

DETERMINING ENERGY CONSERVATION DURING TORPOR FOR
THREE *MYOTIS* SPECIES AND REPSONSE OF *MYOTIS* SPECIES TO HUMAN
DISTURBANCE WHILE DAY ROOSTING

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ABSTRACT

THESIS: Determining Energy Conservation During Torpor for Three *Myotis* Species and Response of *Myotis* Species to Human Disturbance While Day Roosting

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The endangered Indiana bat (*Myotis sodalis*) has been the focus of much research in the past 40 years, primarily with respect to the summer habitat requirements for the species. Recent advances in radio telemetry have allowed researchers to learn about the specific activity patterns for roosting bats. Torpor is an energetic process that bats use to conserve energy through the day. We used an equation that gives a threshold for when the animal enters torpor to quantify the amount of energy conservation among Indiana bats, northern long-eared bat (*M. septentrionalis*) and little brown bat (*M. lucifugus*) and their reproductive stage. Additionally, we used the torpor threshold to determine if researchers were causing disturbances to roosting female *Myotis* bats in the summer.

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CHAPTER 1

INTRODUCTION

Indiana bats (*Myotis sodalis*) were officially listed in 1967 as an endangered species. From the early 1960's until the mid 1990's there was a population decrease of approximately 60% from the approximate estimate of 880,000 bats. More than 85% of the remaining Indiana bats have been hibernating in only nine hibernacula since the mid 1990s (USDI Fish and Wildlife Service 1999). Since Indiana bats form large colonies concentrated in a few hibernacula, there has been extensive research on these hibernacula and the bats within (Hall 1962, Brack 1984, Humphrey 1978). With strong efforts to conserve suitable winter habitats, such as installing bat-friendly gates in caves and abandoned mines to prevent human disturbance and constructing stabilization tunnels in the entrance of some hibernacula (Currie 2002, Johnson et al. 2002), Indiana bat numbers continue to decrease in much of its range (Clawson 2002). Although winter conservation and research continues to be important, this population decline has shifted the focus of much recent Indiana bat research to summer ecology (see Menzel et al. 2001).

The focus of the summer habitat and foraging research has primarily been on female Indiana bats, because they form large maternity colonies in their summer range (e.g. Humphrey et al. 1977, Gardner and Cook 2002, Carter 2006). Indiana bats are associated with multiple forest habitats, including bottomland, riparian, and other hydric habitats (Carter 2006). Attention can now be turned to specific activities and foraging

behavior of Indiana bats within these habitats (LaVal et al. 1977). Suitable roost trees are important in the summer ecology of the Indiana bat (Menzel et al. 2001, Carter and Feldhamer 2005) and further insights into roost selection will help researchers and managers use proper forest management techniques to conserve these critical habitats.

Radio telemetry studies have shed light on Indiana bats' roosting behaviors and daily activity patterns during the spring and summer months (see Menzel et al. 2001). This technology enables researchers to locate an individual bat every day and assess its specific roost requirements. Radio transmitters can also provide information such as temperature of the animal while roosting, time spent in torpor, and disturbances to the roosting animal (Carter et al. 1999).

Torpor is a daily, controlled decrease in body temperature employed by many small mammals and birds (Heinrich and Bartholomew 1971, Wang and Wolowyk 1988). The benefit of torpor for insectivorous bats is a significant energy savings (Wang and Wolowyk 1988), but the cost can be a slower rate of fetal development and an inhibition of lactation in female pipistrelle bats (*Pipistrellus pipistrellus*) and big brown bats (*Eptesicus fuscus*; Racey and Swift 1981, Audet and Fenton 1988, Wilde et al. 1999). Audet and Fenton (1988) proposed that maintaining offspring development is more important than the energy conservation and predicted decreased levels of torpor in reproductively active animals.

Whether common or not, quantifying the amount of energy savings from torpor is complicated by the challenge of obtaining accurate metabolic measurements of bats in the laboratory and the field (Kurta et al. 1987). As endangered species, sacrificing individual animals is not desirable and in some cases sacrifices could represent a significant loss for

the species (Endangered Species Act 1973). Development of a relative index for the amount of energy savings would allow comparison of torpid animals in the wild, without excessive recaptures or sacrificing of the animal (Racey and Swift 1981).

Understanding the dynamics of torpor in small insectivorous bats is made easier by applying an equation created by Willis (2007), which sets a threshold for the onset of torpor in the roosting bats. This equation provides an accurate determination as to when a roosting bat enters torpor, as well as an indication of the depth and duration of torpor. By learning more about the energetic processes of roosting female *Myotis* bats, we can also test for effects of researchers on roosting females.

The cost of human disturbance to nesting birds has been extensively studied (e.g., Gillett et al. 1975, Ellison and Cleary 1978, Wilson and Culik 1995, Giese 1996, Carney and Sydeman 1999, Fowler 1999, Gill 2001, Beale and Monaghan 2004). Götmark (1992) determined that disturbance of nesting birds associated with research increased intra- and inter-specific predation on eggs and young. Nimon et al. (1995) found that Adélie penguins' (*Pygoscelis adeliae*) heart rates increased rapidly in the presence of humans. In contrast to the numerous studies of human disturbance on nesting birds, studies of disturbance of reproductively active female bats has been limited to large scale habitat disturbances (Gehrt and Chelvig 2004, Swihart et al. 2006).

While researching tree-roosting bats, it is common practice to collect data on the roost tree at the time of discovery. Techniques include hammering an identifying tag into the roost tree; measuring DBH, tree height, roost height, and canopy cover; and frequently setting a plot around the roost tree to conduct an analysis of the surrounding vegetation (Gardner et al. 1991, Kurta et al. 1996). It is common in the vicinity of the

roost tree to hear the chatter (vocalizations) of the bats. This alone may be evidence that these studies disturb roosting female bats. And, if the bats are disturbed enough to abandon torpor, the data collection may have caused the animals to waste valuable energy that would have otherwise been conserved. Determining if these common studies have effects on the thermoregulatory processes of reproductively active female bats is important to maintaining healthy populations, and not contributing to the decline of this endangered species.

OBJECTIVES

1. Compare the amount of energy conserved in females of three *Myotis* species, Indiana bats (*Myotis sodalis*), northern long-eared bats (*M. septentrionalis*), and little brown bats (*M. lucifugus*).

Ho: All three *Myotis* species have the same amount of energy conservation.

2. Compare the amount of energy conservation in three different stages of reproduction (pregnant, lactating, and post-lactating) for the three species.

Ho: Every stage of reproduction will have the same amount of energy conservation.

3. Test for an effect of human disturbance on the thermoregulatory processes of three species of reproductively active female *Myotis* bats.

Ho: The amount of human disturbance will not disrupt the thermoregulatory processes and raise their body temperature out of torpor.

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CHAPTER 2

DETERMINING ENERGY CONSERVATION DURING TORPOR FOR
THREE *MYOTIS* SPECIES

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ABSTRACT

Torpor is an important strategy for many endothermic organisms. By decreasing body temperature, animals can conserve energy that would otherwise be needed for internal heat production and other metabolic processes. Quantifying energy savings for animals in a field setting is a difficult task. Our objective was to compare energy conservation for three species of female *Myotis* bats, Indiana bats (*Myotis sodalis*), northern long-eared bats (*M. septentrionalis*), and little brown bats (*M. lucifugus*), during pregnancy, lactation, and post-lactation. Using an equation that provides a standardized threshold for differentiating torpor from normothermia Willis (2007), we accurately calculated the relative amount of energy conservation based on body temperature of the bats. During the summers of 2007 and 2008, we captured and applied temperature sensitive radio transmitters to 96 female *Myotis* bats (34 Indiana bats, 38 northern long-eared bats, and 24 little brown bats). Using telemetry dataloggers, we recorded the signal from the transmitters and converted the data into bat body temperature. By applying Willis' equation as an upper limit of the onset of torpor, we then used body temperatures of the bats in torpor to create a relative index for the amount of energy conservation for the three reproductive stages of each *Myotis* species. Mean energy conservation ($^{\circ}\text{C}$) differed among Indiana bats and little brown bats and among northern long-eared bats and little brown bats ($P = 0.003$), but there was no difference among the three reproductive stages when all three species were combined ($P = 0.833$). Within species, there was a difference between pregnant northern long-eared bats and pregnant little brown bats ($P = 0.035$), but no difference among the three species when lactating ($P = 0.121$). Use of this relative index to measure the amount of energy conserved by roosting

female bats will reveal more about the thermoregulatory processes of pregnancy, lactation, and post lactation for *Myotis* bats and other tree-roosting bats.

INTRODUCTION

Torpor is the controlled reduction of thermoregulatory processes (Hudson 1978, Wang and Wolowyk 1988). Small mammals and birds going into shallow torpor during the day conserved energy that otherwise would be allocated to maintaining high body temperatures (T_b), an important savings during times of inclement weather or low food availability (Wang 1989). The cost of reducing T_b for reproductively active female pipistrelle bats (*Pipistrellus pipistrellus*) is delayed parturition and an inhibition of lactation (Racey and Swift 1981, Wilde et al. 1999).

North American insectivorous bats commonly use daily torpor while in their summer roosts. Conserving energy for female bats is important because of the high energy requirements of reproductive activity; as they spend the majority of the summer months in pregnancy, lactation, or in post lactation (allocation to fat reserves for winter hibernation). However, it is also important to reach a balance between energy conservation and offspring development, since torpor may delay parturition and inhibit lactation in other female bats besides pipistrelle bats (*Pipistrellus pipistrellus*; Racey and Swift 1981, Wilde et al. 1999).

Female Indiana bats (*Myotis sodalis*), northern long-eared bats (*M. septentrionalis*), and little brown bats (*M. lucifugus*) all form summer maternity colonies (Easterla 1968, Humphrey et al. 1977, Fenton and Barclay 1980, Thompson 1982, Foster and Kurta 1999, Caceres and Barclay 2000). These colonies of females and young form

clusters in snags, crevices, or buildings (Gardner et al. 1991, Carter and Feldhamer 2005). One hypothesis to explain this behavior is that formation of maternity colonies and selecting warmer microclimates for roosts allows females to reduce the energy expenditure associated with maintaining a high T_b , resulting in increased rates of fetal development (McNab 1982, Kunz 1982).

Studies of bat torpor are frequently conducted in the laboratory (Kurta et al. 1987) and/or require sacrificing animals (Racey and Swift 1981). However, studying torpor in the natural habitat of forest bats can reduce intrusive behaviors during data collection. Understanding energy conservation in torpid bats can shed light on knowledge of the depth, duration, and frequency of torpor bouts that these species employ daily during the summer months. Additional information regarding the activities of female bats during the summer can aid in the conservation of the species.

We tested the hypothesis that the amount of energy conserved from daily torpor would vary during reproductive conditions (pregnant, lactating, and post-lactating) of the three *Myotis* species (northern long-eared bats, little brown bats, and Indiana bats).

STUDY AREA

Mist-netting and radio telemetry were conducted in seven sites across southern Illinois, southern and central Indiana, and western Kentucky. The sites in southern Illinois are in the floodplains of the Mississippi River and include Oakwood Bottoms in Jackson County and Union County Conservation Area in Union County. Oakwood Bottoms is a bottomland, hardwood forest. Union County Conservation Area has

multiple habitats including bottomlands (similar to Oakwood Bottoms), wetlands, and agricultural fields (Carter and Feldhamer 2005).

The sites in Indiana included Camp Atterbury, in Bartholomew, Johnson, and Brown Counties in central Indiana, Muscatatuck National Wildlife Refuge, Jackson County in southern Indiana and Big Oaks National Wildlife Refuge, Jefferson, Ripley, and Jennings Counties in southern Indiana, and Wilbur Wright Fish and Wildlife Area, Henry County in east-central Indiana. These sites were characterized by continuous tracts of bottomland forest with hydric (e.g., riparian corridors, floodplains, bottomlands, and wetlands) habitats throughout (Carter 2006).

The Kentucky site was Mammoth Cave National Park, which encompasses a large area of continuous forest around the Green River in Edmonson County in western Kentucky. This creates suitable habitat for several bat species including the three targeted *Myotis* species. Mammoth Cave National Park along with five other study areas were selected based on previous Indiana bat captures (Montgomery Watson 1999, Whitaker and Gummer 2002, Carter et al. 2002, Carter 2003, Carter and Feldhamer 2005), while Wilbur Wright Fish and Wildlife Area was selected because of the intact forest and riparian habitat.

METHODS

CAPTURE & RADIO TELEMETRY

Bats were captured from May to August 2007 and 2008, using mist-net systems similar to Gardner et al. (1989). Mist-nets were established in areas of known *Myotis*

activity in southern Illinois, central Indiana, and western Kentucky (Montgomery Watson 1999, Whitaker and Gummer 2002, Carter 2002, Carter 2003, Carter and Feldhamer 2005). Upon capture, temperature sensitive radiotransmitters (Model LB-2N and LB-2NT, Holohil Systems Ltd., Ont., Canada) weighing approximately 0.45 grams were fitted to the backs of the first captured targeted female *Myotis* bats. Temperature sensitive radiotransmitters attached to the skin of bats are a reliable measurement of T_b (Barclay et al. 1996). We used Torbot[®] liquid bonding cement (Torbot Group Inc., Cranston, RI) and Skin Bond Cement[®] (Smith & Nephews United Inc., Largo, FL) to attach the radiotransmitters on a dorsal location between the bats' scapulae (Holohil Systems Ltd. 2007).

Bats with radiotransmitters were tracked to their day roosts using an ATS R420 scanning receiver (Advanced Telemetry Systems, Inc., Isanti, MN) and a 3 element Yagi antenna (Wildlife Materials, Inc., Murphysboro, IL). When roosts were located, waterproof enclosures (45 quart coolers) containing an ATS R4500S Scientific Receiver/Datalogger (Advanced Telemetry Systems, Inc., Isanti, MN), 12-volt battery, and an external antenna (3 or 5 element Yagi, Wildlife Materials, Inc., Murphysboro, IL) were established in proximity to the day roosts. The enclosures were moved as needed when the bats switched day roosts. Data from the datalogger were downloaded as needed onto a laptop computer in the field (Win Rec S v106, Advanced Telemetry Systems, Inc., Isanti, MN).

ANALYSIS

Radiotransmitters were calibrated by Holohil Systems, Ltd. or our team, by exposing the radiotransmitters to different temperatures and recording the associated inter-pulse period (IPP). Linear relationships between the radiotransmitter's IPP and temperature (see Carter et al. 1999) were quantified, allowing conversion of continuously recorded IPP's from the dataloggers into understandable temperature units (°C; see Carter et al. 1999).

After regressions for daily bat body temperature were calculated, we applied an equation that identifies the threshold for the onset of torpor in the animals (Willis 2007) and allowed us to estimate the amount of energy conservation for these bats, using ambient temperature (T_a) and body mass (BM):

$$T_{b\text{-onset}} - 1 \text{ SE} = (0.041)\text{BM} + (0.040)T_a + 31.083$$

The resulting plots provide an indication as to when the animal was in torpor throughout the day (Figure 1).

A relative index was then created to quantify the amount of energy conservation by torpid bats during different reproductive stages across the three species of *Myotis* bats. The x-axis and y-axis of each day of data for every animal were all set to 24 h periods from 0:00 of the first day to 0:00 of the next day, with 30 minute intervals set to quantify the differences between shallow, deep, long, or short bouts of torpor. After the relationships were standardized, we calculated the difference in °C between the threshold of torpor determined by the Willis equation and the observed body temperature of the bat at 30 min intervals recorded for 24-hours. When data was not complete, we extrapolated the data to finish body temperature lines on the graphs. The differences were summed to obtain the total °C below the torpor threshold per day for each bat, even if the animal did

not enter torpor. While the number of degrees conserved per day is not a direct measure of energy savings, we submit that it can be used as a proxy for the energy conservation these animals receive from torpor.

The mean energy conservation per day for all Indiana bats, northern long-eared bats, and little brown bats was compared using a non-parametric Kruskal-Wallis test. Kruskal-Wallis tests were also used to compare the means of bats (all species combined) across different reproductive stages (pregnant, lactating, and post-lactating) as well as within each reproductive stage (e.g. pregnant Indiana bats, pregnant northern long-eared bats, and pregnant little brown bats). If a difference was detected, we used a non-parametric multiple comparison test to identify significantly different means.

RESULTS

Of the 860 bats caught in the summers of 2007 and 2008, 235 were the targeted female *Myotis* species (38 Indiana bats in 2007 and 14 in 2008, 56 northern long-eared bats in 2007 and 11 in 2008, and 38 little brown bats in 2007 and 78 in 2008). Radiotransmitters were applied to 81 female *Myotis* bats in 2007 and 15 female *Myotis* species were fitted with radio transmitters in 2008. For the comparisons of the three species we used 17 Indiana bats, 19 northern long-eared bats, and 11 little brown bats due to incomplete data sets for some of the transmitted bats. We used 20 pregnant individuals, 17 lactating individuals, and 10 post-lactating individuals for comparisons of reproductive conditions regardless of species.

Mean energy conservation differed for the three species ($P = 0.003$). Indiana bats ($n=17$) had significantly greater energy conservation ($\bar{x}=63.2^\circ$) than little brown bats

($n=11$; $\bar{x}=14.96^\circ$) and similarly, northern long-eared bats ($n=19$) had significantly higher energy conservation ($\bar{x}=77.0^\circ$) than little brown bats ($\bar{x}=14.96^\circ$). No difference was found in the comparisons of northern long-eared bats and Indiana bats. There was also no difference in mean energy conservation between the three reproductive stages, pregnant ($n=20$), lactating ($n=17$) and post-lactating ($n=10$) with all species combined ($P = 0.833$).

During pregnancy, there was no difference among female Indiana bats ($n=13$) northern long-eared bats ($n=3$) or little brown bats ($n=4$). However, pregnant northern long-eared bats ($\bar{x}=92.7^\circ$) had significantly greater energy savings than pregnant little brown bats ($\bar{x}=6.59^\circ$). During lactation, there was no difference in energy conservation among Indiana bats ($n=4$), northern long-eared bats ($n=6$), or little brown bats ($n=7$; $P = 0.122$). We only obtained data for northern long-eared bats during post-lactation and therefore were unable to make comparisons across species in this reproductive stage.

DISCUSSION

Our data partially supported our hypothesis energy conservation would differ across the three bat species regardless of reproductive state. Indiana bats and northern long-eared bats are similar in their daily thermoregulatory processes, while little brown bats exhibited less overall energy conservation. With respect to energy conservation within reproductive stages, only pregnant northern long-eared bats and little brown bats differed, again with little brown bats having a significantly lower mean of energy savings overall. All three species shared similar thermoregulatory processes during lactation.

And no difference was found in energy conservation across reproductive stages for the species combined.

It is interesting that the significant differences in energy conservation we detected were always in comparisons of little brown bats with at least one of the other species. In laboratory studies, little brown bats consistently used torpor only during severe energetic stress (Kurta et al. 1987; Kurta and Kunz 1988). Given no apparent severe energetic stresses during our summers of research, it is possible that the little brown bats in our field study did not consistently use torpor. While little brown bats cluster to form maternity colonies, a behavior similar to northern long-eared bats and Indiana bats, the maternity colonies of little brown bats can be larger than those formed by northern long-eared and Indiana bats (Barbour and Davis 1969; Humphrey and Cope 1976). Little brown bats also have increased fidelity to one roost compared to the other two species (Barbour and Davis 1969; Gumbert et al. 2002, Sichmeller et al. 2008). Burnett and August (1981) concluded that colonial roost occupation for little brown bats consistently raised the maximum daily roost temperature by as much as 7°C. Little brown bats are unlike other *Myotis* species. Their behavior of forming these larger maternity colonies may effectively raise the temperature of the maternity roost and thus forego the need for long and deep bouts of torpor to save energy which can slow fetal development and inhibit lactation (Racey and Swift 1981; Wilde et al. 1999). Willis and Brigham (2007) showed that energy conservation by individuals in clusters is not only the result of the elevated temperature, but in addition, formation of a group results in lower metabolic heat loss.

When we compared the energy conservation by reproductive states for the three species, we expected at least one difference in comparisons by reproductive states. Our results suggest that the reproductive state of a female myotine bat is not a factor in the degree to which torpor is used to conserve energy within an individual. Other factors are necessary to determine depth, frequency, and duration for an animal in torpor. These factors may include the previous nights foraging success, the severity of the ambient conditions, or the abundance of conspecifics within the maternity roost tree (Humphrey and Cope 1976; Burnett and August 1981; Willis et al. 2006).

Additionally, the variations in mean amount of body temperature conservation among individuals are noteworthy. For example, we observed two pregnant Indiana bats that both roosted in trees within the same vicinity (~ 10 m) for one day but exhibited different energy conservation mechanisms. One exhibited a long and deep bout of torpor conserving 215.25°C of body temperature (Figure 2a), while the other exhibited a short and shallow bout of torpor, conserving only 40.5°C throughout the day (Figure 2b) despite experiencing the same ambient conditions. This suggests that the depth, length, and frequency of torpor are based on a factor(s) relating to the individual or the microclimate of the roost rather than environmental conditions or specific reproductive state.

MANAGEMENT IMPLICATIONS

From our study, we recommend that researchers use northern long-eared bats as a surrogate species for Indiana bats when conducting summer energetic studies. By doing this researchers can focus their attention on two bat species that have similar energetic

processes during the summer. Little brown bats frequently roost in large colonies and we documented significantly lower energy savings from this behavior. This suggests that these larger roosts that house larger colonies may provide better microclimate which may reduce the need for these bats to use torpor to conserve energy. This may explain why Indiana bats are known to select large snags as roosts when available (Humphrey et al. 1977, Gardner et al. 1991). Others have proposed that these large roosts provide these microclimate resources that promote fetal development and lactation (Racey 1973, Callahan et al. 1997). This leads us to suggest to land managers to when possible providing large roost trees and perhaps large man-made bat houses when managing for Indiana bats. Larger roosts can hold more bats, resulting in structures and trees that offer improved thermoregulatory conditions to not only little brown bats but to the federally endangered Indiana bat. We also recommend conducting larger scale energetic studies with increased numbers of individuals. Since we found that energy conservation is based more upon the individuals and the different environmental factors that they encounter, a larger scale study is needed to accurately depict individual energy saving techniques.

Using this relative index for energy conservation can be an important technique that can expand our knowledge of the day to day energy budget for not only bats but other small mammals and birds that employ torpor as an energy savings mechanism. By closely studying these thermoregulatory processes, researchers can begin to analyze what other environmental factors are effecting the targeted animal. Foraging success, size of a maternity cluster, or roost tree characteristics are all factors that may affect the ability to successfully conserve energy. By developing this relative index as a way to evaluate the amount of energy conservation of torpid bats, researchers will be able to learn more about

the energy requirements of reproduction during the summer, not only for *Myotis* bats but for all tree roosting bats. With this relative index, we are now able to accurately document the energy conservation habits of three different *Myotis* species, in particular the federally endangered Indiana bat.

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Figure 1. An activity graph showing a pregnant Indiana bat arriving at her roost, then entering torpor, raising her body temperature out of torpor, and then leaving the roost.

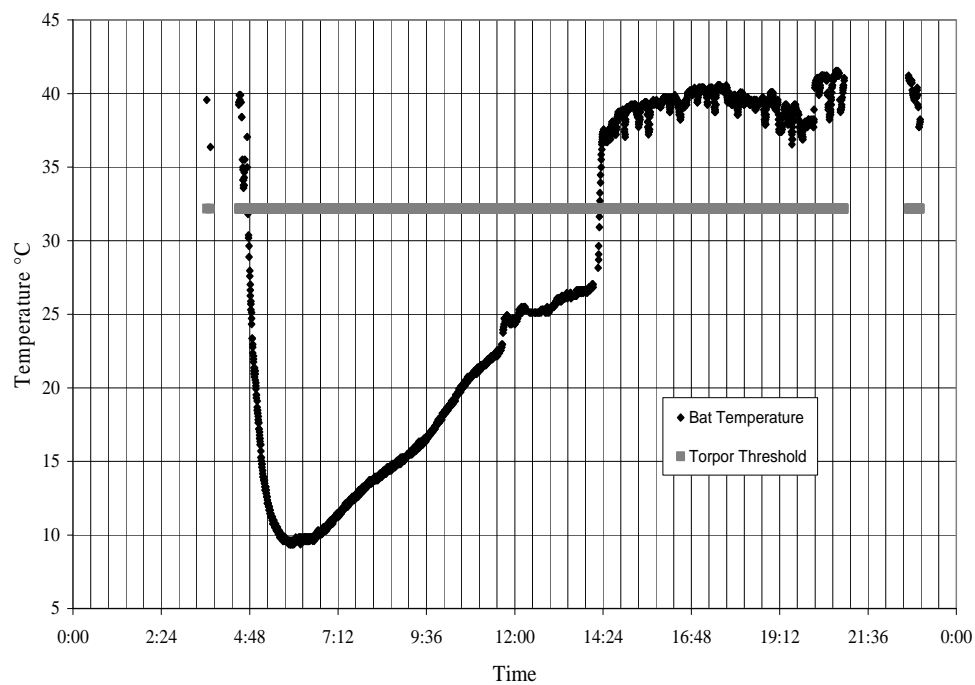


Figure 2a. Body temperature conservation in two lactating Indiana bats roosting in the same area on the same days. Note difference in energy conservation between the two bats.

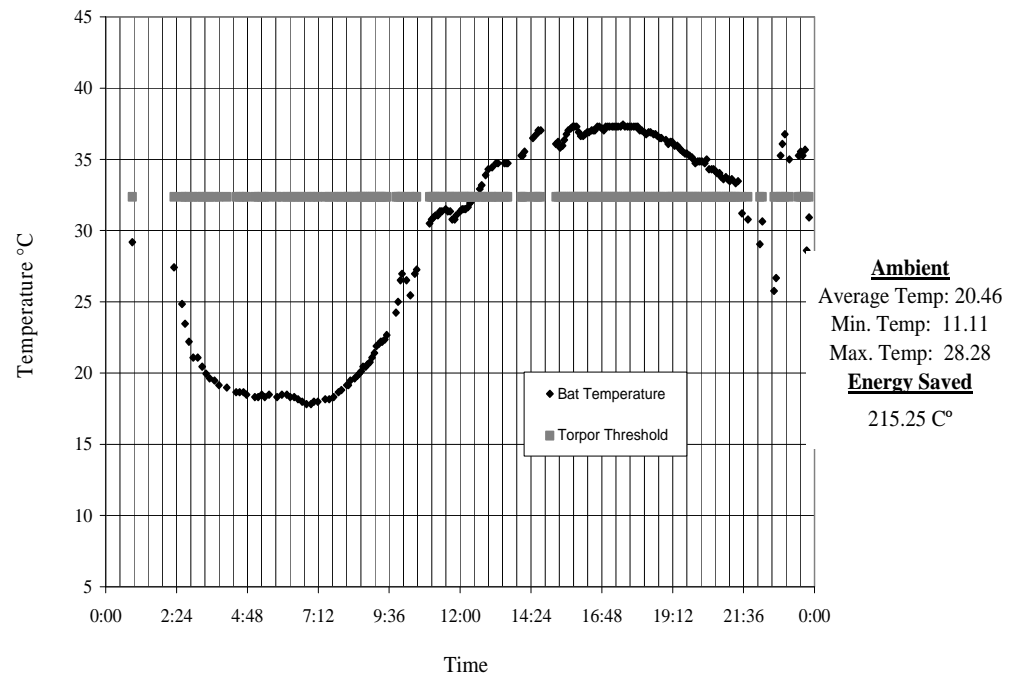
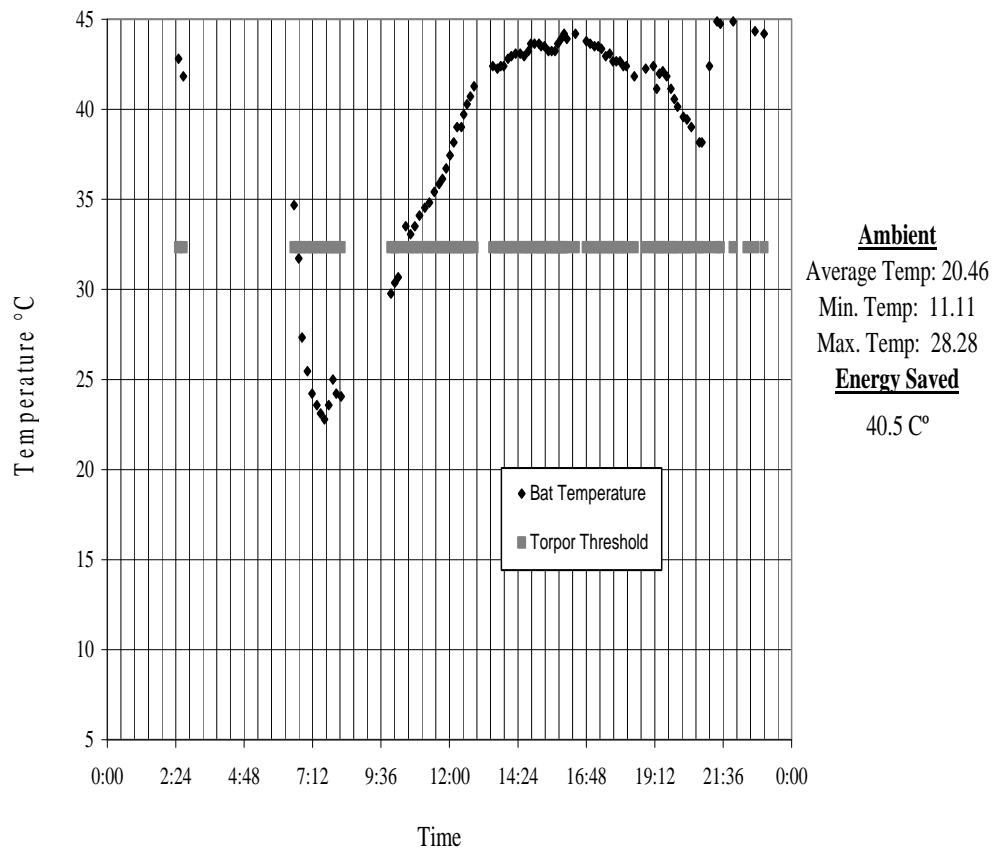


Figure 2b.



CHAPTER 3

RESPONSE OF *MYOTIS* SPECIES TO HUMAN DISTURBANCE
WHILE DAY ROOSTING

Sichmeller, T. J., T. C. Carter, and M. G. Hohmann

ABSTRACT

Advances in technology have allowed bat researchers to increase knowledge of summer roosting over previously available information. It is now possible with telemetry for a researcher to locate numerous roosting bats or colonies in a single day. While advances in telemetry have led to a rapid increase in our understanding of bat roost ecology, the possible negative impact of disturbance to these roosting bats by researchers has not been examined. When tracking bats, audible sounds from the roost are often used to help locate the specific roost tree. It is not known if these animals are vocalizing as a result of the disturbance from the researcher or if it is a common behavior exhibited throughout the day. Over the course of two maternity seasons we used temperature sensitive transmitters combined with continuously recording receiving equipment to monitor daily activity patterns and body temperature of roosting bats. Using these data we are able to assess possible disturbance events when researchers approach and work around roost trees. We used 175 roosting days to determine if an individual bat's thermoregulatory processes were disrupted by our presence. In only 13 instances was there a distinct increase in body temperature and only 8 of these increases resulted in the bat coming out of torpor. We suggest that the presence of humans at a maternity tree causes no thermoregulatory disturbances to a female *Myotis* bat.

INTRODUCTION

Human activities can impact wildlife populations in a variety of ways. Wildlife geographic distribution, habitat use, dispersal patterns, fecundity, survival, and energy

budgets can all be affected by human activity (Knight and Cole 1991). The effects of these disturbances can impact the entire population or select individuals with the result of reduced fitness (Holmes et al. 1993).

The costs of human disturbance to nesting birds have been extensively studied throughout the years (Gillett et al. 1975, Ellison and Cleary 1978, Wilson and Culik 1995, Giese 1996, Carney and Sydeman 1999, Fowler 1999, Gill et al. 2001, Beale and Monaghan 2004). These studies provide information about the effects of general human activity on breeding populations, and the effect that scientific researchers can have on individuals and or populations. Fetterolf (1983), Götmark (1992), and Sandvik and Barrett (2001) all looked at how scientific studies can become disturbances on nesting birds. Fetterolf's (1983) findings showed that the presence of scientific investigators near nesting ring-billed gulls (*Larus delawarensis*) caused adults and chicks to become more active and more aggressive towards each other. Birds that were more aggressive and active, survived less than those that were not disturbed by the researchers. Nimon et al. (1995) were able to determine that in the presence of humans, Adélie penguins (*Pygoscelis adeliae*) rapidly increased their heart rate, marking a disturbance to their normal heart rate patterns.

Though there has been extensive studies conducted on human disturbance to nesting birds, the limited work on human disturbance to bats has been done on large scale habitat disturbances (Gehrt and Chelsvig 2004, Swihart et al. 2006) and winter hibernacula disturbances (Hardin and Hassell 1970, Thomas et al. 1990, Speakman et al. 1991, Richter et al. 1993, Thomas 1995, Johnson et al. 1998). No work has been done on

the possible effects of human investigators disturbing maternity colonies during summer research projects.

While conducting research on bat roosting habits, it is common practice to collect data on the roost tree and apply a unique identifying aluminum tag which is often nailed into the roost tree, information such as DBH, tree height, roost height, canopy cover, and tree species is regularly collected and often times a vegetation sample plot is established surrounding the roost tree (Gardner et al. 1991, Kurta et al. 1996). These studies can be potentially disturbing to roosting female bats that occupy that tree. This disturbance can range from elevating stress levels from researcher proximity, causing animals to temporarily abandon torpor and therefore cause the animal to waste valuable energy that was otherwise being conserved (Wang 1989), or in extreme cases immediate abandonment of the tree causing the animals to become vulnerable to diurnal predators that they would not normally confront.

We looked at reproductively active female Indiana bats (*Myotis sodalis*), northern long-eared bats (*M. septentrionalis*), and little brown bats (*M. lucifugus*) to determine the affect of human disturbance on their thermoregulatory processes. Determining the affects of common research techniques will be valuable in understanding how they affect activity patterns or behaviors of these bats and in turn allow researchers to better interpret their results and design future studies to minimize researcher impact.

Our hypothesis was that our research conducted around roost trees containing reproductively active female *Myotis* bats would not disturb their thermoregulatory processes enough to raise their body temperature (T_b) out of torpor.

STUDY AREA

Bats were captured and tracked in seven different sites within Indiana bat, northern long-eared bat, and little brown bat distribution. In 2007, we captured and tracked bats at Camp Atterbury, Bartholomew, Johnson, and Brown Counties, located in central Indiana, Muscatatuck National Wildlife Refuge, Jackson County and Big Oaks National Wildlife Refuge, Jefferson, Ripley, and Jennings Counties, both in south-central Indiana, and Mammoth Caves National Park, Edmonson County, located in western Kentucky. Sites were selected based on previous *Myotis* captures in the areas (Montgomery Watson 1999, Whitaker and Gummer 2002).

In the summer of 2008, we captured and tracked bats in three different sites, Oakwood Bottoms, Jackson County and Union County Conservation Area, Union County both located in southern Illinois and Wilbur Wright Fish and Wildlife Area, Henry County, located in east-central Indiana. The sites in southern Illinois were selected based on previous surveys conducted (Carter et al. 2002, Carter 2003, Carter and Feldhamer 2005). Wilbur Wright FWA was chosen because of the presence of the Little Blue River running through the forested property which provided suitable Indiana bat habitat (Carter 2006).

METHODS

CAPTURE & RADIO-TELEMETRY

During the summer months of 2007 and 2008, bats were captured using monofilament mist nets (Gardner et al. 1989). Mist net height and width was selected

based on the specific location of the set. Previous surveys and preferred habitat types allowed us to select sites that targeted the three *Myotis* species (Montgomery Watson 1999, Whitaker and Gummer 2002, Carroll et al. 2002, Carter 2002, Carter 2003, Carter and Feldhamer 2005). Mist nets were erected near known roosts, over water sources and flight corridors (Gardner et al. 1989).

Temperature sensitive radiotransmitters (Model LB-2N and LB-2NT, Holohil Systems Ltd., Ont., Canada) were calibrated by either Holohil Systems, Ltd. or by our team of researchers by exposing the radiotransmitters to different temperatures (C°) and recording the corresponding inter-pulse period (IPP). The temperature sensitive radiotransmitters weighed approximately 0.45 grams and the first captured female Indiana bats, northern long-eared bats, and little brown bats were fitted with a transmitter. Torbot® liquid bonding cement (Torbot Group Inc., Cranston, RI) or Skin Bond Cement® (Smith & Nephews United Inc., Largo, FL) was used to attach the temperature sensitive radiotransmitter between the scapulae of the selected female *Myotis* bats (Holohil Systems Ltd. 2007).

Using a telemetry receiver (ATS R420 scanning receiver, Advanced Telemetry Systems, Inc., Isanti, MN) and a 3 element Yagi antenna (Wildlife Materials, Inc., Murphysboro, IL), bats were tracked daily to their roost tree until the transmitter was shed or the transmitter battery died. Upon locating the roost tree, data collection including DBH of the tree, tree height of the roost, roost height, percent canopy cover, roost tree species, type of roost tree (live, partially alive, or dead), and the type of roost (exfoliating bark, tree cavity, or tree crevice) were collected. To identify the roost tree for future reference a GPS waypoint was established (Garmin International, Inc., Olathe,

KS) and a unique aluminum numbered tree tag was nailed into the roost tree itself.

Collecting this information took approximately 10-20 min.

At the same time that we collected information for bat daily roosts and roost trees, waterproof enclosures (45 quart coolers) containing a datalogger (ATS R4500S Scientific Receiver/Datalogger, Advanced Telemetry Systems, Inc., Isanti, MN) fitted with a 3 or 5 element Yagi antenna (Wildlife Materials, Inc., Murhysboro, IL) were placed near the roost tree. The dataloggers continuously recorded the signal from the temperature sensitive radiotransmitters. Recorded data were downloaded via a laptop in the field as needed. If a bat moved to a roost tree that was out of range of the datalogger, the datalogger set-up was moved to a site nearer to the new roost tree.

ANALYSIS

Graphs depicting the relationship between IPP and temperature were generated by Holohil Systems, Ltd. or by our team for each individual radiotransmitter (See Carter et al.1999). A form of the quadratic equation derived from the graphs was used to convert the recorded IPP's into body temperatures of the animal (T_b). This relationship between temperature and IPP allowed us to convert the continuously recorded IPP's by the dataloggers into comprehensible units of temperature (Carter et al.1999).

Functions for T_b of the bats were calculated each day the radiotransmitter was operating. An equation created by Willis (2007) that sets a threshold for the onset of torpor in the animal was used to determine when the animals were and were not in torpor. This formula based on ambient temperature (T_a) and body mass (BM), allowed us to delineate the onset of torpor for the animal:

$$T_{b\text{-onset}} - 1 \text{ SE} = (0.041)BM + (0.040)T_a + 31.083$$

The resulting functions document when a bat entered and exited torpor throughout the day (Figure 1).

Functions for each bat were examined to identify disturbances and if disturbances occurred, if that disturbance of T_b resulted in a shift out of torpor for the animal. Sudden spikes in T_b of the animal were noted as a disturbance event, whether the bats stayed out of torpor or eventually went back into torpor after the disturbance. Additionally, we compared mean T_b function during times that researchers were present at the roost trees to other times to test if our presence resulted in a rise of T_b out of torpor.

RESULTS

Between the summer months of 2007 and 2008 a total of 96 radio transmitters were applied to female *Myotis* bats in 2007 and 2008, 81 total transmittered animals in 2007 and 15 in 2008. We located 199 roost trees using radio-telemetry in 2007 and 16 roost trees in 2008. From the 199 roost trees found in 2007, we recorded 591 trips to the roost trees throughout all the sites in 2007. The trips were defined as a researcher approaching a roost tree, regardless for whether this was the first or second incidence. In 2008 we recorded 72 trips to sites.

Out of the total 96 animals with temperature sensitive radiotransmitters, we recorded 175 total days of measurable T_b to measure disturbance. Of the total 175 days recorded, there were 162 days that resulted in no disturbance of the thermoregulatory processes of the roosting bats with transmitters (e.g. Figure 1). Of the 13 general T_b disturbances, only 8 resulted in the bat coming out of torpor (e.g. Figure 2). Only one

individual Indiana bat was disturbed, however her body temperature did not rise above torpor levels. We found 10 northern long-eared bats were disturbed with 6 coming out of torpor, from the 6 disturbances there were only 2 instances where the disturbance was at the same time as the researchers were present at the roost. Two little brown bats were disturbed sufficiently to come out of torpor. Indiana bats experienced a total of 56 days of no disturbance to their T_b , northern long-eared bats had 92 total days of undisturbed roosting, and little brown bats had 14 days of no disturbance to their T_b .

Using the 175 total days of data and finding the difference of the 26 days of no disturbance while researchers' exact time was known, we found there to be 149 days of data without our exact time known. With this number we used the total number of disturbances (13) and identified a general disturbance rate of 8.72%. From the days we know our exact time at the roost tree (26) we found there to be a 7.69% rate of disturbance when our researchers were present.

DISCUSSION

There were few instances where T_b of the female bats rose to above torpor levels in a short period of time. This suggests some form of disturbance. While the 13 instances of disturbances in the females could be due to the researchers being present at the roost tree, during the 26 times that the exact time that our researchers were at the tree was noted only twice did it result in a disturbance event for the roosting bats. This resulting 7.69% disturbance rate while our researchers were present is lower than the general 8.72% disturbance rate that occurred while the exact time of our researchers'

presence was not known. Additionally, the large number of days without thermoregulatory disturbance during researcher visits suggests that the female bats are not wasting valuable energy to raise their body temperature in response to our research activities.

The location of these maternity roost trees was typically within the forest interior, where human activity is typically low, and we sought to determine if the minimal contact bats had with humans while roosting affected their ability to maintain torpid temperatures in their daily roost. Other studies find that many species of wildlife have different reactions to human activity, from adverse reactions to no apparent responses. Negative reactions in wildlife to human presence, such as increased heart rate in mountain sheep (*Ovis canadensis*) and Adélie penguins (MacArthur et. al 1982, Nimon et. al 1995), displacing chamois (*Rupicapra rupicapra*) from high nutrition areas (Hamr 1988), and decreasing moose (*Alces americanus*) abundance in the presence of human trails (Ferguson and Keith 1982) show that humans can negatively alter wildlife behavior and ecology. Human activity does not always cause negative disturbance to wildlife. There may not be an immediate effect as with American martens (*Martes americana*), that do not respond to off-highway vehicles (Zielinski et al. 2008). Another way humans can negatively impact one aspect of a population and not have an effect is for common sandpipers (*Actitis hypoleucos*), where a reduced breeding population size occurs in the presence of human disturbance, but through redistribution there is no effect to the breeding success of the remaining breeding individuals (Yalden 1992).

During the summer months when female bats are pregnant, lactating or post-lactating, conserving energy for the development of young is of vital importance. If our

activities near the roost tree cause females to expend additional energy by causing them to come out of torpor prematurely, we could be negatively impacting the adult females and potentially their reproductive success. Our results for disturbance rate while at the roost tree is lower than a general rate for an animal coming out of torpor. Thus, we suggest that researchers' activities around the roost tree may continue without having an effect on the reproductive females' thermoregulatory processes. Since these bats are roosting within the forest where other wildlife may happen to pass near the roost tree and where birds such as wood peckers may make noises and vibrations on roosts, the bats within the maternity colony may regard our movements and activities below their roost as no more than that of the native wildlife common to their forest.

MANAGEMENT IMPLICATIONS

By conducting this study we were able to determine that the common research techniques conducted on daily maternity roost trees had no negative thermoregulatory effects on reproductively active female *Myotis* bats. With these results, researchers can continue needed research without fear of ill effects to breeding females and their reproductive success.

To determine if there are any other types of negative effects, besides thermoregulatory processes, on roosting female bats it is our recommendation that behavioral response studies such as, heart rate analysis and roost switching due to disturbances be conducted to fully understand the true effects of our presence and activities near the roost tree have on pregnant, lactating and post-lactating female bats, along with their reproductive success.

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Figure 1. An activity graph showing a pregnant Indiana bat (*Myotis sodalis*) arriving at her roost, then entering torpor, raising her body temperature out of torpor, maintaining an active state, and then leaving the roost.

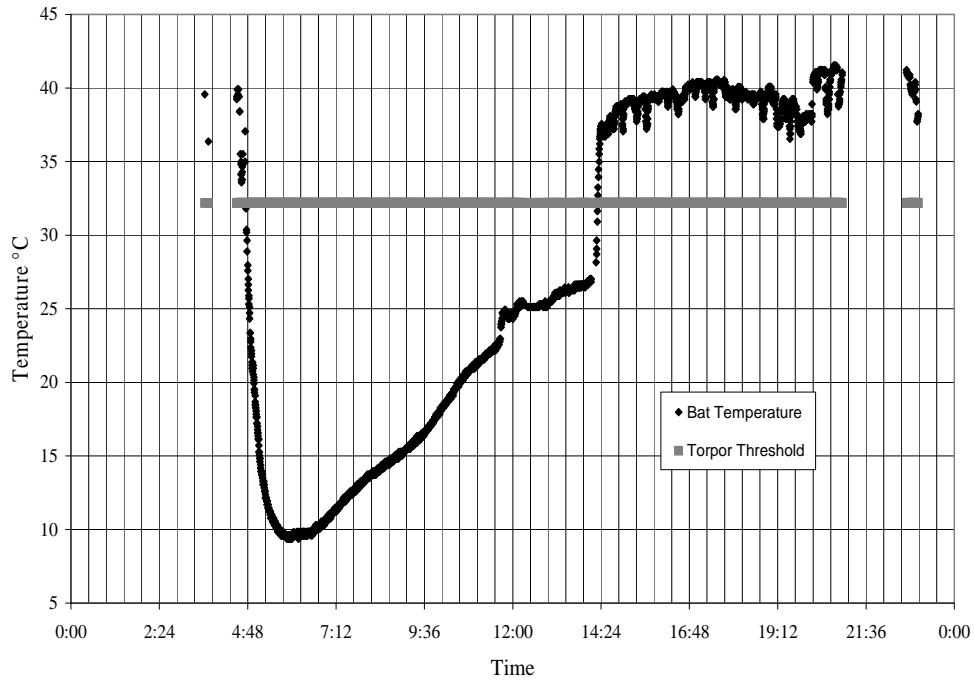


Figure 2. An activity graph showing a lactating northern long-eared bat (*Myotis septentrionalis*) at her roost, then raising and lowering her body temperature above and below the torpor threshold during a 24 h period.

