Chapter 9
White-Nose Syndrome in Bats

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Abstract White-nose syndrome (WNS) is an infectious disease of hibernating bats that has killed millions of bats since it first emerged in eastern North America in 2006. The disease is caused by a pathogenic fungus, *Pseudogymnoascus* (formerly *Geomyces*) *destructans* that was likely introduced to North America by human trade or travel, demonstrating the serious problem of global movement of pathogens by humans in the Anthropocene. Here, we present a synthesis of the current state of knowledge on WNS, including disease mechanisms, disease ecology, global distribution and conservation and management efforts. There has been rapid research response to WNS and much about the disease is now well understood. However, critical gaps in our knowledge remain, including ways to limit spread, or effective treatment options to reduce disease mortality. There are several hibernating bat species in North America that are threatened with extinction from WNS. Protecting those species has become a race against time to find and implement creative solutions to combat the devastating impacts of this disease.

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9.1 Introduction

In late winter of 2007, biologists at the New York State Department of Environmental Conservation encountered a macabre scene during their annual winter surveys of hibernating bats in caves and mines in northern New York State: heaps of dead bats piled on cave floors (Fig. 9.1) (Veilleux 2008). Bats were also seen flying out in the middle of winter onto the snowy landscape and the number of citizen reports of dead bats found in backyards was much higher than normal. A white fuzzy growth was observed on muzzles and wings of the few remaining live bats, which led to the name white-nose syndrome (WNS) (Veilleux 2008; Reeder and Turner 2008; Turner and Reeder 2009). WNS is now recognized as one of the most devastating wildlife epidemics in recorded history and has caused the death of millions of bats in eastern North America. The research and management response to WNS has been rapid and we know much more about WNS than when those first dead bats were observed in New York, although there is still a great deal about this wildlife disease that is yet to be resolved.

The first evidence of WNS in North America is dated to a photograph taken by a caver at Howe's Cave in 2006 (Turner and Reeder 2009). Howe's Cave is a popular tourist attraction that receives hundreds of thousands of visitors each year, many of whom visit from other parts of the world. The white fuzzy growth visible on bats is caused by a pathogenic fungus, which was described as *Geomyces destructans* (Gargas et al. 2009; Blehert et al. 2009), but was recently re-named *Pseudogymnoascus destructans* after closer evaluation of its taxonomic allies (Minnis and Lindner 2013). The fungus infects the skin tissues, including the wings and tail membranes, and causes bats to arouse too frequently from torpor

Fig. 9.1 Bats that died from WNS during winter at Aeolus Cave in Vermont, USA. *Photo* by Al Hicks

Fig. 9.2 A hibernating little brown myotis (*Myotis lucifugus*) with typical WNS infection visible on skin tissues. *Photo* by Ryan von Linden

during hibernation (Lorch et al. 2011; Warnecke et al. 2012) (Fig. 9.2). Bats die before spring brings warmer weather and insects for food.

WNS has spread rapidly and by 2014 was found in 25 U.S. states and 5 Canadian Provinces (Fig. 9.3). A confirmed case of WNS is defined by the presence of cupping erosions on the skin caused by infection by *P. destructans*, which is determined by histopathological examination (Meteyer et al. 2009). There are currently seven hibernating species in North America that have been confirmed with infections characteristic of WNS, including *Myotis lucifugus, Myotis septentrionalis, Myotis sodalis, Myotis leibii, Myotis grisescens, Eptesicus fuscus and Perimyotis subflavus*. There are several additional species for which *P. destructans* has been detected on skin tissues using swab sampling and quantitative PCR methods (Muller et al. 2013), but that have not been confirmed with characteristic skin lesions that define the disease.

Two of the species confirmed with WNS (*M. sodalis, M. grisescens*) were already listed as federally endangered under the US Endangered Species Act before WNS emerged and several other species have been predicted to go globally or regionally extinct due to mortality from WNS (Frick et al. 2010; Langwig et al. 2012; Thogmartin et al. 2013). The US Fish and Wildlife Service listed *M. septentrionalis* as federally threatened in 2015 due to the risk of extinction

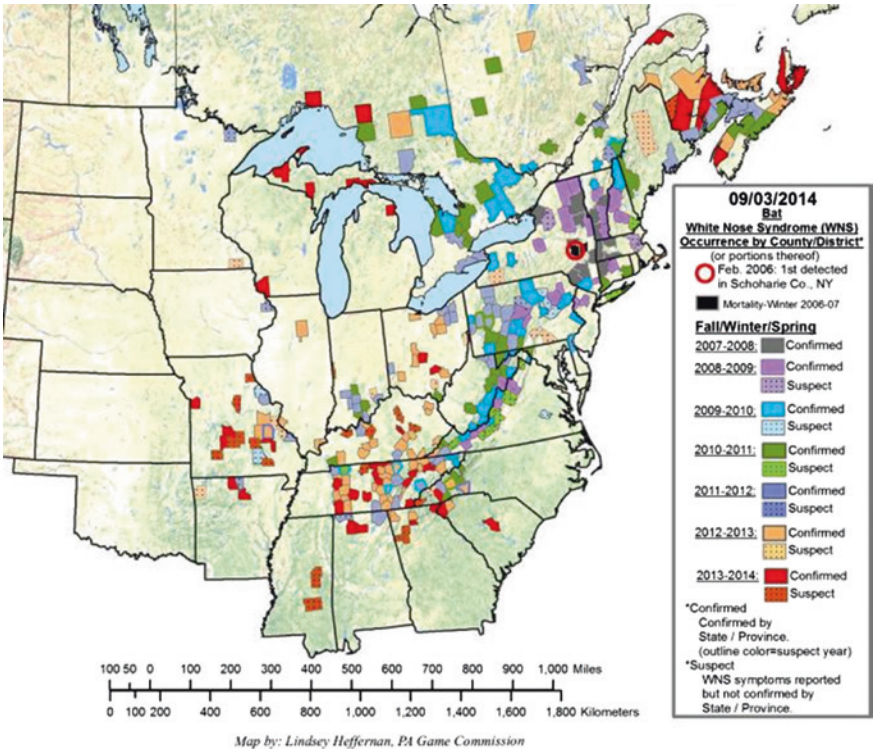

Fig. 9.3 Map of current distribution and past spread of WNS across North America. Confirmed WNS cases are those where disease has been confirmed by histological examination of tissues. Suspect cases are those that are either a molecular detection of *Pseudogymnoascus destructans* by quantitative PCR (Muller et al. 2013) or by visual signs and/or aberrant behaviour consistent with WNS disease at a site. Updated versions of this map are made publically available at whitenosesyndrome.org

from WNS-associated mortality. In addition, a status review of *M. lucifugus* is being conducted to determine whether listing as federally endangered is warranted of this once common species (Frick et al. 2010). In Canada, three species, *M. lucifugus*, *M. septentrionalis* and *P. subflavus* were listed as endangered in 2015. The rapid spread and extensive mortality associated with WNS raise serious concerns about population viability for species that are being impacted by this disease.

In this chapter, we review what is currently known about WNS, focusing on mechanisms of disease, disease ecology, global distribution patterns and conservation and management. We first explain why WNS belongs in a volume addressing bats in the Anthropocene. We review what is known about disease mechanisms, including what we currently understand about the physiology of the disease and immune response in bats. We then review what is currently known about disease ecology of WNS, including the population impacts to species, and then highlight

unanswered questions about transmission dynamics. We discuss global distributions patterns, focusing on what is known about WNS in Europe. We conclude by discussing current conservation and management strategies.

Wildlife disease is increasingly recognized as a major conservation threat (Daszak 2000). Global movements of humans increase the probability and rate at which we introduce pathogens into naïve ecosystems (Cunningham et al. 2003). This human-mediated spread of pathogens has been dubbed "pathogen pollution" to highlight the role of human trade and travel in the spread of wildlife pathogens (Cunningham et al. 2003). The fungus *P. destructans* was presumably introduced to North America from Europe by people, most likely from someone who had visited caves in Europe and subsequently visited Howe's Cave with contaminated boots or gear (Puechmaille et al. 2011c; Leopardi et al. 2015). No bats are known to migrate between the Americas and other continents, implicating human trade or travel in the trans-Atlantic arrival of the fungus (Wibbelt et al. 2010). Ironically, bats are often seen as reservoirs of diseases with consequences to human health (e.g. rabies, SARS, etc.). In the case of WNS, humans were most likely the unwitting transcontinental carrier of a pathogen that has killed millions of bats and now threatens species with extinction.

The emergence of WNS has dramatically changed conservation planning and population monitoring of temperate bats in North America (Foley et al. 2011). On the positive side, this crisis prompted collaborative research efforts among bat conservationists in North America and in Europe. Although mortality from WNS is currently restricted to North America, the pathogen is a potential threat to hibernating bat populations in other parts of the globe and is a global concern for bats in the Anthropocene (Puechmaille et al. 2011c).

9.2 Disease Mechanisms

Challenge or inoculation studies (e.g. Lorch et al. 2011; Warnecke et al. 2012; Wilcox et al. 2014) and comparative studies of bats from affected versus unaffected hibernacula (Moore et al. 2011; Storm and Boyles 2011; Reeder et al. 2012; Brownlee-Bouboulis and Reeder 2013) have led to progress in our understanding of mechanisms underlying WNS. The wings of bats are physiological active tissue involved in gas exchange and fluid balance. In general, results of physiological studies are converging on a consensus that cutaneous infection of the wings accounts for the physiological and behavioural effects of WNS (Cryan et al. 2010).

Lorch et al. (2011) experimentally inoculated the wings of healthy *M. lucifugus* with *P. destructans* for comparison to sham-inoculated controls. They housed bats in temperature- and humidity-controlled incubators that maintained environmental conditions approaching natural hibernacula [82 % relative humidity (RH) at 6.5 °C]. This experiment resolved a critical question by demonstrating that experimental infection with *P. destructans* caused the defining characteristics

of WNS (e.g. cupping erosions in the epidermis associated with fungal growth, Meteyer et al. 2009). They also found that *P. destructans* spread from infected to un-infected bats housed in the same cages but did not spread between cages in the same incubator confirming contact but not airborne transmission of the causal pathogen under laboratory conditions. Lorch et al. (2011) did not detect differences in survival between infected and un-infected bats possibly because the experimental duration was shorter than a typical hibernation season and/or because humidity in this experiment was lower than that of hibernacula used by *M. lucifugus* in the wild, potentially influencing hibernation patterns of both control and infected bats. Warnecke et al. (2012) repeated aspects of Lorch et al.'s (2011) experiment but increased ambient humidity to >97 % RH at 7 °C and ran the experiments for 120 days (vs. 102 days in Lorch et al. 2011). In Warnecke et al.'s (2012) experiment, all sham-inoculated bats survived four months of hibernation, while infected bats exhibited a significant increase in the frequency of periodic arousals, reduced fat reserves and reduced survival, thus confirming that infection with *P. destructans* alone causes the pathology that defines WNS, altered torpor behaviour and mortality. A field study comparing arousal frequency of bats in affected versus unaffected caves (Reeder et al. 2012) also reported a difference in arousal frequency similar to that observed by Warnecke et al. (2012). Together these findings suggest a strong role for increased arousal frequency and altered energy balance in WNS pathophysiology.

Comparisons of control and infected bats have also provided insight into immune responses (or lack of responses) of bats during and after hibernation. Hibernators generally exhibit down-regulated immune function during winter and bat species affected by WNS appear to be no exception (Meteyer et al. 2009, 2012; Moore et al. 2011). During hibernation, there is little evidence of initiation of an inflammatory response or recruitment of immune cells in bats infected by *P. destructans* based on histopathology (Meteyer et al. 2009, 2012). Despite the absence of an inflammatory response, however, variation in other aspects of cellular immunity may have a role to play. Moore et al. (2013) found differences in immunological responses of *M. lucifugus* in affected versus unaffected hibernacula, specifically higher leukocyte counts, reduced antioxidant activity and lower levels of interleukin-4 (an important precursor for differentiation of T-cells) in bats from WNS-affected caves. Although comparisons between populations of bats in different hibernacula are challenging to interpret because of the potential for underlying differences between bats independent of infection, these findings suggest that even the hardest-hit bat species attempt some, albeit weak, immune response to *P. destructans* infection. This also raises the possibility that some bats may be better equipped to resist infection than others (Puechmaille et al. 2011c) with the potential for directional selection on immune function if these differences are heritable and provide a survival advantage.

Immune responses of bats to WNS could be as much a disadvantage as an advantage. Meteyer et al. (2012) recently reported the disheartening paradox that some survivors of WNS exhibit characteristic signs of immune reconstitution inflammatory syndrome (IRIS). When infected bats emerge from hibernation and their immune

function resumes, they exhibit a massive neutrophilic inflammatory response to the fungal infection. This response appears to dramatically increase tissue damage and may reflect an over-reaction to infection because euthermic body temperatures in spring would likely be sufficient to combat the fungal infection (Chaturvedi et al. 2010; Puechmaille et al. 2011b; Verant et al. 2012). The response is likely energetically expensive and the resulting wing damage could compromise flight ability and, therefore, spring energy balance by increasing healing and immunity costs, while reducing potential foraging efficiency at a time when energy balance is critical to support reproduction. Further studies of the role of IRIS in the ecology of WNS are essential for understanding the potential for populations to recover from WNS.

A down-regulated immune response in hibernating bats generally, combined with increased arousal frequency (Boyles and Willis 2010; Reeder et al. 2012; Warnecke et al. 2012) and possibly increased metabolic rate and body temperature during torpor following infection (Storm and Boyles 2011; Verant et al. 2014), appears to result in premature fat depletion and starvation. However, why fungal infection would increase arousal frequency is still not fully understood. Cryan et al. (2010) proposed the hypothesis that fungal damage to the wings of bats could lead to increased evaporative water loss (EWL) across damaged epidermis. Rates of EWL during torpor are a strong predictor of arousal frequency in hibernators (Ben-Hamo et al. 2013; Thomas and Cloutier 1992; Thomas and Geiser 1997) so an increase in EWL or fluid loss due to skin damage from infection by *P. destructans* could lead to the observed effects on arousals. Willis et al. (2011) used data on water loss and arousal frequency in healthy bats, combined with an individual-based model quantifying survival of hypothetical populations of bats, to demonstrate that even a small increase in EWL resulting from infection could cause the same patterns of arousal and mortality observed for infected bats, thus highlighting the plausibility of the dehydration hypothesis.

Two independent datasets from both captive and free-ranging bats also support a role for dehydration and fluid loss in WNS pathophysiology (Cryan et al. 2013; Warnecke et al. 2013). In addition to high hematocrit levels consistent with dramatic fluid loss, Cryan et al. (2013) and Warnecke et al. (2013) both found evidence of electrolyte depletion (with no evidence of renal pathology), consistent with hypotonic dehydration due to fluid loss across damaged wings. Presumably infected bats lose fluid containing both water and electrolytes across injured wing tissue but can only replenish or partially replenish water stores by drinking, because electrolytes are not available in hibernacula. Warnecke et al. (2013) also found preliminary evidence of a respiratory response to metabolic acidosis in infected bats which they hypothesized reflect reduced perfusion of infected tissues, localized anaerobic metabolism and acidosis, and increased respiratory rate to increase CO_2 excretion and counter acidosis. In addition to increased arousal frequency, these physiological responses also predict increased metabolic costs and elevated body temperature during torpor. To date, measurements of torpid body temperature with enough precision to test this hypothesis are unavailable but these would be valuable, especially alongside measurements of metabolism during torpor and arousal in infected versus un-infected bats.

Other physiological mechanisms could also be at play. Willis and Wilcox (2014) reviewed three (of many potential) hormone systems that could be influenced by WNS, both within individuals and via selection on traits which could favour survival. For example, the lipostat hormone leptin is strongly associated with winter energy balance and pre-hibernation fattening. Bats must enter a state of leptin resistance during fall to accumulate adequate fat stores to survive the winter. If, as the evidence suggests, WNS represents a challenge for hibernation endurance, bats with the greatest leptin resistance (and therefore potential fat stores) in autumn may be best equipped to survive increased arousals associated with WNS (Willis and Wilcox 2014). Interactions between WNS and other hormone systems important for seasonal energetics, body temperature regulation and energy and fluid balance (e.g. glucocorticoids, melatonin, thyroid hormone, vasopressin, androgens) could also play important roles in disease dynamics and evolution of remnant populations and are worth further study.

In addition to physiological research, recent studies have also examined behavioural mechanisms associated with WNS that could reflect either adaptive responses to disease or maladaptive pathological responses. Langwig et al. (2012) reported that a much greater proportion of the *M. lucifugus* surveyed in WNS-affected caves after the emergence of the disease were hibernating solitarily (i.e. without clustering) compared to bats surveyed before WNS. This could reflect a behavioural change by individuals following infection or selection by WNS for bats which tend to roost individually (Langwig et al. 2012). Wilcox et al. (2014) reported behavioural observations of bats inoculated with *P. destructans* and found evidence supporting the former hypothesis. Infected bats gradually reduced their clustering behaviour as hibernation progressed. Wilcox et al. (2014) also observed a reduction in behavioural activity during arousals, in general, for affected bats. Taken together, reduced clustering and reduced activity by infected bats could reflect general patterns known as "sickness behaviour", a coordinated response to infection characterized in part by lethargy presumably to save energy for immune responses (Adelman and Martin 2009). These behaviours could also reduce the potential for transmission among individuals in a social group within a hibernaculum. Even bats that have already been infected with *P. destructans* could benefit by reduced subsequent exposure to other infected individuals because new contacts could lead to additional areas of infection in the wings, exacerbating disease severity. On the other hand, reduced clustering behaviour could increase energy expenditure and EWL leading to negative consequences for survival. More work is needed to understand the survival consequences of a range of physiological and behavioural responses to WNS.

9.3 Disease Ecology of WNS

One of the defining characteristics of WNS is that it is a multi-host disease, meaning that *P. destructans* infects multiple bat species. Although all hibernating bat species in northeastern North America can be infected with *P. destructans* and

develop the cupping erosions in their skin tissues that characterize the disease, population impacts from WNS vary widely among species (Langwig et al. 2012; Turner et al. 2011). Prior to the emergence of WNS in North America, all six hibernating bat species that occur in the northeastern United States had positive population growth trends (Frick et al. 2010; Langwig et al. 2012). With the emergence of WNS, four of these six species suffered severe population declines (*M. septentrionalis, M. lucifugus*, *M. sodalis* and *P. subflavus*) (Langwig et al. 2012). Two species (*M. leibii* and *E. fuscus*) have experienced less severe impacts from disease (Langwig et al. 2012). In addition, species of the genus *Corynorhinus* do not appear to get sick and die from WNS, despite occurring in WNS-affected caves in states in the mid-Atlantic region, such as West Virginia and Virginia. Why some species suffer higher mortality than others is an important area of current research, but there are no clear-cut answers yet. Langwig et al. (2012) showed that differences in roosting microclimates (temperature and RH) were correlated with differential impacts among sites for some species. For example, sites with warmer roosting temperatures had the highest declines for *M. lucifugus* and sites with highest RH had the highest declines for *M. sodalis*, suggesting that roosting microclimates could play an important role in WNS impacts (Langwig et al. 2012). Differences in environmental conditions as well as exposure, transmission, susceptibility, torpor physiology and immune response among species could contribute to observed differences in mortality. Future research focusing on differences in these factors among species will be critical for identifying the risks to particular species.

Understanding whether transmission is dependent on the density of hibernating populations is key to determining whether WNS will cause bats to go extinct or whether bat populations will stabilize at low numbers. For diseases where transmission is density-dependent, the probability of extinction is much lower because transmission rates decline as populations become smaller (De Castro and Bolker 2004). Langwig et al. (2012) showed that for bats that hibernate in dense clusters (e.g. *M. lucifugus* and *M. sodalis*), there was no evidence for density-dependent declines, meaning that declines from WNS were equally severe in populations that ranged from 100 to 100,000 bats. In contrast, there was evidence that declines were smaller in smaller populations for species that roost solitarily (e.g. *P. subflavus* and *M. septentrionalis*). Although the declines were density-dependent in *M. septentrionalis*, declines were not predicted to stabilize before populations went extinct in this species, suggesting that this species is at serious risk of extinction from WNS.

Determining whether a pathogen can persist in an environmental reservoir is also important for understanding disease transmission dynamics and extinction risk from disease (De Castro and Bolker 2004). Pathogens that can persist in an environmental reservoir are more likely to drive species extinct because hosts can get infected from the environment even if only a few individuals remain. Studies have shown that *P. destructans* is found in sediments and environmental substrates in hibernacula (Puechmaille et al. 2011a; Lindner et al. 2011; Lorch et al. 2013a, b). Lorch et al. (2013b) demonstrated that viable *P. destructans* can be cultured

from samples taken during late summer when bats have been absent for several months, suggesting that *P. destructans* persists in the environment between hibernation seasons. An unpublished experiment conducted by Al Hicks at the New York Department of Environmental Conservation demonstrated that naïve bats that had never been exposed to *P. destructans* could contract disease and die from WNS when placed in an infected hibernaculum with no access to other infected bats (Hicks, pers. comm.). The evidence to date suggests that hibernacula are environmental reservoirs for *P. destructans*, which has potentially dire consequences if the environment proves a major source of transmission.

WNS is a seasonal disease and recent work by Langwig et al. (2015) describes how the seasonal patterns of transmission of *P. destructans* are driven by hibernation. Bats begin to become infected in the fall when they return to hibernacula during fall swarm and transmission spikes in early winter once bats begin hibernating. Infection intensity increases during hibernation and peaks in late winter at which time most bats have become infected. These seasonal patterns are similar to temporal prevalence of visual signs of *P. destructans* growth on bats at sites in Europe as described by Puechmaille et al. (2011a), where a peak of infection was also observed in late hibernation when most individuals present were infected. In Langwig et al.'s study, most bats cleared infection during summer and prevalence of infection fell to zero by late summer at maternity roosts. The seasonal timing of infection suggests that mortality occurs at a time of maximal impact for populations (before the birth pulse). However, a peak in transmission after bats begin hibernating in early winter may reduce the rate of spread among hibernacula since bats presumably move among sites less frequently once they start hibernating compared to during the fall swarm period.

9.4 Status of *P. Destructans*/WNS in Europe

In contrast to the severe impacts WNS has on North American bat species, *P. destructans* is commonly found on bats in Europe but is not associated with mass mortality (Wibbelt et al. 2010; Puechmaille et al. 2011a). Europe is a putative source of the pathogen and the pathogen likely arrived in North America by some means of human trade or travel. Ongoing studies on global distribution of *P. destructans* (S.J. Puechmaille and J.R. Hoyt, unpublished data), including surveys in temperate Asia, may reveal important insights about the global distribution of the pathogen.

Pseudogymnoascus destructans was first reported in Europe by Puechmaille et al. (2010) who sampled a hibernating *Myotis myotis* from southwestern France showing the typical powdery white fungal growth on its nose. Since then, the fungus has been morphologically and genetically confirmed in 14 countries in Europe (France, Portugal, Belgium, The United Kingdom, The Netherlands, Germany, Switzerland, Austria, Slovakia, Poland, Hungary, Ukraine and Estonia) and

convincing photographic evidence further supports its presence in an additional four countries (Luxembourg, Denmark, Romania and Turkey [the European part]) (Martínková et al. 2010; Puechmaille et al. 2010, 2011a; Kubátová et al. 2011; Simonovicová et al. 2011; Mestdagh et al. 2012; Wibbelt et al. 2010, 2013; Burger et al. 2013; Paiva-Cardoso et al. 2014; Sachanowicz et al. 2014). At the continental scale, most European reports are from northeastern France through Belgium, the Netherlands, Germany and the Czech Republic, but it remains unclear whether this pattern of higher prevalence of the fungus is real or reflects sampling bias (Puechmaille et al. 2011a). Studies conducted in Italy, Slovenia and Sweden, where *P. destructans* was not detected (Voyron et al. 2010; Nilsson 2012; Mulec et al. 2013), support the hypothesis that *P. destructans* occurrence and/or prevalence varies between different geographic regions in Europe (Puechmaille et al. 2011a).

Puechmaille et al. (2011a) demonstrated that the prevalence of visible signs of *P. destructans* on bat wings and nose drastically varied through the hibernation period with the first cases appearing around mid-January. The number of cases increased to reach a peak in March and declined as bats emerged from hibernation. This pattern further complicates comparisons of prevalence of visual signs of fungal growth on bats between sites, regions or years unless surveys are carried out at the same time. Work done in the Czech Republic and Slovakia detected differences in prevalence of bats suspected to carry *P. destructans* (based on visual observations) between sub-mountain humid to mesic regions (higher prevalence) and mountainous and limestone regions (lower prevalence) (Martínková et al. 2010), supporting the idea that *P. destructans* is not equally abundant across Europe. Nevertheless, the differences in sampling strategy (spatio-temporal), sampling intensity (number of sites, number of samples), nature of the samples collected (e.g. swab from the bat vs. environment vs. guano) and analysis techniques (e.g. culture, PCR detection) between different European studies make quantification of these fine- and large-scale patterns challenging (Puechmaille et al. 2011a).

All confirmed cases of *P. destructans* infection come from fungal material collected on bats with the exception of a case from Estonia where the fungus has been isolated and cultured from the walls of the hibernation site, representing the first published isolation of viable spores from the environment in Europe or North America (Puechmaille et al. 2011a). In terms of species, available data suggest that *M. myotis* is the most commonly infected species (ca. 66 % of cases) with *P. destructans* in Europe (Martínková et al. 2010; Puechmaille et al. 2011a). The fungus is known to also infect another nine species of European *Myotis* (ranked by decreasing order of prevalence): *M. dasycneme, M. mystacinus, M. blythii, M. daubentonii, M. brandtii, M. emarginatus, M. nattereri, M. bechsteinii* and *M. escalerai*/sp. *A*. The list of species with *P. destructans* infection is likely to increase as sampling intensity increases as illustrated by the recent Zukal et al. (2014) study which reported infection of a few individuals from three more species of the family Vespertilionidae, *Eptesicus nilssonii*, *Plecotus auritus* and *Barbastella Barbastellus*, as well as on a single individual of *Rhinolophus hipposideros*, of the family Rhinolophidae.

Owing to the protection of bats across Europe and the absence of mass mortality, only three studies with limited to moderate numbers of samples have investigated the pathology of *P. destructans* during the hibernation period (Pikula et al. 2012; Wibbelt et al. 2013; Bandouchova et al. 2015). In Europe, *P. destructans* invasion of the wing membrane is generally restricted to the epidermis and adnexae without deep invasion into the underlying connective tissue but with occasional formation of neutrophilic pustules, contrasting with the common and extensive invasion of dermal connective tissue in bats from North America (Pikula et al. 2012; Wibbelt et al. 2013; Zukal et al. 2014; Bandouchova et al. 2015). Based on investigation of two euthanized individuals, *P. destructans* invasion in the skin of the muzzle seems to be more pronounced than invasion of the wing membrane (Pikula et al. 2012; Wibbelt et al. 2013). As damage to the skin of the muzzle may not be as physiologically important for homeostasis as damage to the wing membranes (Cryan et al. 2010; Reeder et al. 2012; Warnecke et al. 2013), we suggest that it may be important to differentiate the pathology of *P. destructans* on the wing and on the muzzle. If dehydration and fluid loss play an important role in WNS pathophysiology, quantifying wing damage consistently (e.g. following Reeder et al. 2012 or an alternative scoring system) alongside physiological measures of disease severity will be critical for a better understanding of the disease, its progression and species-specific attributes, compared to the commonly reported dichotomous presence/absence of the disease.

The term WNS was originally used to describe the symptoms associated with bats in the field before the disease was fully characterized as a cutaneous infection of skin tissues by the pathogenic fungus, *P. destructans* (Blehert et al. 2009; Meteyer et al. 2009). As such, the name ‘WNS’ has changed from referring to a set of symptoms, including visible fungal growth on skin surfaces, depletion of fat reserves, altered torpor patterns and aberrant winter behaviour (Blehert et al. 2009) to referring to the presence of disease as defined by the presence of cutaneous infection characterized by cupping erosions (Meteyer et al. 2009). This has led to confusion and some debate about whether the term WNS should be used to describe infections occurring in Europe, which are pathologically similar to those in North America but which do not include mass mortality or aberrant winter behaviour (Puechmaille et al. 2011a). Despite its original definition as a syndrome (Veilleux 2008; Reeder and Turner 2008; Turner and Reeder 2009), the term WNS is now routinely used to refer the cutaneous infection caused by *P. destructans*, which have been documented in Europe (Pikula et al. 2012; Wibbelt et al. 2013; Zukal et al. 2014). Some have advocated a name change to clarify a difference between a ‘syndrome’ and a ‘disease’ caused by fungal infection (Chaturvedi and Chaturvedi 2011). Inconsistency in the literature could lead to confusion but recent use of the term white-nose disease (WND; Paiva-Cardoso et al. 2014) could clarify the situation by providing terminology reminiscent enough of WNS to avoid confusion but technically consistent with the definition of a disease.

Recent work comparing colony sizes of hibernating vespertilionid bats in North America before and after the emergence of WNS, to current colony sizes in Europe, reveals an intriguing pattern. Before WNS emerged in North America,

colony sizes of hibernating bats were, on average, about 10-fold larger than those of similar species in Europe (Frick et al. 2015). However, after the emergence of WNS, colony sizes in eastern North America are no longer statistically different from those in Europe (Frick et al. 2015), raising the following question: Were hibernating bat colonies in Europe once much larger prior to the emergence of WNS there? If WNS is indeed acting as a hidden force on bat populations in Europe, then small winter colony sizes in eastern North America may become the norm for species in North America that manage to persist. However, Frick et al. (2015) also show that 69 % of winter colonies of *M. septentrionalis* were entirely eliminated within 7 years of WNS detection, suggesting that this species is rapidly disappearing from the landscape. The predicted extinction of *M. septentrionalis* from WNS begs the question whether past extinctions of bat species may have also occurred in Europe.

9.5 Conservation and Management

Conservation and management strategies for WNS in North America have focused primarily on preventing spread of the pathogen to new areas through decontamination protocols as well as cave closures to limit the potential for human-mediated spread. Decontamination of gear used in hibernacula by both recreational cavers and bat researchers is an important management strategy to reduce the risk of spread of *P. destructans* by humans. *P. destructans* spores have been found on field gear after use in infected sites and therefore utmost precaution is needed to reduce the chance that researchers and cavers spread *P. destructans* to new areas. Cave closures have been controversial and have been met with some resistance by some members of the caving community. Some cave closures have subsequently been relaxed in parts of the western United States where *P. destructans* has not yet spread. Determining whether cave closures are effective can be challenging given that the absence of spread in areas is hard to measure. Bats are capable of spreading the fungus, but the primary focus of closing caves and advocating decontamination was to slow spread by people, especially to distant locations.

Finding a treatment for infected bats has proved elusive and difficult. Several studies have examined the efficacy of treating bats with anti-fungal chemicals, such as terbinafine, but none have shown any promise. There has also been interest in alternative forms of treatment, including use of naturally occurring bacteria (Fritze et al. 2012; Hoyt et al. 2015) or volatile compounds (Cornelison et al. 2014). Recent work by Cornelison et al. (2014) showed that a volatile organic compounds (VOCs) inhibited growth of *P. destructans* in vitro. Similarly, a recent study by Hoyt et al. (2015) showed that *Pseudomonas* bacteria that naturally occur on hibernating bats inhibit growth of *P. destructans* in vitro. Other strains of *Pseudomonas* found in Europe have shown similar results (Fritze et al. 2012). Research on these biological control treatment options is still in early stages and although early lab results have shown promise, experimental and field trials will

need to be conducted before the efficacy of these approaches is fully evaluated. The WNS research and management community is developing standards and protocols for evaluating the safety and efficacy of biological treatment options.

Other ideas for active management have included building artificial hibernacula that can be cleaned and decontaminated each summer between hibernating seasons. An experimental artificial hibernacula was built in Tennessee and existing military bunkers have been used as artificial hibernaculum in the northeastern US. The goal of these structures is to provide a place for bats to hibernate that does not serve as an environmental source of transmission when bats re-enter the hibernaculum in fall. To date there have been no studies to determine whether bats will use these artificial hibernacula naturally and whether survival will be improved in these sites.

Given what we know about the potential role that electrolyte depletion plays in the physiology of the disease, some researchers have also explored the potential for electrolyte therapy for hibernating bats by providing access to electrolyte supplements during hibernation. Experimental trials to test this are underway. Finally, bats are very difficult to breed in captivity and, while the prospect of captive breeding and management of bats has been explored, it remains doubtful whether this approach could be useful as a management tool for bat species affected by WNS. However, if breeding programmes could be developed, they could provide a supply of animals for laboratory studies to reduce potential impacts of research on wild populations.

9.6 Conclusions

Although we have learned a great deal about WNS in the past seven years, there are still many unanswered questions about disease mechanisms, ecology, transmission dynamics, long-term impacts, global distribution patterns and potential treatment options that will be important for managing WNS and its impacts on bats. The US Fish and Wildlife Service has been pivotal in terms of coordinating meetings for information exchange among researchers and state biologists as well as directly funding much of the research on WNS in both the US and Canada. Research priorities for management and conservation of species have focused on topics such as establishing that *P. destructans* was the causative agent of infection, trying to identify potential treatment of infection, the physiology of infection and mechanisms of mortality, characterizing the environmental reservoir and understanding transmission and immunological response.

For many of us, working on WNS is a grim business. There is nothing quite like the experience of going underground and entering a chamber that was formally home to thousands of bats and seeing empty walls and a few straggling survivors covered in white fungus. However, the sense of commitment within the WNS community and the dedication of researchers and managers to try and find new ways to understand and solve this crisis provide a certain hope. We have yet to find

a way to stop bats dying from WNS, but we are trying hard to do so. Whether we are able to prevent species extinctions may rely, in part, on the creativity to find solutions before it is too late and the willingness of agency biologists to implement creative solutions without clear assurances of outcomes.

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