### White-Nose Syndrome of Bats

by Jessie A. Glaeser, Martin J. Pfeiffer and Daniel L. Lindner

Devastating. Catastrophic. Unprecedented. This is how white-nose syndrome of bats (WNS) is characterized. It is one of the deadliest wildlife diseases ever observed and couldhave significant impacts on outdoor recreation, agriculture and wildlife management.

White-nose syndrome was first described during the winter of 2006/2007 from a massive die-off of bats in four caves near Albany, NY. Photographs from the previous year showed affected bats in Howes Cave, a large tourist cave in upstate New York. The term "white-nose syndrome" was used because dead bats usually had white, fuzzy fungus growing over their nose, mouth and face (See photo to the right). Later people realized that the same fungus also caused large, damaging lesions in the wings (See photo p. 47).

The disease initially took the familiar path down the Appalachians, following the track of chestnut blight over 100 years before. However, it also spread into the eastern provinces of Canada and is on track to move from western Ontario to Manitoba soon. Currently, the disease is reported from 30 states and 5 provinces (see map p. 47). The estimated mortality of bats now stands at over 6 million. It seems that all species of hibernating bats in eastern North America are affected, but the highest mortality rates are found with little brown bats (Myotis lucifugus), the most common bat in eastern North America, and tricolored bats (Perimyotis subflavus). Recently, the Northern long-eared bat (Myotis septentrionalis) was federally listed as "threatened" because of WNS. Bats that were already endangered, such as the gray bat (Myotis grisescens) and Indiana bat (Myotis sodalis), may become extinct (Frank et al., 2014), while previously "common" species - such as the little brown bat - may face regional extinction in as little as 16 years (Frick et al., 2010).



Little brown bat (*Myotis lucifugus*) showing signs of whitenose syndrome (WNS) on its nose. Photo Credit: A. Hicks, New York Department of Environmental Conservation.

#### The Fungus

The fungus associated with white-nose syndrome is *Pseudogymnoascus destructans*, an ascomycete in the family Pseudeurotiaceae. The sexual stage of the fungus is likely very small (1-2 mm) cotton balls, technically known as "gymnothecia" or "open perithecia." This stage has not yet been observed in nature or in the laboratory, although genetic analyses indicate that the fungus is

capable of sexual reproduction, at least in Europe where both mating types are present (Palmer et al, 2014). In North America, only one mating type of the fungus has been found. To reproduce asexually, the fungus makes large numbers of crescent-shaped conidia from the tips of branched conidiophores. These spores are rather large  $(5-12 \times 2-3.5 \,\mu\text{m})$  – not like the small, easily airborne conidia of Penicillium or Aspergillus. The fungus is considered psychrophilic (cold loving) for it can survive at temperatures higher than 20°C (68° F) but does not actively grow above 19°C (66°F). Its optimum temperature for growth is between 12-14° C (54-57° F) (Figure 5). At temperatures above 12° C (54° F), the hyphae and conidia become atypical and may appear deformed (Verant et al., 2012). Among all mammals, the fungus is only known to cause disease in hibernating bats, likely because their body temperatures drop to those of the ambient environment – often as low as 2-14° C (36-57° F) (Blehert et al, 2009). The fungus survives in cave sediments and metabolizes a variety of carbon- and nitrogen-containing compounds, thus surviving in hibernation sites in the absence of bats (Lorch et al., 2013).

#### The Disease

The disease mechanisms are very unusual and not entirely understood. Most dermatophytes, such as the athletes' foot fungus or ring worm fungus (species of *Trichophyton*), produce superficial lesions that do not penetrate deeply into underlying tissues. In contrast, *P. destructans* forms significant lesions, especially in the wing tissue. It is thought that this sets off a cascade of physiological events that ultimately results in the death of the host bat (Verant et al., 2014).

Elevated fat utilization occurs early in the disease process, when bats show only mild to moderate skin lesions. The bats also undergo chronic respiratory acidosis with low blood pH (i.e., high acidity) and high levels of dissolved carbon dioxide, bicarbonate, and potassium. Once the carbon dioxide level is elevated, bats begin to hyperventilate, which results in increased arousals from hibernation to expel excess carbon dioxide and return the blood pH to its normal level. The arousals from hibernation require more and more energy, further depleting fat levels and water loss. Damage to wing membranes interferes with water absorption, leading to further dehydration and loss of electrolytes. These disturbances ultimately lead to mortality unless the bat has enough energy reserves to last until spring.

Larger bat species that have higher levels of fat reserves often survive the disease more frequently than the



Conidia of *Pseudogymnoascus destructans*, 1000X, stained with Congo blue. Photo credit: Jessie A. Glaeser, U.S. Forest Service

smaller bat species (Verant et al., 2014). Bats that survive until spring, when insects are plentiful, can frequently overcome the infection when normal metabolism is reestablished. However, even if bats survive until spring, their immune systems may "overreact" when coming out of hibernation, leading to death from an acute inflammatory response. This phenomenon is similar to the "IRIS" effect first observed in human HIV patients when the immune function is quickly restored through anti-retroviral therapy (Meteyer et al., 2012).

#### Origin of the Disease

When white-nose syndrome was first observed, there was much speculation as to whether it was due to a complex of factors – such as pesticides, climate change, or viruses – with the fungus being merely an opportunistic pathogen colonizing a weakened host. If the disease was caused by a single pathogenic fungus, was it an introduced, non-native fungus or a native fungus that had mutated or recombined to become a more lethal pathogen?

In 2011, researchers showed that exposure to a single fungus – at that time named *Geomyces destructans* – was enough to cause the disease (Lorch et al., 2011). Later the fungus was determined to be significantly different from "true" *Geomyces* species, and it was transferred to the genus *Pseudogymnoascus* (Minnis and Lindner, 2013). Many undescribed species of *Geomyces* and *Pseudogymnoascus*  have been found in cave sediments (Lindner et al., 2011; Lorch et al., 2013), highlighting the need for more taxonomic research in this family (Figure 6).

Recent molecular comparisons show strong genetic similarities between North American and some European fungal populations (Leopardi et al., 2015). The fungus was recently discovered in China as well and is now known to have a Eurasian distribution (Hoyt et al, 2016). Bats with fuzzy noses have been observed in Europe, even in museum collections, but there has been no observation or documentation of massive mortalities in Europe. The European fungus was shown to cause typical white-nose syndrome disease expression in North American bat species (Warnecke et al., 2012), and it is now widely assumed that the North American fungus originated from Europe.

The European strains are more variable genetically compared to the North American strains, and increased diversity is often indicative of an older population. The genetic uniformity of the North American fungus is typical of an introduced, clonal population. The North American fungus exhibits a single mating type, preventing – to date – sexual reproduction, while two mating types are present in Europe (Palmer et al., 2014).

Introduction of the other mating type to North America could have severe consequences because a sexually reproducing population of the fungus could produce strains with higher levels of pathogenicity or that could perhaps grow at higher temperatures. Therefore, policies that limit the inter-continental spread of this fungus (e.g. decontamination of equipment and gear used in caves) are of crucial importance. Since bats do not migrate between Europe and North America, it is highly probable that human activity brought the pathogen to North America. An infected bat could have entered the Port of New York in a shipping container or fungal spores might have been transported on boots, equipment or clothing. The original site of introduction in North America is a big tourist attraction with many international visitors. We will never know the actual mechanism of introduction, but preventing the introduction of additional mating types is essential.

#### Effects on Agriculture and Forestry

Bats provide a diverse set of ecological services, including pollination, seed dispersal, and pest control. In the continental United States alone, 44 species of bats are known insectivores (Fleming et al., 2003), consuming a large volume of insects. Insectivorous bats can consume 50% or more of their body weight in insects every night (Harvey et al., 1999). Economic benefits from this consumption include reductions in insect damage and reduced insecticide usage on agricultural crops and other environments. One widely-cited study estimates that Brazilian free-tailed bats consume sufficient insects to save \$100,000 in annual insecticide costs for 10,000 acres of cotton plantings in southcentral Texas (Cleveland et al., 2006). Another study extrapolated these findings and estimated that the loss of bats in North America could lead to economic costs of up to \$3.7 billion (Boyles et al., 2011).

#### Saving the Bats

This is a devastating disease, but some bats have survived (Reichard et al., 2014). Are these bats somehow resistant to the pathogen or did they just randomly escape exposure? Approaches to increase survival rates include building "sanctuary" artificial hibernation sites that are pathogenfree and implementing management strategies that increase the overall health and body weight of bats before they go into hibernation. Several types of biological control are also being explored. Many nonpathogenic fungi and bacteria, as well as nonlethal opportunistic pathogens, are found in bat hibernacula and on the bats themselves (Lorch et al., 2015). Some of these might be able to compete with the pathogen for nutritional resources during its saprotrophic phase. Other microorganisms produce fungitoxic compounds that might kill the fungus before significant disease develops (Hoyt et al., 2015; Zhang et al., 2015; Cornelson et al., 2013). In a promising treatment, researchers from the U.S. Forest Service exposed small numbers of infected bats to the bacterium Rhodococcus rhodochrous to combat the fungus (http:// www.nature.org/ourinitiatives/ regions/northamerica/unitedstates/ tennessee/success-in-treating-whitenose-syndrome.xml).

Decontamination protocols have been put into effect to prevent human-induced transmission of the disease. Scientists who conduct field research and members of the caving community follow a specific set of protocols for cleaning their clothing, boots, and equipment. These protocols include soaking the materials in hot water (55oC for 20 minutes) or specific cleaning agents, such as Professional Lysol® Antibacterial All Purpose Cleaner, dilute chlorine bleach or Formula 409® Antibacterial All Purpose Cleaner for 10 minutes. Protocols are revised and refined annually as

we learn more about the efficacy of cleaning agents against the spores of the fungus (Shelley et al, 2013; Glaeser, 2015). The caving community, including recreational cavers, are very supportive of efforts to contain the disease. Current recommendations on decontamination can be found at: https://www.whitenosesyndrome. org/topics/decontamination.

#### Will the bats survive?

It is very unusual for a pathogen to totally eradicate its host, but it is probable that some of the endangered bat species may drop below a genetic threshold where the species can no longer survive. Certainly the skies of eastern North America have far fewer little brown bats than in years past. Selection pressure favors bats that are larger and heavier, both within a species and among species. In Europe, bats hibernate in small groups rather than in large, compact hibernation clusters as is typically observed in North America. Large, dense colonies of bats create perfect conditions for transmission of the fungus. Scientists, cavers and naturalists are working tirelessly to develop treatments and to control the spread of the disease. Even if they are successful, due to the slow reproductive rate of bats, this disease will have a lasting effect on bat populations and the humans that depend on their ecosystem services. Next time you swat a mosquito or encounter a leaf-eating beetle in your garden, you should think of bats and their struggle against the fungus that is threatening their extinction.

For more information and for what you can do to help, see:

Bat Conservation International (http://www.batcon.org/)

National Speleological Society (https://caves.org/WNS/) U.S. Fish and Wildlife Service, Department of the Interior (https:// www.whitenosesyndrome.org/)

U.S. Forest Service (http://www. fs.fed.us/research/invasive-species/ terrestrial-animals/white-nosesyndrome.php)

U.S. Geological Survey, National Wildlife Health Center

(http://www.nwhc.usgs.gov/ disease\_information/white-nose\_ syndrome/)

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Near right: Phylogenetic tree showing the large number of fungi closely related to *P. destructans* from cave sediments, many of which have not been named and are referred to by letters and numbers. *P. destructans* itself is indicated by the bat icon. From Minnis and Lindner, 2013

Far right: Little brown bat (*Myotis lucifugus*) showing fungal growth and lesions on wings. Photo Credit: A. Hicks, New York Department of Environmental Conservation.

Bottom right: Current range of white-nose syndrome as of November, 2015. Note the 2015 discoveries (in red) at the leading edge of the infected area. Map by Lindsey Heffernon, PA Game Commission



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Cover Photo: Stereum ostrea with rainwater, photographed by Deniz Tezcan.

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