2012 Abstracts of Presented Papers and Posters

WHITE-NOSE SYNDROME SYMPOSIUM

5th Annual White-Nose Syndrome Symposium

June 4-7, 2012,
Madison, Wisconsin

All abstracts are listed in alphabetical order

Sponsors of the Symposium:

Population viability analysis of an endangered species: Indiana bat (*Myotis sodalis*)

S. K. Amelon[1], L. Eggert[2], A. Isabelle[2], and K. Traylor-Holzer[3]

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Population viability analysis (PVA) is widely applied in conservation biology to predict extinction risks for threatened species and to compare alternative options for their management. As a step in assessing the effects of geomycosis (WNS) and other factors on the viability of Indiana bat populations we developed a population viability analysis model using VORTEX (http://www.vortex9.org). VORTEX is a Monte Carlo simulation of the effects of deterministic forces as well as demographic, environmental, and genetic stochastic events on wild populations. We established a stable baseline condition for an admixed population prior to processing scenarios that range from a single admixed population across the entire range and mortality rates as estimated in hibernacula currently affected with WNS through four sub-populations with high mortality in the northeastern region and lower mortality across southern and western populations. This evaluation will serve as a tool to promote a better understanding of the population dynamics of the species and the expected impact of future geomycosis scenarios and management options. (Oral)

Assessing the risk of *Geomyces destructans* transmission by bats that occupy contaminated hibernacula in late summer

A. Ballmann, D. Blehert, C. Meteyer, and R. Russell

USGS-National Wildlife Health Center, Madison, WI

Collaborating agencies: Kentucky Division of Fish & Wildlife Resources; Tennessee Wildlife Resources Agency; USFWS-Cookeville, TN; Tennessee Nature Conservancy; USFS-Wayne National Forest; Ohio Dept of Natural Resources; Indiana Dept of Natural Resources; West Virginia Dept of Natural Resources; New Jersey Division of Fish & Wildlife; Vermont Fish & Wildlife Dept; Quebec Ministere des Ressources naturelles et de la Faune

Geomyces destructans (Gd), the causative agent of white-nose syndrome (WNS) [Lorch et al. 2011], persists in contaminated hibernacula and it remains possible for bats occupying contaminated sites in the summer to come into contact with viable fungus. Fungal spores picked up from the environment could then be carried on the bat's skin and serve as a source of transmission to other bats as they disperse from the contaminated sites and come into contact with unexposed bats from other locations during fall swarm interactions or trapping activities. This cross-sectional study will investigate if late summer occupants of contaminated sites are potential sources of viable Gd spread in the fall and if the proportion of bats harboring Gd increases for sites suspected to have higher levels of environmental contamination. Bats captured exiting 8 sites (3 contaminated sites with reduced winter populations, 3 contaminated sites with stable, pre-WNS winter population counts, and 2 "clean" hibernacula within the WNS confirmed region) will be tested for Gd presence and viability. Estimation of environmental contamination loads of Gd will be attempted from samples collected from within each site. The results will have important management implications for focusing future potential treatment strategies to reduce the spread of Gd as well as evaluating the risk of fall swarm trapping surveys, particularly at contaminated sites. Additionally, this study will improve our knowledge of the timing of Gd/WNS infection among bats, evaluate the potential for bats at contaminated sites to become exposed to Gd, and provide valuable ecological insights regarding summer bat use of known winter hibernacula. (Poster on WNS RFP Award)

Update on Submissions to the North American WNS Diagnostic Laboratory Network

A. Ballmann

North American WNS Diagnostic Laboratory Network (USGS- National Wildlife Health Center, Southeastern Cooperative Wildlife Disease Study, NY State Animal Health Diagnostic Lab, Colorado State University Diagnostic Center, and Canadian Cooperative Wildlife Health Centres; presented by Anne Ballmann (Diagnostic Working Group Leader)

Since White-nose Syndrome (WNS) was first recognized in hibernating bat populations in North America in Winter 2006/2007 over 5.5 million cave bats are estimated to have died from the disease (USFWS, 2012). During this time, several laboratories in collaboration with numerous wildlife management agencies have been actively involved in characterizing and diagnosing WNS as well as documenting the spread of the causative agent, *Geomyces destructans*. This presentation will summarize 5 years of data obtained from over 1500 bats submitted to the North American WNS Diagnostic Laboratory Network. Bats representing 24 species from 41 states and 7 Canadian provinces collected throughout the calendar year have been tested. Information from bat submissions provides helpful guidance for future disease surveillance efforts. In addition, results from last summer's Diagnostic Lab Usage questionnaire which explored resource managers' WNS diagnostic needs will be discussed. (Oral)

The Physiology of *Geomyces destructans*

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In order to understand the pathology, potential for transmission, and life history of *Geomyces destructans* (*Gd*), we must understand its physiology. The physiology of *Gd* will enable us to understand how the pathogen to exploit its environmental niche, including understanding its mechanisms of nutrient acquisition and defense. Such data will not only help us understand whether the potential for *Gd* propagation in hibernacula and whether it can survive using a saprophytic lifestyle, but where 'hot spots' for infection and contamination may be found. It may also allow us to understand environmental resistance and survival under a number of conditions and the likelihood of transmission pathways. By understanding *Gd's* primary nutrient acquisition strategies, we can also understand the pathology of *Gd* and how it establishes a successful infection on bats, where other *Geomyces sp.* cannot. We will present our work on the physiology of *Geomyces* isolated from bats and the cave environment, their physiologic comparison with *Gd,* and how we can shed light on the growth and survival of this pathogen. (Oral)

An overview of ongoing bat white-nose syndrome research at the U.S. Geological Survey – National Wildlife Health Center

David S. Blehert[1], Melissa Behr[2], Justin G. Boyles[3], Paul M. Cryan[4], Jonathan Epstein[5], Daniel L. Lindner[6], Jeffrey M. Lorch[7], Carol U. Meteyer[1], Kevin J. Olival[5], David N. Redell[8], Jennifer A. Schehr[8], John Speakman[9], Michelle L. Verant[10], William Waldrep, Jr.[11], and Gudrun Wibbelt[12]

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We provide updates on three projects designed to address mechanistic aspects of progression of white-nose syndrome (WNS) in hibernating bats. An understanding of disease mechanisms may provide a basis for development of mitigation strategies. It has been hypothesized that wing damage caused by infection with *Geomyces destructans* disrupts water balance in hibernating bats. To evaluate the potential role of dehydration as a factor in WNS mortality, we experimentally infected little brown bats (*Myotis lucifugus*) with *G. destructans* and injected them with doubly labeled water ($^2H_2{}^{18}O$). An overview of the experimental design will be presented. We also describe temperature dependent growth performance and

morphology for six independent isolates of *G. destructans* from North America and Europe. Optimal temperatures for growth were between 12.5 and 15.8°C, and the upper critical temperature for growth was between 19.0 and 19.8°C. Above 12°C all isolates displayed atypical morphology that may have implications for proliferation of the fungus. These results demonstrate that small variations in temperature affect growth performance of *G. destructans*, which may influence temperature-dependent progression and severity of WNS in wild bats. However, temporal variation in climatic conditions within and among bat hibernacula remains poorly documented. Thus, characterization of the role of these parameters in environmental loading of *G. destructans* and in the progression of WNS will be useful to develop models both to predict spread and severity of WNS and to assess potential efficacy of management actions. A recently initiated effort to define the role of climatic parameters in the proliferation of *G. destructans* will be described. (Oral)

Characterization of climatic parameters within bat hibernacula, their influence on environmental loads of *Geomyces destructans*, and implications for the mitigation of white-nose syndrome in bats

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The fungus *Geomyces destructans*, the causative agent of bat white-nose syndrome (WNS), persists in soil and on rock surfaces of bat hibernacula affected by this disease, indicating that environmental reservoirs likely play an important role in the epidemiology and ecology of WNS. Although active growth of *G. destructans* is restricted to cold temperatures, recent analyses demonstrate substantial differences in temperature-dependent growth performance of the fungus over the range of temperatures at which bats hibernate. Thus, climatic variation (temperature and/or relative humidity) within underground bat hibernacula may considerably influence distribution and proliferation (environmental loads) of *G. destructans* and the resulting prevalence and severity of WNS in bats.

Despite these implications, temporal intra- and inter-hibernaculum variations in climatic conditions are inconsistently documented. In order to fully understand the epidemiology and ecology of WNS, it will be necessary to define the influences of both intra- and inter-hibernaculum climatic parameters on environmental loading of *G. destructans* and on the progression of disease in bats. We propose to precisely characterize conditions within six representative bat hibernacula in the eastern United States to: **1)** monitor temporal and spatial patterns of microclimate variation and to quantify loads of *G. destructans* from environmental samples; **2)** quantify loads of *G. destructans* from skin swab samples of bats collected during late hibernation to assess correlations between temporal differences in environmental variables and fungal infection of bats; and **3)** develop and test predictive models of *G. destructans* distribution and abundance within hibernacula based on microclimate and site-specific characteristics to forecast WNS progression both within a hibernaculum and across the landscape.

Climatic parameters (temperature and relative humidity) will be measured at regular intervals for two years by deploying data-loggers at 75 microsites within each of six study sites. Paired environmental samples (soil or cave-surface swabs) will be collected at each data-logger micro-site at three annual sampling intervals, including early hibernation, late hibernation, and summer. Skin-swab samples will also be collected from bats during late hibernation. Together, these data will help us to better understand the role of the environment in WNS epidemiology and provide a basis by which to assess the effectiveness of WNS management strategies, including manipulation of environmental conditions, cave closures, and/or other approaches. (Poster on WNS RFP Award)

Pacific Northwest (PNW) Interagency White Nose Syndrome (WNS) Response Plan

P. Ormsbee[1], S. Bautista[1], J. Burco[2], D. Campton[3] (presenter), A. Hanson[2], R. Huff[4], L. Ray[1], T. Rodhouse[5], E. Rowan[6], E. Rybak[1], G. Throop[1], G. Visconty[1]

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Federal and state agencies in the PNW have drafted a plan to manage for the potential spread of WNS on public lands in the region. The PNW Plan tiers to the National Response Plan (2011) and includes the same seven categories: *Communications and Outreach, Data and Technical Information Management, Diagnostics, Disease Management, Etiology and Epidemiological Research, Disease Surveillance, and Conservation and Recovery*. A key component of the PNW Plan is a WNS risk assessment for each *HUC-5* watershed. The risk of introducing WNS within each watershed was scored as *high, medium,* or *low* based on criteria such as presence of show caves, abundance of caves and abandoned mines, documentation of wintering bats in those caves and mines, and proximity to urban areas (pop. $\geq$ 50,000). Watersheds without caves or abandoned mines were excluded from the assessment. Risk levels were projected to a GIS map to focus geographic priorities and optimize efficient use of funds for disease surveillance and public outreach. The Plan identifies two geographic *trigger zones* for action: Trigger Zone 1 includes the southern portion of British Columbia, western Montana, Idaho, Nevada, and California; Trigger Zone 2 encompasses all of Oregon and Washington. Management guidelines become more restrictive if *Geomysis destructans (Gd)* or WNS is detected in Trigger Zone 1 and more restrictive yet if *Gd* or WNS is detected in Trigger Zone 2. A step-by-step protocol for responding to suspected cases of WNS is also provided for field personnel. (Poster)

Identifying the natural habitat of *Geomyces destructans*, the etiologic agent of bat geomycosis (White Nose Syndrome)

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Background: The psychrophilic fungus *Geomyces destructans* causes geomycosis (White Nose Syndrome), which has been implicated in the catastrophic decline of bat populations since 2006. Appropriate disease control measures are difficult to implement as the natural habitat of the fungus remains undefined. We describe the results of extensive surveys carried out in affected caves and mines to identify the natural habitat of *G. destructans*.
Materials: The 2010 - 2011survey started in Williams mine complex in Ulster County, NY where geomycosis was first discovered. Additional sampling was done in other heavily infested sites - Barton Hill, Graphite and Hitchcock mines, and Hailes cave, NY and Aeolus cave, VT. The samples included sediments with and without decomposed bat remains, soil, swabs of walls, crevices and floors. The samples were aseptically transferred to the laboratory in ice coolers and processed for isolation of fungi at 6°C. The samples were also directly tested by a real time PCR specific for *G. destructans*. All fungi recovered in primary cultures were purified and identified using morphological and molecular criteria.
Results: Forty three of 86 environmental samples yielded *G. destructans* in cultures. The positive samples originated from mine and cave sediments and swabs of above ground surfaces. Twenty eight other genera of fungi were isolated from these samples including closely related *G. pannorum*. The most abundant species included dematiaceous *Cladosporium laxicapitulatum* and *C. phaenocomae* oleaginous *Mortierella alpina* and *M. amoeboidea, M. elongatula, M.*

fimbricystis, M. gamsii, M. minutissima, M. parvispora, and psychrophilic *Penicillium chrysogenum*, *P. concentricum*, *P. dipodomyis*, *P. expansum*, *P. gladicola*, *P. griseofulvum*, *P. swiecickii,* and *P. vulpinum*. Forty one of 86 environmental samples also tested positive for *G. destructans* by direct real time PCR although there was no concordance between culture positive and PCR positive samples. Conclusions: We report the largest isolations of *G. destructans* from affected sites independent of diseased bats. Our results suggest that the natural habitat of *G. destructans* is most likely in the sediments of affected caves and mines. The data would be useful for designing environmental remediations and disease control measures. (Oral)

Fungal Biocontrol Agents for Alleviation or Remediation of *Geomyces destructans*

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Geomyces destructans is a newly emerging pathogen that spreads rapidly in the susceptible populations. An effective remedial measure will be to break its disease cycle by reducing or eliminating the environmental sources of the pathogen, which lowers the rates of new infections. Fungal biocontrol agents refer to fungi that destroy plant pathogenic fungi, nematodes, insects and invasive plant species. These agents are effective, cheap, and environment friendly and they do not infect native plants and animals. The central hypothesis of this study is that the biocontrol fungi are a viable tool to alleviate or remediate *G. destructans* in natural sites. Our preliminary observations indicate that biocontrol fungus *Trichoderma atroviride* impedes the growth of *G. destructans* in the laboratory. Therefore, the main objective of this study is to identify most effective fungal biocontrol agent (s) against *G. destructans*. A number of experimental approaches are proposed for challenging *G. destructans* with fungi obtained from systematic surveys in New York and Vermont and also with well-known strains of biocontrol fungi. Both dual cultures and experimental seeding of soil and rock samples will be investigated. Additionally, the culture-free fungal products will be tested for bioremediation. The chemical nature of the effective compounds will be determined. The fate of *G. destructans* and biocontrol agent will be monitored by culture, microscopy, and molecular tests. The results will be used to develop a 'cave or mine in a lab' model for testing the efficacy of other promising biocontrol agents by USFWS. Finally, the most promising agent from laboratory studies will be identified for future field trials at a select location (s). The proposed study will leverage a FWS-funded systematic survey of *G. destructans* currently underway in New York and Vermont. The proposal has high potential to become a showcase for the integration of natural survey with remediation measures for controlling the spread of *G. destructans*. (Poster on WNS RFP Award)

Antifungal skin microbes as tools for WNS management

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In wildlife disease, microbial therapy is proving to be a viable, bio-friendly, and effective method for disease management. These methods utilize naturally occurring host microbes that exhibit competitive interactions against microbial pathogens. We propose to determine the efficacy of microbial therapy treatments for bats afflicted by White-nose Syndrome through a combination of in vitro challenge experiments and laboratory treatment trials with captive bats. In particular, we will focus on at least one bacterium, *Pseudomonas fluorescens*, which is currently used as an effective biocontrol against root disease in plant agriculture and against bacterial infection in farmed trout. We will also explore competitive interactions between *G. destructans* and other *Geomyces* spp. Finally, we will test the viability and effectiveness of microbial therapy on captive bats to measure effects of treatment on survival after exposure to *G. destructans*. Ultimately, the results from our study will inform managers on the viability of microbial therapy as an effective method for managing WNS in wild populations. (Poster on WNS RFP Award)

Lights, camera, action: Behaviors of hibernating bats before and after WNS revealed by surveillance video

Paul Cryan[1], Justin Boyles[2], Gary McCracken[2], Kevin Castle[3], Dave Dalton[4], Juan Yanez[4], Jenny Beeler[5], Amy Wilson[5], Al Hicks[6], Carl Herzog[6], Ryan vonLinden[6], Scott Johnson[7], Cassie Hudson[7], Timothy Shier[7], and Jeremy Coleman[8]

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White-nose syndrome (WNS) kills bats while they hibernate during winter. Because most bats hibernate in unlit places that must be free of disturbance, we are still largely in the dark when it comes to understanding the hibernation behaviors of even healthy bats in natural settings. More importantly for conservation, few details are known about how infection by *Geomyces destructans* might dangerously alter hibernation. Since autumn of 2009, we have been studying hibernation behaviors of bats before and/or after the arrival of WNS using video surveillance (both near-infrared and thermal). Imagery has now been gathered from hibernacula in New York, Tennessee, Ohio, Indiana, and Virginia. Video of hibernating little brown bats (*Myotis lucifugus*) for three winters prior to the arrival of WNS offers a first glimpse into the long-term behaviors of these bats during winter. Preliminary analysis reveals that most individuals composing hibernation clusters remain in deep torpor throughout the winter and are generally not sensitive to disturbance by active bats, including frequent mating attempts by males. Arousals from hibernation by entire clusters of presumably uninfected *M. lucifugus* during winter in the Virginia cave are rare. In contrast, video from hibernacula of infected Indiana bats (*M. sodalis*) in New York and

Indiana show more activity during mid-winter and greater proportions of active individuals, including arousals involving entire clusters. It is not yet clear whether some of these patterns may be related to species differences in hibernation behaviors. Behaviors of hibernating bats observed thus far shed light on potential mechanisms of increased winter activity in bats affected by WNS, as well as have implications for disease transmission and spread. (Oral)

White-Nose Syndrome: Lessons learned at Fort Drum Military Installation

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We monitored a maternity colony of little brown myotis (*Myotis lucifugus*) on Fort Drum Military Installation in northern New York in 2009 - 2011 for impacts associated with white-nose syndrome (WNS). Declines in colony numbers presumed to be attributed to WNS were initially discovered in the spring 2009. Colony numbers initially declined at a rapid rate in 2009 and 2010, but seemingly leveled off in 2011. The presence of *Geomyces destructans* (the causative agent of WNS) has been documented at the maternity colony in 2009 and 2010 using multiple techniques. Dorsal surfaces of wing and tail membranes were streaked directly onto culture plates, which were then monitored for *G. destructans* growth. Surfaces of wing and tail membranes were also sampled using moistened sterile swabs. These samples were subsequently checked for the presence of the morphologically distinctive conidia of G. destructans. Additionally, guano was collected from the colony and tested by PCR analysis for the presence of G. destructans DNA. Positive samples were cultured to determine viability of any *G. destructans* present. Although tests are ongoing, preliminary results suggest that *G. destructans* may be able to persist in the maternity colony throughout April-August. Despite exposure to G. destructans and WNS, we have determined that some individual female little brown myotis can survive over multiple years. We also provide evidence that some individual female little brown myotis are able to heal from wing damage and infection associated with WNS within 30-90 days of arrival at the maternity roost. Further, almost all recaptured bats within individual years that had suspected wing damage from WNS when emerging from hibernation also later showed evidence of recent lactation, suggesting that some impacted bats can partition energy into healing, while maintaining a seemingly normally reproductive cycle. (Oral)

Highly sensitive qPCR assay for the detection of *Geomyces destructans* in biological and environmental samples

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We report the development of a dual-probe TaqMan real-time quantitative PCR assay for the detection of *Geomyces destructans*. This assay is based upon a single nucleotide polymorphism in the ITS1 region of the ribosomal RNA operon. It is specific enough to distinguish between *Geomyces destructans* and closely related non-pathogenic *Geomyces* spp. allies. Furthermore, mixtures of pathogenic *G. destructans* and non-pathogenic *Geomyces* spp. common to most bat biological samples and hibernaculum

environmental samples can be determined. The sensitivity of the assay is 292 copies of the ITS, the equivalent of less than one *G. destructans* genome, and has been demonstrated to detect subclinical White-Nose Syndrome (WNS) infections. It is at least an order of magnitude more sensitive than other *G. destructans* molecular assays. The assay has been optimized to overcome inhibitors present in clinical and environmental samples, and has been used to successfully detect target in soil and fecal DNA extracts. The assay itself is rapid, requiring only 2 hours from PCR set-up through data analysis. Coupled with a variety of high-throughput DNA extraction methods, hundreds of bat skin swabs, skin biopsies, fecal samples, and hibernaculum sediment, guano, and surface swabs can be processed in as few as 2 days. This assay has the potential to become a critical diagnostic tool for agency and academic researchers to track the spread of WNS, and for the early detection of the WNS etiologic agent in previously unaffected bat populations. (Oral)

The origin and spread of *Geomyces destructans* in North America

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Genetic analysis of the fungal pathogen, *Geomyces destructans*, will be critical to understanding the origin, transmission, and spread of white-nose syndrome in bats. We have taken two different approaches to assess genetic variation among independent isolates of *G*. *destructans*, microsatellite analysis and whole genome sequencing. We developed PCR assays for 140 microsatellite loci in *G*. *destructans* and genotyped a diverse set of 21 isolates from throughout the U.S. and Canada, and 13 isolates from eight different European countries. We found no genetic variation in isolates from North America but considerable variation among isolates from Europe. We also applied whole genome sequencing to assess genetic variation among a subset of 28 isolates of *G. destructans*. Overall, whole genome sequencing results were consistent with microsatellite analyses, with limited genetic variation among North American isolates and substantial variation among European isolates. We did, however, find sufficient variation among North American isolates of *G. destructans* to assess their dispersal. (Oral)

Transmission dynamics of *Geomyces destructans* - what we're learning and why it matters

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Transmission is a critical force driving dynamics of infectious diseases, yet very little is known about transmission dynamics of White-Nose Syndrome (WNS). To date, there are no comprehensive studies that address the prevalence and intensity of infection and transmissibility among individuals in wild bat populations, limiting our ability to determine effective management strategies for controlling the spread and mitigating the impacts of WNS. A major objective of our research is to track spread of *Geomyces destructans* at a continental scale to understand the factors associated with risk of arrival of *G. destructans*, directionality and rate of spread, and disease progression. In collaboration with agency personnel, we sampled over 55 hibernacula in 16 states this winter across enzootic, epizootic, and leading edge regions and along a latitudinal gradient in North America. We sampled over 800 bats from 8 species during winter hibernation. Surveys involved swabbing bats to look for presence and intensity of *G. destructans* infection. Swabs were analyzed using a quantitative PCR developed by Jeff Foster at NAU to determine prevalence of bats infected with *G.destructans* at each site. We will present preliminary results and discuss findings and future objectives. We will give special attention to how empirical data collection is necessary for better understanding critical aspects of disease transmission and highlight ways in which these results matter for effective management of WNS. (Oral)

Epicenter Regional Update

C. Herzog[1] and R. Reynolds[2]

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White-Nose Syndrome was first documented in NY in 2006. Since then it has spread to all Northeast states and beyond. Although some states in the region are at different points in the temporal progression of the disease, nearly all hibernacula in the Northeast are now believed to harbor *Geomyces destructans* fungus and infected bats. Losses due to the disease vary by species and these trends are mostly consistent among states, with northern, little brown and tri-colored bats the most severely affected, Indiana bats typically less so, and big brown and small-footed bats the least affected. Although there are weaknesses in estimates of the disease impact based on hibernacula surveys for some species, in general corroborating evidence from outside the hibernation season supports trends observed during winter counts. There is some evidence that losses of little brown bats vary among winter sites in ways that are not explained by length of time the disease is present at a site or any other recognized variable. There is some evidence that apparent severity of fungal infections decreases in bats that are not killed during the first 2-3 years of infection at a hibernation site. (Oral)

White-nose Syndrome and Illinois Bat Hibernacula

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We are monitoring the invasion of *Geomyces destructans* in bat hibernacula in Illinois. Our team includes 3 mammalogists, a mycologist, a microbial ecologist, a wildlife veterinary epidemiologist, and a cave biologist, and is assisted by several resource managers. We use molecular and culture-based approaches to evaluate dead and live-caught bats and cave and mine substrates for the presence of G. destructans, and describe the microbial and fungal communities of sampled animals and caves. Beginning in winter 2012, we will visit about 8 hibernacula per year for 3 years, and sample active bats during summer. We are collecting swab and wing-punch samples from asymptomatic and symptomatic bats; soil, air, and various other substrate samples from hibernacula; and temperature, humidity, and light data to characterize cave environments. Our study will provide data on the occurrence and distribution of G. destructans in hibernacula on the leading edge of the spread of white-nose syndrome, and the microbial ecosystems in which it becomes established. Circumstances permitting (i.e., timing and extent of the invasion), we hope to better understand potential competing or synergistic interactions between *G. destructans* and microbial communities on bats and in caves that influence the establishment of *G. destructans*. (Poster)

Banding begins to determine if WNS survivors occur in the Northeast

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Surveys suggest that the number of *Myotis lucifugus* in some Northeast hibernacula has recently stabilized or increased after four or more years of White Nose Syndrome (WNS). While commonly referenced as examples of survivors, the causes of these trends are unknown. To determine if this represents long term survival of individuals, or simply changes in distribution within a still declining population, we have initiated a study in the Northeast to band and subsequently recover animals in these hibernacula. A concurrent Midwestern project is determining band retention rates and band-related morbidity and mortality. In both studies we marked *M. lucifugus* using a banding plier recently designed by Vesper Environmental LLC, and 2.9 mm aluminum alloy bands by Porzana Ltd. Single bands were applied to 324 animals within 12 Northeastern hibernacula in late March and early April 2012. In late March 2012 we banded 1,467 animals in 7 WNS free hibernacula in the Midwest, alternately applying a band to one or both wings. Colonies in both studies will be revisited next winter (2012-2013) and banded individuals will be identified. In the Midwest, we will determine band loss rate (losses of single bands among double banded animals), band related mortality (recoveries of single vs. double banded animals), and band related injury. If recovery rates in the Northeast are high, after adjusting for the Midwest results, then that will suggest that survivors are a major component of these stable colonies. Low recovery rates could have multiple explanations. (Poster)

Ecology of geomycosis in Central Europe

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In the Czech Republic and Slovakia, a systematic search for geomycosis on hibernating bats started in 2008. From 2009, it has been implemented into the standardized monitoring of bat hibernacula running since 1969. About one tenth of surveyed sites were checked repeatedly in February and March with microscopic and genetic identification of *Geomyces destructans*. Thus, now we have reliable data on geomycosis in Central European hibernacula from three successive winters enabling to test various effects, including hypotheses on temporal trends and pattern of interregional differences. By March surveys, geomycosis was present in 62-64% of ca. 100 major hibernacula. The mean prevalence ranged between 7 and 11% in different seasons. The prevalence was lowest in lowland and karst areas while in the submountain and mountain hibernacula, non-carbonatic rocks and appearance of structural scree in the vicinity of the site there was nearly 100% incidence and prevalence often exceeding 50% for the most often affected species, *Myotis myotis*. Despite that, no mass mortality related to geomycosis was recorded, and winter flight activity within hibernacula showed only minor differences before and after detection of geomycosis at the site, though mean cluster sizes showed a decrease in some sites. The post-hoc analyses of photographs revealed occasional presence of fungus on bats already in the 1990's and further indirect evidence suggests that geomycosis is a resident phenomenon in Central Europe and the bats are capable to prevent devastating cascade effects of white-nose syndrome in North America. (Oral)

Has white-nose syndrome been underreported?

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We used seasonal occupancy models to estimate occupancy, colonization, extinction, and detection probabilities for white nose syndrome. Data were incidences of visibly infected bats from winter bat count surveys in West Virginia, Tennessee, Pennsylvania, and New York from 2004 through 2011. Our results suggested that detection probabilities for white–nose syndrome were relatively high, when compared to wildlife detection probabilities presented in the occupancy literature. However, detection probabilities were not as high as might be hoped. The expected value for detection was 80% in 2007, and dropped to under 64% in 2011. Incomplete detection of white-nose syndrome has consequences for surveillance strategies that are based on collection of visibly infected specimens. Occupancy probabilities were strongly dependent on distance from the disease's point of discovery, reflecting expansion of range through colonization. At distances median for the survey sample the expected value for probability of occupancy by white-nose syndrome had increased to over 82% by 2011. (Poster)

Swabbing Bats to Determine the Prevalence of *Geomyces destructans*

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White-Nose Syndrome is a large-scale epizootic in hibernating bats in the United States and Canada. The causal agent of WNS has been identified as the psychrophilic fungus, *Geomyces destructans*. Detection of *G. destructans* in the field is limited by the ability of researchers to visually document fungal growth on affected bats. Because most major bat hibernacula are entered only once a year or less, the presence of *G. destructans* may be missed at the early stages. This limits our understanding of the arrival, rate of spread, and prevalence of *G. destructans*, and the transmission dynamics of the pathogen in bat populations. To determine the presence and quantify the fungal load of *G. destructans*, bats were swabbed on their muzzle and forearm with polyester-tipped swabs dipped in sterile water. From June 1 - August 15, 2011, mist-netting and swabbing was focused on eleven sites in eight states: Michigan, Illinois, Indiana, Ohio, Missouri, Kentucky, Tennessee, and North Carolina. From September 2011 - April 1, 2012, surveys in hibernacula were conducted and bats were swabbed at the same sites in Missouri and Tennessee multiple times during the hibernation season. This is crucial for understanding the seasonality of *G. destructans* transmission. Thirteen sites have been visited once this winter and will be visited again before bats emerge. Swabs are awaiting quantitative PCR to determine the number of *G. destructans* spores, if present. This study should provide a sensitive analysis for *G. destructans* where it may not otherwise be detected, and be useful for understanding differences in susceptibility among bat species and hibernacula. (Poster)

Using long-term mist-netting to assess the impact of white-nose syndrome on summer bat populations

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The bulk of information about the spread and impact of White-nose Syndrome (WNS) has come from winter hibernacula, where baseline counts had often been conducted prior to the onset of WNS. Little information is available regarding infected bat populations in summer, in part because of a lack of such long-term, baseline data. However, the Monongahela National Forest in West Virginia has been conducting summer mist-netting annually since 1997, with many sites identified for long-term monitoring where consistent mist-net locations, protocols, and levels of effort have been used across multiple years. Mist-net capture data from 2003-2011 (n = 8,350) were analyzed to assess changes in bat communities pre- and post-WNS. Results showed significant declines in *Myotis lucifugus*, *M. septentrionalis*, and *Perimyotis subflavus*, with a corresponding increase in the relative proportion of species such as *Lasiurus borealis* and *Eptesicus fuscus*. Specific demographic parameters also differed between pre- and post-WNS survey data for some species, particularly *M. lucifugus*; these included changes in the reproductive status of adults in the population, as well as differences in body condition indices early in the season. While these results are preliminary given the relatively short time period since WNS began to spread across WV, such long-term demographic information may be critical in better understanding the overall impact of WNS on bat populations and informing conservation and recovery strategies. (Poster)

The use of tissue explants as an alternative for experimental infections of bats in white-nose syndrome research

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Experimental infection trials are necessary to answer many basic questions about white-nose syndrome. However, such trials have inherent challenges associated with variable host response, husbandry of bats and constraints imposed by the timing of hibernation. It is also becoming increasingly difficult to identify populations of bats that are certain to be naïve to Geomyces destructans and many researchers are reluctant to sacrifice a sufficient number of bats to fulfill the requirements for infection trials. As an alternative, we developed protocols for the use of tissue explants, consisting of skin biopsies, which enable us to limit many variables inherent in experimental infections and to limit the number of bats that must be sacrificed for such experiments. Small biopsies can be taken from live bats, or many samples can be collected from recently euthanized animals. Each sample collected can be used as a replicate in applicable experiments. As many as 50 explants can be collected from a single tri-colored bat (Pipistrellus subflavus). We have been able to maintain the tissues on nutritive media, with minimal changes, for up to two months. The most significant change has been the slight accumulation of fluid between epithelial cells. However, within the epidermis, no changes were observed in cell membranes, cell junctions, organelles, keratin layers or the basement membrane. Geomyces destructans spores applied to skin explants germinated and colonized the tissues. We continue to perfect this technique and have begun trials to determine the suitability of explants from non-hibernating bats for infection experiments. (Oral)

Hibernacula of Bats in Michigan

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The states of Michigan and Wisconsin are perhaps the last great bastions of uninfected little brown bats (*Myotis lucifugus*) and northern bats (*M. septentrionalis*) on the continent, due to the isolation of the hibernacula from major areas of karst and barriers presented by the Great Lakes. In Michigan, 179 different potential hibernacula, including, caves, mines, and other manmade sites, have been examined since 1978; most have been visited since 2005. The Lower Peninsula is geologically unsuitable for underground hibernators, but a significant colony (16,000–20,000 bats) occupies a hydroelectric dam. Most hibernacula are located in abandoned copper and iron mines of the western Upper Peninsula, where populations of up to 55,000 animals occur. Total number of bats hibernating in underground sites is about 267,000, although many sites likely remain undiscovered. This report will identify sites where bats are found and where they are not found and make a preliminary analysis of the physical and environmental features seemingly preferred by bats in this region. Knowledge of where and under what environmental conditions bats hibernate will give the Michigan Department of Natural Resources the opportunity to protect sites that seem desired by the bats and that may be less conducive to the growth of *Geomyces destructans*. (Poster)

Seasonal Patterns in Infection Prevalence of *Geomyces destructans*

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Hibernating bat species have seasonally variable behavior that may influence prevalence of the pathogen *Geomyces destructans*. Although mortality from white-nose syndrome (WNS) occurs primarily during winter hibernation, seasonality in disease prevalence can increase pathogen transmission as contact rates and likelihood of infectious contacts change with host behavior. Presence of *G. destructans* has been documented on nine species of hibernating bats, but impacts from WNS vary among these species. We investigated seasonal patterns in pathogen prevalence and intensity on bat species in the eastern United States. Swabs from exposed wing and muzzle tissue of bats were collected and analyzed using real-time quantitative PCR. Prevalence differed significantly over time, and among sites and species. Prevalence of *G. destructans* was highest on little brown myotis during late hibernation, with both eastern small-footed myotis and big brown bats, species that may be less impacted by WNS, with lower average prevalence. Pathogen prevalence during the summer maternity season was very low, suggesting that bats in the active season likely cleared infections shortly after emerging from hibernation, with little to no transmission between mother and offspring. Understanding seasonal changes in *G. destructans* transmission on multiple host species will allow for targeted disease management that may reduce the consequences of WNS on bat populations. (Oral)

Surveillance for White Nose Syndrome in Canada

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* On behalf of Canada's Interagency White Nose Syndrome Committee

Surveillance for White Nose Syndrome (WNS) began in Canada in the winter and spring of 2008-09 in areas adjacent to affected regions of the northeastern United States: Nova Scotia, New Brunswick, Quebec and Ontario. Wildlife officials and the public were asked to report and collect specimens where day-flying and dead bats were observed on the landscape, and the interiors or entrances of some known hibernacula were inspected. In 2008-09, a small number of bats with external lesions compatible with WNS were examined in Ontario but WNS was not confirmed. In 2009-10, WNS was confirmed in Little Brown Bats (LBB) and Northern Long-eared Bats (NLEB) at 9 of 12 sites under surveillance in Ontario and in 2 regions of Quebec. Substantial mortality (hundreds of dead bats) was detected at one Ontario site. Eight hibernacula were monitored in New Brunswick and six in Nova Scotia, but WNS was not detected. In 2010-11, WNS was detected at 9 new locations in Ontario, at least one with substantial mortality, 8 new locations in Quebec, and at 13 locations in Nova Scotia and New. Massive mortality was reported from one hibernaculum in New Brunswick that had been under study prior to arrival of WNS. In 2011-12, WNS was detected at 3 new locations in SE Ontario but no evidence of further westward spread was found. In Quebec, WNS was detected at a new site in the Saguenay-Lac-St-Jean area, east of previous locations. Day-flying bats also were reported near Quebec City but no specimens were secured for diagnosis. Affected bats were found at 17 different areas in New Brunswick and Nova Scotia and at 5 known hibernacula. The bat population at the New Brunswick site of mass mortality was 6000 bats in the fall of 2010, prior to WNS, fell to 300 bats after the WNS in the fall of 2011 and contained 5 bats in

spring 2012, all with signs of WNS. So far, there is no evidence of WNS in Newfoundland or west of Marathon, Ontario. In this survey, both histological lesions with intra-epidermal fungi of appropriate morphology and a positive PCR test were the criteria used to identify WNS. A more specific set of PCR primers for *G. destructans* was developed and is now in use at the Canadian WNS reference laboratory to reduce the probability of false-positive PCR results. In February 2012, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) recommended to the federal Minster of Environment that LBB, NLEB and Tri-colored Bat be officially designated as *endangered* because of Canadian and continental population declines due to WNS. (Oral)

Finding a fungal needle in a mycological haystack: Why is it so difficult to design sensitive and specific DNA-based tests for *Geomyces destructans* in environmental samples?

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Development of accurate, rapid and sensitive DNA-based techniques capable of detecting *G. destructans* in environmental samples has been hindered by the recent discovery of a diversity of closely related and uncharacterized *Geomyces* species. In order to better understand the diversity of these *Geomyces* species, we used culture-based techniques to investigate the fungal community found in soil from bat hibernacula. We cultured fungi from soil samples collected from 24 bat hibernacula (caves and mines) in the eastern U.S.A. Based on differences in colony morphology, 332 fungal isolates were obtained and regions of ribosomal DNA were sequenced to identify isolates. *Geomyces* species were one of the most abundant and diverse groups, representing approximately 34% of all isolates while being recovered from 83% of sites. Many of these *Geomyces* isolates appear to represent undescribed taxa and many were genetically similar to *G. destructans*. We were able to culture *G. destructans* from samples from three sites, suggesting that cave soil may serve as an environmental reservoir for the pathogen and play an important role in the lifecycle and transmission dynamics of this fungus. Understanding the diversity of fungi found in hibernacula will lead to improved techniques for detecting *G. destructans* relative to the many *Geomyces* species commonly associated with bats and their environments. (Oral)

Geographic distribution and environmental persistence of the pathogenic bat fungus, *Geomyces destructans*, in the United States

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White-nose syndrome is an emerging wildlife disease that has caused deaths of over 5.5 million hibernating bats in eastern North America. However, little is known about the causative agent, *G. destructans*, including the origins of the fungus in the U.S. and its persistence in the environment. *G. destructans* has been found on bats in Europe, but without apparent detriment to European bat populations, prompting two hypotheses as to the source of pathogenic *G. destructans* in North America: 1) the fungus is an exotic species introduced to North America; or 2) *G. destructans* is endemic to North America, but a virulent strain spontaneously emerged. To investigate the second hypothesis, we analyzed soil samples collected in winter 2008-2009 from 55 hibernacula both within and outside the known range of WNS at that time using a newly developed real-time PCR assay. *G. destructans* was only detected from sites within the known range of WNS at the time the samples were collected. These results indicate that fungal distribution correlates with manifestation of disease in hibernating bats and further suggests the fungus was not widely distributed in North American bat hibernacula prior to the emergence of WNS. We also investigated whether *G. destructans* can persist in infested bat hibernacula throughout the summer when bats are largely absent. Soil samples were collected from 14 WNS-positive hibernacula in winter 2010-2011, late summer 2011, and winter 20112012, and samples were cultured to screen for viable *G. destructans*. The results of this ongoing experiment will be presented. (Oral)

From phylogeography to multispecies genetic landscapes: A case study of *Perimyotis subflavus* and an argument for collaboration

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White-nose syndrome (WNS) is an epidemic affecting hibernating bats across eastern North America. Observed mortality rates are extremely high in several species, including *Myotis lucifugus*, *M. septentrionalis*, *M. sodalis*, *M. leibii*, *Perimyotis subflavus*, and *Eptesicus fuscus*. As WNS spreads across North America, analyses of the phylogeography and genetic demography of these known host species and other potential future hosts are increasingly important in providing baseline data regarding population size, demographic trends, patterns of population substructure, and estimates of gene flow. From these data, we may evaluate the importance of observed population declines from a historical perspective and infer likely paths of disease spread. For example, phylogeographic analyses of *P. subflavus* indicate that the predominant pattern of gene flow mirrors the spread of WNS along and out from the Appalachians, suggesting that similar analyses in populations along and ahead of the disease front may allow us to predict future patterns of disease spread. Several research groups are currently engaged in such studies of individual species. While we agree that such analyses are important for understanding and predicting the impact of WNS, we argue that a multispecies approach is necessary for a better understanding of the spread of a disease that is remarkably broad in its host preference. We encourage increased collaboration across research groups and urge the development of multispecies phylogeographies, as well as better integration of ecological and genetic data to model both intra- and inter-specific transmission. (Oral)

White-nose syndrome diagnosis in Europe

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Geomycosis is known from Europe since 2008, but white-nose syndrome (WNS) was not confirmed until recently. We found histopathological changes in greater mouse-eared bats (*Myotis myotis*) from the Czech Republic associated with infection of *Geomyces destructans* that are diagnostic of WNS. The fungus invaded the skin, forming cupping erosions filled with dense aggregations of spores. This confirms presence of WNS in Europe, but without mass mortality observed in North America. Long-term population survey data of the greater mouse-eared bat show population fluctuation within the predicted confidence interval. The diagnostic examination of other bats species and additional sites affected with geomycosis was not attempted due to the conservation status of all bat species in Europe. (Poster)

The Need for Cave Closures on Western Federal Lands to Prevent the Spread of White-Nose Syndrome

M. Matteson

Center for Biological Diversity

Despite compelling scientific evidence for anthropogenic spread of white-nose syndrome to North America from Europe, precautionary measures have not been put in place across most western U.S. federal public lands, including millions of acres of bat habitat and likely thousands of bat hibernacula and roosts that have yet to be exposed to the disease. Broad-based cave closures, combined with mandatory decontamination procedures, stepped-up inventories of bat resources, and education of cavers and cave visitors, are together the best means of diminishing the risk of human transport of Geomyces destructans into uninfected regions beyond the range of natural bat dispersal, including the western U.S. In particular, swift implementation of administrative closures and decontamination requirements, and educational outreach to the public could buy time for labor-intensive cave surveys to occur, and vital bat sites to be identified and permanently and effectively protected. In January 2010, the Center for Biological Diversity petitioned the Bureau of Land Management, which controls 182 million acres in the 11 western states, and the U.S. Forest Service, which oversees 142 million acres in the West, to take such action, but to date precautionary measures have only been taken in a few jurisdictions. The measures enacted thus far will be discussed and a comprehensive strategy to prevent anthropogenic spread of this devastating disease will be proposed. (Oral)

What do western bats do in winter? Implications for WNS risk assessment and response planning in the West

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Western states face unique challenges when it comes to assessing the potential spread of WNS and developing prevention and response strategies. In many areas of the west, extensive surveys of caves and mines have revealed that very few are used by bats as hibernacula; those that are typically contain only small numbers of individuals. Rather, preliminary studies suggest that western bats exhibit a diversity of overwintering strategies that vary both geographically and by species.

Because bats in eastern North America hibernate in caves and mines, most response planning to date has been site-based and focused on hibernacula, an approach that will not be feasible or adequate in the west. This presentation will summarize what is known about western bats in winter, discuss the implications for the spread of WNS to the west and the surveillance and monitoring of western bat populations, and outline the important research questions from a western perspective. (Oral)

Pathology Associated with Wing Fluorescence in Bats with White-nose Syndrome

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Characteristic skin erosions seen in histologic sections of the wing membrane, muzzle, and ears of bats are the current 'gold standard' for diagnosing white-nose syndrome (WNS). Surface illumination and transillumination of wings with long-wave ultraviolet light (UV) causes a characteristic fluorescence of the wing membrane from bats with WNS. When small points of fluorescence were sampled for histopathology, the fluorescent areas correlated with defined sites of fungal skin erosion diagnostic for WNS. The fluorescence of bat wing membranes infected with WNS provides a non-lethal field technique to screen bats for WNS and to optimize sample selection of wing tissue for histopathology confirmation. (Oral)

Immunological responses to *Geomyces destructans* in experimentally infected little brown myotis (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*)

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Understanding the role of the immune system in the development of white-nose syndrome (WNS) requires the knowledge of infection status in affected individuals, the use of unaffected control animals held under identical conditions for comparison, and descriptions of the temporal variation in immune responses post-infection. For this reason, we are conducting experimental infections of little brown myotis (*Myotis lucifugus*) and big brown bats (*Eptesicus fuscus*) and are measuring multiple aspects of immune function at different times post-infection (3, 7, 11, and 15 weeks). We focus on these two species to describe how a highly susceptible species (*M. lucifugus*) and a relatively more resistant species (*E. fuscus*) differ in their ability to respond immunologically to invasion by *Geomyces destructans* (Gd). Our assessment of immune responses includes (1) measuring splenocyte proliferation in response to lipopolysaccharide and concanavalin A to estimate the responsiveness of B and T lymphocytes, (2) testing phagocytosis of *Escherichia coli*, *Staphyloccocus aureus*, and zymosan, (3) estimating microbicidal ability against *E. coli*, *S. aureus* and *Candida albicans*, (4) analysis of blood smears for total and differential leucocyte counts, (5) measuring total and anti-Gd antibodies, and (6) describing circulating levels of multiple cytokines. All measures of immune response are being considered in the context of relative body condition, patterns of torpor versus arousal, sex, age and reproductive stage. This presentation will cover all completed analysis to date and discuss our current understanding of how inter-species differences in immunological responses against Gd may help explain differential rates of mortality. (Poster)

Use of echolocation call recording techniques to monitor bat populations in Connecticut in the wake of white-nose syndrome.

K. Moran

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Since its discovery in the 2006 in eastern New York, white-nose syndrome (WNS) has caused significant bat mortality in eastern North America. In January, 2012 the US Fish and Wildlife Service (USFWS) announced that between 5.7 and 6.7 million bats have died as a result of WNS. To limit the potential spread of WNS, the USFWS advises researchers to minimize activities (such as mist-netting or entering hibernacula) that might facilitate disease transmission or cause unnecessary disturbance to bats that may already be infected. Advances in echolocation call recording techniques, however, have made it possible to collect large amounts of data on bats without posing such threats. In 2011 the Connecticut DEEP Wildlife Diversity Program (WDP) initiated an acoustic monitoring study that seeks to document bat activity across the state and throughout the active season. Nine 20-mile driving transects were monitored twice per month from May to October, 2011. Vehicles equipped with ultrasonic recording devices were slowly driven along the transects while WDP staff and volunteers recorded bat vocalizations and GPS coordinates. Analysis of this data will provide important baseline information on species presence, distribution, and seasonal activity levels, while contributing to the broader regional understanding of bat populations in the wake of WNS. (Poster)

Individual variation in immune responses in a species only moderately affected by WNS: Characterizing the primary humoral immune response to a novel antigen in big brown bats, *Eptesicus fuscus*

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Due to the rapid population declines associated with White-nose Syndrome, it has become increasingly important to understand the fundamental question of who will live and who will die. Resistance to infection by Geomyces destructans may in part be explained by phenotypic variation in different survival traits, including differences in immune competence. The purpose of the present study was to investigate the intra-species variability of the adaptive immune response in a captive colony (n=68) of *Eptesicus fuscus*. We characterized the primary humoral antibody profile in response to an exposure to the novel antigen 2,4-dinitrilphenol (DNP) conjugated to bovine serum albumin (BSA). Each individual was subcutaneously inoculated on day 0 and received a booster injection on day 21. In order to assess the temporal changes in the antibody titer throughout the course of the primary immune response, blood sample collection continued through 8 weeks following the initial injection. Immunoglobulin levels for each time point were quantified using an enzyme-linked immunosorbent assay (ELISA) that was modified to specifically detect bat antibodies against DNP-BSA. Preliminary and expected results will be presented. (Poster)

Potential vaccine for white-nose syndrome and other bat diseases

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White-nose syndrome (WNS) caused by *Geomyces destructans* (Gd), has decimated bat populations of eastern North America with mortality rates exceeding 90 percent in some hibernacula. Because the Gd organism survives long periods in the environment, the feasibility of using environmental antifungals or post-infection therapeutic interventions is highly questionable. Environmental control of pathogens also often raises ecologic concerns due to secondary effects. Vaccine campaigns have been highly effective in reducing disease incidence in wild animals (e.g. rabies). Using poxvirus vectors, we are developing an efficient, effective, and practical vaccine against WNS in free-ranging bats. In our studies we are: 1) assessing the ability of various poxvirus vectors to induce protective immune responses in bats via a nonparenteral route; 2) developing a vehicle for efficient topical administration and delivery of the vector to groups of bats; 3) identifying potential Gd gene targets that could be useful to ultimately develop a WNS specific vaccine; and 4) developing an alternative WNS-animal model for testing of vaccine candidates. Our approach of exploring the feasibility of vaccination against WNS could result in a targeted intervention of the disease with minimal environmental impact. Identifying a suitable vaccine vector for bats may also be highly valuable for mitigation of other important bat-borne diseases such as rabies. (Oral)

Biochemical analysis of *Geomyces* proteases to understand putative function in pathogenicity of White-Nose Syndrome

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Proteins secreted by the fungus *Geomyces destructans* may function as pathogenicity factors in White-Nose Syndrome (WNS) of bats. We hypothesize that *G. destructans* secretes specific proteases that enable hyphael penetration of host tissue to mobilize carbon and nitrogen from integumentary substrates for its metabolic needs. This presentation will address our research objective to isolate, identify, and characterize those proteins primarily mediating host-pathogen ecology in WNS. We are using an in vitro approach to culture *G. destructans* on proteinaceous substrates to facilitate this objective. Minimal aqueous media containing structural integumentary proteins or trypsin digested proteins were screened for overall protease production and activity. Protease activity was readily detected in culture medium concentrates with fluorescent casein substrate and displayed a broad optimal activity range of pH 6 to 9. Crude enzyme extracts are greatly inhibited with henylmethylsulfonyl fluoride and ethylenediaminetetraacetic acid, thus suggesting a dominant serine and metallo- protease activity. Native PAGE with casein zymogram indicated multiple activities are present. SDS-PAGE was used to generate total protein profiles and provided resolution of major protein bands accumulated in *Geomyces* cultures. These gels were used subsequently for peptide-mass finger printing of select protein bands with MALDI-TOF mass spectrometry. This proteomics approach is being used in attempt to directly identify individual proteins based on structure (sequence) properties. Results from these studies will provide baseline data on the identity of secreted *Geomyces* enzymes and offer insights into their in vivo role in pathogenesis. (Poster)

USFWS Analysis of Captive Bat Management Strategies in Response to White-Nose Syndrome

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U.S. Fish and Wildlife Service

In fall 2010, the U.S. Fish and Wildlife Service convened a team of agency biologists to develop recommendations for captive management of insectivorous bats as a possible response to the white-nose syndrome crisis. We considered the management objectives and alternative strategies, including no captive management (no action), that were identified during the St. Louis Captive Management Feasibility Workshop. To promote transparency and deal explicitly with the uncertainties surrounding the need and potential for WNS-related captive bat management, the Service team employed a structured decision-making (SDM) process for seven selected bat species: the eastern small-footed bat, gray bat, Indiana bat, little brown bat, northern long-eared bat, Ozark big-eared bat, and Virginia big-eared bat. This poster presentation illustrates results and recommendations stemming from the SDM process. Results include ranking of strategies for each species relative to management objectives, and identification of research needs. Management recommendations include cautiously pursuing a holding strategy for three of the seven species as well as identification of conditions under which captive management might be considered in the future. An initial approach of holding hibernating bats over one winter was the generally preferred strategy. Management precautions include foregoing more ambitious propagation strategies unless and until we have a better understanding of their ramifications, implementing one or more pilot projects to ascertain best practices and likelihood of success, and careful project monitoring and evaluation. Research recommendations focus on determining the resistance/potential immunity of bat species to WNS, investigating the susceptibility of bat species to WNS (as one of the conditions for considering captive management), conducting pilot studies as adaptive management experiments, and determining the efficacy of cryopreservation/cell line establishment as a potential conservation tool. The central lesson of this effort has been that decisions about WNS-related captive management should be made on a species-specific basis within the dynamic context of our most current understanding of the species and the disease. (Poster)

Temporal variation in bat wing damage in the absence of white-nose syndrome

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Wing damage has been documented in bats with WNS and could become a useful detection method for use in post-hibernation seasons, but because there are no historical records of wing damage prior to the emergence of WNS, it is unknown what types of damage are specific to WNS. To address this knowledge gap, we inspected the wings of hundreds of bat carcasses collected in Illinois from 2005 to 2010, and modeled wing discoloration scores using age, sex, year, and season as predictors in *Eptesicus fuscus.* Season and year were included in our top model for wing discoloration. We found that wing discoloration scores peaked in early summer. This is consistent with temporal changes in wing damage observed in WNS-positive bats both in the field and in captivity, and suggests that some of the wing damage observed in WNS-positive bats is due to normal seasonal fluctuations. (Poster)

Predicting the potential distribution of *Geomyces destructans* in North America and Eurasia using ecological niche modelling

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White-nose syndrome (WNS), an emerging infectious disease caused by the fungus *Geomyces destructans* (Gd), has been expanding year after year in North America, suggesting a recent introduction of the fungus (probably from Europe). Given the massive mortalities associated with WNS in North America, it is of prime importance to predict areas suitable for its causative agent, *Geomyces destructans*. Using the niche occupied by Gd in Europe, we will predict its distribution in North America and Asia. To achieve this, we will be using species distribution modelling, models that have been proven to accurately predict current species distributions. In the last years, the scope of this technique has widened greatly, nowadays being frequently used to predict suitable areas for species reintroductions, potential areas of invasive species or forecasting of range shifts under climate changes (past and future). We will also explore the similarities/differences in niche occupied by Gd between North America and Europe and highlight potential adaptations of Gd in North America. Finally, we will determine potential dispersal routes that Gd could use to expand its current distribution. (Oral)

Understanding WNS Survivors: Exploring Resilience and Resistance to Variable Levels of *Geomyces destructans* Exposure in the Context of Mitigation and Conservation

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White nose-syndrome (WNS) has decimated many bat colonies, but it does not appear to affect all species or all populations equally. The fundamental question of why and how some bats survive infection with the WNS-causative agent *Geomyces destructans* (Gd) is one of the most pressing issues at hand. This study will combine field and laboratory approaches to determine whether survivors are resilient or resistant to Gd infection (or both) – and to understand the role that the environment plays in bat-pathogen interactions. By exposing naïve little brown bats (*Myotis lucifugus*; collected from outside the current WNS-zone) to variable doses of Gd under two different growth conditions (2°C and 10°C), we will simultaneously determine the minimum infective dose, the physiological consequences of different infection levels (those bats that survive infection will be considered 'survivors'), and the inter-relationship between infectious dose, environmental conditions, and bat biology. The expression of immunologically and metabolically important genes will be compared between these laboratory-generated survivors, control uninfected captive animals, and free-ranging survivors. Furthermore, the ability of these survivors to withstand infection in subsequent years will be tested. The knowledge gained will allow for focused management and educated scientific decision making. For example, if surviving bats are resilient and/or immunologically resistant, decontamination of existing hibernacula and equipment carried into those hibernacula is less important than if they have simply not yet experienced Gd, in which case it is only a matter of time before they may succumb. Likewise, if resilience or resistance is found (and presumably has heritable underpinnings), remaining populations should be carefully guarded to ensure that natural selection has the capacity to act. Finally, if the currently proposed studies, which are able to control for infection load, demonstrate greater survivorship at lower temperatures (as has our previous investigations of naturally-infected bats), cooling hibernacula may be a viable and important mitigation strategy. (Poster on WNS RFP Award)

Surviving WNS: Species Differences in Susceptibility

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Now that a number of areas have been affected by WNS for multiple years, it is evident that there are dramatic species and individual differences in susceptibility to WNS. Differences in susceptibility are likely caused by life history differences such as body size, time in hibernation, and microclimate preference and by physiological differences such as immune competence and differences in thermoregulatory behavior. We have shown that WNS affected little brown bats (*Myotis lucifugus*) hibernating at colder temperatures survive significantly longer than those hibernating at warmer temperatures – and these findings inform survivability in relation to microclimate preference in other species. Current studies in my lab are examining species differences in susceptibility and in the immunological response to WNS using naïve animals from outside the WNS zone that have been inoculated with Gd. Preliminary findings will be presented – illustrating clear species and individual differences.

These findings, in relation to our upcoming studies of WNS survivors will be discussed as will the concept of a survival phenotype and predictions of susceptibility of those species not yet impacted by WNS. (Oral)

Enhancing survival of WNS infected bats; Update on antifungal treatment trials

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We report on 2 studies in which we tested the hypothesis that treating *Geomyces destructans* (Gd) infected bats with antifungals drugs early in hibernation will reduce infection loads and increase overwinter survival. In one study, survival, Gd infection load, and tissue terbinafine levels were measured in Gd infected little brown bats (*Myotis lucifugus*) given varying doses of injected terbinafine for 10 days. In this study, there was no significant difference in survival time between terbinafine treated bats and control bats, and average survival was 57 days post-treatment. However, terbinafine tissue levels in the higher dosage groups maintained expected therapeutic levels in wing tissue for up to 109 days, suggesting the potential efficacy of short term drug treatment in hibernating bats. Additionally, terbinafine had no adverse effects on bats below the highest dose tested. We compare these results to a second survival study in which injected voriconazole (V-Fend®, Pfizer), and topical terbinafine (Lamisil Once®, Novartis) ointment were administered to Gd infected bats. Voriconazole treated bats exhibited adverse drug effects after the first dose, indicating that this drug has toxic effects when used in bats even at the lowest dosages. Gd infected controls, sham injected, and topical terbinafine treated bats had similar survival of 25 to 33% to the study endpoint of 101 days. These experiments suggest that timing of treatment application and varying hibernation conditions may increase survival. Further study of captive hibernation conditions and antifungal drug therapies and protocol timing are needed to provide intervention strategies for this devastating disease. (Oral)

Assessing status and trend of bat populations at broad geographic scales with dynamic distribution models

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In an effort to increase capacity for evaluating bat population status and trends in the wake of White-nose Syndrome across broad geographic extents, we analyzed capture and acoustic detection records for *Myotis lucifugus* across Oregon and Washington obtained from the Bat Grid monitoring program. Using a Bayesian auto-regressive approach, we developed dynamic distribution models for the species that accounted for imperfect detection and provided estimates of annual turnover and trend in occurrence patterns. Drawing on species-energy theory, we included measures of net primary productivity and forest cover in models, predicting that *M. lucifugus* occurrence probabilities would covary positively along those gradients. Despite its common status, *M. lucifugus* was only detected during ≈50% of the surveys in occupied sample units. Our models provide evidence of an association between NPP and forest cover and *M. lucifugus* distribution. Turnover, the probability that an occupied sample unit was a newly occupied one, was estimated to be low (≈0.04 -0.14), resulting in flat trend. We mapped the predicted occurrence probabilities and corresponding prediction uncertainty across the study area. Our results provide a much needed baseline against which future anticipated declines in *M. lucifugus* occurrence can be measured. The dynamic distribution modeling approach offers a coherent and flexible framework for using bat monitoring data to assess regional population status and trends. The ability to map these results facilitates communication among scientists, land managers, and policy makers. (Poster)

The effect of White-nose Syndrome on bat activity patterns for two winters across Pennsylvania

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White Nose Syndrome (WNS) has spread rapidly since 2006. Current methods of detecting WNS require human entry into hibernacula, increasing the risk of spreading the disease to uninfected sites. A non-invasive passive surveillance technique that could identify infected sites without entering the hibernacula would increase survey efficiency, reduce risk of disease transmission, and reduce disturbance to hibernating bats. Infected bats have been observed breaking torpor more frequently and during anomalous times, e.g. low temperatures, daylight. We report on a preliminary study to assess whether increased and anomalous activity outside the hibernaculum can be used as an indicator of disease presence. In winter 2009-2010 we installed acoustic monitors at the entrances of 7 hibernacula - 3 sites thought to be infected for 2 years, 2 for 1 year and 2 assumed uninfected. Six of these sites were monitored again in 2010-2011. We used quantile regression to contrast diurnal and nocturnal activity and to estimate the proportion of activity occurring below certain threshold temperatures. We evaluated whether these values differed systematically with years of infection. In 2009-10, a comparison of sites in their second year of infection to those with 20% of activity occurred diurnally vs 10% of activity occurred when temperatures. (Poster)

Large-Scale Analysis of Correlates of Bat Susceptibility to White-Nose Syndrome and Consequences for Bat Communities

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White-nose syndrome poses a severe emerging threat to hibernating bats across North America. Researchers and managers have rapidly mobilized to respond to this threat, but our traditional approaches to research and management, in particular, small-scale studies, a single-species focus, and management responsibility divided by political boundaries are challenged by the rapid geographic spread of the disease and its effects on multiple bat species. Statistical analyses of data compiled from across species and geographic regions can complement such approaches and facilitate understanding of large-scale spread and impacts from the disease. We compiled published and unpublished data on the impacts of white-nose syndrome on bat colonies, and factors that may correlate with disease impacts across hibernacula. We then used model estimation and selection, multiple regression, and multivariate analysis approaches to identify key factors correlated with bat susceptibility to white-nose syndrome and the impacts of white-nose syndrome on bat communities. Our analyses suggest that bat susceptibility to white-nose syndrome varies by bat species and geographic region, and that white-nose syndrome has substantially changed the composition and diversity of hibernating bat communities in affected areas. These findings suggest species and hibernacula that may be most vulnerable to future spread, and highlight the community-level consequences of white-nose syndrome. Further multi-site analyses of bat colony changes and their correlates could enable a more comprehensive understanding of bat susceptibility to white-nose syndrome and its effects on bat communities. (Oral)

Efficacy of a terbinafine impregnated implant in the prevention of white-nose syndrome in little brown bats (*Myotis lucifugus*)

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Terbinafine has been shown to be effective against *G.destructans (Gd);* previous attempts to treat infected bats have been unsuccessful. This study evaluated the efficacy of a terbinafine impregnated sub-cutaneous implant in the prevention of disease development after little brown bats (*Myotis lucifugus*) were exposed to *Gd*. The implant was designed to deliver terbinafine over the course of 4-6 months following application; animals only needed to be handled once. Forty bats were collected from two caves and appeared healthy with no lesions characteristic of WNS. Bats were divided into four groups of 10 with each group assigned to a different treatment (2 mg implant, 4 mg implant, 8 mg implant, control implant). After implants were placed subcutaneously over the dorsum, each animal was inoculated topically on one wing with *Gd* and then placed into hibernation for approximately 3 months. Animals were monitored for lesions associated with infection. Wing biopsies were collected to determine terbinafine concentrations. Fourteen bats survived until the end of the study (two control implants, five 2mg implants, four 4mg implants, three 8mg implants); all bats that were alive at the end of the study were euthanized and samples were collected. The average survival time for bats that died was 72 days after inoculation with *Gd*. A total of 8 control implant animals died during the trial (mean survival = 75 days), five 2mg implant animals died (mean survival = 74 days), six 4mg animals died (mean survival = 74 days), and seven 8mg animals died (mean survival = 65 days). Few animals had gross lesions consistent with *Gd* infection. Further analysis of samples including ultraviolet image analysis, histopathology and pharmacology are still being performed. (Oral)

Small population consequences of white-nose syndrome: A sensitivity analysis

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White-nose syndrome causes severe declines in the abundance of eastern species of hibernating bats. What is unclear is whether these much-reduced populations are capable of sustaining themselves after the initial pass of this disease, let alone eventually recovering to former levels of abundance. Extinction risk is the integration of trend, variability in trend, and population size. With a stochastic population model, we examined the consequences of small population size and environmentally induced variability in abundance to identify the underlying risk these species face when they are forced to low abundance by white-nose syndrome. Preliminary results of our population model indicate that risk of extinction for Myotis spp. in the face of white-nose syndrome is sensitive to the size of the population and winter survival rates of adults post-initial WNS exposure. Small populations are at greater risks of extinction simply because of stochastic variability in the environment. Uncertainty regarding how winter survival rates of adult bats are affected by smaller population sizes, and long-term exposure to WNS contribute to uncertainty in predicting the extinction risk of hibernating bats. If adult winter survival recovers post-WNS exposure (i.e., bats develop resistance or the smaller post-WNS populations are less affected by WNS), the risk of extinction declines. Conversely, if smaller post-WNS populations maintain lower survival rates because of, say, an inability to thermoregulate in winter hibernacula, extinction risk is increased. Understanding post-WNS population dynamics are critical to answering whether WNS is an agent capable of extinction. (Poster)

Metabolic and hygric aspects of *Geomyces destructans* infection in experimentally inoculated *Myotis lucifugus*

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The mechanisms underlying mortality in bats affected by white-nose syndrome are still poorly understood. However, altered torpor patterns of infected bats during hibernation have recently been confirmed and disruptions of water and electrolyte balance have been implicated as contributing factors. We studied the effects of experimental infection with *Geomyces destructans* (Gd) on energy requirements, evaporative water loss, thermoregulation and blood physiology of hibernating little brown bats *Myotis lucifugus*. Experimentally inoculated bats (n= 21) and sham-inoculated control bats (n = 21) were held in two separate cages within an artificial hibernaculum kept at 7°C and 99% relative humidity over the 2011/12 hibernation season. All individuals were outfitted with temperature-sensitive data loggers. To investigate the progression of Gd infection, we sub-sampled animals from each group at four-week intervals and measured metabolic rates and evaporative water loss. Following respirometry, for each bat we analyzed blood samples, retrieved skin temperature data and conducted a necropsy and histopathologic examination. These data will contribute to our understanding of physiological mechanisms underlying mortality from white-nose syndrome. (Oral)

Ectomycota associated with hibernating bats in Eastern Canadian caves after the emergence of white-nose syndrome

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In the winter of 2010, 117 fungal species were isolated from the external surface of apparently healthy hibernating bats (*Myotis* spp.) in eight caves and mines in New Brunswick (Eastern Canada). The most common taxa isolated were *Geomyces pannorum sensu lato, Penicillium* spp.*, Mortierella* spp.*, Mucor* spp.*, Cephalotrichum stemonitis, Leuconeurospora* spp.*, Penicillium solitum, Cladosporium* spp.*,* and *Trichosporon dulcitum*. White-nose syndrome (WNS) was first detected in the province in Berryton Cave, March 2011, with ~84-90% mortality in the first winter. By late fall 2011, WNS had spread to an additional three sites in New Brunswick, although no significant mortality was observed. We continued to monitor the spread of WNS and bat mortality rates in New Brunswick during the winter of 2012. The 2010 fungal study was replicated in the winter of 2012 in caves with and without WNS to compare the fungal community on hibernating bats pre and post WNS. Changes, if any, in the external mycoflora of bats in infected caves may indicate that *Geomyces destructans* interacts with the native fungal community. (Oral)

Temperature-dependent variation in growth of Geomyces destructans

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White-nose syndrome (WNS) is an emergent disease of hibernating insectivorous bats that has caused severe declines in bat populations of eastern North America. The causative agent, *Geomyces destructans*, is a psychrophilic (cold-loving) fungus that can only grow in cold environments such as bat hibernacula. Thus, temperature may be an important disease-mediating factor for WNS. To explore this hypothesis, we measured radial expansion of colonies of six isolates of *G. destructans* (three from North America and three from Europe) grown in laboratory culture at temperatures ranging from 0 to 21°C weekly for five weeks. Data were used to define thermal performance curves and growth parameters for each isolate. For all isolates, maximum growth performance was attained at approximately 14°C, and the upper limit for growth was approximately 19°C. Over a range of temperatures relevant to hibernating bats (1 - 16°C), there were substantial differences in measured growth rates for all isolates. Following incubation above 12°C, G. destructans showed atypical morphology and production of altered reproductive structures resembling arthrospores and chlamydospores that may be biologically important for propagation and persistence of the fungus at warmer temperatures. In summary, laboratory experiments indicate that variations in temperature consistent with those commonly found in bat hibernacula strongly influence growth performance of *G. destructans* in ways that may influence progression and manifestation of WNS. (Poster)

Test of a biocompatibile, biodegradable, widely available and inexpensive anti-fungal agent on the growth of *G. destructans*, the causative agent of White-nose Syndrome, on experimentally-infected bats under controlled laboratory conditions

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As white-nose syndrome (WNS) continues to increase in prevalence and expand across North America, there is an urgent need to develop mechanisms and strategies to reduce mortality rates and limit transmission to new regions. While synthetic antifungal medications have been shown to inhibit the growth of *Geomyces destructans* (Gd) *in vitro*, these compounds have a number of significant drawbacks, including toxicity, expense, and logistical considerations, that limit their usefulness as general treatments that could be widely implemented. We propose to test the efficacy of the non-chemical, naturally occurring biopolymer chitosan for the prevention and treatment of WNS. Chitosan is a powerful antimicrobial agent that also has extensive wound-healing properties. Importantly, it is fully biocompatible, biodegradable, and nontoxic. It is inexpensive and commercially available in preparations that can be mass distributed through spraying or aerosolization. We argue that these characteristics (low cost, commercial availability, potential ease of delivery, biocompatibility, biodegradability, and combination of antimicrobial and wound healing properties) of chitosan make it an ideal candidate for testing on bats as a biological means to decrease infection rates and limit the extent of tissue damage incurred by WNS-affected bats. Our preliminary tests of two different chitosan preparations showed that one preparation exhibited strong antifungal properties against Gd (84% inhibition) at the highest concentration we tested. Based on these initial and encouraging results we propose to 1) continue testing different chitosan preparations to maximize antifungal efficacy; and 2) test the preparation with maximal efficacy on experimentally-infected little brown bats *in vivo* to determine its influence on fungal growth, bat arousal behavior, and the histopathology of WNS. Our proposed research will confirm whether the antifungal and wound healing properties of chitosan are sufficient to limit growth of the fungus *in vivo*, and hence limit or prevent the associated tissue damage and disruption of physiology that cause the mortality of infected bats. (Poster on WNS RFP Award)

Using phylogeography to understand the spread of white-nose syndrome in *Myotis lucifugus*

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As White-nose Syndrome (WNS) continues to spread across North America and cause high rates of mortality among some affected species, it has become clear that we lack the necessary information to place levels of mortality in context with respect to baseline population estimates and demographic trends. For most bat species we have no knowledge of the size of populations, their demographic trends, and the degree of structuring into genetically discrete subpopulations. To be able to predict the full impact of WNS, it is important to know whether affected species exist in single panmictic populations, or series of discrete units that may or may not be connected by gene flow and that may be experiencing independent demographic trends. Molecular markers can be used to examine levels of population differentiation within a species, highlight demographic connections among populations that may allow us to predict transmission pathways, and estimate relative effective population sizes. We are utilizing mitochondrial and nuclear genetic markers to address these questions across the range of the little brown bat (Myotis lucifugus), one of the species most highly affected by WNS. Utilizing >500 samples from 22 populations across the United States and Canada, we will identify potential barriers to gene flow, estimate dispersal rates among differentiated populations, and provide estimates of effective population size. Our results will help us to place current patterns of mortality and disease spread in eastern North America in the broader context of population-level processes across the range of this species. (Oral)

To be or not be breached- do we need to re-asses our understanding of Gd lesions?

Gudrun Wibbelt

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White-nose syndrome (WNS) has claimed the death of millions of hibernating insectivorous bats in North America. The etiologic agent *Geomyces destructans* (Gd) which causes skin lesions that are the hallmark of the disease. The fungal infection is characteristic with white powdery growth around the muzzle, on ears and wing membranes. For the diagnosis of WNS certain criteria have to be met: Aberrant hibernation behaviour, confirmation of Gd via PCR, mass mortality events and histologic confirmation of the typical lesions like erosions and necrosis by fungal hyphae. The hyphae invade deeply into the living tissue resulting in areas of necrosis. While these changes have repeatedly shown for deceased bats in North America, European bats have only been recently investigated by histo-pathology as nature conservation regulations formerly prohibited the invasive sampling on hibernating bats. These investigations in Gd affected hibernating European bats revealed skin lesions intriguingly similar to what is seen in North American bats. Whilst investigated wing biopsies revealed only a superficial involvement of the epidermal layers, the penetration of the epidermal basement membrane by fungal hyphae and subsequent invasion into the dermis was shown in two bats from Czech Republic. Unfortunately, these animals' carcasses had already started to decompose and it remains elusive whether the progression of Gd hyphae into the deeper tissue had occurred ante or post mortem. In general, one of the crucial structures in assessing skin diseases is the basal membrane, which separates the outer epidermis with its multiple cell layers from the connective tissue of the dermis. The basal membrane is regarded as one of the most important defence borders of the body against injuries. Once this structure is breached by an infectious agent severe damage will result and the

physiologic function of the skin, like body fluid homeostasis, is in danger. Many studies have shown that hibernating bats in North America suffer from extensive necrosis of their wing membranes and resulting scarring can be observed in animals surviving the infection. In regard to the findings of superficial epidermal colonization of Gd in European bats it appears that a new or extended discussion is urgently needed to evaluate our current view on how lesions caused by Gd should be evaluated. (Oral)

Laboratory Studies of Host-Pathogen Interactions between *Geomyces destructans* and Bats

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Despite recent progress in understanding white nose syndrome (WNS), critically urgent questions remain. We still have no understanding of differences between European and North American bats that could explain the lack of mortality in Europe, we know little about potential mechanisms underlying altered torpor-arousal cycles in infect bats, and we have little understanding of the efficacy of possible interventions that could enhance survival in the wild. We propose experiments that will address questions in all of these areas and address two specific objectives relevant to priorities of the RFP: 1) Assess the potential susceptibility of a common European bat species to infection with isolates of North American (NAGd) and European (EUGd) Gd and identify physiological differences in response to infection between European and North American bats; and 2) Test the hypothesis that hypotonic dehydration affects torpor arousal cycles and survival while also testing the feasibility of electrolyte supplementation as a non-chemical option to decrease mortality. We will address these objectives with experimental inoculation studies in a biosafety containment facility at the Western College of Veterinary Medicine (WCVM), in Saskatchewan Canada. Following an inoculation study we conducted on little brown bats (*Myotis lucifugus*) during 2010/2011, we will address Objective 1 by experimentally inoculating European greater mouse-eared bats (*Myotis myotis*) concurrently with little brown bats to test the hypothesis that European bats have innate characteristics which allow them to survive infection. Our preliminary evidence suggests from previous experiments suggests that hypotonic dehydration (i.e., low electrolyte levels) may play a mechanistic role in symptomatic disease and mortality from WNS for little brown bats. To address this hypothesis and Objective 2, we will test whether providing an electrolyte supplement, commonly used in wildlife rehabilitation, reduces the severity of infection and improves survival rates of bats exposed to Gd.

(Poster on WNS RFP Award)