Abstracts Related to White-nose Syndrome for the International Bat Research Conference (IBRC)and North American Symposium for Bat Research (NASBR), San Jose, Costa Rica, August 11-15, 3013

(Note: these are abstracts of research in progress. Some will ultimately be published in peer-reviewed journals, but others will not. These papers are presented by professors and students alike, as well as other professional biologists and technicians, and the conferences afford the ability for questions and constructive criticism from peers. Nonetheless, this is probably the single focused source of ongoing scientific research related to WNS. - Peter Youngbaer, NSS WNS Liaison)

The Science and Management of Emerging Wildlife Diseases: *Gd* in *Bd*'s Slipstream

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Emerging infectious diseases in wildlife appear to be on the rise, with fungal pathogens implicated in mass mortality events among diverse taxa including amphibians, reptiles, and mammals. Mass mortality events from fungal pathogens appear to be a relatively novel concern for wildlife, either due to their being a cryptic scourge relative to our notice in the past or due to new levels of virulence or transmission. Fungi can spread quickly within populations, decimating local abundances, and may be able to move easily among populations, rapidly dispersing across broader regions. Furthermore, human globalization patterns may assist fungal migration. For instance, Chestnut Blight (*Cryphonectria parasitica*) is believed to have been introduced to North America from Europe. Amphibian trade, including species like the African Clawed Frog *(Xenopus laevis)* and American Bullfrogs *(Lithobates catesbeianus*), has been linked to transportation of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis, Bd*) around the world. Recent evidence indicates that the fungus *Geomyces destructans*, *Gd*, in bats arrived in a New York cave via human-mediated transport from Europe. Hence, in several regards, fungal pathogens present a very different wildlife problem than other microbes, and their ecology and epidemiology is relatively little known. What can herpetologists and mammalogists learn from each other that may contribute not only to dealing with these current threats; but to those that may arise in the future. We examine the similarity and contrasts between *Bd* and *Gd* in pathogen characteristics as well as scientific and management approaches to understanding and arresting the diseases they cause.

Cave Environment Influence on the Microbial Symbionts of Four Bat Species

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Bats are a critical component of ecosystems across the globe and are found in a wide variety of environments including man-made structures, caves, and tree roosts. The microbial communities that exist in these habitats can be very diverse and may play a role in the development of the symbiont microbiome of the animal hosts. The influence of environment on the skin-associated microbes of hosts is an under-explored area of research, particularly in wildlife. The aim of this study was to survey the skin microbial populations of four common bat species. Bats and their hibernacula were sampled using a sterile skin swabbing technique in two states within the White Nose Syndrome (WNS) epidemic area. Samples were sequenced with an Illumina Hi-Seq at the University of Colorado-Boulder targeting the 16S rRNA gene, and the microbial community was analyzed with the QIIME bioinformatics pipeline. Cave site is the strongest predictor of the composition of microbes living on bat skin, indicating that the environment has a strong influence on the microbes that colonize bats. Secondarily, there is also a strong influence of host species identity on shaping the skin microbial communities and bat skin communities are significantly different than the cave surface. These results suggest that it will be important to consider the hibernacula environment of the bat as we progress towards understanding how the WNS pathogen interacts with the host skin community and begin to develop microbial profiling tools for predicting disease risk.

Changes in Roosting Behaviour of Hibernating *Myotis lucifugus* Inoculated with *Geomyces destructans*

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Pathogens and parasites can change the behaviour of hosts and, in the case of white-nose syndrome (WNS), understanding changes in behaviour of bats infected with *Geomyces destructans* (*Gd*) could be important for management. Infection with *Gd* appears to cause reduced clustering behaviour in some of the affected species which leads to two possible hypotheses: 1) Bats cluster less because it improves individual survival (e.g., by reducing

energetic costs or affecting local microclimate to slow fungal growth; or 2) Bats cluster less to reduce pathogen transmission. The first hypothesis predicts that individual bats, which cluster less, should exhibit reduced severity of clinical signs and increased survival. We evaluated this prediction by examining video recordings of captive, hibernating little brown bats. Bats were outfitted with temperature and humidity controlled incubators for the winter. Their behaviours were recorded by motion-activated cameras during periodic arousals from torpor. After several months in hibernation, wing tissue samples were obtained from infected and control bats and scored to provide a measure of disease severity. The roosting positions of each individual were recorded after each arousal throughout the duration of the study for each individual. Our results indicate that bats infected with *Gd* reduce clustering but we found no evidence of a relationship between reduced clustering by individuals and severity of disease. Thus we found no support for the hypothesis that behavioural changes of infected bats provide a survival benefit. Altered clustering could, therefore, reflect a behavioural mechanism to reduce pathogen transmission, similar to behaviours that occur in other wildlife diseases. Further studies of wild and captive bats are needed to understand the role of behaviour in transmission of *Gd*.

Life-history Traits of Bat Species at Risk from White-nose Syndrome and Histopathologic Fndings in Multiple European Bat Species

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Despite pan-European distribution of *Geomyces destructans*, mass mortality has not been observed in hibernating bats in Europe. Dermatohistopathology, however, revealed fungal infection with cupping erosions and skin invasion diagnostic for white-nose syndrome (WNS) in a *Myotis myotis* specimen hibernating in the Moravian Karst, Czech Republic and eight bat species have already been reported positive for the WNS fungus in Europe. The objective of this study was to examine life-history traits of European bat species reported positive for *G. destructans* and identify other bat species at risk. To do so, we explored life-history traits linked with hibernation to group bat species with similar characteristics, and proposed a model of species-specific potential for infection. Then, we verified the prediction model by screening species with unknown infection status in Czech hibernacula**.** Over two hundred specimens of 14 bat species were monitored for the WNS and its causative agent *G. destructans* in the Czech Republic in spring 2012 and 2013. Sampling procedures employed to screen bats for WNS and *G. destructans* included punch biopsy of WNS-suspected skin lesions, skin swabs for fungal culture and polymerase chain reaction, and histopathologic examination of skin samples. We provide evidence on multiple species positive for the WNS diagnostic features on histopathology. Importantly, the broadening spectrum of affected species may have implications for the current knowledge on the epidemiology of this fungal disease.

The North American Bat Conservation Alliance: Finding Our New Direction

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Although the bat conservation network in North America was one of the first to formalize its relationships, our coordinated efforts have varied in structure and effectiveness over the years. Canada, Mexico and the US all have dedicated biologists and local networks (regions or states) operating effectively to respond to localized priorities. However, the international network between these three countries has been inactive for many years. The early coordinated North American network faltered for a number of reasons, but we are currently launching an effort to revitalize this network and establish clear priorities and goals to address bat conservation challenges that warrant our combined attention. We are working under a new draft charter, developed during a 2008 meeting in Tucson, Arizona, and are taking steps to develop a functioning team with working objectives, key deliverables, and a specific mandate. Independent of an overarching network, several important collaborations are moving forward including the development of a North American bat monitoring effort and a multinational response to White-nose Syndrome. While these efforts will continue, we believe a coordinated and effective North American Bat Conservation Alliance would add value by enhancing cooperation and consideration of the full suite of concerns, threats and issues facing bats in North America. The alliance would help to establish continental bat conservation priorities and encourage efforts to address priorities that are not already being considered. It could also help elevate bat conservation in other existing conservation partnerships between our three countries and generate support and funding for critical projects.

Changes in Winter Activity of Bats in the Great Smoky Mountains National Park, Tennessee, Due to Whitenose Syndrome

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The White-nose syndrome (WNS) epizootic, characterized by the psychrophilic fungus *Geomyces destructans* (Gd), has caused unprecedented mortality of hibernating bats throughout the Northeastern United States. Gd was first recorded in the Great Smoky Mountains National Park (GSMNP) during winter 2009/2010 and bats were confirmed WNS positive in winter 2011/2012. By January 2013, Park rangers began receiving numerous reports regarding daytime activity of bats within the Park, including contact with a Park visitor. The goal of this research is to evaluate possible behavioral changes of bats during winter hibernation following progression of WNS. We hypothesize that WNS affected caves in southern latitudes may experience delayed mortality due to warmer ambient temperatures during hibernation. Using ultrasonic detectors and temperature data we have established that bats in GSMNP are active throughout the winter. Bat calls were recorded during each acoustic survey night (140 sample nights) regardless of low nightly temperatures. By year three of WNS confirmation, we see a significant increase in activity levels during two of the coldest months of the year (January: t-ratio -4.36, $p < 0.000$ and March: t-ratio 2.7, $p < 0.011$), suggesting a time lag in mortality due to WNS when compared to affected regions in the Northeast. Although we have documented this trend in GSMNP, we have yet to see a precipitous change in winter activity levels of bats at other caves in Tennessee.

Torpor Patterns of Hibernating Gray Bats: Implications for WNS

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White nose syndrome (WNS) has resulted in the death of over 5 million hibernating bats across 7 species since it was first discovered in 2006. While the exact cause of mortality is unknown, WNS infected little brown bats (*Myotis lucifugus*) arouse more frequently than uninfected bats. While gray bats (*M. grisescens*) have been shown to be infected with WNS, for unknown reasons, extreme mortality events have not yet been observed. Factors such as hibernation behavior are being proposed as possible explanations. To investigate the hibernation ecology of gray bats, we attached temperature sensitive radio transmitters onto 58 female gray bats across 4 hibernacula and recorded body temperature at approximately 15 minute intervals for over 90 days. Preliminary analysis of temperture data shows hibernation temperatures of between 10 and 15°C. Torpor bout lengths varied substantially between individuals (range: 6.36-16.56 days). Or results indicate similarity in torpor bout lengths between gray and little brown bats. Additionally, as gray bats are hibernating at temperatures in which *Geomyces destructans* (the fungus that causes WNS) grows faster, lack of impacts of WNS-induced mortality cannot be explained through differences in hibernation ecology.

What Proportion of the Bats that Occur at the Entrance of Caves and Abandoned Mines Hibernate There?

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In the late summer and early fall in temperate areas, large numbers of bats congregate at the entrances of caves and abandoned mines which are used during the winter as hibernacula. There are several hypotheses to explain these swarms including courtship and mating, and information transfer about potential hibernating sites or migration routes. It is known that some bats that visit a site during swarming do not necessarily hibernate at that same site, although some certainly do. Characterization of the proportion of bats that occur at the entrance of underground sites during the spring and also during the fall swarming period that eventually hibernate there is not well known and could provide insight into the movement dynamics, site fidelity and population structure of temperate hibernating species. Thus, our objective was to compare the proportion of bats (males and females) that were tagged during spring emergence and fall swarming that actually hibernate inside the mine. At an abandoned gold mine at Rawdon, NS, Canada, we trapped and PIT-tagged 338 *M. lucifugus* and 224 *M. septentrionalis* during emergence in the spring or swarming in the fall. We have actively searched for PIT- tagged bats during each of 1-3 winter counts inside the mine from 2008-2012 where we expect that we were able to count and scan >90% of the □1200 bats present (pre-WNS). We have 660 individual relocation records of tagged bats during hibernation. Generally, bats that were tagged in the spring were more likely to be recaptured at the site during hibernation, *M. lucifugus* were more likely to be recaptured during hibernation than *M. septentrionalis* and, for both species, males were more likely to be recaptured than females.

Separately Measuring Cutaneous and Pulmonary Gas Exchange and Water Loss of Hibernating Bats
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The "dehydration hypothesis" postulates the causative fungal agent (*Geomyces destructans; Gd*) of Whitenose syndrome (WNS) damages the wings in such a way as to disrupt water balance, which is a crucial challenge for bats. Unfortunately, due to methodological limitations, past studies have only speculated about gas exchange and water loss across even healthy wings. To test the dehydration hypothesis and further elucidate the pathogenesis of WNS we must understand physiological processes during hibernation. To this end, I tested various respirometry chambers to investigate which design best allowed me to separate cutaneous and pulmonary gas exchange and water loss of hibernating bats. Measurements were obtained using three chamber designs: masks, body chambers, and wing chambers. Masks were secured over rostrums with bands. Body chambers involved placing bats in a small cylindrical chamber with their heads protruding out through a fitted opening. This design separates pulmonary exchange (and the minute cutaneous exchange from the head) from the rest of the body. Wing chambers were made out of malleable plastic, flexible enough to allow bats to fold their wings. The body chamber was the most successful design for separately measuring pulmonary and cutaneous respiration. Now relative contributions of pulmonary and cutaneous water loss and changes due to WNS-associated wing damage can be estimated. Not only will this chamber design allow us to expand our understanding of the physiological complexities of hibernation, but also the WNS epidemic. Moreover, this study advances our ability to study hibernation as a whole and is applicable across disciplines.

Seasonal and Reproductive Effects of Flight Membrane Wound Healing in Captive Big Brown Bats
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Research on bat flight membranes has revealed several physiological functions essential to bat survival and conservation; however, injuries to the flight membranes of bats are commonly seen in the wild. In many cases such injuries heal completely, although little is known about wound healing during times of energy restrictions and high demand (e.g. hibernation and reproduction). Because wound healing is an energy-dependent process, it is expected to vary across seasons and reproductive status. We used an 8 mm diameter circular biopsy tool to punch the wing membranes of individuals in a captive research colony of big brown bats, *Eptesicus fuscus*, and compared differences in healing rates between summer and winter seasons, and between reproductive (lactating) and nonreproductive (non-lactating) females. We also compared the Body Condition Index (BCI=mass/forearm length) of biopsy and control bats to infer if wing damages results in faster depletion of fat reserves and/or slower healing rates as a function of reproductive stage. Preliminary results indicate the wing membranes heal rather quickly regardless of season. Moreover, so far we have not observed differences in BCI scores between membrane biopsy and control groups. This study provides bat researchers with important baseline data that can be used to assess the health status of bat populations and to develop strategies important for bat conservation given the threat of white-nose syndrome to bats.

Persistence versus Extinction: Are Disease Dynamics of White-nose Syndrome Changing?
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The emergence of the novel pathogen, *Geomyces destructans* (Gd), which causes the disease White-nose syndrome (WNS) in bats, has driven dramatic population declines in North America and is predicted to cause the local extinction of several bat species. However, after severe initial declines in New York, the oldest infected hibernacula now seem to be exhibiting changes in disease dynamics leading to apparent population stabilization for *Myotis lucifugus*. We present prevalence and infection intensity data using swab sampling and real-time PCR for localities where populations are suspected to be stabilizing and compare these to localities experiencing continued decline. We propose potential mechanisms that would lead to stabilization for some populations. Understanding persistence versus extinction processes is critical for determining long-term impacts of WNS, and informing conservation decisions for species and localities that continue to be pushed towards local extinction.

Clustering, Energetics, and Phenology of Hibernation in a Cold-Temperate Climate
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Hibernation consists of energy-saving torpor bouts (controlled reductions in body temperature (T_b)), which are interrupted by periodic arousals to normothermic T_b, presumably to correct physiological imbalances that occur during torpor. Arousals account for the majority of hibernation energy expenditure and arousal timing varies among individuals. Many hibernating bats are unusual among hibernators in their tendency to cluster in large numbers during hibernation and this could influence torpor arousal cycles, energetics and emergence timing. We tested the hypothesis that clustering is a strategy for energy conservation which also influences torpor bout duration and emergence timing. We predicted that bats with the smallest fat reserves at capture in early winter would be most likely to cluster and use the longest torpor bouts but would emerge earliest in the spring once conditions warmed

enough to support flying insects. We used temperature telemetry to quantify the frequency and duration of torpor bouts and arousals, infrared camera traps to record the tendency of individuals to cluster and cluster size, and passive transponders (PIT tags) to determine emergence timing of free-ranging bats hibernating in central Manitoba, Canada. We outfitted 7 adult females, 11 young-of-the-year females, and 28 adult males from two caves. This study will add to our understanding of hibernation behaviour and help determine links between clustering, energetics, and hibernation phenology.

The Structure and Function of Hibernating *Myotis myotis*'s Constitutive Immune System: Are Eurasian Bats Special?

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Mammalian hibernation consists of prolonged periods of reduced basic metabolic rate and body temperature, which are interrupted by intermissions of arousal when most of the physiological functions are temporarily restored. Hibernation affects as well the immune system, and it has been hypothesized that arousals may activate the dormant immunity to combat accumulated pathogens. Despite a plethora of studies on hibernating rodents and insectivores, we know virtually nothing about the immunocompetence of hibernating bats. We collected blood from greater mouse-eared bats (*Myotis myotis*) in order to compare the structure (white blood cell counts) and the functionality (bacterial killing capacity of the blood) of the pre-hibernating, hibernating and aroused individuals' constitutive immune system. In accordance with studies on non-Chiropterans, hibernating bats showed reduced numbers of total leukocytes, both agranulocytes being affected. After arousal, the numbers of these cells were restored, while the number of neutrophils was not affected as their values remain similar for all three physiological conditions. When compared with euthermic conspecies, hibernating individuals had the lowest bacterial killing capacity, although their immune system function at both 37°C and 9°C. The constitutive immune function is reduced during torpor, but contrary to all animal species studied so far, hibernation of greater mouse-eared bats has no influence on circulating neutrophils indicating a continuous preparedness of the immune system against pathogens. Further comparative studies are needed to test whether this is a general pattern among hibernating bats and if dissimilarities occur, to explain the apparent continental differences in susceptibility to colonization with *Geomyces destructans*.

Immune Responses in Hibernating Bats

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We are developing tools to better understand the immune physiology of bats and how it may differ from that of other mammals. Compared to other mammals, bats appear to have delayed or reduced immune responses to some pathogens that may allow them to be disease carriers. The state of immune suppression that accompanies torpor in hibernating bats renders them susceptible to the psychrophilic fungus, *Geomyces destructans*. The epizootic disease, white-nose syndrome (WNS) that results may be due, in part, to immune pathology that accompanies restoration of immune competence upon arousal from hibernation. In order to address whether immune responsiveness in hibernating bats is a contributing factor in WNS or in the ability of bats to serve as zoonotic disease carriers, we wish to establish the levels of normal immune function in bats and how they are affected by torpor. We have developed methods to measure levels of specific immune cells, cytokines, and antibodies within *Myotis lucifugus* and *Eptesicus fuscus*. We are comparing these molecular measures to classical ecoimmunological assays to determine which measures are correlated. We will also present our plans to develop gene expression assays to quantify and compare individual immune and metabolic mechanisms. Together, these methods should allow us to better understand the differences in immune physiologies between WNS-susceptible and WNS-resistant bat species and between bats and other mammals.

Phylogeography of *Geomyces destructans* in North America and Europe

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Geomyces destructans is the fungus responsible for White-Nose Syndrome in bats. Prior to its discovery and description in the past several years this fungus was unknown to science. Detailed genetic work using microsatellites and genomic work with whole genome sequences on fungal isolates from bats and sediments in North America suggests a recent introduction and rapid spread across the eastern part of the continent. Using these same genetic and genomic approaches we have been searching for the likely source of *G. destructans* using isolates and swab samples collected from a variety of European sources. We describe the discovery of potential sources of *G. destructans* from Europe and the apparent introduction routes of this devastating pathogen.

Patterns of *Geomyces destructans* Infection across North America

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Transmission dynamics of *Geomyces destructans* in wild bats remain poorly understood. Determining prevalence, intensity of infection, and transmissibility among individuals in wild bat populations aids development of effective management strategies for controlling spread and mitigating impacts of WNS. We assessed prevalence of *Geomyces destructans* infection by swab sampling bats in 20 states across enzootic, epizootic, and leading edge regions in North America during the 2011-2012 and 2012-2103 winter seasons. Prevalence varies by species in highly impacted regions even within the same hibernacula and by region depending on time since WNS first detected. We also compare infection prevalence and intensity on bats to hibernacula wall substrates. By noninvasive swab sampling of multiple individuals at sites, we are able to provide early detection methods of presence of *G.destructans* before disease symptoms or mortality and visible infection are apparent. Our results are useful to track spread of *G. destructans* at a continental scale and determine factors associated with risk of arrival of *G. destructans*, disease progression, and impacts to populations.

Spring Arrival Sequence and Summer Activity of Bats at Schmeeckle Reserve and Kemp Wildlife Station, Wisconsin, U.S.A.

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Wisconsin currently has a healthy population of bats. However, cave-hibernating bats and migratory treedwelling bats are threatened by White Nose Syndrome (WNS) and wind farms in Wisconsin. White Nose Syndrome is due to a fast-growing fungal disease that arouses bats during hibernation causing the depletion of energy stores, exposure, and potentially death. The disease has spread quickly since its discovery in New York in 2006, threatening some species with extinction, and is expected to reach Wisconsin within two years. Migratory tree-dwelling bats are experiencing high mortality rates around rapidly growing wind farms throughout North America. Data were collected from acoustic detectors (Anabat) permanently mounted by the Wisconsin Department of Natural Resources (WDNR) at two sites in the state: Schmeeckle Reserve in Stevens Point and Kemp Wildlife Station on Lake Tomahawk in Woodruff. Echolocation calls were collected and analyzed from each site for April through August 2009–2012 to determine the spring arrival sequence and overall summer activity patterns of individual species. Analook software was used to identify individual species of bats and to record time and date of activity. *Myotis lucifugus, Eptesicus fuscus/Lasionycteris noctivagans,* and *Lasiurus borealis* are the most common species with variations in prevalence between the two sites. Little activity was detected in April with the majority of activity occurring in July and early August. This research project contributes to baseline distribution, migratory, and hibernation data being collected by the WDNR to further the understanding of threats to these ecologically and economically important resident bat populations.

Year Round Activity of Peripheral Bat Populations in the North Carolina Coastal Plain, USA

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When there is a major threat to core populations shifting conservation efforts to peripheral populations can be an effective way to mitigate core population loss, such is the case with White Nose Syndrome (WNS). Warm coastal temperatures along the Atlantic Coastal Plain, USA may allow peripheral bat populations there to remain active through winter, thus decreasing their susceptibility to WNS. Therefore, the objective of our study was to determine the distribution and activity of peripheral bat populations in the North Carolina Coastal Plain, USA. We set up four mist-netting and recording stations along a 295 kilometer north-south transect in the North Carolina Coastal Plain to monitor bat activity year round in intensively managed pine or bottomland hardwood forests. We had 62 nights of mist-netting and captured 467 bats of 9 species. Additionally, in the summer of 2012, we established a Song Meter SM2Bat+ detector station to record bat ultrasound from sunset to sunrise for two years at each site. Total number of bats captured, species richness, and number of call sequences recorded was higher in bottomland hardwood forests than intensively managed pine forests. Recorded calls are currently being analyzed to determine presence and seasonal activity of bat species. Preliminary acoustic results suggest that on warm winter nights, winter activity of bats is comparable to summer activity. We will discuss significance of these results in relation to WNS.

Contamination by Polychlorinated Biphenyls (PCBs) and Bat Activity along the Hudson River, New York

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The upper Hudson River, New York, is contaminated with polychlorinated biphenyls (PCBs) because of historical discharges. Although elevated levels of PCBs have been found in the tissues of bats, it is not clear whether this contamination has affected bat activity along the Hudson River. We monitored bat activity at sites with varying levels of PCB contamination along the Hudson River between May and August of 2008 and 2009. To assess possible effects of PCB contamination on bat foraging behaviour, we measured *Myotis lucifugus* (little brown bat) and *Lasiurus cinereus* (hoary bat) activity along the Hudson River, as well as foraging times and distances covered by radio-tagged *M. lucifugus*. Activity of *M. lucifugus* and *L. cinereus* varied along the Hudson River, and activity of both species was lower in 2009 than 2008. Compared to two sites on the Hudson River, *Myotis lucifugus* foraged longer and farther at a site away from the Hudson River. Comparisons of bat activity levels with differing approximate PCB concentrations indicate that the variation in bat activity along the Hudson River is not related to PCB concentrations. Our data indicate that PCBs have not significantly affected bat activity or foraging behaviour along the Hudson River.

Beneficial Bacteria Present on Hibernating Bats in the Eastern United States

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White-nose syndrome threatens several hibernating bat species with extinction, and at present, there are no known effective treatments for this disease. Bacteria are broadly used as biocontrols against fungal diseases in plants, and more recently, in fish, and amphibians. Using culture based techniques, we isolated bacteria from bat skin and challenged it against the fungus that causes white-nose syndrome, *Geomyces destructans*. We found that *Pseudomonas spp.* present on bats can effectively inhibit the growth of *Geomyces destructans* in vitro. The natural occurrence of *Pseudomonas spp.* on hibernating bats, as well as their ability to successfully inhibit *Geomyces destructans*, make them promising candidates as biocontrol agents for reducing impacts of white-nose syndrome.

Cryptic *Geomyces destructans* Infections in the Southeastern United States

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White-Nose Syndrome is an epizootic in hibernating bats and the causal agent has been identified as the 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 and submit these bats for laboratory testing. Swabbing bats for fungal DNA provides non-invasive sampling for *G. destructans*, and the opportunity to document the accuracy of visual observations for detecting the fungus. To determine the presence of *G. destructans*, bats were swabbed on their muzzle and forearm, and it was noted whether any fungus was visible. qPCR was used to detect *G. destructans* DNA, if present. As part of a continent-wide study, 665 bats of 7 species were swabbed at 18 hibernacula in 3 states (TN, MO, AL) in the winter of 2011-2012, and 624 bats of 10 species were swabbed at 19 hibernacula in 4 states (TN, MO, AL, KY) in the winter of 2012-2013. *G. destructans* DNA was detected by swabbing as early as mid October and as late as the end of May on bats mist-netted near cave entrances. Results suggest the occurrence of “cryptic” infections; 109 bats of 6 species from the first winter of swabbing tested positive for *G. destructans* DNA without showing any signs of visible fungus. Only one little brown bat (*Myotis lucifugus*) tested negative for *G. destructans* DNA while showing signs of visible fungus. These data are useful for management purposes in

documenting the presence of *G. destructans* in hibernacula.

The Effect of Temperature and Infectious Dose on White-nose Syndrome in Captive *Myotis lucifugus*

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Since the discovery of white-nose syndrome (WNS) in North America in 2006, much has been learned about how infection with the fungus *Geomyces destructans* (*Gd*) leads to large-scale mortality among cavehibernating bats. Much remains unresolved, however, including how variation in environmental conditions and level of exposure to the fungal pathogen promote differences in mortality in infected bats. To better understand WNS mortality rates, we collected 71 male and 77 female little brown bats (*Myotis lucifugus*) from the wild and placed them into artificial hibernacula. Captive bats were randomly placed into control and experimental groups artificially hibernated at 4° and 10° C. At each temperature, 14-15 bats were inoculated with a control solution or a solution containing 500, 5,000, 50,000, or 500,000 fungal spores. Bats were left in hibernation for 21 weeks to assess the influence of temperature and infectious dose on the progression of WNS and resultant mortality. Data presented will include analyses of mortality, decline in body condition, rate of periodic arousals from hibernation, and wing damage as influenced by sex, body condition entering hibernation, hibernacula temperature, and infectious dose. These data will provide important insights into patterns of mortality among wild populations of little brown bats in eastern North America, and help to understand future population declines as WNS continues to spread.

Transmission of *Geomyces destructans* between Bats and the Environment

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Understanding the role played by different species in the transmission of multi-host pathogens is necessary to determine which species are reservoirs and drive transmission, and to determine the impact of a disease on species as they decline in abundance. White-nose syndrome has devastated many populations of hibernating bats over the past six years and threatens some species with extinction. We examined patterns of infection with the fungus that causes white-nose syndrome among five species and the environment in hibernacula in the eastern USA to determine the extent to which transmission is predominantly within species, and to examine whether some species play disproportionate roles in contaminating the environment. We found that transmission was primarily within species and some species appeared to play larger roles in contaminating the environment. These results highlight the importance of social interactions among bats during hibernation for the transmission of *G. destructans*.

Infection Loads of *Geomyces destructans* Influence Disease Impact

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Understanding *Geomyces destructans* transmission within multiple host species will allow for targeted disease management that may reduce the consequences of white-nose syndrome on bat populations. High pathogen loads on individuals may enhance transmission, and potentially drive mortality from disease. Seasonal changes in host behavior, as well as temperature and humidity differences among hibernacula, may also affect fungal growth, causing variation in mortality among species and individuals. We investigated transmission of the etiological agent of white-nose syndrome, *G. destructans*. Swabs from exposed wing and muzzle tissue of bats were collected and analyzed using real-time quantitative PCR. We found strong evidence of intense transmission during the winter, and by late winter nearly all bats were infected. For some species, pathogen loads increased significantly throughout hibernation, and females were more likely to be infected than males, possibly due to earlier entry into torpor. Species with high prevalence in early hibernation had the highest increases in loads, and the greatest mortality. In contrast, loads on big brown bats and Indiana myotis did not increase as greatly over the course of hibernation, and this may account for reduced mortality in these species. Pathogen prevalence and loads on little brown myotis decrease rapidly upon emergence from hibernation, suggesting that hibernacula serve as the source of the yearly epidemic. Changes in pathogen loads coupled with early timing of infection may be important factors in WNS mortality, and be important drivers of local transmission.

Unravelling the Mysteries of Winter Bat Ecology in Western Canada

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White Nose Syndrome (WNS) kills bats during hibernation; increased winter flights have been associated with WNS, and dehydration and starvation have been implemented in mortalities. We wanted to study normal hibernation behaviour in western Canadian bat species to begin to understand how this disease may unfold in the West, and which species may be more or less at risk. Our goals are to: 1) Determine species diversity and ecology during winter across British Columbia (B.C.); 2) Locate and describe hibernacula. We used both acoustic monitoring and radiotelemetry to examine winter distributions/movements of bats, habitat use, and behaviour. British Columbia has 16 species of bats, 14 of which may hibernate in the province. We present results from the first 2 years of this 4+ year project. We found late fall (November) activity of at least 8 species, suggesting they likely overwinter in B.C. During winter, we acoustically recorded and observed/captured 6 species of free-flying bats: big browns (*Eptesicus fuscus*), silver-haired (*Lasionycteris noctivagans*), Townsend's big-eared (*Corynorhinus townsendii*), Californian (*Myotis californicus*), Western small-footed (*M. ciliolabrum*), and Yuma (*M. yumanensis*). Hibernacula were located for these species, and roosts included rock crevices, mines, trees, snags, and buildings. We captured free-flying bats in winter at study sites across southern B.C., with the most active species being silverhaired and Californian. Silver-haireds mated in winter and frequently switched roosts, alternating between mine and tree hibernacula. Californians appeared to be foraging on dormant insects in low elevation mines, as determined through plastic laid in mines and acoustic monitoring.

Tracking White-nose Syndrome and Other Threats: A Population Monitoring Program for North American Bats

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Bats in North America are facing unprecedented threats including White-Nose Syndrome, wind energy development, habitat loss and fragmentation, and climate change. Yet, there is currently no coordinated monitoring program to track changes in their populations in response to these threats. A group of scientists and statisticians from the U.S., Canada, and Mexico is developing the North American Bat Monitoring Program (NABat), a framework for bat monitoring across North America. NABat will provide the statistical, biological and administrative architecture for coordinated bat population monitoring that will promote effective decision-making and long-term viability of bat populations across the continent by providing robust data on changes in bat distributions and abundance. The sampling framework will likely be comprised of a nested grid consisting of 50 km cells as primary units, 10 km cells as secondary units, and 5 km cells as tertiary units; 1 km cells can be used for intensive research and local monitoring projects. A spatially balanced design will be used so that unequal and changing survey efforts can be accounted for in design-based and model-based population estimates of status and trend. The primary data sources will be maternity and hibernacula counts and acoustic data collected along driving transects or at stationary points across the landscape. Data will be housed and managed by the Bat Population Data (BPD) Project, an initiative of the USGS Fort Collins Science Center to foster data sharing and collaboration through a web-based bat population data management application.

Phylogeny of Vespertilionid Bats in Relation to White-nose Syndrome

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The white-nose syndrome (WNS) caused an epizootic in North America that could lead to multiple local extinctions of hibernating bats in the near future. While North American bats recognised with WNS belong to the genera *Myotis*, *Perimyotis* and *Eptesicus*, *Myotis* species are the most severely affected. Likewise, in Europe the *Geomyces destructans* infection (geomycosis), as well as WNS, were diagnosed in *Myotis* species. We hypothesized that closely related bats are more susceptible to the infection. We tested this on a maximum likelihood phylogeny based on DNA sequences from 13 mitochondrial and nuclear genes. Pruning the phylogeny to species distributed in North America and Europe, we tested the phylogenetic representation of geomycosis and WNS on 55 taxa. The results showed that the previously diagnosed taxa were significantly phylogenetically clustered (net relatedness index > 0) and the nearest relatives were more likely to be infected with *G. destructans* than expected (nearest taxon index > 0). However, the manifestation of WNS in nearest relatives was indistinguishable from random distribution of the trait on the phylogeny. Adding three taxa newly diagnosed with geomycosis, the phylogenetic relatedness indices decreased without changes in significance. We confirmed that bats with geomycosis are phylogenetically clustered and those with WNS are significantly clustered on the phylogeny, but the disease need not manifest in the nearest relatives. Our data indicate that the disease might affect distantly related taxa albeit the most likely ones to suffer would be related.

Summer Bats to Winter Bats: Preparing Captive *Myotis lucifugus* for Hibernation

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Captive assurance populations can be a critical last resort for maintaining imperilled species. When chytridiomycosis threatened amphibians, researchers were skeptical of the potential for captive assurance because of captive maintenance and breeding difficulties. However, technical advances improved outcomes and many threatened amphibian species are now successfully maintained. Hibernating insectivorous bats present large and unique challenges for captive assurance but, given the enormous declines for many species due to white-nose syndrome, active research in this area is critical. Wildlife rehabilitation experts routinely keep little brown bats (*Myotis lucifugus*) in short, shallow hibernation over winter and some research groups have maintained little brown bats captured from hibernacula in long-term hibernation using temperature and humidity controlled incubators. An outstanding challenge is transitioning captive bats from summer active periods into hibernation. Recently, we brought little brown bats into captivity during the summer in preparation for hibernation experiments that winter. Attempting to transition bats from summer active season to winter hibernation, we provided an *ad libitum* diet high in polyunsaturated fatty acid. We then progressively exposed bats to cold temperatures of increasing duration and concurrently reduced food availability, hypothesizing that these factors mimic natural conditions experienced during autumn. We compared arousal frequencies over the first month of hibernation from these bats to bats studied in previous years where individuals were collected directly from hibernacula. To maintain captive assurance populations effective methods to transition bats from summer active periods and winter hibernation are needed. Based on our experience, we propose improvements which could enhance this transition.

Nature's Options Values: the Ecological-economics of Insect-eating Bats

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If bats read the biological control literature they would learn that the most effective insect pest control agents are specialist predators that attack pests at early stages of the pest's life cycles and are capable of driving pest populations to near extinction. Bats would find that generalist predators traditionally have been eschewed as effective agents of biological control, and that eating a pest insect does not equate to providing an ecosystem service (ES). Indeed, bats would read that the reproductive capacities of insects are so great that eating huge numbers of pests need not suppress pest populations. We argue that bats know better. The few economic estimates of the ES of insectivorous bats suggest that the services of bats are much greater than previously appreciated, or even imagined. As highly mobile, generalist predators that persist in the environment on alternate food when pest populations are low and recruit rapidly to exploit pests when pest populations surge, much of the "natural capital" of bats exists not only in avoided costs but as "option values" resulting from the bats' presence in contemporary agro-ecosystems. Furthermore, the existing E estimates for bats are "snapshots" in time and space. We illustrate how the E values of bats fluctuate in time and space due to natural and socio-economic factors and due to investments in manufactured substitutions for natural capital (e.g. pesticides) and the depreciation of this manufactured capital (i.e. resistance evolution). As ES values fluctuate, option values are realized and the natural capital of bats enhanced.

Fluorescence, Fungal Load, and Severity of Disease

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Most studies of white-nose syndrome (WNS) to date report presence/absence of *Geomyces destructans* (Gd) (i.e., control vs. inoculated in the lab; affected vs. unaffected in the field) but we have little understanding of relationships between fungal loads, disease severity and potential infectivity. Quantitative PCR and histopathology allow for estimates of fungal load but are costly and require specialized expertise for interpretation. Ultraviolet (UV)

fluorescence has potential as an inexpensive alternative but relationships between fluorescence, histopathology and fungal load need to be determined. We used data from experimental inoculations with *Myotis lucifugus* and *Myotis myotis* to test the hypothesis that Gd load correlates with severity of clinical signs and quantitative estimates of UV fluorescence. We outfitted bats with temperature dataloggers, inoculated them with *G. destructans* and held them in temperature and humidity controlled hibernation chambers. For experiment 1, bats were kept at 7°C and one of four humidity treatments (85, 90, 95, or 99% RH). For experiment 2, bats were sampled from the incubators at monthly intervals to assess disease progression. Whenever bats were sampled we took UV photographs of flight membranes, swabbed forearms following a standard protocol, and preserved wing tissue for histopathology. We quantified torpor/arousal patterns based on skin temperature, fungal load using qPCR, scored histopathology using a 5-point scale, and calculated the area of each wing fluorescing in digital photographs. Our results provide new information on the relationship between fungal load and severity of WNS and the potential of UV fluorescence as an alternative/complement to other diagnostic methods.

Fitness Consequences of White-nose Syndrome

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Little brown myotis (*Myotis lucifugus*) have experienced significant population declines in the face of white-nose syndrome (WNS). Although infection with *Geomyces destructans* (Gd, the causative agent of WNS) does not always result in death, little brown myotis survivors may experience fitness consequences. We collected 71 male and 77 female little brown myotis believed naïve to Gd from the wild and placed them into artificial hibernacula. Captive bats were either sham inoculated or inoculated with variable doses of Gd and left to hibernate for 21 weeks. Bats that survived were trained to eat a captive diet and maintained in a flight cage; differences in body mass, wing wound healing, and metabolic rate will be presented in relation to severity of infection and sex. These data are important in assessing the future survival abilities of little brown myotis.

The Effect of White-nose Syndrome Mortality on the Genetic Structure of New York's *Myotis lucifugus* Population

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White-Nose Syndrome (WNS), caused by the cold-temperature tolerant fungus, *Geomyces destructans*, is an emerging infectious disease that affects hibernating bat species in North America and Europe. Since the initial 2006 outbreak in Howes Cave, New York (NY), WNS has spread to all major hibernacula in NY, leading to unprecedented mortality in several bat species. The WNS-induced mortality has resulted in an estimated 95% decline in the NY *M. lucifugus* population alone. Given the devastating outcome, the impact of WNS on the genetic diversity of bat populations has not been fully evaluated. We propose to establish the genetic structure of the NY *M. lucifugus* population prior to the onset of WNS, and determine if the spread of WNS westward from the epicenter of infection results in a loss of genetic diversity, both spatially and temporally. We utilized 11 autosomal microsatellite regions and the HVI and HVII regions of mitochondrial DNA to sample genetic diversity. Preliminary results indicate that the pre-WNS population (2003 - 2005) has genetic structure and the population as a whole maintains some genetic diversity across NY in subsequent years (2008 - 2011). Any loss of genetic diversity will decrease the likelihood that this species will return to its former effective population size in NY. Not only will this study provide wildlife managers with information vital to the development of recovery plans, but it also is a unique opportunity to study the impacts of an emerging infectious disease on a wildlife population.

Hibernation Phenology and Survival of *Myotis lucifugus* in Central Canada

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Phenology and energy allocation should reflect both extrinsic (e.g., weather, climate, predation risk) and intrinsic (e.g., reproductive timing, age/sex class) factors. Males of hibernating species which mate following hibernation (e.g., ground squirrels) both immerge into and emerge from hibernation before females, which may reduce predation risk in late summer and increase access to mates in spring. Bats, which mate prior to hibernation, should exhibit opposite patterns, with females emerging and immerging earliest. Climatic severity should also affect phenology by extending hibernation duration. This, in turn, is expected to increase pressure on animals to accumulate large fat stores and/or lead to decreased survival. I used a short-term dataset based on passive transponders (PIT tags) to examine the influence of reproductive timing on the hibernation phenology of *Myotis* *lucifugus* and a 20-year banding dataset to test the hypothesis that weather conditions and winter duration influence survival of little brown bats in central Canada. Adult females emerged earlier than both adult males and subadult females, and adult males were active significantly later in the fall than adult females. Annual survival was constant and extremely high at all sites (>0.90), despite inter-annual variability in precipitation. High survival may result from the tendency of bats in central Canada to obtain a large pre-hibernation fat reserve and hibernate for long periods which could reduce extrinsic mortality. Bats from my study site are between 24-37% heavier at hibernation onset than bats in eastern North America which may lead to an increased ability to tolerate White-Nose Syndrome.

Indiana Bat Roost Habitat Selection in the Southern Appalachian Mountains
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The federally endangered Indiana bat (*Myotis sodalis*) is being impacted by white-nose syndrome and habitat loss across much of its range, and climate change may pose additional threats. A better understanding of roost ecology of the species may facilitate conservation of healthy populations and could be critical to the overall survival of the species. Our goal was to identify the multi-scale characteristics of roost habitat for Indiana bats in the southern Appalachian Mountains. From May–August 2008–2012, we attached 0.32–0.42 g radio transmitters to adult females and juveniles, and measured characteristics of trees and 0.1 ha plots for 86 day roosts and associated random trees. We used 76 roost locations to identify important landscape-scale predictors with the presence-only modeling approach of MaxENT. Most (76%) roosts were yellow pine (*Pinus* subgenus *Diploxylon)* snags, but white pine (*P. strobus*) and hemlock snags were also used. Roosts were taller (18.7 m), larger in diameter (37. 7 cm), and in a lower state of decay than random trees. There was similar canopy closure in roost (58%) and random (73%) plots, but closure was low (<30%) directly above roosts. The spatial model validated our field observations that, in the southern Appalachians, optimal roosting habitat for Indiana bats is near the ridgetop in a south-facing mixed pine forest at elevations from 260–700 m. Management activities that promote these conditions and create or preserve large snags should aid in the management and recovery of the Indiana bat.

Lipid Profiling of Bats and *Geomyces* to Understand Trophic Interactions in a Host/Pathogen System
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Host integument supplies nutrients to the microbial community colonizing the skin. Mechanisms responsible for microbial acquisition of integumentary molecules may provide targets for disruption of pathogen infection. Lipid profiling provides evidence at a molecular level of initial nutrient levels in a host/pathogen system. Furthermore, differences in lipid profiles contribute to understanding of taxonomy, life history, and clinical signs of disease. We obtained lipid profiles for 13 species of bats and 2 species of *Geomyces* (*G. destructans* and *G. pannorum*). Bat sebaceous lipids are a complex mixture of glycerolipids, sterols, wax/sterol esters, squalene, and free fatty acyls (FFAs). Bat fatty acid methyl ester profiles are species specific. *Geomyces* produced primarily triacylglycerides (TAGs), FFAs, and sterol with minor amounts of mono/diacylglycerides and sterol esters. *G. destructans* lipids are more unsaturated compared to *G. pannorum*. Proportion of TAG to FFA increased when *G. destructans* was cultured in stressed conditions. These results provide baseline information on host lipids that may interact with *G. destructans* during infection. *Geomyces* have different lipid profiles, which can be used in disease diagnostics and detection. *G. destructans* may alter lipid ratios to lower cellular toxicity or stimulate reproduction in stressed environments. Increases in lipid unsaturation may also partially explain the psychrophilic life history of *G. destructans*. Further experimentation should highlight differences among lipid profiles of additional *Geomyces* species and metabolic processes involved in digesting bat integumentary lipids.

Does *Geomyces destructans* Infection Impair Reproductive Capacity in *Myotis lucifugus*?
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White-nose syndrome (WNS) is a disease caused by a cold-adapted fungus, *Geomyces destructans* (*Gd*), that results in devastating population declines of North American cave-hibernating bats. Rates of decline at most sites are estimated by annual counts, so it is uncertain whether declines are due entirely to increased mortality or if reduced fecundity also occurs. Female little brown bats (*Myotis lucifugus*) store sperm and a single Graafian follicle throughout hibernation, and will not produce any offspring if storage fails before spring emergence. Bats with WNS become emaciated and dehydrated, which could compromise their ability to maintain the stored follicle and sperm. We conducted a histological study of reproductive tissues from 58 hibernating female *M. lucifugus*, including both

Gd-positive and *Gd*-negative individuals. We hypothesized that *Gd*-positive females would be less likely than *Gd*negative females to maintain a Graafian follicle and stored sperm. We found that *Gd*-positive females were less likely to maintain a Graafian follicle, but results were not significant. If *Gd* infection reduces fertility in hibernating *M. lucifugus* females, the effect is small. Additional studies of reproductive capacity of females at maternal colonies will determine if *Gd* infection reduces the probability of maintaining pregnancy in the post-hibernation season.

Modelling *Geomyces destructans* Distribution in North America and Eurasia Using Ecological Niche Modelling: What Can We Learn?

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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, Gd. By modelling the occupied niche by Gd in Europe, we predicted its distribution in North America, and which ecological factors could be limiting its distribution. To achieve this, we used a species distribution modelling technique – maximum entropy modelling - that has been proven to accurately predict current species distributions. To reduce uncertainties in models' projections we used the reciprocal modelling approach. This way the full niche of the species is considered (including data from both Europe and North America). We also calculated the similarities/differences in niche occupied by Gd between North America and Europe and highlight potential adaptations of Gd in North America. Finally, we determined potential dispersal routes that Gd could use to expand its current distribution. Results indicated that Gd distribution was limited by temperature variables. Model predictions comprised the currently known distribution of Gd in Europe but also areas where Gd has not yet been detected. Results showed a mismatch between realised niche in Europe and North America. This indicates that Gd is probably not occupying its entire potential ecological niche in Europe or that it recently adapted to North American conditions.

The Influence of Environmental Variables on the Demography of *Myotis lucifugus*

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Given the variety of threats to bat populations in North America and around the world, accurate information on the population dynamics of bat species are necessary to generate effective conservation policies. We have used markrecapture data from a long-term monitoring study of a maternity colony of little brown myotis (*Myotis lucifugus*) in Peterborough New Hampshire to generate estimates of some of the primary demographic variables (adult survival rates, juvenile survival rates, and reproductive rate) and secondary demographic variables (birth sex ratio, reproductive timing, reproductive synchrony, and post-natal growth rates) necessary to inform population viability analyses and recovery models. These data suggest that adult female survivorship has the highest impact on population growth rates overall, with juvenile survivorship strongly influenced by reproductive timing and environmental conditions. There was no evidence of an overall biased birth sex ratio but strong suggestion that more females were born early in the reproductive season compared to males. The data also suggest that primiparous females give birth significantly later than experienced females and have a male-biased sex ratio. Data collected from this population also suggest that few females reproduce in their first year after birth, and that cohort-specific reproductive rates become age-independent after three years. These data should prove valuable for generating and validating population models in this species, and highlights the value of mark-recapture studies to understand the role of environmental variables on the population growth of long-lived species.

Winter Activity of Four Bat Species at Three Desert Hibernacula in Idaho

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Documenting activity patterns of hibernating bats in western North America is important for understanding the behavioral ecology of these mammals, especially before white-nose syndrome potentially affects some of these species. Activity patterns of bats during winter in western North America, however, are poorly understood. We acoustically monitored 3 caves (Middle Butte, Rattlesnake, and Aviator caves) that are important hibernacula (up to 705 hibernating bats) in southeastern Idaho during winter (November to March) from 2011 to 2013. At those caves, AnaBat detectors were set for 234 sampling nights during 2011 to 2012 and for 320 sampling nights during 2012 to

2013. We used filters in AnaLookW to quantify bat passes (sequences ≥ 2 calls separated by ≥ 1 second), and documented western small-footed myotis (*Myotis cilolabrium*), Townsend's big-eared bat (*Corynorhinus townsendii townsendii*), big brown bat (*Eptesicus fuscus*), and the little brown myotis (*Myotis lucifugus*) flying outside of caves during mid-winter. Activity was highly sporadic and differed among species, with western small-footed myotis being most active during winter (bat passes 2, 0), followed by Townsend's big-eared bat (bat passes = 694). We recorded the highest diversity (4 species) and the most activity (3,051 bat passes) at Middle Butte Cave. Our study documents activity patterns outside of caves for several species of bats during hibernation. These results improve our understanding of the behavioral ecology of these species in western North America prior to the potential arrival white-nose syndrome, and the possibility of this disease altering winter behavior of these mammals

Crucial Hibernacula for Bats in Southern Idaho: Implications for Conservation and Management

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Bat populations are being impacted by human disturbance and modification of hibernacula. Identifying important hibernacula and counting hibernating bats are effective ways to conserve these mammals. We compiled periodic counts of hibernating bats during winter (November to March) in 36 caves from 1984 to 2013 to document the number of caves used by bats, as well as to investigate if the number of bats hibernating varied by colony size. Researchers counted 24,919 bats representing 6 species. Townsend's big-eared bats (*Corynorhinus townsendii townsendii*) comprised 95.8% (23,874 individuals) of those bats and used 35 caves, and western small-footed myotis (*Myotis ciliolabrum*) comprised 4.1% (1,014) of those bats and used 19 caves. Twenty caves were substantial hibernacula (≥ 20 individuals) for Townsend's big-eared bats, and five caves were substantial hibernacula (≥ individuals) for western small-footed myotis. The largest hibernating colony of *C. t. townsendii* occupied Kid's Cave ($\bar{x}$ = 1,446, *SD* = 516.3, range = 619 to 1,994 individuals). The largest hibernating colony of *M. ciliolabrum* occupied Fool's Wading Pool ($\bar{x}$ = 87, *SD* = 51.4, range = 32 to 146 individuals). Smaller hibernating colonies varied more in the number of bats counted than did larger colonies. We document one of the largest densities of caves used by hibernating bats in the western USA, as well as possibly the largest reported hibernacula for Townsend's big-eared bats and western small-footed myotis in their distributions. This information provides important context regarding hibernating bats prior to major threats (i.e., white-nose syndrome) occurring in southern Idaho.

Phylogeography of Little Brown Myotis Using RAD-Seq: Genome-Wide Patterns of Genetic Diversity and Divergence

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Myotis lucifugus is among the most widespread and well-studied bat species. Information on its demographic history and possible adaptive differences among regional populations is particularly valuable, given the precipitous decline of populations in eastern North America due to white-nose syndrome (WNS). We used restriction site-associated DNA sequencing (RAD-Seq) to generate thousands of short sequences scattered throughout the genome from individuals sampled across the species' range. Clustering analyses distinguish eastern and western populations with admixture east of the Rocky Mountains. West of the Rockies, *M. lucifugus* exhibits an isolation-by-distance pattern in which genetic similarity declines with geographic distance; east of the Rockies, genomic data suggests that the population is essentially panmictic (or has been in the recent past). This pattern at autosomal loci contrasts with that of the maternally inherited cytochrome *b* gene, which is geographically structured in both the East and West, suggesting male-biased dispersal. We fit several candidate demographic models using δaδi, a program that simulates the expected allele frequency spectrum (AF) under a given model. The observed AFS best fits a model where, early in the Pleistocene, part of the ancestral population split off and then expanded in the East. A genome scan using RAD loci identified ~12 "outlier" loci that may differ between East and West due to divergent selection. Potentially unique adaptations to the WNS-threatened region warrant further exploration because preservation of functional diversity is critical to species conservation, and adaptive differences may have important implications for repopulation of WNS-affected regions by migrants.

Citizen Scientists and Acoustic Technology Team Up

Anna Zack, Sybill Amelon, and David Riggs
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Bats are currently faced with a suite of threats, including roost disturbance, habitat loss, wind development, white-nose syndrome and other diseases, as well as, synergistic and interacting effects. Localized bat mortality from these threats is well-documented in some cases, but long-term changes in regional populations of bats remain poorly understood. Acoustic detectors are a tool available for assessing bat status on the landscape and can provide indices of change over time at large spatial scales. Various approaches are currently being used, including driving and walking transects and point monitoring. Evaluation of tradeoffs in spatial and temporal distribution, sampling investment, logistics and potential for statistical analysis associated with these methods is needed by natural resource managers. Our study includes each of these acoustic methods and teamed researchers, software developers and citizen scientists to quantify the tradeoffs associated with each and to provide a visual interpretation of the results. The Miramiguoa Chapter of Missouri Master Naturalists (MMN) conducted acoustic surveys within the Ozark Highlands ecological region of Missouri while researchers conducted acoustic surveys across a larger geographic area. Equipment and training were provided by USFS Northern Research Station and MMN volunteered their time. MYOTISOFT software was used for the visual interpretation. Our team approach resulted in 10 walking, 10 driving transects, 10 driving with point counts and 32 passive points of monitoring data each repeated a minimum of 2 times to assess detection probability. Each transect or monitoring station can be accessed visually in Google Earth or ArcMap© using MYOTISOFT Transecticizer or TransectPro. We discuss tradeoffs associated with sample design, analysis, occupancy status and how this team approach offers multiple benefits for a regional bat monitoring program.

Flying or Sleeping: Influence of WNS on Flight Activity of Bats during Deep Hibernation

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Several bat species are threatened by White-nose Syndrome (WNS) in North America and Europe. WNS affected bats typically have visible white cover on the muzzles, wings and ears, and exhibit abnormal hibernation behavior. Our objective was to test the influence of WNS on hibernation behavior and flight activity of European bats. We predicted that affected bats will exhibit abnormal flight activity (higher level and sooner onset of activity) and different general pattern of hibernation. Hibernation behavior of bats was studied in a limestone cave during two winter seasons 2006/07 (before WNS detection) and 2010/11 (after WNS). Only data from „deep hibernation" period were analyzed. We realized biweekly visual monitoring of hibernating bats and observation of bat flight activity in the cave with a night-vision scope. Bat movements through the hole in the gate were monitored continuously by a custom-made IR barrier. We did not registered abnormal changes in bat hibernation behavior, activity level or its seasonal pattern after WNS occurrence. Flight activity was generally low and temperature remained the best predictor of its level. The higher ambient temperatures increased flight activity of bats, made its onset more desynchronized and bats were leaving the cave even during January. Low prevalence of WNS registered at locality under study combined with stable hibernation behavior support the hypothesis that the fungus has been present in Europe for a long time and has only recently invaded North America. This study was supported by the grant of GACR No. 506/12/1064 and institutional support RVO:68081766.