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Review

Investigating and Managing the Rapid Emergence of White-Nose Syndrome, a Novel, Fatal, Infectious Disease of Hibernating Bats

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Abstract: *White-nose syndrome (WNS) is a fatal disease of bats that hibernate. The etiologic agent of WNS is the fungus* Geomyces destructans*, which infects the skin and wing membranes. Over 1 million bats in six species in eastern North America have died from WNS since 2006, and as a result several species of bats may become endangered or extinct. Information is lacking on the pathogenesis of* G. destructans *and WNS, WNS transmission and maintenance, individual and site factors that contribute to the probability of an outbreak of WNS, and spatial dynamics of WNS spread in North America. We considered how descriptive and analytical epidemiology could be used to fill these information gaps, including a four-step (modified) outbreak investigation, application of a set of criteria (Hill's) for assessing causation, compartment models of disease dynamics, and spatial modeling. We cataloged and critiqued adaptive-management options that have been either previously proposed for WNS or were helpful in addressing other emerging diseases of wild animals. These include an ongoing program of prospective surveillance of bats and hibernacula for WNS, treatment of individual bats, increasing population resistance to WNS (through vaccines, immunomodulators, or other methods), improving probability of survival from starvation and dehydration associated with WNS, modifying hibernacula environments to eliminate* G. destructans*, culling individuals or populations, controlling anthropogenic spread of WNS, conserving genetic diversity of bats, and educating the public about bats and bat conservation issues associated with WNS.*

Keywords: emerging infectious disease, extinction, fungal disease

Investigando y Manejando la Rápida Emergencia del Síndrome de Nariz Blanca, una Enfermedad Infecciosa, Nueva, Fatal, en Murciélagos Invernantes

Resumen: *El síndrome de nariz blanca (SNB) es una enfermedad fatal en murciélagos que invernan. El agente etiológico del SNB es el hongo* Geomyces destructans*, que infecta la piel y las membranas alares. Desde 2006 más de 1 millón de murciélagos de 6 especies han muerto de SNB, y como consecuencia varias especies de murciélagos pueden estar en peligro o extintas. Se carece de información de la patogénesis de* G. destructans *y SNB, la transmisión y mantenimiento de SNB, los factores individuales y de sitio que contribuyen a la probabilidad de una epidemia de SNB y de la dinámica espacial de la dispersión de SNB en Norte América.*

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Consideramos cómo la epidemiología descriptiva y analítica podrían contribuir a llenar esos vacíos de información, incluyendo una investigación de la epidemia, aplicación de un conjunto de criterios (de Hill) para evaluar las causas, modelos de compartimiento de la dinámica de la enfermedad y modelado espacial. Clasificamos y criticamos las opciones de manejo adaptativo que se han propuesto anteriormente para SNB o que fueron útiles para atender otras enfermedades emergentes en animales silvestres. Estas incluyen un programa de vigilancia prospectiva de murciélagos y sus sitios de hibernación para detectar SNB, tratamiento de murciélagos individuales, incremento de la resistencia a SNB (mediante vacunas, inmunomoduladores u otros métodos), incremento de la probabilidad de supervivencia a la inanición o la deshidratación asociadas con SNB, modificación de los ambientes de hibernación para eliminar G. destructans*, sacrificio de individuos o poblaciones, control de la dispersión antropogénica de SNB, conservación de la diversidad genética de murciélagos y campañas para educar al público sobre murciélagos y temas de conservación asociados con SNB.*

Palabras Clave: enfermedad fúngica, enfermedad infecciosa emergente, extinción

Introduction

White-nose syndrome (WNS) is a fatal disease of insectivorous bats that hibernate (hereafter hibernating bats), and it is presumed to be caused by a newly discovered psychrophilic (cold adapted) fungus, *Geomyces destructans* (Blehert et al. 2009). The genus *Geomyces* contains other psychrophilic saprophytic fungi that can colonize skin (Marshall 1998; Gianni et al. 2003), but *G. destructans* is the only species that invades and destroys the skin of hibernating bats (Cryan et al. 2010). WNS is the first epizootic documented in bats, and the disease has caused unprecedented reductions in the abundance of hibernating species in eastern North America, with up to 95% mortality in some hibernacula (Frick et al. 2010*a*). As a result, over 1 million bats are estimated to have died due to WNS (Frick et al. 2010*a*), and species may become endangered or extinct if the disease maintains its virulence and continues to spread across North America.

WNS was first documented in photographs taken in winter 2005–2006 in Howes Cave, and subsequently dead and dying bats were found with WNS in four nearby caves 30 km west of Albany, New York, in winter 2006–2007. By July 2010, DNA of *G. destructans* or WNS characteristic lesions were detected in hibernating bats in New York, Vermont, Massachusetts, New Jersey, Connecticut, Pennsylvania, New Hampshire, Delaware, Virginia, West Virginia, Tennessee, Missouri, and Oklahoma, and Ontario and Quebec (Fig. 1). Species in which WNS lesions or *G. destructans* DNA have been detected are: the endangered gray and Indiana bats (*Myotis grisescens* and *M. sodalis*), little brown bat (*Myotis lucifugus*), northern long-eared bat (*M. septentrionalis*), eastern small-footed bat (*M. leibii*), southeastern bat (*M. austroriparius*), cave bat (*M. velifer*), tricolored bat (*Perimyotis subflavus*), and big brown bat (*Eptesicus fuscus*). In Europe infection with *G. destructans* has been confirmed in at least five species: greater mouse-eared bat (*M. myotis*), Daubenton's bat (*M. daubentonii*), pond bat (*M. dasycneme*), Brandt's bat (*M. brandtii*), and Monticelli's myotis (*M. oxygnathus*) (Martínková et al. 2010; Puechmaille et al. 2010; Wibbelt et al. 2010). Nevertheless, monitoring has not documented major mortality events associated with *G. destructans* on bats in Europe.

G. destructans Biology and WNS Pathogenesis

G. destructans is detected consistently in skin of bats with characteristic lesions of WNS (Blehert et al. 2009; Meteyer et al. 2009; Lorch et al. 2010). This fungus grows at temperatures 3–15 °C and >90% relative humidity, conditions similar to bat hibernacula and bodies of hibernating bats (Cryan et al. 2010). Transmission occurs through direct bat-to-bat contact (D. Blehert et al., personal communication), but other routes (e.g., exposure to environments in which the fungus is present, human or animal vectors) are also possible (Lindner et al. 2010). Illness occurs mostly in winter, and WNS lesions and aberrant behaviors are most detectable after January. In autumn hibernating bats build up fat reserves and then at the onset of winter hibernate in sites that are cold and damp where food is scarce (Davis 1970; Ransome 1990). The metabolic rate of a hibernating bat is low and its body temperature is within a few degrees of the ambient temperature for extended periods (Geiser 2004; Speakman & Thomas 2003). Every few weeks bats must arouse from hibernation to restore homeostatic balance (e.g., drink, urinate, relocate, and probably induce immune functioning) (Thomas & Geiser 1997; Speakman & Thomas 2003). Over the winter this periodic arousal consumes most of the stored body fat (Thomas et al. 1990). Bats with WNS may arouse from hibernation more frequently or for longer periods than average and thereby prematurely expend fat reserves (Boyles & Willis 2010). Direct mortality from infection of the wings with *G. destructans* may also occur (Cryan et al. 2010). Aberrant behaviors associated with WNS observed in large numbers of bats include movement to roosting areas near cave entrances or other exposed sites and flying during the day from hibernacula in mid winter; fatalities often occur inside the hibernacula and/or near the entrance. In spring, a few affected

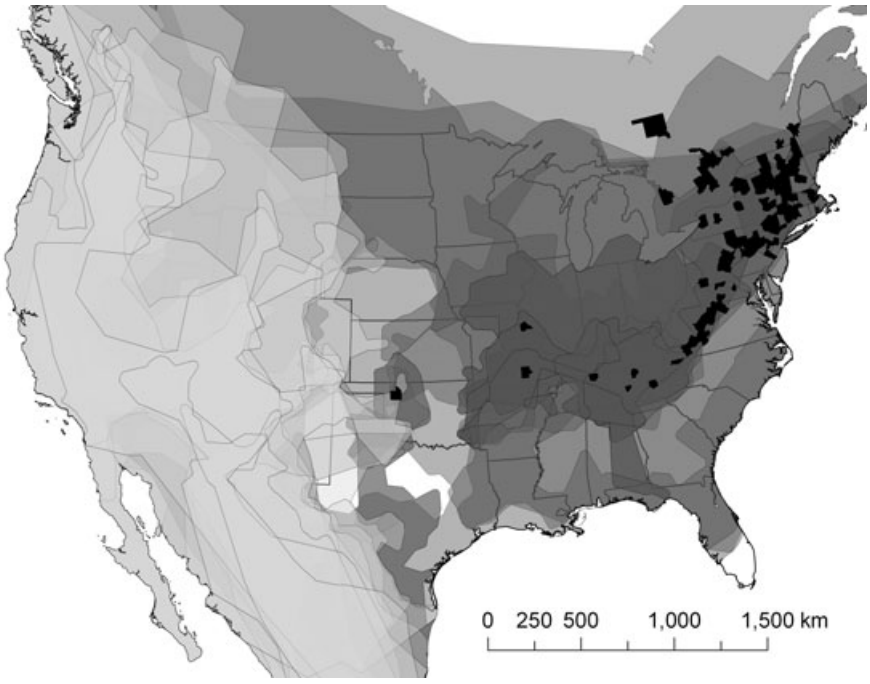

Figure 1. Areas in North America where white-nose syndrome or Geomyces destructans *has been detected in bats (black) superimposed on the overlapping distributions of bat species known to be infected with* G. destructans *(darker grays;* n = *9 species) and of hibernating species of bats that are not yet known to be affected by the* G. destructans *(lighter grays;* n = *13 species). Fungus distribution is based on maps created by C. Butchkowski, Pennsylvania Game Commission (http://www.fws.gov/whitenosesyndrome/). Bat distributions are based on data from U.S. Geological Survey and Bat Conservation International and available through a national atlas (http://www.nationalatlas.gov/mld/bat000m.html).*

animals may recover but with wing damage (Reichard & Kunz 2009).

More than half of the 45 species of bats that occur in the continental United States hibernate in caves, mines, and/or deep rock crevices, including four species and subspecies listed as endangered under the U.S. Endangered Species Act (Indiana, gray, Virginia big-eared [*Corynorhinus townsendii virginianus*], and Ozark big-eared bats [*C. t. ingens*]). In North America all species of bats that hibernate could be susceptible to WNS, and it is unknown whether WNS will be a major source of mortality in bats that rarely occur in caves, such as migratory tree-dwelling species (e.g., silver-haired bats [*Lasionycteris noctivagans*], hoary bats [*Lasiurus cinereus*], and eastern and western red bats [*Lasiurus borealis* and *L. blossevillii*]).

Certain characteristics of hibernating bats may affect the dynamics of WNS. Sociality and group formation in vespertilionid bats differ among seasons and between sexes. In general, both sexes occur in winter hibernation sites, but in spring females move to maternity colonies, where synchronized births of young occur. Males tend to spend spring and summer away from females and roost alone or in smaller groups at cooler sites (Weller et al. 2009). The sexes reunite during autumn swarming, when mating begins and multiple species of bats often congregate and interact at cave entrances before hibernation (Barbour & Davis 1969). Bats generally have lower survival in their first year, after which adult survival is high relative to similarly sized mammals (Frick et al. 2010*b*). High annual adult survival and low fecundity result in modest population growth rates and abundances that do not fluctuate widely over time (O'Shea et al. 2010). Although most adult females breed, they typically have only one offspring per year (Tuttle & Stevenson 1982). In addition to survival effects, reproduction may be adversely affected by WNS (Frick et al. 2010*b*). Volant mammals have a high capacity to spread and transmit infectious disease. Many of the species affected by WNS migrate tens to hundreds of kilometers between winter and summer habitats and can travel tens of kilometers per night (Barbour & Davis 1969; Griffin 1970). The seasonal sex differences in behaviors of hibernating bats, life-history characteristics that favor longevity and low fecundity, and the extreme vagility of bats may strongly influence WNS disease dynamics.

Knowledge Gaps

Although knowledge of WNS disease ecology is accumulating, it is unknown whether *G. destructans* is the only pathogen involved and, if so, how it causes mortality. Means of transmission and spread are unknown, and there is no information on management actions that might reduce mortality and be specific to hibernating bats.

Ecology of Bats and *G. destructans*

Locations of most roost sites and details of the movement of individuals are largely unknown for many species of bats. Other gaps in knowledge include in-depth information on feeding and roosting behaviors; nightly, seasonal, and annual flight distances; population carrying capacities; age-specific survival and reproductive rates, and potential thresholds for Allee effects. There are few

long-term data on abundance, and even fewer data collected with mark-recapture methods or that account for age classes and recruitment (sensu O'Shea et al. 2004).

Little is known about *G. destructans*, but it is the only species of the genus known to infect the living skin tissues of bats (Cryan et al. 2010). Congeners, such as *G. pannorum* (which infects fur and feathers of various species), *G. sulphureus*, and *G. asperulatus* are saprophytic. It is not known whether *G. destructans* coevolved virulence with bats and requires an animal host or whether it originated as a saprophyte in cold environments but had virulence factors facilitating host infection ("accidental virulence") (sensu Casadevall & Pirofski 2007). The residence time of the fungus in North America is unknown. Nevertheless, recent sampling of sediments from caves and mines within and beyond the area affected by WNS revealed DNA of *G. destructans* only in regions where WNS had been observed (Lindner et al. 2010). The breadth of its host tropism, whether it has vectors, how long it survives without a host, how it interacts with soil or host microbiota, and many other details of its ecology are unknown.

Investigation of Outbreaks

An outbreak investigation framework (Gordis 2000) helps prioritize information needs specific to disease. The first step in such an investigation is to synthesize existing information and address logistical considerations, including biosecurity for field workers. The second step is to verify the diagnosis. Histopathologic examination is used to diagnose WNS (Meteyer et al. 2009). Blehert et al. (2009) used histopathologic methods to confirm the presence of the fungus in 105 of 117 bats with clinical signs of WNS (89.7%). Histopathologic examination, however, is time consuming, expensive, and most useful for diagnosing disease in dead bats. Biopsy lacks sensitivity (the ability to detect characteristic lesions if present) because relatively large samples are required for diagnosis. Culture and polymerase chain reaction (PCR) are less useful as diagnostic tests because the presence of viable fungus or fungal DNA does not equate to disease caused by *G. destructans*. Nevertheless, Lorch et al. (2010) report that PCR detected 96% histopathology-positive samples, whereas culture detected 33%. In their study, specificity was 100% for both methods. The low success rate of culturing is due in part to the difficulty of excluding other fungi from cultures. Published PCR primers for *G. destructans* react with other species of *Geomyces* found in cave sediments (Lindner et al. 2010). Nevertheless, PCR as a diagnostic test is 100% specific for *G. destructans* when bat tissues are tested. Until more-specific primers are found, PCR samples that are positive for *G. destructans* should be genetically sequenced to confirm that *G. destructans* is involved. Establishing guidelines to ensure consistency across laboratories in protocols and interpretation of results is critical.

The third step of an outbreak investigation is to establish what constitutes a suspect or confirmed case (i.e., case definitions). Draft case definitions for suspect and confirmed cases of WNS have been developed (http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/wns_definitions.jsp). During hibernation, WNS is suspect if consistent clinical signs are observed or an individual bat is found emaciated or dead in the vicinity of bats with confirmed WNS. Cases are presumptive if there are consistent clinical signs with positive *G. destructans* fungal culture or PCR, and cases are confirmed on the basis of histopathologic examination. Whether WNS is present in a hibernation site or other location can also be analyzed as a "case." Suspected case hibernacula have animals with apparent WNS clinical signs. Confirmed hibernacula have at least one dead, histopathology-positive bat.

On the basis of case definitions, an outbreak can be confirmed by determining whether suspected cases of a disease are real, that there is an actual increase in cases above previous baseline mortality, and that cases are related to each other or some causal factor. It is possible that unidentified WNS cases existed prior to 2007. WNS qualifies as an outbreak because mass mortality from this disease did not occur until recently and strong evidence indicates most cases are real (i.e., a diagnosis has been made) and that they are related in time and space.

Descriptive and analytical epidemiological statistics have not yet been compiled for individual bats and for bat populations and hibernacula. We suggest that data be collected from individual cases on sex, species, site, age class, clinical signs, ectoparasite load, season, and other possible factors that increase the probability of differences in susceptibility and transmission. Hibernacula can be classified by such characteristics as WNS prevalence, bat density, species richness of bats, location, and microclimate (e.g., humidity, temperature). A case-control epidemiologic study could be performed at the hibernaculum level if randomly chosen uninfected sites were evaluated. In contrast, bats and hibernacula evaluated to date have been ad hoc and have not been compared rigorously with controls. Final steps in the outbreak investigation are to implement control and prevention measures and communicate findings.

Establishing Causation of WNS

The evidence that WNS is associated with *G. destructans* implies but does not prove that this fungus is causal, and other factors likely contribute to disease. In addition to establishing causation of WNS by *G. destructans*, we recommend assessing the causation of the common clinical findings, such as emaciation and dehydration. Hill's nine criteria for causation are applicable in this situation, and

Table 1. Application of epidemiologic framework and Hill's (1965) criteria to assess *Geomyces destructans* as the cause of white-nose syndrome in bats.

Criterion	*Definition*	*Evidence whether criterion is met*
Strength of association	Stronger association implies agent under study is more likely to be causal for disease.	There is ample evidence for a strong association of *G. destructans* with WNS in North America. This may not be the case in Europe.
Consistency	Repeated observations of causal factors by "different persons, in different places, circumstances, and times."	As reports of WNS accumulate and affected bats are evaluated histopathologically and through PCR and culture, the relation between *G. destructans* and disease appears increasingly consistent.
Plausibility	Association under study is consistent with currently accepted understanding of pathological processes.	Skin infection by *G. destructans* is a plausible primary cause of mortality associated with WNS. Fungal infection of bat wings may disrupt the energy balance or cause life-threatening disruption of homeostasis.
Coherence	Association under study is compatible with existing theory and knowledge.	The postulated relation of *G. destructans* and WNS fits well with "known facts of the natural history and biology of the disease" (Hill 1965).
Experimental evidence	Disease can be prevented or ameliorated by an experimental regimen.	Very early experimental attempts to prevent or ameliorate effects of WNS were not successful.
Analogy	For analogous disease agents and diseases, similar outcomes have occurred.	Several diseases similar to WNS have emerged rapidly, been attributed to a fungus or oomycete, and resulted in substantial declines in abundance of their host species. These include the amphibian disease chytridiomycosis, attributed to the fungus *Batrachochytrium dendrobatidis,* sudden oak death, caused by *Phytophthora ramorum*, chestnut blight, caused by *Cryphonectria parasitica*, and crayfish plague, caused by *Aphanomyces astaci.*
Specificity	Factor or disease agent specifies a particular outcome or condition.	*G. destructans* has been implicated in essentially all cases of WNS evaluated to date.
Temporality	Exposure to disease agent precedes disease.	The temporal relation between *G. destructans* and WNS is not well established.
Biological gradient	Disease occurs after a threshold pathogen level is exceeded or disease is more severe if there is a higher dose of pathogen.	This has not been established for WNS.

we suggest they would be useful because they are general and flexible. Hill's criteria are strength of association, consistency, plausibility, coherence, experimental evidence, analogy, specificity, temporality, and biological gradient (Table 1). No single criterion is definitive, but evidence in support of each increases the probability that a factor is causal (Hill 1965; Plowright et al. 2008). In light of Hill's criteria, existing knowledge of WNS is consistent with *G. destructans* as the causal agent, but we think additional contributing factors need to be assessed (Table 1).

WNS Disease Ecology

The population dynamics of bats drive enzootic and epizootic WNS. Nevertheless, almost all critical details (or, in a modeling framework, parameter values) needed to understand and model the ecology of WNS in bats are unknown. We outline a WNS model, consider relevant parameters, and determine gaps in knowledge that can be filled through research.

Compartment modeling is commonly used to model disease dynamics. In such models groups of host individuals move among compartments designated as susceptible (S), infected or infective (I), and recovered or resistant (typically immune, R) to a disease (Kermack & McKendrick 1927; Bailey 1982). If recovered individuals can lose immunity and become susceptible again, the disease model is denoted as SIRS. If there is no immunity but animals recover, then the disease model is SIS. If infection persists without recovery, the disease model is SI. Differential equations describe how individuals move among the compartments with the parameters infection rate, recovery rate, and rate at which immunity is lost. If the time span of disease dynamics is long relative to host life span, then it is necessary to include functions for dynamics of host population growth independent of disease. Depending on the duration of the disease relative to host life spans, parameters for host birth, death, and population regulation (e.g., density dependence) may be included. Other modifications to compartment models

allow for addition of parameters on demographic and environmental stochasticity, exposed but not yet infective (e.g., fungus not reproducing) classes (E), vector transmission, and an environmental reservoir (e.g., fungus persists in hibernacula without a bat host). A generic set of SIRS differential equations is

$$\frac{dS}{dt} = bN - \beta SI - dS,$$

$$\frac{dI}{dt} = \beta SI - \gamma I - dI,$$

and

$$\frac{dR}{dt} = \gamma I - dR,$$

where N is the total population size ($= S$ individuals $+I$ individuals $+R$ individuals), b is host birth rate, d is host death rate, β is the rate of disease transmission, and γ is the rate of host recovery. These equations assume disease transmission is density dependent (i.e., each infected individual transmits infection to an a priori proportion of the available S individuals). It alternatively could be assumed that disease transmission is frequency dependent, in which case I individuals transmit to an a priori number of S individuals. Frequency-dependent transmission can lead to the infection of every S animal in a population. Whether WNS is frequency or density-dependent is unknown.

It is also unknown whether individuals that are exposed to, or recover from, the disease are resistant and whether individuals that recover become susceptible to or act as a source of infection. The existence of recovered individuals might seem unlikely, given the apparent high mortality observed to date. Nonetheless, some animals may recover if they had a mild case of the disease late in the winter (C. Meteyer, personal communication) or if mild winter weather increases probability of survival. The accumulation of recovered individuals could constitute herd immunity. All parameter values must be estimated, which also means the routes and rates of transmission must be determined, such as whether *G. destructans* is spread by direct contact among bats, through contact with contaminated roost sites, or through exposure to human or other animal vectors. The model may require substructuring that includes different bat species or age classes if bats have different levels of disease susceptibility, mortality, and recovery. Because males roost individually or in small groups in colder locations than females, they may function as reservoirs. Substructuring according to species or age could cause the model to predict longer-lasting endemic disease (Bolker & Grenfell 1996). The presence of reservoirs or vectors of WNS (which could include bat ectoparasites) may need to be included in the model. If animals can be medically treated, then recovery parameters can be adjusted.

Spatial modeling also may be useful for examining the pattern, rate, and direction of spread of WNS. The locations of some hibernacula of bats with WNS are known. We recommend that cases confirmed pathologically be considered separately from those identified through either culture- or PCR-only evidence of infection. This differentiation will allow for testing of two hypotheses: WNS and *G. destructans* infection are synonymous and thus overlap in time and space and *G. destructans* is already present in caves or perhaps spreading ahead of WNS. Spatial modeling with, for example, nearest-neighbor or moving-window analyses (Alexander & Boyle 1996) would facilitate examination of potential clusters of WNS and patterns of spread. Because bats often occur in groups, cluster analysis should be conducted at the hibernaculum level and separately for winter hibernacula and summer roosts. Such analyses would help determine whether the disease is spreading locally in clusters typical of regional contagion or more erratically, with new infections far from known infections. Approaches used to examine diffusion of, for example, plague (Noble 1974; Adjemian et al. 2007) and rabies (Moore 1999) also might be appropriate for determining the directions in which WNS is spreading, whether the speed of the diffusion front is increasing, and whether expansion of the disease is constrained by geological features (e.g., Appalachian Mountains with their associated caves and abandoned mines).

Network theory and cellular automaton models (del Rey et al. 2006) might also be useful in exploring possible patchiness and lack of spatial homogeneity of the probability of the spread of WNS. If limited data are available, individual-based simulation models may be useful (e.g., Kindlmann & Burel 2008; Lookingbill et al. 2010). Simulation models have been used to examine spread of rabies virus (Deal et al. 2000).

Science-Based Strategies for Adaptive Management of WNS

In the absence of well-validated strategies to reduce the spread of WNS and its effects on bat populations, we considered the following: disease surveillance, treatment of individuals, increasing population resistance to WNS (through vaccines, immunomodulators, or other methods), improving survival from starvation and dehydration associated with WNS, modifying hibernacula environments to eliminate *G. destructans,* culling individuals or populations, controlling anthropogenic spread of WNS, conserving genetic diversity of bats, and educating the public about bats and bat conservation.

Targeted epidemiological surveillance programs to detect disease occurrence that reduce bias from passive detection of disease are optimal, but data can also be

acquired through judicious use of convenience samples (e.g., suspected rabid bats submitted to public health departments) and reports from citizens. Ideally, surveillance is minimally invasive and does not disturb bats. Regardless of the approach, surveillance is improved by clear and consistent case definitions, consistent sampling protocols, and centralized data entry, management, analysis, and reporting. Descriptions of ideal sample quality and storage, including storage of voucher specimens, should be standardized. There are currently no targeted epidemiological surveillance programs for WNS, but such surveillance is essential for knowing where and when to take actions to minimize WNS effects.

Treatment of infected bats may prevent death and reduce the incidence of fungus. Treatment options under consideration include chemical or biological agents, especially fungicides. *G. destructans* is susceptible to treatment in vitro, but treatments (e.g., drugs) and delivery mechanisms proven safe for bats have not been developed. A major obstacle is delivery of treatment. Fogging caves with fungicide almost certainly would affect microbial flora in the cave. Unless bat populations decline to very low abundances, hand delivery of treatment to individual bats would not be feasible. It is unknown whether bats would require repeated treatment. Treatment with fungicide during passage in and out of hibernacula or roosting sites may be possible. Affected bats could be treated in captivity but issues of quarantine, handling, and release would need to be addressed. The proportion of a population that would need to be treated to reduce sufficiently the "infected" compartment of a population to reduce enzootic disease levels and spread is unknown.

Focusing recovery actions on increasing population resistance to *G. destructans* may be a useful component of WNS management. Little is known about immunity to WNS, whether some bats become resistant after exposure and to what extent immunity could be induced (e.g., through vaccination). If one assumes WNS is maintained and spread primarily bat to bat, it is possible to calculate the fraction of the population that, if immune, would lead to local abatement of the disease. Increased resistance in local populations of bats might interrupt transmission from infected to susceptible populations and curtail spread. There are precedents for vaccination against fungal disease, including recombinant vaccines for humans against fungal disease (Wuthrich et al. 2000), novel vaccines against valley fever for humans (caused by *Coccidioides immitis*), a vaccine for cats to speed recovery from ringworm (caused by dermatophyte fungi), and a phosphorus prophylactic treatment for oak trees against sudden oak death (Garbelotto et al. 2007). All possible means to ensure the good health of bat populations should be applied, such as maximizing habitat quantity and quality and reducing the effects of synergistic stressors (e.g., toxins) that reduce resistance.

Reducing starvation and dehydration during hibernation may reduce mortality. The cause of death in WNS is thought to be either starvation, major disruption of homeostatic balance, or impaired survival due to wing damage. Some obvious actions to prevent death, for example supplemental feeding or watering, pose challenges because hibernating insectivorous bats will likely not learn to feed from novel food sources during winter and their gut physiology may not adjust to availability of winter food.

Treatment of or modification of hibernacula may eliminate *G. destructans.* WNS treatments have been proposed that would deliver chemical or biological control agents into a cave or mine. There are several likely obstacles to this approach. First, many affected caves and mines occur on private land, where access may be restricted. Second, many caves and mines used by bats have great internal volume and structural complexity that would render complete coverage extremely difficult. Third, treatment may not meet its objectives if transmission is from bat to bat, rather than from cave surfaces to bats. Fourth, antifungal treatment in caves would almost certainly change resident species composition, possibly even increasing the probability of WNS if resident invertebrates or microbes are already competing with or somehow limiting transmission of *G. destructans.* It may be possible to manipulate the temperature and humidity of hibernacula so that they are less conducive to growth or transmission of *G. destructans* or to mitigate the effects of fungal infection on bats. Although a model suggests that localized warm areas within hibernacula could increase survival of infected bats (Boyles & Willis 2010), this approach has yet to be tested. Certain hibernating bats have evolved to survive winter in the very conditions at which *G. destructans* grows (Davis 1970; Cryan et al. 2010), and altering hibernacula to discourage growth of the fungus could also reduce survival of bats.

Although culling of infected individuals or populations may seem a viable approach to reducing pathogen load, the incidence of WNS within populations, and the probability of transmission to other populations, we suggest its potential effectiveness must be considered carefully and critically. For culling to be effective, the following are necessary: little or none of the pathogen should originate from fomites (objects that may be contaminated with the pathogen); most cases should be clinical or diagnosed after death; a sufficiently high proportion of affected individuals should be removed (this proportion can be calculated with SIRS models once a realistic model and model parameters are obtained); and the remaining population of individuals must be isolated to prevent spread and reintroduction. Culling in wild animal populations is less successful than culling of livestock because of difficulties and delays in diagnosis; vagility of animals, particularly in volant and potentially migratory species such as bats; and inability to control environmental factors and ongoing disease exposure. Culling of animals in the

wild for disease control has been either ineffective (e.g., control of Tasmanian devil [*Sarcophilus harrisii*] facial disease [Lachish et al. 2010]) or implicated in the exacerbation of disease (e.g., badger [*Meles meles*] tuberculosis [Jenkins et al. 2010]). Culling also may be perceived negatively by the public, may remove individuals with resistance to the disease because field indications of WNS are ephemeral (e.g., white noses) and often difficult to detect; and may lead to local extinction. For bats, culling to separate affected from unaffected bat populations (i.e., construction of a *cordon sanitaire*) would be difficult. Recent data document extensive spread of WNS, which increases the likelihood that a *cordon sanitaire* would be breached. Should culling be considered, we believe population and disease models should inform and justify decisions to cull, and concurrent research should assess key features of WNS disease ecology, such as the presence of reservoirs and alternate hosts, means and levels of disease transmission, possibilities of disease recovery and immunity, and different levels of susceptibility among different host species.

Even though the spread of WNS probably occurs mostly through contact among bats and possibly among bats and other animals, preventing the anthropogenic spread of *G. destructans* from cave to cave (most likely explanation for intercontinental spread) and from bat to bat during capture and handling could prevent some disease transmission. We think it is reasonable to require humans entering uninfected sites to disinfect their clothes and equipment. People studying or monitoring bats can also implement strict protocols for disinfecting equipment and preventing cross-species infection (Constantine 1986). In places where large numbers of humans and bats are likely to co-occur, caves could be closed to humans. If bats in a cave are uninfected, prohibiting human entry might slow *G. destructans* introduction, and if bats are infected, this prohibition might reduce spread from that cave as a nidus (center of infection).

Increased efforts to maintain genetic diversity of bats may become necessary to reduce spread of and mortality to bats from WNS. Decreases in the abundance of bats are likely to be followed by decreases in genetic diversity. Captive propagation or captivity during the winter could be initiated for critically endangered species; certain species of bats have been reared in captivity successfully (but see results of work with Virginia big-eared bats, http://www.fws.gov/WhiteNoseSyndrome). Nevertheless, such captive populations would only sustain relatively low levels of genetic diversity.

Monitoring populations of bats, although difficult (O'Shea & Bogan 2003), will provide important information on which species of bats are most susceptible to WNS and whether management actions are reducing mortality in bat populations. Newer quantitative methods, such as open population models (e.g., quantifying survival and reproductive rates [O'Shea et al. 2004]) and occupancy modeling (e.g., tracking occurrence of species over time at affected hibernacula [MacKenzie et al. 2006]), may offer promise for assessing the viability of bat populations exposed to WNS, prioritizing species on which to focus management, and gauging the effectiveness of management actions.

Education of the public may encourage people to report cases of WNS, avoid inadvertent spread of the fungus, and avoid disturbance of hibernacula. Education may also minimize reactive and ineffective killing. Public health departments responsible for surveillance of rabies could be educated about WNS, given they may be the first agencies to respond to bat-mortality events. State and federal land management agencies could opportunistically educate the public about bats and WNS. In situations such as high-traffic tourist caves with few hibernating bats, the potential benefits of educating the public about bats and WNS may be greater than the probability of human transmission of *G. destructans* to and from such sites.

In the 3 years since its discovery, WNS has changed the focus of bat conservation in North America. Prior conservation strategies for bats in North America sought to alleviate human-associated mortality (Weller et al. 2009), but WNS is a much less tractable natural threat. In contrast to diseases for which national response plans have been developed (e.g., chronic wasting disease, highly pathogenic avian influenza), WNS affects nongame species and poses no known direct threats to humans or domestic animals. Because WNS affects a number of species designated as endangered under the U.S. Endangered Species Act, some responsibility for coordinating a response to WNS rests with federal and state agencies charged with preventing extinction of listed species. Some of these agencies may have little or no experience dealing with epizootics. Our epidemiological roadmap is intended to supplement and inform emerging national and state plans for coordinating management activities directed at WNS in the United States.

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