WHITE-NOSE SYNDROME: AN OVERVIEW OF ONGOING AND FUTURE RESEARCH NEEDS

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Abstract

White-nose syndrome (WNS) is an emerging infectious disease that is causing unprecedented mortality of hibernating bats in eastern North America and is threatening regional extinction of formerly common species. The rapid rate of spread and high mortality associated with WNS makes this epizootic one of the most threatening wildlife diseases ever reported for bats. Current estimates indicate that over one million hibernating bats among six North America bat species have died from this disease since its first discovery in New York in 2006. These six species are the little brown myotis (*Myotis lucifugus*), northern long-eared myotis (*M. septentrionalis*), Indiana myotis (*M. sodalis*), eastern small-footed bat (*M. leibii*), tricolored bat (*Perimyotis subflavus*), and big brown bat (*Eptesicus fuscus*). A recent study predicted that if current assumptions about mortality rates and spread persist, *M. lucifugus*, the species that currently is the most severely affected by WNS, will experience regional extinction within 16-20 years. *Geomyces destructans* (*Gd*), the putative fungal pathogen associated with WNS, was recently isolated from three additional species, the southeastern myotis (*M. austroriparius*), gray myotis (*M. grisescens*), and cave myotis (*M. velifer*), but to our knowledge, evidence of *Gd* infection based on histopathology (the "gold-standard") has not been confirmed in *M. grisescens* from Missouri or *M. velifer* from Oklahoma. To date, no evidence of mass mortality has been reported for the latter three species. Researchers and wildlife managers are challenged by lack of sufficient knowledge on transmission dynamics and disease resistance in bats, which is limiting the ability of researchers to develop effective mitigation and management strategies. Research support is needed to investigate seasonal and geographic variation in fungal prevalence and loads, differences in species susceptibility and infectiousness to *Gd* infection, and mechanisms, routes and intensity of *Gd* transmission at different colony and geographic scales, with the purpose of identifying effective mitigation strategies to reduce mortality of affected bats and to implement protocols to protect populations at risk.

Key words: Chiroptera, *Geomyces destructans*, hibernating bats, North America, research needs, White-nose syndrome

Introduction

White-nose syndrome (WNS) is one of the most devastating diseases in recorded history to affect wildlife in North America (Figure 1). Since its discovery in upstate New York in February 2006, estimates indicate that over one million hibernating bats have died from this disease (Blehert et al., 2009; Frick et al., 2010a), with losses averaging 73%, but with decline of up to 100% in some hibernacula and maternity colonies in eastern North America. To date, six species are known to be affected by WNS, including the most severely affected little brown myotis (*Myotis lucifugus*), northern long-eared myotis (*M. septentrionalis*), Indiana myotis (*M. sodalis*), tricolored bat (*Perimyotis subflavus*), and the apparently less affected eastern small-footed bat (*M. leibii*) and big brown bat (*Eptesicus fuscus*). Three other species, including the southeastern bat (*M. austroriparius*), the Federally Endangered gray bat (*M. grisescens*), and the cave myotis (*M. velifer*) have been diagnosed using PCR tests indicating presence of *Geomyces destructans,* but to date, infection from this fungal pathogen has not been confirmed based on histopathology for *M. griscescens* from Missouri or *M. velifer* from Oklahoma (USFWS, 2011)

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Figure 1. A hibernating little brown myotis (*Myotis lucifugus*) infected with *Geomyces destructans,* a fungus associated with white-nose syndrome (Photo credit: A.C. Hicks, New York Department of Environmental Conservation).

The earliest research on bats affected by WNS identified cutaneous fungal infections caused by *Geomyces destructans* (*Gd*), a previously unknown, cold-adapted fungus that grows optimally between 5 ° and 10°C, within the 2 ° to 14°C temperature range that is characteristic of hibernacula in North America affected by WNS (Blehert et al., 2009). Based on morphological and genetic (PCR) analyses, *Gd* has been reported from hibernating bats in 17 states (Connecticut, Delaware, Indiana, Maryland, Massachusetts, New Hampshire, New Jersey, New York, North Carolina, Ohio, Pennsylvania, Vermont, Virginia, West Virginia, Tennessee, Missouri, and Oklahoma), and four Canadian provinces (New Brunswick, Nova Scotia, Ontario, and Quebec) (Meteyer et al., 2009; Chaturvedi et al., 2010; USFWS, 2011; Figure 2). However, to date, mass mortality has only been reported from seven northeastern states (Connecticut, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, and Vermont) and one Canadian province (New Brunswick).

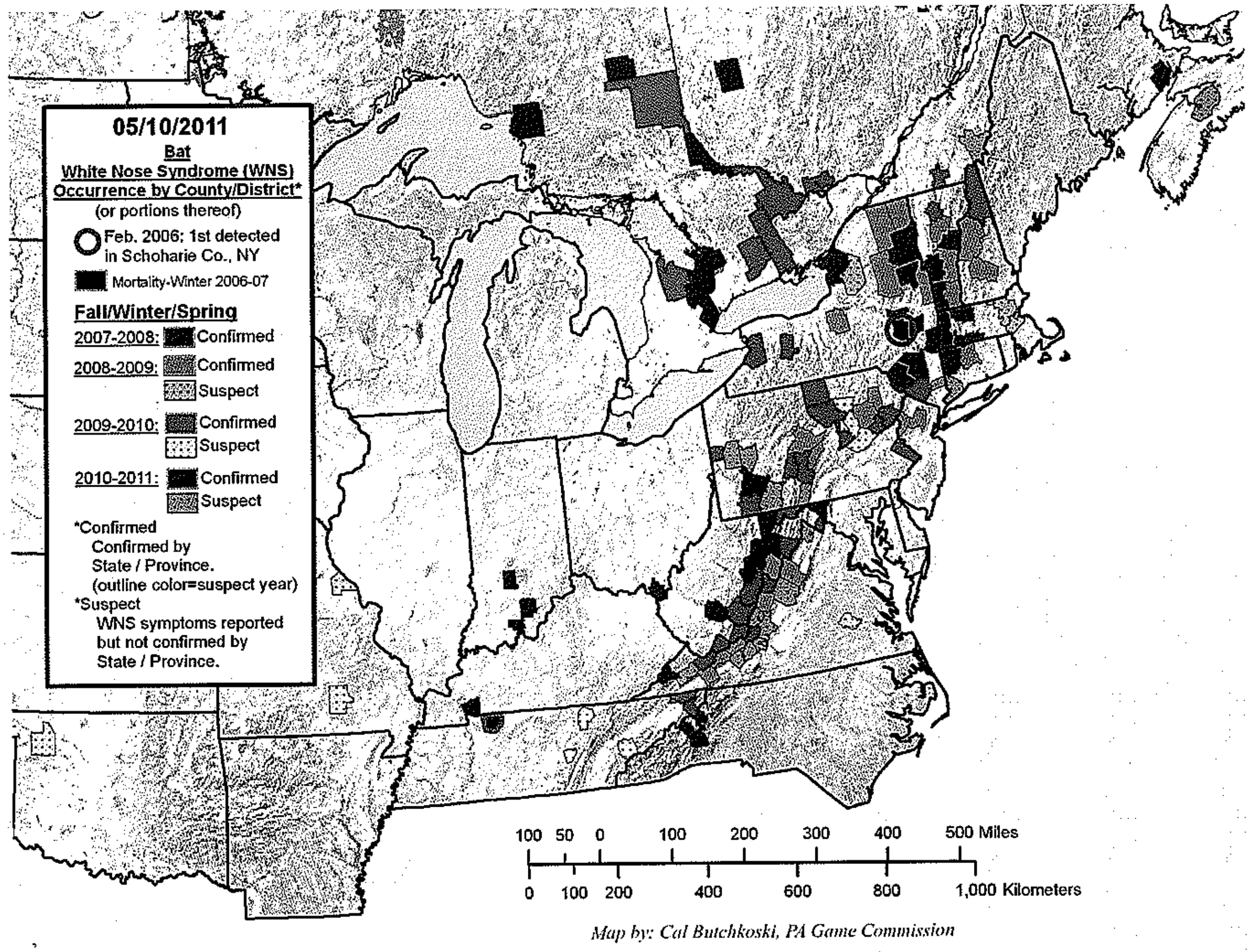

Figure 2. Map showing the distribution of the fungal pathogen *Geomyces desctuctans* (*Gd*) and locations of bats manifesting symptoms of white-nose syndrome in North America http://www.fws.gov/whitenosesyndrome/maps/WNSMap_040411_300dpi_DS.jpg (accessed May 10, 2011).

Research and monitoring studies on hibernating bats in eastern North America have revealed that bats affected by WNS are characterized by the following symptoms: white fungal growth on exposed skin tissues, such as nose, ears, tail and wing membranes (Blehert et al., 2009; Gargas et al., 2009); prematurely depleted fat reserves (Blehert et al., 2009; Gargas et al., 2009; Courtin et al., 2010; J.D. Reichard, unpubl. data); immunological changes (M.S. Moore, unpubl. data; D.M. Reeder, unpubl. data); altered arousal patterns during hibernation (D.M. Reeder et al., unpubl. data); atypical flight behavior in mid-winter (A.C. Hicks, pers. comm.); and ulcerated, necrotic, and scarred wing tissue (Reichard and Kunz, 2009; Cryan et al., 2010).

Recent evidence suggests that *Gd* is a pathogen that was introduced to the U.S from Europe by human trade or traffic. Researchers have established that *Gd* is present in nine species of European bats, although no evidence of mass mortality has been reported (Puechmaile et al., 2010; Wibbelt et al., 2010; Martínková et al., 2010). Fungal isolates of *Gd* from selected hibernacula in the U.S. (Lorch et al., 2010; Lindner et al., 2010) appear to be derived from a single clone (Chaturvedi et al., 2010), suggesting a relatively recent introduction. Research on WNS in North America suggests that there is no difference in susceptibility caused by potential environmental toxins because they were similar in bats both affected and unaffected by WNS (Courtin et al., 2010; Kannan et al., 2010), although more work is needed in this particular context. Mass die-offs resulting from WNS (Figure 3; Frick et al., 2010b) are consistent with the hypothesis of an introduced pathogen in a naïve wildlife population (Cunningham et al., 2003). Notwithstanding, the origins of *Gd* in the U.S. will not be known until comparative genomic analyses of isolates from North America and Europe are complete.

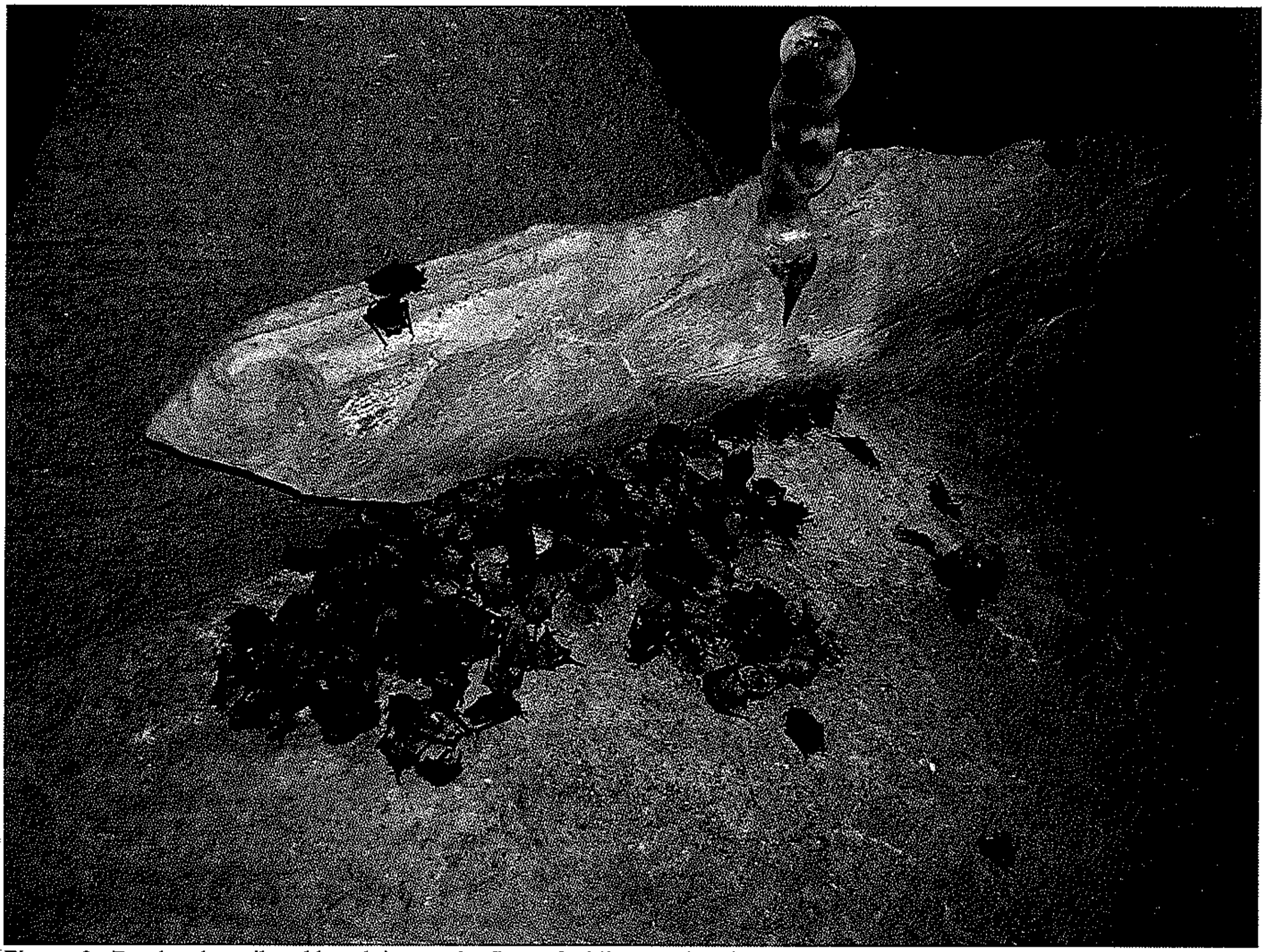

Figure. 3. Dead and moribund bats lying on the floor of a hibernaculum in Vermont caused by white-nose syndrome (Photo credit: M.S. Moore, Boston University)

Several mitigation strategies have been proposed, including installation of heated roosts as "thermal refugia" in caves to reduce energy expenditure of aroused bats (Boyles and Willis, 2009), and culling to reduce the spread of *Gd* (Arnold Air Force Base, 2009). However, attempts to deploy heated roosts have not been successful (C. Willis, pers. comm.), and a recent modeling study demonstrates that culling would be ineffective in stopping the spread of *Gd* (Hallam and McCracken, 2011). Additionally, proposals for reducing the spread of *Gd* by closing caves and mines to human traffic are being practiced by some state and federal agencies, and protocols for decontaminating clothing and field equipment are being implemented (USFWS, 2010). However, comprehensive understanding of WNS epidemiology remains elusive (Foley et al., 2011).

Ongoing and Future Research Needs

Since 2008, wildlife biologists from state and federal agencies, non-government organizations, and academic researchers have participated in several regional WNS strategy meetings and conferences in an effort to identify research and monitoring needs (Bat Conservation International, 2009). Both non-government and academic scientists have developed and presented proposed budgets for research and conservation management activities at congressional hearings, but limited funding has been made available from federal sources or state governments to address this devastating and rapidly spreading disease. In October 2010, the U.S. Fish and Wildlife Service proposed a draft National Plan (http://www.fws.gov/whitenosesyndrome/nationalplan.html) to coordinate surveillance and monitoring efforts (Coleman, 2011), but as of this writing, this plan has not been implemented.

State and federal agencies, non-government organizations, and academic institutions have established partnerships (Waldien et al., 2011) to help develop and address key questions related to understanding and managing WNS (Bat Conservation

International, 2009). Examples of these questions include: (1) What are the mechanisms of disease-caused mortality in hibernating bats? (2) What are the physiological, behavioral and immunological responses of individuals to *Gd* infection? (3) How is *Gd* transmitted among individuals and across sites? (4) How does disease-related mortality from *Gd* affect population dynamics and viability of affected populations and species? (5) What is the origin of *Gd* and how is it spread? (6) Is there variability in the susceptibility of different bat species to *Gd*? (7) Does the rate of disease progression in bats vary in relation to microclimate of hibernacula? (8) Can quantitative diagnostic tools be developed for identifying *Gd*? (9) Can selected chemical compounds be used to reduce or eliminate *Gd* on skin surfaces? (10) Should anti-fungal compounds be used as a management strategy to reduce the effects of or spread of Gd? and (11) How can knowledge of ecosystem services be used to convey the value of bats to humankind and to help raise funding levels to support research and management of WNS?

Ongoing Research

1. Physiological and immunological responses to WNS infection

Changes in body composition of bats affected by and unaffected by WNS—Early field and laboratory observations in the northeastern U.S. have shown that bats affected by WNS have severely depleted fat reserves in mid-winter, a condition that is expected to compromise successful hibernation and ultimately reduce chances of survival. Studies have been designed to test hypotheses that reduced fat reserves (white adipose tissue, WAT) are caused by failure to deposit sufficient WAT during the prehibernation period or premature depletion of WAT reserves during hibernation, due in part to frequent or extended bouts of arousal. Other hypotheses state that over-winter survival and subsequent reproductive success of hibernating bats also requires sufficient quantities and qualities of WAT deposited during the pre-hibernation period (Kunz et al., 1998; Humphries et al., 2003), and that these reserves include sufficient quantities of essential saturated and polyunsaturated fatty acids (PUFAs) that can be obtained only from dietary sources because they cannot be synthesized by hibernating mammals, including bats (C.L. Frank, pers. comm). The latter hypothesis predicts that dietary deficiencies of certain PUFA's will affect the depth and duration of deep torpor during hibernation.

To date, analysis of body composition, including PUFA's, of little brown myotis (*M. lucifugus*) during the pre-hibernation period at sites affected and unaffected by WNS suggests that bats deposit adequate reserves of WAT in autumn (J.D. Reichard, unpubl. data). However, by mid-winter, WNS-affected bats have markedly less WAT compared to unaffected bats. As WAT reserves approach critical thresholds, bats affected by WNS appear to adopt behaviors causing them to emerge from hibernation prematurely in attempts to feed or gain access to water (J.D. Reichard, unpubl. data). At some WNS-affected sites, bats have also been observed roosting near mine or cave openings long before spring emergence (A.C. Hicks, pers. comm.). Such activities may reflect attempts by bats to sample outside conditions for early detection of spring warming or insect availability. If bats adopt these behaviors when WAT reserves are low, they may be responding to some minimum threshold of fat needed to initiate other physiological processes (e.g. immune responses or female ovulation).

Data collected to date have provided valuable insight for testing proposed hypotheses to help explain why hibernating bats are dying prematurely at hibernacula in the northeastern U.S. and also suggest directions for future study to better understand the etiology of WNS. Low reserves of WAT at the end of hibernation may reduce reproductive success of females, especially when leptin levels are low (Kunz et al., 1998). Current evidence suggests that little brown myotis affected by WNS have poorer body condition in spring and summer than unaffected individuals during the same period (Reichard and Kunz, 2009). Some stored fat reserves at the end of winter are needed to fuel spring migration and early foraging bouts and to sustain early gestation while energy sources transition from winter to the active season when insects and other arthropod prey become available.

Immune function of hibernating bats affected and unaffected by WNS—Understanding the immunological status of bats affected with WNS is essential to assess their ability to resist pathogenic or opportunistic infections. Effective immunological defenses against tissue-invading fungi generally include the activity of soluble complement proteins, direct killing through phagocytosis (e.g. by neutrophils, macrophages, dendritic cells), cellular inflammatory responses, T lymphocyte mediated responses, and antibody dependent cell-mediated cytotoxicity (Blanco, 2008; Shoman, 2005; Speth, 2004, 2008) with optimal resistance to fungi occurring at typical euthermic body temperatures (Bergman and Casadevall, 2010). However, because bats use long periods of deep torpor during hibernation, their ability to resist infection may be significantly decreased relative to the active season when bats are mostly euthermic. During the hibernation period, optimal temperature conditions are available for growth of *Gd* (Blehert et al., 2009). Numerous aspects of immune response are known to become depressed in other hibernating mammals (Jaroslow, 1972; Kurtz, 2007; Larsen, 1971; Manasek, 1965; Maniero, 2000; Maniero, 2002; Bouma et al., 2010). Additionally, several experimental studies have shown that immunological stimulation alters patterns of torpor and arousal (Burton and Reichman, 1999; Prendergast, 2002). Investigations are currently focused on multiple aspects of innate, adaptive, and cellular inflammatory immune responses in *M. lucifugus* affected by WNS, as well as research designed to investigate relationships between these responses, body temperature, stage of arousal, and body composition (M.S. Moore, unpubl. data; R. Jacob and D.M. Reeder, unpubl. data).

Relationship between body composition and immune competence of bats during hibernation—While several aspects of the immune response have been described, it is important to understand the relationship between levels of immune competence and the amount of energy available to hibernating animals in the form of fat reserves. In addition to fueling a variety of physiological processes and behaviors (Humphries et al., 2003), WAT is essential for proper immune function. Immune function exhausts energy reserves in two important ways. First, to restore and mount an immune response, animals must arouse from torpor to a euthermic state (Burton and Reichman, 1999; Humphries et al., 2003; Prendergast et al., 2002; M.S. Moore and J.D. Reichard, pers. obs.). At this time, bats may relocate to warmer parts of their hibernacula where the cost of maintaining elevated body temperature is reduced (Boyles and Willis, 2009), although periodic arousals also account for most of the depletion of WAT during hibernation (Thomas et al., 1990). Second, an immune response requires considerable amounts of energy following arousal. Limited reserves of WAT may adversely affect immune competence directly (Demas et al., 2003) and indirectly through leptin-mediated pathways, as has been shown in hibernating rodents (Demas and Sakaria, 2005). A similar pattern is expected in bats (M.S. Moore, unpubl. data). Metabolic rates may increase by up to 60% in animals that mount immune response to severe infections (Lochmiller and Deerenberg, 2000). Moreover, while some hibernators are able to upregulate immune mechanisms during their prolonged periods of euthermy (arousal bouts lasting ~24 h), arousal bouts of bats typically last only 70-90 minutes (Britzke et al., 2010; D.M. Reeder, C.L. Frank, et al., unpubl. data), which quite likely confers few immunological benefits. Lastly, given the fact that hibernating bats affected by WNS experience severely depleted WAT reserves by mid winter, they may also have reduced immune function owing to this deficit.

Quantifying arousal frequencies during hibernation—Periodic arousals from torpor during mammalian hibernation typically account for 80-90% of the energy expended throughout this period (Kayser, 1965). Thus, the premature depletion of WAT observed in WNS-affected little brown myotis may be due to more frequent arousals (thus, shorter torpor bouts). Increased arousals from torpor are postulated to occur in response to infection with *Gd*. Ongoing investigations are examining patterns of hibernation in hundreds of affected and unaffected little brown myotis in several hibernacula across the northeastern and midwestern U.S. (D.M. Reeder, C.L. Frank, E.R. Britzke, A. Kurta, G.G. Turner, A.C. Hicks, S.R. Darling, C.W. Stihler, in progress). How the behavior of WNS-affected little brown myotis differs from that of unaffected bats during these arousals is also the subject of an ongoing study (S.A. Brownlee, unpubl. data). Limited studies of hibernation patterns in the WNS-affected tricolored bat (*P. subflavus*), the moderately affected big brown bat (*E. fuscus*), and the as of yet unaffected Virginia big-eared bat (*Corhynorhinus townsendii virginianus*) are also underway.

2. Testing the Efficacy of Selected Chemical Compounds to Reduce or Eliminate Gd

The severe impact of white-nose syndrome on bat populations requires unusual intervention to explore possible treatment strategies for both captive and wild populations. Testing both pharmaceutical and non-pharmaceutical compounds for their capacity to safely combat *Gd* infection in bats is useful in that it may lead to the development of mitigation strategies for both free-ranging bats and captive bats. While a number of antifungal agents successfully kill *Gd*, many of them are undesirable for their other actions, including endocrine disruption. Thus, only a subset of pharmaceutical compounds is likely to be viable for treating bats. A number of non-pharmaceutical compounds also hold promise for treating free-ranging bats (H.A. Barton, pers. comm.) without having significant ecological consequences. Testing of agents on bats under captive conditions is ongoing in several laboratories (e.g.,. H.A. Barton, University of Northern Kentucky; D.M. Reeder, Bucknell University; and A.H. Robbins, Cummings School of Veterinary Medicine, Tufts University).

To date, several compounds have been identified using *in vitro* testing that effectively kill *Gd* grown in culture (H.A. Barton, pers. comm., M.A. Ghannoum, unpubl. data). The antifungal drug terbinafine has good fungicidal activity against *Gd* in culture, and has a long safety record in humans and domestic animals. A study of the safety and efficacy of terbinafine in WNS infected bats held in captivity is currently underway. Studies using other compounds to treat bats in natural hibernacula are also underway (D.M. Reeder, unpubl. data). However, it is important to emphasize that any compound or compounds used to treat bats must be effective, environmentally safe to use, and easily applied with minimal handling or disturbance.

3. Quantitative Diagnostic Tools for Identifying Gd and Assessing Transmission Dynamics

A quantitative PCR (qPCR) assay that reliably detects low-level amounts of *Gd* on bats is a fundamental tool needed to assess disease epidemiology of WNS. A recently developed qPCR assay will be used to identify infected bats, quantify *Gd* fungal load, and assess transmission. The challenge for any DNA-based assay for *Gd* is the abundance of closely related species of *Geomyces* in cave environments (Lindner et al., 2010). A similar challenge exists for histopathological studies in identifying *Gd* from closely related species. Thus, a diagnostic tool, based on qPCR, must be both highly sensitive and specific to provide reliable identification of *Gd.* Initial screening against >100 *Gd* isolates, *Geomyces* isolates, and PCR clones from cave soil extracts indicates that use of these two qPCR assays combined provides the most promising diagnostic tool for detecting *Gd* (J.T. Foster, unpubl. data).

Detection of *Gd* presence and quantification of fungal abundance will have broad applicability to the WNS research and management community for addressing questions such as testing the efficacy of disinfection techniques on field equipment, testing and quantifying infectious loads from skin swabs or fecal samples, and testing for efficacy of antifungal treatments on bats. In particular, these tools will make it possible to quantify the number of infectious particles on individual bats and to enable comparisons among infection levels of individuals of different species, at different times of the year, and to quantify transmission dynamics. This information could also be used to help identify infection stages in which interventions could reduce transmission or increase survival rates.

Future Research Needs

While the above studies are ongoing, additional research is needed to: (1) assess transmission dynamics of *Gd* and epidemiology of WNS; (2) determine optimal environmental conditions for growth and transmission of *Gd*; (3) determine variation in host susceptibility to *Gd*; (4) determine pathogen origin and factors driving spread of *Gd*; (5) assess population genetic structure and gene flow in bats at local and continental scales; (6) assess the population dynamics of maternity colonies affected and unaffected by WNS; (7) assess impact of wing damage from *Gd* on foraging ability and reproductive success; (8) evaluate and implement appropriate mitigation strategies; and (9) quantify the economic importance and ecosystem services of bats affected by WNS.

The results of the proposed research, highlighted below, are critical to understanding the causes and consequences of WNS.

1. Assess Transmission Dynamics of Gd and Epidemiology of WNS

Determining whether transmission is frequency or density-dependent and how contact rates vary among species that vary in social behavior are critical to understanding the impact of WNS on bat populations. Transmission of *Gd* may increase with bat density, if per capita contact rates increase with colony size. Transmission may also vary among species as a function of contact rates during arousal bouts, when bats are euthermic, active, and switching to other roost sites. Alternatively, transmission of *Gd* within hibernacula may be frequency-dependent, if social clustering of bats during hibernation eliminates the effect of overall density in a hibernaculum. If transmission is frequency-dependent, the main drivers of differences in transmission among sites may be due to variation in microclimate because of its effect on fungal growth (Blehert et al., 2009; Chaturvedi et al., 2010). The crucial reason it is important to determine whether transmission is frequency or density-dependent (or more generally, how it depends on density) is that a purely density-dependent disease will die out once bats reach low numbers, but if it operates as frequency-dependent because of clustering of remaining bats, it could cause extinction. It could also be density-dependent at high bat densities through several mechanisms, but frequency-dependent effects at lower densities would also make extinction possible. In addition, even if *Gd* is density-dependent and therefore less likely (but not impossible) to cause extinction and if the density transmission relationship could be quantified, one could predict at what density the populations would level out, which would be extremely valuable from a management perspective.

Seasonal variation in social behaviors and environmental conditions can both influence transmission rates (Bjornstad et al., 2002; Hosseini et al., 2004; Shaman and Kohn, 2009). Bat aggregations vary substantially from large mixed-species colonies in winter to smaller more species-specific and sexually-segregated groups in summer. In contrast, contact rates may be highest during fall when bats are mating and interacting in potentially infected environments (swarming sites and hibernacula). Microclimate conditions for *Gd* growth (but not necessarily transmission), such as moderately low cave and mine temperatures and high humidity occur most commonly in winter (Blehert et al., 2009; Chaturvedi et al., 2010).

2. Determine Optimal Environmental Conditions for Growth and Transmission of Gd

Seasonal variation in environmental conditions (e.g., temperature and humidity) can potentially influence growth, transmission rates, and prevalence of *Gd* in hibernacula (K.E. Langwig, pers. comm.). The highest rates of prevalence can be expected in late winter, after *Gd* has had the opportunity to grow and spread in hibernacula. Microclimate conditions for *Gd* growth are also expected to affect survival and transmission rates of *Gd*.

3. Determine Variation in Host Susceptibility to Gd

Multi-host pathogens demand increased theoretical and empirical understanding for planning conservation efforts for species at risk from emerging infectious diseases (Daszak et al., 2000). *Geomyces destructans* is a multi-host pathogen that infects bats with widely varying distributions and social systems. The rapid spread of *Gd* from its epicenter in upstate New York, southward to Tennesee and North Carolina, and westward to Oklahoma and beyond, provides an opportunity to empirically assess factors that influence the mode and rate of spread at both local and continental scales. Variation in pathogen-host interactions may be especially important for understanding transmission and levels of infectivity. Additionally, whether WNS will affect other hibernators, including hibernating ground squirrels (family Sciuridae) and bats that hibernate in trees rather than caves, remains to be determined (C.K.R. Willis, pers. comm.). Multi-host pathogens pose greater risks to endangered species because one species can serve as a reservoir to support persistent transmission while a more vulnerable species may go extinct. Measurements of contact rates, prevalence, and infection intensity among individuals of different species that vary in sociality (e.g., group size and composition) and environmental conditions are needed across different life stages at local and regional scales to better understand the transmission dynamics of *Gd*. Known species-specific preferences for particular microclimates in hibernacula (temperature, humidity, and airflow) will strongly influence which species are most likely to experience significant mortality. For example, the big brown bat (*E. fuscus*) prefers to hibernate at low relative humidity and at temperatures that are below the optimal growth rate of *Gd*, which may explain their apparent relative resistance to this fungus (L.E. Grieneisen, pers. comm.).

4. Determine Pathogen Origin and Factors Driving Spread of Gd

Recent advances in the speed and accuracy of whole genome sequencing of microbes using Next-Generation Sequencing provides a viable alternative to traditional cloning and sequencing methods (Mardis, 2008). This is particularly relevant to *Gd* where few genetic differences are expected between isolates due to the recent emergence of the fungus and most genetic methods may not be able to distinguish *Gd* isolates. With a reference genome for comparison, phylogenetic relationships between isolates from bats in the U.S., Canada, and Europe, as well as from closely related *Geomyces* species, can now be made using whole genomes. Studies are underway to sequence closely related congeners to identify unique characteristics of *Gd* and determine whether differences between North American and European isolates contribute to pathogenesis in bats of the U.S. With adequate variation in microsatellites, *Gd* can also be used to analyze spatial spread of *Gd*. Thus, understanding the genetics of *Gd* is essential for assessing disease epidemiology.

The rate of spread of *Gd* across North America may be affected by colony size and species richness of bats and regional prevalence of *Gd*. Alternatively, the spread of *Gd* across North America may be influenced primarily by abiotic factors (e.g., temperature and humidity) and traits of different bat species unrelated to social behavior. The rate of geographic spread may also depend on the distribution and density of hibernacula (T.G. Hallam, unpubl. data). The probability of invasion of a pathogen should be a function of propagule pressure, which, in a disease context, is the force of infection (i.e. the density of infected individuals moving into uninfected populations). The product of colony size and prevalence summed across species richness of bats could be used as a correlate of propagule pressure. However, the diffusion of a multi-host pathogen may also be influenced by traits of different host species (e.g. differential movements or rates of infectiousness), the permeability of the landscape, and climatic effects.

5. Assess Population Genetic Structure and Gene Flow in Bats at Local and Continental Scales

The identification of gene flow corridors and barriers to major bat hosts of *Gd* could be used to identify populations most at risk and to inform decisions on WNS surveillance, prevention, and mitigation. Geographic or other landscape features that pose barriers to, or facilitate dispersal of bats, could create complex patterns of gene flow. Previous studies of host-parasite relationships have shown that host population structure is reflected in gene flow, along with dispersal of a dependent parasite or pathogen (McCoy et al., 2005; Nadler et al., 1990; Mulvey et al., 1991; Blanchong et al., 2008).

Little brown myotis is relatively abundant and currently shows the highest prevalence of infection, and thus is likely to be the primary mode of dispersal for the fungus. Thus, the potential spread of *Gd* via dispersal might be predicted by using historical patterns of gene flow in *M. lucifugus* across North America, and knowledge of the population connectivity of this species is critical to predicting routes of spread and populations most at risk of WNS introduction (A.P. Wilder, pers. comm). Previous studies of little brown myotis sampled during summer months have found little genetic differentiation in populations, indicating that the species is wide-ranging and that dispersal is common. From this we can expect that an isolation-by-distance pattern, and spread of WNS from infected populations to uninfected populations will be highly correlated with spatial distance between colonies (A.P. Wilder, pers. comm.).

Samples of bats when they are breeding (fall swarming sites) or hibernating (fall and winter), may reveal more population structure than when populations have dispersed to maternity roosts (spring and summer). If populations of little brown myotis are structured, but the geographic pattern of WNS expansion is not predicted by patterns of gene flow, then other bat species may be playing the dominant role in the spread of *Gd* (A.P. Wilder, pers. comm).

6. Assess the Population Dynamics of Maternity Colonies Affected and Unaffected By WNS

While most prior research on WNS has focused on factors affecting mortality in hibernating bats, the impacts of this disease on bats during the active season have not been fully evaluated. Observed declines of bat populations in winter should be manifested by comparable declines during the active period in the same region. The little brown myotis has experienced severe winter mortality in the northeastern U.S. (Frick et al., 2010b) but could also be a valuable species for determining population-level impacts of WNS during the non-hibernating period. Relative to most other bat species affected by WNS, maternity colonies of little brown myotis can be readily monitored because this species roosts in relatively large numbers in a variety of anthropogenic structures (Kunz and Anthony, 1996; Kunz and Reynolds, 2003; O'Shea and Bogan, 2003). Long-term monitoring studies are crucial for obtaining demographic data needed for assessing both population dynamics (Frick et al., 2010a) and viability (Frick et al., 2010b). Similarly, acoustically monitoring the activity of bats during the warm season can also provide valuable information on a broader landscape scale (Brooks 2011). The studies by Dzal et al. (2010) and Brook (2011) confirms that the overall observed decline of 73% based on surveys of bats in hibernacula (Frick et al., 2010) is consistent with their data based on acoustic monitoring of bats in northwestern New York and west-central Massachusetts, respectively. Data derived from such studies should facilitate the development of strategies that will aid in informing future management decisions.

7. Assess Impact of Wing Damage from Gd on Foraging Ability and Reproductive Success

Little brown myotis at maternity colonies in spring and throughout early summer have shown moderate to severe wing damage associated with WNS (Reichard and Kunz, 2009). Such damage could adversely affect the abilities of these bats to forage efficiently and thus maintain normal body condition (Reichard and Kunz, 2009; S.A. Brownlee, unpubl. data; N.W. Fuller, unpubl. data). Moreover, reduced feeding efficiency could lead to lower survival and lower reproductive success (Reichard and Kunz, 2009). Thus, field-based studies are needed to assess the consequences of WNS-related wing damage, including the influence of wing damage on navigational ability, foraging success, and postnatal growth (a surrogate of reproductive success). If foraging success is compromised, one would predict that postnatal growth rates and survivorship of pups born to mothers with damaged wings will be greatly reduced (N.W. Fuller, pers. comm.).

8. Evaluate and Implement Ecologically-Sound Mitigation Strategies

Additional experimental research is needed to test the efficacy and safety of antifungal compounds to increase survival of bats infected with *Gd*. Protocols should be identified to rid individual bats of *Gd*, especially those targeted for captive studies and to create assurance colonies (see below). If an effective compound or compounds and treatment protocols are identified, research will be needed on delivery methods to treat large numbers of free-ranging bats in field settings, with minimal handling or disturbance to bats and their cave ecosystems. Drug safety and efficacy testing requires large numbers of animals. Research is also needed to develop an animal model of WNS to increase the pace of drug development studies and to reduce the lethal experimental use of dwindling bat populations (H.A. Barton, pers. comm.; D.M. Reeder, unpubl. data; A.H. Robbins, unpubl. data).

Research and management strategies that provide secure, protected maternity roosts are needed to promote long-term use by maternity colonies at risk of extirpation. This could be accomplished by installing thermally and structurally-enhanced bat houses and roost modules to promote reproductive success of surviving individuals in small, remnant colonies (T.H. Kunz, unpubl. data). For example, installation of roost modules in buildings that were previously occupied by little brown myotis could also be used for long-term population monitoring programs. Data derived from installing roost modules could be used to inform future management strategies needed to sustain populations and to promote recovery of bats currently being affected by WNS.

Protection of hibernating bat colonies will continue to be of paramount importance. Caves and mines not yet gated should be considered for gating to protect the small numbers of bats that may be resistant to WNS. Disturbance to hibernating bats must be kept to a minimum. If current studies of survival in relation to microclimate at hibernacula (L.E. Grieneisen, in progress) indicate significant survival of bats at sites outside the optimal growth range for *Gd* (i.e., caves and mines below 4°C), one possible mitigation strategy might be to alter the microclimate of mines and other human-made structures (e.g., abandoned military bunkers and artificial caves) to help promote the survival of hibernating bats. Such temperature modifications are currently being used by the Pennsylvania Game Commission (D.M. Reeder, unpubl. data).

Another mitigation strategy currently under discussion is the creation of captive colonies of affected species, or so-called 'captive assurance populations'. This strategy has been employed in the amphibian conservation community in response to chytridiomycosis in frogs, another fungal infection with significant mortality in multiple species and extinction in an estimated 165 species (www.amphibianark.org). Dozens of species of amphibians have been brought into biosecure "amphibian arks" with the eventual goal of reintroduction into the wild. However, while protocols for amphibian husbandry are fairly well established, housing bats, and most especially establishing successful breeding colonies of bats in captivity, is not likely to be practical for most insectivorous species. Captive breeding has been proposed as a last ditch effort to protect against extinctions; however, maintaining breeding populations of insectivorous bats is difficult and labor intensive and sustaining sufficient numbers for gene pool integrity is a daunting prospect. The best prospect for pursuing such efforts is to engage the talented and dedicated services of the community of animal rehabilitators who specialize in maintaining bats in captivity (Barnard, 2010). Whether captive bats could ever successfully be reintroduced to the wild is highly questionable. Notwithstanding, in the final analysis, the scale and multi-species nature of WNS may ultimately call for such novel efforts.

9. Quantify the Economic Importance and Ecosystem Services of Bats Affected by WNS

The severe decline in numbers of bats in areas affected by WNS is likely to have significant impacts on agriculture, forest ecosystems, human health, and the economy in the forms of reduced crop yield, decreased forest production, increased pesticide use in agriculture, increased exposure of humans to these pesticides, and increased numbers of arthropod-borne pathogens. Little brown myotis can eat upwards of 100% of its body mass during peak lactation (Kurta et al., 1989) and at least one-half of its body mass, on average, during the warm season from mid-April to mid-October. Because over one million bats have already died from WNS in the northeastern U.S., this translates to approximately 660-1320 metric tons of insects have gone uneaten each year since mass die-offs from WNS have been reported (Boyles and Willis, 2009). Increased attention should be given to quantifying nightly food (Kurta et al., 1989; Kunz et al., 1995) and assessing dietary habits using molecular markers to identify potential insect crop pests, forest pests, and arthropods vectors of human diseases consumed by this and other species (Claire et al., 2010; G.F. McCracken, unpubl. data.). Partnerships between bat biologists, agricultural and forest land managers, disease ecologists, and economists should be established to explore relationships between population declines of bats and crop damage and yield in both traditional agriculture settings and where organic gardening is being practiced, and the possible transmission of insect borne diseases. This type of information, along with estimates of crop damage and pesticide costs, can then be used to more effectively assess the economic value of bat populations (Cleveland et al., 2006; Federico et al., 2008; Boyles et al. 2011; Kunz et al., 2011).

Conclusions

While one cannot foresee the future, it seems certain that researchers and wildlife managers are still in the early stages of assessing the WNS disease epidemic in bats. The spread of *Gd* is expanding geographically at an accelerating rate. As it does so, it continues to involve different bat assemblages and in landscapes that differ in climatic, topographic, and physiographic features. The current state of knowledge of the *Gd* pathogen, while still meager, has expanded enormously in a very short time, and the emergence and threat WNS, the disease associated with *Gd*, has motivated an enormous body of new and challenging research into poorly known and previously unknown aspects of bat biology. Our hope for mitigating the further spread of this devastating disease depends on increased levels of funding and additional research that stretches the limits of existing knowledge and technologies. Promoting the economic value of insectivorous bats to agriculture and to humankind for their cultural and aesthetic value are important steps toward educating the general public and government decision-makers that a relatively small investment now is preferred to much larger investments that will be needed later when ecosystems collapse and an increasing number of endangered bat species become listed.

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