

BREEDING BEHAVIOR OF THE TRI-COLORED BAT (*PERIMYOTIS SUBFLAVUS*):
RELEVANCE FOR DEVELOPMENT OF A CAPTIVE BREEDING PROGRAM

by

KIERSTEN KATHELEEN GIBIZOV

(Under the Direction of Dr. Sharon Crowell-Davis)

ABSTRACT

Since the discovery of White-nose Syndrome (*Geomyces destructans*) in the United States in 2006, the populations of several cave dwelling bat species have greatly decreased. One of the species affected by White-nose syndrome is the Tri-colored bat (*Perimyotis subflavus*). Due to the drastic decline in bat populations, researchers and conservationists are discussing possible actions that can be taken to ensure that affected species can survive. Captive breeding programs have been successful in saving threatened and endangered species in the past and this course of action has been proposed for *P. subflavus*. The largest obstacle to this proposal is that little information exists on how *P. subflavus* copulates. The goal of this thesis is to describe the breeding behavior and associated environmental parameters for *P. subflavus* so that this information may be used as a foundation for development of captive breeding programs.

INDEX WORDS: *Perimyotis subflavus*, Tri-colored bat, chiropteran social behavior, copulation, hibernation, *Geomyces destructans*, captive breeding programs

BREEDING BEHAVIOR OF THE TRI-COLORED BAT (*PERIMYOTIS SUBFLAVUS*):
RELEVANCE FOR DEVELOPMENT OF A CAPTIVE BREEDING PROGRAM

by

KIERSTEN KATHELEEN GIBIZOV

B.A., Texas A&M University, 1998

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

© 2013

Kiersten Katheleen Gibizov

All Rights Reserved

BREEDING BEHAVIOR OF THE TRI-COLORED BAT (*PERIMYOTIS SUBFLAVUS*):
RELEVANCE FOR DEVELOPMENT OF A CAPTIVE BREEDING PROGRAM

by

KIERSTEN KATHELEEN GIBIZOV

Major Professor: Sharon Crowell-Davis

Committee: Steven Castleberry
John Maerz

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2013

DEDICATION

This endeavor is dedicated to my family, who helped me accomplish the next level of education in hopes of a brighter future. Thank you to my father, Roger Anderson, who didn't say no when I asked him to keep a bargain made fifteen years earlier. I would not be here without his generosity. Thank you to my husband, Georgiy Gibizov, who knew I would not quit once I'd started. Thank you to my mother, Donna Anderson, who listened to all my doubts and assured me that perseverance always pays off.

ACKNOWLEDGEMENTS

Thank you to Mason Rountree, the owner of White River Cave, without which I would not have had a tri-colored bat hibernaculum within which to conduct this research.

Thank you to Dr. Crowell-Davis for taking me on as a graduate student. Thank you to Dr. John Maerz, who helped me determine my thesis project and also to Dr. Steven Castleberry, who allowed me use of equipment that aided me in my observations as well as providing guidance with my chosen species.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS.....	v
LIST OF TABLES.....	viii
LIST OF FIGURES.....	ix
CHAPTER	
1 INTRODUCTION.....	1
Background on White-nose Syndrome.....	2
Life Cycle of <i>Perimyotis subflavus</i>	5
Purpose of the Study.....	8
Expected Results.....	9
References.....	10
2 BREEDING BEHAVIOR OF THE TRI-COLORED BAT (<i>PERIMYOTIS SUBFLAVUS</i>): RELEVANCE FOR DEVELOPMENT OF A CAPTIVE BREEDING PROGRAM.....	16
ABSTRACT.....	17
Introduction.....	18
Materials and Methods.....	21
Study Site.....	21

Materials.....	22
Methods.....	23
Results.....	25
Discussion.....	32
References.....	40
3 CONCLUSIONS.....	51

LIST OF TABLES

	Page
Table 2.1. Temperature and humidity levels in cave chambers.....	49
Table 2.2. Measurements, in inches, between roosting individuals.....	49
Table 2.3. Dates copulations, vocalizations, and grooming were observed or recorded.....	50

LIST OF FIGURES

	Pages
Figure 1.1 <i>Perimyotis subflavus</i>	15
Figure 3.1. Bat gate of White River Cave.....	46
Figure 3.2. Behaviors in hibernaculum.....	47
Figure 3.3. Typical aggregation pattern of <i>P. subflavus</i> seen in White River Cave, chamber 2.....	48

CHAPTER 1

INTRODUCTION

Conservation programs contribute to the survival and recovery of endangered species, especially those threatened by external factors, such as disease introduced by human activity, habitat destruction, and food resource depletion, bringing many well known species back from the brink of extinction. The California condor (Alagona, 2004), the bald eagle (Marcovitz, 2003), and the American alligator (Horton and Weissgold, 2005) are some of the most famous examples of successful conservation efforts. These successes would not have been possible without understanding the obstacles to their continued survival and understanding their life cycle. With these species as a template, we must expand our knowledge of threats to native bats to provide a foundation for successful conservation efforts (Weller et al., 2009). Captive breeding programs are one of the options for reversing the fate of endangered, or potentially endangered, species of bats (Foley et al., 2011).

White-nose syndrome (WNS) is one of the obstacles to survival that cave hibernating bats in the United States are facing. To further conservation efforts, we must understand what the cause of WNS is and what the effects are on the various hibernating bat species. For a captive breeding program to be a viable option, a better understanding of all phases of their life cycle must be obtained from observations of unaffected wild populations (Weller et al., 2009).

Background on White-nose syndrome

White-nose syndrome (WNS) was first seen in the northeastern United States at Howe Cave in upstate New York in 2006 (Foley et al., 2011). Hibernating bats within these caves were presenting with a white fuzzy substance, now known to be a fungus, on the nose and other areas of exposed skin. Large numbers of dead bats were also found in caves nearby in which live bats infected with the white substance were also found. Mortality in these caves reached up to 99% in some hibernacula (Frick et al., 2010; Jonasson and Willis, 2011).

In 2008, the causative agent of WNS was identified as a psychrophilic (cold-loving) fungus in the genus *Geomyces* (Blehert et al., 2009). Fungi in the genus *Geomyces* are adapted to growth in colder temperatures and the species cultured from bats displaying WNS lesions grew best in temperatures from 5°C - 10°C (41°F-50°F) but could still grow in temperatures of 15°C (59°F), although not optimally (Blehert et al., 2009). Growth terminated at $\leq 3^{\circ}\text{C}$ (37.4°F) and $\geq 20^{\circ}\text{C}$ (68°F). The fungus associated with WNS is called *Geomyces destructans* and is found in colder, more humid portions of caves that certain species of hibernating bats frequent.

How *Geomyces destructans* causes death is still undetermined but it is associated with premature arousal during hibernation, aberrant behavior during hibernation such as exiting the hibernaculum during winter temperatures, and premature loss of the fat reserves needed to survive the hibernation period (USFWS, 2009; Frick et al., 2010; Foley et al., 2011). The white fuzzy substance on the muzzle and wings is composed of

the conidia (spores) from the fungus. Research initiated in 2009 revealed that the hyphae of the fungus penetrate the skin, replacing epidermal cells, hair follicles, sebaceous glands, sweat glands, and even the deeper dermal layers of the skin, especially in the wing membrane (Blehert et al., 2009; Cryan et al., 2010). Altering the integrity of the skin severely alters the bat's ability to maintain proper temperature levels and water retention to survive hibernation (Neuweiler, 2000). Because the fungus is capable of invading, eroding, and digesting the skin of the hibernating bats (Cryan et al., 2010), presumably irritating the bats enough to trigger arousal at inappropriate times, scientists hypothesize that the increased alterations in their physiological homeostasis during hibernation causes them to deplete their fat stores before there are enough available food resources to replenish them, which may be sufficient enough to cause emaciation and/or dehydration, leading to death.

Gross lesions associated with White-nose syndrome consist of white fungal growths on the muzzle, ears, and wings of hibernating bats (Blehert et al., 2009). The fungus can be seen on any exposed skin. Clinical signs are harder to casually identify but the most commonly observed with WNS are emaciation due to loss of fat reserves, aberrant behavior, such as exiting the hibernaculum in freezing temperatures, and death (USFWS, 2009; Frick et al., 2010; Foley et al., 2011; Jonasson and Willis, 2011). The mortality pattern appears to follow a seasonal cycle, with lesions and aberrant behavior detected mostly in winter, often after January (Foley et al., 2011).

As of 2010, it was estimated that almost one million bats have died as a result of WNS, which far exceeds any mortality event previously seen in bats, whether natural or

anthropogenically caused (Cryan et al., 2010). U.S. Fish and Wildlife estimates of deaths attributable to WNS increased to 5.5 million individuals. Researchers are still unsure of how the fungus is transmitted, but it is thought that bat to bat transfer is the most likely cause; although, transfer from location to location may also be facilitated by inadvertent human contact (USFWS, 2009; Foley et al., 2011). It is also possible that roost sites that an infected bat has used and that are subsequently visited by multiple uninfected bats is a mechanism of transfer (Puechmaille et al., 2011). By July of 2010 WNS lesions had been detected on hibernating bats in thirteen states and two Canadian provinces including New York, Vermont, Massachusetts, New Jersey, Connecticut, Pennsylvania, New Hampshire, Delaware, Virginia, West Virginia, Tennessee, Missouri, Oklahoma, and Ontario and Quebec (Foley et al., 2011). As of April 2013, WNS has been confirmed in seven more states including Maine, Ohio, Illinois, North Carolina, South Carolina, Georgia, and Alabama, as well as two additional Canadian provinces including Nova Scotia and New Brunswick (whitenosesyndrome.org).

WNS is a severe threat to any hibernating bat found within the optimum environmental parameters of *G. destructans*, i.e. temperatures between 3°C (37.4°F) and 20°C (68°F) with high humidity. To date, nine bat species in the United States and Canada have been found with WNS lesions, including the gray bat (*Myotis grisescens*), the Indiana bat (*Myotis sodalis*), the little brown bat (*Myotis lucifugus*), the northern long-eared bat (*Myotis septentrionalis*), the eastern small-footed bat (*Myotis leibii*), the southeastern bat (*Myotis austroriparius*), the cave bat (*Myotis velifer*), the tri-colored bat (*Perimyotis subflavus*), and the big brown bat (*Eptesicus fuscus*) (Foley et al., 2011). *M.*

lucifugus was previously the most widely spread and commonly found bat in the United States. Since WNS was discovered it has been virtually wiped out in the northeast where hibernacula counts once numbered in the hundreds of thousands. *M. grisescens* and *M. sodalis* were already endangered pre-WNS. Now WNS threatens them with extinction (Langwig et al., 2012). *P. subflavus* may not be facing imminent extinction, but their numbers have decreased significantly since WNS has been detected in hibernacula where they are found (Langwig et al., 2012) and their numbers were never as large as *M. lucifugus*.

Unfortunately, WNS is not the only threat to temperate bat species. Continued pressure from humans in the form of habitat destruction, roost destruction, and poisoning through overuse of pesticides also threatens their survival (Swift, 1998). For any conservation effort to succeed, we must acknowledge all facets of the situation and learn as much as possible about the life cycle of the species we wish to help before what threatens them prevents us from ever doing so (Weller et al., 2009).

Life Cycle of *Perimyotis subflavus*

The tri-colored bat, *Perimyotis subflavus*, is classified in the Order Chiroptera, Suborder Microchiroptera, Family Vespertilionidae (Vesper or Evening bats) and is found throughout the eastern United States, from northern Maine to southern Florida and as far west as central Texas. Outside the US they can be found in Nova Scotia, Canada, and south into southeastern Honduras (Fujita and Kunz, 1984). Previously, this species was classified in the Genus *Pipistrellus* as *Pipistrellus subflavus*. In 2003, Hooper and Van

Den Bussche undertook the challenge of investigating, via mitochondrial DNA sequencing, the relationship of various vespertilionid bats. It had been thought for years that some species were incorrectly classified. Two of the species in question were the Eastern pipistrelle, *Pipistrellus subflavus* and the Western pipistrelle, *Pipistrellus hesperus* (Hooper et al., 2006). Through phylogenetic analyses it was confirmed that these two bats do not share a most recent common ancestor with other pipistrelles or even each other. Menu (1984) suggested twenty years ago that *P. subflavus* should be reclassified into the Genus *Perimyotis* and Hooper and Van Den Bussche's results supported the change. Currently they are the only bat classified in the Genus *Perimyotis* (Hooper et al., 2006).

Tri-colored bats can be distinguished from other Vesper bats of the same size by their distinctive tricolored hairs. The individual hairs are dark brown or black at the base, a lighter yellow-brown in the middle, and dark brown at the tip. Their overall coloration can vary from yellowish-brown to dark reddish brown both dorsally and ventrally (Fujita and Kunz, 1984; Schober and Grimmberger, 1997). Measurements are a total body length of 77-89 mm, a tail length of 34-41 mm, a hind foot length of 7.3-9.9 mm, an ear length of 12.4-14.1 mm, a forearm length of 31.4-34.1 mm, and weight of 4.6-7.9 g (Fujita and Kunz, 1984). The tragus is blunt, the calcar is not keeled, and the forearms of live bats are pink in color (Schober and Grimmberger, 1997) (Fig. 1.2).

Many bats in the Family Vespertilionidae divide habitat selection into spring and summer roosts and fall and winter roosts. *P. subflavus* is no exception. During the spring and summer seasons, male and female tri-colored bats will separate and live apart from

each other (Fujita and Kunz, 1984; Foley et al., 2010). The males will live solitary lives roosting in tree foliage and hunting for insects, while females gather in tree foliage (Veilleux et al., 2003), caves, and manmade structures (Sandel et al., 2001) forming maternity roosts where the young are born and raised. When the fall season begins both sexes will migrate to hibernacula, typically caves and mines, where they roost together throughout the winter season (Cockrum, 1955).

Before long term torpor begins, many species of vespertilionid bats will participate in an activity called swarming. Swarming is characterized by “intense chasing flights” (Glover and Altringham, 2008) that typically occur at cave or mine entrances. Whether *P. subflavus* participates in this activity is still unclear, but they are found sharing hibernacula with *Myotis* species, which do swarm. It is thought that swarming may be important in identifying appropriate mates and determining the suitability of hibernacula (Parsons et. al, 2003). Within winter hibernacula, the sexes of *P. subflavus* roost together in loose colonies where individuals rest close together but usually are not touching each other (Langwig et al., 2012). During the autumn, before temperatures drop to near freezing and freezing, the bats will exit the hibernaculum to continue hunting and building up their fat reserves.

Copulation has been observed to occur during this season, as both sexes are together, making this the optimum time for reproductive activities (Cockrum, 1955). As colder temperatures evolve into the winter season, the bats spend more time in torpor, and females’ reproductive status stalls before fertilization which halts the reproduction cycle until the warmer weather in spring (Neuweiler, 2000). When temperatures warm the

females, harboring stored sperm retained from fall and winter copulation activities, once again travel to maternity colonies where they will birth and raise offspring while males travel to their solitary lifestyle.

Hibernacula can house many species of bats and *P. subflavus* has been found in the company of other vespertilionid bats such as the little brown bat, the Indiana bat, the southeastern bat, the gray bat, and the big brown bat (Fujita and Kunz, 1984). Within the hibernaculum each species prefers specific temperatures and humidity levels, thereby minimizing interspecies competition for roosting space. *P. subflavus* prefers warmer areas with high humidity (Cryan et al., 2010) ranging between 9-12°C (49-54°F) (Brack Jr., 2007; Briggler and Prather, 2003) and 95% humidity. Compared to other cave hibernating bats, the temperatures preferred by *P. subflavus* are considerably warmer and found in more stable chambers, which allows them to stay in torpor for longer periods of time than other species (Vincent and Whitaker, 2007). Due to their choice of winter roosts, *P. subflavus* is within the optimum growth range of *Geomyces destructans* and is one of the species affected by white-nose syndrome (Langwig et al., 2012).

Purpose of the Study

Minimal research has been conducted on *P. subflavus* in the past and none has focused specifically on breeding behavior. The purpose of this study is to understand the normal mating behavior of the Tri-colored bat, in order to facilitate development of a successful captive breeding program for this species. This study focuses on pre-copulatory, copulatory, and post-copulatory behavior of the tri-colored bat and the

parameters within which these behaviors occur. Knowledge of the copulation habits and the environmental factors that contribute to this behavior are integral to development of a successful captive breeding program. It is more than just bringing males and females together in the same place. Temperature, humidity, population size, and availability of food and water resources may have an impact on copulatory behavior (McNab, 1974; Lourenço and Palmeirim, 2003; Wojciechowski et al., 2007). Hopefully, observations provided by this study will offer a strong foundation upon which an effective captive breeding program can be designed.

Expected Results

Based upon previous reproductive research involving *P. subflavus* and other temperate Vespertilionid bats, breeding during the beginning of the hibernation period was expected (Wimsatt, 1945; Cockrum, 1955), and possibly breeding throughout the hibernation period; although reports of copulation throughout hibernation are rare (Boyles et al., 2006). The males and females of many temperate bat species only fraternize during the fall and winter seasons; therefore, this would be the obvious time to copulate. It was also expected that, like other colonial hibernating bats, such as the little brown bat, swarming, periods of intense chasing during flight, typically seen at the mouths of hibernacula or within hibernacula chambers, would be an important period in which copulation would occur and this behavior was expected (Thomas et al., 1979; Parsons, Jones, and Greenaway, 2003). Periodic arousal for feeding, drinking, and copulation has been recorded for other Vespertilionid bats; therefore we did expect to see

some individuals in flight during the hibernation period (Boyles et al., 2006). Individuals roosting in areas of the cave with warmer, more stable temperatures, ranging between 10°C to 13°C (50° F-56° F), was also expected (McNab 1974; Fujita and Kunz, 1984; Vincent and Whitaker Jr., 2007).

References

- Alagona, Peter S. 2004. Biography of a “Feathered Pig”: The California Condor Conservation Controversy. *Journal of the History of Biology* 37, 557-583.
- Blehert, David S., Alan C. Hicks, Melissa Behr, Carol U. Meteyer, Brenda M. Berlowski-Zier, Elizabeth L. Buckles, Jeremy T. H. Coleman, Scott R. Darling, Andrea Gargas, Robyn Niver, Joseph C. Okoniewski, Robert J. Rudd, and Ward B. Stone. 2009. Bat White-Nose Syndrome: An Emerging Fungal Pathogen? *Science* 323, 227.
- Boyles, Justin G., Miranda B. Dunbar, and John O. Whitaker Jr. 2006. Activity following arousal in North American vespertilionid bats. *Mammal Review* 36, 267-280.
- Brack Jr., Virgil. 2007. Temperatures and Locations Used by Hibernating Bats, Including *Myotis sodalis* (Indiana Bat), in a Limestone Mine: Implications for Conservation and Management. *Environ Manage* 40, 739-746.
- Briggler, J. T. and J. W. Prather. 2003. Seasonal use and selection of caves by the eastern pipistrelle bat (*Pipistrellus subflavus*). *American Midland Naturalist* 149, 406-412.

- Cockrum, E. Lendell. 1955. Reproduction in North American Bats. Transactions of the Kansas Academy of Science 58, 487-511.
- Cryan, Paul. M., Carol Uphoff Meteyer, Justin G. Boyles, and David S. Blehert. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology 8, 1-8.
- Foley, Janet, Deana Clifford, Kevin Castle, Paul Cryan, and Richard S. Ostfeld. 2011. Investigating and Managing the Rapid Emergence of White-Nose Syndrome, a Novel, Fatal, Infectious Disease of Hibernating Bats. Conservation Biology 25, 223-231.
- Frick, Winifred F., Jacob F. Pollock, Alan C. Hicks, Kate E. Langwig, D. Scott Reynolds, Gregory G. Turner, Calvin M. Butchkoski, and Thomas H. Kunz. 2012. An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species. Science 329, 679-682.
- Fujita, Marty S. and Thomas H. Kunz. 1984. Mammalian Species. *Pipistrellus subflavus*. Am. Soc. Mammal. 228, 1-6.
- Glover, Anita M. and John D. Altringham. 2008. Cave selection and use by swarming bat species. Biological Conservation 141, 1493-1504.
- Hoofer, Steven R., Ronald A. Van Den Bussche, and Ivan Horacek. 2006. Generic Status of the American Pipistrelles (Vespertilionidae) with Description of a New Genus. Journal of Mammology 87, 981-992.
- Horton, Clif and Bruce Weissgold. 2005. Partnerships for Alligator Recovery and Trade. Endangered Species Bulletin 30, 10-11.

- Jonasson, Kristin A. and Craig K. R. Willis. 2011. Changes in Body Condition of Hibernating Bats Support the Thrifty Female Hypothesis and Predict Consequences for Populations with White-Nose Syndrome. *PLoS one* 6, 1-8.
- Langwig, Kate E., Winifred F. Frick, Jason T. Bried, Alan C. Hicks, Thomas H. Kunz, and A. Marm Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 15, 1050-1057.
- Lourenço, Sofia, I. and Jorge M. Palmeirim. 2004. Influence of temperature in roost selection by *Pipistrellus pygmaeus* (Chiroptera): relevance for the design of bat boxes. *Biological Conservation* 119, 237-243.
- Marcovitz, Hal. 2003. *Bald Eagle*. Mason Crest Publishers, Bromall, Pennsylvania.
- McNab, Brian K. 1974. The behavior of Temperate Cave Bats in a Subtropical Environment. *Ecology* 5, 943-958.
- Neuweiler, Gerhard. 2000. *The Biology of Bats*, Oxford University Press, New York.
- Parsons, K. N., G. Jones, and F. Greenaway. 2003. Swarming activity of temperate zone microchiropteran bats: effects of season, time of night and weather conditions. *Journal of Zoology, London* 261, 257-264.
- Parsons, Katharine N., Gareth Jones, Ian Davidson-Watts, and Frank Greenaway. 2003. Swarming of bats at underground sites in Britain - implications for conservation. *Biological Conservation* 111, 63-70.
- Puechmaile, Sébastien, J. Gudrun Wibbelt, Vanessa Korn, Hubert Fuller, Frédéric Forget, Kristin Mühldorfer, Andreas Kurth, Wieslaw Bogdanowicz, Christophe

- Borel, Thijs Bosch, Thomas Cherezy, Mikhail Drebet, Tamás Görföl, Anne-Jifke Haarsma, Frank Herhaus, Guénael Hallart, Matthias Hammer, Christian Jungmann, Yann Le Bris, Lauri Lutsar, Matti Masing, Bart Mulkens, Karsten Passior, Martin Starrach, Andrzej Wojtaszewski, Ulrich Zöphel, and Emma C. Teeling. 2011. Pan-European Distribution of White-nose Syndrome Fungus (*Geomyces destructans*) Not Associated with Mass Mortality. PLoS ONE 6: e19167, 1-11.
- Sandel, Jody K., Grant R. Benatar, Kyle M. Burke, Christopher W. Walker, Thomas E. Lacher Jr., and Rodney L. Honeycutt. 2001. Use and Selection of Winter Hibernacula by the Eastern Pipistrelle (*Pipistrellus sibflavus*) in Texas. Journal of Mammology, 82, 173-178.
- Schober, Wilfried and Eckard Grimmberger. 1997. The Bats of Europe and North America. T. F. H. Publications, Inc., New Jersey.
- Swift, Susan M. 1998. Long-Eared Bats. T&A D Poyser Ltd., London.
- Thomas, Donald W., M. Brock Fenton, and Robert M. R. Barclay. 1979. Social Behavior of the Little Brown Bat, *Myotis lucifugus*: I. Mating Behavior. Behavioral Ecology and Sociobiology 6, 129-136.
- U.S. Fish and Wildlife Service. 2009. White-nose syndrome in bats: Frequently Asked Questions.
- Veilleux, Jacques Pierre, John O. Whitaker, and Sherry L. Veilleux. 2003. Tree-roosting Ecology of Reproductive Female Eastern Pipistrelles, *Pipistrellus subflavus*, in Indiana. Journal of Mammology 84, 1068-1075.

- Vincent, Elizabeth A. and John O. Whitaker Jr. 2007. Hibernation of the Eastern Pipistelle, *Perimyotis subflavus*, in an abandoned mine in Vermillion County, Indiana, with some information on *Myotis lucifugus*. Proceedings of the Indiana Academy of Science 116, 58-65.
- Weller, Theodore, J., Paul M. Cryan, and Thomas J. O'Shea. 2009. Broadening the focus of bat conservation and research in the USA for the 21st century. Endangered Species Research 8, 129-145.
- Wimsatt, William A. 1945. Notes on Breeding Behavior, Pregnancy, and Parturition in some Vespertilionid Bats of the Eastern United States. Journal of Mammalogy 26, 23-33.
- Wojciechowski, Michal S., Malgorzata Jefimow, and Eugenia Tegowska. 2007. Environmental conditions, rather than season, determine torpor use and temperature selection in large mouse-eared bats (*Myotis myotis*). Comparative Biochemistry and Physiology, Part A 147, 828-840.



Figure 1.1. *Perimyotis subflavus* taken at White River Cave in 2012 by author.

CHAPTER 2

BREEDING BEHAVIOR OF THE TRI-COLORED BAT (*PERIMYOTIS SUBFLAVUS*): RELEVANCE FOR DEVELOPMENT OF A CAPTIVE BREEDING PROGRAM¹

¹ Gibizov, Kiersten. To be submitted to *Journal of Veterinary Behavior: Clinical Applications and Research*

ABSTRACT

The key to any successful captive care and breeding program is knowing and understanding the environmental parameters in which a given species survives and thrives in the wild. Once this information is obtained it can be replicated as closely as possible in captivity to provide the captive population with the best chance of survival. The tri-colored bat, *Perimyotis subflavus*, is a temperate bat species for which large gaps of knowledge concerning winter breeding behaviors exist. Aggregations of *P. subflavus* were observed in a hibernaculum in Northern Georgia for two winter seasons and various behaviors, including copulation were recorded. Within this hibernaculum, temperature and humidity, roosting distance between individuals, post-torpor activity, and pre- and post-copulatory behavior was recorded, revealing information previously unknown about *Perimyotis subflavus*. Temperature means were 12.7-13C, humidity means were 84-88.7%, and roosting distance was between 0 cm to 86.4 cm. Flying, grooming, drinking, and copulation were the post-torpor activities recorded. Sniffing preceding copulation was recorded. Copulation was recorded throughout the hibernation season.

INDEX WORDS: *Perimyotis subflavus*, Tri-colored bat, chiropteran social behavior, copulation, hibernation, *Geomyces destructans*, captive breeding programs

INTRODUCTION

Understanding portions of a species' life cycle is important to promote recovery and survival of a species in a captive breeding program. The specific differences between similar species is what makes them unique and can make attempts to house them in captivity difficult. Sometimes species are similar enough that knowledge of one is sufficient for creating suitable housing for another, but often one wrong parameter can lead to failure. With 930 different species in the Suborder Microchiroptera, this is especially important (Feldhamer et al., 2007). Captive breeding programs are proposed for species that are threatened with extinction. The tri-colored bat is a species with many pressures placed on its survival such as White-nose syndrome, a fungal disease causing large numbers of deaths in temperate bats, habitat destruction, roost scarcity, and reduction in food resource availability.

One of the most well studied temperate bats in the Family Vespertilionidae is *Myotis lucifugus*, the little brown bat. Through previous research, seasonal life cycle changes (Fenton and Barclay, 1980), roost selection, including temperature preferences (Brack Jr., 2007), and winter hibernacula activity (Boyles et al., 2006) including copulation behavior (Wimsatt, 1945; Thomas et al., 1979; Fenton, 1985) is well known. Many species of temperate bats, including *M. lucifugus*, winter in hibernaculum, caves with appropriate temperatures and humidity to induce torpor, where males and females will come together after a spring and summer apart. Mating occurs at this time since the

sexes are in close proximity. Two forms of breeding have been observed with *M. lucifugus*, including active, where males are joined by females and copulation occurs, and passive, where active males copulate with torpid females (Wai-Ping and Fenton, 1988). *M. lucifugus* arrive at hibernacula in the fall and are often seen swarming at cave entrances. It is thought that swarming is used to learn about the hibernaculum and to find appropriate mates (Parsons, Jones, and Greenaway, 2003; Glover and Altringham, 2008).

A literature search for details on the breeding behavior of the tri-colored bat, *Perimyotis subflavus*, also a species in Family Vespertilionidae, resulted in very little information. Since these two species are classified in the same taxonomic family, using *M. lucifugus* behaviors as a template for *P. subflavus* behaviors provides a platform from which researchers can begin to discover the true behaviors of *P. subflavus*. It is unknown whether active or passive copulation, as reported for *M. lucifugus*, is utilized by *P. subflavus*. It is also unknown whether swarming is an important part of the life cycle of *P. subflavus*.

Within roosts, *M. lucifugus* cluster in close contact colonies, whereas *P. subflavus* roost in loose aggregations where members have little physical contact with each other (McNab, 1974). ‘Agonistic’ calls or ‘irritation buzzes’ have been described for *M. lucifugus* by Barclay, Fenton, and Thomas (1979) and were stated to be a short ‘squawk’ given during times of jostling during cluster formations and by non-torpid females struggling during copulation attempts. A long ‘squawk’ given by torpid bats during jostling and copulation attempts was also reported. A situation-specific copulation call has also been described for *M. lucifugus*, in which the male produces a vocalization that

increases in intensity and repetition the more agitated the female becomes during passive copulation (Barclay and Thomas, 1979). ‘Copulation calls’ have not been reported for *P. subflavus*.

Development of a captive breeding program is one component of several options set forth by US Fish and Wildlife Service to manage the threat of White-nose syndrome to populations of insectivorous bats (Traylor-Holzer, 2010). For a program such as this to work, an understanding of how bats copulate and what parameters must be reproduced in captivity is essential.

As of 2010 a survey of zoos around the world reported only 17 institutions having any experience holding *P. subflavus* (Traylor-Holzer, 2010). The maximum number of individuals held at one time was eight with a life span of only two years, which is well below their expected age of 25 to 30 years in the wild (Neuweiler, 2000). When assessing the feasibility of long-term captive management options for *P. subflavus*, whether high intensity or low intensity, the common factor in the section on knowledge gaps is “reproduction and reproductive behavior, social behaviors, and husbandry needs” (Traylor-Holzer, 2010).

One does not have to look far for evidence in support of preserving insectivorous bat species in North America. The impact that insectivorous bats have on insect populations is immense. Studies with *M. lucifugus* showed that 85% of the feces sampled from the local bat population included mosquitos (Wilson, 1997), which spread diseases such as malaria (Eckhoff, 2011) and West Nile Virus (Hofmeister, 2011), in their diet. A large colony of Brazilian free-tailed bats, *Tadarida brasiliensis*, that resides in Bracken

Cave in Texas can eat 225 metric tons of insects in a single night (Wilson, 1997).

Insectivorous bats that roost near farmlands feed mostly on crop pests, such as cucumber beetles, successfully protecting crop yields from complete destruction. The loss of insectivorous bats from the landscape could bring about large scale crop infestations, costing farmers and consumers millions of dollars.

Determining the specific needs of each species in nature is the only way to ensure the best chance of success when developing a captive care and breeding strategy (Kingston, 2011). The objective of this thesis is to ascertain the parameters surrounding copulation behavior of the tri-colored bat in order to facilitate the establishment of a captive breeding program in the future.

MATERIALS AND METHODS

Study Site - White River Cave located in Bartow County, Georgia near the town of Cartersville was the site used for the research presented in this thesis. This privately owned cave has been listed with Georgia Department of Natural Resources as a hibernaculum for the Tri-colored bat, *Perimyotis subflavus*, for the last 2 years. Two chambers within the cave were favored by *P. subflavus* during winter roosting. The front chamber measured 18.3 m (60 ft) in length, varied in width from 1.5 m (5 ft) to 7.6m (25 ft), and had a maximum height of 42.7 m (140 ft). The second chamber measured 7.6 m (25 ft) in length, 9.4 m (31 ft) wide, and 61 m (200 ft) at its' maximum height. The cave formation is primarily limestone and the walls are a rough texture, providing multiple foot holds for roosting bats. There is a cast iron bat gate enclosing the entrance to prevent

human admittance without permission (Fig. 2.1). The gate does not appear to have significantly prohibited any natural behaviors from occurring since it was installed in 2009 and is not close to the chambers where research was done (Spanjer and Fenton, 2005).

An assistant and I visited the cave every week or every other week, as scheduling permitted, beginning in December 2011 to early March 2012 and then again the next season, beginning in late October 2012 and continuing to late December 2012. Human traffic through the cave was minimal, with a few visits by the owner and his family during the period of my research, as well as a visit on January 25, 2012 by the Georgia Department of Natural Resources for a yearly bat count. Previous research with *P. subflavus* and other temperate hibernating bats *in situ* (on site) has shown that they are not greatly disturbed by human movement through hibernacula (Thomas, 1995; Traylor-Holzer, 2010). We attempted to keep our impact upon their torpor and natural behaviors as minimal as possible, because some disturbance from observers has been reported, and to optimize our chances of recording behaviors that most closely reflected natural activities.

Materials - Because the cave has no natural lighting and bats roosted from 1.5 m (5 ft) off the cave floor to 61 m (200 ft) off the cave floor, recordings were made with a Sony® digital HandyCam HDR-CX700 video camera equipped with Infrared NightShot®. External infrared lights, two IRLamp6, were used to broaden the range of the infrared. The camera was mounted on a tripod, as well as used in hand to record copulation behavior. Infrared lighting has been used for years to capture bat behavior

(Altenbach, 1988). A La Crosse Technology dual thermometer/hygrometer was used to measure temperature and humidity at random within the two chambers.

Methods - Prior researchers have found that most bat activity, at roosting sites, in spring and summer occurs in the evening after sunset (Barbour and Davis, 1969; O'Farrell and Studier, 1975; Fujita and Kunz, 1984; Jones and Rydell, 1994) and just before dawn (Hamilton Jr. and Whitaker Jr., 1979; Hill and Smith, 1984); therefore, those times were chosen to visit the cave during winter, assuming that if there was any activity it would be at the same time. Morning visits were between 0700 hrs and 1100 hrs with most of the bat activity happening before 0900 hours. Evening visits were between 1730 hrs and 2130 hrs with activity fairly constant the entire time. Visits were limited to two to three hours so as to disturb the bats as little as possible. The bats were observed a total of 30 hrs and 15 mins.

Each time we visited the cave, temperature and humidity levels were recorded in the chambers we observed that day (Table 2.1)

Video recording was used because on site observations were less clear due to the dark conditions of the cave and the distance at which the individuals were observed. Recording activity with the video camera allowed us to play the behavior on a larger computer screen to determine details of the activity captured. After the initial visit, it was determined what noises indicated a possible copulation and we followed that noise on foot or through zooming the camera until we could see the active pair. We then filmed the process until completion and reviewed the details later. In February 2012, we caught our first approach of a male to a female before copulation began. Subsequently, we altered

our filming strategy to following bats in the view finder of the camera as they flew, watching them until they landed.

Seven behaviors were recorded during both seasons and in both chambers. These include flight, grooming, drinking, a state of immobility assumed to be some phase of torpor, climbing, copulation, and scenting or sniffing.

Flight is defined as the action of flying through the air.

Grooming is defined as the licking of various parts of an individual's own body including hair and patagia (wing membrane) (Fig. 2.2).

Drinking is defined as ingesting water in flight or from a surface.

Torpor is defined as a state of immobility, with the eyes closed and no visible signs of breathing or other movement, whether the pelage was covered in dew or dry. No diagnostic testing was done on any individual to determine if they were in true physiological torpor, but it was assumed that complete immobility indicated torpor at some level since this was the winter hibernaculum (Fig. 2.2).

Climbing is defined as using thumbs of the forelimbs and feet to maneuver over the wall of the cave (Fig. 2.2).

Sniffing or scenting Sniffing is defined as drawing scent from a specific individual with the nose pointed directly at a portion of the body (Fig. 2.2). Scenting is defined as lifting the head dorsally and quickly bobbing the head in an up and down motion to allow air to be drawn in through the nose or mouth. Sniffing and/or scenting was assumed based on the movement of the head. In sniffing, one individual moved their nose towards another individual for a brief period of time. Scenting was assumed based

on the quick head bobbing motion of an individual while the nose was pointed into the air.

Copulation is defined as the male positioning himself on the posterior dorsal portion of the female with his ventral side to her dorsal side, grasping the scruff of her neck or dorsal side in his mouth with his thumbs touching, possibly holding, on either side of her head, and his caudal aspect contracting convulsively in a motion indicative of pelvic thrusting. We were not able to determine whether penetration or successful insemination was achieved in any copulations observed due to the distance of the individuals and the fact that we did not want to interfere with any natural behaviors (Fig 2.2).

On two days in January 2012, a Stanley LeverLock 7.6 m (25 ft) retractable tape measure was used to record distances between roosting individuals. Individuals were chosen at random based on ease of accessibility. The bats were not marked in any way for individual identification; therefore, there was no way to determine if the ones touching each other on one visit were the same on a subsequent visit.

Small flying insects were present in the cave during all visits. No specimens were ever collected.

RESULTS

Study Site - The site at which this research was done did not have a history of swarming behavior by the residents; therefore, it was a possibility that no breeding behavior would occur at all if swarming was a requirement. Upon our first visit, we found

bats flying, grooming, drinking, and making a lot of noise which we later determined were vocalizations associated with copulation. Our first visit also allowed us to visually observe copulation behavior indicating that, at least in this population of *P. subflavus*, swarming is not a mandatory prerequisite to passive mating. These observations were important in determining the suitability of this site for our research. Three species of bat were found at White River Cave during both seasons including the tri-colored bat, *Perimyotis subflavus*, the big brown bat, *Eptesicus fuscus*, and the Northern long-eared bat, *Myotis septentrionalis*.

Season - This cave located in Northern Georgia begins accumulating bats in October when outside temperatures begin to cool into the low 20s°C (70s°F). Individuals continue to gather through the end of December, when the population numbers stabilize. Bats remain in the cave through the winter until temperatures warm in March, with most of them leaving by the end of March. The first season temperatures immediately outside the cave in December and January usually ranged between 0°C (32°F) and 7°C (45°F) with one day of 18°C (65°F). February and March warmed to 11°C (52°F) to 14°C (58°F). Most bats had departed by March 15th. Temperatures in the second season were atypically warm. October's temperature on the day we first visited, late in the month, was 22°C (72°F) and the coldest day was in late December with a temperature of 10°C (50°F). The second season's temperatures were unseasonably warm for Northern Georgia.

Temperature and humidity - In the front chamber (Chamber 1), the lowest temperature, recorded February 20, 2012, was 11°C (52°F) and the highest temperature,

recorded December 17, 2011, was 15°C (59°F). Chamber 1 had a mean of 12.7°C (55°F) and a mode of 13.9°C (57°F). The humidity level for the front chamber ranged between 74% and 94% with a mean of 84% and a mode of 89%. In the back chamber (Chamber 2), the lowest temperature, recorded on January 2 and 16, 2012, February 4, 2012, and March 6, 2012, was 12°C (54°F) and the highest temperature, recorded on October 20, 2012, was 19°C (67°F). Chamber 2 had a mean of 13°C (56°F) and a mode of 12°C (54°F). Humidity ranged from 61% to 96% with a mean of 88.7% and a mode of 92%.

Roosting distance - We saw only a few individuals touching while in torpor and never more than two individuals at a time, with the majority roosting from 7.6 cm (3 in) to 86.4 cm (34 in) apart (Table 2.2). The most common distances recorded were 17.8, 20.3, 22.9, and 25.4 cm (7,8,9, and 10 in) apart in the front and back chambers. The first season a total count of 901 tri-colored bat individuals was recorded by the Georgia Department of Natural Resources. The second season had almost 200 fewer individuals with a count of 746 tri-colored bats, also obtained through the Georgia Department of Natural Resources.

Activities - Flying was seen in thirteen out of fifteen visits throughout both seasons. It is unknown whether the individuals were male or female. We observed one to three individuals at a time flying in the two chambers. It was not possible to determine if it was the same individual when there were multiple flights during an observation period or if there were multiple individuals.

Grooming behavior was also observed on thirteen of the fifteen visits (Table 2.3). Some individuals would autogroom after relocating at the end of a copulation. We knew

these were males since it was the bat in the male position that would fly away, then groom, while the female that had been left did not groom. Some individuals were observed in the middle of a grooming session and continued grooming until they were finished, then settled quietly where they were, not moving and possibly returning to some state of torpor. Six instances of autogrooming were recorded via camera. Based upon the six recorded groomings, three phases of autogrooming appear. Each individual began with licking either the right or left hind foot and using the same foot to comb the fur around the face and head. The second phase consisted of moving to the uropatagium (tail membrane) of the right or left side, corresponding to the foot used to comb the fur. Five of the individuals licked the ventral side of the uropatagium, while one licked the dorsal side. All individuals licked from the interior of the patagia toward the end of the patagia. The third phase consisted of licking the right or left plagiopatagium (wing membrane below the forearm), beginning at the wrist and terminating at the ends of the phalanges. The phases do not indicate sequence; although, all instances recorded began with foot licking and fur combing. No times are reported for each phase because no recordings captured the autogrooming behavior from start to finish.

Drinking was observed only twice, once after a few days of rain caused the cave to fill enough to create a shallow pool in the first chamber. One bat was seen flying over the water, dipping close to the surface. The other instance was in the back chamber, in which a bat was observed licking water from seepage from the cave wall.

Sixteen instances of climbing were recorded with the camera. Nine incidents involved climbing to an individual roosting on the wall near where the previously flying bat landed. The other seven instances recorded involved no obvious goal.

Sniffing or scenting was recorded via camera sixteen times with three occurrences directed toward a female preceding a copulation. Sniffing was an unexpected behavior and, according to literature searches, has yet to be reported for this species. Four incidents of sniffing directed toward a second individual were recorded, three aimed toward females that ended in mounting and copulation and one aimed toward an individual of unknown sex that ended with no attempt to mount. Sniffing, associated with the three attempts ending in mounting, ranged in time from 19 seconds for the February observation to 1 minute 12 seconds for the March observation and 19 seconds for the November observation. These times indicate how long the male sniffed the female before mounting. The sniffing that was not followed by an attempt to mount lasted 33 seconds before the male became uninterested and settled nearby to autogroom.

Another unexpected behavior was vocalizations that were audible to the human ear. These vocalizations were recorded during copulation and during instances in which one bat was jostling another individual it was roosting near. Two distinct vocalizations were produced by the female during copulation. One was a longer, louder raspy vocalization while the second was a quick, quieter chirp. Both of these vocalizations were also heard during instances in which one individual was autogrooming too close to another individual and the groomer was occasionally bumping the other as it groomed.

These descriptions are based on the unaided human ear as no specialized equipment was used to determine frequency, tone, or inflection.

Thirteen out of fifteen visits included observations of copulations or detection of auditory vocalizations associated with copulation. Twenty copulatory events were recorded via camera, two from approach to disengaging, one from approach, and one an unsuccessful attempt to mount (Table 2.3). The two copulations witnessed from approach to departure measured 3 minutes and 52 seconds on February 20, 2012 and 3 minutes and 6 seconds on November 26, 2012. As briefly outlined in the ethogram, copulation consists of the male mounting the female with his ventral side to her dorsal side. In every copulation filmed the female was roosting with her ventral side touching the cave wall and was still and silent until the male touched her to mount. One copulation was with a female covered in dew who remained mostly unresponsive throughout the encounter, only making a few chirps. The one unsuccessful attempt to mount was seen with a female roosting in the center of the ceiling. The male appeared to have difficulty getting into a position in which he could properly mount.

As the male is mounting, the female will generally arouse from torpor and produces the raspy call until he is in position and has grasped her neck or back in his mouth. Once he has grasped her, movement of his tail and uropatagium can be seen as he begins pelvic thrusting. At this time the female stopped the raspy vocalization and began producing the quieter chirp. Just before this intromission phase ends the male makes one more thrust, contracting his back into extension and remaining still for a few seconds. The female is also quiet during this time. Copulation appears to be complete

when the male releases the female's neck and she begins the raspy call again. This period lasts for only a few seconds and the female keeps producing the raspy call until the male flies away or climbs far enough away from her that he is no longer touching her. Based on filming and onsite observation, once copulation is complete and the male has flown away, the female is not approached again for a minimum of ten minutes. Once the male is gone the female becomes quiet again and appears to return to a state of torpor. There appeared to be no obvious evidence of injury to the female; although, we were not able to handle any females for closer inspection.

We recorded two instances in which a male copulated with one female and then moved directly to another female nearby. In both instances the male sniffed the female's side, in front of her back legs and near her uropatagium, before mounting. This behavior was also filmed as one male landed near a single female and proceeded to sniff her before mounting. The females began the raspy call as soon as the male was close enough to touch her even while he was only sniffing.

One instance of what may be described as male-male competition was also recorded. During the November 26, 2012 copulation, as the male moved from one female to the second roosted nearby, another bat of unknown sex landed behind the pair as the male initiated mounting. Eight seconds lapsed before the male who was mounting turned to face the recently landed individual. Once face to face, the new bat took a slight step back with his right forelimb and flew away, leaving the initial male to copulate.

DISCUSSION

Upon undertaking this research project I had no idea what I would discover in a hibernaculum mainly filled with tri-colored bats. Much research has been done on the life cycle and behaviors of the little brown bat (Thomas et al. 1979; O'Farrell and Studier, 1974; O'Farrell and Studier, 1975; Brack Jr., 2007; Kurta and Kunz, 1988; Frick et al., 2010), and I expected to see similar behaviors. Both species are in the same family taxonomically, Vespertilionidae, and both have a similar life cycle in which they are frequently found in caves for the winter hibernation season and sexually segregating themselves in the spring and summer. They have also been reported to roost within the same hibernacula (Fenton and Barclay, 1980; Fujita and Kunz, 1984). Within White River Cave, I have confirmed *P. subflavus* does enter a period of torpor during the seasons of fall and winter in Northern Georgia beginning in October and ending in March. I have confirmed that this period of inactivity is accompanied by temperatures previously reported for other *P. subflavus* aggregations in different hibernacula locations ranging from 11°C (52°F) to 15°C (59°F) (McNab, 1974; Brack Jr., 2007). High humidity levels, ranging from 61% to 96%, may also be a preference of *P. subflavus*, which has been suggested in other research but never directly recorded. Individual roosting distances were measured, supporting reports that *P. subflavus* are not close contact roosters (Fujita and Kunz, 1984; Langwig et al., 2012). Winter time post-arousal behaviors were also recorded, broadening our understanding of what may be typical behaviors for *P. subflavus* throughout time spent in winter hibernacula. Flying, grooming, drinking, and copulation were all seen in this hibernaculum during two winter seasons.

Within this hibernaculum, even though insectivorous bats of this size are capable of long term torpor lasting up to seven months (Neuweiler, 2000), given the parameters seen here *P. subflavus* will remain active and mate throughout the winter season. Many reasons for the behavior we saw may be explained by previous research with *M. lucifugus* and other insectivorous vespertilionids. Flying and drinking behaviors observed within the hibernaculum may be spurred by the search for water. It is thought that thirst is the most likely reason for arousal, since bats can lose large amounts of water through evaporative loss due to their wing membrane and sizable lungs (Neuweiler, 2000). Respiration is reduced during torpor but is not completely terminated.

Grooming is a behavior bats perform regularly to maintain patagia elasticity and health (Neuweiler, 2000), remove parasites and debris (ter Hofstede and Fenton, 2005), and improve overall body fitness. I did see individuals flying, a behavior that requires healthy, flexible wings, at every visit. This could explain why grooming was observed at almost every visit as well, because grooming helps maintain patagia health even though grooming uses much needed energy reserves. The autogrooming behavior recorded in this hibernaculum does imply that a pattern may be followed, but due to the small sample size, further research is needed.

Copulation behavior is seen in the winter because insectivorous, hibernating bats have evolved a life cycle that separates the sexes until fall and winter seasons (Fenton and Barclay, 1980; Fujita and Kunz, 1984). This is the time when intraspecific interaction between the sexes is possible, although as the season progresses and temperatures become colder the activity usually wanes. Breeding throughout the hibernation period is

rarely seen in temperate bats that engage in a winter torpor period and has only been reported in *M. lucifugus* (Boyles et al., 2006) until now. The life cycle of these bats includes an adaptation called delayed fertilization or delayed ovulation, which means follicular growth has occurred within the ovary but ovulation, the egg descending into the fallopian tube, has not happened at this time (Feldhamer et al., 2007). This phenomenon has been shown to occur in the tri-colored bat (Wimsatt, 1945). Females are able to store sperm in the uterus for an extended period of time until a few days before arousal from winter torpor (Neuweiler, 2000). The males produce high levels of testosterone in late summer to produce sperm which is then stored in the epididymis and is used during copulation in fall and winter arousal periods (Neuweiler, 2000). Previous research with *M. lucifugus* reviewed by Cockrum (1955) intimated that copulation after arousal may have been caused by human disturbance within the hibernaculum. This was a concern for me because I wanted to record the most natural behaviors possible because the purpose of this research was to establish a foundation upon which a captive breeding program could be based, but I believe our presence did not trigger copulation activity because vocalizations, similar to those heard during recorded copulation events, could be heard from the entrance of the cave, which was approximately 244 m (800 ft) from the first chamber where *P. subflavus* was roosting. Although it could be argued that if I could hear them they could hear me, previous research indicated that the presence of humans within the roosting area itself, rather than humans outside the roosting area, was the disturbing factor (Wai-Ping and Fenton, 1988).

All of the copulatory incidents observed were with active males and torpid females. As the winter season progressed activity of any kind was expected to taper and be replaced by torpor and occasional arousal to drink at the walls that were consistently wet with seepage. Instead, copulation and grooming behavior lasted throughout the winter season into spring. Copulation throughout the period of hibernation has only been previously reported in *M. lucifugus* (Boyles et al., 2006). White River Cave does offer access to water and some insects continuously throughout the year, even when the temperature outside is below freezing. This may be why this hibernaculum is active all winter, since one reason for torpor is absence of food availability (Neuweiler, 2000). The internal chambers of the cave, where the majority of roosting occurred, also remained fairly stable, approximately 12.7°C (55°F) with high humidity, which are conditions favored by *P. subflavus* (Fujita and Kunz, 1984).

Mating systems are strongly influenced by sexual selection which in part is driven by female preference (Ryan, 1994). The novelty of the mating system observed within the population of tri-colored bats studied in White River Cave is that females displayed no obvious signs of preference. No female was seen leaving or attempting to leave once a male initiated mounting. This lack of apparent female choice is unusual in mammalian mating systems even in comparison to other mammals in which the life cycle includes a hibernation or torpor period. The brown bear, *Ursus arctos*, mate in late spring and early summer before they begin to prepare for the winter hibernation period (Steyaert et al., 2012). Because bears are typically solitary animals throughout most of their lives, males must seek out females for mating. Both sexes display a roam to mate behavior, but

females signal their receptivity before mating can occur (Steyaert et al., 2012). A similar process is also observed in the European hedgehog, *Erinaceus europaeus*, that has a similar life cycle, breeding in spring and late summer with a period of winter hibernation, and a roam to mate behavior (Riber, 2006). Ryan (1994) states that it is easy to understand female preference when it is obviously adaptive, but it is harder to understand why a female chooses when the male has nothing to offer but sperm. This population of tri-colored bats may be an instance in which the male has nothing to offer but sperm; therefore, the female just doesn't choose.

Four recordings revealed a sniffing behavior not previously described. The males climbed to the female, sniffed their flank, in front of the back legs, and then in the uropatagium area before proceeding to mount. Many mammalian species rely on olfactory cues to recognize conspecifics and determine appropriate mates (Bloss et al., 2002; Caspers et al., 2009). Sniffing an individual before mounting may be an important step in determining appropriate mates for *P. subflavus*. The fourth encounter recorded began with the male approaching and sniffing an unidentified individual in the uropatagium area and, for an undetermined reason, not mounting. Whether the roosting individual that was approached was a male or a female lacking the appropriate scent is unknown. It is possible that this individual, if a female, was previously mated by another male and his scent was left behind, obscuring the female's scent, or her scent did not indicate that she was currently biologically receptive (Brennan and Kendrick, 2006). During the hibernation period females are in a state of sub-estrus and the Graafian follicles are enlarged (Wimsatt, 1945). It is feasible that females give off some odor to

indicate their sex or reproductive status and the males use this as an indicator that this is an individual toward which they should direct their energy (Brennan and Kendrick, 2006). Conversely, the individual approached might have been a male whose scent indicated an inappropriate mate (Bouchard, 2001). Based on recordings, it appears there are no proceptive behaviors, or active attraction behaviors, used by females to encourage males to mount, but it seems that olfaction may play an important role. Since the cave is in complete darkness and even nocturnal animals need a low level of light to see, it is possible that olfaction may be involved in determining an individual's location and sex.

Once the male came into contact with the female, she began to produce a raspy call, also never before described for *P. subflavus*, which she continued to make until the male mounted and grasped her scruff with his mouth. A copulation call was described for *M. lucifugus* by Barclay and Thomas (1979), but that was said to be produced by the male. None of our recordings revealed any vocalizations made by the male at any time before, during, or after the copulation.

A second vocalization was then produced by the female, a short, quiet chirp. Throughout the period in which pelvic thrusting can be seen, the female continues to produce the chirp vocalization until the male becomes still, possibly with a last thrust indicative of ejaculation, at which time she also becomes quiet. Once the male releases her scruff and looks around she begins the raspy call again until he flies away or climbs far enough away to not touch her.

Most of the vocalizations heard were associated with copulation behavior; although, a few times we followed the noise associated with copulation only to find one

bat too close to another grooming itself and bumping its neighbor. Both the raspy call and the chirp were recorded during copulation activity and during moments of agitation.

‘Agonistic’ calls or ‘irritation buzzes’ have been described for *M. lucifugus* by Barclay, Fenton, and Thomas (1979) and were stated to be a short ‘squawk’ given during times of jostling during cluster formations and by females struggling during copulation attempts. A long ‘squawk’ given by torpid bats during jostling and copulation attempts was also reported. Both of these descriptions sound similar to what we heard *P. subflavus* make during this study; therefore, these vocalizations may be merely agitation calls. More detailed examination of the vocalizations themselves may determine whether they are different in frequency, which could reveal the purpose of the vocalizations.

Many areas of future research can be proposed by data collected throughout this research. Only one hibernaculum was visited; therefore, to establish whether this mating system is normal for tri-colored bats unaffected by WNS more hibernacula need to be researched. Are the temperature and humidity parameters the same in other hibernacula? Does the fact that this cave has food and water available throughout the hibernation season affect copulation behavior? What has prompted females to abandon attraction signals and female preference? Is this actually the case or are the receptive signals just not obvious enough to be easily observed? Is the sniffing behavior observed an integral part of choosing an appropriate female mate? Does the female emit an olfactory scent or a pheromone that indicates she is ready to mate? Are the vocalizations heard proceptive or receptive signals given by the females or simply agitation calls?

With the data obtained through the current research project some of the needs that are important to incorporate into a captive habitat for *P. subflavus* can be addressed. Based on the results of this study, a constant temperature of 12.7°C (55°F) to 13°C (56°F) and humidity levels of 84% to 88.7% during a five to six month long period should be offered to help promote hibernation activity in captivity, including arousal for copulation to occur. Water should be available at all times for rehydration and, possibly, insects as a food resource. An area large enough for individuals to roost a minimum of eight inches from any other individual if they choose should be provided. The walls should be rough enough to allow for foot holds for roosting and for males to land near a female and climb to her, but not so rough as to irritate exposed skin of the patagium and prevent females from hanging with their ventral side parallel to the wall. A chamber large enough to promote swarming may not be necessary for this species to copulate successfully. Infrared lighting and small flash lights for human movement should be included in housing design; although, husbandry practices must take human disturbances into consideration. The possibility that olfaction may play an important part in mate detection must also be reflected in husbandry procedures. Minimizing entrance to the artificial hibernaculum and reducing introduction of odors from cleaning substances may be essential to successful captive propagation. The ability for the males and females to separate from each other after the hibernation period should also be included in a housing design.

The argument can be made that because we do not know how to prevent WNS from infecting bats or spreading across populations, there is no point in learning how to

breed them in captivity. Is it a waste of time and resources to develop a plan to keep them alive in captivity when, upon release, they will just succumb to WNS in the wild? There is new evidence that *Geomyces destructans* has been identified in Europe where bats appear to experience no mass mortality attributable to the psychrophilic fungus (Puechmaille et al., 2011). It has been hypothesized that the fungus co-evolved with temperate bat species of Europe years before it was discovered in the United States and North America, and that native bats of North America may, with time, develop adaptations that may allow them to live with the fungus. If, given time, native bats of North America can develop an immunity to the effects of *G. destructans* and we can provide them that time through a captive breeding program, the effort and resources employed are well worth the time.

References

- Altenbach, J. Scott. 1988. Techniques for Photographing Bats. *In: Ecological and Behavioral Methods for the Study of Bats*. Kunz, Thomas H. (Ed.); Smithsonian Institution, Washington D.C. 125-140.
- Barbour, Roger W. and Wayne H. Davis. 1969. *Bats of America*. University Press of Kentucky, Lexington.
- Barclay, Robert M. R. and Donald W. Thomas. 1979. Copulation Call of *Myotis lucifugus*: A Discrete Situation-Specific Communication Signal. *Journal of Mammalogy* 60, 632-634.

- Barclay, Robert M. R., M. Brock Fenton, and Donald W. Thomas. 1979. Social Behavior of the Little Brown Bat, *Myotis lucifugus*: II. Vocal Communication. Behavioral Ecology and Sociobiology 6, 137-146.
- Bloss, Johanna, Terry E. Acree, Janelle M. Bloss, Wendy R. Hood, and Thomas H. Kunz. 2002. Potential Use Of Chemical Cues For Colony-Mate Recognition In The Big Brown Bat, *Eptesicus fuscus*. Journal of Chemical Ecology 28, 819-834.
- Bouchard, Sylvie. 2001. Sex discrimination and roostmate recognition by olfactory cues in the African bats, *Mops condylurus* and *Chaerephon pumilus* (Chiroptera: Molossidae). Journal of Zoology, London 254, 109-117.
- Boyles, Justin G., Miranda B. Dunbar, and John O. Whitaker Jr. 2006. Activity following arousal in North American vespertilionid bats. Mammal Review 36, 267-280.
- Brack Jr., Virgil. 2007. Temperatures and Locations Used by Hibernating Bats, Including *Myotis sodalis* (Indiana Bat), in a Limestone Mine: Implications for Conservation and Management. Environ Manage 40, 739-746.
- Brennan, Peter A. and Keith M. Kendrick. 2006. Mammalian social odours: attraction and individual recognition. Philosophical Transactions: Biological Sciences 361, 2061-2078.
- Caspers, Barbara A., Frank C. Schroeder, Stephan Franke, W. Jurgen Streich, and Christian C. Voigt. 2009. Odour-based species recognition in two sympatric species of sac-winged bats (*Saccopteryx bilineata*, *S. leptura*): combining chemical analyses, behavioral observations and odour preference tests. Behav Ecol Sociobiol 63, 741-749.

- Cockrum, E. Lendell. 1955. Reproduction in North American Bats. Transactions of the Kansas Academy of Science 58, 487-511.
- Eckhoff, Philip A. 2011. A malaria transmission-directed model of mosquito life cycle and ecology. Malaria Journal 10:303, 1-17.
- Feldhamer, G. A., L. C. Drickamer, S. H. Vessey, J. F. Merritt, and C. Krajewski. 2007. Mammology: Adaptation, Diversity, and Ecology. Third edition. Johns Hopkins Press, Baltimore, MD.
- Fenton, M. Brock and Robert M. R. Barclay. 1980. *Myotis lucifugus*. Mammalian Species 142, 1-8.
- Fenton, M. Brock. 1985. Communication in the Chiroptera. Indiana University Press, Bloomington.
- Frick, Winifred F., D. Scott Reynolds, and Thomas H. Kunz. 2010. Influence of climate and reproductive timing on demography of little brown bat myotis, *Myotis lucifugus*. Journal of Animal Ecology 79, 128-136.
- Fujita, Marty S. and Thomas H. Kunz. 1984. Mammalian Species. *Pipistrellus subflavus*. Am. Soc. Mammal. 228, 1-6.
- Glover, Anita M. and John D. Altringham. 2008. Cave selection and use by swarming bat species. Biological Conservation 141, 1493-1504.
- Hamilton Jr., William J. and John O. Whitaker, Jr. 1979. Mammals of the Eastern United States, 2nd. Edition. Cornell University Press, Ithaca.
- Hill, John E. and James D. Smith. 1984. Bats: A Natural History. University of Texas Press, Austin.

- Hofmeister, Erik K. 2011. West Nile virus: North American Experience. *Integrative Zoology* 6, 279-289.
- ter Hofstede, Hannah M., and M. Brock Fenton. 2005. Relationships between roost preferences, ectoparasite density, and grooming behavior of neotropical bats. *Journal of Zoology, London*, 266, 333-340.
- Jones, Gareth and Jens Rydell. 1994. Foraging Strategy and Predation Risk as Factors Influencing Emergence Time in Echolocating Bats. *Philosophical Transactions: Biological Sciences*, 346, 445-455.
- Kingston, Tigga. 2011. Roosting Ecology and the Captive Environment. Pages 321-338 in Susan M. Barnard, editor. *Bats in Captivity: Volume 3-Diet and Feeding, Environment and Housing*. Logos Press, Washington D.C.
- Kurta, Allen and Thomas H. Kunz. 1988. Roosting Metabolic Rate and Body Temperature of Male Little Brown Bats (*Myotis lucifugus*) in Summer. *Journal of Mammalogy* 69, 645-651.
- Langwig, Kate E., Winifred F. Frick, Jason T. Bried, Alan C. Hicks, Thomas H. Kunz, and A. Marm Kilpatrick. 2012. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 15, 1050-1057.
- McNab, Brian K. 1974. The behavior of Temperate Cave Bats in a Subtropical Environment. *Ecology* 5, 943-958.
- Neuweiler, Gerhard. 2000. *The Biology of Bats*, Oxford University Press, New York.

- O'Farrell, Michael J. and Eugene H. Studier. 1974. Reproduction, Growth, and Development in *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae). *Ecology* 54, 18-30.
- O'Farrell, Michael J. and Eugene H. Studier. 1975. Population Structure and Emergence activity Patterns in *Myotis thysanodes* and *M. lucifugus* (Chiroptera: Vespertilionidae) in Northeastern New Mexico. *American Midland Naturalist* 93, 386-376.
- Parsons, K. N., G. Jones, and F. Greenaway. 2003. Swarming activity of temperate zone microchiropteran bats: effects of season, time of night and weather conditions. *Journal of Zoology, London* 261, 257-264.
- Puechmaille, Sébastien, J. Gudrun Wibbelt, Vanessa Korn, Hubert Fuller, Frédéric Forget, Kristin Mühldorfer, Andreas Kurth, Wieslaw Bogdanowicz, Christophe Borel, Thijs Bosch, Thomas Cherezy, Mikhail Drebet, Tamás Görföl, Anne-Jifke Haarsma, Frank Herhaus, Guénael Hallart, Matthias Hammer, Christian Jungmann, Yann Le Bris, Lauri Lutsar, Matti Masing, Bart Mulkens, Karsten Passior, Martin Starrach, Andrzej Wojtaszewski, Ulrich Zöphel, and Emma C. Teeling. 2011. Pan-European Distribution of White-nose Syndrome Fungus (*Geomyces destructans*) Not Associated with Mass Mortality. *PLoS ONE* 6: e19167, 1-11.
- Riber, Anja B. 2006. Habitat use and behavior of European hedgehog *Erinaceus europaeus* in a Danish rural area. *Acta Theriologica* 51, 363-371.

- Ryan, Michael J. 1994. Mechanisms Underlying Sexual Selection *In*: Behavioral Mechanisms in Evolutionary Ecology. Real, Leslie A. (Ed.); University of Chicago Press. 190-215.
- Spanjer, Genevieve R. and M. Brock Fenton. 2005. Behavioral Responses of Bats to Gates at Caves and Mines. *Wildlife Society Bulletin* 33, 1101-1112.
- Steyaert, Sam M. J. G., Anders Endrestol, Klaus Hacklander, Jon E. Swenson and Andreas Zedrosser. 2012. The mating system of the brown bear *Ursus arctos*. *Mammal Review* 42, 12-34.
- Thomas, Donald W., M. Brock Fenton, and Robert M. R. Barclay. 1979. Social Behavior of the Little Brown Bat, *Myotis lucifugus*: I. Mating Behavior. *Behavioral Ecology and Sociobiology* 6, 129-136.
- Thomas, Donald W. 1995. Hibernating Bats are Sensitive to Nontactile Human Disturbance. *Journal of Mammalogy* 76, 940-946.
- Traylor-Holzer, K., R. Tawes, M. Bayless, A. Valenta, N. Rayman, and N. Songsasen (eds.). 2010. Insectivorous Bat Captive Population Feasibility Workshop Report. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley.
- Wai-Ping, Virginia and M. Brock Fenton. 1988. Nonselective Mating in Little Brown Bats (*Myotis lucifugus*). *Journal of Mammalogy*, 69, 641-645.
- Wilson, Don E. 1997. *Bats in Question*. Smithsonian Institution Press, Washington.
- Wimsatt, William A. 1945. Notes on Breeding Behavior, Pregnancy, and Parturition in some Vespertilionid Bats of the Eastern United States. *Journal of Mammalogy* 26, 23-33.



Figure 2.1. Bat gate of White River Cave. Photo taken by author.

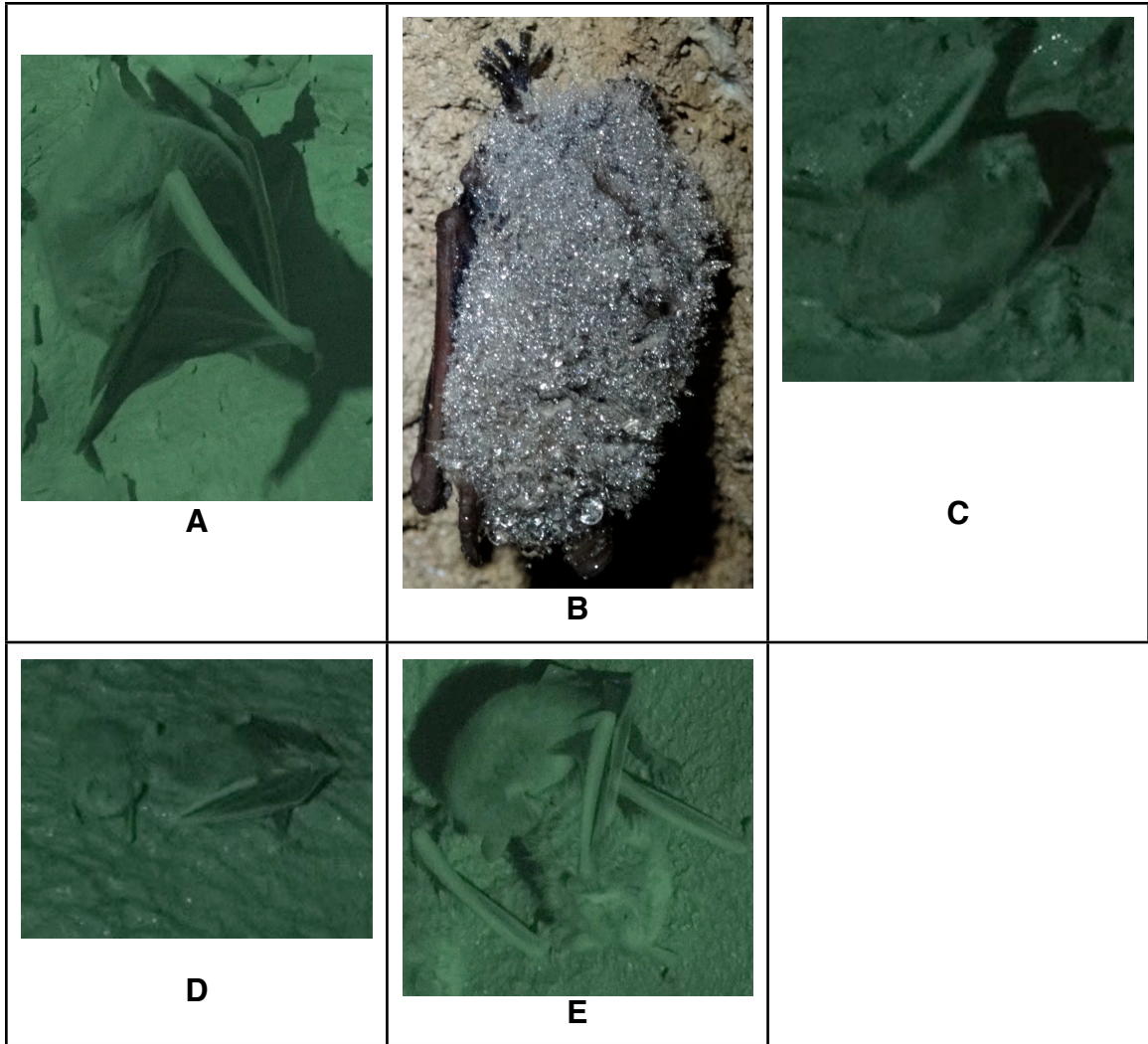


Figure 2.2. Behaviors in hibernaculum. (A) Grooming of the wing membrane. (B) Bat in typical torpor position covered with moisture. (C) Left wing is extended in climbing behavior. (D) Sniffing behavior of female by male. (E) Copulation mount. All photos taken by author.



Figure 2.3. Typical aggregation pattern of *P. subflavus* seen in White River Cave, chamber 2. Photo taken by author.

Table 2.1. Temperature and humidity levels in cave chambers. ND indicates no data was recorded for that chamber on that date.

Date	Temp °C		Humidity %	
	Chamber 1 / Chamber 2	Chamber 1 / Chamber 2	Chamber 1 / Chamber 2	Chamber 1 / Chamber 2
12/17/2011	15 / ND	76% / ND		
12/24/2011	ND / 13.9	ND / 95%		
1/2/2012	13.3 / 12.2	88% / 96%		
1/16/2012	13.3 / 12.2	83% / 92%		
1/28/2012	11.7 / 12.8	74% / 90%		
2/4/2012	ND / 12.2	ND / 92%		
2/20/2012	11.1 / ND	94% / ND		
3/6/2012	ND / 12.2	ND / 90%		
10/22/2012	13.9 / 19.4	89% / 61%		
11/5/2012	12.8 / ND	91% / ND		
11/26/2012	13.9 / 13.3	74% / 84%		
12/3/2012	ND / 13.3	ND / 93%		
12/24/2012	12.2 / 12.8	89% / 94%		

Table 2.2. Measurements, in inches, between roosting individuals.

Date	Distance in inches
1/16/2012	0(touching), 4", 7", 9", 10", 11", 13", 16", 17", 19", 20", 28", 34"
1/28/2012	3", 3", 5", 5", 5", 5", 7", 7", 7", 8", 8", 8", 9", 9", 10", 10", 10", 12"

Table 2.3. Dates copulations, vocalizations, and grooming were observed or recorded.
 * indicates recorded grooming sessions.

Dates	Copulations recorded	Vocalizations heard	Grooming
12/10/2011	1	Y	Y*
12/17/2011	1	Y	Y
12/24/2011	1 attempted	Y	N
1/2/2012	3	Y	Y*
1/16/2012	1	Y	Y*
1/28/2012	0	Y	Y*
2/4/2012	0	Y	Y*
2/20/2012	5	Y	Y*
3/6/2012	4	Y	Y
9/24/2012	0	Y	N
10/22/2012	0	Y	Y
11/5/2012	2	Y	Y
11/26/2012	2	Y	Y
12/3/2012	0	Y	Y
12/24/2012	0	Y	Y

CHAPTER 3

CONCLUSIONS

The impetus behind this research was a need to establish knowledge of the details of copulation in preparation for establishment of a captive breeding program if tri-colored bat populations fall to critical enough levels that extinction becomes a threat to their existence. The overall objective of this thesis was to ascertain the parameters surrounding copulation behavior of the Tri-colored bat. Chapter 1 presents information which was used as a foundation to formulate the approach to obtain the data outlined in Chapter 2. To acquire unknown knowledge, a place to start must be ascertained. Based upon research established with closely related temperate bat species, such as *Myotis lucifugus*, I knew to concentrate efforts during fall and winter within an established hibernaculum site. Recording the behaviors using infrared lighting and a digital camera dove-tailed with my decision to impact my chosen research population as little as possible. Finding a local hibernacula site and obtaining permission to use the site was the last step.

In Chapter 2, I report the behaviors seen within this aggregation of *P. subflavus*. Focusing on the environmental parameters within the hibernaculum, I established temperature and humidity requirements for copulation to occur, confirmed roosting preferences of individuals within the hibernaculum, and confirmed seasonality of copulation activities. It was established that *P. subflavus* does not need to participate in

swarming for passive copulation to occur during the hibernation period. A completely unique mating system in which the female is torpid directly before mating occurs was recorded. For the first time, evidence of olfaction as a possible determining factor of mate selection within bats was caught on tape during passive mating behavior. Vocalizations produced during copulation were recorded which may or may not be unique to this activity. Combining all the data collected provides us with a better understanding of the factors surrounding *P. subflavus* breeding behavior. I believe that incorporating this information into a captive breeding program plan will increase its chances of success.