

**Body temperature and body mass of hibernating little brown bats *Myotis lucifugus*
in hibernacula affected by White-nose syndrome**

Jonathan J. STORM* and Justin G. BOYLES

Abstract

Populations of hibernating bats in the northeastern United States are being decimated by White-nose Syndrome (WNS). Although the ultimate cause of death is unknown, it may be related to the premature depletion of fat reserves. Previous research has suggested the cause of starvation is the namesake white fungus of WNS, *Geomyces destructans* Blehert and Gargas, 2009. During hibernation, the immune system is suppressed; however, it is possible that some immune function may be maintained by retaining an elevated body temperature (T_b) during hibernation. Although an elevated T_b may facilitate an immune response, it also accelerates the depletion of fat stores. We sought to determine if little brown bats *Myotis lucifugus* Le Conte, 1831 hibernating in WNS-affected hibernacula have an elevated T_b and reduced fat stores, relative to bats not affected by WNS. We found that WNS-affected *M. lucifugus* maintain a slightly, but significantly, higher skin temperature (T_{skin}), relative to surrounding rock temperature, than do WNS-unaffected Indiana bats *M. sodalis* Miller and Allen 1928 from Indiana. However, the difference in T_{skin} is very small and we argue that it is unlikely to explain the premature starvation seen in WNS-affected bats. We also report that WNS-affected *M. lucifugus* weigh significantly less than *M. lucifugus* from a hibernaculum outside of the WNS region.

(JJS) Division of Natural Sciences and Engineering, University of South Carolina Upstate,
Spartanburg, 29301 USA

(JGB) Center for North American Bat Research and Conservation, Department of Ecology and
Organismal Biology, Indiana State University, Terre Haute, 47809 USA

Present Address for (JGB): Department of Zoology and Entomology, University of Pretoria,
Pretoria, 0002, South Africa

Corresponding Author: *jstorm@uscupstate.edu

Keywords:

Geomyces destructans; hibernation; immune response; psychrophilic fungus; thermal preference

Running Title: White-nose syndrome in bats

Introduction

Populations of hibernating bats in the northeastern United States are being decimated by White-nose Syndrome (WNS). The characteristic feature of WNS is a white fungus that covers the muzzle, ears, and/or wing membranes of affected bats. The fungus was first discovered at 4 caves during the winter of 2006-2007 in New York State and since then, has spread rapidly to additional caves throughout most of the northeastern and mid-Atlantic states (Blehert et al., 2009). The fungus directly associated with WNS, *Geomyces destructans* Blehert and Gargas, 2009 (Gargas et al., 2009), is a recently described psychrophilic (cold-loving) species that invades the living tissue of hibernating bats (Meteyer et al., 2009). Even for WNS-affected bats that survive winter, infection with *G. destructans* can inflict lasting damage to the wing membranes and impair summer foraging (Reichard and Kunz, 2009). The proximate cause of death in many WNS-affected bats may be starvation, but the ultimate cause is yet to be determined. Although preliminary, infection by *G. destructans* likely plays a role in this starvation, either directly or indirectly. Three possible links between the fungus and starvation have been hypothesized: 1) WNS affects summer feeding or physiology, causing bats to enter hibernation underweight, 2) affected bats alter the expression of hibernation to mount an immune response, or 3) epithelial irritation associated with the fungus causes bats to alter hibernation patterns, thereby increasing energy expenditure (Boyles and Willis, 2010).

Using a modeling approach, Boyles and Willis (2010) concluded that mortality from WNS is likely due to changes in hibernation physiology, not pre-hibernal body condition. During hibernation, the immune system of most hibernators is partially or completely arrested (Carey et al., 2003). Following an immune challenge, the pattern of arousal from hibernation suggests a need to periodically raise body temperature (T_b) to mount an immune response against pathogens

(Prendergast et al., 2002; Luis and Hudson, 2006). Assuming the immune system of hibernating bats is suppressed as it is in other hibernating mammals, WNS may trigger a tradeoff in hibernation strategies. On one hand, affected bats must remain torpid to conserve energy, while on the other hand, they must maintain a sufficiently high T_b , or arouse often enough, to mount an immune response. Fighting an infection likely presents a more immediate priority than conserving energy; therefore, WNS-affected bats may reduce the expression of hibernation to mount an immune response.

Several hypotheses have been proposed for how changes in hibernation physiology lead to mortality. WNS-affected bats may: 1) arouse more frequently than unaffected bats; 2) remain euthermic for longer periods during each arousal; or 3) increase T_b during hibernation. The first two hypotheses have been modeled and discussed elsewhere (Boyles and Willis, 2010). The purpose of this study was to evaluate the likelihood of the third hypothesis. We sought evidence about whether WNS-affected bats thermoregulate at a higher T_b relative to the surrounding rock temperature than unaffected bats. An elevated T_b would lead to a higher torpid metabolic rate and thus could lead to an increased rate of fat metabolism during hibernation.

There is evidence that a majority of, but not all, WNS-affected bats are underweight at the time of death (Blehert et al., 2009); however, there are no published data on whether living WNS-affected bats are underweight during hibernation. If WNS-affected bats are underweight during hibernation, this would suggest that body fat is being used too quickly throughout winter, not just immediately before death. Thus, we also compared energy (fat) reserves of affected and unaffected bats by comparing the mass of WNS-affected bats in New York State to unaffected bats from Ohio (Boyles et al., 2007). If WNS-affected bats are underweight, but survive hibernation, they may still be susceptible to death following spring emergence from hibernation.

Material and methods

Skin Temperature of Myotis lucifugus

We took thermal images of naturally hibernating clusters of little brown bats *Myotis lucifugus* Le Conte, 1831 in 2 mines (Williams Lake Mine and Williams Hotel Mine) in Ulster County, New York, USA on 12-13 February 2008. All images were taken using a ThermaCAM PM575 (FLIR Systems, North Billerica, MA, USA) thermal imaging camera. Images were recorded as digital files with ± 0.1 °C resolution. Upon entering hibernacula, we took images of clusters from below so the bats' lightly-furred faces comprised the majority of most images. To ensure that only hibernating individuals were included, we avoided clusters with individuals that were noticeably active or had much warmer skin temperatures (T_{skin}) than surrounding bats. We used matching photographic images to determine the number of individuals in each cluster. We followed the methods of Boyles et al. (2008) so that our data are comparable to those collected on Indiana bats *M. sodalis* Miller and Allen 1928 in hibernacula within southern Indiana, USA. *Myotis sodalis* and *M. lucifugus* are closely related (Stadelman et al., 2007) and share many physiological and ecological traits (Whitaker and Hamilton, 1998), so we suggest that a comparison between the two species, while not perfect, still provides valuable data regarding WNS. We recorded whether any bats in a cluster were visibly affected by WNS, but subsequent research has shown visible clues to be a poor indicator of WNS status (Meteyer et al., 2009) and that most bats in affected caves likely have WNS.

We analyzed thermal images using IRwin Research 2.01 software (FLIR Systems, North Billerica, MA, USA). We calculated the mean and standard deviation of cluster temperature within a polygon traced around each cluster. Because the lightly-furred faces of bats constituted the majority of the picture, we were essentially measuring T_{skin} of the bats in the cluster. Skin

temperature closely reflects T_b in small mammals (Dausmann, 2005), including torpid bats (Willis and Brigham, 2003), so we considered the mean temperature within the polygon to be a proxy of the mean T_b of individuals in the cluster (Boyles et al., 2008). We calculated mean rock temperature in an irregularly-shaped polygon adjacent to, but not touching, each cluster. The size of each polygon could not be standardized because thermal images of clusters were taken at varying distances. We used the difference between mean cluster and mean rock temperature as the variable of interest in our analyses (Boyles et al., 2008).

We used an analysis of covariance (ANCOVA) in Statistica 6.0 to determine if *M. lucifugus* in WNS-affected hibernacula maintained a higher T_b relative to rock temperature than WNS-unaffected *M. sodalis* in Indiana. In the ANCOVA, we considered population (Indiana or New York) as the independent variable, difference between cluster temperature and rock temperature as the dependent variable, and cluster size and absolute rock temperature as covariates. Cluster size was log-transformed to account for the large variation in cluster size. If WNS-affected bats maintain a higher T_b relative to ambient (rock) temperature, this could explain (in part) why bats prematurely exhaust energy reserves.

Mass of Myotis lucifugus

On 13 February 2008, we weighed a sample of *M. lucifugus* from the Williams Lake Mine using a digital balance (Scout II; Ohaus, Pine Brook, NJ, USA) accurate to 0.1 g. Mass alone (or mass corrected for forearm length) is not always an effective indicator of fat mass, but body mass and fat mass are correlated in *M. lucifugus* (Kunz et al., 1998). Following data collection, we released bats on a surface near where they were obtained. Data were compared to measurements taken on *M. lucifugus* from a WNS-unaffected limestone mine in Preble County,

Ohio, USA, on 12 February 2007 (Boyles et al., 2007) and 9 February 2008. During autumn and winter of 2007-2008, the average temperature at the New York and Ohio sites were comparable (Fig. 1). Thus, *M. lucifugus* hibernating in New York and Ohio should experience a similar length of hibernation, making comparisons of fat mass between sites feasible and biologically relevant. We tested for differences in body mass of WNS-affected bats from New York and WNS-unaffected bats from Ohio using *t*-tests. All procedures were approved by the Indiana State University Animal Care and Use Committee (Protocol: JOW/JB 9-18-2006).

Results

We took thermal images of 97 individuals or clusters of *M. lucifugus* in 2 WNS-affected hibernacula. The average cluster size was 10.8 ± 25.3 individuals (mean \pm SD) with a range of 1-169. The average rock temperature at the site of hibernation was 4.8 ± 1.5 °C. We compared data from these images to thermal data collected on 202 hibernating *M. sodalis* clusters during January-February 2007 in Indiana (Boyles et al., 2008). Interestingly, the difference between T_{skin} and rock temperature was significantly greater for WNS-affected *M. lucifugus* than for unaffected *M. sodalis* ($p < 0.0001$) suggesting they were defending a higher T_b relative to the surrounding rock than were *M. sodalis*. *Myotis lucifugus* maintained a T_{skin} 0.15 ± 0.31 °C above rock temperature while the T_{skin} of *M. sodalis* was, on average, -0.25 ± 0.29 °C below rock temperature. A T_{skin} below rock temperature is typically caused by air movement in the hibernaculum or variable ambient temperatures and differential rates of heating and cooling between clusters and the surrounding rock.

We weighed 100 WNS-affected *M. lucifugus* (52 males: 48 females) in New York during February 2008, 443 (169 males: 274 females) WNS-unaffected *M. lucifugus* in Ohio during

February 2007, and an additional 64 in Ohio during February 2008 (34 males: 30 females; Fig. 2). Averaged across years, males from Ohio had a body mass of 7.2 ± 0.5 g (mean \pm SD), while males from New York weighed 6.2 ± 0.5 g. Females from Ohio had a mean body mass of 8.0 ± 0.7 g and females from New York weighed 6.5 ± 0.5 g. Both male and female *M. lucifugus* from New York had significantly less mass than *M. lucifugus* from Ohio (*t*-tests; $p < 0.0001$ in all comparisons).

Discussion

Our data represent the first published assessments of T_b and body mass during hibernation for WNS-affected bats. We found that *M. lucifugus* with WNS had a significantly higher T_{skin} relative to surrounding rock temperature than WNS-unaffected bats. Although we did not collect microclimate data, we think the most likely cause of this T_{skin} difference is microclimate differences between hibernacula in New York and Indiana. For the WNS-unaffected bats in Indiana caves, cluster temperatures were often below rock temperature, suggesting the microclimate within these hibernacula is more variable than in New York mines. Although the elevated T_{skin} of WNS bats may result from adaptive physiological mechanisms aimed at boosting the immune system, we argue that the difference between T_{skin} and rock temperature in WNS-affected bats is far too minor (the largest difference we recorded was 0.7 °C) to explain the mortality seen in WNS-affected populations. Unfortunately, available data on metabolic rates and torpor patterns have low resolution (i.e., measurements usually taken every 5 °C). Therefore, any attempt at modeling the effect of such a small increase in T_{skin} on survival would be dubious. Thus, we are left making relatively qualitative comparisons between the likelihood of WNS causing an increased T_b during hibernation and changing patterns of arousals

and torpor bouts (Boyles and Willis, 2010). Given the relatively small increase in T_{skin} relative to rock temperature in WNS-affected bats, we suggest it is unlikely that bats challenge the WNS infection by maintaining an elevated T_b . However, this is not based on a quantitative comparison with results from other analyses (Boyles and Willis, 2010), so our conclusions are preliminary.

Our body mass measurements for *M. lucifugus* represent some of the first empirical evidence that hibernating bats affected by WNS are underweight during hibernation. We found that mean mass of hibernating *M. lucifugus* in Ohio was 1.03 and 1.43 g higher than New York bats for males and females, respectively. These results are in agreement with reduced fat stores found in necropsied WNS bats (Blehert et al., 2009). The drastic differences in body mass between populations, in addition to reduced fat stores found in newly dead bats (Blehert et al., 2009), solidify the suspicion that WNS leads to diminished energy reserves in affected bats. Our mass data suggest that WNS-affected bats expend energy too quickly throughout the winter as opposed to expending their fat reserves in a short time period prior to death. Thus, in the absence of a total “cure” for WNS, mitigation efforts aimed at reducing the rate of energy expenditure in WNS-affected bats (e.g. Boyles and Willis, 2010) may be the most direct method to increase survival. Our data should be viewed with caution for several reasons. First, we did not measure the body mass of WNS-affected bats in New York at the start of hibernation. Thus, we cannot rule out the possibility that WNS-affected bats were simply lighter at the beginning of the hibernation season. Likewise, while environmental temperatures were similar between the 2 sites (Fig. 1), we do not know whether the hibernation period began near the same time for WNS-affected bats in New York and non-WNS bats in Ohio. Finally, we do not have data on the fatty acid composition of foods consumed by WNS-affected bats prior to hibernation. This may be important, as previous work has shown that the level of polyunsaturated fatty acids in the diet

can influence the expression of torpor in hibernating mammals (Frank, 2008). Although these constraints may limit the interpretation of our data, we argue that comparisons of WNS-affected bats to individuals outside the range of WNS may be the best method of comparison until effective methods for detecting WNS in the field are developed.

Future laboratory studies will likely examine the relationship between WNS and T_b (or T_{skin}) during hibernation using more controlled experiments than our preliminary field research. Our data suggest that starvation in WNS-affected bats is likely not the result of elevated T_b during hibernation. We suggest instead that changes in hibernation patterns (e.g. increased arousal frequency and/or duration of euthermia; Boyles and Willis, 2010) are more likely to explain starvation of WNS-affected bats. While our non-manipulative methods mean the results are not conclusive, we think they can still offer valuable information on the areas where limited funds should be spent as we move forward with research on this rapidly spreading disease. As such, we suggest that future studies of WNS-affected bats focus on arousal frequency, level of activity during arousals (e.g. feeding) and the duration of euthermia over an entire hibernation period (e.g. thermal imaging cameras placed permanently in hibernacula).

Acknowledgements

We thank A. Hicks of the New York Department of Environmental Conservation (NYDEC) for organizing trips into WNS-affected mines, and A. Hicks, J. Reichard, and M. Moore for assistance collecting data. Funding was provided by the Indiana State University Center for North American Bat Research and Conservation.

References

- Blehert, D. S., Hicks, A. C., Behr, M., Meteyer, C. U., Berlowski-Zier, B. M., Buckles, E. L., Coleman, J. T. H., Darling, S. R., Gargas, A., Niver, R., Okoniewski, J. C., Rudd, R. J., and Stone, W. B. 2009. Bat white-nose syndrome: An emerging fungal pathogen? *Science* 323: 227-227.
- Boyles, J. G. and Willis, C. K. R. 2010. Could localized warm areas inside cold caves reduce mortality of hibernating bats affected by white nose syndrome? *Frontiers in Ecology and the Environment* 8: 92-98.
- Boyles, J. G., Dunbar, M. B., Storm, J. J. and Brack, V. 2007. Energy availability influences microclimate selection of hibernating bats. *Journal of Experimental Biology* 210: 4345-4350.
- Boyles, J. G., Storm, J. J. and Brack, V. 2008. Thermal benefits of clustering during hibernation: a field test of competing hypotheses on *Myotis sodalis*. *Functional Ecology* 22: 632-636.
- Carey, H. V., Andrews, M. T. and Martin, S. L. 2003. Mammalian hibernation: Cellular and molecular responses to depressed metabolism and low temperature. *Physiological Reviews* 83: 1153-1181.
- Dausmann, K. H. 2005. Measuring body temperature in the field - evaluation of external vs. implanted transmitters in a small mammal. *Journal of Thermal Biology* 30: 195-202.
- Frank, C. L., Carpovich, S. and Barnes, B. M. 2008. Dietary fatty acid composition and the hibernation patterns in free-ranging arctic ground squirrels. *Physiological and Biochemical Zoology* 81: 486-495.
- Gargas, A., Trest, M. T., Christensen, M., Volk, T. J. and Blehert, D. S. 2009. *Geomyces destructans* sp nov associated with bat white-nose syndrome. *Mycotaxon* 108: 147-154.

- Hall, E. R. 2001. The Mammals of North America, 2nd Edition. Blackburn Press, Caldwell: 1-1280.
- Kunz, T. H., Wrazen, J. A. and Burnett, C. D. 1998. Changes in body mass and fat reserves in pre-hibernating little brown bats (*Myotis lucifugus*). *Ecoscience* 5: 8-17.
- Luis, A. D. and Hudson, P. J. 2006. Hibernation patterns in mammals: a role for bacterial growth? *Functional Ecology* 20: 471-477.
- Meteyer, C. U., Buckles, E. L., Blehert, D. S., Hicks, A. C., Green, D. E., Shearn-Bochsler, V., Thomas, N. J., Gargas, A. and Behr, M. J. 2009. Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 21: 411-414.
- Prendergast, B. J., Freeman, D. A., Zucker, I. and Nelson, R. J. 2002. Periodic arousal from hibernation is necessary for initiation of immune responses in ground squirrels. *American Journal of Physiology – Regulatory, Integrative and Comparative Physiology* 282: R1054-R1062.
- Reichard, J. and Kunz, T. 2009. White-nose syndrome inflicts lasting injuries to the wings of little brown *Myotis* (*Myotis lucifugus*). *Acta Chiropterologica* 11: 457-464.
- Stadelmann, B., Lin, L. K., Kunz, T. H. and Ruedi, M. 2007. Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Molecular Phylogenetics and Evolution* 43: 32-48.
- StatSoft Incorporated v6 2003. Statistica. StatSoft Inc., Tulsa, OK, USA.
- Whitaker, J. O. and Hamilton, W. J. 1998. Mammals of the Eastern United States, 3rd Edition. Cornell University Press, Ithaca. 1-583.

Willis, C. K. R. and Brigham, R. M. 2003. Defining torpor in free-ranging bats: experimental evaluation of external temperature-sensitive radiotransmitters and the concept of active temperature. *Journal of Comparative Physiology B* 173: 379-389.

Figure 1. Ambient temperature in western Ohio and southern New York during autumn and winter of 2007-2008. Notice the similarity in temperature between the two sites used in the body mass experiment. Data taken from the United States Historical Climatology Network (http://cdiac.ornl.gov/epubs/ndp/ushcn/daily_doc.html)

Figure 2. Body mass of hibernating female and male little brown bats (*Myotis lucifugus*) from Ohio (OH) and New York (NY). Data were collected during February 2007 and 2008 for Ohio and February 2008 for New York.

Figure 1.

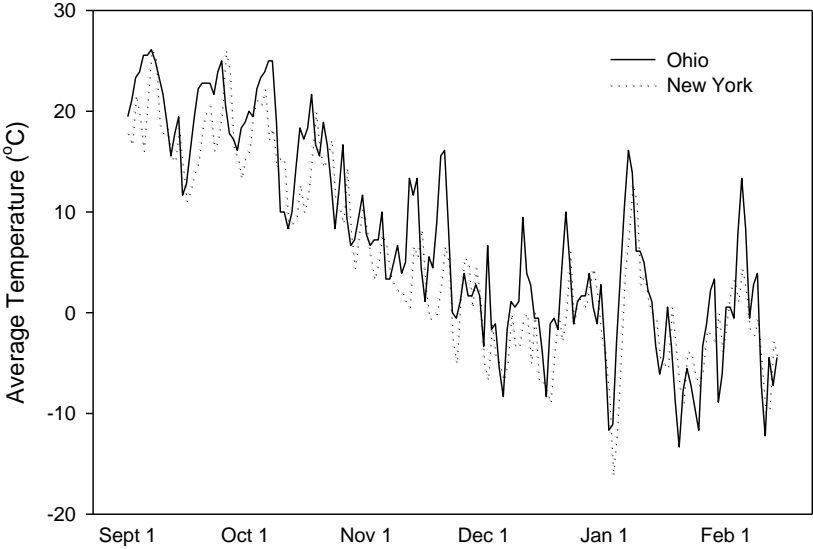


Figure 2.

