2008
NSS WNS RAPID RESPONSE FUND
GRANT SUMMARY

2008-1. Title: *"Death by Starvation: An Hypothesis-Based Investigation of White Nose Syndrome in the Little Brown Myotis (Myotis lucifugus)"*
Award Recipient: Dr. Thomas Kunz, et al, Boston University
Grant Amount: $14,000

PROJECT SUMMARY

White-Nose Syndrome (WNS) has been identified as the cause of unprecedented morbidity and mortality in six of nine species of bats in the northeastern United States (US). First observed in one hibernaculum and subsequently documented in five other hibernacula in New York State in the winter of 2006-2007, reports in the winter of 2007-2008 from at least 33 caves and mines in four states (New York, Vermont, Massachusetts, and Connecticut) indicated mortality as high as 95% at some sites.

This proposal addresses one of the primary goals established at a recent conference on WNS that was convened in Albany, New York (June 9-11, 2008), and attended by over 100 research scientists and natural resource managers from most of the eastern US, representing several state and federal management agencies, federal and state laboratories, and academic institutions. Several hypotheses were proposed and discussed by the body of participating scientists, including those advanced in this proposal. The consensus among those in attendance is that the hypotheses proposed herein are among the highest priorities needed to address why hibernating bats are dying in such large numbers in the northeastern US.

We postulate that a reduction in the quantity and quality of insects during the pre-hibernation period in autumn may limit the deposition of adequate stores of white adipose tissue (WAT) and thus compromises successful hibernation, and ultimately reproductive success. WAT is the primary source of energy that sustains bats (and other hibernators) throughout the winter, when they have no access to food. Over-winter survival and subsequent reproductive success requires a sufficient quantity and quality of WAT deposited during the pre-hibernation period, and sufficient fat reserves to sustain deep torpor and periodic arousals throughout the winter, and a final arousal in spring. Because insectivorous bats cannot synthesize polyunsaturated fatty acids (PUFAs), deficiencies in dietary PUFAs during the pre-hibernation period in autumn may reduce the duration and depth of torpor during hibernation. Frequent arousals during hibernation may result in premature depletion of WAT before the end of the hibernation period. Depleted WAT at this time also may contribute to a decrease in leptin production (necessary for ovulation and successful reproduction by females), or, in the worst cases, the inability to arouse from torpor or inability to mount an immune response to possible pathogens.

Analyses of body composition and dietary composition (including PUFAs) of bats and the biomass and quality (i.e., PUFA content) of insects available to little brown myotis (*Myotis lucifugus*) during the pre-hibernation period will be conducted at sites affected by and unaffected

by WNS to test five hypotheses that may help reveal or rule out causes of premature deaths or compromised reproductive success in these hibernating bats. Destructive body composition analysis provides the most accurate and reliable estimates of total body water, WAT, and lean dry mass, and analyses of PUFAs in WAT collected from bats, stomach contents, and insects during the pre-hibernation fattening period. Data from these analyses promise to provide valuable insight for testing proposed hypotheses to help explain why hibernating bats are dying prematurely at hibernacula in the northeastern US, and in turn suggest directions for future study to better understand WNS.

TIMELINE

July 2008

Assemble and purchase needed equipment supplies. Identify field sites that represent localities affected and unaffected by WNS. Coordinate our research efforts with those being conducted in other laboratories (e.g. Cornell, USGS, New York State Department of Environmental Conservation).

August-November 2008

Pre-hibernating bats will be captured and processed (sexed, aged, measured, weighed, and banded) at bi-monthly intervals from mid-August through mid-November as they emerge nightly from and return to selected pre-hibernation swarming sites at caves and mines where WNS has been identified and where it has not (controls). Samples of bats will be euthanized and frozen in the field for subsequent laboratory analysis. Complete body composition analysis will be conducted using destructive sampling in the PI's laboratory. Stomach contents and samples of subcutaneous WAT deposits of bats will be collected for analysis of PUFAs in Frank's laboratory. Insects captured in Malaise and Hook rotator traps will be identified to Order and frozen for laboratory analysis of PUFAs.

December 2008-May 2009

Hibernating bats will be collected from selected mines and caves in December and in April and processed as described above. In addition, before bats are euthanized, 50 μL of blood will be collected from each bat for analysis of plasma leptin concentration (analyzed in Widmaier's laboratory), and immune responses during hibernation, in collaboration with DeeAnn Reeder (Bucknell University), Beth Buckles (Cornell University), and Marianne Moore (Boston University). Biopsies of WAT will be collected from hibernating bats for analysis of PUFAs in Frank's laboratory. Body composition analysis of bats collected during the pre-hibernation and hibernation periods will be conducted in Kunz's laboratory. Statistical analyses and manuscript preparation will be conducted during this period both in the PI's and Co-PI's laboratories.

As part of our collaborative efforts, we will be coordinating our sample collections with investigators from other laboratories who will be assessing frequency and duration of torpor bouts using temperature-sensitive radio transmitters. The latter data will be compared with our results from our analyses of WAT and PUFA at affected and unaffected sites. We will also coordinate our research with studies investigating the role of brown adipose tissue on arousal rates and immune functions.

2008-2. Title: *"Geographic Distribution of the Psychrophilic Fungus (Geomyces sp.) Associated With White-Nose Syndrome"*
Award Recipient: David S. Blehert, Ph.D., et al, USGS National Wildlife Health Center (fiscal agent: Northeastern Cave Conservancy)
Grant Amount: $5,000

PROJECT SUMMARY

White-Nose Syndrome (WNS) has been associated with unprecedented morbidity and mortality in six of the nine species of bats in the northeastern United States. First observed in six hibernacula in New York State in the winter of 2006-2007, WNS has been subsequently reported from at least 30 caves and mines in four states (New York, Vermont, Massachusetts, and Connecticut) in the winter of 2007-2008, with mortality reported as high as 95% at some hibernacula.

This proposal is designed to document the geographic distribution of the fungus that has been directly associated with WNS, by collecting samples of organic matter—the substrate on which the psychrophylic fungus (*Geomyces* sp.) is known to colonize--and using geographic information systems (GIS) to evaluate its distribution with respect to available landscape and environmental variables in the eastern US over a three-year period. This study is designed to test the hypothesis that the fungus associated with WNS is restricted to the northeastern states, as currently known. This is one of the key research needs identified by scientists who participated a recent conference on WNS was convened in Albany, New York (June 9-11, 2008), and attended by over 100 research scientists and natural resource managers (Science Strategy Meeting, 2008). Several questions, hypotheses, and research needs were proposed and discussed by this body of participating scientists, including those advanced in this proposal. The consensus among those in attendance is that knowledge of the geographic distribution of the fungus associated with WNS is critical to understanding its epidemiology.

TIMELINE

Winter 2008-2009
Collect 10 samples of organic matter from each of 100 mines and caves in the eastern U.S. and analyze these for the presence of molecular markers that characterize *Geomyces*. Analyze samples as they are received by the USGS laboratory in Madison, Wisconsin.

Spring and Summer 2009
Analyze geographic distribution of fungal samples and landscape and environmental variables using GIS.(Note: this is envisioned as a three-year project, with out-year sampling to be determined by the results of year one. The sampling portion has been completed, and came in under budget. $1,975.18 has been returned to the NSS and is available to be re-granted. The USFWS has funded the analysis portion of the project, with results expected in Sept.)

2008-3. Title: *"Winter Energetics of Little Brown Bats with White Nose Syndrome"*

Award Recipient: Tom Tomasi & Amanda Janicki, Missouri State University

Grant Amount: $2200

PROJECT SUMMARY

Research Goals: We propose to keep Ta constant, and quantify the torpid metabolic rates and Tb of hibernating little brown bats in early-, mid-, and late-hibernation. We will do this for bats/caves clearly affected by WNS (NY), bats/caves well removed from WNS (MO), and bats/caves that are possibly affected by WNS (PA). When combined with continuous long-term Tb data being collected on hibernating bats by other researchers, these data will allow us to compose a good picture of the energetic changes associated with WNS. The "condition" of bats in this study will also be evaluated and compared among sites via measurement of body mass and plasma leptin concentrations.

While this will not identify the ultimate cause, energetic changes will point us towards both the causal agent and possible interventions. A leading candidate for the ultimate cause of the suspected higher metabolic rates while hibernating is the fungal infection seen in many of the bats. This fungus is only dermal in penetration, but grows well at hibernation temperatures (David Blehert, personal communication). Such an infection might interfere with normal hibernation energetics by acting as an irritation that prevents the bats from entering deep torpor. Alternatively, it may cause them to arouse from torpor too often in an attempt to re-boot their immune system or induce a "fever" to get rid of the pathogen. Other possible causes for an elevated winter metabolism include as yet unidentified pathogens, a direct effect of some unknown contaminant, an indirect effort of a contaminant (pesticide?) acting as an endocrine disruption, or some combination of these factors. We hope that the knowledge gained in this study will help determine the ultimate cause of WNS by eliminating some of the possibilities and narrow the focus of subsequent investigations.

This was deemed a high priority because it is a major component of the model for addressing the question "*Why are bats starving*?" While WNS is defined by a fungal growth on the bats' faces, not all dead/dying bats show the fungus. It has not yet been determined that this fungus is even the cause of the bat mortality (directly or indirectly). However, all dead bats do show depleted fat reserves (Chenger 2008). Regardless of the contributing factors, this depletion of energy reserves (body fat) seems to be a consistent observation that any theory of the WNS pathology must address/explain.

TIMELINE

Task	September 2008	October 2008	January 2009	March 2009	April 2009	May 2009	Summer 2009
Site selection	X						
Early hibernation data		X					
Mid–hibernation data			X				
Late hibernation data				X			
Leptin assays					X		
Data analysis						X	
Writing							X

2008-4. Title: *"Are Hibernating Bats Affected with 'White Nose Syndrome' Immunocompromised?"*

Award Recipients: Marianne S. Moore, and Thomas Kunz, Boston University

Grant Amount: $6,133

PROJECT SUMMARY

To help describe the potential causes and correlates of 'White Nose Syndrome'(WNS) in North American hibernating bat species, we propose a field-based study to assess various aspects of relative immune function. Multiple methods will be used to measure different arms of the immune system in bats from affected and unaffected sites. Additionally, a time course of blood samples will be collected from torpid bats and bats that have incrementally higher body temperatures, or stages of arousal. These approaches will test several hypotheses associated with WNS. First, measuring relative immune function in bats from affected and unaffected sites will help elucidate whether or not bats with WNS are experiencing immunosuppression. If they are, this study will identify if reduced immune defenses are solely due to physiological constraints associated with deep torpor or due to some other cause, such as an immunosuppressive infectious agent, a contaminant, or a lack of sufficient energy reserves. Second, measuring immune function in bats at different stages of arousal will specifically test the ability of hibernating bats, in general, to mount an immune response. Based on research in hibernating ground squirrels, it is possible that bats must arouse from torpor to mount an effective immune response (Prendergast 2002) and, in support of this hypothesis, preliminary analysis of a timed blood collection series that we performed in February 2008 suggests that as bats arouse from torpor, their ability to kill *Escherichia coli* increases. Our winter 2008 preliminary study also suggests that there may be a statistically significant difference in immune function between bats with visible signs of fungal infections and those without. Moreover, it is possible that WNS affected bats are both immunocompromised due to an extrinsic factor or factors as well as constraints associated with the physiology of torpor.

TIMELINE

November 2008
Conduct timed blood collection series at affected and unaffected sites for assessment of immune responses in bats during early hibernation.

December 2008
Conduct timed blood collection series at affected and unaffected sites for assessment of immune responses in bats during mid-hibernation.

January 2009
Conduct timed blood collection series at affected and unaffected sites for assessment of immune responses in bats during late hibernation.

December 2008 – March 2009
Assay plasma samples for multiple immune function measures.

April 2009-September 2009
Analyze data, prepare manuscripts and submit for publication.