Abstracts of Presented Papers and Posters For

2010 White-nose Syndrome Symposium

May 25-27, 2010, Pittsburgh, Pennsylvania

All abstracts are listed in alphabetical order

Sponsors of the Symposium:

Generation of *Geomyces destructans*-specific Monoclonal Antibodies – Project Update

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Project start date: January 2010
Anticipated project completion: March 2011

White-nose syndrome in bats is caused by infection of a newly discovered pathogen, *Geomyces destructans* (G.d.). The fungus is currently diagnosed in bats by histological exam, culture and PCR identification, all time consuming and labor intensive techniques. Currently, there is no non-invasive method available to rapidly detect G.d. in live bats, before visible fungal hyphae are present, and there is no method available to specifically detect G.d. in environmental samples. In this project we focus on generating mouse monoclonal antibodies (MAbs) specific to G.d. The production of G.d.-specific antibodies will greatly enhance our ability to produce more rapid and simple diagnostic tools, including field-based diagnostic assays. These MAbs can be used to isolate G.d. from the bat cave environmental samples, which can then be subjected to PCR for genotyping analyses. The MAbs can also be used to develop a 'dipstick' method for detecting G.d. from bat or environmental samples. Furthermore, these antibodies can be used for other epidemiological and laboratory investigations. These G.d.-specific antibody producing hybridomas represent a long-term source of the MAbs, or large quantities of antibodies can be produced from either mouse ascites or from concentrated culture supernatants. These antibodies should be relatively stable for years if properly stored. Additionally, these monoclonal antibodies will be valuable reagents to other scientists and can be used for other applications. [oral]

A Potential Model Framework to Investigate Disease Progression

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Project start date: March 2010
Anticipated project completion: Phase 1, September 2010

Epidemic models are designed to predict disease outcome, each individual is a host population classified according to their disease status. Individuals are either susceptible (S), infected (I) or recovered (R). Recovered individuals can become immune to further infection or may only recover and subsequently be susceptible to re-infection. We are developing and testing both SIS and SIR models to compare modeled disease outcomes to those that have been observed in areas of white-nose syndrome (WNS) mortalities. We explore two classes of infected individuals, those exposed but no apparent evidence of disease development, and those exposed that immediately progress to full disease status. Our approach may allow us to predict the lag time between initial exposure to *Geomyces destructans* and high levels of pathogen that result in all individuals developing non-recoverable WNS. [oral]

Identification of Microflora Communities Inhabiting Bat Skin

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Project start date: November 2009
Anticipated project completion: March 2011

Animal skin serves as an environment for the controlled growth of a number of bacteria and fungi. The term 'normal microflora' is commonly used when defining the microbial collection inhabiting the body. Current research on the complex interplay between the skin microbiota and the innate immune system of the skin indicates that the microbiota have a beneficial role, much like that of the gut microflora. There are numerous examples of indigenous nonpathogenic microbes being used to protect animals as well as plants from invasion by infectious agents. Bacteria found naturally on the skin of amphibians have been shown to inhibit the fungus responsible for chytridiomycosis. Similarly, a large variety of indigenous bacteria and some fungi have been shown to protect plant surfaces against disease by infectious agents. We hypothesized that bats also harbor nonpathogenic microbes that would naturally protect them from invasion by pathogens. We collected bat skin swabs from both known infected individuals (Vermont) and from believed white-nose syndrome unaffected individuals (Missouri). We will provide our preliminary findings and progression towards testing these microbes for antagonistic activity towards *Geomyces destructans*. [oral]

Assessing the Genetic Viability of Indiana Bats (*Myotis sodalis*)

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Project start date: November 2009
Anticipated project completion: October 2011

The critical conservation situation facing Indiana bats (*Myotis sodalis*) with the advance of white-nose syndrome (WNS) indicates the need to understand the spatial organization of genetic variation, gene flow, and relationships to landscape isolation. Small or rare populations commonly face problems of inbreeding, genetic drift and high susceptibility to catastrophic disease events. Each of these problems increases the potential for unrecoverable genetic loss. Understanding the combined genetic viability impacts from current population losses in northeastern states and the potential loss from hibernacula in the Midwest and Ozark-Central Recovery Units is vital for evaluating strategies to conserve this species and prevent extinction. We are examining the relative susceptibility of this species to inbreeding depression, outbreeding depression, and catastrophic population loss (due to WNS). We will model population viability examining the potential effects of several management options on long term genetic viability of this species. We provide an overview of preliminary findings and the development of a population viability assessment to provide a basis for a genetic management plan to initiate or support a security population. [oral]

The GateKeeper Beam-break System in Kentucky

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The GateKeeper beam-break system allows automated counting of bats as they enter and leave hibernacula using paired infrared beams for long-term, continual monitoring with remote reporting. Once calibrated, the system can measure overwinter mortality and summer fecundity and provide unbiased population census data with confidence intervals, all information that has never-before been available - and all of this is done with no disturbance to the bats. These capabilities are valuable for sites without white-nose syndrome (WNS) – and may become invaluable for WNS-affected sites. The GateKeeper beam-break system can provide a wide range of valuable data to managers through programmable ‘alarms’ in text messages in ‘real-time’ and can be archived for later analyses. The system can be powered by solar/batteries or standard current. Remote reporting can be achieved through GSM cell networks or by an Iridium satellite-based transceiver for $300 or $360 per year, respectively. These systems can be removed from abandoned sites – and moved to sites that continue to house bats. The GateKeeper beam-break system, designed by David Redell and Daniel Shurilla while at the University of Wisconsin, Madison and Milwaukee, respectively, consists of components that are relatively easily tested and replaced in-the-field. The system is intended to be long-term, durable infrastructure and should function for many years with occasional maintenance. Each paired beam is powered by roughly 1 watt of electricity. The first installation in Kentucky was in late January 2010 at Saltpetre Cave in Carter County, a Priority 2 hibernaculum for the Indiana myotis. We installed systems at James and Coach Caves in Edmonson Co., KY in March and April, respectively, of 2010. These two Priority 1 sites together house roughly 300,000 gray myotis during winter and James Cave houses a bachelor colony of gray myotis in summer. Bat Conservation International has current plans to fund additional installations at sites in eastern Kentucky and southern Indiana. We are working with state and federal partners in Kentucky and Indiana on these plans and are working with state and federal partners in Tennessee, Illinois, and Arkansas to jointly plan and fund GateKeeper beam-break system installations at Priority hibernacula in these states. [poster]

Research Update from USGS-NWHC

ANNE BALLMANN, DAVID BLEHERT, CAROL METEYER, JEFF LORCH, AND LAURA MUELLER

U.S. Geological Survey, National Wildlife Health Center, Madison, WI

Plan to discuss preliminary findings from various research projects conducted by the National Wildlife Health Center (NWHC) or in which we provided collaborative diagnostic assistance (infection trials, effects of supportive care vs. topical vinegar treatment of WNS bats, etc.); will also discuss summer samples submission guidelines to NWHC that address persistence and viability of *G. destructans* at caves/mines used by bachelor colonies during the summer or bats at maternity colonies in late summer. [oral]

WNS: Bugs, Caves and Decon

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While there is no specific evidence for the anthropomorphic spread of *Geomyces destructans,* the causative agent of white-nose syndrome (WNS), limiting the potential spread of this pathogen by human activity is a critical factor in controlling the WNS epidemic. We have been working on developing decontamination protocols that are effective against the spores of *G. destructans,* use easy to obtain household disinfectants, and can be successfully implemented without formal training in microbiology. To test the effectiveness of compounds, we are using *Geomyces pannorum* as a model organism, with follow-up work in *G. destructans*. Spores of these species were tested due to their inherent resistance to chemical and environmental conditions. Initial screening was carried out using disk diffusion assays, wherein over 80 chemical compounds were tested for their effectiveness against *G. pannorum.* Specificity was determined by comparing effectiveness against cultures of the fungi *Aspergillus* niger and *Penicillium chrysogenum* (nee *notatum*). Of these agents, eight were chosen for additional screening on fabrics used by recreational cavers; the resistance of *G. pannorum, A. niger* and *P. chrysogenum* spores to these agents was tested with colony counts, germination and total kill assays. The results suggested a number of reagents that are both selective and effective against *G. pannorum.* These agents were then used in confirmatory assays using *G. destructans* spores. Due to the chemical constraints and difficulty carrying out these identified treatments under certain field conditions (specifically field surveys of bat populations), alternative methods of decontamination are also being tested. Subsequent to this effort, we are examining both the selectiveness and effectiveness of a number of naturally derived compounds and fungal cultures to determine if any of these agents can be used *in situ* to protect bat populations. The results of these studies, along with our current understanding of the ecology of *Geomyces* species in cave environments will also be presented. [oral]

Using Acoustic Transects to Monitor Bat Population Trends in the Eastern United States

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Bats are difficult to survey and except for a few species that are readily trackable in caves during hibernation, researchers have little idea of their population levels. This inability to monitor bats makes assessment of the impacts of white-nose syndrome difficult for many species. In 2009, we initiated a bat monitoring program using ultrasonic detectors to sample bats along transects. The initial year's efforts resulted in sampling over 200 transects throughout the eastern United States. While not able to assess monitoring efforts with only a single year of data, presence of existing data helps confirm potential utility of this technique. Results from a single transect sampled in 2006 and 2009 showed a dramatic decrease in *Myotis* activity. Data management issues were present early in the process, but were manageable after additional planning and technological improvements to the analysis system. This project establishes a foundation on which regional efforts can be established to assess population-level impacts to bats that cannot be easily assessed in other ways. [oral]

Behavior of Bats with White-Nose Syndrome

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In 2006, a fungus was found growing on the muzzles and forearms of bats in New York. This fungus was later found to be connected to the death of a million bats in the Northeastern United States. The relationship between the fungus, *Geomyces destructans,* and the disease, known as white-nose syndrome (WNS), are now at the forefront of bat research. Behaviorally, research on healthy hibernating bats is limited, let alone on bats with WNS. Previous work has shown that bats with WNS are arousing more frequently than normal from hibernation. This research provides valuable information into the question of what they are doing during these arousals, which has been identified as a topic of high research priority. To monitor the behavior of these bats, infrared motion-sensitive cameras and acoustical monitoring equipment were deployed in WNS affected and unaffected caves, and in captivity. The duration and frequency of behaviors was measured, including grooming, non-flight locomotion and flying. Bats with WNS were shown to change locations within the hibernacula more so than healthy bats and WNS bats also had higher levels of activity. The systems will be deployed again next year for more data collection. [poster]

Histology of the Temporal Progression of WNS and Comparison of the PHA Response of Bats with and without WNS

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In order to better characterize the pathogenesis of white-nose syndrome (WNS) and the involvement of *Geomyces destructans*, two histopathology based studies were undertaken. In the first study, wing membranes from bats in known WNS and WNS free areas were evaluated for histologically detectable lesions. Wings were given a numeric score between 0 and 4 based on four microscopic categories: amount of *G. destructans*, invasiveness of *G. destructans*, amount of inflammation and amount of tissue necrosis. Using these data we have been able to document increasing prevalence of G. *destructans* infection throughout the hibernation cycle, differences in *G. destructans* prevalence in different states, and temporal correlation of *G. destructans* infection with the development of wing damage. Furthermore, we have documented superficial fungal infections in bats from areas without WNS. These infections are distinct from *G. destructans* infections and are considered incidental. The aim of the second study was to determine if the morphology of the inflammatory response differed between groups of hibernating bats with and without WNS. These results were compared to euthermic bats in order to determine if hibernating bats could mount a similar inflammatory response to their active counterparts. Bats were given subcutaneous injections of PHA, a known immune stimulant, and the injection areas biopsied at different times post-injection. Biopsies were scored based on type of inflammatory cells, amount of edema and amount of vascular reaction. Preliminary results reveal that the euthermic bats tended to mount a more robust inflammatory response than hibernating bats. Furthermore, the main inflammatory cells detected in the active bats were neutrophils and eosinophils, while hibernating bats tended to have more lymphocytes. The results of these two histopathology studies will be combined with the results of body condition and laboratory based studies of immunocompetence in the bats. Taken together, these data will yield important information on the epizootiology and pathogenesis of WNS and eventually provide information that can be used to better understand and manage the spread of the disease. [oral]

WNS and the National Park Service; What Have We Got to Lose and What Are We Doing About It?

KEVIN CASTLE

National Park Service, Fort Collins, CO

White-nose syndrome emerged as a devastating new disease of North American hibernating bats over the past four winters. Thousands of caves and mines are administered by the National Park Service, and many units contain tunnels and other natural or man-made structures that serve as summer bat roosts or winter hibernacula. Bats testing positive for white-nose syndrome or its causative agent, *Geomyces destructans* have been detected at three sites in the National Park System thus far. The National Park Service has been preparing for the spread and effects of white-nose syndrome through a proactive national program of response coordination, research support and interpretation, and education. National park areas across the nation are uniquely situated to help understand white-nose syndrome and its ecosystem impacts, and assist in the conservation and recovery of affected bat species. [oral]

The White-Nose Syndrome National Plan – an Overview

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U.S. Fish and Wildlife Service, Hadley, MA

Since the discovery of white-nose syndrome (WNS) in 2007 State and Federal biologists have been working with academic researchers, NGOs, and private partners to investigate and manage the threat presented by this disease to hibernating bat species in North America. At a June 2008 meeting in Albany, NY, biologists and managers developed informal working groups and a coordination structure to guide the collective response to the disease. By spring 2009 the rapid spread and devastating impacts of WNS made it apparent that a coordinated response to the disease was warranted at the national level. A multi-agency writing team was convened by the U.S. Fish and Wildlife Service (USFWS) to draft a plan to provide guidance for the response of Federal, State, and Tribal agencies to WNS. The writing team was composed of representatives from: USFWS, U.S. Geological Survey, National Park Service, U.S. Forest Service, Department of the Army - Corps of Engineers, Animal and Plant Health Inspection Service, Bureau of Land Management, St. Regis Mohawk Tribe, and biologists with state wildlife conservation agencies in the following six states – Kentucky, Missouri, New York, Pennsylvania, Vermont, and Virginia. The WNS National Plan (hereafter the Plan) formally establishes seven working groups to address critical needs for the national response: 1) Communications, 2) Data & Technical Information Management, 3) Diagnostics, 4) Disease Management, 5) Etiology & Epidemiological Research, 6) Disease Surveillance, and 7) Conservation & Recovery. The new working groups will incorporate existing efforts and guidance produced prior to the implementation of the Plan. We anticipate having a draft of the Plan available for public comment through the Federal Register in July 2010, but the implementation of elements from the Plan will begin as necessary. The Plan will provide guidance and coordination at the national level, and is intended to mesh with, but not replace, state or regional planning efforts that target critical response activities at the local level. [oral]

Update on White-nose Syndrome (WNS) Research and Response Activities at the USGS Fort Collins Science Center

PAUL CRYAN

U.S. Geological Survey (USGS), Fort Collins Science Center, Fort Collins, CO

Biologists at the USGS Fort Collins Science Center have been involved in the response to WNS since it was first discovered in 2007. This talk will provide an overview of associated research and support activities including: development and deployment of an infrared surveillance camera system capable of monitoring the behaviors of infected and uninfected bats hibernating deep within remote caves and mines; assessing the potential role of homeostatic imbalance as a proximate cause of mortality during winter; developing a specimen tracking system for monitoring and studying the progression of the disease; and working with scientists at Colorado State University to try and predict the potential spread of WNS across the continent. Collaboration has been extremely important. [oral]

Population Genetics of *Geomyces destructans* from Whole Genome Sequences

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Project timeframe for genome sequencing: July 2009 – July 2010
Project timeframe for whole genome resequencing: May 2010 – May 2011
The genome sequencing of the fungus *Geomyces destructans*, the apparent cause of white-nose syndrome, is nearing completion. This genome will provide the basis for research into the genetics of the fungus, including population genetics, phylogenetics and comparative genomics. Because of the recent emergence of the disease and rapid spread within only a few years, little genetic differentiation is expected. Thus, we will use whole genome comparisons from resequencing of 15-20 isolates from diseased bats collected throughout the current range of the disease in the U.S. We expect genome coverage of at least 100X, enabling in-depth comparison of isolates to assess genetic changes across the landscape. We will also compare these genomes to three isolates from Europe to evaluate differentiation and the potential for the fungus to have emerged from European bats. The ultimate goal is to use genomics to assess spread, source populations, transmission, pathogenicity, detection and evolutionary history of the fungus. [oral]

The Relationship between Dietary Fatty Acids and WNS Susceptibility at Both the Species and Population Levels

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The mortality associated with white-nose syndrome (WNS) is due to the depletion of depot fat reserves before spring feeding. This in turn is caused by greatly reduced torpor bout lengths during hibernation in bat populations with WNS. The cause of the abnormal torpor patterns in WNS populations is unknown. Numerous studies on other species of hibernating mammals have revealed that moderately high levels of polyunsaturated fatty acids (PUFAs) are required in the diet during the fall for proper torpor. PUFAs cannot be synthesized by mammals, but these fatty acids accumulate in their membrane and storage lipids when present in the diet. Two PUFA types have been previously shown to influence torpor: linoleic and α-linolenic acids. We thus predicted that the fall diets of bat species that are not susceptible to WNS have relatively higher levels of PUFAs than those of species that are susceptible to WNS found in the same habitat. We also predicted that the fall diets of bat populations where WNS does not occur have relatively higher PUFA contents than the diets of populations where WNS occurs. We tested these hypotheses by analyzing white adipose tissue samples from: a) little brown bats (*Myotis lucifugus*) collected from different hibernation sites, and, b) big brown bats (*Eptesicus fuscus*), a species not strongly affected by WNS. The fall diets of *E. fuscus* contain significantly more linoleic acid than those of *M. lucifugus* collected from the same mine with WNS. Our analyses also revealed that *M. lucifugus* populations prone to WNS have significantly less α-linolenic acid in their fall diets than those where WNS does not occur. Our findings thus support the hypothesis that bat species not susceptible to WNS have fall diets containing relatively higher levels of PUFAs than those of species feeding in the same area that are susceptible to WNS. Our results also support the hypothesis that bat populations not suffering from WNS have higher levels of PUFAs in their fall diets than those with WNS. It thus appears that dietary PUFA content affects WNS susceptibility at both the species and population levels. [oral]

Impacts of White-Nose Syndrome on Population Viability of Little Brown Myotis (*Myotis lucifugus*) in North America

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White-Nose syndrome (WNS) is an emerging infectious disease causing mass mortality and precipitous population declines of hibernating bats in eastern North America. Six species are currently affected, but WNS now extends into the range of at least nine species of hibernating bats that could be vulnerable to infection, including several endangered species. The little brown myotis (*Myotis lucifugus*), one of the most abundant, widely distributed, and best-studied species of bat in North America, has suffered some of the highest observed mortality at hibernacula infected with WNS. Annual decreases in numbers of hibernating little brown myotis range from 30% to 99% at sites with WNS. Using population viability analysis (PVA), we estimated the probability of regional extinction of little brown myotis from disease-associated mortality to determine the impact of introduction of WNS on this species. We compared trends in pre- and post-WNS populations and simulate 100 years of post-WNS population dynamics to assess the consequences of introduction of the disease on regional population viability. We evaluated the potential for disease fade-out and test hypotheses regarding the influence of density and time since infection on population growth rates at infected hibernacula. There is little evidence of density-dependence in disease mortality, but data suggest that population declines may lessen with time since infection. Our model predicts that even if disease-mortality ameliorates with time that the regional population of little brown myotis in the northeastern U.S. could face extinction within 20-40 years and experience a population crash to less than 1% of its pre-WNS population size within 20 years. We will discuss these results in the context of potential management actions and conservation strategies. [oral]

A Wing and a Prayer: Little Brown Myotis (*Myotis lucifugus*) Recover from Wing Injuries Associated with White-Nose Syndrome

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Project start date: May 2009
Anticipated project completion: Manuscript in preparation

Since the appearance of white-nose syndrome (WNS) in North America, researchers have observed discolored and necrotic wings on little brown myotis (*Myotis lucifugus*) at maternity colonies. Damaged wings are hypothesized to result either from fungal and bacterial infections or from frostbite during atypical winter activity. Over the course of the summer, the apparent abundance of bats with severely damaged wings decreases, leading to speculation that reduced flight ability makes bats susceptible to increased predation or reduced foraging success. We tested the hypothesis that reduced frequency of severe wing damage results from healing rather than mortality. We trapped bats weekly at the time of nightly emergence, alternating between two maternity colonies in Massachusetts and New Hampshire. Bats were assessed for age, sex, reproductive condition, length of forearm, and wing damage index (WDI). Wings were transilluminated with a lightbox and photographed before bats were banded and released. Digital photographs were analyzed for changes in pattern and severity of wing damage. As previously documented, the relative proportion of bats with severe and moderate wing damage decreased as the summer progressed. Body mass index [BMI = body mass (g) / length of forearm (mm)] for adult females varied among different WDIs. Bats with moderate wing damage had significantly lower BMI than bats with minor or negligible scarring. We recaptured 36 bats, 50% of which experienced improved wing conditions, including at least two individuals whose wings improved from the most severe condition (WDI = 3) to a lesser one (WDI ≤ 2) in less than three weeks. Our results suggest that wings of little brown myotis can heal quite rapidly from injuries sustained during winter. Thus, decreased occurrence of severely damaged wings later in the summer does not necessarily lead to increased mortality. Further studies are needed to investigate the long-term survival of individual bats and population trends at WNS-affected hibernacula and associated maternity colonies. [poster]

Hibernacula Microclimate and White-nose Syndrome Susceptibility

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Anecdotal evidence suggests that bats that hibernate in colder and drier caves and mines are less affected by the emerging infectious disease white-nose syndrome (WNS) and that WNS-affected bats shift roosts in early-mid winter to cave entrances. To test the notion that colder microclimates offer protection against WNS, affected little brown myotis (*Myotis lucifugus*) were housed in captivity at different temperatures (4^{o}C, 7^{o}C, and 10^{o}C). Likely due to energy savings and slowed growth of the putative fungal pathogen, bats hibernating at lower temperatures lived longer. To determine why WNS-affected bats shift to cave entrances, the thermal preference of WNS-affected and unaffected captive and free-ranging bats was tested. We predicted that WNS-affected bats hibernating at the front of the hibernacula would prefer colder temperatures than WNS-affected bats hibernating in ‘normal’ roosts deeper in the cave, but all bats selected relatively warm roosting sites (8.06^{o}C ± S.E. 0.46). This suggests that WNS-affected bats are moving to the entrances of hibernacula not to select colder roosts and thus conserve energy, but for some other reason. The results from this study will help predict which hibernacula are more likely to be infected and whether altering microclimate properties of mines may mitigate the disease. [poster]

The Temporal Dispersal of White-nose Syndrome - Preliminary Report

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The data on dispersal of white-nose syndrome (WNS) infected bats is both frightening due to the rapid rates of the dispersal and striking in the patterns of distribution. Our initial efforts focus on an analysis of Pennsylvania data because of the quality and breadth of available data. We will discuss a conjecture that dispersal is bimodal in that there are both short distance dispersals and long distance dispersals of infected bats. We are currently working with the data for the United States and Canada to determine if this conjecture remains valid for the range of dispersal of *Geomyces destructans*. Mathematical models currently under development are agent based. One model is formulated to understand the implications of the bimodal distribution with affected hibernacula used as agents. Another more complex model with bats as agents focuses upon movement of infected bats during life history stages and will be used to predict probabilities of dispersal of infected bats. [oral]

Munitions Bunkers Potentially Available as Bat Hibernacula for WNS Research and Management

ALAN C. HICKS

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Many of the research needs associated with white-nose syndrome (WNS) would benefit from having previously unoccupied hibernacula in which air flow, temperature, and humidity could be manipulated and where bats would have as close to a natural hibernating environment as captivity can allow. Having disease free hibernacula (or sites that should be easily decontaminated) within the WNS zone would allow researchers to work with *Geomyces destructans* without fear of infecting the surrounding landscape or native bats. These possibilities exist within munitions bunkers at the former Seneca Army Depot in Central NY, now operated by the Seneca County Industrial Development Agency. Access to these facilities might be arranged through DEC for the appropriate projects. Depending on the requirements of each study, bunkers might be available free of charge. If there is interest in these facilities, DEC will work to secure access. [poster]

Investigations into the Environmental Transmission of WNS to Hibernating *Myotis lucifugus*

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Project start date: October 2010
Project completion: April 2010

Addressing the white-nose syndrome (WNS) related decline in bats requires in part, an understanding of the role played by environmental transmission of the disease during the hibernating season. To further that understanding , we transferred 79 *Myotis lucifugus* from a WNS free mine in northern Wisconsin and released them into two WNS positive mines in Vermont (Greely and Bridgewater), where native bats had been excluded. Wing bands and uniquely marked data loggers or similar masses were attached to each bat. We took elaborate precautions to make sure that the introduced bats would not escape and return to Wisconsin. Bats were released in each mine on October 26, 2009, and were subsequently visited on December 16, January 27, February 18, March 18, and April 9, 2010. On each visit we recorded the location of all bats encountered, collected dead, and severely moribund, individuals for necropsy and photographed all animals on roost so that we could check for visible evidence of *Geomyces destructans (G.d.)* infection (the presumed causative agent of WNS). Twenty one bats (12 of 41 at Bridgewater, 9 of 38 at Greely) were never observed alive during these visits and presumably died from non-WNS causes. Among the living observed on 12/16 (8 weeks post-release), 15 of 24 (Greely) and 1 of 28 (Bridgewater) showed visible evidence of a G.d. like infection. In Greely, all bats that had ever been observed alive were dead by 2/18/2010. Four bats were still alive at Bridgewater on 3/18/2010 one of which was still alive, but moribund on 4/8/2008. We have not completed the examinations, or analysis of data, so we cannot yet state how many mortalities can be attributed to WNS. We discuss the likelihood of a positive finding and what it could mean for disease management and the future of affected bat species. [oral]

Initial Tests of the Anti-fungal Agent Terbinafine on the Little Brown Bat (*Myotis lucifugus*) in WNS Infected Hibernacula

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Project start date: February 2010
Project completion: April 2010

If recent downward trends in bat populations continue as a result of white-nose syndrome (WNS), it may eventually be necessary to treat animals with anti-fungal agents to prevent species extirpations, or extinctions. Doing so will require an efficient means of testing candidate products in the field. We describe preliminary results in the application of three topical formulations of the anti-fungal agent

Terbinafine to *Myotis lucifugus* in two hibernacula. The study was initiated to both test the efficacy of the drug against the fungus *Geomyces destructans* under field conditions *and* to develop an efficient field testing procedure. Animals within reach were placed into one of six treatment groups in the order that they were collected. These included three control groups (not handled, band only, or aqueous delivery vehicle) and three experimental groups (aqueous Terbinafine, aqueous Terbinafine and Citral, or Terbinafine ointment). All handled animals were processed in the same manner (including swabbing, banding, and release) and were isolated from one another throughout processing. On February 3, 2010, we processed all 123 *M. lucifugus* present in the Cranberry Mine, Orange County, NY. When we revisited the site on March 24, 2010, only 2 *M. lucifugus* (both unbanded) were present. On February 9, 2010, we processed 300 of the 450 *M. lucifugus* counted at the Hooper Mine, Washington County, NY. On April 1, 2010, we found approximately 250 live *M. lucifugus* of which roughly 42 were wearing bands. Although not yet finalized, we are confident that the results will show no significant differences in survival among the three groups treated with antifungal drugs and the two control groups that involved handling. However, bats that were not handled (including those beyond reach) clearly survived at a higher rate than handled bats. We discuss the processing procedure, conditions that may have influenced the study results, and recommendations for future actions. [oral]

The Characteristics of *Geomyces destructans* Infection of Bat Skin as Demonstrated by Scanning Electron Microscopy of Naturally Infected Tricolored and Little Brown Bats

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Project start date: February 2010
Anticipated project completion: July 2010

The skin of two little brown bats (*Myotis lucifugus*) and four tricolored bats (*Perimyotis subflavus*), with grossly visible lesions consistent with white-nose syndrome, were examined by scanning electron microscopy. All bats were euthanized and immediately fixed in 10% neutral buffered formalin then post-fixed in glutarldehyde. Skin samples from the muzzle, patagium and trunk of each bat were dehydrated by critical point drying and sputter-coated with gold prior to microscopy. Growth of hyphae on the muzzle was generally diffuse, but proliferation on the patagium was much more multifocal. Hyphae were tightly adhered to the surface of the stratum corneum and occasionally infiltrated the stratum corneum, extending under the edges of individual cornified epithelial cells. Hair follicles of the muzzle and patagium were frequently invaded by hyphae. Areas with more dense hyphae had some hair shafts with adherent clumps of fungal elements, but invasion or damage of the hair shafts was not observed. Densely furred areas from the trunk had little growth of fungus. The characteristics of the infection did not differ appreciably between the little brown bats and tricolored bats examined. Erosion or inflammation of the epidermis was not evident in any of the bats. Sporogenesis was observed on all the bats examined and conidia were very numerous in some individuals. The epidermis of the muzzle in some individuals was paved by dense mats of conidia. Our findings illustrate the tight adherence of hyphae to the skin, paving of epidermal surfaces by conidia, the lack of damage to hair shafts and only mild damage to the stratum corneum itself. Adherence is a critical factor in the pathogenesis of dermatophytosis and might also be an important factor in infections by *G. destructans*. [oral]

A Remote Internet-based Video System for Assessing Colony Size and Seasonal Dynamics of Little Brown Myotis (*Myotis lucifugus*) Affected by White-nose Syndrome

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Project start date: February 2008
Anticipated project completion: November 2010

Assessing colony size and seasonal dynamics of bats are among the most important challenges facing ecologists. These variables are especially relevant when bats are experiencing threats from emerging diseases, anthropogenic disturbances, and global climate change. We describe a network-based infrared video system (BatCam I) capable of censusing bats during nightly emergences and transmitting these digital data to a local laptop computer over the Internet to remote stations. BatCam I consists of a small, weatherized web-based camera connected to a local video server and gateway for Internet access. Using the BatCam I, we successfully recorded emergence activity and censused a small colony of little brown myotis (*Myotis lucifugus*) that roosted in a specially-designed bat house at Moore State Park, Paxton, Massachusetts, from mid-April through late September in 2008 and 2009; years following the appearance of white-nose syndrome (WNS) in New England. Emerging bats in each video frame were first detected by an algorithm using background filtering, in which an observed image frame was compared with an estimate of the background images without bats. A second algorithm tracked each newly detected bat through the camera's field of view. An estimate of colony size was determined from the number of bats that were tracked throughout the video sequence recorded during each emergence. This novel, remote-controlled BatCam and subsequent analytical approach promises to be a valuable tool for assessing nightly, seasonal and interyear changes in colony size, and may be especially important for assessing changes in maternity colonies of bats affected by WNS. Bats first began to arrive in mid-April, and reached a peak colony size of 210 adult bats in early- to mid-June, before young were fledged. Most of the bats had emigrated by late September. Based on nightly video recordings, peak adult colony size decreased by 70% from 2008 to 2009, which we attribute to high winter and spring mortality associated with WNS. At present, remote video recordings provide a cost-effective and reliable method for monitoring colony size and nightly emergence behavior of bats, and can be deployed in multiple locations without committing abundant man hours each night. [oral]

Declines of Six Hibernating Bat Species from White-Nose Syndrome in the Northeastern United States

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Variation in susceptibility to pathogen infection among species can provide valuable insight into the causes and consequences of emerging infectious diseases. White-nose syndrome (WNS) is thought to be associated with the newly described psychrophilic fungus *Geomyces destructans*, with documented infections in all six hibernating bat species in the northeastern United States. The greatest prevalence of WNS infection has been observed in this region, with overall declines at hibernation sites ranging from 70 to 100%. Despite a progressive lessening of mortality rates at some hibernacula affected by WNS, there is no clear evidence of resistant colonies or decreased susceptibility of infection by survivors. Based on surveys conducted at hibernacula during pre- and post-WNS periods, the little brown myotis (*Myotis lucifugus*), northern long-eared myotis (*M. septentrionalis*), and tri-colored bats (*Perimyotis subflavus*) have shown the largest population declines. Substantial declines in northern long-eared myotis at hibernacula have caused serious concern among natural resource managers that this species may be in immediate danger of regional extinction. Despite its highly gregarious winter roosting habits, the Indiana myotis (*M. sodalis*) has experienced less severe decline as compared to little brown myotis, northern long-eared myotis, and tri-colored bats. The relatively rare eastern small-footed myotis (*M. leibii*) is found in a small number of hibernacula, and while overall region-wide decline has been observed, the impact WNS on this species, as well as the relatively abundant big brown bat (*Eptesicus fuscus*), is not yet clear. Variation in disease susceptibility among the six species of bats affected by WNS in North America suggests that behavioral, environmental, physiological, and immunological factors may contribute to differences in susceptibility to infection from *G. destructans*. [oral]

Genetic Approaches to Understanding the Spread of White-nose Syndrome in Little Brown Bats (*Myotis lucifugus*)

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Project start date: October 2008
Anticipated project completion: August 2011

White-nose syndrome (WNS) is an emerging disease that primarily affects hibernating bats. Between 75-100% mortality has been reported in affected colonies, and little brown bats (*Myotis lucifugus*) have experienced the highest documented mortality rates. Recent evidence has demonstrated that the disease may be spread through direct bat-to-bat contact. Therefore, although recent cave closings may effectively stop the spread of WNS via humans, it will not control WNS transmission through the bats themselves. Future strategies aimed at mitigating the spread of WNS will thus require advance knowledge of which naïve colonies are likely to be most at risk for infection, based on patterns of seasonal bat movements across the landscape. This information is currently lacking, and our research aims to fill this gap. The goal of this project is to employ population genetic analyses to describe the genetic population structure of *M. lucifugus* across its entire range. This will enable us to determine the geographical scales over which colonies share individuals through gene flow, with the aim of predicting likely routes of bat-mediated transfer of WNS throughout North America. In addition, this study will quantify current levels of genetic diversity in the *M. lucifugus* population, and monitor this through the predicted WNS-induced bottleneck. This will enable us to estimate the long-term potential of the *M. lucifugus* population to recover from this devastating disease. Our research is critical for understanding the future spread of WNS, for the development of management or mitigation strategies to limit transmission to new regions, and to facilitate recovery of affected populations. [oral]

Immunological Correlates of 'White-nose Syndrome' in Hibernating Little Brown Myotis (*Myotis lucifugus*)

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Project start date: February 2008
Anticipated project completion date: December 2010

We are conducting research on the immune system of the little brown myotis (*Myotis lucifugus*) to test the following hypotheses: 1) bats from white-nose syndrome (WNS) affected sites have weakened immune responses, 2) bats with visible signs of fungal infection have reduced immune responses when compared to those without visible infections from the same sites, 3) euthermia is necessary for elevating immune responses, 4) bats have different strengths of response to various pathogens, 5) variation in immune function is related to hibernation physiology, and 6) immunological responses are constrained in hibernating WNS-affected bats due to a depletion of fat reserves. We collected bats from WNS-affected and unaffected sites in early-, mid-, and late-hibernation during the winter of 2008-2009. We used bactericidal (*Escherichia coli* and *Staphylococcus aureus*) and fungicidal assays (*Candidia albicans* and *Geomyces destructans*), total immunoglobulin enzyme-linked immunosorbant assays (ELISA), colorimetric total antioxidant power assays, analysis of blood smears and C reactive protein ELISAs. We also used injections of phytohemagglutinin (PHA) and histological analysis of responses in bats challenged during the active season and during hibernation. Bats from affected sites had either significantly elevated or reduced innate immune responses depending on the microbe assayed and stage of hibernation. Affected bats had higher circulating immunoglobulins compared to bats from unaffected sites during late hibernation and antioxidant levels were lower in affected bats throughout the hibernation period. Compared to bats without visible WNS signs, bats with visible fungal infections had lower total immunoglobulin levels during early hibernation and reduced bactericidal ability and lower antioxidant levels throughout hibernation. Our results also indicate that euthermia is necessary for sufficient immune responses. We observed significant decreases in immune function and associated proteins as the hibernation period progressed, and significant relationships between body mass index (body mass/length of forearm) and immune responses. We found that the ability of blood from *M. lucifugus* to lyse microbial cells differs as follows: *E. coli* > *S. aureus* > *C. albicans* > *G. destructans*. Overall, our results suggest that the relationship between energetics and immune function in hibernating bats facilitates the development of WNS. [oral]

Searching for the Conidia of *Geomyces destructans* with a Microscope - Protocols and Suggestions

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The readily recognizable conidia of *Geomyces destructans* present an opportunity for low-tech investigations of white-nose syndrome (WNS). Requirements include only a good compound microscope, a few inexpensive supplies, and a search image. The latter can quickly be acquired with practice on known positive samples. Fiber-tipped swabs can be used to sample live or dead bats or hibernaculum surfaces. Examination of samples is conducted at 400-500X magnification. Findings can be documented simply with an internal focusing point and shoot digital camera. Possible applications of this approach include preliminary confirmation of WNS at new sites, investigation into the ecology of *G. destructans* in hibernacula, and, as a very non-invasive way of monitoring *G. destructans* in live bats, a fecal pellet examination. A hands-on opportunity to look at preserved samples through a microscope will be included. [poster]

Detection of the Conidia of *Geomyces destructans* in Northeast Hibernacula, at Maternal Colonies, and on Gear – Some Findings Based on Microscopy and Culture

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Geomyces destructans, the apparent causal agent of white-nose syndrome (WNS), produces enormous numbers of conidia (asexual spores). These conidia can be readily identified with light microscopy and can be cultured on standard media. In the last year we have employed these techniques to detect the presence of *G. destructans* in various investigations. Using a portable Burkhard sampler which deposits airborne particles on two-sided tape mounted on a microscope slide, we collected air samples (0.09 m^3/sample) from WNS-affected hibernacula in New York and Vermont. To date, examination of 36 samples from six hibernacula collected during the hibernation season has yielded a total of seven positive results from three sites. Five of the positive samples captured only one or two conidia. The highest conidia count (109) was collected less than 0.5 m below a small group of WNS-affected bats. All of the 33 samples collected at six hibernacula outside of the hibernation season have been negative. Microscopic searches of swab samples collected from surfaces in hibernacula on which airborne conidia are likely to be deposited have, so far, yielded mostly negative results. In contrast, swab samples from drill-holes at one mine (where direct contact with bats is likely) were mostly positive. Attempts to culture *G. destructans* from swabs of the same surfaces failed due to rapid growth of other fungi. Conidia can frequently be found on decomposed bat remains in WNS-affected hibernacula, although numbers decline rapidly with time and the growth and activity of other organisms. Findings at necropsy suggest that a lot of conidia are

swallowed in grooming during arousal bouts. These conidia can comprise the bulk of material in fecal pellets produced during hibernation. Conidia from the colon have been found to be viable on culture. The fate and importance of this concentration of conidia in fecal material awaits investigation. We have not yet found *G. destructans* growing on anything in hibernacula except live or freshly dead bats. At maternal colonies, swabbing of bats and direct media inoculations collectively yielded positive results in both May (3/15) and August (3/17) at Fort Drum, and in one of four bats at a colony in the upper Hudson Valley in June. Sampling at a colony near Lake Champlain (July, n=21) and another in the Finger Lakes (August, n=11) was negative. Conidia of *G. destructans* were observed in swab or rinse samples of apparel and a gear used in WNS-affected hibernacula. [oral]

The Bat Grid: a Model for WNS Surveillance and Population Monitoring for Bats

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Monitoring and inventory strategies associated with species presence benefit from spanning large portions of a species range, and from having a standardized sampling frame and protocol. Standardization facilitates confidence in temporal and spatial data analyses that support management decisions. In 2002, we initiated a sampling frame and protocol in the Pacific Northwest known as “The Bat Grid” that uses a grid-based sampling design and standardized methodologies for sampling morphologic, acoustic, and genetic data from bats. Sampling in Oregon and Washington targets fifteen bat species, spans nine years, and covers multiple land ownerships that allows for exploration of insular population distributions at an unprecedented resolution. Species occurrence and detection statistics and trends in these are fundamental outputs of this program and essential tools for species conservation efforts. These data and sampling efforts benefit surveillance efforts for white-nose syndrome (WNS) as well as establish a baseline for monitoring potential WNS-effected populations. [oral]

Fungal Digestion of Chiropteran Integument

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Project Need: This proposal directly addresses the need to protect bats from white-nose syndrome (WNS). The United States Fish and Wildlife Service (USFWS) draft framework (8 September, 2009) for assisting states, federal agencies, and tribes in managing WNS in bats includes the need for research that leads to a greater understanding of WNS pathogenesis, host and disease ecology, and eliminating or containing the disease in outbreak areas. This study will directly address these specific draft framework goals: E.3. *Action Items 1 and 3. Research on biology and pathology of WNS and disease management/host ecology*. We will monitor optimal growth conditions of *Geomyces destructans* on bat tissues and analyze how the host (bat hair and skin) provides essential nutrients for fungal growth and fungal metabolites. Currently, there is very little understanding of the pathogenicity of *G. destructans*. One of the most obvious symptoms of WNS is degradation of wing membranes. **Hypothesis:** 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 overall goal of this study is to establish a system to determine the damage by the pathogen on host integumentary tissue. **Objectives:** To assess *in vitro* the time-course for integumentary damage to occur and the amount of damage that occurs over time. To determine how *G. destructan s*and *G. pannorum* infection affects the mechanical integrity of the integument. This project will provide for the first time knowledge on the time course of disease progression from initial fungal colonization of bat integument and subsequent tissue damage. [poster]

The Potential Threat of White-nose Syndrome to European Bats: An Action Plan

Sebastien J. Puechmaille on the behalf of the White-Nose Syndrome Consortium consisting of 65 co-authors from 30 countries.

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A new disease, white-nose syndrome (WNS), is causing unprecedented mortality of six species of bats in the Eastern USA. The supposed agent of WNS is a newly described fungus, *Geomyces destructans*. Recent publications have confirmed the presence of *G. destructans* colonizing several species of European bats in France and other European countries (Puechmaille et al. 2010; Wibbelt et al. under review), and ongoing studies of samples from Belgium, the Czech Republic, Hungary, Italy, the Netherlands, Poland and Slovakia are being undertaken to confirm whether a suspected fungus growing on hibernating bat snouts and wings is indeed *G. destructans*. Despite finding colonization of *G. destructans* in European bats, to date no case of WNS has been found in Europe (Barlow et al. 2009; Puechmaille et al. 2010). In contrast to the American bats, European bats exhibiting *G. destructans* growth were in good body condition. These recent findings suggest four possible hypotheses regarding the emergence of WNS, (1) *G. destructans* has only recently arrived in Europe, (2) a non-lethal strain of *G. destructans* has been present in Europe and North America for many years; however, in New York State, a virulent strain of *G. destructans* has emerged that can override the immunological responses of its hosts, (3) *G. destructans* is not the primary mortality agent but acts as an opportunistic pathogen in immunocompromised individuals, and some other agent is killing North American hibernating bats or, (4) the fungus has been present in Europe for a long time and has only recently arrived in North America. Each of these hypotheses will be described and discussed. Finally, ongoing monitoring and research as well as future research needs and conservation strategies will be presented. [oral]

Mitigating White-nose syndrome: Results of Captive Treatment Trials, Behavioral Observations, Microclimate Variation, and Explorations of Immune Responses to WNS.

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A variety of approaches may eventually be taken to mitigate the effects of White-nose Syndrome (WNS) in bats, ranging from drug treatments to alterations in hibernacula characteristics. Understanding if and how these strategies will be successful requires greater knowledge of the epizootiology of WNS, including the physiological and behavioral effects of WNS on bats and the ecological factors underlying disease progression. We will report the results of several studies performed in the Reeder lab during 2009 and 2010, in collaboration with a number of other researchers. (1). Immune studies: Beyond the 'normal' immune suppression that occurs during hibernation, WNS affected bats appear to have even lower immune competence than unaffected bats, particularly in relationship to B-cell mediated immunity. Arousals from hibernation appear to upregulate immune mechanisms, but the brief duration of these arousals is likely insufficient for mounting a significant response to *Geomyces destructans* (Gd). Difficulties in developing an assay to detect antibodies against Gd have been encountered, and it is not yet known whether bats specifically respond to infection with Gd. (2) Captive treatment trials: 110 WNS infected bats were brought into captivity and tested with several antifungal compounds. While several of the treatments appeared to cause mortality, no bats appeared to have increased survivorship compared to controls. (3) Behavior and Microclimate: The behavior of WNS affected and unaffected bats was tracked in captivity and in the field. WNS bats displayed higher levels of behavior and *survived significantly longer when housed in colder conditions*. [oral]

Patterns of Fat Accumulation and Depletion in Little Brown Myotis (*Myotis lucifugus*) Affected by White-nose Syndrome

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Project start date: February 2008
Anticipated project completion: August 2010

Emaciation in bats affected by white-nose syndrome (WNS) is a frequently stated and commonly accepted condition. However, a thorough examination of fat accumulation during swarming and depletion during prehibernation at affected and unaffected sites may provide greater insight into the role of energy stores in the development of WNS. We used destructive body composition analysis to quantify patterns of fat accumulation during prehibernation at Aeolus Cave and depletion during hibernation at affected and unaffected hibernacula to test the following hypotheses: 1) bats at WNS-affected sites do not deposit sufficient fat reserves during the prehibernation period; 2) bats at affected sites begin hibernation with lower fat reserves than bats at unaffected sites; 3) bats at affected sites deplete fat reserves earlier in hibernation than bats at unaffected hibernacula. Our analyses indicate that body mass index [BMI = body mass (g) / length of forearm (mm)] provides a reasonable estimate of total body fat (TBF) in little brown myotis through hibernation. Although bats at the affected Aeolus Cave deposited similar amounts of fat in autumn compared to unaffected bats, TBF in early winter was lower on adult bats at affected sites than at unaffected sites. At affected sites, BMI decreased between early and mid-winter, but remained relatively stable between mid- and late-winter. However, BMI appears to decline more gradually at unaffected sites. In early winter, mean percent body fat at unaffected sites was 28.1% and 24.9% for adult females and males, respectively. Mean percent body fat was significantly lower at affected sites in early winter: 20.1% and 19.4% for adult females and males, respectively. At Aeolus Cave, mean percent body fat of adults declined from 17.1% in early winter to 8.9% and 5.5% in mid- and late-winter, respectively. For juvenile bats at Aeolus Cave, mean percent body fat declined from 18.0% in early winter to 7.7% and 7.0% in mid- and late-winter, respectively. Bats swarming at WNS affected sites appear to deposit sufficient fat reserves during fall, but these reserves appear to be depleted relative to unaffected bats both during the final stages of prehibernation and the early stages of hibernation. At affected sites, hibernating bats appear to reach critically low fat reserves by late January, which is about halfway through the hibernation period. [oral]

Banding for White-nose Syndrome Surveillance and Research

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Although white-nose syndrome (WNS) may be spread by humans, it is generally agreed that bat-to-bat contact is the major avenue of transmission. Predicting the spread of the disease and containing WNS will require a better understanding of bat movements across the eastern U.S. and Canada. However, very little is known about bat movements, either between summer and winter ranges or among hibernacula, and obtaining this information will require intensive monitoring of individually-marked bats. Banding can provide valuable information on many aspects of bat biology and ecology including migration, movements among hibernacula, and demography. Extensive bat banding in the U.S. from 1932 to 1972 resulted in considerable information about migration of bat species currently impacted by WNS, but most of these efforts were abandoned when banding was implicated in the decline of several populations. A review of the banding injury rates suggests that much of the population-level impact of banding was due to the methodology and not to direct injury. New metal alloy lipped bands have substantially lower injury rates than historic bands, and recent studies have proven the value of banding for collecting data on population demographics and migration patterns that are critical for the surveillance of WNS and the ultimate population recovery. Specific questions regarding the transmission, spread, and dynamics of WNS that can be addressed using banding include movements of bats between summer and winter ranges, movement of bats among swarming sites and hibernacula, transmission of WNS through capture and handling, survival of bats in WNS sites, response to treatments, survival relative to wing damage, and genetic and microbial factors related to immunity. Although banding and other marking techniques are not risk-free, the data gathered using these techniques may be worth the risk if they contribute to the long-term survival and recovery of WNS-affected species. [oral]

Biometric Changes Associated with WNS Progression in Virginia, Preliminary Results

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We collected biometric data (species, age, sex, reproductive condition, Wing Damage Index, presence/absence of flaking or fungus, RFA, and mass) on 1690 bats at 12 sites between October 2008 and May 2010. Individuals were captured during the swarming period (autumn) using harp traps or mist nets and handled within the caves during early and post-hibernation periods (torpid bats). Twenty-six adult *M. lucifugus* (22 males, 4 females) were banded at four sites in October 2008 and forty-one adults (37 males, 4 females) were banded at these same sites in October 2009. The mean body mass for males appeared greater in 2008 (9.1 g ± 1.0 SD), but was not significantly different (T = 475, P = 0.19) than in 2009 (8.5 g ± 1.8 SD). Eighteen adult *M. septentrionalis* were banded at three sites in October 2008 (16 males, 2 females) and forty-nine adults were banded at these same sites in October 2009 (40 males, 9 females). The mean body mass for males in 2008 (8.5 g ± 0.6 SD) was significantly different (T = 586.5, P = 0.018) than 2009 (7.8 g ± 1.5 SD). Sixteen adult *P. subflavus* were banded at two sites in October 2008 (10 males, 6 females) and sixty adults were banded at these same sites in October 2009 (51 males, 9 females). The mean body mass for males in 2008 (7.5 g ± 0.7 SD) was significantly different (T = 472, P = 0.002) than in 2009 (6.7 g ± 0.7 SD). *M. lucifugus* in early hibernation were captured and banded inside caves between late October and late November 2009. Cave comparisons were made in reference to assumed WNS negative (Cudjo's Cave) and WNS positive caves (Breathing, Newberry-Bane, and Clover Hollow) in 2009. The mean body mass for males at Cudjo's Cave (8.3 g ± 0.7 SD) was significantly different than at Breathing (7.4 g ± 0.6 SD; t = 11.76, P = <0.001), Newberry-Bane (7.9 g ± 0.8 SD; T = 4939.0, P = <0.001), and Clover Hollow (7.7 g ± 0.7 SD; T = 741.5, P = 0.001). Fourteen *M. lucifugus* banded (5 of which had fungus) in May 2009 at Breathing Cave were recaptured in autumn 2009; bats with fungus in May showed mass changes from -9 to 15% compared to 5 to 35% for those without fungus. [poster]

Amphibian Chytridiomycosis: Epidemiologic and Conservation Parallels to White-Nose Syndrome in Bats

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Though much is still unknown about white-nose syndrome, there are a number of close parallels to amphibian chytridiomycosis (Bd), a fungal epizootic that has caused the extinction of more than 100 frog species in recent decades: Both WNS and Bd are likely to have resulted from the introduction of a novel fungal pathogen, the epizootics affect multiple host species, both are associated with population-wide mortality rates that may reach 100%, mortality is seasonal and affected by the temperature tolerances of the fungal pathogen, and, in both cases, the fungal pathogen can probably persist as a saprobe. Despite a decade of research on the disease ecology and epidemiology of chytridiomycosis since it was identified in 1998, it has thus far been impossible to either stop the spread of the disease on contiguous land masses or to eliminate the fungus from the environment. Recent progress on chytridiomycosis research and frog conservation relevant to possible research avenues for white-nose syndrome will be discussed. [oral]

Testing the Effectiveness of a Passive Acoustic Monitoring Technique to Detect WNS-infected Sites

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White-nose syndrome (WNS) has spread rapidly across the eastern United States. Despite the importance of conducting surveillance for this devastating disease, resource managers are overwhelmed by the number of hibernacula that need monitoring. Additionally, there is concern over human transmission of WNS and disturbance to bats by entering hibernacula. Therefore, a passive technique that requires little human intervention would greatly improve efficiency to conduct WNS surveillance while reducing disturbance. Abnormally high bat activity has typically been documented at WNS infected sites during the hibernation season, but there have been few efforts to quantify activity levels at hibernacula entrances. We tested the ability of the Anabat II detectors to detect changes in activity at WNS symptomatic (infected) and asymptomatic (assumed WNS-free) hibernacula. We deployed Anabat systems, 21 December to 15 April 2009-2010, to automatically record bat activity at seven hibernacula (3 - second-year infected sites, 2 – first-year infected sites (one only PCR confirmed), and 2 - asymptomatic sites). Second-year WNS sites showed abnormally high activity levels even at the beginning of our sampling period. Second-year sites showed higher activity than both first-year and asymptomatic sites, similarly first-year sites showed higher activity levels than asymptomatic sites, indicating that it may be possible to detect differences in behavior even in the first year of infection. Differences were most pronounced in the months of Dec-Feb especially for second-year sites. The patterns of activity were more similar in March and April for all sites, probably due to the natural emergence period for hibernating bats. Another possible indictor of infection was mean activity during the day which ranged from 6-23 calls/day for Dec-Mar for second-year sites, similarly our visually confirmed first-year infected site had a 200-fold increase in mean daytime activity for Dec-Mar (0.09-19.58 calls/day). Alternatively asymptomatic sites demonstrate low mean daytime activity (0.2-1 calls/day). Acoustic monitoring appears to offer an effective, affordable, and less invasive approach for surveying multiple hibernacula for WNS. [poster]

Potential Role of Emerging Contaminants in White-nose Syndrome of Bats

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The U.S. Fish and Wildlife Service is investigating the potential role of environmental contaminants, particularly emerging contaminants, in white-nose syndrome (WNS) in bats. This syndrome has decimated bat populations in the northeastern United States. It is characterized by unusual behavior, weight loss, mortality and a unique fungus that may sometimes be apparent on the nose, wings and tails of affected bats. This project is being conducted by U.S. Fish and Wildlife Service offices in New York, Pennsylvania, New England and Indiana. We have performed chemical analysis of bats collected from a number of locations in New York State for legacy chemicals that have historically been associated with adverse impacts in mammals, including bats. We have also analyzed bat samples for those pesticides that are most commonly used in New York today. Fairly low concentrations of tDDT, PBDEs, chlordane, dieldrin, chlorphenoxy herbicides (dalapon and chlorpyralid) and the neonicotinoid insecticide, thiamethoxam, were detected. We found no PCBs, PCTs, pyrethroids, carbamates, organophosphates, or triazines in excess of detection limits. We propose additional chemical analysis for PBDEs, neonicotinoides, and substituted benzenes, as well as analysis for emerging chemicals that are becoming more relevant in the environment. [poster]

Challenges in Establishing an *Ex Situ* Colony of the Virginia Big Eared Bat *(Corynorhinus townsendii virginianus)*

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Due to the occurrence of white-nose syndrome in the habitat of the endangered Virginia big-eared bat (VBEB), the feasibility of establishing a 'security', *ex situ* population of this subspecies was explored. On 9 November 2009, 40 (20.20) VBEBs were collected from a cave in Pendleton County, West Virginia, and transported to the Smithsonian Conservation Biology Institute (SCBI) in Front Royal, Virginia, with a single mortality occurring upon arrival. The remaining bats were weighed, separated by sex, and each group placed in an indoor enclosure. Initially, bats were weighed twice daily and hand-fed to ensure adequate food intake and hydration. Veterinary care was provided as needed. After onset of self-feeding, a given bat was handled only once daily (with weight as an indicator of general well-being) unless veterinary care was required. VBEB were slower to self-feed than predicted, and the time to transition varied among individuals. Of the 39 bats, two individuals readily ate whole mealworms (hand fed) on the day of arrival. By using a 'crate confinement' method, a total of 21 bats (7.14) successfully self fed within 3.5 months after being in captivity. Medical issues encountered included dermatitis (on face and neck related to feeding overly hydrated mealworms), digit and carpal abrasions (due to the need to land and walk on surfaces to feed) and unexpected deaths with no clear pathological cause for mortality. Fifty percent of the males and 50% of females (n = 34) died within 2 and 4 months, respectively. Most deaths were attributed to septicemia related to dermatitis and digit or carpal lesions, although some mortalities were inexplicable. As of 17 May 2010, five (2.3) bats survived in the colony, more than 6 months from date of capture. Mean body weights at this time were no different from time of collection from the wild (males, 12.6 ± 0.2 g vs. 11.5 ± 0.3 g; female, 12.6 ± 0.2 g *vs* 12.4 ± 0.2 g). The primary challenge to maintaining the VBEB in captivity was the protracted interval encountered in training to self-feed and physical debilities developing from the unnatural need to land and walk on surfaces. Nonetheless, a few bats adapted. Future studies should explore the impact of (1) collecting specimens at a more active time (i.e., during summer) and (2) subspecies stress sensitivity to even subtle perturbations in the captive environment. [oral]

White-Nose Syndrome Visitor Education and Intervention Efforts to Minimize the Risk of Spreading the Disease via Anthropogenic Means at Mammoth Cave National Park

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To date, white-nose syndrome (WNS) has not been found in Kentucky or Mammoth Cave National Park. There are over 400 caves within the park; one of those is >590-km (367-mile) Mammoth Cave. All park caves are closed to human access, except via ranger-led tours or by permit. The park has not issued permits for recreational caving since the late 1980s. Colonial bat roosts are located more than 16 km (10 miles) (subsurface) away from toured areas, although individual bats are occasionally seen along cave tour routes. Nearly one year ago the park initiated an outreach program to: 1) educate cave tour participants about WNS in bats, and 2) intervene to help prevent human-assisted spread of WNS. Education includes *non-personal* communication (such as a park WNS webpage, an educational video on WNS in the Visitor Center, and large posters and fact sheet handouts on WNS), as well as *personal* communication (such as announcements to groups before their tours depart and direct ranger-to-visitor contact). Intervention efforts include screening tour participants for previous cave visitation, preventing potentially contaminated items from being worn or taken on a tour, placing potentially contaminated items in a sealed plastic bag, decontaminating potentially contaminated footwear by soaking in a disinfectant bath, and requiring the use of park-supplied gear. In terms of potential exposure to cave surfaces and required intervention measures, the park distinguishes general walking cave tours (on trail) from "wild cave" type tours (off groomed trails). Since June 2009, park staff have made direct ranger-to-visitor contact with approximately 1,900 visitors who needed intervention. The soles of almost 1,600 pairs of shoes/boots have been decontaminated prior to cave entry, and over 450 potentially contaminated items have been kept sealed in a plastic bag or out of the cave. The park's intervention efforts have been overwhelmingly well-received by visitors. Assuming the arrival of WNS in the local area may be imminent, park staff are formulating strategies to minimize the risk of spreading WNS from park caves if/when it arrives. Some post-arrival measures have been implemented on a trial basis (for "wild cave" type tours), while others are being explored. [oral]

Update from Central Canada: Thermal Refugia, between Individual Variation in Torpor Expression, and Large-scale Movements of Bats across Ontario and Manitoba

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We report on three projects from central Canada: 1) In one small Manitoba hibernaculum, during winter 2009/2010, we field-tested solar-powered, heated bat-boxes as a means to help bats save energy during periodic arousals. No PIT-tagged bats read our proposal or used the design we tested, possibly because we positioned it close to the cave entrance to ensure we did not disrupt normal microclimate. In 2010/2011 we will test smaller prototypes positioned closer to bats prior to the onset of hibernation; 2) Although band-recapture studies for eastern populations of little brown bats have a long history, few data exist for western North America. We have begun analysis of a 20-year band-recapture dataset (n > 10,000 bats) from northwestern Ontario and Manitoba. As in the east, most recaptures occurred at the initial site of capture. However, a few individuals (n = 19) traveled more than 250 km between winter and summer, and six individuals traveled 440 - 570 km. Bats from the largest hibernaculum in Manitoba are, therefore, as little as "two degrees of separation" from the affected area. Multiple movements on this scale provide a mechanism to explain how quickly white-nose syndrome (WNS) may spread across its northern front; 3) Although critical to determining if bats can rebound from the current bottleneck, it is not known if any inherited trait might provide a survival benefit to some individuals over others. Such traits could provide the basis for strong positive selection and a natural recovery of WNS-resistant or tolerant populations. Our temperature telemetry data show that, during winter, considerable variation in torpor expression is explained by sex, with females exhibiting shorter arousals and slower rates of fat depletion than males. However, respirometry experiments conducted on wild bats in spring confirm inherent, individual differences in torpor expression after controlling for body condition, diet, and sex. This could lead to individual variation in overwinter energy balance and/or susceptibility to *Geomyces*, both of which could have a major impact on survival. These results highlight the need for studies addressing potential survival benefits and heritability of many physiological and behavioral traits, including thermoregulatory traits, microclimate selection, and clustering. [oral]

White-nose Syndrome and Winter Energetics of Little Brown Bats

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Even though the ultimate cause of death with white-nose syndrome (WNS) is related to a fungal infection, the proximal cause seems to be depletion of fat reserves before hibernation is over. We hypothesized that little brown bats (*Myotis lucifugus*) affected with WNS would have shallower torpor depth and elevated torpid metabolic rates. Body temperatures (with iBBat temperature-sensitive dataloggers) and oxygen consumption rates were measured throughout the 2008-2009 hibernation season at a Williams Lake Hotel mine in NY, at Woodward Cave in PA, and at Brooks Cave on Ft. Leonard Wood military base in MO. Fat reserves were also measured indirectly by body mass and serum leptin levels (via RIA). The metabolic rates of bats in NY were two to three times higher that of bats in PA ($P < 0.0005$). Torpid metabolic rates of bats in PA were similar to rates measured in other bat species. This is consistent with our hypothesis, even though WNS was detected in Woodward Cave by March 2009. Bats in MO had intermediate rates of metabolism, possibly due to geographic differences. The higher metabolic rates during torpor (not accounting for changes in arousal patterns) in NY bats are calculated to utilize an additional 0.7 grams of fat over the winter, and this may be part of the reason why affected bats are starving to death. Body mass and leptin concentrations are correlated ($P = 0.038$) and decreased throughout hibernation as expected. [oral]

Use of Ultraviolet Light in Field Diagnosis of WNS Bats

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The flight membrane of bats is composed of only a thin layer of skin, and therefore, presents a unique opportunity to examine how light may be viewed and photographed as it passes through the membrane. We examined the wings of bats from three sites that appeared to be unaffected by white-nose syndrome (WNS) and were greater than 80 miles from any confirmed site, along with six sites confirmed to have WNS using both ultraviolet light at 368 nm and white light. Light was placed over top of bats as well as behind to back-light wings. Bats observed with WNS symptoms including visual fungus displayed areas of intense yellow fluorescence seen only with UV light, while the bats from apparently unaffected sites did not display any similar fluorescence. The areas of fluorescence correlated highly to the areas of visible fungus, but were typically more extensive. Using ultraviolet light and an indelible marker, we delineated areas that fluoresced heavily as well as those that did not fluoresce at all on individual bat wings. These bats were submitted to the USGS National

Wildlife Health Center for microscopic evaluation. Analysis confirmed that fungal invasion was the cause of the yellow fluorescent areas. Specific bats displaying visible signs of WNS fungus were collected from a hibernating state and kept euthermic from this point forward. Examinations are currently underway, but it can be concluded that the yellow fluorescence is visible for an extended period of time following initial collection while the visible fungus (representing aerial hyphae) disappeared in less than five minutes, suggesting that the fluorescence represented the fungal hyphae as it invaded wing tissue. Other projects underway, such as visual surveys and photo documentation of a site that was PCR positive for the fungus but clinically negative and examination of a confirmed site interior surface walls, will be discussed. [oral]

Winter Surveys and Their Effect on Bat Mortality at WNS Hibernacula

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Counts of bats within hibernacula are, to date, the most effective means of monitoring wintering bat colonies. In recent years, these surveys have provided the critical data needed to track declines resulting from white-nose syndrome (WNS). Prior to the onset of WNS, winter surveys were regularly conducted at numerous hibernacula across the states of NY, VT and PA. Given the consistent trend of increasing bat numbers at these hibernacula, it is reasonable to conclude that the disturbances caused by these surveys were having little, if any, negative effect on bat populations. With the development of WNS and the associated stress upon affected individuals, it is prudent to question whether disturbances at hibernacula are now more likely to represent a cause of increased mortality. Survey data from 35 hibernacula in these states were reviewed to determine total population losses post-WNS. Additionally, for those sites where it existed, data from the second year of infection was reviewed in an effort to standardize results across all sites. All sites were classified based on the perceived amount of winter disturbance by humans since the first year of WNS infection as either low (confident of one or fewer trips per winter), medium (between two and four trips a winter) or high (more than five trips per winter). Preliminary review of the data has shown cumulative and 2nd year only WNS declines ranging from roughly 30% to 99% and 34% to 97%, respectively, with as much variation within disturbance groups as between them. Meaningful differences in mortality associated with winter surveys are not detectable using this data set. If such differences do exist, their detection will require a more rigourous investigation that would include more accurate bat counts, and more accurate assessments of the frequency and intensity human activity. We discuss data that has currently been reviewed, difficulties in its interpretation, and variables that need to be addressed before a true understanding of the effects of disturbance on bat mortality at WNS sites can be reached. [oral]

Evaluating Changes in Bat Activity and Species Composition from White-nose Syndrome at Fixed Acoustic Monitoring Locations in Vermont

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White-nose syndrome (WNS) was first documented in southeastern Vermont during the winter of 2007/2008. This syndrome has spread throughout the region and has caused unprecedented mortality over the past two winters. While this mortality has been documented at hibernacula, we were unsure of the magnitude of change in bat activity during the summer season. It is vital to verify and model expected declines in northeastern bat populations due to the possible future extirpation of local or regional populations. Intensive acoustic monitoring surveys along the Grandpa's Knob ridgeline have been conducted at five sites over the past three years (from the start of WNS in 2007 till present). Overall average bat activity varied among the five sites, but for four of the five sites, activity decreased significantly among years. This decrease was not only for overall activity but was also for species with high frequency echolocation calls. The consistency between these two trends suggests that the high frequency bats are the ones responsible for the observed trends. Species-specific responses were similar with some species, such as northern long-eared bats (*Myotis septentrionalis*), being impacted more severely. Studies conducted in 2007 and 2008 represent the most intensive acoustic survey conducted in Vermont to date. Because data collection began before WNS spread into Vermont, these studies provide an opportunity to compare pre- and post-WNS acoustic activity. [poster]

Geomyces destructans in Europe

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White-nose syndrome (WNS) is an emerging disease causing dramatic declines of hibernating bats in North America. Although the etiology of WNS remains unclear, the newly identified fungus *Geomyces destructans* causes the skin lesion that is hallmark of the disease. Since its first detection in 2006 mortality events have spread for a remarkable distance covering almost the entire northeastern U.S. and bordering Canada, comprising a pattern characteristic for the spread of an infectious agent. Alerted by early WNS press postings, European bat researchers discussed the implications of the findings in the U.S. and a possible occurrence of WNS in Europe. Discussions were controversial as a large number of bat researchers and bat activists had never experienced fungal growth on hibernating bats, despite being involved in annual hibernacula census for decades. Still, a number of people remained who had seen hibernating bats with similar white patches around the nose and on wing membranes, some even on a regular base. But as such animals had always arisen from hibernation uneventfully without any losses, no effort was made to identify the fungus. During annual hibernacula census in winter 2008/2009, collaborative efforts of bat researchers from four different European countries resulted in the detection of individual hibernating bats colonized by fungus in a similar fashion to WNS bats from the U.S. Touch imprints with adhesive tape as well as clipped hairs were submitted to the Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany, for further investigation. Single hairs from each batch were examined by scanning electron microscopy for the presence of characteristic *G. destructans* conidia, touch imprints were examined by light microscopy. All samples were subject to isolation attempts and molecular investigations, for touch imprints areas with high conidia density were cut out and subsequently processed. Details and results of these investigations will be published in *Emerging Infectious Diseases* by July 2010 (E-publication). Similar to a case report of a hibernating bat in France published by Puechmaille *et al.* (2010) genetic comparison of the samples revealed complete

homology of selected fungal genes between strains from Europe and the U.S. With this, two possible scenarios have to be taken into consideration: besides the homology in distinct parts of the genome, it could be that important virulence factors differ between the U.S. and the European strains, hence explaining the marked differences in regard to mortality events at either side of the Atlantic Ocean. However, in a second scenario European bats could have a different immune response towards the fungus resulting in superficial fungal colonization without deteriorating effects. [oral]

An Assessment of Population Genetic Structure in the Little Brown Myotis (*Myotis lucifugus*) to Predict the Spread of White-nose Syndrome

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Project start date: August 2009
Anticipated project completion: June 2013

White nose syndrome (WNS) is an emerging infectious disease that has caused unprecedented mortality of bats in the northeastern United States. Because little brown myotis (*Myotis lucifugus*) is the most common and most affected bat species, and thus may be the primary mode of dispersal for the fungal pathogen, knowledge of the connectivity of little brown myotis populations may aid in predicting routes of spread and determining which populations are at greatest risk from WNS. We recently initiated a study of the population genetic structure of little brown myotis to identify possible corridors and barriers to migration, allowing an assessment of the likely routes of introduction of WNS from affected to unaffected regions. Previous genetic studies of little brown myotis have found limited regional differentiation, suggesting substantial historical gene flow among populations. For our study, individuals will be genotyped using restriction site-associated DNA (RAD) markers and next-generation sequencing to generate data for several thousand loci per individual. We expect this approach to provide much greater resolution of current population structure and subtly varying levels of gene flow among locations. We hypothesize that WNS is transmitted primarily within hibernacula, therefore, sampling of bats has focused on fall and winter swarming sites and hibernacula. The majority of samples from hibernacula have been acquired from other researchers to minimize disturbance to bats and maximize sample size. Because of the rapid spread and high mortality associated with WNS, substantial effort should be made to prevent the introduction of WNS to unaffected locations. Knowledge of population risk is critical for the implementation of surveillance and control strategies, and historical gene flow among little brown myotis populations may be an important predictor of the future spread of WNS. [oral]