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ADAPTIVE MOVEMENTS AND THERMOREGULATION IN BIG-EARED BATS

A Thesis  
presented in partial fulfillment of requirements  
for the degree of Master of Science  
in the Department of Biology  
The University of Mississippi

by

CODY S. JORDAN

December 2015

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## ABSTRACT

There are a variety of (non-exclusive) reasons to explain the presence of group-living, but clustering or huddling is especially important for small endotherms with high surface-area-to-volume ratios. Clustering is interesting because variations within clustering are seldom investigated despite anecdotal evidence that bat clustering varies widely. I studied a colony of Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) to learn more about clustering behavior using infrared video. I predicted that temperature would be the primary driver of how and when bats cluster while roosting. The actual relationship was not as predicted by an energetic model. High density clusters of bats were common across a wide range of ambient and roost temperatures, and substantial clustering variation exists. The bats I captured (79 individuals) showed no sign of the causal agent of WNS. I found that areas of the roost used by bats were less variable in temperature but not warmer than areas not used. Also presented are preliminary nighttime foraging locations for bats at this roost site. These results provide insight into energetics, clustering behavior, and general ecology for an uncommon species in a part of its range where it has not been previously studied. These data should be useful for future behavioral and/or energetic investigations as well as for conservation decision-making. Resampling of variation in bat numbers suggested that building roosts require at least 3 visits to confirm bat absence and 16 visits to count the maximum number of bats using the site. Finally, I discuss considerations and ideas for future research.

## DEDICATION

I wish to dedicate this thesis to my wife, Jade Maharrey, for her unwavering support despite [a tiny bit] of complaining and procrastination on my part. She helped me maintain perspective, optimism, and, at times, sanity. I also wish to thank my mother and my wife's family for putting up with me during this time and for all their words of encouragement. A thesis is so much more than a paper!

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For assistance with information and photos, I wish to thank George Harris, Andrea Schumann, John Abbott, and Mitch Withers. A huge thank you to fellow graduate students Hak-Chul Lee (감사!) for his time and assistance and Reese Worthington for his help and always letting me borrow equipment. Finally, I was lucky to have such great undergraduate field assistants that worked diligently despite the heat, darkness, snakes, mosquitoes, biting flies *ad infinitum*: Seth Cappleman, Will Ross, Ramsey Salloum, Bethany Summerford, and Sylvi Tinsley.

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## **CHAPTER I: GENERAL INTRODUCTION: COSTS AND BENEFITS OF GROUP-LIVING**

All decisions that an animal makes have both costs and benefits. We should expect, however, for the costs to be outweighed by the benefits in order for the behavior (or suite of behaviors) to be considered adaptive (i.e., increase the animals' relative fitness via survival and/or reproduction; Davies et al. 2012). One such decision that animals must make is whether to live in a group. Many animals live a solitary existence; they come together only when necessary (e.g., at concentrated foraging areas or for mating purposes). However, for some species, being around others is integral to their nature.

Group-living is distinct from sociality (sociality implies social interactions among individuals). Although sociality does typically involve group-living, group-living does not necessarily indicate sociality (see Slobodchikoff and Shields 1988; Blumstein and Armitage 1997). Group-living is common in vertebrates: examples of many individuals living together for extended periods of time can be found in fish, birds, and mammals (Rubenstein and Kealey 2010). Group-living is seen outside the Vertebrata subphylum, too, including in ants (Hughes et al. 2002), spiders (Uetz et al. 2002), and brittle stars (Broom 1975). Living in a group should provide fitness benefits for the individuals within that group that outweigh any costs that members incur by joining the group. Examples of potential benefits include: reduced risk of predation, easier access to scattered or uncommon resources, reduced parasite loads, increased mating opportunities, and reduced energy expenditure (Rubenstein and Kealey 2010).

There may be multiple reasons driving the presence of group-living in any animal species. Additionally, there are also notable costs that can be associated with group-living. For

example, even though mates might be easier to locate when living in a group, increased competition for mating opportunities can occur (Dobson 1982). While the many-eyes hypothesis and the dilution effect may explain benefits for grouped animals in avoiding predation, it is also worth noting that large aggregations of animals can also be more conspicuous to predators (Lima and Dill 1990). Two other costs may include increased parasite/disease burdens (Godfrey et al. 2009) and competition for food resources (Majolo et al. 2008). While I will focus primarily on benefits, it is important to acknowledge that many costs may also exist. In reality, adaptive functions of group-living depend on the species' physiology, anatomy, and behavior, as well as the habitat quality, and climate. Below, I review seven common explanations (benefits) for animals to live in groups.

### *Relatedness and inclusive fitness*

An individual can pass on some of its genes, even if it does not produce offspring, by facilitating its relatives' survival or reproduction. In vertebrates, all of an individual's full siblings are as related (coefficient of relationship = 50%) to one another as they would be to their own offspring. Clearly, for more distant relatives (i.e., coefficient < 50%), the fitness benefit is less than if an individual reproduces (i.e., directly passes on genes), but indirectly passing on genes still acts as a net fitness benefit. However, accrued fitness benefits of helping distant relatives could be as valuable as reproducing if the individual helps numerous relatives. Examples include alloparenting of related offspring as in prairie voles (*Microtus ochrogaster*; Stone et al. 2010) or 'self-sacrifice' in some eusocial insects where many individuals do not reproduce—but they exhibit a high degree of relatedness to those that they help reproduce (Queller and Strassmann 1998).

### *Reduced risk of predation*

Individual animals may group together because grouping decreases the chances of predation. This can be as simple as the dilution effect, in which the probability of predation for any particular individual decreases as the group size gets larger (even if the presence of a large group makes detection of the entire group easier for predators). The dilution effect has been used to at least partially explain group-living in many taxa, including stream invertebrates, fish, and insects (Wrona and Dixon 1991; Wisenden and Keenleyside 1995; Creswell 1994). This idea is related to the concept of the ‘selfish herd’ (Hamilton 1971).

Group-living can more actively reduce predation risk through increased vigilance (e.g., the ‘many-eyes’ hypothesis) and/or alarm calls. For example, Thomas’ langurs (*Presbytis thomasi*) emit alarm calls in the presence of conspecifics when a predator is spotted (Wich and de Vries 2006). Whether group-living yields passive, ‘selfish’ or more active benefits regarding predation, the benefit should outweigh known costs such as decreased foraging efficiency or increased probability of aggressive or antagonistic behavior from conspecifics (Molvar and Bowyer 1994).

### *Influence of ecological niche/specializations*

Animals may live in groups due to aspects of their ecology: perhaps food is sparse or patchily distributed. Individuals may garner information from other group members that gives them information about foraging sources. Starlings (*Sturnus vulgaris*) were observed to use cues from other starlings regarding the quality of experimentally-placed food resources in deciding whether to visit that patch (Templeton and Giraldeau 1995). For some animals, their

environment poses unique challenges which facilitate (or necessitate) group-living. For example, a phylogenetic analysis found that many rodents that reside underground live in groups or have at some point in their evolutionary history. Generally, group-living is seen in subterranean rodents unless the species developed specialized morphology (e.g., for digging; Smorkatcheva and Lukhtanov 2014).

### *Reduced parasite loads*

It was widely assumed that individuals living in groups would necessarily have higher rates of parasite transmission for both endo- and ectoparasites, although this relationship is perhaps not as strong as previously thought, nor is it universally true (Rifkin et al. 2012). The dilution effect may also apply to mobile parasites. For example, shoaling fathead minnow (*Pimephales promelas*) individuals were less likely to contract trematodes than non-shoaling minnows (Stumbo et al. 2012). However, the dilution effect requires fairly specific constraints and pertains primarily to mobile ectoparasites (like flies) or other parasites which can sometimes be avoided via change in behavior (e.g., some of the shoaling minnows were able to detect and ‘dodge’ trematodes in the water column). However, the relationship between group-living and vulnerability to parasites depends on both the ecology of the host and the parasite (Monello and Gompper 2010). With parasites that are highly contagious, living in groups is generally a disadvantage (Côté and Poulin 1995).

There is another, perhaps more intuitive way in which living in groups could reduce parasite loads, particularly for ectoparasites: allogrooming. Others can search for and remove ectoparasites in locations on the body which an individual may not be aware of or may be unable to reach. However, benefits of allogrooming may be greater than simply the removal of

ectoparasites (e.g., increased social bonds). There are also notable costs to allogrooming individuals. While allogrooming, impalas (*Aepyceros melampus*) become less vigilant for predators than those not allogrooming (Mooring and Hart 1995). It seems likely that group-living provides some benefits for parasite avoidance, but these benefits may be outweighed by other costs in most cases. A notable exception is in leaf-cutter ants (*Acromyrmex* sp.), where a combination of antibiotic secretions (which increase as ant density increases) and allogrooming appears to offer substantial resistance to parasitic fungi (Hughes et al. 2002).

### *Cooperative breeding*

Cooperative breeding (i.e., helping rear or provide for offspring) can also occur among non-related individuals. Assisting with unrelated offspring can be helpful because of a large benefit simply to living in a group and/or due to experience gained for one's future brood. For example, pied kingfishers (*Ceryle rudis*) have been observed to have non-related helpers at the nest. Non-related helpers do not help as much as related helpers (presumably because any benefit to being a non-related helper is smaller and less direct), but non-related helpers may have higher mating success as well as gain experience for the future if they have not reared offspring previously (Reyer 1980 and 1984).

### *Reciprocity*

Living in groups can also allow individuals to help each other (e.g., during times of resource scarcity) provided that helped individuals will return the favor at a later date. The benefit of being helped (even if delayed for the original helper) should outweigh the cost of helping. A well-known example of this sort of reciprocity has been documented in vampire bats

(*Desmodus rotundus*). In both kin and non-kin, vampire bats have been observed to regurgitate blood meals for individuals who did not have recent foraging success (Wilkinson 1984). This is of great benefit to the hungry bat because a few nights without a blood meal (which can be difficult to obtain) can result in starvation. Such systems appear to be relatively uncommon but are robust to ‘cheaters’ because the animals typically can discriminate between and repeatedly encounter the same individuals. In other words, if a vampire bat accepts help but then does not reciprocate, it should be harder for that bat to receive assistance in the future. Similarly, Thomas’ langurs (*Presbytis thomasi*; which I noted earlier as alerting conspecifics to the presence of a predator), appear to remember which individual gave the alarm call (Wich and de Vries 2006).

#### *Thermoregulation and energy conservation*

Keeping appropriately warm or cool is important to most animals, whether they are endotherms or ectotherms. Thermoregulation is the process by which an animal uses physiological and behavioral mechanisms to maintain a relatively constant body temperature. When the ambient temperature is near or inside the thermoneutral zone (TNZ) of the animal (the range of temperatures within which an animal’s normal metabolic rate is lowest), metabolic energy expenditure required for thermoregulation is lower than in ambient temperatures outside the thermoneutral zone. Animals can use behavioral means to reduce energy expenditure associated with thermoregulation. Such behavioral means may include fanning, panting, shivering, or seeking shade. In environments or microclimates where temperature is substantially outside the thermoneutral zone, these behaviors become very energetically costly (Terrien et al. 2011; Kingma et al. 2012).



Grouping together is another way that animals can regulate their temperatures. Red wood ants (*Formica rufa*) with higher numbers of individuals inside the nest were able to buffer against cool outside temperatures—times when smaller colonies had to wait for exogenous warming (e.g., high levels of solar radiation on the outside of the nest; Rosengren et al. 1987). Other examples include alpine marmots (*Marmota marmota*) and Andean toads (*Rhinella spinulosa*), both of which huddle together closely when temperatures are low (Arnold 1988; Espinoza and Quinteros 2008). Grouping together in hotter climates is generally not as helpful for thermoregulating (i.e., cooling off; Dausmann and Glos 2015), but is still useful in warm climates with periodic cool temperatures. For example, red-fronted lemurs (*Eulemur rufifrons*) in Madagascar will huddle together to stay warm during cool nights (cool is relative in this case at  $< 15^{\circ}\text{C}$ ). This is in an environment where temperatures during parts of the year can exceed  $40^{\circ}\text{C}$  (Ostner 2002).

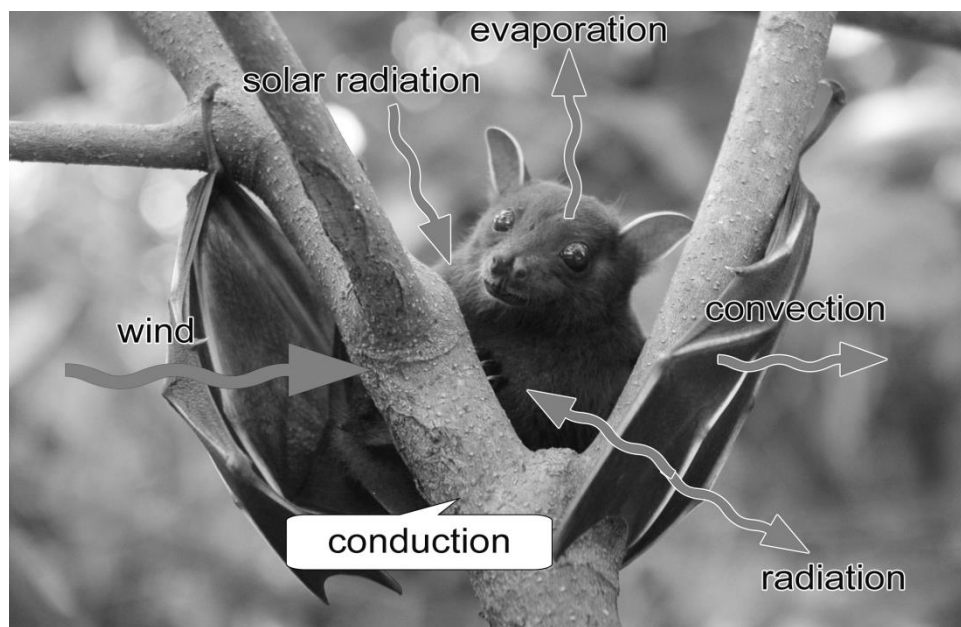
#### *What about bats?*

Bats are no exception to group-living: many of the 1,100+ species of bats are highly social and live in large groups (Dechmann et al. 2010). While little data exist for the occurrence of group-living across the entire Chiroptera order, bats that live solitarily are relatively uncommon in the United States. In fact, this feature is a fairly useful identifying characteristic (e.g., Craven and Iwen 1996). Just 25% of the species occurring in Indiana—all of which also occur in Mississippi—are considered solitary (Indiana DNR 2000). Group-living in bats has received less study than many other groups of animals, but numerous ideas have been proposed to explain why bats might live together. Bats may experience many of the potential benefits of group-living covered above: allogrooming (Kerth et al. 2003), information transfer regarding

foraging opportunities (Dechmann et al 2009; Wilkinson 1992), food-sharing (Wilkinson 1984), predator avoidance (Russ et al. 1998), and a host of other reasons (e.g., Chaverri and Gillam 2010). Kin selection may also play a role in explaining group-living, although bat colonies often have low levels of relatedness (Kerth et al. 2002; Rossiter et al. 2002).

### *Bats and thermoregulation*

One of the most important benefits of group-living for bats in temperate locations, however, is thermoregulation. In endotherms, the body temperature is not generally affected by the ambient temperature, but ambient temperature affects thermoregulation by influencing the energy required to maintain the animal's metabolic rate. Thermoregulation requires balancing heat production by the body with heat loss to the environment; the latter is known as thermal conductance (Feldhamer et al. 2003 p. 115; see Figure 1).



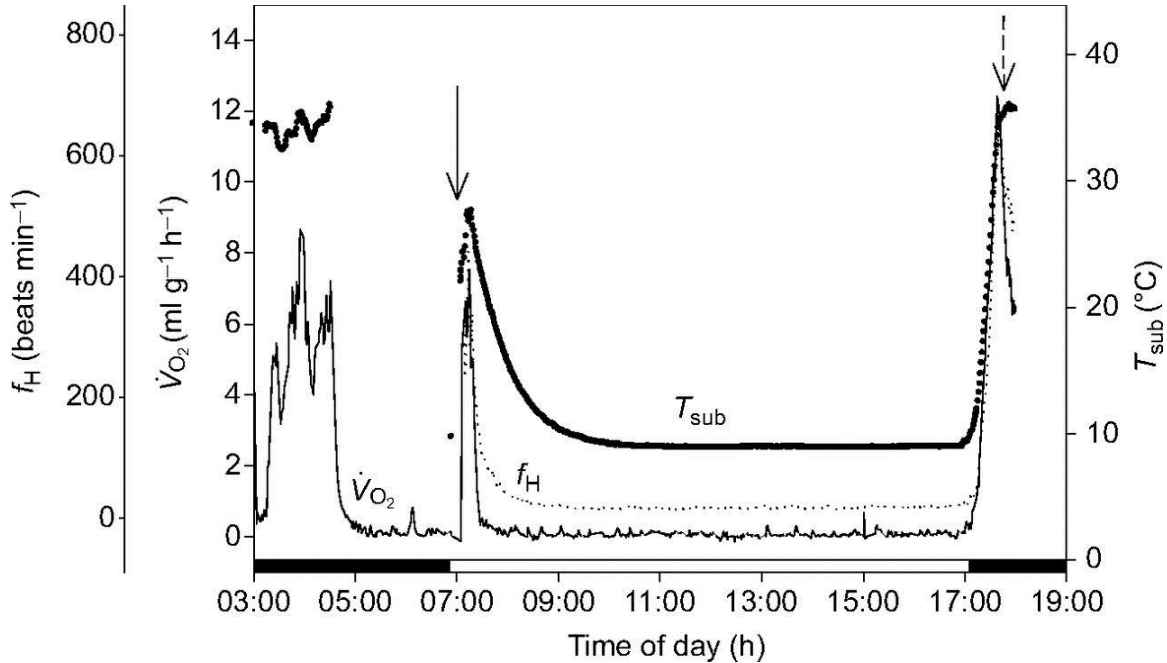
**Figure 1.** Various ways that heat is gained or lost by a bat's body. Note that conduction (transfer of heat between body and objects in contact) frequently lowers the body temperature, but if the surface is warmer compared to the body,

the surface can actually warm the body (e.g., a lizard may bask in the sun on a warm rock). Because they are nocturnal, bats do not generally receive solar radiation (or substantial amounts of reflected solar radiation). Thermoregulation via groups (also known as social thermoregulation or 'huddling') reduces convection because less surface area of each individual bat is exposed to the air. Figure created from original photo taken by Anton Croos, used with permission.

An animal's thermoneutral zone is the range of environmental/ambient temperatures inside which the body's normal metabolic rate is approximately balanced with thermal conductance. Inside their thermoneutral zone, animals do not have to alter their metabolic rate because they can use behavioral means of increasing or decreasing thermal conductance. Outside the thermoneutral zone, endotherms must increase their metabolism to maintain body temperature. For small animals, this increase is quite energetically costly. Because bats have a high surface area-to-volume ratio, heat loss (i.e., thermal conductance) is a major concern. Bats have a very limited amount of expendable energy, particularly in winter when insects and other seasonal food items are scarce. Couple a temporally-variable food source with bats' energetically-expensive mode of locomotion, small body sizes, and high surface-area-to-volume-ratios, and it becomes clear that energy balance (which is important to all animals) is of paramount importance to bats (Kurta et al. 1989).

In order to survive during periods of low energy intake and high energy loss, bats may seek thermal shelters where they are inactive. In cooler temperatures, bats typically undergo bouts of torpor, a state where the metabolic rate is substantially decreased for several hours to several days (extended torpor is referred to as hibernation). Torpor can reduce the metabolic rate of bats by 15x (Wojciechowski et al. 2007). Torpor allows bats to reduce metabolic upkeep (i.e., reduce heat production by the body that requires energy via food) in the presence of cold air. Cooler air greatly exacerbates thermal conductance (i.e., heat lost to the surrounding environment). One simple proxy for energy expenditure is an animal's heart rate (Weimerskirch

et al. 2002). A bat's torpid heart rate can be as low as 10 bpm (beats per minute) despite a normal resting heart rate of 450 bpm and an active rate of 1,000 bpm (Brunet-Rossini and Austad 2004). Torpor thus provides a means for reducing the resting heart rate by almost 98% (see Figure 2).



**Figure 2.** Heart rate, oxygen consumption ( $\dot{V}O_2$ ), and subcutaneous body temperature of Gould's long-eared bats (*Nyctophilus gouldi*) at an ambient temperature of 10°C. The two arrows represent the beginning and end of torpor. Data and graph from Currie et al. 2014, used with permission.

A downside of torpor is that torpid bats are vulnerable (e.g., to predation or to energetically costly arousal due to disturbance). Therefore, choosing the appropriate location for torpor is of paramount importance. Clearly, temperature and thermoregulation should be the basis for much decision-making in bat species that undergo torpor. A large body of research supports the idea of a relationship between bat behavior and temperature/thermoregulatory needs. For example, use of synthetic bat houses has been linked to temperature regimes. Lourenço and Palmeirim (2004) reported that bats were more likely to use bat houses that had

been painted a dark color because these houses had higher internal temperatures. The use of day roosts by female Bechstein's bats was found to be largely explained by temperature. Females chose cooler roosts before parturition and favored warmer roosts after giving birth (Kerth et al. 2001).

I acknowledge several different potential benefits of group-living in bat species, but thermoregulation is probably the most fundamental. It could be that group-living was initially selected solely as a means of more efficient thermoregulation, and other auxiliary benefits subsequently arose from group-living (exaptations). Some explanations for group-living do not apply very well to bats; bats could probably survive without roost mates alarm-calling in response to predators (Russ et al. 1998) and without other bats recruiting them to a roost (Chaverri and Gillam 2010). However, in environments where temperatures drop below freezing and roosts are not well-insulated from outside temperatures (unlike caves), bats probably require behavioral reduction of thermal conductance. One major way in which this can be possible is through close proximity to other individuals. My research focuses on the ways in which Rafinesque's big-eared bats may use grouping behavior to respond adaptively to thermal and energetic conditions.

Decision processes are an integral part of an animal's life history. An animal responds to stimuli in the environment and should typically change its behavior according to decision rules for a variety of tasks: foraging, avoiding predation, mating, intraspecies conflict, and parental care among others (Feldhamer et al. 2007; Coleman et al. 1985; Enquist and Leimar 1983). A decision simply means that one option is selected from two or more possible 'choices,' although these decisions need not be (and often are not) consciously made.

In behavioral ecology, it is assumed that animals make decisions in an attempt to maximize fitness (i.e., reproductive success or survival) by making trade-offs within ecological constraints (Sih 2013). Identifying and quantifying every piece of information that an animal could possibly use to make decisions is typically impossible. Moreover, which decision in a particular scenario is most beneficial (in terms of fitness) is not always clear. Despite these difficulties, the study of animal decision-making can be a powerful method to inform evolutionary and ecological theory as well as increase the efficiency and success of conservation and management actions (e.g., see Westneat 2013:275).

Like all animals, bats (Class Mammalia, Order Chiroptera) routinely make decisions. Bats must decide when to enter torpor or hibernation (see Table 1), when and with whom to mate, where to roost, how to navigate the landscape, and where to forage. In each case, multiple options are available to the individual bat.

**Table 1.** Definitions of common bat biology terms.

<b>torpor</b>	The lowering of body temperature and metabolic rate used to conserve energy. During torpor, bats are immobile.
<b>hibernation</b>	Essentially an extended version of torpor. Hibernation is longer (several weeks to months) and is typically associated with winter.
<b>echolocation</b>	Production of very high frequency sound (20-200kHz) from the mouth (in some species, the nose) and subsequent listening to echoes of that sound. Microchiropteran bats use echolocation to locate and discern prey objects as well as for navigation. Because it is based entirely on sound waves, echolocation allows bats to "see" in complete darkness (bats also have normal vision).
<b>roosting</b>	The act of resting by a bat. Can occur for short intervals during nighttime foraging, but typically refers to daily resting. Roosting can take place in a variety of places including caves, trees, bridges, or buildings.

Bats use a variety of signals and cues to make decisions. For example, Geoffroy's tailless bats (*Anoura geoffroyi*) used information from both echolocation calls and vision to navigate a maze (Chase 1983), and brown long-eared bats (*Plecotus auritus*) used both visual information and echolocation to locate prey items (Eklöf and Jones 2003). Microchiropteran bats choose

whether or not to pursue a prey item at least in part based on sounds reflected from echolocation (Simmons and Kick 1983). Female cave myotis bats (*Myotis velifer*) tend to choose maternity roost locations with environmental conditions favorable to pup growth (Buecher and Sidner 1999). Female greater sac-winged bats (*Saccopteryx bilineata*) appear to use both male odor and male 'songs' when deciding on a mate (Voigt and von Helversen 1999; Behr et al. 2006). Little brown bats (*Myotis lucifigus*) choose habitats for foraging which are likely to yield higher foraging success, even if that habitat may be more difficult to navigate due to its complexity (Kalcounis and Brigham 1995).

It seems clear that bats make many decisions regarding many different aspects of the environment using an array of information. Memory retention from previous experiences also likely influences decisions that bats make (Ruczyński and Siemers 2011). Nevertheless, we should expect that in all decisions a bat makes, some pieces of information are more important than others.

Because of their physiology and anatomy (e.g., small mass, high surface-area-to-volume ratio combined with high metabolic rates), conserving energy is crucially important to bats. Bats in temperate areas of the world have a useful tool for coping: the ability to raise and lower the body temperature. Body temperature can approach ambient temperature even in sub-freezing temperatures. Torpor and arousal from torpor thus provides advantages for bats, but it also has downsides (e.g., increased susceptibility to predators).

As night approaches, each individual bat has a decision to make regarding its individual energetic status: should it remain in torpor, arouse from torpor and move to a different roosting location, or should it leave the roost and forage? A bat must consider the energetic costs and benefits of each of these options in order to make a choice that maximizes its relative fitness.

Roosting and thermoregulation are integral to bat biology; insights into how bats respond to external conditions are crucial for understanding the evolutionary history and ecology of bats and other mammals as well as for conservation and management decisions.



## CHAPTER II: CLUSTERING BEHAVIOR RELATIVE TO THERMAL CONDITIONS IN A FOREST BAT

### Introduction

For endothermic animals, maintaining a warm body temperature at low ambient temperatures presents a substantial energetic challenge. Low ambient temperatures represent extreme departures from an animal's thermoneutral zone (TNZ), the range of ambient temperatures within which a resting animal has the lowest euthermic metabolic rate. Such an energetic challenge is heightened by low food availability in seasonally cold habitats (Nuñez-Villegas et al. 2013). To combat this, many animal species exhibit clustering (sometimes called huddling). Clustering is an "active and close aggregation of animals" (Gilbert et al. 2010) widely recognized to be an effective way to reduce heat loss and total metabolic expenditure (Gilbert et al. 2010; Canals 1989; Vickery and Millar 1984).

Clustering facilitates energy savings in at least three ways. (a) Convective heat loss is lower because clustering reduces the body surface area on an individual that is exposed to moving air. For example, emperor penguins (*Aptenodytes forsteri*) use 32% less energy due to reduced convection when they cluster (Gilbert et al. 2008). (b) The insulation provided by contact with cluster-mates also reduces evaporative water loss. The reduced evaporation from clustered animals reduces the need to drink water (Boratyński et al. 2015). This can have indirect energetic benefits in hibernating and aestivating species because arousal to normothermy is expensive. (c) The radiative heat loss to a cluster-mate is less than the animal would lose when radiating to the cooler sky, trees, or other structures in their environment. Clustering effectively

lowers the exposed surface area, thus reducing radiative heat loss (Alberts 1978; Blumberg 2001). Consequently, individuals typically have lower metabolic rates when clustering (Putaala et al. 1995; Brown and Foster 1992).

Clustering definitions are often imprecise given that a wide range of variation can exist within a cluster. For example, Takahashi (1997) defined huddling in wild Japanese macaques (*Macaca fuscata*) simply as “mutual physical contact.” Clusters can vary in at least four ways: number of individuals congregated in the cluster, position of an individual within the cluster, the body orientation of the clustering animals, and the density or closeness of the cluster-mates. The number of individuals in a group is often seen to increase at lower temperatures (e.g., Takahashi 1997). However, other studies report that there is a threshold amount of animals above which there are no additional thermoregulatory benefits to the group members (Canals et al. 1989). Rarely have authors considered the energetic benefit of clustering variables other than the number of cluster-mates.

Position within a cluster might substantially alter the energetic scenarios faced by individuals. Previous studies have found that such differences are often negligible either physically or due to dynamic behavioral changes, such as routine re-shuffling within the cluster (Bautista et al. 2008; Waters et al. 2012). In voles (*Microtus agrestis*), local heating of the surroundings by the cluster might offset heat loss by individuals located on the periphery of the cluster (Hayes et al. 1992).

The body orientation of clustering animals differs depending on species' morphology and behavior. For example, Alberts (1978) referred to rat pups in ‘piles,’ that is, the animals could lay side-by-side, and/or on top of each other. This means that the amount of exposed surface area per individual can vary widely and is difficult to estimate. Gilbert et al. (2008) did not have this

issue with emperor penguins (*Aptenodytes forsteri*) because those authors investigated males which were incubating eggs on their feet and thus were largely limited to lateral contact with other penguins within their cluster. Even if cluster-mates are not in contact, the distance to another individual should affect energy loss.

Clustering density (sometimes referred to as intensity; Gilbert et al. 2008) refers to the distance between individuals (i.e., how closely are they grouped to one another). Despite a long-standing recognition that clustering density is likely important for estimating energetics (Mount 1960), relatively few studies have investigated densities of animal groups. Surprisingly, many studies on animal clustering make no mention of group densities whatsoever (Schradin et al. 2006; Boix-Hinzen and Lovegrove 1998; Roverud and Chappell 1991; Yahav and Buffenstein 1991; Alberts 1978). These studies typically count only the number of individuals (e.g., Nuñez-Villegas et al. 2013) and either ignore density entirely or assume that density is always constant (e.g., Mitchell 1998).

Canals et al. (1998) wrote that “temperature probably affects” the density of clustering, although this prediction was not investigated. Studies that have considered cluster density include that of Sugita and Ueda (2013) who reported that both clustering occurrence and density in Bonin flying foxes (*Pteropus pselaphon*) decreased with increasing ambient temperatures. However, these authors do not clearly define clustering density or explain how it was measured in their study. Gilbert et al. (2008), on the other hand, used light and temperature sensors to determine whether emperor penguins, were in loose or tight huddles, and documented the number of penguins per m<sup>2</sup>. They reported that tight clusters were more common than loose or non-clusters as temperature decreased. After bioenergetics modelling showed that clustering would be more common at lower temperatures (Canals et al. 1998), Canals and Bozonovic

(2011) used a grid system to estimate clustering density in lab mice (*Mus musculus*). They found that animals grouped more closely at lower ambient temperatures.

In many wild species, it may be difficult to assess cluster density. Animals may cluster side-by-side or on top of other individuals, and do so in burrows or other places with low visibility. In this regard, many bat species offer an advantage in that bat roosting morphology places more limitations on their clustering and resting orientations because the hind feet must remain attached to a substrate. While bat roosts are usually dark and may be in remote locations, the roosts are often large enough to facilitate easier observations than for small animals that cluster in burrows or tree cavities.

In their recent review of clustering behavior, Gilbert et al. (2010) identified three characteristics of species for which clustering might be particularly important: 1) low seasonal or environmental ambient temperatures, 2) social group living, or 3) poor insulation or high surface-area-to-volume ratios. Many bat species, including the focal species of this study, meet all three of these criteria. However, not all social species form clusters (and not all bat species are social). Interspecific variation in clustering indicates that there must be some fitness trade-offs for bats roosting in clusters.

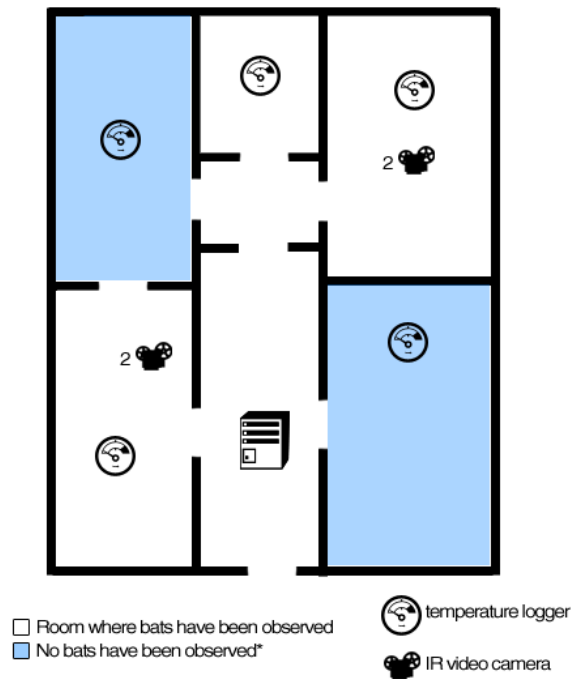
In this study, I used infrared video cameras to record bat behavior and estimate parameters of clustering, including density, in Rafinesque's big-eared bats (*Corynorhinus rafinesquii*). I used these data to test the hypothesis that clustering behavior can be predicted by an energetic model. Additionally, I predicted that a) roosting rooms would be warmer and exhibit less variance than non-roosting rooms, b) bat activity would be positively correlated with ambient temperature, c) high density clusters would be most common, and d) clustering would be common at all temperatures yet decrease as ambient temperatures rose.

Investigating variation in social thermoregulation over a range of environmental conditions yields useful information for modeling energetics and predicting behavior in bats. Propensity to cluster and density of clustering is integral to understanding the ecology of bats, many of which are threatened and endangered bat species. For example, we could use such information to ascertain when bats are most prone to human disturbance (Thomas 1995) or how some species are apparently resistant to the deadly white-nose syndrome epidemic (Turner et al. 2015; Johnson et al. 2012).

## Methods

### *Study species and site*

Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) is an uncommon bat species in the southeastern United States for which relatively little natural history or population trend information is known. I studied a colony that roosted in a partially collapsed building (approximately 130m<sup>2</sup> in total area, abandoned in the 1960s) constructed of concrete blocks and located in the Holly Springs National Forest in north-central Mississippi, USA (34°30'N, 89°21'W). The building consists of an entryway and five rooms, two of which were never used for roosting, and one very small room which was used on occasion (Figure 3). The site around the building is mixed pine-hardwood forest with hilly terrain and consists mostly of secondary growth with few trees of adequate size for roosting.



**Figure 3.** Rooms not used by bats are shaded. Temperatures were recorded in all rooms, and two infrared video cameras recorded bats in each of the two main roosting rooms.

This research was approved by the University of Mississippi Institutional Animal Care and Use Committee (application #13-019) and the Mississippi Department of Wildlife, Fisheries, and Parks (permit #0325132).

*Video recording and analysis*

Temperature data loggers (HOBO H01-001-01; ONSET software, Bourne, MA, USA) were deployed throughout the building and ambient weather data were obtained from a weather station (WINM6) managed by the US BLM ~10km from the colony site. Video was recorded by four infrared cameras (IRCAM-BW models; Polaroid Industries, Minnetonka, MN, USA) securely mounted on wooden shelves with metal mounting brackets and covered with 4 mil plastic sheeting to prevent condensation on the lenses. Behavioral recordings took place from January through September 2014. Cameras were connected via BNC cables to a security DVR (K808AV500GB model; CIB Security, Sunnyvale, CA, USA). The cameras were each set to record one hour of video at 06:00, 12:00, 18:00, and 24:00. These times were chosen to give an equal representation of bat activity across the circadian cycle. To reduce autocorrelation of temperature effects (Brown et al. 2011), every sixth day of video was analyzed because this was the smallest interval which showed no significant autocorrelation. Hourly ambient temperatures did not have different variances when compared daily versus every 6 days (Welch's one-way ANOVA,  $F_{8644,1458}=1.05$ ,  $p=0.23$ ). For statistical analyses regarding behavioral data, ambient temperatures independent of date were used from the residuals of a temperature and Julian date regression. These residuals were used for statistical analyses regarding behavioral data so that temperature would be independent of date ( $T_{ai}$ ) as date could interact with the species' biology in ways irrelevant to the present study.

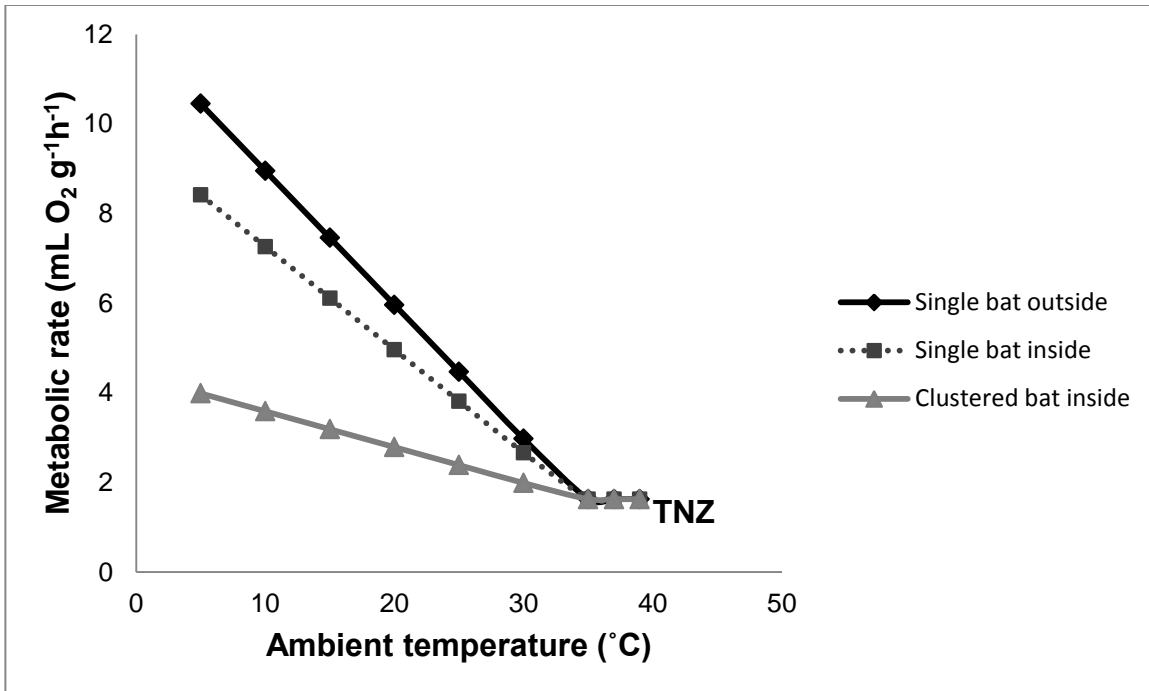
Data extracted from the video recordings included: estimated number of bats present in the field-of-view, whether bats were flying, whether bats were inactive (a proxy for torpor), and

presence and density of clusters. I adopted the bat cluster density categories of Codd et al. (2003): tight (most bats touching) or loose (separated by less than the width of one bat, approximately 30mm), and dispersed (bats separated by more than the width of one bat). Any bat located 0.5m or more from other bats was not considered part of a cluster (i.e., roosting singly). Any number of bats greater than 1 was considered a cluster if they met the conditions described above.

Defining torpor in bats has been the subject of much debate (e.g., Barclay et al. 2005; Willis and Brigham 2003), but typically involves reduction of body temperature. I used a behavioral definition: lack of any discernible movement ('inactivity') for at least 30 minutes. This definition is consistent with previous behavioral definitions of torpor in mammals (Wang and Wolowyk 1988).

I assumed that the behavior of the bats in the video view were representative of any other bats in the colony at-large. Some bats were necessarily not sampled by the fact that they occurred outside the field-of-view for the cameras. I also assumed that the four, one-hour intervals were representative of overall bat behavior.





**Figure 4.** This graph compares energy expenditure at all temperatures below the thermoneutral zone (TNZ) of a bat roosting singly outside a roost, inside a roost, and clustering with other bats inside a roost. The energy savings of a clustered bat inside a roost is 2.8x greater than a single bat inside the same roost. Roosting inside provides a slight reduction in metabolic rate, but forming a cluster provides a much greater energetic advantage.

### *Energetic modelling*

In order to understand the functional significance of clustering to bat energetics, I constructed a model (Figure 4) to illustrate energy expenditure of bats in the scenarios: outdoors with no roost (unprotected), a single bat within a roost, and a bat tightly clustered within a roost. The model is based on the euthermic energy expenditure ( $E_{eu}$ ) equation from Humphries et al. (2002):

$$E_{eu} = RMR + (T_{lc} - T_a)C_{eu}$$

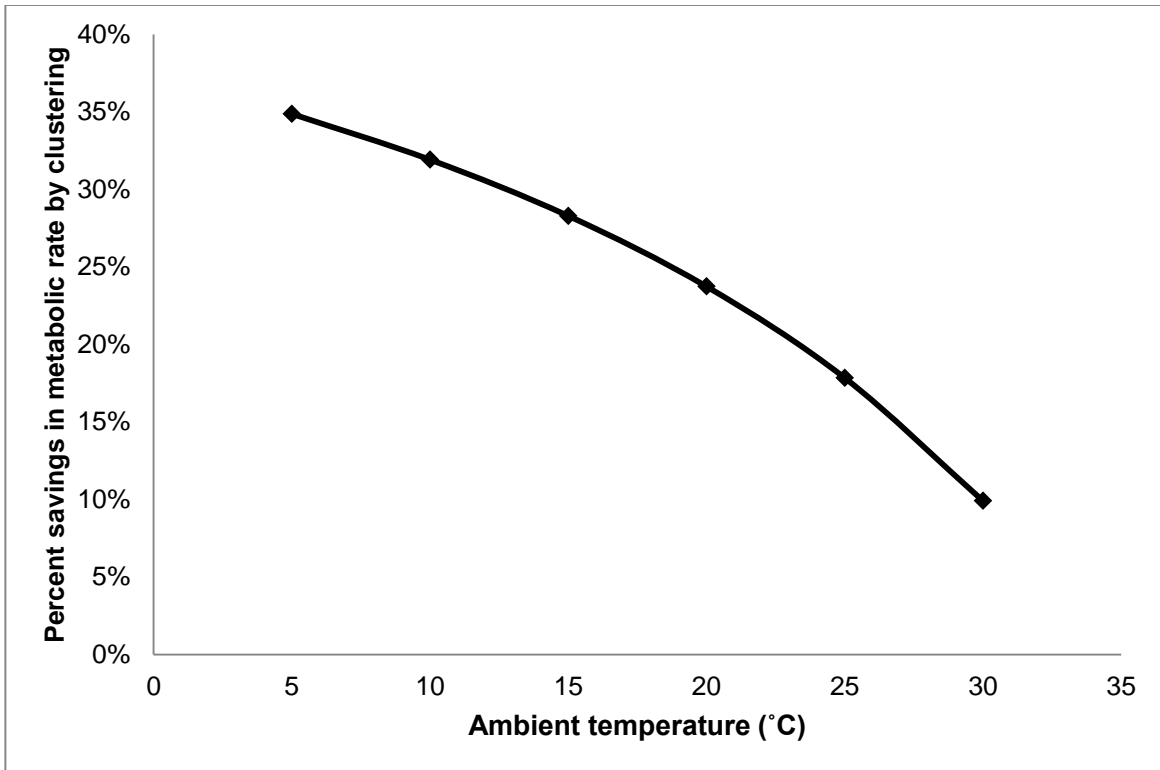
This model does not incorporate torpor physiology and thus represents a first attempt at modeling hibernation of this species. To represent *C. rafinesquii*, I used a resting metabolic rate (RMR) value reported for *Plecotus auritus* by Webb et al. (1992). *P. auritus* has a very similar morphology, diet, and ecology to *C. rafinesquii* and was previously classified as a congener

(Johnson et al. 2012).  $T_a$  represents ambient temperature and  $T_{lc}$  is the lower critical temperature (the lower bound of the TNZ). The euthermic conductance value (rate of heat loss;  $C_{eu}$ ) for a single bat (mass 11.42g) outside a roost was calculated from the mammalian equation provided by Herreid and Kessel (1967) and is similar to one reported for another bat species by Humphries et al. (2002). To obtain conductance values for the three modeled conditions (outside alone, inside alone, and inside clustered), the *C. rafinesquii*  $C_{eu}$  value was adjusted to reflect the proportional relationship that Kurta (1985) found for *Myotis lucifugus* in those roosting conditions. Values, units, and sources are reported in Table 2.

**Table 2.** Values used to model the effect of ambient temperature and clustering on metabolic expenditure for *Corynorhinus rafinesquii*. Abbreviations include resting metabolic rate (RMR), thermoneutral zone (TNZ), and euthermic conductance ( $C_{eu}$ ).

Parameter	Value	Units	Source
RMR	1.63	mL O <sub>2</sub> g <sup>-1</sup> h <sup>-1</sup>	Webb et al. 1992
TNZ	34.5 - 39	°C	Webb et al. 1992
$C_{eu}$ – single, outside	0.30	mL O <sub>2</sub> g <sup>-1</sup> °C	Calculated
$C_{eu}$ – single, inside	0.23	mL O <sub>2</sub> g <sup>-1</sup> °C	Calculated
$C_{eu}$ – clustered, inside	0.08	mL O <sub>2</sub> g <sup>-1</sup> °C	Calculated

Predicted energy savings provided by clustering in relation to  $T_{ai}$  were fitted to a polynomial equation ( $y = -0.002x^2 - 0.0013x + 0.36$ ,  $R^2 = 0.99$ ; Figure 5). To test if this model explained variation in roosting behavior, I investigated whether values predicted by this equation showed a similar relationship to  $T_{ai}$  as the observed values. Predicted and observed values were standardized for comparison. When comparing the probability of observed clustering density,  $T_{ai}$  was binned into 5- and 10-sample groups for analyses. Probability of tight clustering was calculated for each bin, and the mean  $T_{ai}$  was calculated for each bin. Probabilities were standardized and compared to standardized probabilities predicted by the model.



**Figure 5.** The polynomial equation ( $y = -0.002x^2 - 0.0013x + 0.36$ ) describing energy savings due to clustering. The equation was derived from the RMR difference between a single bat inside and a bat clustering inside.

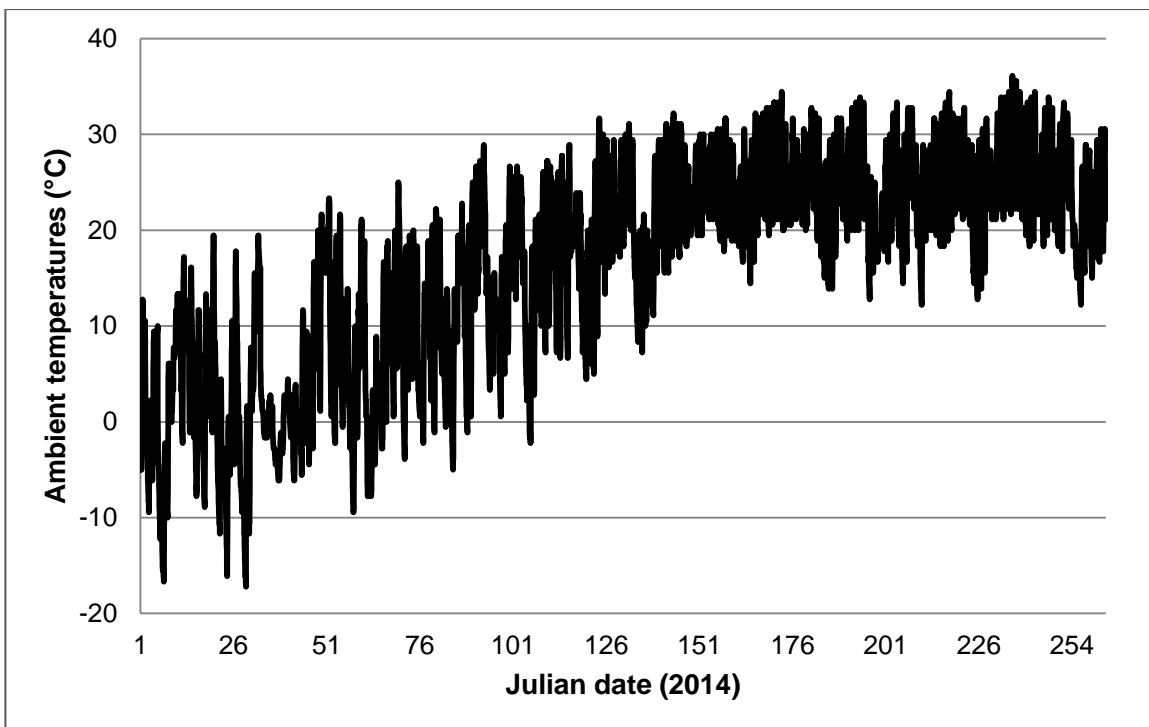
Welch’s one-way ANOVAs and Games-Howell post-hoc tests were used because of unequal variances. Spearman correlations were used due to frequent non-normality. Firth bias-reduced logistic regressions (FBRLR) were performed due to unequal distribution of binary data. Data analysis was primarily performed in *R* (The R Foundation; [www.r-project.org](http://www.r-project.org)). The box plot was made using Plotly ([www.plot.ly](http://www.plot.ly)).

## Results

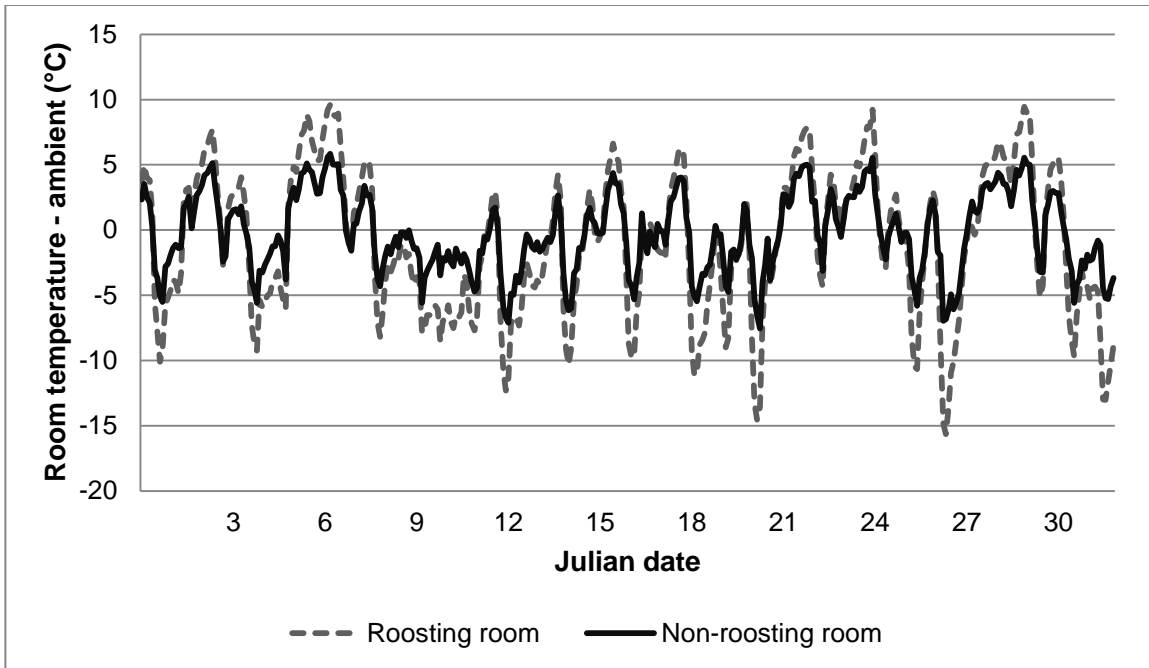
### *Thermal conditions*

Ambient temperatures during the study period ranged from  $-17^{\circ}\text{C}$  to  $36^{\circ}\text{C}$  (Figure 6).

Unexpectedly, roosting rooms were colder than non-roosting rooms during 5 of the 11 months monitored (Table 3). I expected differences in temperature variance between roosting and non-roosting rooms. In all months, the variance in temperature was lower in roosting rooms than non-roosting rooms. In all months, the variance in temperature was lower in roosting rooms than non-roosting rooms (which, in turn, were lower than ambient; Table 3), but only in January, February, March, and April were variances in roosting and non-roosting rooms significantly different. Figure 7 demonstrates the relative temperature fluctuations in roosting and non-roosting rooms during the coldest month.



**Figure 6.** Ambient temperature data recorded hourly for 2014 near the study site. Behavioral observations began on Jan. 1 and continued until 9/20/14.



**Figure 7.** Time series of January 2014 temperature differences in roosting and non-roosting rooms relative to ambient temperatures. Roosting rooms were more protected from swings from ambient temperature than non-roosting rooms.

**Table 3.** Indoor compared to ambient temperatures (°C) at bat roosting site. Significant differences between ambient, non-roosting room and roosting room means (within a row,  $\pm$  standard deviation) are indicated by different letters (Tukey's test,  $p \leq 0.05$ ) where the ranking of letters corresponds to the ranking of means. Significant differences in the temperature variance of the room types are indicated by an asterisk.

Month (days)	Ambient ( $\mu \pm s$ )	Non-roosting rooms ( $\mu \pm s$ )	Roosting rooms ( $\mu \pm s$ )
January (31)	1.25 <sup>a</sup> $\pm$ 7.93	1.00 <sup>a</sup> $\pm$ 5.29	0.27 <sup>a</sup> $\pm$ 3.26*
February (28)	4.31 <sup>a</sup> $\pm$ 7.27	3.76 <sup>ab</sup> $\pm$ 4.90	3.20 <sup>b</sup> $\pm$ 3.89*
March (31)	8.84 <sup>a</sup> $\pm$ 7.47	7.94 <sup>b</sup> $\pm$ 4.73	7.08 <sup>c</sup> $\pm$ 3.69*
April (28)	15.81 <sup>a</sup> $\pm$ 6.61	14.37 <sup>b</sup> $\pm$ 4.23	13.43 <sup>c</sup> $\pm$ 3.29*
May (31)	20.60 <sup>a</sup> $\pm$ 6.14	18.71 <sup>b</sup> $\pm$ 3.74	17.91 <sup>c</sup> $\pm$ 3.09
June (30)	24.28 <sup>a</sup> $\pm$ 4.21	22.55 <sup>b</sup> $\pm$ 1.75	22.09 <sup>b</sup> $\pm$ 1.31
July (24)	23.67 <sup>a</sup> $\pm$ 4.89	21.92 <sup>b</sup> $\pm$ 2.37	21.53 <sup>c</sup> $\pm$ 1.89
August (24)	25.22 <sup>a</sup> $\pm$ 4.87	23.16 <sup>b</sup> $\pm$ 2.17	22.49 <sup>c</sup> $\pm$ 1.67
September (20)	21.19 <sup>a</sup> $\pm$ 5.18	20.13 <sup>b</sup> $\pm$ 2.75	19.89 <sup>b</sup> $\pm$ 2.20
October (31)	16.73 <sup>a</sup> $\pm$ 6.24	15.87 <sup>b</sup> $\pm$ 4.06	15.79 <sup>b</sup> $\pm$ 3.53
November (18)	6.10 <sup>a</sup> $\pm$ 7.06	6.01 <sup>a</sup> $\pm$ 4.70	6.37 <sup>a</sup> $\pm$ 3.95

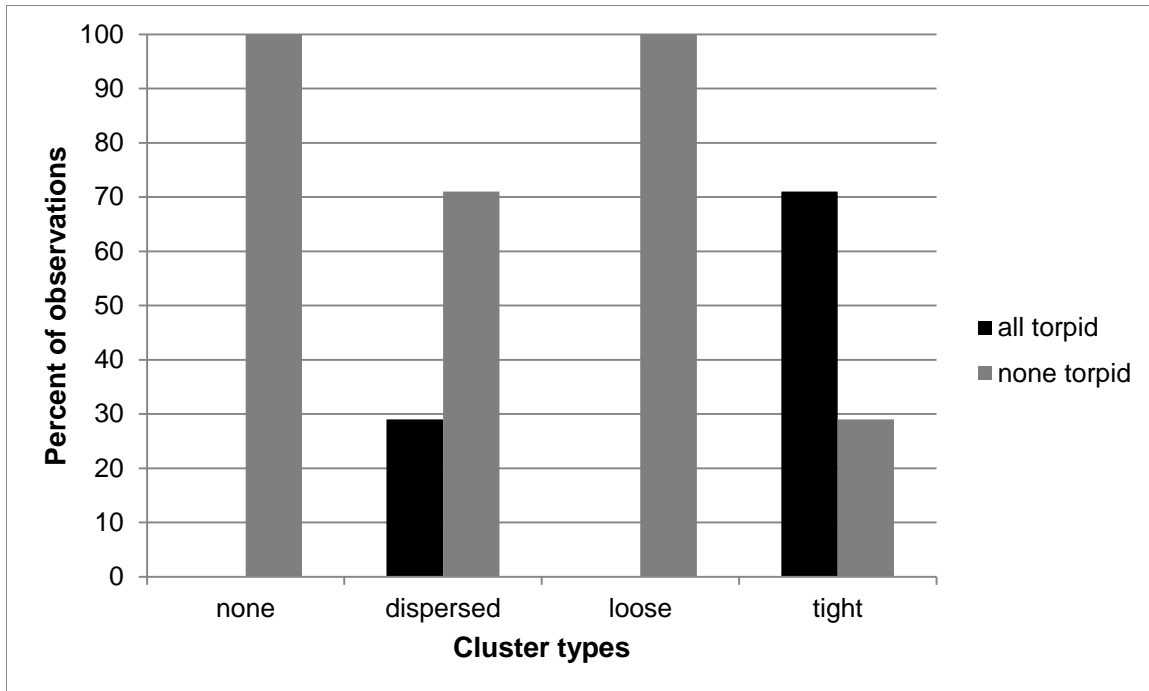
### *Bat occupancy*

Across the study, bat numbers varied with time of day (Welch's one-way ANOVA  $F_{3,143}=3.77$ ,  $p=0.01$ ). Numbers were highest at 06:00 ( $22\pm 21.45$ ) and lowest at 24:00 ( $7.11\pm 12.18$ ). For 12:00, the mean number of bats was  $16.62\pm 21.09$  and  $18.08\pm 21.85$  for 18:00. Only the differences between 06:00 and 24:00 (Games-Howell  $p<0.01$ ) and 18:00 and 24:00 ( $p=0.05$ ) were statistically significant.

Overall, the number of bats observed was not correlated with  $T_{ai}$  (Spearman's  $\rho=0.15$ ,  $n=128$ ,  $p=0.10$ ). When the number of bats present  $\geq 10$ , however,  $T_{ai}$  and number of bats were positively correlated as predicted (Spearman's  $\rho=0.37$ ,  $n=56$ ,  $p<0.01$ ). Bat occupancy was positively correlated with  $T_{ai}$  at 18:00 (Spearman's  $\rho=0.36$ ,  $n=37$ ,  $p=0.03$ ), nearly so at 06:00 (Spearman's  $\rho=0.30$ ,  $n=37$ ,  $p=0.09$ ), but not at 12:00 (Spearman's  $\rho=0.20$ ,  $n=37$ ,  $p=0.26$ ) or 24:00 (Spearman's  $\rho=0.03$ ,  $n=36$ ,  $p=0.88$ ).

### *Bat torpor*

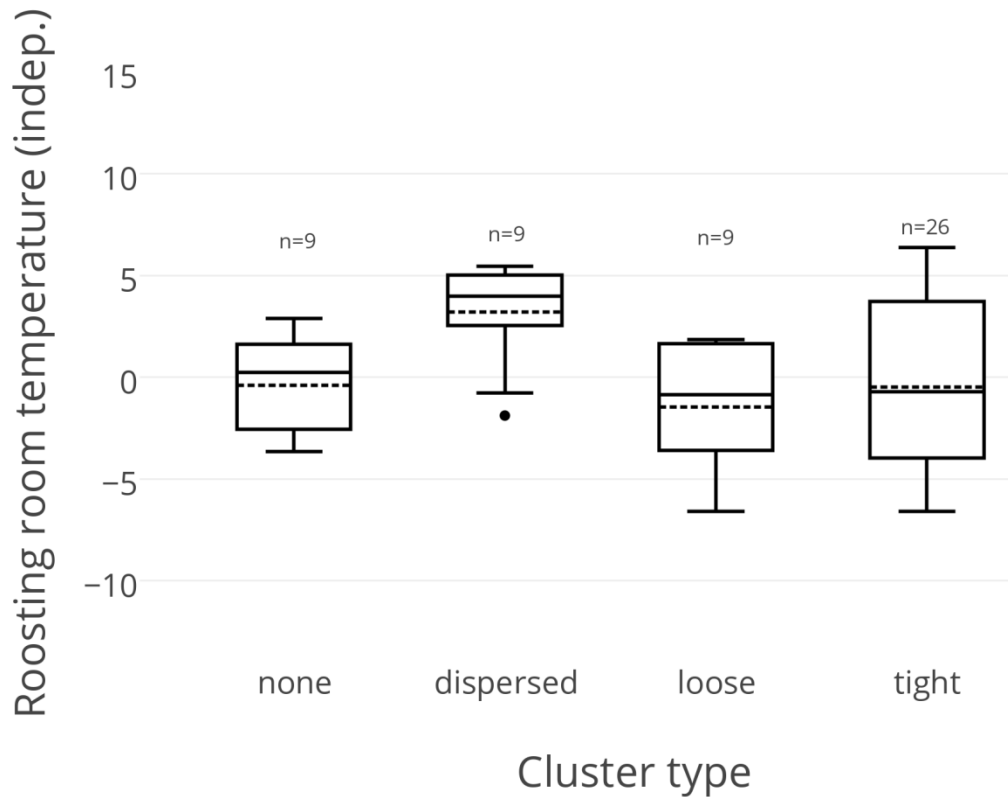
Not surprisingly, occurrence of torpor was reflected in the percent of bats flying (Spearman's  $\rho=-0.58$ ,  $n=43$ ,  $p<0.01$ ). Occurrence of colony-wide torpor (compared to complete absence of torpor) of roosting bats was significantly different across the four cluster categories (Fisher's exact test with Freeman-Halton extension,  $p<0.01$ ; Figure 8). Notably, tight clusters were very common when all bats were in torpor.  $T_{ai}$  did not predict colony-wide torpor (Firth bias-reduced logistic regression [FBRLR] coefficient=0.01,  $t=0.19$ ,  $n=33$ ,  $p=0.85$ ).



**Figure 8.** The proportion of all-torpid to none-torpid observations varied across cluster categories.

### *Clustering*

Approximately 49% of observations where the total number of bats  $\geq 5$  exhibited tight clustering; tight clustering was expected to be the most common type of clustering. However,  $T_{ai}$  was not a predictor for the presence of tight clusters (FBRLR coefficient=0.03,  $t=0.80$ ,  $n=56$ ,  $p=0.42$ ), and the logistic model fit the data well (Hosmer-Lemeshow  $\chi^2=9.78$ ,  $df=8$ ,  $p=0.28$ ). Curiously,  $T_{ai}$  did not differ across cluster densities (Welch's one-way ANOVA,  $F_{3,20.5}=1.1$ ,  $p=0.37$ ), but mean roosting room temperature (independent of date) did differ across cluster densities ( $F_{3,20.8}=4.64$ ,  $p=0.01$ ; Figure 9). A post-hoc Games-Howell test indicated a significant difference in roosting room temperatures between dispersed and loose ( $p=0.02$ ), dispersed and none ( $p=0.04$ ), and dispersed and tight ( $p=0.03$ ).



**Figure 9.** Box plot of roosting room temperatures (independent of date) in each cluster density. Means are represented by a dashed line and modes by a solid line.

#### *Comparisons to energetic model*

While not statistically significant, the relationship between observed behavioral responses to  $T_{ai}$  and those predicted by the model were quite unexpected. The slope of the polynomial equation (predicted energy savings due to clustering) tended towards a slight negative correlation with observations of clustering density across  $T_{ai}$  (Spearman's  $\rho=-0.22$ ,  $n=56$ ,  $0.11$ ). Similarly, probability of clustering tended towards a negative correlation with  $T_{ai}$  in both 10-observation bins (Spearman's  $\rho=-0.67$ ,  $n=6$ ,  $p=0.15$ ) and 5-observation bins (Spearman's  $\rho=-0.51$ ,  $n=11$ ,  $p=0.11$ ). Neither observations of clustering density (Spearman's  $\rho=0.14$ ,  $n=56$ ,  $p=0.30$ ) nor observed probabilities of tight clustering had any obvious relationship to  $T_{ai}$  (Spearman's  $\rho=0.11$ ,  $n=11$ ,  $p=0.75$ ). Linear regression yielded  $R^2$  values of 0.01 (10-observation bins) and 0.02 (5-observation bins). Additionally, none of these correlations were significant with unadjusted  $T_a$ .



## **Discussion**

### *Thermal conditions*

There was a clear difference in temperature regimes between roosting and non-roosting rooms. Both were more stable than outside temperatures. Buffering of roosts from ambient temperatures has been widely reported for bats (Chruszcz and Barclay 2002; Zahn 1999). Notably, mean temperatures were often slightly lower in roosting rooms despite reduced temperature fluctuation. During January-April, this reduced variation was significant (Figure 7). Low fluctuations during colder times may be beneficial for maintaining torpor when arousal would be undesirable due to low food availability and low ambient temperatures (Webb et al. 1996). It should be noted that roost thermal conditions in this case were unlikely to be influenced by the presence of bats as tree roosts may be (Willis and Brigham 2007) because the roost structure is very large compared to the bats.

### *Bat occupancy*

Low numbers at 24:00 are not surprising given that bats are nocturnal foragers. Differences in bat occupancy or visibility at different times of day may warrant further investigation for censusing purposes (see also Hayes 1997), especially given that the association of occupancy was positively correlated with ambient temperature at some times of day but not others. Higher numbers of bats (when total  $\geq 10$ ) at warmer temperatures across all times indicates that temperature would be important for censusing, too.

### *Bat activity and clustering*

Even though ambient temperature did not predict colony-wide torpor, differences over the clustering categories suggest that clustering plays an important role in bat energetics. Almost all observations of colony-wide torpor were tight clusters. At virtually all observed times and ambient temperatures with  $\geq 15$ , bats formed a cluster of some type. Given that the ambient

temperature almost never approached the estimated TNZ, this is not surprising. Constantly being below the TNZ would indicate that these small bats are in a constant state of energetic struggle and must seek ways to reduce heat loss.

Despite a moderate positive correlation between ambient and roosting room temperature, roosting room temperatures were different across cluster densities while ambient temperatures were not. This could be due to microclimate differences not measured in this study or to the physical properties of the roost structure. For example, the roost building is constructed of cement blocks which are relatively poor at conducting heat. Low thermal conductivity is one likely reason why roosting room and ambient temperatures are not more highly correlated.

In this study, clustering was extremely common across ambient temperatures. Clustering is likely of great importance for energetic balance. Because virtually all observations were below the TNZ, reducing heat loss (and perhaps evaporative water loss) is still critical for these small mammals. While probably more complex than a simple response to ambient temperature, variation in bat clustering clearly translates to different energetic scenarios (Hristov et al. 2008). Some previous studies indicating a more direct relationship between ambient temperature and clustering in other species (e.g., Alberts 1978; Mount 1960) may be because variables like relative humidity and temperature variability were controlled, and food availability was not an issue for those animals. Further, bats exhibit torpor whereas rats and pigs do not. Complex environmental interactions in the ‘real world’ are well-known (Calisi and Bentley 2009), so perhaps it should be expected that animals in the field do not respond in the same way as those in a laboratory setting. Numerous additional potential reasons for animals to group together exist, including reduced predation risk (Turchin and Kareiva 1989), reduced parasite loads in some cases (Stumbo et al. 2012), or reciprocity (Wilkinson 1984). It should be noted that multiple

reasons may apply for any one species, and this does not necessarily preclude thermoregulation being the primary reason.

#### *Energetic model comparisons*

The constructed model did not predict likelihood of cluster across ambient temperature. Modelling is an iterative process by which an investigator refines the model component variables and their values. I estimated  $C_{eu}$  and/or RMR values from *P. auritus*, but this may not be appropriate for *C. rafinesquii*. Intraspecific variation in  $C_{eu}$  and TNZ have also been reported in different geographical areas for some bat species (Willis et al. 2005). I also assumed that change in euthermic conductance with clustering for *C. rafinesquii* was as predicted by Kurta (1985) for a different bat species. In addition, I did not consider evaporative water loss (EWL) in my calculation of heat loss. Boratyński et al. (2012) reported that water vapor pressure predicted cluster sizes in greater mouse-eared bats (*Myotis myotis*) in winter, presumably due to bats trying to reduce EWL. Finally, animals can alter  $C_{eu}$  in other behavioral ways such as piloerection or vasoconstriction, although these are not known to be significant in most bat species (but see Betts 2010). Future studies of clustering should use body temperature of bats to identify torpor while also considering how the life stage affects metabolic demands of bats preparing for reproduction.

#### *Conclusions*

My study is notable because the local environment and ecology are different from most previous studies of *C. rafinesquii*. For example, several studies (e.g., Johnson and Lacki 2014; Johnson and Lacki 2011) have taken place in Kentucky where temperatures are usually lower and bats commonly roost in caves. Caves have different thermal and humidity profiles and exhibit airflow and barometric pressure differently than other types of roosts (Tuttle and Stevenson 1977). Other studies of *C. rafinesquii* have taken place in bottomland hardwood areas

where large trees ( $\geq 100\text{cm dbh}$ ) are readily available for roosting (Lucas et al. 2015). Such trees are very rare in my study area. Scarcity of roosts in the present study area may mean that this population has higher levels of roost fidelity. Differences in roost fidelity have been documented across different roost types and habitats for *C. rafinesquii* (Trousdale et al. 2008) and other bat species (Lewis 1995). It stands to reason that roosting behavior may also vary across roost types.

Energetics of bats is a complex topic. Temperate bats, unlike most endothermic animals, can drastically alter their metabolic rate through torpor and hibernation. They have a variable food source (insects) and an expensive mode of locomotion (flight). These features make bats an interesting study system, but bats are also small, nocturnal, often cryptic and/or rare, and prone to disturbances by human visitation. Another important aspect which has only just begun to be investigated is radiative heat loss during flight (Reichard et al. 2010). The energetic costs associated with various types of bat flight and foraging are not well understood. Energetics may even play a role in the continued nocturnality of bats from an evolutionary perspective (Voigt and Lewanzik 2011).

While thermoregulatory needs are likely an important driver of roosting and clustering behavior, numerous other influences may exist: reducing evaporative water loss (Boratyński et al. 2015 and 2012), parasite avoidance (Reckardt and Kerth 2007), predator avoidance, room for maneuverability (e.g., for pups learning to fly), spatiotemporal variation in food availability, and frequency and magnitude of human disturbance (Trousdale et al. 2008). Although likely limited in scope and benefit, some winter foraging likely occurs in this species, as does winter mating (Johnson et al. 2012). The occurrence of these behaviors may contradict expectations of bat behavior that assume bats do little during winter except hibernate.

Rafinesque's big-eared bats are a federal species of concern and threatened or endangered in most of the states they occupy. Knowledge about their roosting behaviors is especially important for conserving existing roosts as well as the creation of artificial roosts. This study suggests that *C. rafinesquii* prefers more stable roost thermal regimes even if they are slightly cooler. Bat occupancy was positively correlated with ambient temperature around sunset, indicating that temperature may influence foraging behavior.

The relationship between clustering behavior with ambient temperature was not as expected. One likely contributing factor was that the model I used did not account for torpor which can substantially alter energy expenditure. This is, of course, an important feature of the bats' biology which can be incorporated into the model in the future. Nevertheless, this study demonstrates three important observations: **1)** torpor is almost always associated with clustering; **2)** roost temperatures/microclimates may more directly influence clustering behavior than ambient temperature; and **3)** tight clustering is relatively common across all temperatures and seasons, but much variation in clustering exists.

I also documented variation in the number of individuals in a cluster, but this is likely due to bat occupancy rather than clustering behavior per se. Other clustering parameters also exist. Previous research indicated that position within a cluster does not significantly alter an individual's insulation (Kurta 1985) although thermal imaging studies can revisit this topic. Body orientation of bats is difficult to quantify but may expose bats to different levels of convection and conduction. Such variation should be taken into consideration for all future studies of bat energetics and behavior, and we should strive to further understand the mechanisms driving clustering behavior. To understand these mechanisms, we must first acknowledge that clustering is much more versatile than typically assumed.

## CHAPTER III: OTHER OBSERVATIONS OF RAFINESQUE'S BIG-EARED BAT BIOLOGY

### Introduction

Relatively little is known about most aspects of natural history and basic biology of the Rafinesque's big-eared bat (*Corynorhinus rafinesquii*). With my study, I sought to document occupancy, size, foraging locations, and roosting behavior for a colony roosting in an abandoned building in north-central Mississippi. This area of the species' range has received virtually no attention in the literature and also appears to be a portion of the range where population numbers and trends are not documented. Similarly, colonies roosting in buildings have typically received less attention than those roosting in trees and caves. This lack of data in many respects has substantially hindered our understanding of the species and what we should do from a conservation and management perspective. Here, I will give a brief overview of what is currently known about the species before describing some of the basic natural history findings of my study.

The Rafinesque's big-eared bat is a small vespertilionid bat ranging in mass from 6-14g. Individuals have large ears which measure 30-37mm and the total body length is approximately 90-105mm. The wingspan is typically around 30cm (Jones 1977). Dorsal fur is reddish brown to dark gray with ventral fur being black at the base and having grayish white tips; this gives the bats a distinctly lighter colored underside. In addition to the large ears, these bats have two parahinal glands between eyes and nose (*Corynorhinus* translates to 'lump-nosed').

Although previously described with 2 subspecies (Handley 1959), recent molecular studies have discounted such a division (e.g., Piaggio et al. 2011). Congeners include the Mexican big-eared bat (*Corynorhinus mexicanus*) and the Townsend's big-eared bat (*Corynorhinus townsendii*), the latter having five subspecies. Collectively, these are known as the North American big-eared bats. *C. rafinesquii* appears to have diverged from the rest of the genus over two million years earlier than the divergence of *C. mexicanus/townsendii*. The most recent *Corynorhinus*-like ancestor diverged during the warm periods of the Pliocene (5.0-2.5 mya; Lack and Van Den Bussche 2009).

At present, the Rafinesque's big-eared bat occurs throughout the southeastern United States from southern Florida, north to the southern half of Virginia, and westward to Arkansas and the eastern edge of Texas. Known populations are scattered throughout this range with the exception of the piedmont physiographic region in Georgia, South Carolina, and North Carolina apparently being devoid of the species (Bennet et al. 2008). This species has been documented in many different forest types across its range, but the densest concentrations of bats occur in mature stands of cypress (*Taxodium*) and tupelo-gum (*Nyssa*) trees near permanent water (Lacki and Bayless 2013).

*C. rafinesquii* typically lives in groups. The largest known colonies are associated with caves in the Appalachian Mountains or in central Kentucky. A handful of these colonies number into several hundred individuals but the majority of known colonies are less than 100 with <50 not uncommon. However, severe paucity of data seriously impedes population estimates and trends (Bayless et al. 2011). *C. rafinesquii* may roost in cavities of large trees (>100cm DBH; Lucas et al. 2015), in caves, under bridges, and in abandoned buildings (Bayless et al. 2011).

The species is non-migratory. Winter hibernation is prevalent but intermittent. In warm weather, these bats typically forage within 4km or less of the roost site regardless of location and habitat type (Johnson and Lacki 2011). They are typically gleaners while foraging, plucking prey items from the surface of foliage or other surfaces and appear to be more agile fliers than many sympatric species (Barbour and Davis 1969). They use relatively low intensity echolocation calls which can make them difficult to survey with acoustic detectors (Lacki and Bayless 2013). Rafinesque's big-eared bats consume a variety of insects which may vary across time and habitat. Most rigorous dietary studies have taken place in Kentucky, but the species appears to be a moth specialist (Lacki and Ladeur 2001).

Roosting behavior may vary by location and roost type. For example, Rafinesque's big-eared bats tend to show higher levels of fidelity to anthropogenic roosts—whether this is due to properties inherent to those structures or a lack of alternate roost choices remains unclear (Lacki and Bayless 2013). Roost availability and use also may vary geographically: large roosting trees are available and preferred in parts of the range like Congaree National Park (Lucas et al. 2015) or the Noxubee Wildlife Refuge (Fleming et al. 2013), caves are used in parts of the range where they occur (e.g., Johnson et al. 2012), and use of anthropogenic structures is prevalent in habitats that lack both caves and roosting trees (Trousdale 2008).

Mating occurs in the fall and at least sporadically throughout winter. Females typically give birth (after delayed fertilization) in late May or early June. Pups measured at birth in eastern Texas were ~2.5g (Mirowsky 1998). Pups have permanent dentition and are volant after 3 weeks. They may reach adult body mass at one month of age but retain a characteristic dark pelage for several more weeks (Jones 1977).



Records of longevity are rare, but recapture of banded individuals suggests at least a 10 year lifespan (Paradiso and Greenhall 1967). Similarly sized, sympatric species have been documented living 30 years in the wild (Keen and Hitchcock 1980). Little is known about mortality or predation rate, although potential predators include rat snakes (*Scotophis* sp.; Clark 1990) and other snake species, raccoons (*Procyon lotor*), domestic cats (*Felis catus*), and Virginia opossums (*Didelphis virginiana*; Jones 1977). Some authors speculate that human disturbance at roosts and loss or degradation of roost sites likely constitute greater concerns to the species (Clark 2000; Lacki 2000).

Prevalence and diversity of both diseases and parasites in the species is virtually unknown except for a few internal helminth parasites (McAllister et al. 2005). Only one individual of the species has been confirmed rabid (Sasse and Saugey 2008). *C. rafinesquii* has not been shown to suffer white-nose syndrome, although they may harbor the causal fungus (*Pseudogymnoascus destructans*, Bernard et al. 2015). The species' apparent resistance to the disease may be due to its relatively shallow use of torpor/hibernation (Johnson et al. 2012). It has long been reported that Rafinesque's big-eared bats appear more active and alert during winter than other bat species (Jones 1977).

While the species has a large range, concern exists because of its patchy distribution and apparently low numbers. The species has never been considered common (Handley 1959) and has been reported to be declining for several decades (Jones and Suttkus 1975). The species is currently having its status reviewed by the US Fish and Wildlife Service. Rafinesque's big-eared bats are listed as vulnerable by both NatureServe and the US Forest Service Southern Region, and are considered threatened, endangered, or of special concern in almost all the states they occupy (Lacki and Bayless 2013). Paradoxically, the International Union for the Conservation of

Nature (IUCN) lists the current population trend as increasing despite also stating that total population size is not known to exceed 10,000 mature individuals and reporting that the species is known or suspected to be declining in more than half of the states in its range. In the remaining half of the states, "data are unavailable to determine trends" (Arroyo-Cabrales and Álvarez-Castañeda 2008). Clearly, much remains to be learned about the species' biology, its current status, and future population trends. The lack of information needed to inform conservation and management decision-making was the primary motivation for my investigations into a colony of Rafinesque's big-eared bats.

## **Methods**

### *Trapping, marking, and biometric data*

Bats were captured using handheld butterfly nets (16" diameter, deep net) or custom-fitted mist nets. Each bat (79 total individuals) was fitted with a split aluminum band (initially, 3mm colored aluminum split bands [#1602, Frisky Finches, Castaic, California USA], later switched to bat 'rings' [2.9mm alloy narrow bat rings, Porzana Ltd. Icklesham, E. Sussex, UK]). This size band has been used in previous studies of the species (Joe Johnson, Alison McCartney, personal communications).

When used carefully, butterfly nets successfully catch both mobile and stationary bats with no known ill effects (Trousdale 2008). Upon capture, usually of multiple bats, they were taken from the net one at a time and each placed into an individual, unused paper bag (standard lunch bags). The top of the bag was folded down and secured with a paper clip to prevent escape while working with other bats. All bagged bats were given a temporary label (e.g., A through Z) in order to make recording handling time easier. The bagged bats were kept safely inside the roost building to remain at approximately the same temperature they were experiencing prior to capture. Bats were weighed while in the bag using a 50g Pesola spring scale; afterwards the mass of the bag and paper clip was subtracted from the total mass. After removal from the bag, forearm length (radial bone; Adams and Pedersen 2000) was measured using a digital caliper (Neiko 01407A), bats were visually inspected for ectoparasites and wounds and then fitted with a band. Males were banded on their right forearm and females banded on the left. Bats were released into the structure or area from which they were captured.

My research was approved by the University of Mississippi Institutional Animal Care and Use Committee (application #13-019) and the Mississippi Department of Wildlife, Fisheries, and Parks (permit #0325132).

### *Temperature monitoring*

Temperature data loggers (HOBO H01-001-01 models, ONSET software; Bourne, MA, USA) were deployed throughout the roost. These data loggers have an accuracy of  $\pm 0.7^\circ\text{C}$  and a resolution of  $0.1^\circ\text{C}$ . Each device was configured and launched using BoxCar Pro 4.3 (ONSET software) using a serial-USB conversion cable. The devices were set to record temperature in degrees Celsius every 2 hours. Ambient temperature readings were used from the Interagency Remote Automated Weather Stations (RAWS) network's WINBORN (WINM6) weather monitoring station located ~10km from the study site. Data were obtained from the University of Utah MesoWest website (<http://mesowest.utah.edu>).

### *Radio telemetry*

In accordance with the '5% rule' (Aldridge and Brigham 1988), bats weighing less than 9g were not fitted with radio transmitters. Bats chosen for radio telemetry were fitted with a 0.46g VHF radio transmitter (A2415; Advanced Telemetry Systems [ATS], Isanti, MN, USA). After trimming a small section of fur between the scapulae (using small scissors or a small cordless beard trimmer) and cleaning the exposed skin with an alcohol pad, transmitters were attached using Perma-Type waterproof surgical cement (AC103; Perma-Type Products, Plainville, CT, USA). Great care was taken not to cut or pinch the skin. As a precaution,

antiseptic liquid skin bandage was on hand (New-Skin liquid bandage; Prestige Brands, Tarryton, NY, USA).

Transmitter attachment methodology was similar to that proposed by Carter (2008). Bats were held for 10-15 minutes after the transmitter was glued on to allow the cement to set. Surrounding fur was pushed around the transmitter and a small amount of corn starch baby powder was applied to keep the glue from being ‘tacky.’ All bats were visually verified to be able to fly normally post-release. Figure 10 demonstrates attachment of radio transmitter to a bat.

**Figure 10.** Photograph of a Rafinesque’s big-eared bat shortly after attachment of a radio transmitter between the scapulae using surgical cement.



Simultaneously transmitter signals were obtained by two people with VHF scanning receivers (ATS R410) and 3-element Yagi antennas (ATS 13860). Using handheld, two-way radios (Uniden GMR1636-2C; Uniden America, Irving, TX, USA), it was verified that both observers were receiving a signal from a particular transmitter. At approximately the same time, the compass bearing for the signal was recorded along with GPS coordinates (via a handheld unit) for each person. Readings were taken and recorded every 2-5 minutes as possible, moving through the landscape as necessary to follow the transmitter signal or locate a new one.

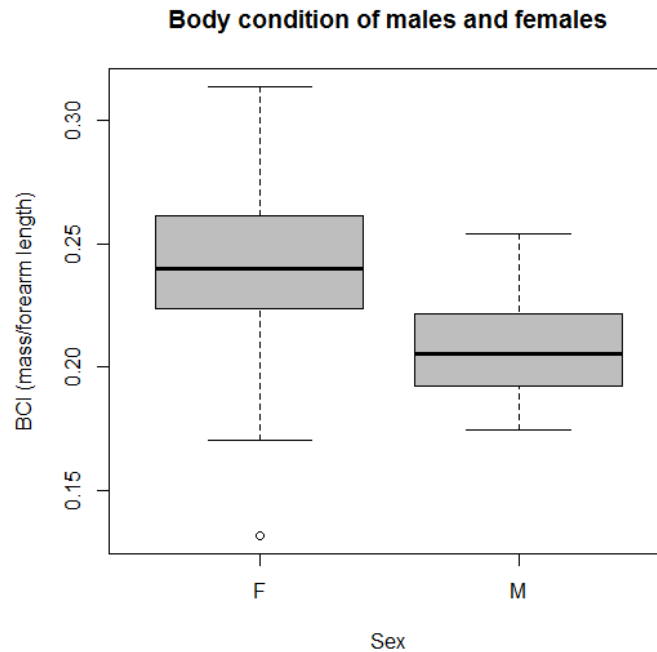
Data were entered into a CSV file. Using *R* (<http://www.r-project.org>), the Bindings for the Geospatial Data Abstraction Library (*rgdal*) package was installed. A custom *R* script was used to 1) convert latitude and longitude (WGS84) coordinates to UTM coordinates, 2) convert degrees to radians, 3) create a line segment from each observer location at the appropriate bearing, and 4) calculate the intersection point of the two lines. The estimated locations were then exported to a new CSV file and imported as a layer into QGIS Desktop (<http://www.qgis.org>). These points were viewed on top of satellite imagery imported via the Python console. Using QGIS, a convex hull enclosing the set of points was created as well as distances and areas calculated. The heatmap plugin for QGIS allowed for generation of a graphical representation of kernel density estimation for all bat detection points. While not quite as intuitive and user-friendly as some other methods, this allowed for similar results without additional costs or investment (both QGIS and *R* are free, open source programs available on a variety of platforms).

### *Pd surveys*

Surveys were done for the presence of *Pseudogymnoascus destructans*, the causal fungal agent of white-nose syndrome because of its recent spread into the state and also because its growth is highly regulated by temperature (Verant et al. 2012). Sterile swabs were dampened with distilled water and used to swab a variety of surfaces and substrates in and around the main bat roost building. After sample collection, swabs were stored in sterile plastic containers and transported back to the laboratory. Once in the laboratory, samples were streak-plated directly onto plates containing Sabouraud dextrose agar. Plates were sealed with plastic paraffin film (Parafilm®) and incubated inverted in the dark at approximately 10°C for two weeks (as in Puechmaille 2011). Plates were visually inspected for fungal growth and some samples observed microscopically.

## Results

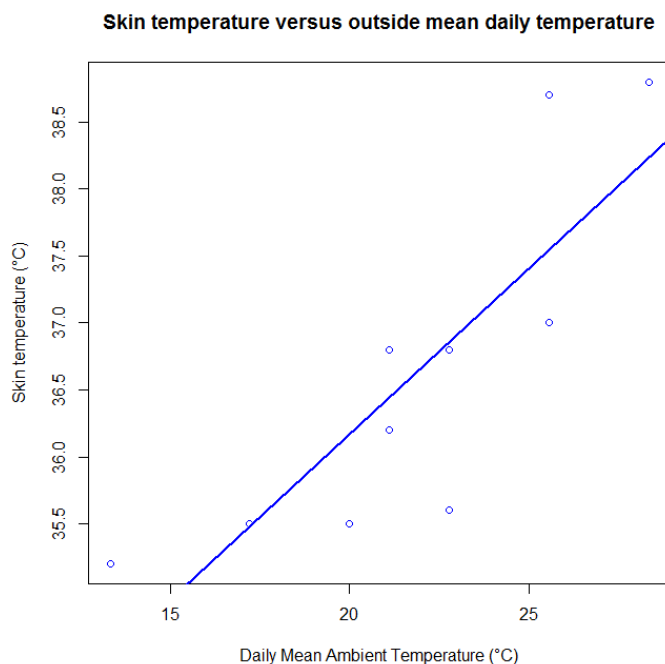
I made 109 captures and banded 79 unique individuals (sex ratio of females:males=3.3:1). For males (n=19), mass averaged  $8.9\pm 0.87\text{g}$ . Male forearm length average was  $42.95\pm 1.5\text{mm}$ . For females (n=60), mass averaged  $10.5\pm 1.42\text{g}$ . Average female forearm length was  $43.60\pm 1.35\text{mm}$ . Body condition indices between males and females were different (Welch's t-test  $t=4.99$ ,  $df=41$ ,  $p<0.01$ ; Figure 11). Body condition indices (mass/forearm length) of both sexes combined were normally distributed (Shapiro-Wilk  $W = 0.989$ ,  $p=0.73$ ). Most measurements (89%) were taken between the months of April and July with the remaining taken through early October.



**Figure 11.** Box-whisker plot of body condition indices (mass/forearm length) for females (n=60) and males (n=19).



Using a thermocouple probe (model HH23, Omega Engineering, Stamford, CT, USA) and type T thermocouple wire, non-torpid abdominal skin temperatures were recorded for some bats (n=10) in-hand during biometric data collection at the main roost site. Abdominal skin temperatures ranged from 35.2°C to 38.7°C. Mean skin temperature for males (n=4) was 36.02°C (s=0.80) and 37.03°C (s=1.44) for females (n=6). Averages for males and females were not significantly different (Welch's unequal variances t-test,  $t(8) = 1.49$ ,  $p=0.17$ ). When sexes were combined, skin temperature readings were positively correlated with outside mean daily temperature (Spearman's  $\rho=0.93$ ,  $n=10$ ,  $p<0.01$ ; Figure 12).



**Figure 12.** Correlation of skin temperatures (both sexes combined) with daily mean ambient temperature.

### *Radio tracking*

Ten transmitters were attached to bats. Tracking attempts took place over approximately 20 nights from June through September 2014. Tracking was conducted with 1-3 bats simultaneously. On several nights, bats tagged with radio transmitters either: could not be

located at all, did not leave the roost, or could not be re-located after leaving the roost. A total of 96 telemetry points judged to be reliable (e.g., not >0.5km from either receiver) were calculated from six bats. Three transmitters apparently malfunctioned (e.g., battery died prematurely), became detached from bats and could not be recovered, or the bats left the area and did not return with functional transmitters.

Fixed kernel home range estimates (95% and 50%) using least square cross-validation (Johnson 2012) were calculated in Biotas (Ecological Software Solutions LLC) for animals which had >20 detection points (Table 4). Other animals' detection points were used only in the overall heatmap.

**Table 4.** Mean home range estimates for bats where >20 detections were made (n=3). The first male had the largest number of detections (n=35).

Bat	95% kernel home range (ha)	50% kernel home range (ha)
UMM0048	37.37	4.67
UMM0009	13.37	4.08
UMM0045	2.20	0.12

**Table 5.** Band identification numbers, sex, and number of detections for each of the six bats tracked. Repeated attempts were made over several nights to locate each tagged bat. Home ranges were calculated for bats denoted by an asterisk.

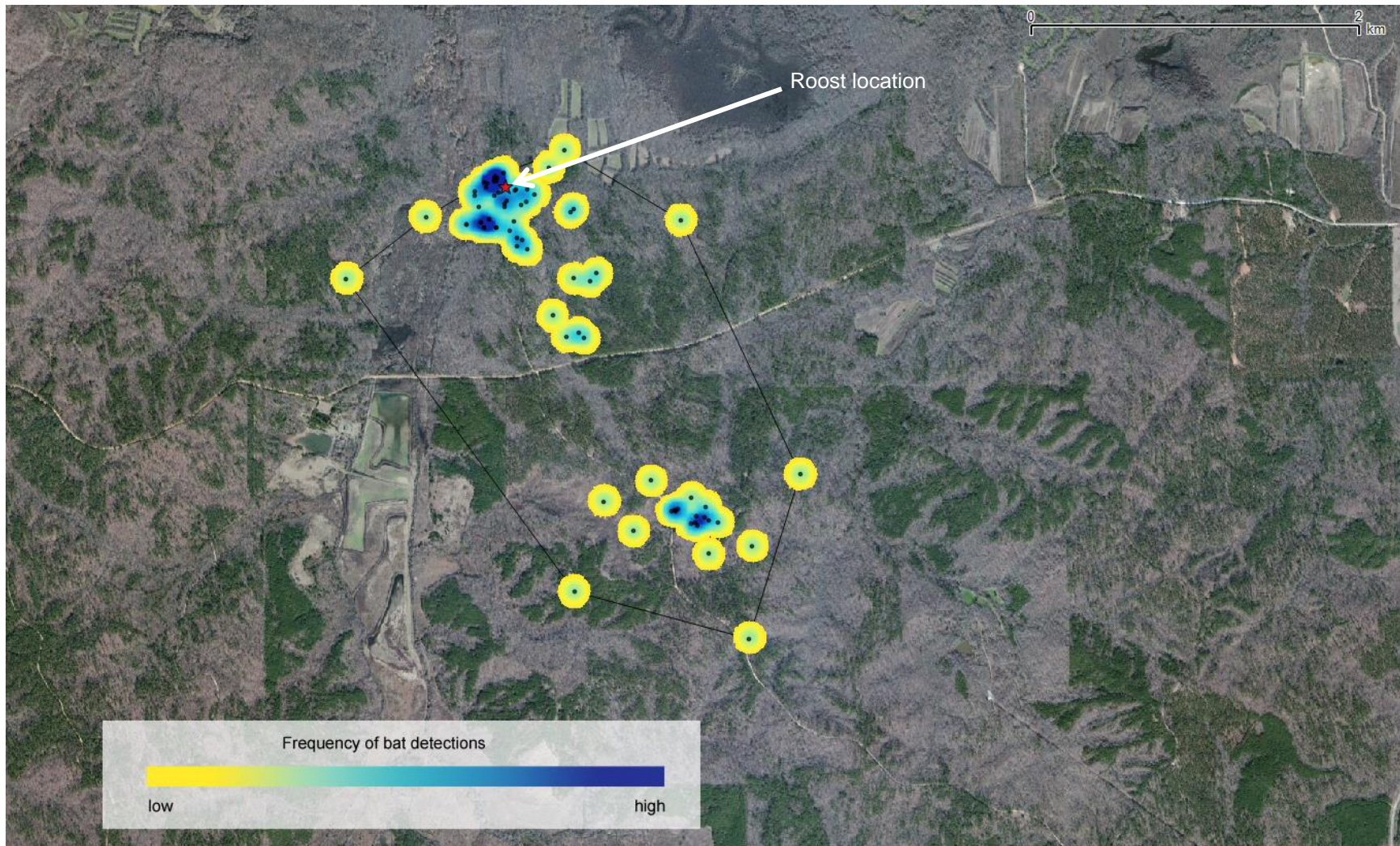
Bat band ID	Sex	Number of successful detections (nights)
UMM0048	M	35* (6)
UMM0045	M	23* (3)
UMM0009	M	22* (1)
UMF0025	F	8 (1)
UMM0002	M	5 (2)
UMF0045	F	3 (1)
UMM0049	M	0
UMF0026	F	0
UMF0046	F	0
UMF0048	F	0

As seen in Figure 13, most nighttime bat activity appeared to be within 2km or less of the roost in a south-southeastern direction. Despite repeated attempts to locate areas that would appear favorable to foraging or roosting for *C. rafinesquii* (e.g., fields, sparse woodlands,

abandoned structures), few detections were made greater than ~1km from the roost. Outside of the immediate roost vicinity, substantial activity was documented in a small area approximately 2km south-southeast of the roost. Finally, two females were observed traveling from the roost in a northeastern direction (i.e., signals disappeared in that direction; towards the agricultural fields and wetland areas northeast roost site), but these bats were not detected again outside of the roost despite repeated attempts.

#### *Pseudogymnoascus destructans* survey

After two weeks of incubation (at 10°C) on Sabouraud dextrose agar, 12 swab samples taken from walls inside the roost (and other structures where bats were observed roosting) showed sparse growth of an unidentified psychrophilic bacteria species. No fungal growth was observed, and there was no indication of *Pseudogymnoascus destructans*, the causal agent of white-nose syndrome. No signs of infection were observed during 109 captures of *C. rafinesquii*. One male southeastern myotis (*Myotis austroriparius*) was also captured inside the roost; likewise, no signs of WNS infection were present on this individual. One dead female *C. rafinesquii* was documented hanging from the ceiling in February 2014, but the body was observed closely and no indications of WNS were present. No obvious trauma or emaciation was observed on this dead individual.



**Figure 13.** Satellite image of the area around the main roost building (indicated by red star) showing detected locations for all tracked bats. A scale bar is located in the top right and a legend identifying frequency of bat transmitter signals detected with confidence is in the bottom left. Yellow indicates few detection(s) and blue indicates a relatively heavy concentration of detections. The black line surrounding the points is a minimum convex polygon (MCP) that encloses all of the detection points. The area of the MCP is approximately  $4.7\text{km}^2$ .

## Discussion

My results indicated a small degree sexual dimorphism as reported by others (e.g., Johnson 2012; Trousdale 2008) for *C. rafinesquii*. Females had 118% larger masses on average, although some of these measurements likely included pregnant individuals. More importantly, males were relatively rare in my study (just 24% of banded individuals were male). It could well be that other, differently-sized males roosted elsewhere and were not documented. Both the largest and smallest individuals were females (5g and 14g). The small females (<6g; n=3) were captured in early April. Their low masses may have been a result of recent awakening from hibernation. For the largest females (>12g; n=9), 89% were captured in May and likely had high masses due to late stage pregnancy.

Bat abdominal skin temperatures (taken during handling) were higher for females than males, but low sample size (and varying times of the year) impeded interpretation of this observation. I recommend further investigation of sex differences in skin temperature as this may be a useful tool for "pregnancy testing" bats. Higher skin temperatures are associated with pregnancy in human females (Butterworth et al. 1990).

It seems obvious that bats prefer roosting in rooms that experience fewer fluctuations in temperature (this is likely one of the main reasons bats frequently roost in caves). However, air flow and humidity levels may also play a role in choosing a roost location, particularly because airflow affects convective heat loss and air humidity affects evaporative heat loss.

Radio tracking yielded important information on summer and fall foraging locations. The number of successfully tracked bats was low, so these locations may not be highly representative of all bats' foraging locations. Nevertheless, bats appeared to forage frequently within 1km of the roost. They did not appear to forage frequently near fields or roads. Investigations into more remote foraging locations, particularly in the northeastern direction from the roost building, should be made because a few female bats appeared to move in that direction and their transmitters were rarely, if ever, detected again.

It should be noted that the number of bat detections in this study was low. Despite reports that small sample sizes in kernel density estimates overestimate animals' home range sizes (Seaman and Powell 1996), the home ranges indicated here are smaller than other studies have reported from male *C. rafinesquii*. Hurst and Lacki (1999) reported a mean foraging area size of  $160.62 \pm 66.45$  ha in Kentucky for three females and two males. Menzel et al. (2001) calculated a mean 95% kernel home range size of 93.15 ha for four males in the coastal plain region of South Carolina (only the mean for all bats was reported). Mean 95% kernel home range size in my study was  $17.65 \pm 17.97$  ha.

At my study site, it has been observed that most bats leave the main roost in this study during the coldest months of the year; this represents a lack of information integral for conservation measures. Furthermore, home range size of bats has been found to vary both with seasonality (de Jong 1994) and, for other mammals, with habitat type (Lucherini and Lovari 1996). These results indicate that bats are using areas near the roost as foraging grounds, but anecdotal evidence (e.g., females often foraging out of range and being difficult to detect again) suggest that areas outside of these home ranges are likely used as well.

Very few nighttime radio telemetry studies have been performed on this species (Johnson and Lacki 2011) and all previous ones have been in substantially different landscapes. It remains to be seen whether these small observed home range sizes are due to biased sampling or if this population covers less area due to some ecological difference from previously studied populations.

Preliminary investigations of other structures (aside from those in the immediate vicinity of the main building) did not uncover new roost locations. It is likely that many males of this population rarely visit the main roost building. It remains unknown where these individuals roost as well as where most of the population roosts during the winter months. Future radio telemetry studies on this population should focus on late fall movements. Given that they may travel several kilometers, autonomous telemetry stations established throughout the area may be helpful for narrowing down alternative roost locations.

No signs of *Pseudogymnoascus destructans* or white-nose syndrome were detected. However, the fungus was recently discovered in Mississippi (US Fish and Wildlife Service 2015). I strongly encourage all researchers at this site to take common sense precautions: clean boots before entering the roost building, handle bats with disposable gloves, and routinely disinfect any equipment which comes into contact with bats (US Fish and Wildlife Service 2015).

Other interesting observations I made included the presence of a wood rat and rattlesnake inside the main building (see Appendix A). Particularly intriguing was the discovery of one southeastern myotis (*Myotis austroriparius*) male roosting with RBEBs (sometimes even in the same cluster). I did not band this individual, but believe that he is a longtime resident.

## CHAPTER IV: DETECTION AND CENSUS BIAS IN FOREST BATS LIVING IN AN ANTHROPOGENIC ROOST

### Introduction

Accurate knowledge of species distribution and abundance is important for conservation. Such information can also help us predict the emergence and spread of zoonotic diseases (Ostfeld and Holt 2004; Keith et al. 2015). Pseudoabsence, the failure to locate a species at a geographic location even though it is actually present, is a serious issue that can hinder ecological understanding and thus impair sound management decisions (Rout et al. 2010). The issue of pseudoabsences is why the terminology ‘detection-nondetection’ is often preferred over ‘presence-absence’ (MacKenzie 2005). Detection-nondetection data can be difficult to interpret because animal detectability is not always constant over time or space. Not surprisingly, detectability can be different between species (e.g., Schieck 1997; Mazerolle et al. 2007), but differences in detectability can also exist between and within individuals of a species over time (Vojta 2005). For example, detectability of horned puffins (*Fratercula corniculata*) at their nesting burrows varied greatly both within and between days (Harding et al. 2005).

Detectability of a species can vary across habitat types, and this problem is often species-specific (Boulinier et al. 1998). Detectability can vary among human observers as well (Diefenbach et al. 2003). Repeated visits for increasing detectability are generally recommended (Geissler and Fuller 1987; Azuma et al. 1990). A distinct but related problem of detectability is the measurement of species abundance. Intuitively, abundance of a species should influence its



detectability (McCarthy et al. 2013; Royle and Nichols 2003). In essence, species that are low in numbers can be difficult to detect, and even species that are abundant can be hard to count if they are hard to detect.

Reliable counts are necessary for making accurate inferences about population trends over time. Harding et al. (2005) recognized that detectability of horned puffins varied across location, time of day, and time of year which significantly impeded their ability to gauge overall population numbers from year to year.

Pseudoabsences and related difficulties with population estimation are particularly problematical for species that are small, rare, cryptic, or nocturnal. Small mammals, such as the 1.8g Etruscan shrew (*Suncus etruscus*) may not trigger traditional small mammal traps which can result in pseudoabsences (Vogel 2012). Throughout much of its range, the endangered saproxylic beetle (*Osmoderma eremita*) is rare and may not be detected unless an array of surveying techniques is used (Chiari et al. 2012). Many animals live in places where they are difficult to observe, such as tiger salamanders (*Ambystoma californiense*) that spend 95% of their post-metamorphic lives underground (Searcy and Shaffer 2014). If animals are difficult to see, such as nocturnal slow lorises (*Nycticebus* spp.) in Borneo, extended survey times may be required (Nekaris et al. 2008). Rafinesque's big-eared bats can be hard to see in their dark roost sites, so count accuracy is improved by the use of multiple observers (Fleming et al. 2013).

Bats exhibit many or all of the characteristics that make detection difficult. They are among the smallest mammals in the United States with most of the 47 species weighing < 20g. Bat species are often difficult to locate due to camouflage (Mormann and Robbins 2007) and inconspicuous roost sites (Weller et al. 2009). Lastly, temperate bat species are predominantly

nocturnal, have vocalizations outside the range of human hearing, and are volant and can evade observers easily (Gorresen et al. 2008; O'Farrell and Gannon 1999).

The focal species of the present study, the Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), is a small, insectivorous bat that occurs in the southeastern United States and is of conservation concern in most of its range. Both the full extent of its distribution and its abundance are not well-known (Lacki and Bayless 2013). The species is reported to be difficult to survey (Clement and Castleberry 2011). Historically, this species roosted in cavities in old-growth bottomland forests, but deforestation has eliminated the majority of these forests (Kress et al. 1996). When caves are available, Rafinesque's big-eared bats may use them for roosting (Johnson et al. 2012). In parts of the species' range where large roosting trees ( $\geq 100$ cm DBH; Lucas et al. 2015) and caves are not readily available, *C. rafinesquii* may use abandoned buildings and the undersides of bridges for roosting (Trousdale et al. 2008; Martin et al. 2011).

Systematic surveys of this species' roosts are uncommon and census methodology has not been standardized. Rafinesque's big-eared bats have been surveyed under bridges in southern Mississippi where occupancy peaked in the late spring and early summer (Trousdale and Beckett 2005). These authors surveyed 90 bridges from March-June. If at least one *C. rafinesquii* was detected on the first visit, bridges were checked again every 2-4 weeks for approximately half the year over a three year period, with a 7 month window where no surveys were done. In a sandstone cave in Kentucky, *C. rafinesquii* numbers also appeared to peak in summer, although emergence counts were done only 9 times over a four year period and one year had no counts (Hurst and Lacki 1999). Fleming et al. (2013) examined probability of detection and accuracy of counts for *C. rafinesquii* roosting in trees by checking tree cavities twice in winter and twice in spring/early summer of the same year. Fleming et al. found that both detection and count

accuracy increased when the first observer's counts were repeated by an additional observer, and they made use of infrared cameras to document bats. Finally, these authors reported that detection can vary throughout parts of a roost (i.e., some parts of a tree cavity may be more readily observed than other parts). In this study, I examine: 1) the times of day and season that are best for detecting and counting these bats; 2) the number of times surveys should be repeated at a potential roost site to detect bat presence; and 3) how often this type of roost needs to be surveyed to provide accurate colony counts.

Anthropogenic roosts may be more accessible to surveyors and have more room for bats than tree roosts, but little is known about how *C. rafinesquii* uses them or how wildlife managers should survey them reliably. My objective is to assess detection probability and variability of bat numbers at a building roost to inform the design of quantitative sampling surveys for this species at similar sites. Repeated surveys decrease the likelihood of reporting pseudoabsences and increase the likelihood of getting accurate counts (Azuma et al. 1990; Nichols et al. 2000). The results of this study will inform surveying techniques to make efficient use of researchers' resources. Finally, these results will facilitate data collection on roost usage and population trends for this uncommon bat species in a frequent yet little studied roost-type.

## Methods

I monitored a colony of Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) roosting in a partially collapsed, one-story building made of concrete blocks located on an inholding in the Holly Springs National Forest in north-central Mississippi, USA (34°30'N, 89°21'W). The building is approximately 13x10x2.4 m (length x width x height) and contains an entryway and five rooms. The site around the building is mixed pine-hardwood forest with hilly terrain and consists mostly of secondary growth with few trees of adequate size for roosting. In 2013 and 2014, 79 bats at this site were banded thus giving a baseline number to compare to video estimates.

Four infrared video cameras (IRCAM-BW models; Polaroid Industries, Minnetonka, MN, USA) connected to a security DVR (K808AV500GB model; CIB Security, Sunnyvale, CA, USA) recorded bat activity and behavior inside the roost during January - September 2014. Cameras were placed only in rooms which were seen to be used by bats, and each camera was mounted to one wall and recorded approximately the same area in its field of view (~4m<sup>2</sup>) on an opposite wall. Video was recorded in four, one-hour intervals (06:00, 12:00, 18:00, and 24:00) every 6 days. Measures of bat occupancy and presence/absence were taken from these videos. To get the total within the roost, the numbers of bats on all 4 cameras for each time period were summed. If no observations of bats were made on any camera for the time period, this was documented as a nondetection (“absence”). For analysis, observations were split into 4 seasons: winter (January - early March), early summer (late May - mid June), late summer (late June - end of July), and fall (August - September) based on the bat natural history expected at these times

(intermittent hibernation, parturition and pup rearing, pup weaning, and mating period, respectively). Recordings were sampled every sixth day to avoid autocorrelation to temperature effects (Chapter II).

Detection/nondetection of bats and number of bats were taken from one time period (18:00) for all seasons except winter and resampled with replacement 500 times using a spreadsheet in Microsoft Excel. This was done with increased sampling size to represent additional visits/surveys. Using these resamples, I estimated the minimum number of times necessary to achieve a 90% success rate at surveying the roost to detect bat presence, to count at least the highest number seen on video, and the annual mean number. Times when no bats were detected were omitted when resampling for how many visits were needed to estimate maximum (70) and annual mean (23) population size. Because of unequal variances, Welch's one-way ANOVA was used to test for differences in numbers of bats present over these seasons and four times of day, and Games-Howell post-hoc tests were performed to check for significant differences. Chi-squared tests were performed to differentiate between probability of detection across seasons and times of day.

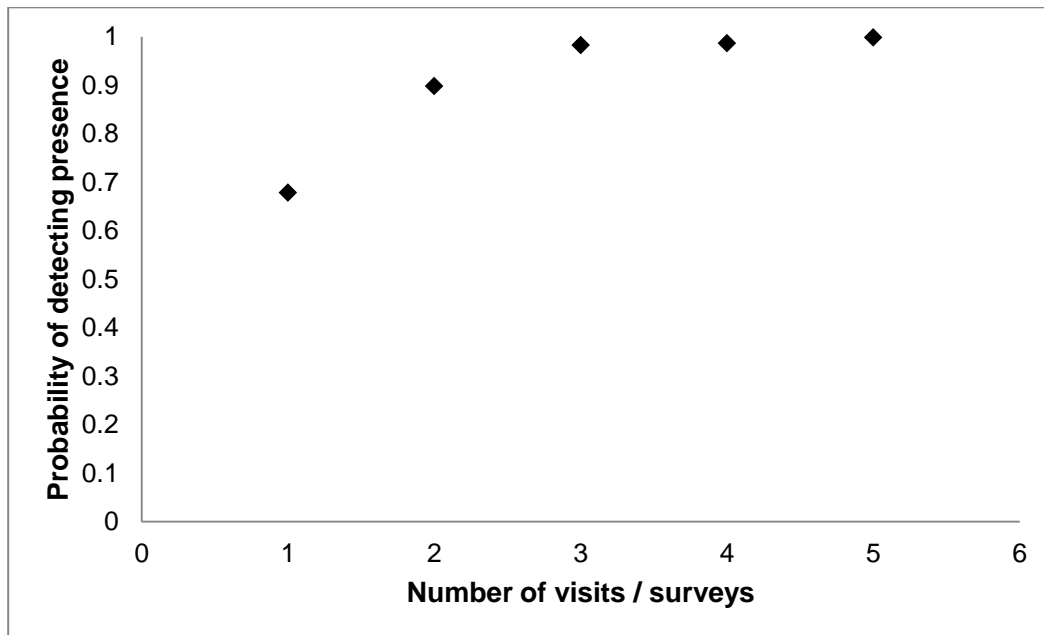
To analyze the effect of movement of bats within the roost building on surveying results, I used bat numbers from 18:00 from the entire study period. Daily bat numbers from the four video areas were randomly resampled without replacement one, two, and three times in order to calculate the probability of detecting the actual roost-wide total number of bats on each day when searching less area than the entire roost.

## Results

In total, 33 days were sampled from January 1 - September 20, 2014. Over this period, ambient temperatures ranged from -17°C to 36°C.

### *Detection*

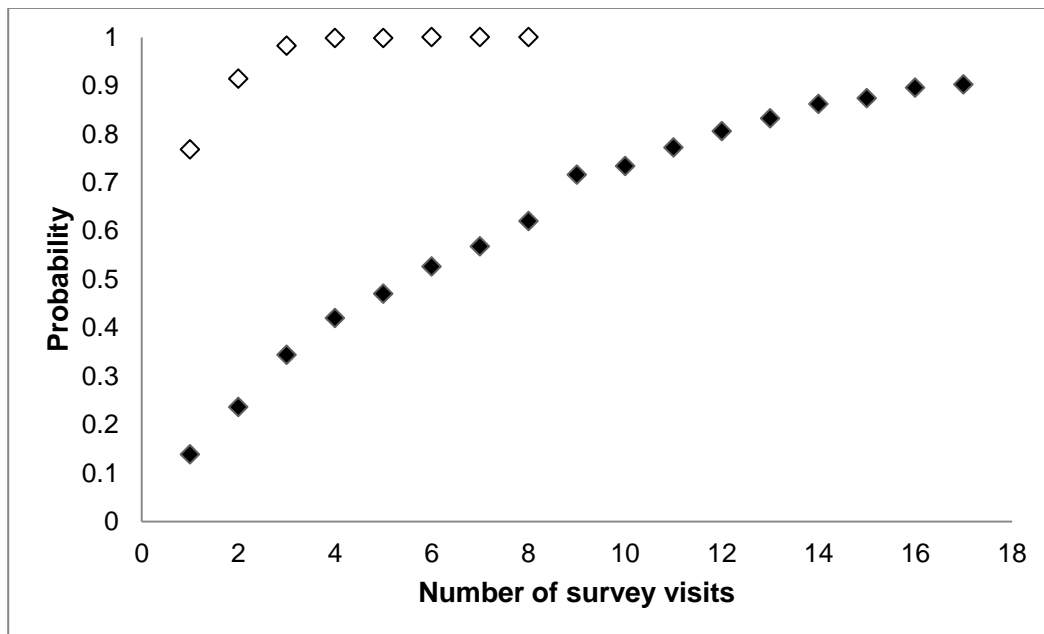
Successful detection of bats was randomly distributed across seasons ( $\chi^2=2.87$ ,  $n=140$ ,  $p=0.41$ ) and times of day ( $\chi^2=1.01$ ,  $n=140$ ,  $p=0.80$ ). The likelihood of bat detection for each season was winter (64%), early summer (71%), late summer (69%), and fall (82%). The likelihood of bat detection for each time of day was 06:00 (66%), 12:00 (60%), 18:00 (71%), and 24:00 (66%). One survey visit (at any time and season) yielded a detection likelihood of 68%, 90% for two visits, and subsequent visits approached a 99% likelihood of detection (Figure 14).



**Figure 14.** Using observations from all times of day and all seasons, the number of surveys required to detect the presence of bats at the roost site was low. Only a few (2-3) visits were required to have a 90% probability of detecting bats.

### Counts

Many more visits were required to estimate population size. If one considers a sampling regime with the highest chance of success, that is ignoring non-detection days, sampling at 18:00 only, and excluding winter when numbers were lowest, the number of survey visits required to have a 90% chance of detecting the maximum number was 15-16 visits (Figure 15). However, only 2-3 visits are required to observe the annual mean in bat occupancy (~23) at this site under the same optimal sampling conditions (Figure 15).



**Figure 15.** With targeted surveys (excluding winter, searching only at 18:00), the number of visits required to observe the approximate maximum number was high (solid points). Fewer visits were necessary to see the yearly mean number of bats (outlined points).

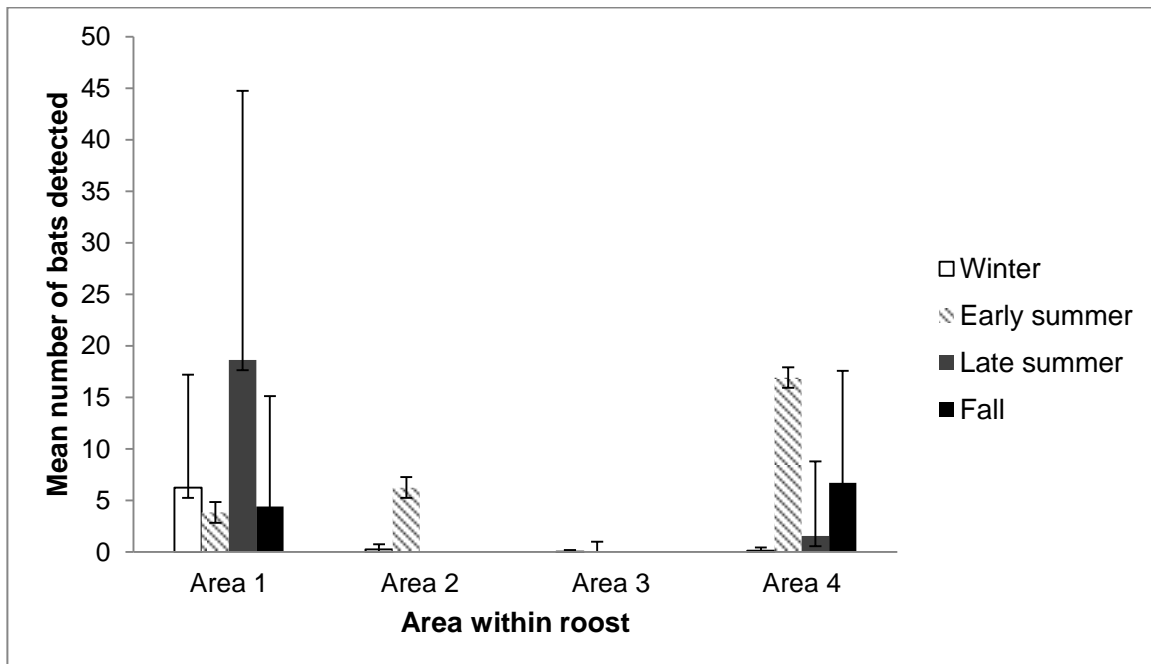
Bat counts did not differ through the day in winter ( $F_{3,17.25}=0.84$ ,  $p=0.49$ ), but were different over the course of the day in early summer ( $F_{3,13}=11.04$ ,  $p<0.01$ ) and late summer ( $F_{3,18}=4.1$ ,  $p=0.02$ ). Post-hoc Games-Howell tests indicated significant differences between 24:00 and 18:00 ( $p<0.01$ ) for early summer and between 24:00 and 12:00 ( $p<0.01$ ) for late summer. Bat counts were highest on average in early summer at 06:00, although observations of

the maximum number of bats were most frequent at 18:00 in early and late summer. Numbers were typically lowest at 24:00, except in winter when there was little variation. Means are reported in Table 6.

**Table 6.** Significant differences in number of bats detected between times of day (within a row,  $\pm$  standard deviation) are indicated by different letters (Games-Howell,  $p \leq 0.05$ ) where the ranking of letters corresponds to the ranking of means.

	06:00	12:00	18:00	24:00
<b>Entire study</b>	17.56 <sup>ab</sup> $\pm$ 22.52	17.03 <sup>ab</sup> $\pm$ 21.94	18.88 <sup>a</sup> $\pm$ 22.69	5.81 <sup>b</sup> $\pm$ 11.18
<b>Winter</b>	4.00 <sup>a</sup> $\pm$ 6.48	7.55 <sup>a</sup> $\pm$ 12.52	7.09 <sup>a</sup> $\pm$ 12.14	7.90 <sup>a</sup> $\pm$ 12.30
<b>Early summer</b>	41.67 <sup>a</sup> $\pm$ 20.41	28.67 <sup>ab</sup> $\pm$ 24.39	36.67 <sup>ab</sup> $\pm$ 29.44	1.00 <sup>b</sup> $\pm$ 1.10
<b>Late summer</b>	25.63 <sup>a</sup> $\pm$ 29.21	22.5 <sup>a</sup> $\pm$ 31.51	25.13 <sup>a</sup> $\pm$ 26.59	7.5 <sup>a</sup> $\pm$ 13.49
<b>Fall</b>	15.00 <sup>a</sup> $\pm$ 10.79	27.71 <sup>a</sup> $\pm$ 15.12	33.00 <sup>a</sup> $\pm$ 15.00	4.71 <sup>b</sup> $\pm$ 12.03

Bat numbers also varied widely throughout areas of the roost. For example, mean number of bats peaked in area 1 in late summer, but peaked in area 4 in early summer (Figure 16). The probability of counting the total number of bats increased as search areas inside the roost increased. By randomly resampling from the four cameras each day (18:00 only), I found that the probability of detecting the actual total number increased as more cameras (1-3) were included: 18%, 41%, and 64%, respectively.



**Figure 16.** Mean number of bats observed in different areas of the roost across seasons. Error bars demonstrate standard deviation around the mean.



## **Discussion**

The ability of researchers to locate species is of paramount importance in ecology and conservation. If researchers ignore imperfect detection and counts, this could lead to sub-optimal or potentially even harmful management decisions (MacKenzie 2005). The idea behind surveying for species diversity and detecting/counting a single species is somewhat similar: more surveying reduces the probability of pseudoabsence and count biases. For example, increasing the intensity of surveys as well as using multiple modes of surveying (e.g., acoustic detection plus trapping) has been shown to increase detections of rare species in Australian forest bats (Mills et al. 1996). Detection bias is even an issue for researchers seeking to quantify the magnitude of bat deaths at wind turbines (Korner-Nievergelt et al. 2011).

Fleming et al. (2013) examined probability of detection and reported that with one observer surveying the same tree cavity twice the probability of bat detection was 95%. These authors also highlight an important point: observations can be biased if the researcher knows about the previous state of the roost. That is, an observer might be more likely to not detect a bat if he or she has previously not detected a bat there or vice versa. However, this problem should be minimal in building roosts where counting individuals may be easier than in tree roosts.

Trousdale and Beckett (2005) found that both detection and counts of Rafinesque's big-eared bats under bridges in southern Mississippi varied throughout the year. They reported that bridges were always surveyed "during daylight hours" but did not appear to analyze numbers or detection probability over time of day. They also found that counts peaked in late spring/early summer which roughly coincides with my findings.

Fleming et al. (2013) reported that bats may occupy parts of tree cavities that are not known to the researcher or that are unable to be surveyed. While inaccessibility may be less common for building roosts, thorough surveys are still a necessity. For example, bats may be hidden inside of concrete blocks if those cavities are available, or bats may retreat into tiny crevices when disturbed (personal observation). I reported that the number of bats varied throughout areas of the roost. Surveying only parts of the roost could easily mislead wildlife managers about actual occupancy. Roost switching between bridges is well-known in bats and has been documented previously in *C. rafinesquii* (Trousdale and Beckett 2005), therefore, it may be unsurprising that bats often move within a large roost such as an abandoned building. The causes of such movements are unknown and worthy of further investigation.

I suggest that 2-3 thorough surveys for buildings that are potential roosts are sufficient for detection. Tree cavities may require more surveys for detection if they are actually used by bats, both because of their greater difficulty of surveying and because bats may switch between tree roosts more frequently than in anthropogenic roosts (Brigham 1991; Trousdale and Beckett 2005).

Three of the four largest known roost sites for Rafinesque's big-eared bats in Mississippi are located in abandoned buildings in upland pine forests (Martin et al. 2011). It is worth noting that my study's roost building is apparently the only known colony of *C. rafinesquii* in a large, mostly rural county with a land area of 1,637 km<sup>2</sup>. Due to fluctuating occupancy levels throughout the year, it is likely that *C. rafinesquii* using abandoned buildings may rely on natural roosts (or other, undiscovered anthropogenic roosts) as well. Because of an absence of caves, buildings may be especially important roosting sites in Mississippi, but they are also used in other parts of the species' range where roosting alternatives exist. For example, in Mammoth

Cave National Park, over 1,000 Rafinesque's big-eared bats are known to hibernate within the park in at least 6 caves. However, they also are found in trees, sandstone outcrops, and abandoned buildings (Johnson et al. 2012). In essence, buildings may be important throughout the range, but especially so in areas where alternatives are substantially limited. So while detection and count probabilities may differ between roost types, even methods suitable only for building roosts would be beneficial for accurately surveying this uncommon species. Roost selection in *C. rafinesquii* is likely to be influenced by both temperature (Hurst and Lacki 1999) and habitat features (Trousdale and Beckett 2005). Scientific investigation of how and why roosts are chosen certainly warrants further consideration, especially for building roosts.

Targeted and repeated surveys will be required to discover accurate information about the species' distribution, particularly in areas that have not been well-surveyed, such as north-central Mississippi (Martin et al. 2011). While some areas of the state do contain tree roosts and surveys for new tree roosts are needed, current knowledge suggests that anthropogenic roosts are particularly important for the species in Mississippi. This is likely primarily because large portions of forested wetlands in Mississippi have been lost or severely fragmented (Martin et al. 2011). Roosts in abandoned buildings may be important for many decades to come as large roosting trees are given time to regenerate. Throughout the species' range, future surveys should investigate such buildings for *C. rafinesquii* roost sites. Due to costs and landowner liability, incentives may be necessary to preserve abandoned buildings.

Surveys of bridges, abandoned buildings, and other anthropogenic structures that are potential roost sites should be made repeatedly and as thoroughly as possible as bats can move throughout the roost. If the goal is to detect the presence of bats, a handful of visits will suffice. However, multiple surveys during warmer months and in midday or late afternoon may be

required to get accurate counts of individuals. Numbers of bats can vary between both seasons and years. While these surveys can be challenging due to time constraints or difficulty in accessibility, they typically do not require special equipment or training. Repeated surveys of anthropogenic structures with detailed record-keeping could be kept in a curated database for monitoring and protecting the Rafinesque's big-eared bat. Finally, if a few surveys of a potential roost structure do not detect bats, it may be prudent to remove this site from routine monitoring. Timed photography or infrared video can also be used in applicable scenarios to reduce the necessary time and effort required to sufficiently survey a site. Resampling these data was informative about surveying the variation in bat presence and abundance at this study site. Similar sampling should be done at other building roosts to see if results from this study site are applicable to other sites.

## CHAPTER V: CONCLUSIONS AND FUTURE CONSIDERATIONS

Relatively little is known about this species (see Chapter III: Introduction). Research is increasing on this species, but a search of the Scopus database found only 26 studies on various aspects of *C. rafinesquii* published in the last 10 years. Studies of *C. rafinesquii* in both Mississippi and roosting in buildings are rare (Table 7).

**Table 7.** I searched the Scopus database for studies of *C. rafinesquii* within the last ten years. While roost selection and conditions are the subject of several of the studies, few focused on anthropogenic roosts.

<b>Topic</b>	<b>Location</b>	<b>Reference</b>
Basic monitoring	Louisiana	Ferarra and Leberg 2005
Dietary	Kentucky	Johnson and Lacki 2013
Dietary	Louisiana	Gregory et al. 2014
Genetics	various	Lack and Bussche 2009
Genetics	various	Piaggio et al. 2011
Genetics	various	Lee et al. 2012
Genetics	various	Meganathan et al. 2012
Habitat use	Arkansas	Medlin and Risch 2008
Landscape population estimation	Georgia	Clement and Castleberry 2013a
Observational report	Mississippi	Wolters and Martin 2011
Parasites	Arkansas	McAllister et al. 2005
Parasites	Georgia	Crossley and Clement 2015
Response to acoustic playback	South Carolina	Loeb and Britzke 2010
Roosting behavior/conditions (bridges)	Louisiana	Ferrara and Leberg 2005b
Roost selection/conditions (bridges)	Mississippi	Trousdale and Beckett 2005
Roost selection/conditions (trees)	Tennessee	Carver and Ashley 2008
Roost selection/conditions (bridges and trees)	South Carolina	Bennett et al. 2008
Roost selection/conditions (trees)	Georgia	Clement and Castleberry 2013b
Roost selection/conditions (trees)	Georgia	Clement and Castleberry 2013c
Roost selection/conditions (trees)	South Carolina	Lucas et al. 2015
Roost selection/conditions (trees)	Kentucky	Johnson and Lacki 2013
Roost selection/conditions (trees)	Kentucky	Johnson and Lacki 2014
Social networks	Kentucky	Johnson et al. 2012
Detection and count error (trees)	Mississippi	Fleming et al. 2013
White-nose monitoring	Tennessee	Bernard et al. 2015
Winter behavior (various roost types)	Kentucky	Johnson et al. 2012b

The population at this roost is >79, likely approaching 100 individuals. My research clearly shows slight sexual size dimorphism (females being larger and heavier), although it

should be noted that were less common at the site. Preliminary investigations into *C. rafinesquii* skin temperatures suggest a possible difference between the sexes which warrants future attention. Temperatures varied considerably inside the roost (presumably offering up a wide array of microclimates for bats to choose from). In all seasons, the roost building offers substantial buffering against fluctuating outside temperatures. Documenting thermal conditions of used rooms versus unused rooms should provide insight into repairing structures used by this species (e.g., the 'Wandering Woods' site at Mammoth Cave National Park, USA) or building structures designed to attract this species (e.g., bat towers).

Radio tracking in this study was arduous and did not provide the quality of data that I had hoped. However, it did shed some light on summer/fall foraging locations. The majority of foraging was in the immediate vicinity of the roost. Future acoustic studies and/or investigations into lepidopteran abundances in the area may provide useful context for these observations.

## **Future considerations and recommendations**

Bats are difficult to study (Burland and Wilmer 2001; Kunz and Fenton 2003; Trimboli 2015). They are nocturnal, volant, small, easily disturbed, and relatively fragile. Many species are found in large numbers only in remote locations. With that in mind, I would like to offer some insight and thoughts on what I might recommend for future studies (both in terms of topics and methods).

### *Roost behavior monitoring*

Using higher resolution cameras, some type of computer-driven analysis might be feasible. Thermal imaging (still or video) would be illuminating but is currently cost-prohibitive. With the current system, clarity is suboptimal. Differentiating between species is difficult (on a few occasions, one *Myotis austroriparius* was observed in the building). In this particular system, that was not a major source of concern, but interpreting more complex behaviors is also difficult because of low visibility.

Another possibility would be to deploy ultrasonic microphones in the roost and use software to analyze frequency and/or amplitude and use those measurements as indicators of bat activity. Devices that might be ideal for this application include the Dodotronic (<http://www.dodotronic.com>, Italy) ultrasonic USB microphones (e.g., ULTRAMIC192K) or a dedicated acoustic recording device by Wildlife Acoustics (e.g., Song Meter SM3BAT recorder; <http://www.wildlifeacoustics.com>, Massachusetts, USA). Of course, much context that is available on video is lost with sound recordings, but analysis should still be useful (in fact,

videos used in my study did not record sound). Analysis of sound only could also be less labor-intensive. One major advantage would be the absence of a limited field-of-view as on cameras. For example, instead of assuming that cameras are a representative sample of bat activity, sensitive ultrasonic microphones should capture sound from bats throughout a roost.

One other desirable yet expensive application of technology would be the widespread deployment of temperature-sensitive radio tracking devices along with a roost-based receiver. For example, previous studies (e.g., Johnson 2012) have used temperature-sensitive trackers to determine when bats descend into and arouse from torpor. A roost-based receiver would allow continuous recording of data from which a researcher could determine what relationship exists between body temperature and time spent inside vs. outside the roost. This could be highly useful in illuminating why some bats remain torpid and some do not at the same temperature. Such devices could even be combined with high-resolution video (that allows for the tracking devices to be easily discerned on video, unlike my setup) to get an idea of individual bat temperatures inside a cluster. This approach may yield similar information to thermal imaging but at a significantly reduced cost.

### *Radio tracking*

In short, I found radio tracking of Rafinesque's big-eared bats at this site to be difficult. Small transmitter size/range, hilly terrain, thick vegetation, and distant or erratic flight patterns often made detection difficult. As such, these data are likely biased—bats that fly farther from the roost exist but were hard to track. More receivers and field assistants would be beneficial. However, that increases not only the investment (receivers and antennas are ~\$600 each), but also coordinating more people could easily become a logistical headache.



Radio tracking did yield useful data from my study. We now know that many of these individuals do frequently forage within the immediate vicinity (i.e., < 0.5km) of the roost. Contrary to what I expected, no significant activity was ever found near several ponds or creeks in the area. Some bats are found to travel in a north/north-easterly direction from the roost, but this terrain is remote (no roads) and access is difficult (swampy or riverine in nature). Radio tracking in these areas would benefit from pre-established monitoring stations and/or tracking by light watercraft. A very high vantage point would substantially increase reception range. Historical maps (from the 1970s and prior) indicate a “Bagley Lookout Tower” in the nearby area, but I could neither pinpoint an exact location nor find this tower. Whether it still exists or is in a condition that would be safe for use is not known. Basic information about plant and animal species I encountered in the vicinity of the study site can be found in Table 8 (Appendix).

It is obvious from observations of the colony (in person and on video) that many of the 100+ bats present in warmer months are roosting elsewhere in colder months. I was unable to locate alternate roost locations (aside from occasional use of other structures in the immediate vicinity of the seismograph building) despite repeated search attempts: checking bridges, old buildings (e.g., silos, barn, dilapidated houses, often discovered using satellite imagery), and trees. Relatively few trees of size reported by others ( $\geq 80\text{cm dbh}$ ) to be used by *C. rafinesquii* are present near the seismograph building. Investigating existing data on forest inventory by the US Forest Service might help focus searches for tree roosts.

Ideally, tracking devices which automatically triangulate a bat’s position using either cellular phone technology or global satellite positioning systems would be of great interest. However, in addition to the high cost of these devices, none (yet?) exists of an appropriate size for use with *C. rafinesquii*. The smallest GPS loggers I found (e.g., the Advanced Telemetry

Systems G10 UltraLITE or the Telemetry Solutions Quantum 4000E small backpack) both weigh approximately 5 grams. The necessary size for use on most Rafinesque's big-eared bats needs to be approximately 0.5 grams, meaning that GPS transmitters currently available are an order of magnitude too large—and these are relatively new devices that are touted for their small size! Furthermore, these devices can also be hundreds of dollars per unit. However, it is an exciting prospect: such devices can have long battery lives, provide much increased accuracy, yield more data points, and are less labor-intensive. Some models even have a feature for remote downloading of data. For now, though, traditional VHF radio trackers remain the only viable option, although clever techniques could enhance detection and data quality.

### *Trapping bats*

In this study, I almost exclusively used 16" diameter, deep butterfly nets to catch bats. This technique requires some practice and finesse, but generally worked well. Great care should be taken when catching bats in flight to reduce risk of injury (particularly with the metal O-ring on the nets). Using a red light on a headlamp (instead of the normal white light) seemed to help somewhat to keep from "spooking" bats. Modified mist nets were sometimes used over entry/exit holes on a concrete bat tower. Single height mist nets or harp traps could be used at the main entrance of the seismograph building, but this would likely be unsuccessful due to numerous other exit points that the bats have due to the poor state of the building. This species is notoriously difficult to capture in mist nets and harp traps (e.g., Loeb 2011).

A homemade harp trap (made of ½" PVC, eye hooks, and two sets of parallel nylon threads with a catch bag at the bottom) was constructed. Translucent plastic sheeting was used to cover other routes inside the building and then the harp trap was placed in the central portion of

the seismograph building. However, most bats either dodged the harp trap entirely or were able to fly through the threads. Slight adjustments (e.g., to thread tension) were made without much success, and even when a few bats did fall into the bag, they were able to quickly regain flight despite the bag being relatively deep and made of slippery plastic. Conventional harp traps are very expensive (baseline model ~\$1000) but would likely be somewhat more successful.

However, there is still the issue of multiple entry/exit points. Researchers at Mammoth Cave National Park (Kentucky, USA) did use a commercial harp trap to catch bats at the Wondering Woods site (pers. comm., Rick Toomey 2013), however, the building at this site is in good structural condition and has only one or two possible points of exit.

#### *Biometric data collection*

In this study, I used a digital caliper for forearm measurements which generally worked quite well. The only consideration should be that caliper prongs can be quite sharp, so care should be taken when using them with bats' delicate wing membranes. For measurements of mass, bats were placed in a paper bag and suspended from a spring scale. For future work, I might suggest a battery-powered digital scale. These can be easily calibrated and also provide more accurate measurements. Keeping a bat inside of a paper bag while measuring would still be useful, and then one simply subtracts the mass of the bag after the bat is released.

For future research, it might be a good idea to use a high-quality digital camera to document the wing membranes of each bat. Such photos could, if standardized, be used by computer software to analyze holes and tears in the wing membrane—such injuries were not uncommon. It would be interesting to know if these types of injuries varied in intensity or frequency throughout the seasons or between sexes.

### *Banding and attachment of radio transmitters*

The Porzana bat bands used in this study were of high quality and no indication of injury or irritation to the bats was observed, even after a year of attachment. Embossed lettering was relatively easy to read (despite the very small size of the bands). My only concern is that the bands were sometimes difficult to apply simply because of their small size and the very delicate nature of bat wing bones. Some type of tool might be useful, but practice in application is also helpful. If a band was accidentally applied too tightly, it was relatively easy to open the band up a little. Banding and all measurements could generally be completed in just a few minutes per bat thus limiting any potential negative effects of handling.

Radio transmitters were a bit more work to attach than forearm bands. I originally used curved tip scissors (“operating scissors”) to trim away fur between the shoulder blades of the bat because the surgical cement only bonds to bare skin. However, due to the very small size of these bats and frequent folding of the skin, I found it difficult (and pain-staking) to trim the fur with these scissors. I experimented with a small, cordless beard trimmer. It worked surprisingly well at clearing away a section of fur without injuring the bat (i.e., cutting the skin). Curiously, rather than being agitated by the buzzing of the trimmer, it actually appeared that most of the bats were “calmed” by it. I had a liquid bandage solution on hand in case of any accidental cuts of bat skin, but thankfully I never needed it.

To apply the surgical cement to the newly-exposed bat skin, I used a long, sterile cotton swab. Great care should be taken to not accidentally drip the surgical cement on other areas of the bat. Also, fumes produced by the cement can be quite strong to the human nose, so I would

not ever recommend placing the bat in a bag after application of the cement (even if the cement has ‘set’). Finally, one should make sure not to leave the surgical cement for prolonged periods in a hot environment (e.g., in a field vehicle). Anecdotal reports suggest that this seriously degrades the adhesive quality of the surgical cement.

### *Temperature data loggers*

Although older models (using a PC serial rather than USB connection), the data loggers used in this study performed well. However, future studies may wish to use models that also record humidity levels or even light levels or air velocity. Most current models that measure temperature also measure humidity levels. Additionally, models that accept thermocouple wires exist (this provides for more precise measurements of a particular area, e.g., an area on a wall or ceiling where bats cluster). Such a thermocouple device could be used to further elucidate differences in microclimates within a single room or small roost. Carbon dioxide loggers might even be useful for estimating air flow and/or number of bats occupying an area.

### *Conclusions*

I reported biometric data, counts, roost thermal conditions, and preliminary foraging locations for *C. rafinesquii* at my study site. This information will be useful for efforts to protect this roost. Building roosts are quite common, particularly in Mississippi, so information from my study may be applied to other colonies that roost in buildings. Comparative studies across roost types and habitats are needed to inform conservation and management decision-making for this uncommon species as well as repeated, detailed surveying and monitoring.

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## APPENDIX

**Table 8.** No concentrated effort was made to inventory any particular taxa (aside from bats) at the field site. However, below is a list of plant and animals species observed within approximately 5km of the site. This table may be useful to those desiring an idea of the area's ecology. An asterisk (\*) indicates direct observation within the roost building

Plants	Mammals	Birds	Reptiles & Amphibians	Arthropods
Trees:				
American beech <i>Fagus grandifolia</i>	eastern gray squirrel <i>Sciurus carolinensis</i>	American crow <i>Corvus brachyrhynchos</i>	common box turtle <i>Terrapene carolina</i>	black widow spider <i>Latrodectus mactans</i>
American elm <i>Ulmus americana</i>	eastern woodrat* <i>Neotoma floridana</i>	barred owl <i>Strix varia</i>	copperhead <i>Agkistrodon contortrix</i>	bumblebee <i>Bombus</i> sp.
American sycamore <i>Platanus occidentalis</i>	nine-banded armadillo <i>Dasypus novemcinctus</i>	eastern phoebe <i>Sayornis phoebe</i>	cottonmouth <i>Agkistrodon piscivorus</i>	camel cricket* <i>Ceuthophilus</i> sp.
green ash <i>Fraxinus pennsylvanica</i>	northern raccoon <i>Procyon lotor</i>	eastern whip-poor-will <i>Antrostomus vociferus</i>	rat snake <i>Pantherophis</i> sp.	eastern pondhawk <i>Erythemis simplicicollis</i>
hickory <i>Carya</i> spp.	southeastern myotis bat* <i>Myotis austroriparius</i>	golden-crowned kinglet <i>Regulus satrapa</i>	southern leopard frog <i>Lithobates sphenoccephalus</i>	eastern tiger swallowtail <i>Papilio glaucus</i>
loblolly pine <i>Pinus taeda</i>		indigo bunting <i>Passerina cyanea</i>	timber rattlesnake* <i>Crotalus horridus</i>	giant lichen orbweaver <i>Araneus bicentenarios</i>
overcup oak <i>Quercus lyrata</i>	striped skunk <i>Mephitis mephitis</i>	pileated woodpecker <i>Dryocopus pileatus</i>		lone star tick <i>Amblyomma americanum</i>
river birch <i>Betula nigra</i>	Virginia opossum <i>Didelphis virginiana</i>	red-headed woodpecker <i>Melanerpes erythrocephalus</i>		lubber grasshoppers Romaleidae
shortleaf pine <i>Pinus echinata</i>	white-tailed deer <i>Odocoileus virginiana</i>	summer tanager <i>Piranga rubra</i>		red wasp <i>Polistes</i> sp.
tulip poplar <i>Liriodendron tulipifera</i>		turkey vulture <i>Cathartes aura</i>		red-spotted purple butterfly <i>Limenitis arthemis astyanax</i>
white ash <i>Fraxinus americana</i>		wild turkey <i>Meleagris gallopavo</i>		spined spiders <i>Micrathena</i> sp.
white oak <i>Quercus alba</i>				
<b>Other vascular plants</b>				
blackberry <i>Rubus</i> sp.				
muscadine <i>Vitis rotundifolia</i>				
orange jewelweed <i>Impatiens capensis</i>				

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