Use of Temperature-sensitive Transmitters to Monitor the Temperature Profiles of Hibernating Bats Affected with White-Nose Syndrome

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Abstract - In temperate ecosystems, hibernation allows bats to survive long periods of limited prey and water availability during colder months. Despite the extended amount of time some bats spend in hibernation, researchers have only recently been able to study the hibernation ecology of bats under natural conditions. With the emergence of white-nose syndrome (WNS), a mysterious disease presently killing large numbers of bats during the hibernation period in the northeastern United States, expanding our knowledge of hibernation ecology and natural history has become more crucial. To collect such data, we used temperature-sensitive radio transmitters and data loggers to monitor the skin temperatures (T_{sk}) of 6 bats (5 *Myotis lucifugus* [Little Brown Bat], and 1 *Myotis septentrionalis* [Northern Long-eared Bat]) hibernating in Mount Aeolus Cave, VT in late winter 2008. We recorded T_{sk} every 14 minutes for the life of the transmitters. We were able to monitor T_{sk} from near ambient temperatures to above 30 ºC. Arousals occurred immediately before the signals were lost and at a time of increased numbers of bats observed on the landscape, thereby suggesting the emergence (and subsequent death) of bats. Our observations provide first data on the hibernating ecology of WNS-affected bats under natural conditions.

Introduction

Bats have the ability to adjust their body temperature in relation to environmental conditions (Hock 1951, Stones and Wiebers 1965). During late fall and winter, when food resources are severely restricted, some temperate-zone bats enter caves and mines for hibernation. During this period, bats can lower their body temperature (T_b) to near ambient temperatures, thereby realizing massive energy savings (Studier and O'Farrell 1976). However, rather than maintaining a low T_b throughout the winter, bats undergo periodic arousals that allows a temporary increase to euthermic body temperature (Brack and Twente 1985, Hardin and Hassel 1970, Twente et al. 1985). Despite the short collective duration of arousal, this warming and subsequent activity may constitute almost 90% of the total energy used by *Myotis lucifugus* Le Conte (Little Brown Bat) over the season of hibernation (Thomas 1995a, Thomas et al. 1990). Although clearly energetically expensive, periodic arousal is

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universal in all hibernators (Lyman et al. 1982). Therefore, any factors that affect the number of arousals may have large influences on the ability to survive the hibernation period (Boyles and Brack 2009).

Although periodic spontaneous arousals are normal, other factors such as human visitation of hibernacula also can elicit arousal from hibernation (Speakman et al 1991, Thomas 1995b). Early conservation efforts of bats focused heavily on reducing or eliminating anthropogenic disturbances during hibernation (US Fish and Wildlife Service 1999). Because of the sensitivity of hibernating bats to disturbance and the logistical challenges associated with accessing caves, previous research on the hibernation ecology of bats has largely been conducted via lab experiments (e.g., French 1985, Thomas et al. 1990). Although laboratory studies have provided important information, captive animals often exhibit significantly different torpor patterns than free-living animals, so field studies are essential (Geiser et al. 2000).

Recently, white-nose syndrome (WNS) has emerged as a serious health issue for bat populations inhabiting the northeastern and mid-Atlantic portions of the United States. Although ultimate causal agents are not fully ascertained, affected bats are characterized by the presence of white fungal growth (Genus: *Geomyces*) on exposed skin during hibernation (Blehert et al. 2009). At impacted sites, hibernating populations have experienced dramatic declines of ≥95%. Physical examination of dead and symptomatic bats has shown that affected individuals have very little remaining body fat (A. Hicks, New York State Department of Environmental Conservation, Albany, NY, 2008 pers. comm.). This observation has prompted some researchers to speculate that low body fat reserves arise as a consequence of bats entering hibernation without sufficient fat reserves to survive the winter. Conversely, it also is hypothesized that bats are exhibiting altered torpor patterns (e.g., more frequent arousals, longer arousal periods, or elevated torpid body temperatures) possibly related to behavioral or immune response to the fungus or other factors associated with WNS (Boyles and Willis 2010; 2008 emergency science meeting on white-nose syndrome—synopsis available online at http://www.batcon.org/pdfs/whitenose/WNSMtgRptFinal2.pdf).

To date, virtually no data on the hibernating body temperatures or frequency and duration of periodic arousals are available for North American bat species hibernating under natural conditions. Although use of temperature-sensitive transmitters and data-logging receivers has enabled researchers to study the ecophysiology of bats during the non-hibernating season under field conditions (Chruszcz and Barclay 2002, Hamilton and Barclay 1994), only Park et al. (2000) has used this technique to research hibernating bats. Therefore, our objectives were to test the effectiveness of the technique with transmitters small enough to be mounted on *Myotis*-sized bats (<10 g). As WNS-affected bats have been shown to not arouse from torpor during disturbance events (J. Boyles, Indiana State University, Terre Haute, IN, 2008 pers. comm.), we also tested the hypothesis that WNS-affected bats are unable to arouse from torpor as a result of reduced fat reserves during late winter.

Field-site Description

Our study was conducted at Mount Aeolus Cave, located in Bennington County, southwestern Vermont. Mount Aeolus Cave is a natural cave with a large number of hibernating bats. Although a count in 2003 estimated 23,000 bats, mortality observed at the site during the winter of 2008–2009 following WNS presence suggested a hibernating population between 200,000 and 300,000 bats. This cave was selected for our project because of the large number of easily accessible bats, presence of WNS-affected bats, and the presence of a cave gate that would minimize potential human disturbance and serve to protect our equipment.

Methods

We calibrated transmitters (Holohil Systems, Ltd., Carp, ON, Canada, model LB2; weight = 0.52 g) for temperature monitoring using a series of five water baths (35.2 ºC, 23.8 ºC, 15.7 ºC, 10.8 ºC, and 3.3 ºC). Transmitters were activated, placed in the weighted fingers of standard latex gloves, and left in each water bath for ten minutes to allow sufficient time to reach equilibrium with the water, after which they were scanned using an ATS R4500 Receiver/Datalogger (Advanced Telemetry Systems, Inc. Asanti, MN) to record the inter-pulse interval. Each transmitter was then moved to another water bath and the process was repeated until the inter-pulse interval of the transmitters had been recorded in each water bath. Our calibration procedure resulted in a relationship between temperature and interpulse interval that was described using linear regression analysis (all r^2 values < 0.99).

Upon arrival at the site on 10 March 2008, we randomly selected 6 bats from throughout the main entry room of the cave. We removed bats from the cave, weighed them with a spring scale, and then sexed and measured each for right forearm length (RFA). Additionally, we noted the presence of fungal hyphae and inability of bats to arouse during the entire handling process (both of which have been linked to WNS-affected bats). After trimming a small patch of fur between the scapula of each bat, we attached transmitters using non-toxic Skin-Bond® cement (Smith and Nephew, Inc., London, UK). We then returned bats to the cluster where they originally were located. We placed a 7th transmitter within 1 m of the ceiling on a rock in the main chamber to monitor cave temperature. The receiver was attached to a 12-volt power source and deployed ≈15 m inside the cave entrance, with an antenna running back to the main roosting chamber. We set the receiver to scan for the frequency of each transmitter. Our initial testing at deployment allowed for detection of bats within the area where they were originally collected; however, detection did not extend appreciably outside the original room. Once a frequency was detected, the receiver recorded the interpulse interval for 2 minutes before searching for the next frequency, thereby allowing a transmitter to be sampled every 14 minutes. If the signal was not detected, the receiver switched to the next frequency for monitoring. As the number of transmitters being detected decreased, the amount of time between monitoring a detected frequency also decreased. All frequencies were scanned

throughout the entire life of the project in hopes of reacquiring signals from bats that were no longer detected.

Every 4–7 days, the receiver and external battery were replaced. We took the receiver to the lab, and the data were uploaded to a computer. These text files included time, interpulse interval, and signal strength for each detected frequency. We then determined values of skin temperature (T_{sk}) using the linear regression equation determined for each transmitter during the calibration process. We removed data from analysis at times when temperature output went below 0 ºC because we viewed this as a dead or dying battery and therefore not an accurate temperature reading. Onset of torpor was defined as a 10 ºC change from normal maximum T_{sk}. Using these endpoints, we determined the time spent in arousal, the time spent in torpor, and the average temperature of torpor.

Results

Five transmitters were attached to Little Brown Bats (2 males: 3 females), and 1 transmitter was attached to a female *Myotis septentrionalis* Trouessart (Northern Long-eared Bat) (Table 1). Bats varied in their body condition and the presence of fungal hyphae on exposed skin surfaces (Table 1). Four of the six bats warmed up after release and then entered torpor within several hours, but one individual (MYLU-M-1) allowed its body temperature to fall close to ambient temperature soon after release (Fig. 1). The time spent in arousal, duration of torpor bouts, and average T_{sk} during torpor varied widely among bats (Table 2). Additional arousals were detected immediately before the signal was lost (Fig. 1). There was no indication that changing the batteries resulted in any disturbance to the bats. Monitoring was terminated on 31 March 2008 when the last bat being detected (bat MYSE-F-1) was found dead on the cave floor.

Discussion

Previous work at a WNS-affected cave in New York showed that some bats did not arouse from torpor despite extensive disturbance (J. Boyles, 2008 pers. comm.); however, we documented multiple arousals from undisturbed bats throughout the study period. Four bats aroused from torpor to T_{sk} greater than

Table 1. Identification and body condition data for the six bats whose body temperatures were monitored during March 2008 at Mount Aeolus Cave, VT. Behavior was noted as whether bats aroused during the handling process. Fungal presence or absence was determined by gross visual examination at the time of first handling. W = weight (g), RFA = right forearm length (mm), BCI = body condition index (mass / FA).

Code	Species	Sex	W	RFA	BCI	Fungal status and behavior
MYLU-F-1	Little Brown Bat	F	6.0	37	0.16	Fungus absent; bat not arousing
MYLU-F-2	Little Brown Bat	F	6.6	38	0.17	Fungus absent; bat not arousing
MYLU-F-3	Little Brown Bat	F	7.0	39	0.18	Fungus absent; bat not arousing
MYLU-M-1	Little Brown Bat	M	6.6	38	0.17	Fungus absent; bat not arousing
MYLU-M-2	Little Brown Bat	M	6.1	38	0.16	Fungus present
MYSE-F-1	Northern Long-eared Bat	F	6.7	32	0.21	Fungus present

30 °C before the transmitter signal was lost. Although the signal loss could have resulted from myriad reasons, the relatively clean temperature profile up to that point, the abruptness of warming indicating an arousal, and the timing coinciding with an increase in reports of bats found flying outside the cave (R. Smith and S.R. Darling, pers. obs.) suggests that bats had likely aroused and emerged from the cave. Our data suggest that bats affected with WNS maintain their ability to arouse, but may adjust the threshold of disturbance that must be attained before an arousal is initiated in response to the need for energy conservation or immune condition. As arousal is a very energetically expensive process,
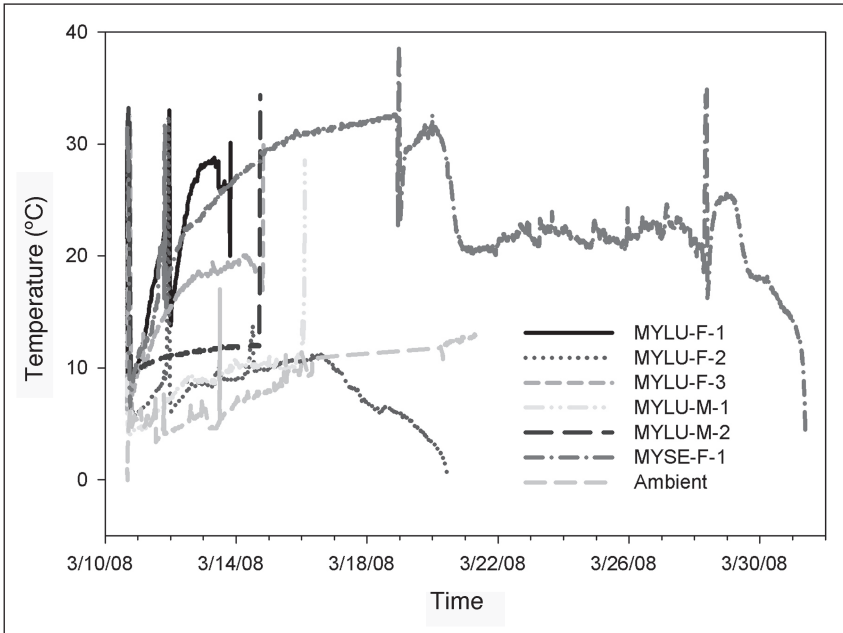

Figure 1. Temperature profiles of 6 bats at Mount Aeolus Cave, VT in March 2008. Temperature-sensitive transmitters were attached to hibernating bats on March 10, 2008, and the temperature of each bat was monitored once every 14 minutes for life of the transmitter. One transmitter was placed in the cave to monitor ambient temperature.

Table 2. Summary of the length of arousal, the lengths of the torpor bout, and the average skin temperature (T_{sk}) during torpor bouts for 6 WNS-affected bats in Mount Aeolus Cave, VT. These bats had temperature-sensitive transmitters attached to their backs, and monitoring occurred in March 2008.

Bat	Length of arousals (hours)	Length of torpor bout (hours)	Average T_{sk} during torpor (°C)
MYLU-F-1	0.93	30.2 and 15.2	14.5 and 20.4
MYLU-F-2	1.4	28.1	7.7
MYLU-F-3	n/a	96.5	16.5
MYLU-M-1	n/a	130.3	8.7
MYLU-M-2	n/a	90.3	11.3
MYSE-F-1	0.93 and 173.7	25.8, 36.9, and 184.47	13.2, 22.6, and 21.7

limiting these periods when only biologically necessary would assist in maintaining fat reserves. Further research is needed to assess the differences in the temperature profiles of affected and unaffected bats.

An individual-based, bioenergetic model has been used to investigate the conditions that mimic the mortality events observed at WNS-affected hibernacula. Results of the model conformed to observed patterns in mortality when arousal lengths were lengthened or torpor bouts were shortened (Boyles and Willis 2010). Our results showed relatively short arousal lengths, thereby providing support for the fact that shortened torpor bouts were responsible for the WNS-affected bat mortality. However, before this can be definitive, we need to determine the torpor-bout length in non WNS-affected bats, and more data are needed from both unaffected and affected bats early in hibernation to see if this trend continues.

We were able to document hibernation physiology of bats using this methodology. Although our sample sizes were small, some interesting trends were observed. First, the length of torpor bouts and arousals was highly variable but typically shorter than the length of torpor bouts observed by Park et al. (2000). Torpor T_{sk} was higher than T_a throughout the study, a finding consistent with Park et al. (2000). It is generally assumed that bats should maximize torpor-bout length and minimize torpor T_{sk} to maximize energy savings. However, our data show that bats are not taking full advantage of the potential benefit of long torpor bouts at cold T_b. Therefore, other factors besides maximizing energy savings are important in determining the hibernation ecology of these bats (Boyles et al. 2007). Future work will determine whether this difference is a result of species differences or if this is an impact of WNS.

Transmitters were attached to bats with various symptoms of WNS. Typically, WNS is determined by the presence of the fungal hyphae on the skin, while other factors such as unresponsiveness to disturbance are less important. While our sample sizes are small, we found no differences in temperature profiles or apparent survivability among bats with varying symptoms of WNS. Apparently, once some bats at a particular site are affected with WNS, it should be assumed that the entire hibernating population is likely to be affected. This assumption does not mean that all bats are affected at these sites; rather it suggests that the status of bats cannot be determined simply by gross visual examination in the field. Future work at hibernation sites should use multiple criteria to determine whether bats are affected with WNS.

This study examined the feasibility of using small temperature-sensitive transmitters (0.52 g) and data-logging receivers to monitor T_{sk} and arousal patterns of hibernating bats. While Park et al. (2000) had demonstrated the ability to use this technique, their study animals and transmitters were much larger than ours. Use of the technique allows T_{sk} to be collected at near real-time levels, thereby reducing the amount and impact of data-loss from bats moving out of the cave. Additionally, transmitters are much smaller than other data loggers, thereby minimizing the impact on studied bats. However, transmitters are currently not available that can monitor the entire hibernating period, thereby requiring successive trips into a site to gain insight into the entire season. Secondly, detection of signals within a cave was much lower than

expected. In appropriate situations (i.e., smaller caves and mines), this system can effectively provide T_{sk} data on bats under natural circumstances.

Small euthermic mammals have high thermal conductance and high T_{sk}, which suggests that temperature-sensitive transmitters glued to the skin should accurately reflect body temperature for small animals (Audet and Thomas 1997, Barclay et al. 1996). However, Willis and Brigham (2003) showed that T_{sk} underestimated high T_b and overestimated low T_b. Additionally, there was no significant difference between T_{sk} and T_b in bats that were clustered during torpor, but these differences were relatively small and the same trends in temperature profiles were apparent in both measures (Willis and Brigham 2003). These limitations of temperature-sensitive transmitters may preclude their use in studies attempting to quantify energy expenditure precisely. However, this method can provide information on the hibernation physiology of bats under natural circumstances; such data will provide useful insight into the studies of the cause of mortality in WNS-affected bats.

This study illustrated the effectiveness of small temperature-sensitive transmitters and data logging receivers for measuring T_{sk} and arousal profiles of hibernating bats. The results showed unexpected hibernating patterns for these bats, with shorter arousals and torpor bouts than expected. Additionally, bats retained the ability to arouse even when immediately before apparent death. Future work can use this technique to address possible differences in the hibernating ecology of bats with and without WNS. Such a comparison might provide insight into the causative agent behind WNS and/or help develop possible mitigation measures for affected bats.

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