Bat White-Nose Syndrome in North America

Since 2007, infections by a previously unrecognized, perhaps imported fungus killed an estimated 1 million bats in North America

David S. Blehert, Jeffrey M. Lorch, Anne E. Ballmann, Paul M. Cryan, and Carol U. Meteyer

In 2007 bats in eastern North America began dying in unprecedented numbers from a previously undocumented disease, now called white-nose syndrome (WNS). Although the ecological and economic impacts of this disease are not fully elucidated, this severe loss of insectivorous bats threatens decreased crop yields, forest defoliation, and a rise in insect-borne diseases. The recent emergence of WNS in bats of eastern North America, its rapid spread, and the severity of the outbreak highlight the importance of wildlife disease as an integral component of ecosystem health.

Summary

- The newly described fungus, *Geomyces destructans*, causes an invasive skin infection in bats and is the likely agent of white-nose syndrome (WNS).
- With immune system functions and body temperatures reduced during hibernation, bats may be unusually susceptible to a pathogenic fungus such as *G. destructans*.
- WNS was first observed in a popular show cave near Albany, New York, leading some investigators to suspect that a visitor inadvertently introduced *G. destructans* at this site, triggering a wider WNS outbreak in North America.
- Biologists trying to manage WNS within North American bat populations face major challenges, including the variety of susceptible host species, incredible dispersal capabilities of bats, difficulties in treating such populations, and persistence of the pathogen in their vulnerable underground habitats.

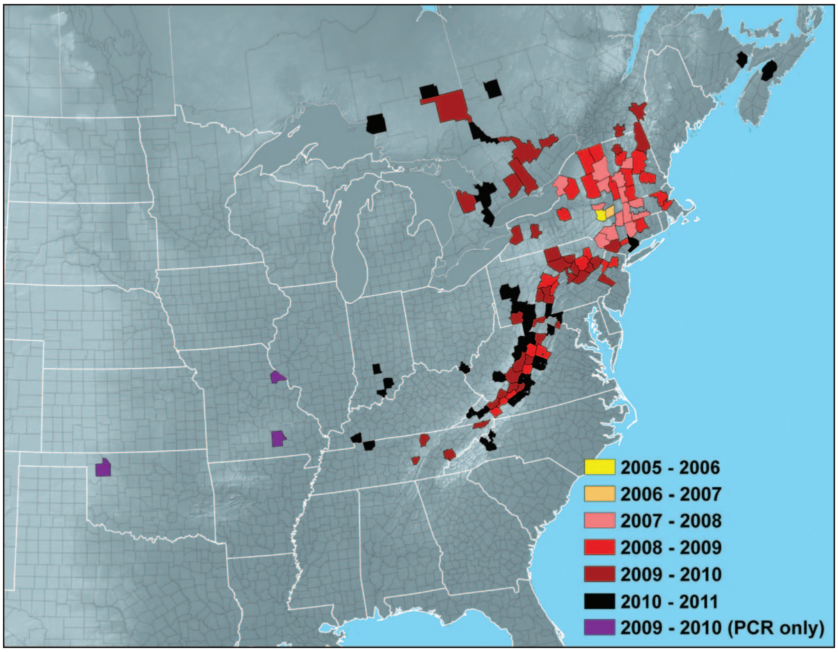
Biologists with the New York State Department of Environmental Conservation first recognized WNS as a problem in late winter 2007 at five hibernation sites near Albany, N.Y. Subsequently, a recreational caver furnished a photograph from February 2006 in nearby Howes Cave depicting bats with clinical signs of WNS, implicating this location as the likely index site and suggesting disease emergence the winter before New York state biologists drew public attention to the disease. By 2011 WNS had spread south along the Appalachian Mountains into eastern Tennessee, as far west as southern Indiana and western Kentucky, and north into the Canadian provinces of Quebec, Ontario, and New Brunswick (Fig. 1). Experts estimate that more than 1 million bats have died from WNS thus far. Modeling studies show that, if such mortality trends continue, one of the most abundant bat species in eastern North America, the little brown bat (*Myotis lucifugus*), could disappear from this region within 16 years. Sustained killing of this magnitude from an infectious disease is unprecedented among the approximately 1,100 species of bats known worldwide.

The Host, Pathogen, and Environment

The likely agent of WNS is a newly described fungus, *Geomyces destructans*, which causes an invasive skin infection that is the hallmark of this disease (Fig. 2). *G. destructans* belongs to the order Helotiales within the phylum Ascomycota. Characteristics that distinguish it from

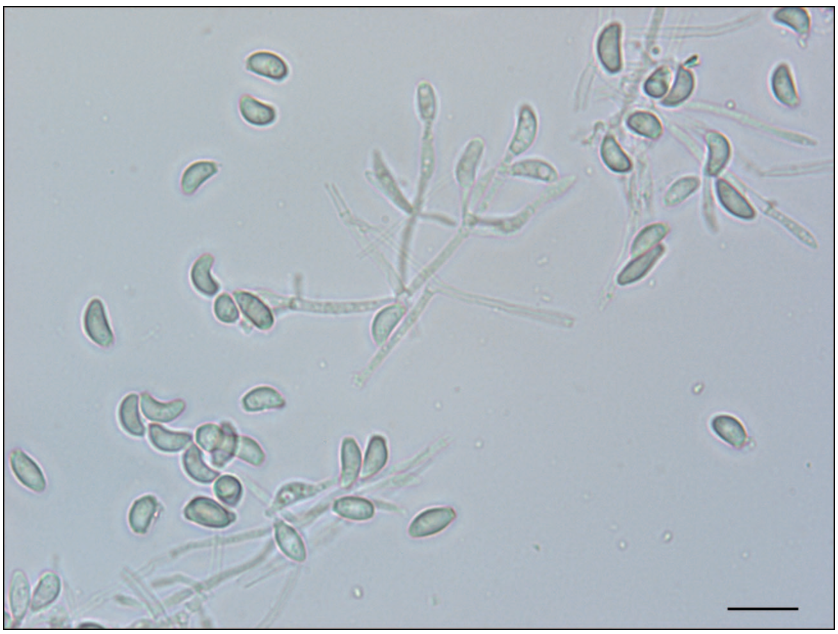
David S. Blehert is the head of the diagnostic microbiology laboratory at the U.S. Geological Survey (USGS)–National Wildlife Health Center, Madison, Wis. (dblehert@usgs.gov), Jeffrey M. Lorch is a graduate student with the Molecular and Environmental Toxicology Center, University of Wisconsin–Madison, Medical Sciences Center, Madison, Wisconsin (jmlorch@wisc.edu), Anne E. Ballmann is a wildlife disease specialist at the USGS–National Wildlife Health Center, Madison, Wis. (aballmann@usgs.gov), Paul M. Cryan is a bat ecologist at the USGS–Fort Collins Science Center, Fort Collins, Colo. (cryanp@usgs.gov), and Carol U. Meteyer is a wildlife pathologist at the USGS–National Wildlife Health Center, Madison, Wis. (cmeteyer@usgs.gov).

FIGURE 1



Occurrence of white-nose syndrome and/or *Geomyces destructans* in the United States (by county) and Canada (by county or district) from winter 2005–2006 through April 2011.

FIGURE 2



Micrograph of *Geomyces destructans* showing distinctive asymmetrically curved conidia either free or borne singly at the tips and sides of branched conidiophores (bar, 10 μm).

other *Geomyces* spp. include curved conidia (Fig. 2), slow growth on laboratory medium, cold adaptation, and pathogenicity to bats. Species of *Geomyces* exist in soils worldwide, especially in colder regions.

Any infectious disease involves interactions among a susceptible host, pathogen, and the environment. To comprehend the ecology of WNS, we must consider the physiological and behavioral aspects of bats that make them susceptible to the disease, the characteristics of the fungus that allow it to act as a pathogen, and the role of underground sites (hibernacula) such as caves and mines in providing conditions conducive to maintaining this pathogen and enabling it to infect these hosts.

WNS appears to occur only in bats, suggesting they possess unique traits that make them a suitable host. Bats are nocturnal and the only mammals capable of powered flight. Their forelimbs are highly modified, consisting of elongated phalanges connected by a thin layer of skin to form wings. This body plan provides bats with selective advantages that allow them to dominate the night skies, making them the second most diverse group of mammals, accounting for approximately 1,100 of 5,400 mammalian species. Of 45 bat species in the United States, at least 6 of the approximately 25 that hibernate have been documented with WNS, including the little brown bat, the northern long-eared bat (*M. septentrionalis*), the eastern small-footed bat (*M. leibii*), the endangered Indiana bat (*M. sodalis*), the tricolored bat (*Perimyotis subflavus*), and the big brown bat (*Eptesicus fuscus*).

All six of those species are insectivorous and cope with winter food shortages by hibernating in cold and humid, thermally stable caves and mines. When hibernating, the animals typically congregate in large numbers, dramatically reduce metabolic functions, and assume a body temperature

Blehert: White-Nose Syndrome Mechanisms in Bats, Mechanics of Vintage Vehicles

If not microbiology, David Blehert might well have studied mechanical engineering instead. While a child, he liked nothing better than to help his father repair cars. When he was in high school, the two of them rebuilt a 1968 sports car and then restored another car from the 1950s. Blehert, 40, continues to collect and repair vintage vehicles, saying this process helps him when "diagnosing and solving problems, understanding how things work, and seeing projects to completion. [It is] a sound basis for conducting laboratory research."

Blehert heads the diagnostic microbiology laboratory within the disease investigation branch of the U.S. Geological Survey (USGS)-National Wildlife Health Center, in Madison, Wis. His lab works with a team that includes wildlife pathologists and other specialists whose expertise ranges from diagnostic virology to parasitology and chemistry. Together, they investigate and find the causes of wildlife illnesses and unexplained deaths. "Essentially, we function as the nation's [Centers for Disease Control and Prevention (CDC)] for wildlife," he says. "Our work provides scientific support for the management of wildlife disease and the promotion of ecosystem health."

Blehert and his collaborators are investigating what led to emergence of bat white-nose syndrome (WNS) in North America, an outbreak that experts attribute to the fungus *Geomyces destructans*. "With bat population declines exceeding 70% in the eastern United States, WNS presents a significant threat to hibernating bat species of North America," he says. "Bats are primary predators of insects, including crop and forest pests. Thus, reduced bat populations could adversely impact agriculture and the health of forests with consequent economic and ecologic repercussions."

Blehert suspects that humans accidentally transported *G. destructans* from Europe to or near a popular tourist cave in New York, enabling the fungus to establish itself among North American bats. "Global travel and trade have effectively eliminated natural barriers, such as mountain ranges and oceans, that once prevented the spread of disease agents around the world, and are today recognized as one of the most significant drivers in the emergence of infectious diseases worldwide," he says. Interest in bat WNS also "serves to highlight the importance of this wildlife disease as part of the 'One Health' concept, that is, the recognition that wildlife health, domestic animal health, human health, and ecosystem health are inextricably linked."

In addition to his work on WNS, Blehert's laboratory is conducting collaborative research with a local company, BioSentinel, to develop rapid in vitro methods for detecting botulinum neurotoxins types C and E, the cause of avian botulism, one of the most significant causes of waterfowl mortality in North America. "The current gold-standard test for detecting botulinum neurotoxins uses live mice, and an in vitro assay would significantly decrease costs, increase throughput, and eliminate the need to use animals for botulinum detection," he says.

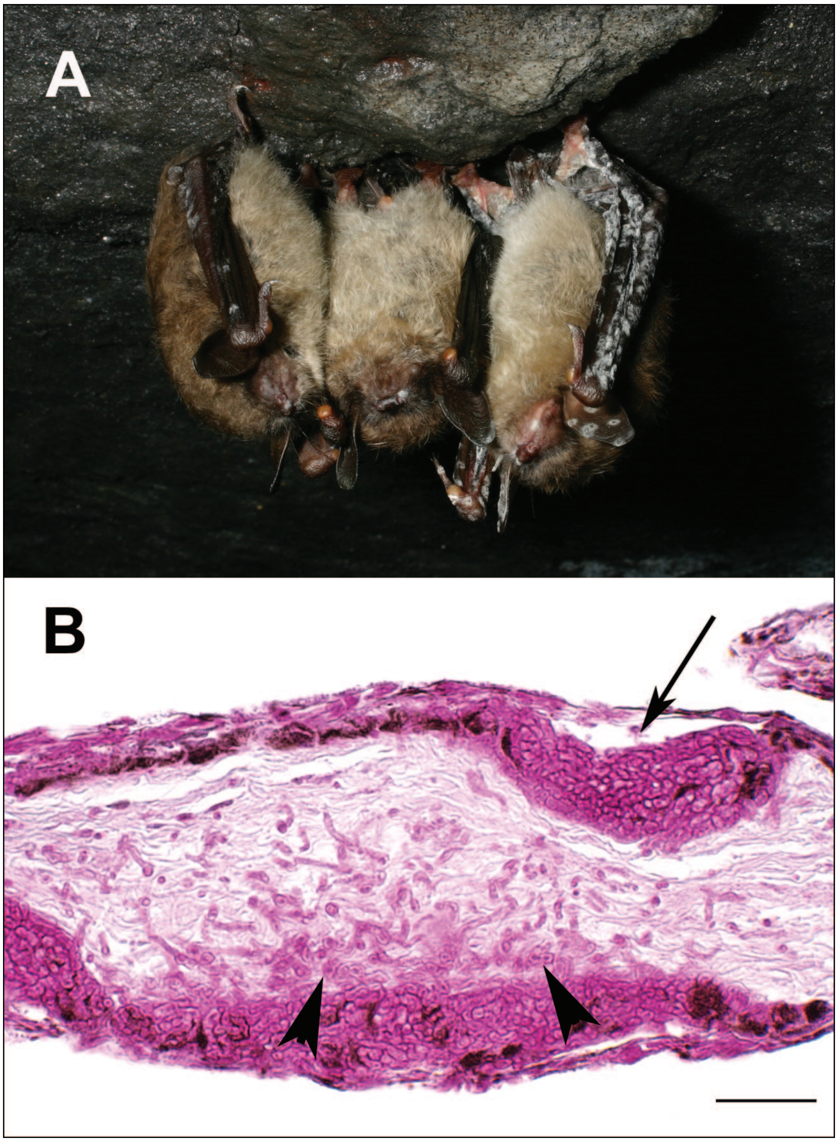
Blehert grew up in Minneapolis. His mother is a teacher, and his father is a mechanical engineer. "My parents strongly support education and encouraged me and my brothers—I have younger identical twin brothers—to take school seriously," he says. "As an engineer, my father especially encouraged us to pursue course work in math and science. I came close to heeding his advice by choosing to study biology."

Long interested in the outdoors, Blehert received a B.S. in biology in 1993 from the University of Minnesota and a Ph.D. in bacteriology in 1999 from the University of Wisconsin, Madison. He did postdoctoral research at the National Institute for Dental and Craniofacial Research at the National Institutes of Health from 1999 until 2003, when he joined the USGS.

Blehert is married to Regina Vidaver, a cellular and molecular biologist who is executive director of the National Lung Cancer Partnership, a nonprofit research advocacy group. They have two children, a daughter, 8, and a son, 5. In his spare time, he indulges his love for the outdoors by hiking, fishing, gardening, ice-skating, skiing, and camping. And he still works on antique vehicles, a collection that now includes two vintage motorcycles and a sports car. "I maintain all of these vehicles in running condition, but given my current work and family responsibilities, finding time to drive and work on them is challenging," he says. "For now, it makes me happy to have them in my garage, complete periodic maintenance and improvements, and collectively drive or ride them a couple of hundred miles per year."

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.

FIGURE 3



(A) Three little brown bats (*Myotis lucifugus*) photographed by Alan Hicks (New York State Department of Environmental Conservation) in Graphite Mine, New York, in November, 2008. Note the white fungus colonizing the muzzles and nostrils of all three bats. Also note the extensive fungal colonization of the skin of the ears and wings of the bat pictured on the right; B) Periodic acid-Schiff (PAS) stained microscopic section of wing membrane from a little brown bat with white-nose syndrome collected in Pennsylvania in February, 2009. Dense colonies of fungal hyphae erode skin and fill the cup-shaped depressions (arrow). Ulceration of epidermis with penetration and replacement of subcutaneous tissue (arrow heads) dramatically alters the integrity of wing membrane. Bar = 25 μm.

close to that of their surroundings (2–7°C). These physiological adaptations and behaviors likely predispose bats to infection by *G. destructans* and consequent development of WNS. Because approximately half the bat species of the United States are obligate hibernators, another 19 species are at risk for infection by *G. destructans* if it spreads beyond its current range.

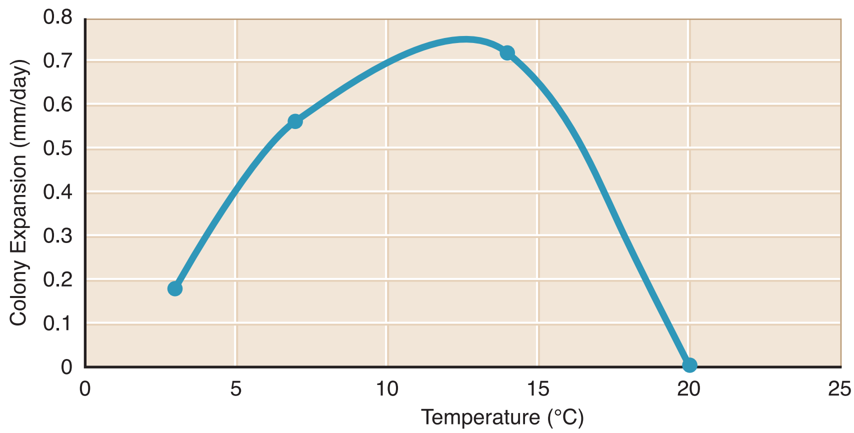
G. destructans colonizes the skin of bat muzzles, wings, and ears, then erodes the epidermis and invades the underlying skin and connective tissues. This pattern is distinctive and is more severe than that caused by typical transmissible dermatophytes. Although the disease was named for the characteristic white growth visible around an infected animal's nose, the primary site of infection is the wing (Fig. 3a). Gross damage to wing membranes such as depigmentation, holes, and tears are suggestive of WNS, but these lesions are nonspecific, and histopathologic examination is necessary to diagnose the disease.

Specifically, fungal invasion of wing membranes ranges from characteristic cup-like epidermal erosions filled with fungal hyphae to ulceration and invasion of underlying connective tissue, with fungal invasion sometimes spanning the full thickness of the wing membrane (Fig. 3b). Fungal hyphae can also fill hair follicles and destroy skin glands and local connective tissue. Bat wings play an important role in the pathogenesis of WNS by providing a large surface area for the fungus to colonize. Once infected, the thin layer of skin that composes the bat wing is vulnerable to damage that may catastrophically disrupt homeostasis during hibernation.

In North America, bat hibernacula range in temperature from approximately 2–14°C, temperatures all permissive to growth of *G. destructans*. Within this temperature range, *G. destructans* exhibits increasing growth rates with increasing temperature (Fig. 4), but the fungus does not grow at temperatures of approximately 20°C or higher. This temperature sensitivity helps to explain why

WNS is observed only among hibernating or recently emerged bats and why the disease is not diagnosed in bats during their active season when body temperatures are consistently elevated above those permissive to growth of *G. destructans*.

FIGURE 4



Colony expansion rates of *Geomyces destructans* when grown on cornmeal agar at 3, 7, 14, and 20°C. The trend line estimates colony expansion rates at temperatures ranging from 3–20°C.

Looking for Other Host and Environmental Susceptibility Factors

Hosts with impaired immune functions tend to be susceptible to opportunistic fungi in their environments. Guided by this concept, some investigators suspected that insults such as exposure to environmental contaminants or infections by viral pathogens compromised bat immunity and made them vulnerable to *G. destructans*. However, neither contaminant exposure nor viral coinfections can be consistently identified in bats infected with that fungus.

Hibernating bats with WNS generally do not exhibit signs of an inflammatory response. However, severe inflammation typifies fungal skin infections of bats aroused from hibernation, providing evidence that such animals are not immunocompromised. Although studies of bat immune functions are in their infancy, studies of other mammalian species indicate that their immune functions are naturally suppressed during hibernation. Thus, rather than suggesting immune-function impairment, the lack of inflammatory response to fungal infection by hibernating bats may reflect an immune suppression that is part of hibernation physiology.

In addition, the body temperature of hibernating bats drops dramatically, providing another vulnerability to infection by *G. destructans*. Fatal fungal diseases are relatively rare among endothermic, or warm-blooded, animals because their tissues are too warm to support the growth of most fungal species. However, fungi are more apt to cause fatal diseases in ectothermic, or cold-blooded, organisms such as insects, fish, amphibians, and plants. Bats and other mammals that hibernate are unique in that they are warm-blooded when metabolically active, but cold-blooded during hibernation—a period when their metabolism and body temperatures are dramatically suppressed. Although lowered body temperatures may predispose torpid bats to infection by *G. destructans*, the mechanism enabling this specific fungus to be a pathogen for bats while other cave-associated fungi remain innocuous is not known.

How *G. destructans* kills bats is under active investigation. One possibility is that fungal infection disrupts how bats behave while hibernating, leading to more frequent or longer arousals from torpor and thus accelerating usage of fat reserves. However, fat depletion is not consistently observed among all bats with WNS. Infected bats also may exhibit other aberrant behaviors midway through the hibernation season, such as shifting from thermally stable roost sites deep within hibernacula to areas with more variable temperatures near entrances. Sometimes, they depart early from hibernacula. Thus, exposure to cold could account for some WNS-associated mortality.

Further, fungal damage to wing membranes, which can account for more than 85% of the total surface area of a bat, may increase fatality rates. In addition to the key role that wings play in flight, wing membrane integrity is essential for maintaining water balance, temperature, blood circulation, and cutaneous respiration. Disrupting any of these functions could increase WNS mortality rates.

As with so many other diseases, the environment affects the progress and transmission of

WNS. Some pathogenic fungi such as *Histoplasma capsulatum*, *Cryptococcus* spp., and *Batrachochytrium dendrobatidis* can persist in the environment without an animal host for survival. This independence contrasts with host-requiring viruses or other pathogens for which transmission dynamics tend to moderate as infected hosts are removed from a population. *G. destructans* likely does not require bat hosts to survive and can persist in caves by exploiting other nutrients.

The cool and humid conditions of underground hibernacula provide ideal environmental conditions for *G. destructans* or other fungal growth. While most *G. destructans* isolates were cultured from skin or fur of bats collected in or near underground hibernacula during winter, DNA from the same fungus is found in soil samples from several hibernacula that harbor WNS-infected bats in the northeastern US. Also, *G. destructans* has been cultured from soil samples from hibernacula in three states where WNS occurs, supporting the hypothesis that bat hibernacula are reservoirs for this pathogen and that bats, humans, or fomites may transport *G. destructans* between hibernacula. How temperature and humidity differences among hibernacula influence *G. destructans* and WNS is not known.

Uncertainties about WNS Emergence

What caused WNS to emerge in a North American cave during the winter of 2005 to 2006? Bats with clinical signs consistent with WNS were first observed in Howes Cave, a hibernaculum connected to a popular North American show cave. Because of its high human traffic, a tourist might have inadvertently introduced *G. destructans* at this site.

Europe might be the source for the fungus causing WNS. Reports dating back several decades describe hibernating bats in Germany with white muzzles resembling bats with WNS in North America. Recent culture and PCR surveys indicate that *G. destructans* is widespread in Europe, including among hibernating bats in hibernacula in the Czech Republic, France, Germany, Hungary, Slovakia, and Switzerland. Unlike in North America, however, mortality rates and population declines remain normal among European bat species. This sharp contrast between disease manifestation among bats in Europe and North America provides an opportunity to investigate how bat species may differ in terms of their susceptibilities to fungal infection, continental variability among fungal strains, and the influence of environmental conditions and bat behavior on this fungal disease.

Challenges in Managing WNS, Conserving Bat Populations

Bat conservation efforts have historically focused mainly on reducing human causes of bat mortality, including habitat destruction, detrimental intrusions into roosts, and intentional extermination of colonies. Bat census figures prior to the emergence of WNS in North America indicate many populations of cave-hibernating bats were stable or increasing. However, the current WNS outbreak brings an even more serious threat to bat populations of North America, confronting biologists with a new set of conservation and management challenges.

Mitigating diseases in free-ranging wildlife populations requires very different approaches from those applied in agriculture for domestic animals. Once established, diseases in free-ranging wildlife are rarely, if ever, eradicated. Biologists trying to manage WNS within bat populations face multiple challenges, including the need to deal with numerous host species, long-distance migrations of infected hosts, poor access to some host populations, impracticalities associated with treating individual wild animals, infected hosts that are sensitive to being disturbed and that inhabit fragile ecosystems, and environmental persistence of the pathogen.

The guiding principle for physicians and veterinarians, "first, do no harm," will help to prevent WNS management efforts from having unintended adverse consequences. For example: depopulating an infected colony would not be effective unless all infectious animals are removed and all hibernacula used by the population are decontaminated—conditions unlikely to be achieved among free-ranging wildlife; using disinfectants to decontaminate hibernacula could have toxic effects on other organisms reliant on those environments; treating individual bats with antifungal agents is labor intensive, is not self-sustaining, and could be toxic for treated animals or their symbionts; and careless intervention could disrupt natural selective processes that might yield behaviorally or immunologically resistant bats.

However, "first, do no harm" does not mean "do nothing." State and federal agencies already are taking measures to combat WNS, including closing caves and mandating decontamination procedures. Such steps are intended to prevent people from disturbing hibernating bats and to reduce the chance that intruding humans will transfer *G. destructans* from one hibernaculum to another. For example, taking a proactive approach prior to the appearance of WNS, state wildlife officials in Wisconsin conferred threatened status on four cave bat species that hibernate within its borders and designated *G. destructans* a prohibited invasive species providing state resource managers with legal authorities to take disease management actions.

Since the first description of *G. destructans* in 2008, its genome has been sequenced, and WNS pathology has been more fully defined. Additionally, hibernacula are being surveyed internationally, and ongoing analyses are revealing much about the biodiversity of fungi associated with bat hibernacula. With these and other advances in understanding WNS, opportunities will arise to better manage the disease cycle. The sudden and unexpected emergence of WNS exemplifies the importance of monitoring, investigating, and responding to emerging wildlife diseases and the ecological and societal threats that they present.

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