2012

NSS WNS RAPID RESPONSE FUND
GRANT SUMMARY

2012-1. Title: "Monitoring Gray Bat (MYOTIS GRISESCENS) Populations at a Priority 1 Hibernaculum in Missouri."

Award Recipients: Ruth Seeliger, Graduate student, Thomas Tomasi, Ph.D., Missouri State University

Grant Amount: $1,800.00

PROJECT SUMMARY

The endangered gray bat requires population monitoring in order to evaluate its status as a protected species. The threat of white-nose syndrome also necessitates an evaluation of the current health of colonies in order to track any progress of the fungal epidemic. One of the most important gray bat hibernacula in Missouri, Coffin Cave, hosts several hundred thousand individuals each winter. In order to cause minimal disruption to the bats, I propose to conduct a census of this cave in April 2012 using thermal infrared video and computer counting software to count the bats as they exit the hibernaculum. This project intends to obtain an estimate of the hibernating population at Coffin Cave, and develop a systematic procedure for the use of this technology for other bat census applications. NSS funds will be matched by $2000 of MSU funds to purchase a specialized thermal infrared camera and travel to and from the study site.

TIMELINE

Data collection will occur March 25 – April 28, 2012. Video recording of colony emergence will be conducted 4-6 nights per week. Insect abundance and weather data will also be collected. In May the data will be analyzed and the final report will be completed by the end of July 2012.

2012-2. Title: "An Investigation and Comparison of Fluorescent Activity Among Three Classes of Bats: Naïve, Presumed Survivors, and European,"

Award Recipients: Gregory G. Turner and Cal Butchkoski, Pennsylvania Game Commission, Carl J. Herzog, New York Department of Environmental Conservation, and Natalia Martinkova, Institute for Vertebrate Biology, Brno, Czech Republic.

Grant Amount: $2,800.00

PROJECT SUMMARY

The novel fungal pathogen, *Geomyces destructans* has recently been confirmed to cause WNS. The disease is diagnosed in the laboratory by examining wing membranes for characteristic lesions where the fungus has invaded the epithelial layer of the skin. A recent technique to determine if bats contain the characteristic lesions and are thus infected with WNS, uses ultraviolet (UV) light to transilluminate wing membranes of bats. When the wings of bats infected with WNS are exposed to the UV light, the

fungus in these lesions will fluoresce (see attached photo, Fig 1.). For the first time, a technique exists to allow non-lethal diagnosis and through photo documentation, the area of wing membrane that will fluoresce can be quantified. Analysis of the technique for confirming WNS (unpublished data, manuscript in prep) is >98% accurate, the same as genetic PCR tests were for the same data set. Having this new, non-lethal technique now opens the door and allows a first examination into survivors of WNS. Our primary goal will be to examine and compare the degree of fluorescent activity among the bats.

The main focus of the project is to use this methodology to compare bats of various species that have been exposed for long periods of time in the U.S.; for short periods of time in the U.S.; and for much longer periods in Europe. The funding for the European component is a particular interest, in that it can inform about the resilience or resistance of bats of varying species to the disease. The NSS funds will cover the European portion of the study.

TIMELINE

1 Feb- 30 Feb 2012	The project would begin with sample design and site selection.
1 Mar- 30 Apr 2012	Sample collection would take place, all samples photo documented
1 May- 31 July 2012	Data would be centralized and entered into computer Technique to quantify wing score will be produced
1 Aug- 31 Dec 2012	Samples will be analyzed, interim report to NSS produced
1 Jan- 31 Dec 2013	Prepare and submit final report to NSS; prepare and submit publication

2012-3. Title: "Effects of temperature on germination of *Geomyces destructans* from WNS affected regions of the United States"

Award Recipients: Marcia Lee, DVM, Yunluan Cui, Miami University (Ohio), Kevin Keel*, PhD, DVM, DACVP, Lisa Last*, DVM, , *University of Georgia.

Grant Amount: $4,247.00

PROJECT SUMMARY

Temperature is likely to have a major impact on the geographic spread of Geomyces destructans (Gd) from the northern to southern United States. Consequently, this project seeks to understand the impact of temperature on Gd germination in isolates from northern and southern sites. Gd conidia, often called spores, are abundant in the white powdery growth on backs, faces and wings of White Nose Syndrome (WNS)-affected bats (Gargas et al., 2009). These spores are largely responsible for the spread of WNS by direct and indirect contact among bats, and their subsequent germination produces wing-invasive hyphae (Chaturvedi et al., 2010; Cryan et al., 2010; Reichard and Kunz, 2009). In May 2012, we conducted initial germination experiments on two Gd isolates from naturally infected bats. Time-course germination assays revealed that germination rates differed significantly between Gd isolates at 10°C, 15°C and 20°C, and provided solid reason to continue this investigation into effects of environmental temperature on Gd germination capacities.

We propose to expand this study to examine Gd isolates from caves along the eastern US, including a 950-mile transect from Howes Cave near Albany, New York, to Russell Cave near Bridgeport, Alabama, and, additionally, to include experiments at 5°C and 25°C We hypothesize that Gd isolates from bats in southern and northern environments require significantly different lag times prior to germination, exhibit differential germination rates, and attain different maximal germination frequencies. This study might help to understand the role of temperature in the spread of WNS southerly in the US. It will expand our knowledge about particular Gd isolates and offer insight regarding potential geographic limitations for WNS.

TIMELINE

August 2012 - October 2012: Perform Gd spore germination assays on Gd isolates from WNS- affected bats caves.
August 2012 - October 2012: Microscopically analyze germination frequencies and young Gd hyphal morphological features using light and fluorescent microscopy.
November 2012: Data analysis. Prepare manuscript and submit final report to the National Speleological Society.

2012-4. Title: "The role of oxidative stress in the development of white syndrome"

Award Recipients: Marianne S. Moore, Ph.D., Mark F. Haussmann, Ph.D., Department of Biology, Bucknell University

Grant Amount: $4,896.00

PROJECT SUMMARY

The main objective of the proposed project is to investigate the role of oxidative stress in white syndrome (WNS) associated bat morbidity and mortality. Although the causative agent of WNS has been identified as *Geomyces destructans* (Gd), the proximate, or direct, cause of death is unknown and no effective control measures have been developed. Results from Moore's Ph.D. Thesis demonstrate reduced antioxidant levels in free-ranging WNS-affected *Myotis lucifugus*, particularly low levels in bats with visible fungal growth, and positive correlations between antioxidant levels and energy stores. These results suggest a possible imbalance between pro-oxidants and antioxidants, also known as oxidative stress. Because pro-oxidants indiscriminately destroy DNA, proteins, fats and tissues, this potential imbalance may be life threatening. We therefore propose to better elucidate the roles of oxidative stress in the development of this devastating disease and to test the following hypotheses: 1) oxidative stress correlates with physiological and energetic reductions and with infection severity in WNS-affected bats and, 2) there are inter-species differences in responses to Gd that are related to the ability to resist oxidative tissue damage and may underlie species-specific differences in rates of mortality. This study will provide considerable new knowledge regarding the proximate cause of death in WNS-affected bats. To the best of our knowledge, no other group is investigating these hypotheses, which if supported, could lead to greater understanding of morbidity and mortality in WNS-affected bats. There are likely multiple underlying mechanisms contributing to morbidity and mortality, however, identifying the role of oxidative stress in this process may indicate a potential use of antioxidant supplementation treatment to mitigate the effects of WNS on individual bats. This could be especially useful for protecting particularly vulnerable species, such as the little brown myotis (*M. lucifugus*) and the most recently observed species with WNS lesions, the critically

endangered grey bat (*Myotis griscescens*). Budget covers the cost of lab assays on 144 bat samples.

TIMELINE

(Note: the samples to be analyzed were already collected for another WNS research project.)

August - December, 2012:	Assay Optimization
January - March 2013:	Assay Performance
April - May 2013:	Data Analysis
June - July 2013:	Manuscript Preparation