the mother's body warmth for survival until they grow their own coat. While they are bald and blind, they can emit audible vocalizations almost immediately after birth (Fujita and Kunz, 1984).

Juvenile Development

Vespertilionid juveniles have the ability to fly when they are about three weeks old. During this time, young are groomed vigorously by the mothers and nursed often. If the mother needs to leave the maternity colony for any reason before the pup is able to fly on its own, the pup will attach itself to the nipple or chest of the mother while in flight (Davis, 1970). The mothers will press muzzles with young present in the maternity colony until their offspring is identified (Shen and Lee, 2000). They are weaned from the mother's milk by seven weeks of age. From the fourth to seventh week, juveniles develop foraging techniques and will begin to hunt independently of their mothers (Koehler and Barclay, 2000). Sexual maturity for both males and females is reached between 2-11 months of age, depending on the species and geographic location (Fujita and Kunz, 1984).

Vespertilionid bats have unique reproductive cycles and characteristics. They are polygamous and promiscuous. Copulation occurs in fall, prior to hibernacula. Ovulation, gestation, and parturition occur when the bats emerge from torpor. Courtship behaviors are minimal, and males do not contribute to post-natal care of the young. The females form maternity colonies with other females in spring and give birth after a 40-50 day time span. After four weeks, the juvenile bats will gain abilities to fly and hunt. Generally, they will reach sexual maturity within the first year. Some species give birth to one offspring per season, such as the Little Brown bat, and some species have twins, or two pups per pregnancy, such as the Eastern pipistrelle (Whitaker, 1998). Some species-specific mating behaviors and details have not yet been described, for instance, details in exact mating time, gestation, and parturition vary among species. However overall, the reproductive cycles of the vespertilionid bats are quite similar.

The information covered in this review is relevant to the understanding and protection of vespertilionid bats, such as the species that are located in DCSNA in Clarksville, TN. Recent observations of mating pairs in this cave system have been observed and recorded (Figure 8); these mating observations are rarely reported in the literature. This brings focus to the reproductive requirements, mating behaviors, and the sex ratios of male and female bats and the need to minimize human disturbances as much as possible during these critical times.

Figure 8. A mating pair of *P. subflavus* in Dunbar Cave taken April 2009.

Emergence of White-Nose Syndrome in the United States

White-Nose Syndrome is a fungal disease associated with temperate-zone hibernating bats in North America. This disease is caused by *Geomyces destructans*, a fungal pathogen associated with the loss of 99% of infected populations of bats in the eastern portion of the United States since its discovery on this continent in 2006 (Frick et al., 2010). Previous studies indicate that bats have exceptionally longer life spans and higher survivorship in adulthood than do other terrestrial mammals (Simmons, 2005). With the recent emergence of White-Nose Syndrome, these conclusions are no longer accurate. Bats make up over 20% of the total mammalian diversity worldwide; it is of great importance to focus conservation efforts to prevent the collapse of bat populations, communities, and associated ecosystems throughout North America and potential extinction of already federally listed endangered species (Simmons, 2005).

White-Nose Syndrome is known to affect cave-dwelling bat populations that hibernate through the winter months. *Geomyces destructans* is a cold-loving fungus newly described in the United States (Frick et al., 2010). The first documentation of infected bats is from February 2006, from Howe's Cave located in New York State, just west of Albany (Blehert et al., 2008). Since its discovery in New York, White-Nose diseased bats have been confirmed in Canada, Vermont, Pennsylvania, Massachusetts, New Jersey, Maryland, West Virginia, Virginia, Tennessee, Missouri, and Oklahoma (Frick et al., 2010). Currently, there is no treatment and no cure for infected bats. Populations collapse after infection with the fungus and have a mortality rate of nearly 100 % at most infected hibernacula. The emergence of the disease has renewed interest in bat conservation across the United States.

Geomyces destructans is a member of the Kingdom Fungi, Phylum Ascomycota, Class Leotiomycetes, Order Heliotiales, Family Myxotrichaceae, Genus *Geomyces*, and Species *G. destructans*. It is a fungus that grows optimally in cold, wet environments, such as in cave systems in the eastern and middle United States. The fungus grows optimally at 5 degrees Celsius to 10 degrees Celsius. This fungus exhibits a moderate growth rate at temperatures as low as 2 degrees Celsius and at temperatures greater than 15 degrees Celsius. Temperatures in most WNS confirmed caves range between two degrees Celsius and 14 degrees Celsius throughout the year (Blehert et al., 2008). This allows the fungus to successfully infect bats hibernating in caves that meet these temperature ranges in the winter, as well as during late fall and early spring months. Although most hibernating bats in the temperate regions of the United States exhibit tree-roosting in the summer and do not roost in the cave environments, the ability of *G. destructans* to survive the spring and summer months in the caves makes these roost sites reservoirs for the fungus (Blehert et al., 2008).

Geomyces destructans causes a cutaneous fungal growth in infected individuals. The term White-Nose Syndrome is a quite descriptive name for the disease as infection presents white fungal growths on the muzzle, ears, and wings. The fungal hyphae attack, grow, and eventually cover the epidermis of the infected region. The fungal hyphae replace the hair follicles, and will fill the sebaceous and sweat glands. The fungus will then invade the underlying tissue of the infected area on the bat (Blehert et al., 2008). The growth of this fungus on the membranous surface of the bat causes irritation and infected individuals frequently will wake and groom during hibernation periods, using critical energy reserves to scratch and groom themselves (Blehert et al., 2008). The fungal pathogen causes a reaction in the immune system of the hibernating bat that further depletes energy reserves of the individual (Frick et al., 2010). Often infected bats are found emaciated, either because of the constant skin irritation caused by the infection or because the infected bat will awake during torpor in seasons that don't harbor sufficient insect populations, as in winter months, in a vain search for food needed to replenish energy used in fighting the disease. Although much is still unknown about the infection caused by *G. destructans*, it has been confirmed that over one million bats in the eastern and middle portions of the United States have died from infections of the fungal pathogen.

During the March 2010 survey, a Little Brown bat was observed roosting in a crevice in the Hallway chamber of Dunbar Cave. The bat's wing membranes and forearm were mottled with what appeared to be a white, powdery substance (Figure 9). Federal and state protocols demand that researchers do not handle the bat, but instead immediately contact state officials. The bat was photographed and documented by researchers and euthanized by wildlife officials. The specimen was sent to a laboratory for diagnostic testing for *G. destructans*. Dr. Ann Ballman, USGS, National Wildlife Health Center, performed the diagnostic testing and confirmed that the bat was infected with *G. destructans*. The fungal pathogen was isolated from the specimen and Dunbar Cave was labeled a White-Nose Syndrome positive site. The cave was closed to the public and to researchers until necessary approval from federal agencies was obtained. The methods for handling and capturing bats in and near Dunbar Cave were revised to meet the new permits. Although researchers were allowed back in the cave in May, surveys were unable to be performed until July due to the extensive flooding of the cave and surrounding area in early May 2010.

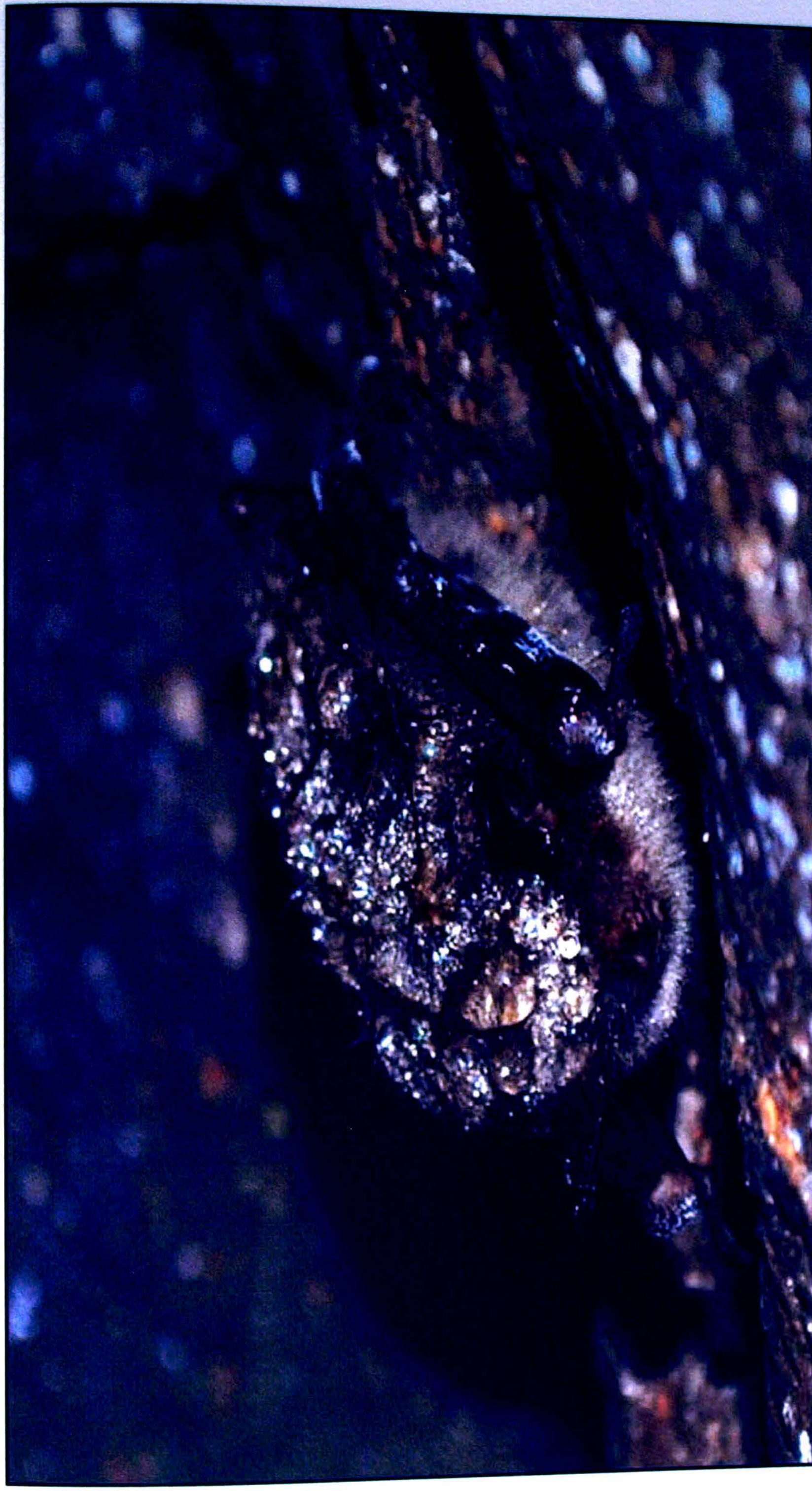

Figure 9. The Little Brown bat in Dunbar Cave that tested positive for *G.destructans*, the causative agent of White-Nose Syndrome. Dr. Ann Ballman, USGS, National Wildlife Health Center, confirmed the identity of the pathogen (personal communication, March 2010).

CHAPTER II

METHODS AND MATERIALS

Methods of Observation

The study was performed at DCSNA, in Clarksville, Tennessee. The observational methods included cave surveys and counts of individuals present within cave chambers and roosting areas throughout the year. Surveys were performed monthly (June 2008 to September 2010), and six banding dates were permitted for each year of the 28-month, agency-permitted observational study period. Surveys lasted for approximately three hours and took place in the morning hours. Individual cave chambers were searched thoroughly for the presence of any bats. Appropriate head-protection gear, flashlights, and cave equipment were used during the surveys to ensure safety of participants and researchers. The species, and when permitted, the sex and number of bats present in each chamber were recorded for each survey date.

It was important to document not only the number of bats in the cave chambers, but also the sex ratio of observed individuals. This information may help to determine if Dunbar Cave is a maternity colony to reproductive females. Both cave-tour exposed chambers and isolated chambers were surveyed for signs of maternity colonization. The highlighted chambers (Figure 10) were also surveyed for signs of bachelor colonies, in which all or most samples are males within the specific chamber. The trapping of both male and female bats in certain cave chambers during early hibernation or late in the spring emergence may indicate late/early mating activities. Observations of mating pairs in such chambers would further indicate that the cave is a hibernaculum used by reproductive bats.

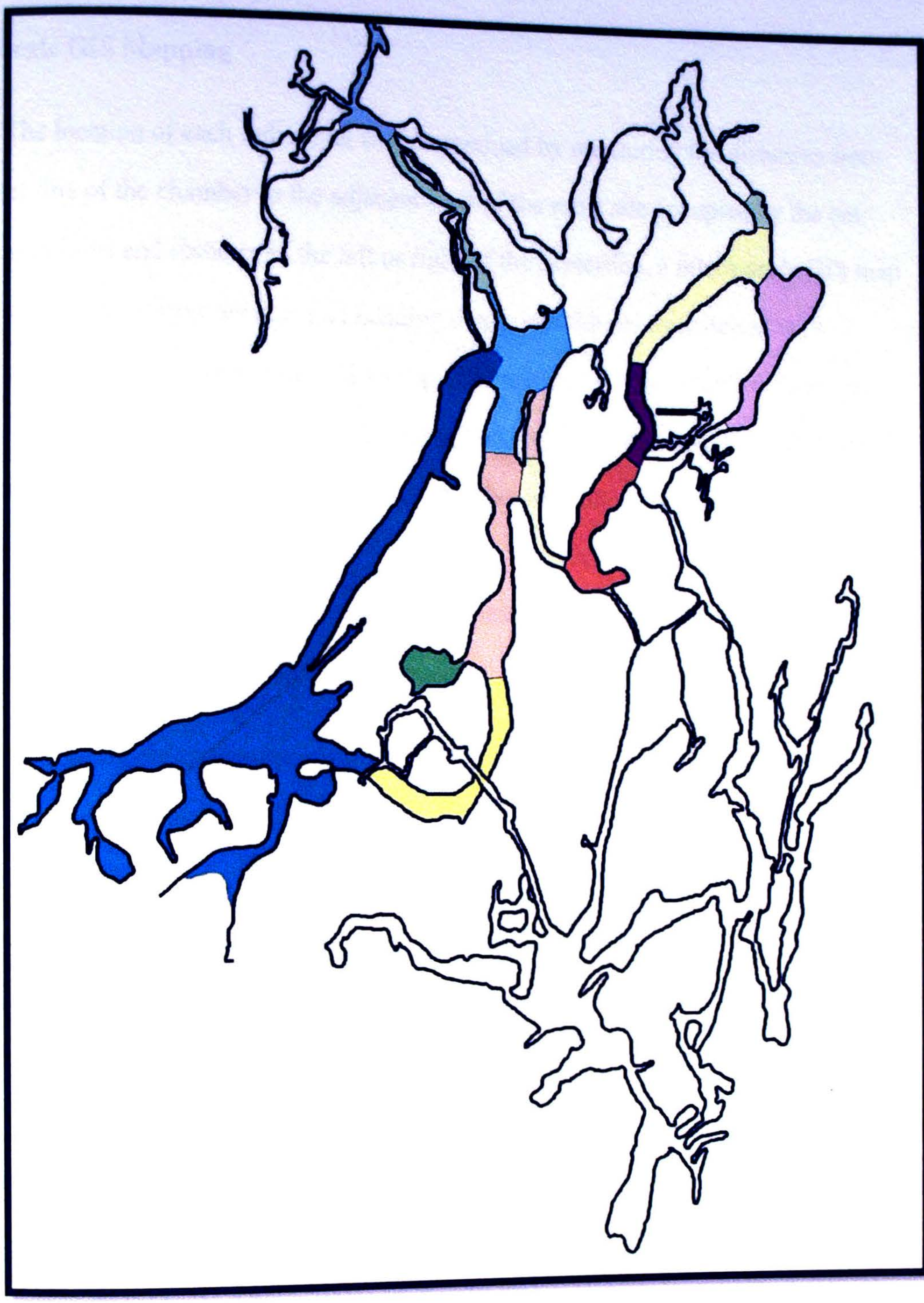

Figure 10. Microscale GIS map of the highlighted survey chambers of Dunbar Cave.

Micro-scale GIS Mapping

The location of each individual was determined by measuring the distances from the center line of the chamber to the adjacent wall of the roost site occupied by the bat. Using the heights and distance to the left or right of the centerline, a micro-scale GIS map was created. During cave surveys and banding dates, individuals were dotted with animal safe paints used to assign individual specific color-codes that identified the sex and the individual. This was done in addition to banding sampled bats. The individuals' roost sites were plotted on the corresponding chamber map, in raw data maps (R. Seth McCormick, personal communication). These data were recorded and used throughout the study to illustrate the site fidelity, species richness, and sex ratio of bats present during hibernation just prior to spring emergence

Fifteen chambers were surveyed during the study, six of which are located on the path of the cave tours given by Dunbar Cave staff. The other nine chambers are isolated from human interferences, other than the surveys performed during this study. There are many water traps, sediment slides, and boulder barriers in Dunbar Cave making it unsafe, and in some cases impossible, to survey some chambers. Such barriers also make the chambers inaccessible to bats. Therefore these chambers were not surveyed.

Field notes were recorded for each survey date including ambient temperatures at four checkpoints, weather conditions, time of day, and the presence of other cave biota. Digital images of the bats were obtained using a Nikon D-60 camera and a Prowler STC-DVIR5 stealth camera which was positioned at the cave entrance. Stealth cameras will take photographic images when the motion trigger has been activated. This allows for documentation to occur while the researchers are not in the field or at the study site. Any mating behaviors or copulatory behaviors observed were recorded for each survey date in the field notes. Audible vocalizations from active individuals were noted throughout the surveys. Any potentially disturbed individuals or erratic movements of bats, as well as accompanying behavior(s), away from research participants were also documented during observation surveys.

Spring and Summer Trapping and Banding Methods

Bats were captured during late spring and early summer months of 2009 and 2010. All spring emergence banding and summer swarming banding took place during the time of evening emergence prior to and during feeding hours. Bats were captured using an eight foot Cave-catcher 36"x44" G5 bat harp trap, acquired from www.batmanagement.com. The harp trap was positioned in front of the cave gate. These traps are effective and cause little harm to captured bats (Alvarez, 2004). Only research assistants that have received the rabies vaccination handled the bats; others involved were responsible for data collection and recording information on the data sheets. Bats were banded using aluminum alloy, 2.9-mm, Porzana rounded-edge bands each with a specific code, for example M0098, and an Austin Peay State University (APSU) label. Bats were weighed in appropriate breathable containers, sexed, and the reproductive state (if any) noted. The descriptive statistics for sampled bats were calculated for the weights of male and female bats per sampling method. Females were banded on the opposite forearm than the males. Males received a band on the right forearm, while females were banded on the left. This allowed researchers to determine the sex of a banded bat that may be roosting out of reach or too high to read the band identification number. Individuals were released at the same location where they were captured. Banding is an improved method for marking as opposed to former methods such as punch-marking individuals. The banding method causes little to no damage and is a more effective marking method because the punch-marking method often scars the wing membrane and the mark is often no longer identifiable after healing (Bonaccoroso et al., 1976). The harp trap was used to sample individuals on seven of the eleven banding dates. These surveys were performed on May 18, 2009; on June 2nd, 8th, and 22nd, 2009; August 12th and 25th 2009; and October 26th, 2009.

Late in the hibernation period of 2010, two dates were selected to trap and band bats that were found roosting within the cave. All dates for trapping and banding were prior approved by the permitting agencies. These banding sessions were performed on 18 and 29 April 2010 following normal survey protocols, which included searching each selected chamber for the presence of any bats. If a roosting bat was sighted but was out of reach of the hand-held net or was still covered in condensation, they were not captured and banded. This method is often used to sample bats in the hibernaculum (Mills et al., 1975). All bats were mapped per chamber. Bats covered in condensation are often still in a deep hibernation or have not been aroused yet for emergence. Bats were also captured and tagged outside the cave during the summer swarming of 2010. Due to the confirmation of Dunbar Cave harboring an individual bat which tested positive for *Geomyces destructans* in May of 2010, a different method of sampling the bat populations was implemented during the swarming sampling period. Instead of using the previously described harp trap, two mist nets were placed in front of the cave gate and parallel to the lake prior to the evening emergence of bats. Some studies have shown that trapping with harp traps is more successful than using mist nets to capture bats (Francis, 1989). However, due to the concern for the spread of White-Nose Syndrome through Dunbar Cave and the surrounding bat populations, mist nets were implemented in order to follow U.S. Fish and Wildlife protocols for White-Nose Syndrome positive caves. Mist nets are useful for capturing bats and assessing the sex ratio, age class, and species richness or diversity of the bat populations present during the late summer swarming (Miller, 2003); (Weller, 2007). There are a number of different sizes of mist nets used to capture bats, ranging from 40'x10' down to smaller hand-held nets referred to as an H-net (Waldien and Hayes, 1999). The mist net that was used was a Hot Foot Mist–net 40 that measured 25'x10'. It was erected with 1.5 inch diameter PVC pipe supports standing

10 feet in height. Iron umbrella bases were purchased and used as a sturdy base for each PVC pipe to ensure stability of the mist net set-up. Holes were drilled two feet apart along both PVC pipes to attach the mist net to the support poles. The mist net was positioned in front of the cave gate and checked for bats every few minutes. Some studies have indicated that checking the nets often can increase the success rate of capturing bats, (MacCarthy et al., 2006). Once a bat was retrieved from the net, it was processed and banded as described above, with the relevant information recorded on the banding data sheet designed for this project (Appendix B). Any bat that was suspicious as a positive White-Nose Syndrome infected bat or was assigned a wing-damage index of two or greater, was subjected to tissue sampling via wing-punch biopsy as recommended by both state and federal White-Nose Syndrome protocols in order to allow diagnostic testing of the infected tissue (United States Fish and Wildlife Service, 2009). Acoustic monitoring of bats present around the cave was performed using the Anabat high frequency recording unit. Anabat units are a commonly used method of taking an acoustic inventory of species present in a specified area (O'Farrell and Gannon, 1999). These recordings were used to assess the species richness and population densities of the bats present at Dunbar Cave during the swarming period in July and August of 2010. One study suggested using both mist nets and ultrasonic call recording units to get a more thorough sample of all species of bats present in the area (Kuenzi and Morrison, 1998).

Statistical Analysis

The numbers of individuals observed during the cave surveys and those per cave chamber were recorded for each survey date. It was expected that the number of bats present in the cave would progressively increase throughout the fall season with the number of individuals present peaking during early spring. The numbers of individuals present on each banding survey were presented in the form of a bar graph using Microsoft Excel analytical software. The numbers of each species found in the cave over the observation period were documented and conveyed in a pie graph using this software. The numbers of each species present during the duration of the study were compared using chi-squared goodness of fit analysis. The data were first evaluated using simple descriptive statistics then compiled into a graph; both pie and bar graphs were created for clear presentation of the results throughout the study.

The sex ratio of sampled *P. subflavus* was evaluated for the total number of male and females banded. The sex ratio of emerging bats was recorded and evaluated using the chi-squared goodness of fit analysis. The data were later compared to the sex ratio of swarming bats in August which was also assessed using the chi-squared goodness of fit analysis. Statistical analysis was performed using Microsoft Excel 2007-2008 software for inferential statistics. Multiple bar graphs were created using this software to illustrate the sex ratio differences throughout the seasons in which the project was conducted.

The number of recaptured individuals was counted and compared to the overall number of bats that were banded throughout the duration of the project. Recaptured individuals allow for evaluation of site fidelity behaviors and also help to determine seasonal population densities (Keen and Hitchcock, 1980). The site fidelity of any individual was documented and specific notes were made on the condition of the identification band and the physical condition of the bat, as well as the location of the bat when it was captured. Comparisons of all data sets, *post hoc,* were compared using nonparametric statistical analyses. Nonparametric analyses were performed because the sample size was different each survey night due to natural fluctuation and the change in sampling method. Additionally, changes in sampling methods were mandated by changes to federal and state permits. Captured individuals per banding survey ranged from one individual to over 50 individuals. Other observational information was collected for each captured bat such as whether the individual had been marked with a paint color code and photographed to document White-Nose Syndrome if present. If the individual was marked with a color code as well as banded, notes were made on the condition of the paint and color code quality and how long it had been since it was originally applied. This was documented in order to make a comparison between the band tagging and the color code marking techniques used during the study.

CHAPTER III
RESULTS

Species Richness and Sex Ratio Data for the Bat Banding Survey

A total of 218 bats was trapped using three trapping methods, twelve of which were recaptured individuals, and one of these which was recaptured on two banding surveys. There were four species of Chiropterans captured throughout the sampling period. A total of 190 *P. subflavus* was captured during the study, 13 of which were recaptures (Table 1). Thirteen banded bats were recaptured at some point during the survey sessions, therefore a total of 205 total individuals (N= 205) was banded throughout the study (Table 2). All recaptured individuals were *P. subflavus.* Recaptured individuals were captured while using all three of the trapping methods. *P. subflavus* were the most frequently observed, trapped, and banded species during the study period. Individual M0198 was recaptured two times after initial banding (Table 3). Therefore, a total of 190 *P. subflavus* was banded during the course of the study. *Perimyotis subflavus* was the only species captured on banding dates with the exception of April 8, 2009; April 22, 2009; August 10, 2010; and August 11, 2010. Big Brown bats *(Eptesicus fuscus)*, Little Brown bats *(Myotis lucifugus)*, and Red bats *(Lasiurus borealis)* were the only other species captured at DCSNA during those sampling sessions. Representing a very small percentage, two Little Brown bats, seven Red bats, and six Big Brown bats were captured and banded during sampling/banding sessions (Figure 11).

There was a total of 143 male *P. subflavus* and 47 female *P. subflavus* banded using the three trapping methods previously described (n=190). There was a total of two male and five female *L. borealis* banded during the study (n=7), all of which were captured using the mist-net technique. Four male *E. fuscus* and two female *E. fuscus* were captured and banded throughout the study (n=6). A chi-squared goodness of fit analysis was performed comparing the observed to the expected ratios of banded individuals per species. When comparing the four observed species, the chi-squared value indicated that the sampled individuals per species did not fit the expected 1:1:1:1 ratio ($x^2_{0.05[3]} = 7.815$; $P<0.001$). The data were substantially skewed toward *P. subflavus*.

Due to the small sample size of banded *M. lucifugus*, *L. borealis*, and *E. fuscus*, statistical analysis was not performed on the sex ratio of individuals of these species. The chi-squared goodness of fit analysis was applied to compare the overall number of male and female *P. subflavus* (n=190). The chi-squared value indicated the *P. subflavus* population that was sampled had a disproportionate number of males ($x^2_{0.05[3]} = 3.841$; $P<0.001$).

Table 1. Total number of bats trapped per survey date and throughout the study at DCSNA.

Date	Number of Individuals
5/18/2009	11
6/02/2009	3
6/8/2009	7
6/22/2009	3
8/12/2009	65
8/25/2009	31
10/26/2009	1
4/18/2010	25
4/29/2010	22
8/10/2010	20
8/11/2010	30
Total Bats	N=218
Recaptures	13

Table 2. Number of bats by species banded at DCSNA.

Species	Total Individuals Banded
P. subflavus	190
M. lucifugus	2
E. fuscus	6
L. borealis	7
Total	205

Table 3. Data for recaptured Eastern pipistrelles, *P. subflavus*. J = juvenile, A = adult.

Date Banded	Date Recaptured	Initial Age	Sex	Initial Weight	Recapture Weight	Band Identification
6/2/2009	6/8/2009	J	F	1.0 g	2.0 g	F0099
8/12/2009	4/18/2010	J	M	2.0 g	4.4 g	M0133
8/25/2009	4/18/2010	J	M	2.0 g	3.6 g	M0198 *
8/25/2009	4/29/2010	A	M	3.6 g	4.3 g	M0198 *
4/18/2010	4/29/2010	A	M	4.1 g	4.7 g	M0157
4/18/2010	4/29/2010	A	M	4.8 g	4.8 g	M0160
4/29/2010	8/10/2010	A	M	4.6 g	6.3 g	M0168
8/25/2009	8/10/2010	J	M	1.0 g	6.1 g	M0109
8/12/2009	8/10/2010	J	M	1.0 g	5.7 g	M0143
8/12/2009	8/11/2020	J	M	2.0 g	4.0 g	M0139
4/29/2010	8/11/2010	A	M	5.6 g	6.4 g	M0166
4/29/2010	8/11/2010	A	F	5.6 g	7.6 g	F0076
8/25/2009	8/11/2010	J	M	1.0 g	6.7 g	M0196

*Indicates duplicate recaptures, n=13

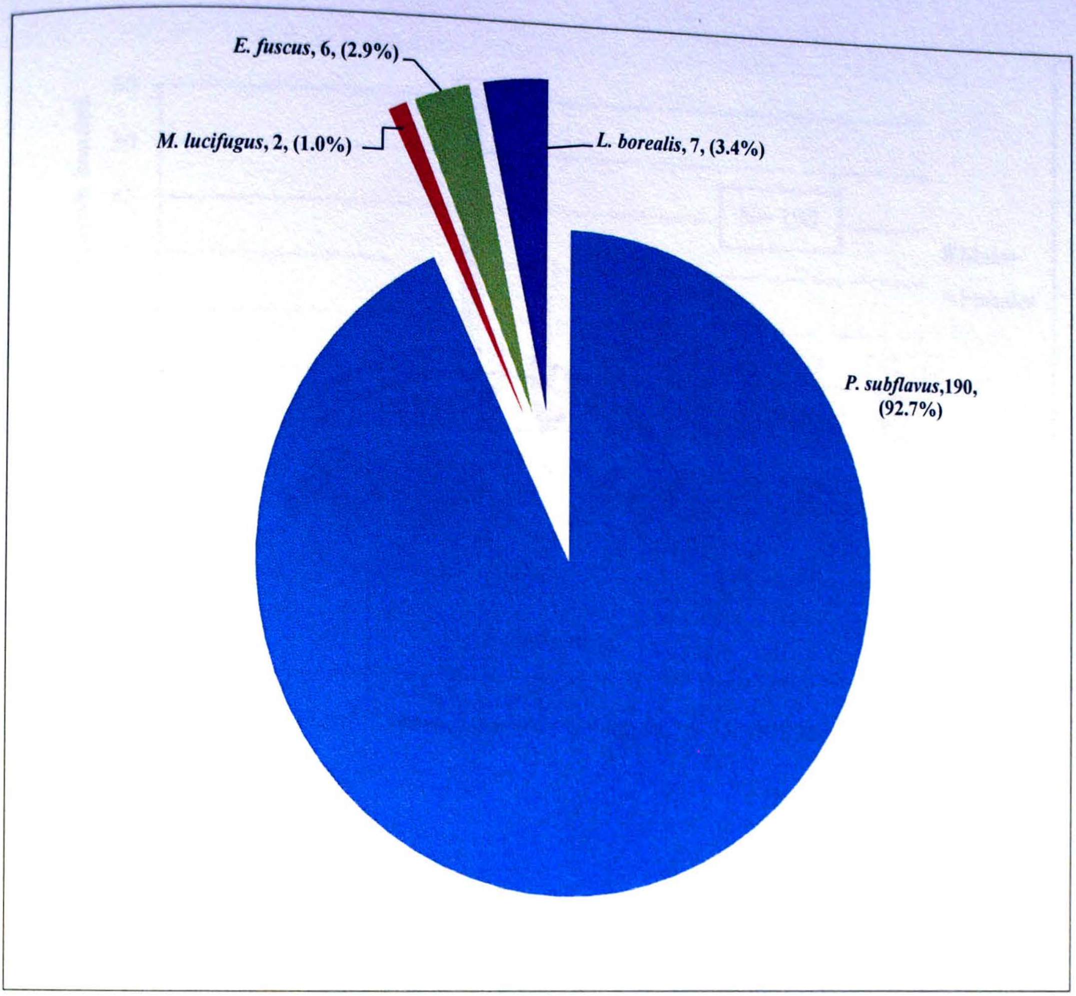

Figure 11. The total number of banded bats per species (N = 205).

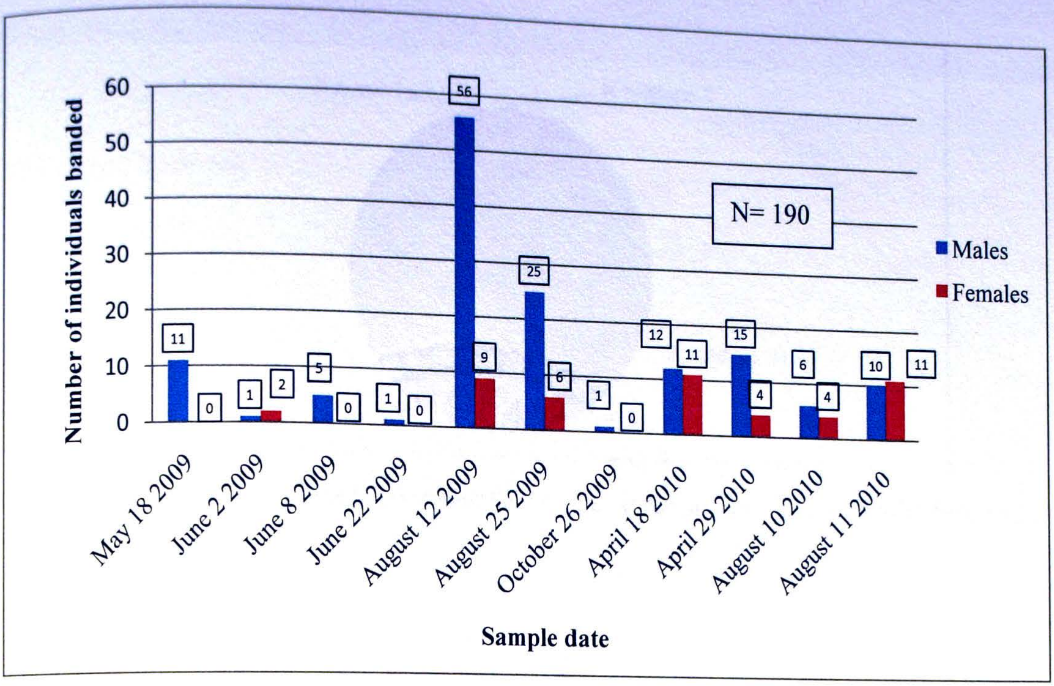

Figure 12. Number of male and female *P. subflavus* sampled per survey date.

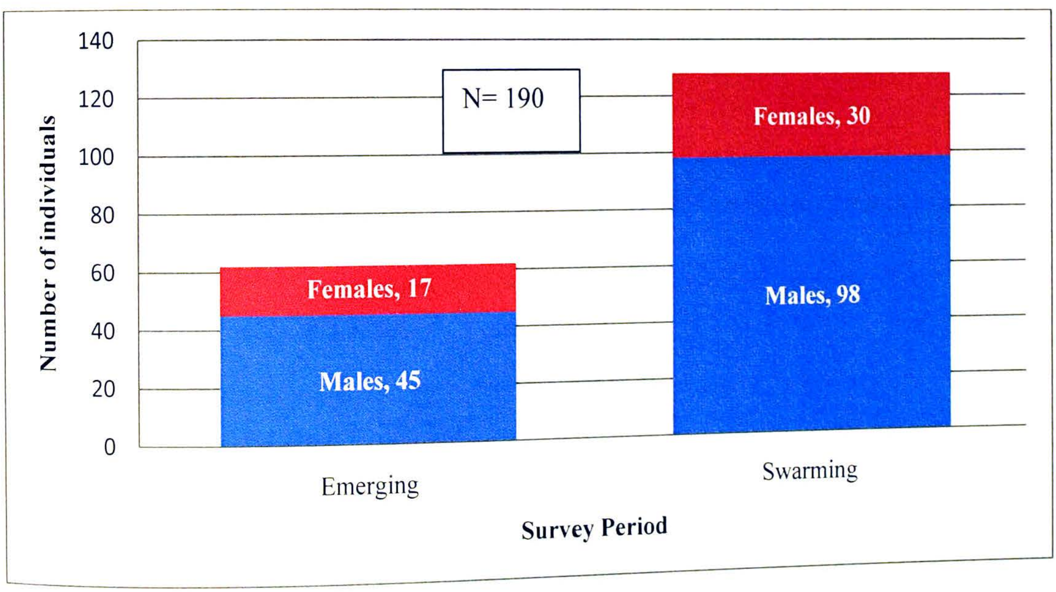

Figure 13. Number of male and female *P. subflavus* banded during emergence and swarming periods.

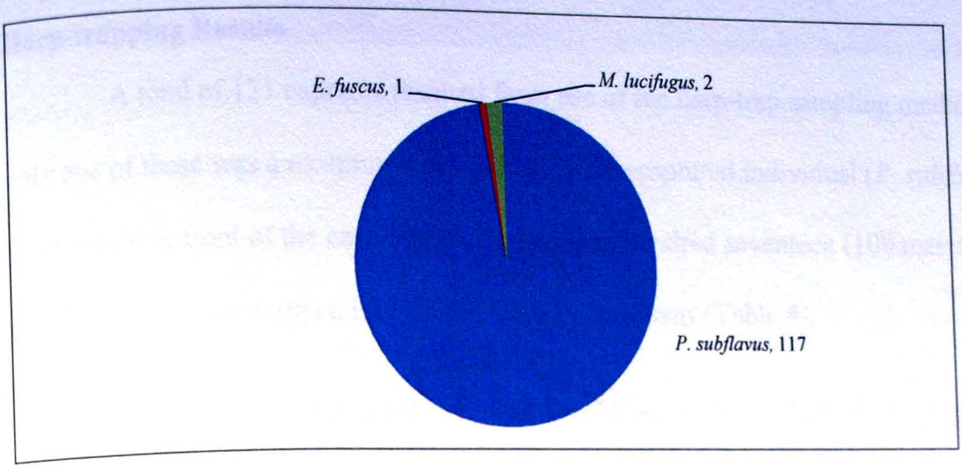

Figure 14. The number of bats banded per species over seven survey nights using the harp-trap method of sampling (n = 120).

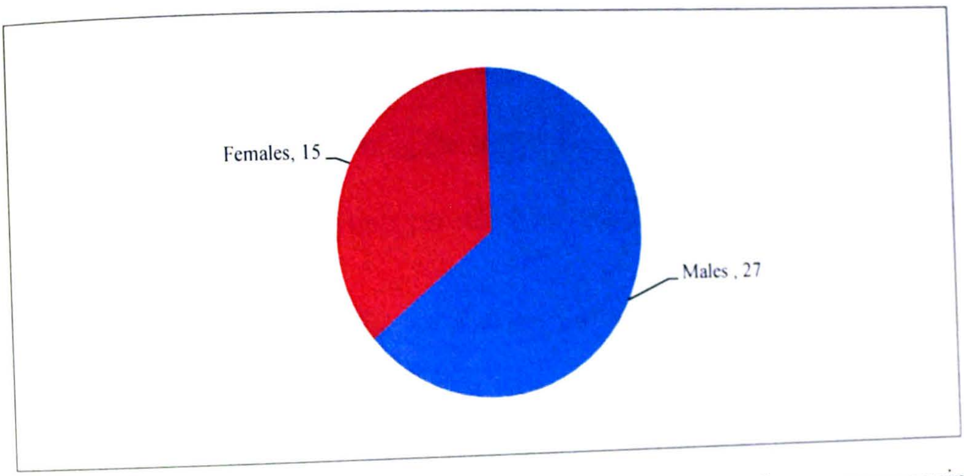

Figure 15. The number of male and female *P. subflavus* banded on the two surveys using the in-cave, hand-removal method of sampling (n=42).

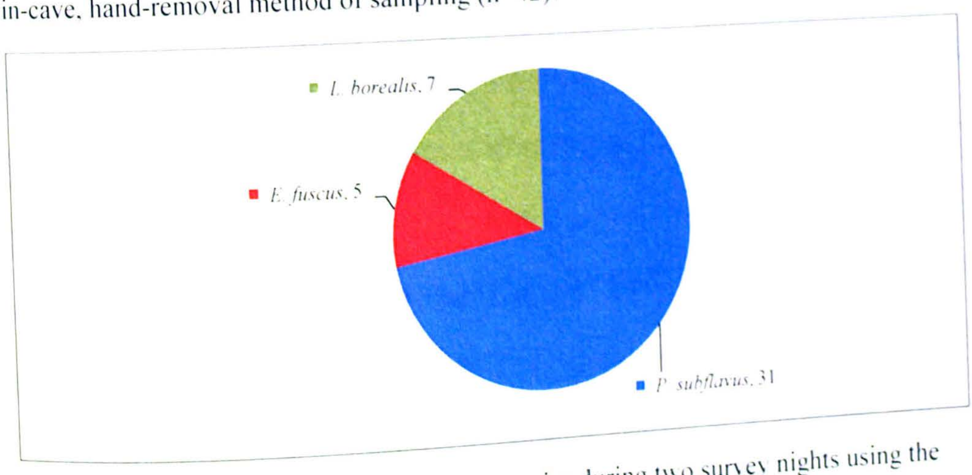

Figure 16. The number of bats banded per species during two survey nights using the mist-net method of sampling (n=43).

Harp-trapping Results

A total of 121 captures resulted from use of the harp-trap sampling method, and only one of these was a recaptured individual. The recaptured individual (*P. subflavus*) was trapped in front of the cave entrance gate. One hundred seventeen (100 males and 17 females) of the harp-trapped individuals were *P. subflavus* (Table 4).

Fifty-six of these bats were juveniles and 61 were adults. Two *M. lucifugus* were captured and banded; both were adult males. One juvenile male *E. fuscus* was captured and banded (Figure 14). The greatest number of harp-trap captures occurred during the late summer swarming period in 2009. More individuals were trapped using the harp trap than any other method used during the study. The harp trap was used on seven surveys while the hand-removal was only permitted for two dates, as was the mist-net sampling.

It was expected that the sampled population would exhibit a 1:1 ratio of male to female *P. subflavus* overall and for each harp-trap banding date and harp-trap surveys during emergence and swarming of 2009. A chi-squared goodness of fit analysis was performed in order to compare the observed sex ratio to the expected 1:1 ratio. The sampled population did not fit the expected 1:1 ratio of male to female *P. subflavus* on all but one survey. The only survey where more female *P. subflavus* were sampled was on June 2, 2009 with a total sample size of only three individuals (n=3) and August 11, 2010 with a total sample size of 21 individuals (n=21). Two of the three individuals were female on June 2, 2009, and 11of the 21 *P. subflavus* were female on August 11, 2010. The sample size on June 2, 2009 was too small to analyze for significance as it was for June 22 and October 26, 2009. Harp-trap samples were analyzed using the chi-squared analysis, and no sample exhibited the expected 1:1 ratio ($x^2_{0.05[1]} = 3.841$). These seven harp-trap samples were skewed towards male individuals. A chi-squared goodness of fit analysis was also used to examine the sex ratio of banded individuals trapped using the harp trap during swarming and emergence periods. The chi-squared analysis indicates the sex ratio did not fit the expected 1:1 ratio for the total number of swarming *P. subflavus*, or for the total number of emerging *P. subflavus*, ($x^2_{0.05[1]} = 3.841$; $P<.001$). Sample data were skewed toward male individuals for both emerging and swarming *P. subflavus*. There was a total of 117 *P. subflavus* banded using the harp-trap method, 100 of which were male and 17 were female. The chi-squared analysis indicates that the sampled population of *P.subflavus* did not fit the 1:1 expected sex ratio and was skewed toward males ($x^2_{0.05[1]} = 3.841$; $P<0.001$).

The descriptive statistics for the weights of bats sampled using the harp-trap sampling method are presented in Table 5.

Table 4. Number of individuals, sex ratio, mean weight (g), and age class ratio of various species captured using the harp-trap method of sampling (n=120).

Statistics	*P. subflavus*	*M. lucifugus*	*E. fuscus*
N	117	2	1
Sex Ratio (M:F)	100:17	2:0	1:0
Mean Male Weight (g)	2.1	3	7
Mean Female Weight (g)	2.7778		
Age Ratio (J:A)	55:62	0:2	1:0

Table 5. Descriptive statistics for the weights of *P. subflavus* banded (n=117) during seven surveys using the harp-trap method of sampling.

Males		Females	
Mean	2.1	Mean	2.778
Standard Error	0.0823	Standard Error	0.2365
Median	2	Median	3
Mode	2	Mode	2
Standard Deviation	0.8227	Standard Deviation	1.003
Sample Variance	0.6768	Sample Variance	1.007
Kurtosis	-0.5988	Kurtosis	0.0013
Skewness	0.2555	Skewness	0.4984
Range	3	Range	4
Minimum	1	Minimum	1
Maximum	4	Maximum	5
Count	100	Count	17

In-cave, Hand-removal Method Results

The in-cave, hand-removal method of capturing bats was utilized for the sampling that took place inside the chambers of Dunbar Cave just prior to spring 2010 emergence. On April 18th and 29th, 2010, chambers were surveyed for roosting individuals that could be removed by hand and individuals that were reachable using a retractable small-mesh. There was a total of 47 captures recorded on these two dates. Five of these (all males) were recaptures; therefore a total of 42 *P. subflavus* were banded on the two surveys using the hand-removal method of sampling *Perimyotis subflavus* was the only species observed roosting in the cave system during these banding sessions (Figure 15). There was a total of 27 males and 15 females among the 42 bats banded (Table 6). All were adults, and none were juveniles. The color codes for the five recaptured bats were no longer distinguishable as a means of individual identification.

The in-cave, hand-removal method of sampling individuals was implemented during the spring 2010 emergence. It was expected that the sampled population would exhibit a 1:1 ratio of males to females for *P. subflavus* overall and for each hand-removal trapping and banding date. A chi-squared goodness of fit analysis was performed to test this hypothesis. Twelve male and 11 female *P. subflavus* were sampled (n=23) using this technique on April 18, 2010. The sampled population did fit the expected 1:1 ratio of males to females ($x^2_{0.05[1]} = 3.841$; $0.75<P= 0.95$). There was a total of 19 *P. subflavus* sampled on April 29, 2010 using the hand-removal technique (n=19). Fifteen of these individuals were male and four were female. This did not fit the expected ratio of 1:1, and was skewed toward male individuals, ($x^2_{0.05[1]} = 3.841$; $0.01<P=0.025$). The sex ratio of male and female *P. subflavus* was analyzed for the total number of hand- removal

sampled individuals. These bats were trapped during the emergence of 2010. The chi-squared analysis indicates the sex ratio of the sample did equal the expected 1:1 ratio of male to female individuals, ($x^2_{0.05[1]} = 3.841$; $0.05<P<0.10$). The descriptive statistics for the weight of bats sampled using the in-cave, hand-removal method of sampling is presented in Table 7.

Table 6. Number of individuals, sex ratio, mean weight (g), and age class ratio of various species captured using the in-cave, hand-removal method of sampling (n=42)..

Statistics	*P. subflavus*
N	42
Sex Ratio (M:F)	27:15
Mean Male Weight(g)	4.5438
Mean Female Weight (g)	5.2733
Age Ratio (J:A)	0:42

Table 7. Descriptive statistics for the weights of *P. subflavus* banded on the two surveys using the in-cave, hand-removal method of sampling (n=42).

Males		Females	
Mean	4.544	Mean	5.273
Standard Error	0.0889	Standard Error	0.1948
Median	4.55	Median	5.6
Mode	4.3	Mode	4.3
Standard Deviation	0.5029	Standard Deviation	0.7545
Sample Variance	0.2529	Sample Variance	0.5692
Kurtosis	0.3342	Kurtosis	-1.166
Skewness	-0.1032	Skewness	0.0687
Range	2.1	Range	2.3
Minimum	3.5	Minimum	4.3
Maximum	5.6	Maximum	6.6
Count	27	Count	15

Mist-net Sampling Method Results

Due to the detection and laboratory confirmation (Dr. Ann Ballman, USGS, National Wildlife Health Center, personal communication, March 2010) of a White-Nose Syndrome-infected bat that was collected inside Dunbar Cave, surveys were halted temporarily. After a multiagency review of the project protocols and decontamination procedures, trapping protocols were abruptly changed to meet federal and state permit requirements. During the late summer swarming period, two previously described mist nets were used to trap swarming bats at Dunbar Cave. One net was positioned approximately eight feet in front of the cave entrance, and the other was placed lakeside approximately 20 feet from the first mist net. The mist-net surveys took place on August 10^{th} and 11^{th}, 2010. Fifty captures (all *P. subflavus*) resulted using the mist-net sampling technique. Seven of these involved recaptured individuals. Six of the recaptured *P. subflavus* were male, and one was a female. There were 31 *P. subflavus*, seven *L. borealis*, and five *E. fuscus* banded (n= 43) during the swarming period using this trapping method (Figure 16).

The sex ratio of *P. subflavus* was skewed slightly toward males. There were 16 male pipistrelles and 15 females banded during the 2010 swarming period using the mist-net sampling method. It was expected that the sampled population would exhibit a 1:1 ratio of males and females overall and for each of the two mist-net trapping and banding dates. A chi-squared goodness of fit analysis was performed in order to compare the observed sex ratios to the expected 1:1 ratio for each of the mist-net samples and the overall sampling data. The chi-squared goodness of fit analysis revealed the population that was sampled had a 1:1 male to female sex ratio for *P. subflavus* ($x^2_{0.05[1]} = 3.841$) on August 10, 2010 ($0.75<P<0.90$) and also on August 11, 2010 ($0.75<P<0.90$).

The chi-squared goodness of fit analysis was also applied to the total number of *P. subflavus* trapped using the mist-net method of sampling. There were four juveniles, and 27 adult *P. subflavus* captured and banded using this method of sampling. Three of the juvenile *P. subflavus* were male, and one was female. There were more female *L. borealis* captured than there were males; five were females and two were males. Both of the male *L. borealis* were juveniles, and all females were adults. Three male and two female *E. fuscus* were captured and banded using the mist-net technique. All mist-net sampled *E. fuscus* were adults (Table 8).

The descriptive statistics for the weight of bats sampled using the mist-net method of sampling are included in Table 9. The descriptive statistics for the weight of *E. fuscus* sampled using the mist-net method of sampling can be found in Table 10. The descriptive statistics for the weight of *L. borealis* sampled using the mist-net method of sampling appears in Table 11.

Table 8. Number of individuals, sex ratio, mean weight (g), and age class ratio of various species captured using the mist-net method of sampling (n=43).

Statistics	*P. subflavus*	*E. fuscus*	*L. borealis*
N	31	5	7
Sex Ratio (M:F)	16:15	3:2	2:5
Mean Male Weight (g)	2.1	14.7	13.8
Mean Female Weight (g)	2.7777	20.75	12.5
Age Ratio (J:A)	4:27	0:5	2:5

Table 9. Descriptive statistics for the weights of *P. subflavus* banded on the two surveys using the mist-net method of sampling (n=31).

Males		Females	
Mean	5.3	Mean	6.131
Standard Error	0.2767	Standard Error	0.2712
Median	5.6	Median	6.25
Mode	5.3	Mode	6.2
Standard Deviation	1.298	Standard Deviation	1.085
Sample Variance	1.684	Sample Variance	1.177
Kurtosis	0.4688	Kurtosis	-0.1290
Skewness	-0.9197	Skewness	-0.5385
Range	5.3	Range	3.8
Minimum	2	Minimum	3.8
Maximum	7.3	Maximum	7.6
N	16	N	15

Table 10. Descriptive statistics for the weights of *E. fuscus* banded on the two surveys using the mist-net method of sampling (n=5).

Males		Females	
Mean	14.7	Mean	20.75
Standard Error	0.9074	Standard Error	2.75
Median	15	Median	20.75
Standard Deviation	1.572	Standard Deviation	3.889
Sample Variance	2.47	Sample Variance	15.13
Skewness	-0.8277		
Range	3.1	Range	5.5
Minimum	13	Minimum	18
Maximum	16.1	Maximum	23.5
Count	3	Count	2

Table 11. Descriptive statistics for the weights of *L. borealis* banded on the two surveys using the mist-net method of sampling (n=7).

Males		*Females*	
Mean	13.8	Mean	12.5
Standard Error	2.7	Standard Error	0.9236
Median	13.8	Median	12.6
Standard Deviation	3.818	Standard Deviation	2.065
Sample Variance	14.58	Sample Variance	4.265
		Kurtosis	-0.5679
		Skewness	-0.6574
Range	5.4	Range	5
Minimum	11.1	Minimum	9.5
Maximum	16.5	Maximum	14.5
Count	2	Count	5

CHAPTER IV

DISCUSSION

Sampling Methods

Three sampling methods were used during the 17-month study. Data were organized and classified per sampling method. Initially, all individuals were to be sampled using harp traps. According to the original protocol, bats would be trapped as they entered or emerged through the bat-friendly entrance gate. This would be done for at least two emergence periods and two swarming periods. This was the method used for the first seven sampling surveys at Dunbar Cave. As mentioned above, during the March 2010 cave survey, a single bat that was covered in patches of a white-powdery substance was observed roosting in the Hallway chamber of the cave system. After diagnostic testing was performed at the national laboratory for White-Nose Syndrome detection, it was confirmed that the individual was infected with the fungal pathogen *Geomyces destructans*, the causative agent of the disease. Harp traps often trap multiple individuals at the same time increasing the potential for an infected bat to come into physical contact with a non-infected individual, spreading the fungus to the healthy bats. It was determined by state and federal agencies that harp traps were not to be used to sample bats (U.S. Fish and Wildlife Service, 2009). Instead, researchers were to implement the hand-removal method of sampling roosting bats during the spring emergence inside the cave, and the mist-net trapping method for swarming bats at the cave mouth. Mist-net trapping is an effective method for determining the bat species inhabiting a specific area (Weller, 2007). Therefore, the harp-trap sampling method was used for the first seven samples, the hand-removal method for the two emergence samples in 2010, and the mist-net sampling method for the swarming surveys in 2010. Because there were an unequal number of harp-trapped samples, hand-removal samples, and mist-net samples, a comparative sampling method analysis was not appropriate.

Species Sampled at Dunbar Cave State Natural Area

Species richness is a fundamental measurement of community and regional diversity that underlies many ecological models and conservation strategies (Gotelli and Colwell, 2001). The species richness of the bat population at Dunbar Cave has been based on cave surveys prior to this study. Acquiring state and federal permits allowed researchers to band sampled individuals throughout the duration of this project and allowed for more accurate species identification. The predominant species banded throughout the duration of the study at DCSNA was *P. subflavus*. Over the past five years, *P. subflavus* and *M. lucifugus* were the two species most often observed roosting in the cave system. Occasionally individual *E. fuscus* were documented utilizing the cave as a hibernaculum. *Eptesicus fuscus* is a species commonly documented to assemble maternity colonies and hibernacula in old or abandoned buildings or attics (Williams and Brittingham 1997). Prior to this study, no members of *L. borealis* have been known to roost in Dunbar Cave, as they are a forest-dwelling species (Mager and Nelson, 2001). During the emergence surveys of 2009 using the harp-trap method of sampling, two *M. lucifugus* and one *E. fuscus* were trapped at the cave entrance. All other individuals trapped during this time were *P. subflavus*. It was expected that the sample size of *M. lucifugus* would have been much larger than it was, but no other *M. lucifugus* were sampled during the study. During the 2009 sampling of emerging individuals, surveys were not permitted by state and federal agencies until late spring, beginning on May 18,

2009. According to survey data from previous years, *M. lucifugus* leave the cave system earlier than *P. subflavus* for summer foraging in late March and early April. Due to the late spring surveys of emerging individuals in 2009, it is possible that the majority of *M. lucifugus* had already left the cave system; therefore, only two individuals were sampled. Early emergence may explain the small sample size of *M. lucifugus* in 2009. In 2010 no *M. lucifugus* were sampled. This could be due to limited human interference during March of 2010 when a WNS infected individual was detected and the cave was closed to the public and to researchers. Cave surveys were prohibited as well as the sampling of roosting bats in the cave. Multiagency permits were required to continue the study in Dunbar Cave, and were not obtained until mid April 2010. No *M. lucifugus* were sampled when the surveys continued. Data from previous years of continued bi-monthly surveys at Dunbar Cave (S. J. Jenkins and R. S. McCormick, personal communication) indicate *M. lucifugus* emigrate from the cave system earlier than *P. subflavus* during spring emergence. Therefore it is possible that members of *M. lucifugus* were not sampled because they had already emerged from the cave during the summer.

Lasiurus borealis is a forest-dwelling, chiropteran species (Saugey et al., 1989). It was expected that sampling in and near the cave would provide a sample of individuals since they are known to utilize cave chambers as a winter hibernacula. *Lasiurus borealis* was detected during the swarming surveys of 2010. This is a deviation from the expected trapping results because these individuals were trapped directly in front of the cave entrance using a mist net. Seven individual *L. borealis* were captured on the cave-side of the net indicating that they were coming from inside the cave. It has been documented that multiple species will utilize the same swarming site and Red bats have been documented roosting in caves (Mager and Nelson, 2001). In other studies, data indicate that more forest bats, in particular *L. borealis*, will utilize caves and mines during the swarming season (Saugey, 1989). Dunbar Cave is located above a large man-made pond surrounded by arboreal habitat. Individuals swarming at Dunbar Cave would have access to a water source, potential food from semi-aquatic and terrestrial insects, a cave with cool and stable temperatures, and potential access to mates that do not overwinter at the cave. Swarming sites are beneficial to bats that exhibit late mating and have an increase of out-breeding potential with nonrelated members of the population (Parsons et al., 2002; Veith et al., 2004). This may account for why forest-dwelling bats were trapped at a cave site. While forest dwelling bats have been documented roosting in caves, never before has a Red bat been observed roosting in or near Dunbar Cave.

According to the chi-squared goodness of fit analysis, the overall sample was skewed toward *P. subflavus*, which has in the past been the predominant species observed in and near Dunbar Cave. All but two banding sessions took place outside the cave; therefore there would be no reason to predict that forest dwelling species would not be sampled. The ratio of banded individuals did not fit the expected 1:1:1:1 ratio of *P. subflavus* to *M. lucifugus* to *E. fuscus* to *L. borealis*. The greatest number of individuals from multiple species was observed during the summer swarming on August 10 and 11, 2010. *Myotis lucifugus* was the only species not observed during the swarming period in 2010. The data indicate that more bat species are present at DCSNA than have been documented prior to this project.

Site Fidelity of Banded Bats

Throughout the duration of the study, 218 bats were trapped using one of the three sampling methods previously described. Thirteen trapped bats had been banded previously by APSU researchers. One individual (M0198) was recaptured twice (Table 3). Therefore, 12 individuals showed some fidelity to DCSNA. All individuals that were recaptured were *P. subflavus*. Recaptured individuals were observed during emergence and swarming surveys. This is not unusual as other studies have recaptured banded bats throughout the entire study period including both hibernation and mating seasons (Jones and Suttkus, 1973). Of the 12 individuals that were recaptured, 10 were male and only two were female. Male *P. subflavus* exhibited more site fidelity to the cave during emergence and during swarming surveys than did female bats. Veilleux and Veilleux (2004) state that, "Fidelity to a roost structure is also variable, with species exhibiting relatively high fidelity to stable rare roosts (e.g., caves) and low fidelity to unstable ubiquitous roosts (e.g., foliage)." During the emergence period in 2010, female *P. subflavus* were documented emerging from the cave for the summer before males. This could be due to the fact that female *P. subflavus* will often join a maternity colony with predominantly female and juvenile individuals in late spring and early summer, often in foliage or dead trees (Veilleux et al., 2003). There is no indication that Dunbar Cave is such a maternity site. However, it is possible that it may develop into a maternity colony site in the future. Further studies and surveys would be required for determining if Dunbar Cave is, or will at some point, become a maternity colony roost site.

Sex Ratios of Sampled Individuals per Survey

The overall sex ratio of *P. subflavus* was skewed toward males. The expected sex ratio was 1:1 for males to females in and near Dunbar Cave. According to the results of a chi-squared analysis, the population that was sampled did not exhibit a 1:1 ratio of male to female *P. subflavus*. A total of 143 male and 47 female *P. subflavus* were sampled during the study (n=190). This amounted to over three times as many males as females. It is not uncommon to observe a male-dominant population of *P. subflavus* (Davis, 1959). During this study, females left the cave system earlier in spring than did the males (Tables 6 and 7). No clustering groups of females were observed inside or outside the cave system. This information indicates that Dunbar Cave is not at this time a maternity colony site. However, as the number of females found roosting in the cave and sampled near the cave has increased from 2009 to 2010 along with the species diversity, it is possible that Dunbar Cave may in the future harbor maternity colonies.

The overall numbers of male and female *P. subflavus* did not fit the expected 1:1 ratio ($X^2_{0.05[1]} = 3.841$; $P<0.001$). However, when the data for survey dates were analyzed individually, four survey dates had sex ratios for *P. subflavus* that did meet the expected 1:1 sex ratio. On June 2, 2009, April 18, 2010, August 10, 2010, and August 11, 2010 there was a 1:1 ratio of male to female *P. subflavus* ($X^2_{0.05[1]} = 3.841$; $P>0.05$). Therefore there were two emergence-period surveys and two swarming-period surveys in which the sampled population did fit the 1:1 expected sex ratio. On June 2, 2009 two females and one male *P. subflavus* were banded for a sample size of three bats. That sample was taken using the harp-trap method of sampling. On April 18, 2010 twelve males and eleven females were banded inside the chambers of Dunbar Cave. The individuals banded on that date were sampled using the in-cave, hand-removal method. All individuals that were reachable and visible were sampled. The number of female *P. subflavus* decreased from 11 individuals on the 18th of April, 2010 to four females on the 29th of April, 2010. Females left the cave system before the males during the spring 2010 emergence surveys. The sex ratio of *P. subflavus* during the swarming period of 2010 did meet the expected 1:1 ratio of males to females. This was the case on both August 10, 2010 and August 11, 2010. On August 10, 2010 six males and four females *P. subflavus* were sampled. On August 11, 2010, ten males and eleven females were banded. This was one of two survey dates where more female than male *P. subflavus* were sampled. The only other date when more females were sampled was on the June 2, 2010.

During the April 18th, 2010 sample of bats roosting in the cave, there was an equal number or greater number of female *P. subflavus* sampled in cave chambers closer to the entrance. The following sample (April 29th, 2010) resulted in no chambers that exhibited a greater or equal number of females. This indicates that female *P. subflavus* are more oriented towards the chambers near the main entrance before emigrating from the cave in the spring. Females are also leaving the cave system before males during the spring emergence. Females will need to forage and find a summer roost before giving birth. This may explain the roosting of females close to the entrance on April 18th, and the decrease in the sampling of females on April 29th. In previous years, public cave tours began in the spring (April) which also may cause reproductive females to emerge early.

Banding Surveys of Emerging Bats in 2009 and 2010

Four surveys took place during the emerging period of 2009. A total of 18 male and two female *P. subflauvs* were sampled during this survey (n=20). The observed sex ratio of the overall sample did not fit the expected 1:1 sex ratio in 2009 ($x^2_{0.05[1]} = 3.841$; $P<0.001$). Three of the four surveys yielded a sample that did not meet the expected 1:1 sex ratio. The June 2, 2009 sample (n=3)was the only one that came close to a 1:1 ratio of males to females for *P. subflavus* but the sample size was too small for analysis. Conversely, the sex ratio of emerging bats in 2010 did meet the expected 1:1 ratio overall ($x^2_{0.05[1]} = 3.841$; $0.05<P<0.1$). Only two survey dates were permitted during the emerging period of 2010, due to the detection of a WNS positive individual earlier that spring. The observed sex ratio did meet the 1:1expected sex ratio on both of the emerging survey dates in 2010.

Banding Surveys of Swarming Bats 2009 and 2010

The swarming period surveys in 2009 (August 12th, August 25th, and October 26th.) yielded 97 *P. subflavus*, of which 82 were male and 15 were female; all were banded following capture in a harp trap. No other species were observed or banded during the swarming period in 2009. No recaptured individuals were observed. However in the swarming period surveys in 2010 (August 10th and August 11th) yielded 47 bats, including 31 *P. subflavus*, seven *L. borealis*, and five *E. fuscus*, all caught in a mist net. There were 16 male and 15 female *P. subflavus*, three male and two female *E. fuscus*, and five female and two male *L. borealis*. The 2009 sampling of swarming bats produced only *P. subflavus*, most of which were male. Conversely, the 2010 samples of swarming bats resulted in the capture of three species: *L. borealis, E. fuscus,* and *P. subflavus*. *Perimyotis subflavus* exhibited the expected 1:1 sex ratio. This indicates that DCSNA is inhabited by more species than previously documented and the sex ratio in 2010 for *P. subflavus* was 1:1, unlike the previous swarming period samples of 2009. Therefore, the overall number of females sampled during the swarming periods has increased throughout the duration of the study. Juvenile *P. subflavus* were observed during both 2009 and 2010 swarming period samples. Juvenile *L. borealis* were observed during the swarming period of 2010, but not in 2009. The two mist nets that were used for both of the 2010 swarming period samples yielded an increased number of species compared to the previous year's samples.

Restoration of Dunbar Cave to Bat Populations

The initial goal of the "Bat Project" at Austin Peay was to determine if the entrance gate at DCSNA was indeed bat-friendly. The erection of cave gates may actually promote usage by cave-dwelling bat species if the gate has an appropriate assembly (Spanger and Fenton, 2005). Prior to this study, the observed number of roosting individuals inside Dunbar Cave never exceeded 90 individuals. However, during the eleven banding dates for this study, a total of 205 bats were banded. Four species of bats were sampled, one of which, *L. borealis*, had never before been documented using Dunbar Cave as a roost site. This indicates that more bats are present in and near Dunbar Cave than had been observed in the past. It also indicates that the species diversity increases during swarming months at Dunbar Cave. This is relevant because there are endangered-bat roosts near DCSNA, and Dunbar Cave has been confirmed to house at least one bat that tested positive for White-Nose Syndrome. If

Dunbar Cave is a swarming site for multiple species during the late summer and early fall months, there could be an increased chance of spreading the fungus to endangered bat populations or to forest-dwelling bats such as *L. borealis*, a species of bat that have not been documented to have WNS. Further research would be necessary to determine the status of WNS in the Dunbar Cave bat populations. Bats are most vulnerable to the fungus when their metabolic activities are at a minimum, such as when hibernating. Therefore, winter cave surveys for 2010-2011 may be a helpful mechanism to determine if the fungus has spread to other individuals roosting in Dunbar Cave. Dunbar Cave is currently closed to the public and no further cave tours will be given until the cave is cleared as a WNS positive site. Cave-closure could be beneficial to the hibernating bat populations in winter 2010-2011. Previous studies have indicated that there has been an increased weight loss in individuals exposed to human visitation during the hibernation season (Johnson, 1998). This may also account for the fact that more females were sampled in the emergence and swarming of 2010. The decrease in human related disturbances may make Dunbar Cave more appealing to reproductive females.

Conclusions

The largest samples were collected during the swarming season in both 2009 and 2010. The greatest number of bat species was observed during the swarming season of 2010. Red bats (*L. borealis*) were documented using Dunbar Cave as a swarming site for the first time. This is a cause for concern as the nationwide spread of White-Nose Syndrome continues. Bat biologists are becoming increasingly concerned with the potential spreading of White-Nose Syndrome from cave-dwelling bats to forest-dwelling bats. Trapping Red bats coming from within the cave increases the potential for the cave-dwelling bats that have been infected with *Geomyces destructans* to expose and infect forest-dwelling bats such as Red bats.

Multiple observations of mating individuals inside the cave and the high number of *P. subflavus* swarming in and near the cave indicate that Dunbar Cave is an important site for mating and swarming activities. Many juvenile individuals and some pregnant females were also documented during the swarming season. Ten of the twelve recaptured individuals were male. Six of these recaptured males were trapped initially as juveniles and recaptured as adults, indicating that some of the bats that hibernate in the chambers of Dunbar Cave also exhibit swarming behaviors during the mating season in or near the cave. Some of the summer-born juveniles will stay at Dunbar Cave and develop into adults (Table 3). Dunbar Cave is not only a hibernaculum for local bat populations, but also an important mating and swarming site. The overall sex ratio of *P. subflavus* did not meet the expected 1:1 ratio for sampled individuals and was skewed toward males. The results indicated that there was a strong affinity toward male dominated reproductive behaviors.

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APPENDICES

APPENDIX A

Banding data

Date	Species	Age Class	Sex	Weight (g)	Band ID	Sampling Method
5/18/2009	EP	J	M	2	M0050	Harp trap
5/18/2009	EP	J	M	2	M0049	Harp trap
5/18/2009	EP	A	M	3	M0048	Harp trap
5/18/2009	EP	J	M	2	M0047	Harp trap
5/18/2009	EP	A	M	3	M0046	Harp trap
5/18/2009	EP	J	M	2	M0045	Harp trap
5/18/2009	EP	A	M	4	M0044	Harp trap
5/18/2009	EP	J	M	2	M0043	Harp trap
5/18/2009	EP	A	M	3	M0042	Harp trap
5/18/2009	EP	J	M	1	M0041	Harp trap
5/18/2009	EP	A	M	2	M0039	Harp trap
6/2/2009	EP	A	M	4	M0038	Harp trap
6/2/2009	EP	J	F	1	F0099*	Harp trap
6/2/2009	EP	J	F	2	F0098	Harp trap
6/8/2009	EP	A	M	3	M0037	Harp trap
6/8/2009	EP	J	F	2	F0099*	Harp trap
6/8/2009	EP	J	M	1	M0036	Harp trap
6/8/2009	EP	A	M	2	M0035	Harp trap
6/8/2009	EP	J	M	1	M0034	Harp trap
6/8/2009	EP	A	M	3	M0033	Harp trap
6/8/2009	BB	J	M	7	M0032	Harp trap
6/22/2009	EP	A	M	2	M0031	Harp trap
6/22/2009	LB	A	M	3	M0029	Harp trap
6/22/2009	LB	A	M	3	M0028	Harp trap
8/12/2009	EP	A	M	3	M0026	Harp trap
8/12/2009	EP	J	M	1	M0025	Harp trap
8/12/2009	EP	A	M	3	M0024	Harp trap
8/12/2009	EP	J	M	1	M0023	Harp trap
8/12/2009	EP	J	M	2	M0022	Harp trap
8/12/2009	EP	J	M	2	M0021	Harp trap
8/12/2009	EP	A	M	3	M0020	Harp trap
8/12/2009	EP	A	M	2	M0019	Harp trap
8/12/2009	EP	A	M	3	M0018	Harp trap
8/12/2009	EP	J	M	2	M0017	Harp trap
8/12/2009	EP	J	M	2	M0016	Harp trap
8/12/2009	EP	A	M	3	M0015	Harp trap
8/12/2009	EP	A	M	4	M0014	Harp trap
8/12/2009	EP	A	M	3	M0013	Harp trap
8/12/2009	EP	J	M	2	M0012	Harp trap
8/12/2009	EP	A	M	3	M0011	Harp trap
8/12/2009	EP	J	M	1	M0010	Harp trap
8/12/2009	EP	A	M	3	M0009	Harp trap

8/12/2009	EP	A	F	3	F0097	Harp trap
8/12/2009	EP	A	M	2	M0007	Harp trap
8/12/2009	EP	A	M	3	M0008	Harp trap
8/12/2009	EP	A	F	4	F0096	Harp trap
8/12/2009	EP	J	M	2	M0006	Harp trap
8/12/2009	EP	J	M	2	M0005	Harp trap
8/12/2009	EP	J	M	1	M0004	Harp trap
8/12/2009	EP	J	M	1	M0003	Harp trap
8/12/2009	EP	A	M	3	M0002	Harp trap
8/12/2009	EP	J	M	2	M0001	Harp trap
8/12/2009	EP	A	M	3	M0150	Harp trap
8/12/2009	EP	A	M	3	M0149	Harp trap
8/12/2009	EP	J	M	1	M0151	Harp trap
8/12/2009	EP	J	M	2	M0148	Harp trap
8/12/2009	EP	A	M	3	M0147	Harp trap
8/12/2009	EP	J	M	1	M0146	Harp trap
8/12/2009	EP	J	M	1	M0145	Harp trap
8/12/2009	EP	J	M	1	M0143*	Harp trap
8/12/2009	EP	J	M	2	M0142	Harp trap
8/12/2009	EP	J	M	2	M0141	Harp trap
8/12/2009	EP	A	M	3	M0140	Harp trap
8/12/2009	EP	J	M	2	M0139*	Harp trap
8/12/2009	EP	J	M	2	M0137	Harp trap
8/12/2009	EP	J	M	2	M0136	Harp trap
8/12/2009	EP	A	F	4	F0094	Harp trap
8/12/2009	EP	A	M	3	M0135	Harp trap
8/12/2009	EP	A	M	2	M0134	Harp trap
8/12/2009	EP	J	M	2	M0133*	Harp trap
8/12/2009	EP	J	M	2	M0132	Harp trap
8/12/2009	EP	A	M	3	M0130	Harp trap
8/12/2009	EP	J	M	2	M0129	Harp trap
8/12/2009	EP	J	M	1	M0128	Harp trap
8/12/2009	EP	A	M	3	M0127	Harp trap
8/12/2009	EP	A	F	5	F0093	Harp trap
8/12/2009	EP	J	M	2	M0126	Harp trap
8/12/2009	EP	J	M	2	M0125	Harp trap
8/12/2009	EP	J	F	2	F0092	Harp trap
8/12/2009	EP	A	M	3	M0124	Harp trap
8/12/2009	EP	J	F	2	F0091	Harp trap
8/12/2009	EP	J	M	1	M0123	Harp trap
8/12/2009	EP	A	F	3	F0090	Harp trap
8/12/2009	EP	J	M	2	M0122	Harp trap
8/12/2009	EP	A	F	3	F0089	Harp trap
8/12/2009	EP	A	F	3	F0058	Harp trap
8/12/2009	EP	J	M	2	M0121	Harp trap
8/12/2009	EP	J	M	1	M0120	Harp trap
8/12/2009	EP	A	M	2	M0119	Harp trap
8/25/2009	EP	J	F	2	F0050	Harp trap
8/25/2009	EP	J	F	2	F0049	Harp trap

8/25/2009	EP	J	M	2	M0118	Harp trap
8/25/2009	EP	J	M	1	M0117	Harp trap
8/25/2009	EP	J	M	2	M0116	Harp trap
8/25/2009	EP	J	M	2	M0115	Harp trap
8/25/2009	EP	J	M	2	M0114	Harp trap
8/25/2009	EP	J	M	1	M0113	Harp trap
8/25/2009	EP	J	M	2	M0112	Harp trap
8/25/2009	EP	A	F	3	F0048	Harp trap
8/25/2009	EP	A	M	4	M0111	Harp trap
8/25/2009	EP	A	M	3	M0110	Harp trap
8/25/2009	EP	J	M	1	M0109*	Harp trap
8/25/2009	EP	J	M	1	M0108	Harp trap
8/25/2009	EP	J	M	2	M0107	Harp trap
8/25/2009	EP	J	M	1	M0106	Harp trap
8/25/2009	EP	J	M	2	M0105	Harp trap
8/25/2009	EP	J	M	1	M0104	Harp trap
8/25/2009	EP	A	M	3	M0103	Harp trap
8/25/2009	EP	J	F	2	F0047	Harp trap
8/25/2009	EP	J	M	1	M0102	Harp trap
8/25/2009	EP	J	M	1	M0101	Harp trap
8/25/2009	EP	J	M	2	M0200	Harp trap
8/25/2009	EP	A	M	3	M0199	Harp trap
8/25/2009	EP	J	M	2	M0198**	Harp trap
8/25/2009	EP	J	M	2	M0197	Harp trap
8/25/2009	EP	J	M	1	M0196*	Harp trap
8/25/2009	EP	A	F	4	F0046	Harp trap
8/25/2009	EP	J	M	2	M0195	Harp trap
8/25/2009	EP	J	M	1	M0194	Harp trap
8/25/2009	EP	A	F	3	F0045	Harp trap
10/26/2009	EP	A	M	3	M0097	Harp trap
4/18/2010	EP	A	F	4.6	F0061	In-cave, Hand-removal
4/18/2010	EP	A	M	3.5	M0193	In-cave, Hand-removal
4/18/2010	EP	A	F	4.3	F0062	In-cave, Hand-removal
4/18/2010	EP	A	F	5.6	F0063	In-cave, Hand-removal
4/18/2010	EP	A	F	4.3	F0064	In-cave, Hand-removal
4/18/2010	EP	A	M	5.1	M0152	In-cave, Hand-removal
4/18/2010	EP	A	F	4.7	F0066	In-cave, Hand-removal
4/18/2010	EP	A	F	5.3	F0067	In-cave, Hand-removal
4/18/2010	EP	A	M	5.2	M0154	In-cave, Hand-removal
4/18/2010	EP	A	F	5.7	F0068	In-cave, Hand-removal
4/18/2010	EP	A	M	4.2	M0155	In-cave, Hand-removal
4/18/2010	EP	A	F	5.9	F0069	In-cave, Hand-removal
4/18/2010	EP	A	F	6.6	F0070	In-cave, Hand-removal
4/18/2010	EP	A	F	4.3	F0071	In-cave, Hand-removal
4/18/2010	EP	A	M	3.6	M0198**	In-cave, Hand-removal
4/18/2010	EP	A	M	4.4	M0133*	In-cave, Hand-removal
4/18/2010	EP	A	M	4.2	M0156	In-cave, Hand-removal
4/18/2010	EP	A	M	4.1	M0157*	In-cave, Hand-removal
4/18/2010	EP	A	M	4.3	M0158	In-cave, Hand-removal

4/18/2010	EP	A	F	6.3	F0073	In-cave, Hand-removal
4/18/2010	EP	A	M	4.6	M0159	In-cave, Hand-removal
4/18/2010	EP	A	M	4.8	M0160*	In-cave, Hand-removal
4/18/2010	EP	A	M	4.9	M0161	In-cave, Hand-removal
4/18/2010	EP	A	M	4.3	M0162	In-cave, Hand-removal
4/18/2010	EP	A	M	4.5	M0163	In-cave, Hand-removal
4/29/2010	EP	A	F	4.6	F0074	In-cave, Hand-removal
4/29/2010	EP	A	F	5.6	F0075	In-cave, Hand-removal
4/29/2010	EP	A	M	5.5	M0165	In-cave, Hand-removal
4/29/2010	EP	A	M	5.6	M0166*	In-cave, Hand-removal
4/29/2010	EP	A	M	4.3	M0198**	In-cave, Hand-removal
4/29/2010	EP	A	M	4.5	M0167	In-cave, Hand-removal
4/29/2010	EP	A	M	4.6	M0168	In-cave, Hand-removal
4/29/2010	EP	A	F	5.6	F0076*	In-cave, Hand-removal
4/29/2010	EP	A	M	4.4	M0169	In-cave, Hand-removal
4/29/2010	EP	A	M	4.3	M0170	In-cave, Hand-removal
4/29/2010	EP	A	M	4.7	M0171	In-cave, Hand-removal
4/29/2010	EP	A	M	4.7	M0172	In-cave, Hand-removal
4/29/2010	EP	A	M	4.1	M0173	In-cave, Hand-removal
4/29/2010	EP	A	M	4.7	M0157*	In-cave, Hand-removal
4/29/2010	EP	A	M	5.2	M0174	In-cave, Hand-removal
4/29/2010	EP	A	M	4.6	M0177	In-cave, Hand-removal
4/29/2010	EP	A	M	3.5	M0178	In-cave, Hand-removal
4/29/2010	EP	A	M	5.1	M0179	In-cave, Hand-removal
4/29/2010	EP	A	M	4.6	M0180	In-cave, Hand-removal
4/29/2010	EP	A	M	4.5	M0181	In-cave, Hand-removal
4/29/2010	EP	A	M	4.8	M0160*	In-cave, Hand-removal
4/29/2010	EP	A	F	5.7	F0077	In-cave, Hand-removal
8/10/2010	EP	A	M	5.3	M0189	Mist Net
8/10/2010	EP	A	M	5.5	M0188	Mist Net
8/10/2010	EP	A	M	6.3	M0082	Mist Net
8/10/2010	EP	A	F	7	F0078	Mist Net
8/10/2010	RED	A	F	12.6	F0079	Mist Net
8/10/2010	EP	A	M	7.3	M0187	Mist Net
8/10/2010	EP	A	M	6.2	M0186	Mist Net
8/10/2010	EP	A	M	6.1	M0109*	Mist Net
8/10/2010	RED	A	F	14.3	F0080	Mist Net
8/10/2010	BB	A	M	15	M0185	Mist Net
8/10/2010	EP	A	M	5.7	M0143*	Mist Net
8/10/2010	EP	A	M	3.4	M0052	Mist Net
8/10/2010	BB	A	M	13	M0053	Mist Net
8/10/2010	BB	A	F	18	F0043	Mist Net
8/10/2010	EP	A	F	5.2	F0020	Mist Net
8/10/2010	RED	A	F	14.5	F0081	Mist Net
8/10/2010	EP	A	F	6.2	F0082	Mist Net
8/10/2010	EP	A	F	6.2	F0060	Mist Net
8/10/2010	RED	J	M	11.1	M0095	Mist Net
8/10/2010	EP	A	M	6.3	M0094	Mist Net
8/11/2010	EP	A	F	5.5	F0087	Mist Net

8/11/2010	RED	A	F	9.5	F0086	
8/11/2010	EP	A	F	4.6	F0085	Mist Net
8/11/2010	EP	J	M	3.5	M0093	Mist Net
8/11/2010	EP	J	M	2	M0092	Mist Net
8/11/2010	EP	A	M	4	M0139*	Mist Net
8/11/2010	EP	A	M	6.2	M0091	Mist Net
8/11/2010	EP	A	M	5.9	M0090	Mist Net
8/11/2010	EP	A	M	5.3	M0089	Mist Net
8/11/2010	EP	A	M	6.4	M0166*	Mist Net
8/11/2010	EP	A	F	7.6	F0084	Mist Net
8/11/2010	EP	A	M	5.2	M0088	Mist Net
8/11/2010	EP	A	F	5.1	F0083	Mist Net
8/11/2010	EP	A	F	6.7	F0059	Mist Net
8/11/2010	EP	A	M	6.1	M0087	Mist Net
8/11/2010	RED	A	F	11.6	F0058	Mist Net
8/11/2010	EP	A	F	7.6	F0076*	Mist Net
8/11/2010	EP	J	M	3.8	M0086	Mist Net
8/11/2010	EP	A	M	4.3	M0042	Mist Net
8/11/2010	BB	A	M	16.1	M0085	Mist Net
8/11/2010	EP	A	F	7.4	F0057	Mist Net
8/11/2010	BB	A	F	23.5	F0056	Mist Net
8/11/2010	EP	A	F	6.4	F0055	Mist Net
8/11/2010	EP	A	M	6.7	M0196*	Mist Net
8/11/2010	EP	A	F	6.7	F0054	Mist Net
8/11/2010	RED	J	M	16.5	M0084	Mist Net
8/11/2010	EP	A	F	5.8	F0053	Mist Net
8/11/2010	EP	A	M	5.1	M0083	Mist Net
8/11/2010	EP	A	F	6.3	F0052	Mist Net
8/11/2010	EP	J	F	3.8	F0051	Mist Net

APPENDIX B

APSU Bat Banding/Tagging Data Sheet

Field Notes:

Date:

Time:

Weather Conditions:

P.I./Researchers

Date	Species	Sex	Age	Weight	Wing Index	Band #	Capture Time	Release Time	Notes

VITA

Morgan Eve Kurz was born in New York, New York on July 4th 1984 to Kathy L. Watts of Clarksville, Tennessee and Arnold W. Kurz of Kassel, Germany. Morgan earned her high school diploma from Clarksville High School in 2002. She enrolled at Austin Peay State University in 2003 and earned her Bachelor of Science in Biology in 2008 with primary interest in wildlife biology and zoology. She lived with her patient and supporting mother throughout her college career.

In 2008, she entered the Master of Science in Biology program as a graduate assistant in the Biology department at Austin Peay State University. She worked under the leadership of Dr. Andrew N. Barrass, a principal investigator in the Center of Excellence for Field Biology and a professor of multiple disciplines in the biological field.

After earning her Master's of Science in Biology, Morgan plans to pursue a position as a Field Biologist with a focus on Wildlife Biology. She also plans to become certified as a teacher for the state of Tennessee for all ages and continue instructing college-level courses.