NSS WNS Rapid Response Fund Grant Summary – Fall Grants, 2009
Peter Youngbaer, January 11, 2010

(Note: This report includes a scope of project amendment to the Virginia DCR grant described in the previous grant summary. It also includes a further description of the supplemental grant awarded to Dr. Hazel Barton.)

2009-2. Hazel Barton, et al, Northern Kentucky University Research Foundation
Title: *"Decontamination Issues and the Natural History of Geomyces Species in the Cave Environment."* Supplemental.
Award: $1,000.

Proposal Summary

This is an amendment to the previously-funded project. It provides additional funds for travel to sample collection sites, as well as additional laboratory consumables, primarily chemicals. A priority identified at the Second Science Strategy Conference in Austin, Texas, was the need to identify compounds aimed at treating bats *in situ.* In order to carry out this research, it is critical that compounds are identified that kill the fungus, but do not decimate the native fungal species found within cave environments. We therefore plan to isolate a comprehensive library of fungal isolates from cave environments to provide a test bank of potential species within caves.

One of the primary problems with *in situ* treatment is finding a chemical that is both safe for bats, provides a convenient mechanism of delivery (each bat does not need to be treated individually), and does not harm the environment. The environmental impact is of particular concern, as we do not know the impact of large-scale, anti-fungal treatment on cave microbial ecosystems; however, we do know that in Lasceaux Cave such treatments were devastating. Given the high number of threatened and endangered arthropod species co-inhabitating U.S. caves with bats, the collapse of fungal populations in these systems could have devastating effects. We will therefore continue to test the efficacy of compounds against *Geomyces* species, but also screen them against natural cave isolates. It is hoped that such work will move us toward isolating a compound that can be used to protect bats during hibernation, but which can also be safely used within caves.
Note: This award, coupled with her earlier award of $1,500, enabled Dr. Barton to have the materials and samples to have conducted a host of decontamination trials, and helped leverage a successful grant award from the U.S. Fish and Wildlife Service for $155,355 for ***"The propogation and decontamination of WNS in the environment (Identifying compounds to stop the fungus associated with WNS)."***

2009-3. Wil Orndorff, et al, Virginia DCR Division of Natural Heritage
Title: *"Fall Swarm Monitoring and Tracking of Virginia Bats Affected by White Nose Syndrome."*
Award: $7,500.

Proposal Summary

This proposal will partially fund efforts to perform studies of cave bat populations in Virginia during the 2009 fall swarm period: August 15 – October 15, 2009. The proposed research consists of using harp traps to capture, assess, and band bats at both WNS positive and WNS negative locations in western Virginia, and will involve at least seven and possibly eight species of bat:

Little brown bat (*Myotis lucifugus)* +,V+
Big brown bat (*Eptesicus fuscus)*, +
Indiana bat (*Myotis sodalist)*, **, +
Eastern small-footed bat (*Myotis liebii)*, +
Northern long-eared bat (*Myotis sepentrionalis)*, +
Tricolored bat, formerly Eastern pipistrelle (*Perimyotis subflavus)*, +,V+
Virginia big-eared bat (Corynorhinus townsendii virginianus) **
Gray bat (Myotis grisescens)*,**

* - not expected during fall swarm, but work will be near Gray bat summer sites
** - federally Endangered species
+ - WNS confirmed to occur in this species
V+ - WNS confirmed in this species in Virginia

Basic biometric measurements – species identification, sex, weight, forearm length, sexual maturity, and reproductive activity – will be collected for each bat. In addition, bat health will be assessed via wing index assessments and physical examination for fungal presence and/or lesions. Each bat will be banded with a unique band. This will facilitate tracking of the progression of WNS in individuals as well as the seasonal movement of bats from swarm site to hibernacula to summer colonies, helping us understand potential geographic vectors for WNS transmission.

During the winter of 2008-2009, researchers found at least three (confirmed) WNS affected bat populations hibernating in Virginia caves. The Virginia occurrences were discovered during a joint monitoring effort between the Department of Game and Inland Fisheries and the DCR Natural Heritage Program. The infected bats in Virginia caves represent the southern extent of the disease. Further spread in Virginia could lead to serious declines in the numbers of three federally listed species – the Virginia big-eared bat, Indiana bat and the gray bat.

There is an urgent need to learn more about survival of individual bats infected by WNS, the spread of WNS in Virginia and the transmission of the disease by infected bats to bats in caves where this disease did not formerly occur. How WNS is spread from bat to bat is poorly understood, and this lack of understanding makes it difficult to develop response strategies.

The WNS positive Virginia caves to be monitored under this project are Breathing Cave, Bath County; Newberry-Bane Cave, Bland County; Clover Hollow Cave, Giles County, and Hancock Cave, Smyth County. Additional significant bat caves throughout western Virginia will be studied as funding allows, but will include at least six additional sites: Rocky Hollow Cave, Wise County; Kelly Cave, Wise County; Stonley Cave, Tazewell County; Repass Saltpetre Cave, Bland County; Hupman's Saltpetre Cave, Highland County; and Gap Cave, Lee County.

The results of this research should better help us assess the condition of Virginia's bats in the first year of White Nose Syndrome, and in WNS free areas will help create a baseline against which future changes can be compared. In addition, it will lay the groundwork for tracking the movements of affected individuals during the 2009-2010 hibernation season and beyond, as well as the progression of the disease in individual animals. This work is particularly important because of the proximity of the Virginia locations to summer bachelor colonies of the Gray bat, which until now has not been known to come into contact with WNS affected bat populations.

Timeline:

August 15 – November 15, 2009: Harp trap capture of bats at eleven or more sites in western Virginia, including four WNS positive sites. Collection of biometric and health parameters and banding of bats.

November, 2009 – April, 2010: We plan to enter several of our hibernacula early in the season to band bats that presumably will be resident throughout the winter and infected with white nose. This will help us to fulfill the objective of tracking individual bats with WNS over the course of the winter. To minimize disturbance of already stressed populations, our handling of bats will be confined to November 2009 and March-April, 2010. The rapid response fund will be covering in part the November 2009 portion.

Budget Summary

The U.S. Fish and Wildlife Service provided $15,000 to conduct the initial WNS surveys that were completed this past winter. This work resulted in the early 2009 discoveries of WNS in Virginia caves. No funding remains from that grant. The U.S. Fish and Wildlife Service will provide $15,000 from the multi-state state wildlife grant to support Virginia WNS surveys during the winter of 2009-2010. Those funds will be helpful to determine the southern advance of WNS and the spread of WNS in Virginia caves. There is no funding currently available to support the proposed research during the fall swarm period of 2009. Requested funds will partially support personnel time, equipment, and supply needs associated with the proposed project.

2009-4. Evan Lacy Pannkuk, et al, Arkansas State University
Title: *"Fungal Digestion of Chiropteran Integument."*
Award: $5,800.

Proposal Summary

We propose a set of experiments that will allow a better understanding the role of the fungus in causing mortality in bats and how physical conditions of infected bats (specifically integumentary damage) are caused by the fungus. Currently, there is very little understanding of the pathogenicity of *G. destructans*. One of the most obvious symptoms of WNS is integumentary degradation, especially of wing membranes. There is no information available on the degree of integumentary mechanical damage due to WNS infection or the length of time for damage to occur. Such information would be invaluable in tracking the amount of time available from initial contact to the presence of gross histological damage. We hypothesize that *G. destructans* infection will deteriorate the epidermis and cuticular layer of hair and decrease the biomechanical strength, toughness, and elasticity of these tissues.

The objective of this project is to quantify the amount of mechanical damage over time caused to bat integument (primarily little brown bats) by the fungus *G. destructans*. The project consist of two parts, the first part is to assess *in vitro* the length of time for damage to the integument to occur and the amount of damage that occurs over time. The second part of the project will explore how *G. destructans* infection affects the mechanical integrity of the integument.

Especially if early detection systems for *G. destructans* are developed, the knowledge of when mechanical damage will occur at the forefront of the disease is of utmost importance. It can decide how quickly management efforts need to be put in place to save bats where WNS is recently introduced. In response to this crisis researchers have been monitoring summer maternity colonies of Indiana bats (*Myotis sodalis*). Unfortunately there is a complete lack of data to assess which infected bats may survive the summer season. The results of this study should add unique and important insights to this question. The most important aspect of this project is that for the wealth of information that can be obtained, the procedure is rather simple and all experiments combined are inexpensive.

Timeline

Permits and equipment will be obtained by January 2010. The initial fungal digestion and ultraviolet/ visible light (UV/VIS) quantification of the oligopeptides will be completed in the spring of 2010. Further fungal digestion of integumentary tissue and mechanical testing will be completed in the spring-summer of 2010.

2009-5. Sarah Brownlee, et al, Bucknell University
Title: *"Behavior of Bats Affected by White Nose Syndrome."*
Award: $6,949.74.

Proposal Summary

One hypothesis that has been put forward to explain White Nose Syndrome (WNS) is that bats are starving to death due to a change in body condition caused by premature depletion of stored fat during hibernation (Reeder and Turner, 2008). Data from the 2008-2009 field season demonstrated that, across multiple field sites, the arousal patterns of WNS bats (monitored non-invasively with temperature-sensitive dataloggers) differed significantly from those of healthy bats (Reeder, unpublished data; Proceedings of the White Nose Science Strategy meeting II). WNS affected bats aroused more often than normal and in some cases remained euthermic for hours or even days. What is not known is the behavior of the bats during these periods of arousal, and information on this will give us a better understanding of the epizootology of the disease, how the animals react to being attacked by the fungus and what they may try to do to stop it. Research into their behavior will give us a wide range of information on the disease and its progression.

The objectives of this research are to:

- Observe bats in their natural hibernacula to see what they are doing while they are euthermic (their 'warm up' periods). Some researchers have suggested a very simple scenario: that the fungus associated with WNS 'irritates' the bats, causing them to warm up in order to groom off the fungus. These warm ups then could explain the loss of body fat and subsequent starvation seen in WNS.
- Observe WNS bats and presumably healthy bats in captivity at three different temperatures and observe their responses. This part will be performed in conjuntion with another experiment, which will be identifying the temperature preference of and physiological changes in WNS affected bats.
- Pilot the use of acoustic monitoring devices on bats within the hibernacula to determine whether aroused bats are echolocating (indicating flight). Although individual bats cannot be identified using this method, linking timestamped calls, timestamped video and timestamped temperature tracking dataloggers attached to individual bats will allow for interpretation of these data.

Timeline

Field Work:

Fall/Winter 2009: Deploy video equipment at two caves, and acoustic equipment at one cave, in Pennsylvania.

Fall '09 through spring '10: Download data from digital video recorders and acoustic monitoring devices deployed in the field and check on status of equipment/batteries on a weekly basis.

Spring/Summer 2010: Analyze video and acoustic data acquired from PA and other sites.

All devices will be placed in as minimally invasive locations as possible so if repairs or maintenance are required throughout the winter it will cause the least disturbance to the bats.

Captive Work:

Fall/Winter 2009: Bring bats into captivity by December and give them time to acclimate to captive conditions. Deploy video equipment into hibernation chambers at three different temperatures.

Fall '09 through spring '10: Download data from digital video recorders (which are kept outside the actual hibernation chamber) on a weekly basis.

Spring/Summer 2010: Analyze video acquired from monitoring the captive bats.

All results will be immediately shared with the WNS community.

October 2010: Final Report

Budget Summary

Rapid Response Funds are for equipment only; personnel, travel, and lab costs are covered from other sources. This grant is being funded in conjunction with the next project, and work being conducted by Dr. DeeAnn Reeder. Brownlee and Grieneisen are Masters level students at Bucknell University.

2009-6. Laura Grieneisen, et al, Bucknell University
Title: *"Hibernacula Microclimate and White Nose Syndrome Susceptibility."*
Award: $6,440.

Project Summary

The objective of this study is to determine the relationship between hibernacula microclimate and White-Nose Syndrome. Microclimate will be examined on a large scale and at the level of the individual bat to determine if there is a difference in microclimate preference between healthy and WNS-infected Little Brown Bats. There is anecdotal evidence that colder, drier hibernacula are less affected by WNS. This will be tested in the field by placing rugged temperature and humidity dataloggers in field sites throughout the Eastern USA and by experimentally determining the response to microclimate differences in captive bats. The results from this study will be immediately applicable to (1) predict which hibernacula are more likely to be infected next winter, (2) determine which hibernacula should be priority gated, and (3) determine if direct mitigation

strategies, such as altering the microclimate of mines, will be effective ways to combat the spread of the fungus.

GOALS AND HYPOTHESES INCLUDE:

1. To examine the relationship between hibernacula microclimate and prevalence and progression of the disease in WNS-affected bats. This will be tested in the field by placing rugged temperature and humidity dataloggers in sites throughout the Eastern USA and by examining historic data. **I hypothesize that because fungi prefer damp conditions and *Geomyces destructans* grows best between 5 and 14^{o}C (Gargas et al 2009), hibernacula that are below that temperature range and have low levels of humidity will have fewer WNS-affected bats and bats whose health is less impacted.**
2. To determine the impact that temperature has on the progression of WNS. This will be tested by placing WNS-affected and non-affected bats in artificial hibernacula (environmental chambers) within the bat vivarium at Bucknell University. Bats will be housed in each of three conditions: 2°C, 6°C, and 10°C. **I hypothesize that because *Geomyces destructans* grows best between 5 and 14^{o}C (Gargas et al 2009), WNS-affected bats housed in the 2°C environmental chamber will exhibit a slower progression of the disease and increased survival rates over those housed in the warmer environmental chambers.**
3. To determine the microclimate preference in WNS-affected and unaffected bats. Bats will be placed individually in a microclimate gradient chamber for twelve hours, allowing them to choose at which temperature they want to hibernate. **Field observations by WNS researchers support the hypothesis that WNS-affected bats will choose to hibernate at colder temperatures than unaffected bats.**

IMPACTS

This project will address the following questions, which were identified as Priority Research Gaps at the 2009 White Nose Syndrome Science Strategy Meeting II:

1. Can the dispersal of WNS be predicted?

   This study will record the microclimate profiles for WNS-affected and unaffected hibernacula. These can be used to predict which caves will be likely to host WNS-affected bats next winter, allowing ample time for conservation efforts.

2. Can WNS-affected bats survive?

   The observation of bats in artificial hibernacula in captivity will not only allow us to determine if WNS-affected bats are able to survive the disease, but also if the hibernacula temperature influences their survival. The progression of the disease in

individual bats will be observed and documented. This may make it possible to examine a bat in the field and determine which stage of the disease it is in and if treatment is possible.

In addition, understanding how *G. destructans* changes bat behavior in respect to temperature preference is a key step in understanding why WNS results in so many mortalities.

Timeline

The data collection schedule for field studies is as follows:

November 2009: Temperature and relative humidity (T/RH) dataloggers (N.I.S.T. calibration certified TransitempII-RH, Madgetech, Warner, New Hampshire, USA) will be placed in cave and mine hibernacula in Pennsylvania, New York, Vermont, New Hampshire, West Virginia, and Massachusetts.

April 2010: T/RH dataloggers will be collected from hibernacula for data download. Caves will be classified following the methods established by Raesly et al (1987).

The data collection schedule for captive studies is as follows:

December 2009: Seventy-five presumed unaffected bats (those taken from presumed clean hibernacula) and 75 WNS-affected bats will be collected from hibernacula in Pennsylvania and transported to the Bucknell University Bat Vivarium.

December 2009, March 2010: To test individual microclimate preferences of bats, individual bats will be tested in a 'microclimate chamber' in which a temperature gradient will be set up following techniques developed by Boyles et al. into torpor. Up to 12 bats from each condition (affected vs. unaffected at each of the three hibernation temperatures) will be tested in December, and an additional 12 bats (different from those tested in Dec) from each condition will be tested during March. Multiple 'microclimate chambers' will be made to facilitate simultaneous testing of these animals.

April 2010: WeeTag dataloggers will be removed from individual bats for data download. Data will be analyzed to determine the thermal preferences of bats from each of the six conditions.

October 2010: Final Report

Budget Summary

As with the Brownlee project, Rapid Response Funds are for equipment only.