2011
NSS WNS RAPID RESPONSE FUND
GRANT SUMMARY

2011-1. Title: *"The impact of wing damage caused by white-nose syndrome in the little brown myotis"*

Award Recipients: Nathanial W. Fuller and Thomas H. Kunz, PhD, Boston University

Grant Amount: $ 6,041.97

PROJECT SUMMARY

This study aims to determine the influence of severe wing damage on foraging effort and reproductive success, while also quantifying how bats are healing early in the active season. By using remote and direct monitoring, we will quantify the differences in foraging effort and reproductive success as they relate to wing damage and construct a temporal model of expected wing healing. These methods will address important questions regarding the impact of WNS during the active season. By measuring foraging effort, we will determine whether bats that have suffered heavy wing damage are equally able to acquire prey as bats with no damage. If foraging success is compromised, then there may also be an effect on the growth rates and survivorship of pups born to mothers with damaged wings. We will also test aspects of relative and specific immune function to determine whether bats with more severe wing damage are less able to mount an effective immune response to bacteria and the presumed causative agent of white-nose syndrome, *Geomyces destructans* (*Gd*).

In addition, the project team will conduct an analysis of insect composition of the guano collected (e.g., do bats with damaged wings feed on different types of prey, such as slower flying but less nutritious insects?). We will attempt to determine whether there are dietary shifts among bats with damaged wings. Because wing damage results in increased wing loading, flight maneuverability will be negatively impacted by wing damage. With reduced flight maneuverability, affected bats will not be able to make quick, sharp turns and may not be able to capture the same insects as bats with unaffected wings. Thus, insect taxa that are especially effective at avoiding bats, such as moths that possess ultrasonic detection and echolocation jamming behavior (Corcoran, 2009), may constitute a smaller proportion of the total volume of insects eaten during a given foraging bout. Through visual analysis of fecal samples collected), we will assess whether bats with damaged wings have diets that are different from individuals with undamaged wings. While there is a trend in dietary analysis toward a DNA bar coding method because of its ability to ID insect fragments to the species level, rather than to order or maybe family level that you get from traditional analysis, this project will at least permit the latter. Samples will be stored for future bar coding.

TIMELINE

January 2011 – March 2011: Install surveillance camera and supporting equipment at PIT tag study site. Visit hibernacula with pre-arranged surveys.

April 2011 – Early June 2001: Trap hibernacula at emergence and begin monitoring at associated maternity colonies. Capture bats at emergence and monitor wing damage patterns, apply PIT tags, deploy and operate insect traps.

Early June 2011 – Mid-July 2011: Postnatal growth study.

Late July 2011 – August 2011: Continue to monitor colonies for wing damage patterns, banding female young of the year at numerous colonies.

September 2011 – March 2012: Data analysis, insect sorting and ID, fecal analysis, develop adjustments to research plans if needed, hibernacula visits.

April 2012 – June 2012: Monitor summer colonies for return of females banded during the previous year, monitor wing damage patterns.

June 2012 – July 2012: Postnatal growth study.

July 2012 – August 2012: Continue to monitor maternity colonies: band female young of the year, recapture previously marked females, and access body condition and wing condition of these females and their mothers.

2011-2. Title: *"Natural Micro-biome of Bats"*

Award Recipients: Kaitlyn Hughes and Diana Northup, PhD., University of New Mexico

Grant Amount: $6,120

PROJECT SUMMARY

This proposal addresses ecological or behavioral questions essential to conservation and management, by investigating the natural microbiota that live on bats to establish baseline information that is currently lacking. Recently, bats across the U.S. became threatened by White Nose Syndrome, putatively caused by *Geomyces destructans*, while the same fungus infects bats in Europe, but does not kill them.

We lack key information about the normal microbiota of bats to allow us to understand the ecology of this new fungus in relation to bats (Foley et al. 2010). Part of this study will document whether species of *Geomyces* live on healthy bats as part of a larger community of microorganisms. The study will take place in El Malpais National Monument (ELMA), located southwest of Grants, NM, where 13 species of bats occur.

Information is generally lacking on local bat populations, roosting habitats, and migration patterns in northwestern New Mexico (Findley et al. 1975). To remedy this lack of baseline data, a USGS investigator, Ernie Valdez, will be conducting a population survey in the ELMA.This study will document the presence or absence of *Geomyces* spp. in cave soils, but will provide little information about the presence of this genus on bats as part of their natural microbiota. Such information is crucial

to our understanding of the role of this fungus in the environments and vis à vis bats. His research provides an opportunity to simultaneously obtain samples of the microbiota associated with different species of bats. Bat biologist Debbie Buecher of Buecher Biological Consulting, who is monitoring cave climate and bat hibernation in the lava caves of ELMA, will also provide samples from these cave roosting bats.

TIMELINE

August 2011: Site selection and bat sampling.

August – December, 2011: DNA extracting and sequencing; Denaturing Gradient Gel Electrophoresis; Sequence alignment.

December, 2011 – June 2012: Data Analysis.

2011-3. Title: *"Evaluation of a terbinafine impregnated subcutaneous implant for the treatment of Geomyces destructans infected bats"*

Award Recipients: Marcy J. Sousa, DVM, MPH, Sherrie Cox, DVM PhD, Kimberly Newkirk, DVM, PhD, University of Tennessee College of Veterinary Medicine

Grant Amount: $11,214

PROJECT SUMMARY

A terbinafine impregnated subcutaneous implant has been designed by the principal investigator with the intention of providing a one-time administration, long term treatment for bats infected with Geomyces destructans (Gd). To date, the PI and Dr Cox have examined the implant in vitro only. During the winter of 2011-2012, little brown bats infected with Gd will be collected and housed at Bucknell University in the laboratory of Dr. DeeAnn Reeder. These bats will be treated with 1 of 4 different implants (control, low dose, medium dose, and high dose) and placed in hibernation for approximately 4 months. Response to therapy will be monitored monthly by documenting disease progression with digital photography using both regular light and ultraviolet light. Additionally, wing skin samples will be collected monthly to determine terbinafine concentrations. Animals that die during the study will have skin samples collected and then the rest of the animal will have histopathology performed to determine extent of disease and evidence of toxicity. All bats will be euthanized at the end of the trial and have histopathology performed.

Funds are being requested for 1) histopathology of tissue samples collected during the clinical trial, and 2) the development of a high performance liquid chromatography assay to determine terbinafine concentrations in skin. Additional funds may be requested in the future (year 2) to analyze skin samples collected during this clinical trial once the HPLC assay is developed and validated.

TIMELINE

Fall/Winter 2011: Infected bats will be captured by various state or federal agencies and given to Dr.

DeeAnn Reeder. 40 bats will be captured, individually identified, and randomly assigned to one of four treatment groups (control, low dose, medium dose, high dose). Lesions will be documented with digital photography prior to treatment. Infection will be confirmed with light microscopy and/or ultraviolet light. Implants will be placed subcutaneously on the dorsum by Dr. Marcy Souza. Animals will be placed into hibernation chambers in the laboratory of Dr. DeeAnn Reeder. This portion of the study will occur at Bucknell University.

Winter 2011-2012: Animals will be monitored by remote cameras and kept in hibernation for approximately 4 months. Once monthly, each animal will be taken from the hibernation chamber, weighed, and have body condition scores recorded. Lesions will be documented with digital photography (with regular and UV light) and a skin sample will be collected by biopsy for evaluation of terbinafine concentrations. Bats will be placed back into the hibernation chamber after lesion documentation and sample collection. Skin samples will be placed in a plastic container and frozen until analysis. Any animals that die during the study will have a skin sample frozen for terbinafine analysis and the rest of the animal will be placed in formalin for histopathology. The UTCVM Pharmacology Laboratory will begin work on the development and validation of an HPLC assay for the determination of terbinafine concentrations in bat skin.

Spring 2012: At the end of the clinical trial, all surviving bats will have lesions documented with digital photography. Animals will then be euthanized , have a skin sample collected for HPLC analysis, and the remaining tissues placed in formalin for histopathology. The UTCVM Pharmacology Laboratory will continue work on development and validation of the HPLC assay.

Summer 2012: Two blinded observers (M. Souza and a summer veterinary student) will score each of the digital photographs according the wing damage index (WDI) scales previously described (Reichard & Kunz, 2009). WDI scores will be averaged and then analyzed to determine if significant differences in clinical progression of disease exist between treatment groups. A similar scale for lesions and colonization visualized with UV light will also be developed and used to score photographs. Dr. Newkirk will begin evaluation of tissues by histopathology. If an HPLC assay is developed & validated, analysis of tissues from clinical trial bats will begin.

Fall - Winter 2012: Manuscript preparation detailing clinical progression of disease following treatment. Continued evaluation of tissues for both HPLC and histopathology. Comparison of ante-mortem evaluation techniques with lesions and toxicity seen on histopathology.

2011-4. Title: *"A proposal to collect and culture fungi from WNS-infected bats to determine if pre and post-WNS fungal communities differ and to identify any fungi present that may have the potential to interact with G. detructans"*

Award Recipients: Donald F. McAlpine, PhD, Karen Vanderwolf

Grant Amount: $6,500

PROJECT SUMMARY

This project proposes to track the emergence of White nose Syndrome (WNS) in New Brunswick (NB), Canada, to collect epidemiological data (mortality, incidence of infection, rate of spread, etc) and most importantly, to examine the broader fungal community present on WNS-infected hibernating bats.

During 2009-10 we cultured at low temperature, and identified, fungi from hibernating bats to develop a pre-WNS fungal dataset in anticipation of WNS arrival in Maritime Canada. We also censused bats to establish baselines and recorded cave microclimatic data. One cave (among 8 bat hibernacula we worked in) experienced a major WNS mortality event (83%+) in 2010-11. We now propose to collect and culture fungi from WNS-infected bats to determine if pre and post-WNS fungal communities differ and to identify any fungi present that may have the potential to interact with *G. detructans*. Some low-temperature fungi co-occurring on hibernating bats may interact with *G. destructans* in ways that have management implications for WNS. We will do this in conjunction with the collection of detailed epidemiological data (which seems to be surprisingly uncommon) from all WNS-infected caves in New Brunswick

TIMELINE

Sept- Nov 2011: Deployment of i-buttons to record temperature in cave sites (and possibly humidity – in the past we have had technical problems with i-buttons recording humidity in caves); locate new hibernacula in anticipation of winter visits.

Nov 2011- Jan 2012: First visits to bat hibernacula to conduct censuses and check for WNS; first public media appeal for records of daSy-flying bats

Feb – March 2012: Second visit to check for WNS; 10 WNS-infected bats will be swabbed for fungal cultures; fungal cultures will be established, monitored, sub-cultured, and identified using morphology or molecular genetic means.

March-May 2012: Third visit to any WNS infected sites to determine mortality; fungal culturing and identification continues.