Qualitative risk assessment:
White-nose syndrome in bats in Australia

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Executive Summary

White-nose syndrome is caused by the fungus *Pseudogymnoascus destructans*. *P. destructans* is a psychrophilic or cold loving fungus that thrives at temperatures below 15^{o}C and ceases growth above 20^{o}C. Since its appearance in North America in 2006 it has killed an estimated five to six million cave-dwelling, insectivorous bats and has spread throughout the eastern USA and Canada. Subsequent surveys have identified the fungus on bats in both Europe and China. While the fungus causes disease in bats in these areas it is not associated with the large mortalities seen in the USA. Reasons for this have not been resolved, but likely involve a combination of species susceptibility, behavior and environmental factors such as temperature and humidity. The fungus appears to cause the death of bats by damaging their wings during hibernation. This results in increased evaporative water loss and dehydration causing the bats to wake more frequently during hibernation, thus consuming all of their energy reserves over the winter period. Dead bats are found dehydrated and emaciated.

P. destructans is believed to be absent from Australia. This qualitative risk assessment examines the likelihood of the fungus entering Australia, and contacting Australian bats, and the consequences should this occur.

Based on available information it seems almost certain that *P. destructans* will enter Australia and contact bats at some point in the future. Bats most likely to be affected by the fungus are the cave-dwelling bats from southern Australia:

- Southern Bent-winged Bat
- Eastern Bent-winged Bat
- Eastern Horseshoe Bat
- Chocolate Wattled Bat
- Large-eared Pied Bat
- Large-footed Myotis
- Finlayson's Cave Bat

While Australia's milder climate should preclude the large mortalities seen in the USA the fungus still has the potential to impact Australia's bat populations, particularly the bent-winged bats. Both subspecies that occur in southern Australia are likely naïve to the fungus and cluster in large groups, an activity associated with high mortality in the USA. As the Southern Bent-winged Bat is listed as critically endangered any additional mortality could have serious consequences for its long term survival. Therefore, the risk posed by white-nose syndrome is high. The Eastern Bent-winged Bat has a more stable population and the risk level is medium.

The other bats listed have stable populations, and roost in smaller groups. The risk of white-nose syndrome to these groups is likely to be low.

Goal, Scope and Focus of the Disease Risk Assessment

This project is a disease risk analysis for white-nose syndrome for Australia. It presents the results of a qualitative risk assessment of the likelihood of incursion and spread of white-nose syndrome in Australian bats. The risk assessment identifies the possible pathways of introduction of the disease into Australia, the likelihood of an incursion via each pathway and the likely consequences. This includes consideration of the differences in climate, bat ecology and immunity that are likely to impact on the epidemiology of the disease in Australia.

It also identifies potential host species and geographic areas at most risk in Australia.

INTRODUCTION

White-nose syndrome has caused the death of five to six million bats in North America since 2006, resulting in a devastating effect upon the conservation status of many species (Turner *et.al.* 2011). The suddenness and scale of this disaster has raised concern about the potential for a similar epidemic to occur in Australian bats. This report was commissioned to assess the risk and consequences of white-nose syndrome for Australian bats.

BACKGROUND

AETIOLOGIC AGENT

The causative agent of white-nose syndrome (WNS) is a fungus called *Pseudogymnoascus destructans* (formerly known as *Geomyces destructans*). The fungal genome has been sequenced and can be viewed at http://www.broadinstitute.org/news/1516. Experimental studies have confirmed that *P. destructans* is a primary pathogen and the cause of WNS (Lorch *et al.* 2011).

In vitro, P. destructans colonies are white marginally, while conidial (spore) masses at colony centres are grey to grey-green. Colony reverse is uncoloured on cornmeal agar and drab to hair brown on Sabouraud dextrose agar. On cornmeal agar asymmetrically curved conidia are borne singly at the tips, on the sides or in short chains on verticillately branched conidiophores (hyphal branches). Conidiophores are smooth, thin-walled and narrow, 1.5-2 µm wide by 35-90 µm or more in length bearing verticils of two to four branches borne at an acute angle to the stalk. Conidia are 5-12 x 2.0-3.5 µm, tapering basally to 1.5-2.0 µm and apically to 0.5-1.5 µm (Gargas *et al.* 2009).

AFFECTED SPECIES

Bat species infected with *P. destructans* have been detected in North America, Europe and China. In North America, seven species of cave-roosting bats, including two endangered species (Gray Bat (*Myotis grisescens*) and Indiana Bat (*Myotis sodalis*)), have been impacted by WNS, with the fungus found on a further five but without clinical signs (Table 1). A recent survey from China examined 385 environmental samples from 12 caves and 215 samples from nine bat species in 10 caves and found *P. destructans* on six species (Table 2). Least Horseshoe Bat (*Rhinolophus pusillus*), Large-footed Bat (*Myotis adversus*), and Rickett's Big-footed Bat (*Myotis pilosus*) tested negative for *P. destructans*. Of the environmental samples nine of the 12 sites tested were positive for *P. destructans* (Hoyt *et al.* 2016). Thirteen species have been recorded with clinical signs of WNS in Europe (Table 3).

The following tables give an overview over the affected species, including their habitat preference and whether they displayed clinical signs associated with WNS.

Table 1. *Pseudogymnoascus destructans* has been found on the following bats in North America (Bernard *et al.* 2015).

Species	Scientific name	Cave-dwelling	Non cave-dwelling	Clinical signs of WNS	No clinical signs of WNS
Little Brown Bat	*Myotis lucifugus*	x		x	
Northern Long-eared Bat	*Myotis septentrionalis*	x		x	
Big Brown Bat	*Eptesicus fuscus*	x		x	
Tri-coloured Bat	*Perimyotis subflavus*	x		x	
Indiana Bat	*Myotis sodalis*	x		x	
Gray Bat	*Myotis grisescens*	x		x	
Eastern Small-footed Bat	*Myotis leibii*	x		x	
Silver-haired Bat	*Lasionycteris noctivagans*		x		x
Virginia Big-eared Bat	*Corynorhinus townsendii virginianus*	x			x
Eastern Red Bat	*Lasiurus borealis*		x		x
Rafinesque's Big-eared Bat	*Corynorhinus rafinesquii*	x			x
South-eastern Myotis	*Myotis austroriparius*	x			x

Table 2. *Pseudogymnoascus destructans* detected in bats in China (Hoyt *et al*., 2016).

Species	Scientific name	Clinical signs of WNS	No clinical signs of WNS
Eastern Water Bat	*Myotis petax*	x	
Eastern Long-fingered Bat	*Myotis macrodactylus*		x
Large Myotis	*Myotis chinensis*		x
Ussuri Tube-nosed Bat	*Murina ussuriensis*		x
Greater Horseshoe Bat	*Rhinolophus ferrumequinum*		x
Greater Tube-nosed Bat	*Murina leucogaster*		x

Table 3. *Pseudogymnoascus destructans* has been found on the following bats in Europe (Zukal *et al*. 2014, Zukal *et al*. 2016).

Species	Scientific name	Cave-dwelling	Non cave-dwelling	Clinical signs of WNS	No clinical signs of WNS
Greater Mouse-eared Bat	*Myotis myotis*	x		x	
Pond Bat	*Myotis dasycneme*		x	x	
Daubenton's Bat	*Myotis daubentonii*	x		x	
Natterer's Bat	*Myotis nattereri*	x	x	x	
Bechstein's Bat	*Myotis bechsteinii*		x	x	
Geoffroy's Bat	*Myotis emarginatus*	x		x	
Northern Bat	*Eptesicus nilssonii*	x	x	x	
Barbastelle	*Barbastellus barbastellus*		x	x	
Brown Long-eared Bat	*Plecotus auritus*	x	x	x	
Lesser Horseshoe Bat	*Rhinolophus hipposideros*	x	x	x	
Mediterranean Horseshoe Bat	*Rhinolophus euryale*	x		x	
Brandt's Bat	*Myotis brandtii*	x		x	
Common Bent-winged Bat	*Miniopterus schreibersii*	x		x	
Lesser Mouse-eared Bat	*Myotis blythii*	x			x
Whiskered Bat	*Myotis mystacinus*	x			x

WORLDWIDE DISTRIBUTION

WNS was first recognised in North America in the state of New York in 2006 and since then has spread along the eastern seaboard with subsequent cases appearing in Alabama, Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, North Carolina, Ohio, Pennsylvania, Rhode Island, South Carolina, Tennessee, Vermont, Virginia, West Virginia, and Wisconsin. In March 2016 the first case of WNS in Washington State was seen in a Little Brown Bat, approximately 2,000 kilometres from the previous westernmost detection of *P. destructans* (Fig. 1). The fungus, but not the disease, has also

been found in Mississippi, Nebraska and Oklahoma (https://www.whitenosesyndrome.org/about/where-is-it-now, http://www.promedmail.org).

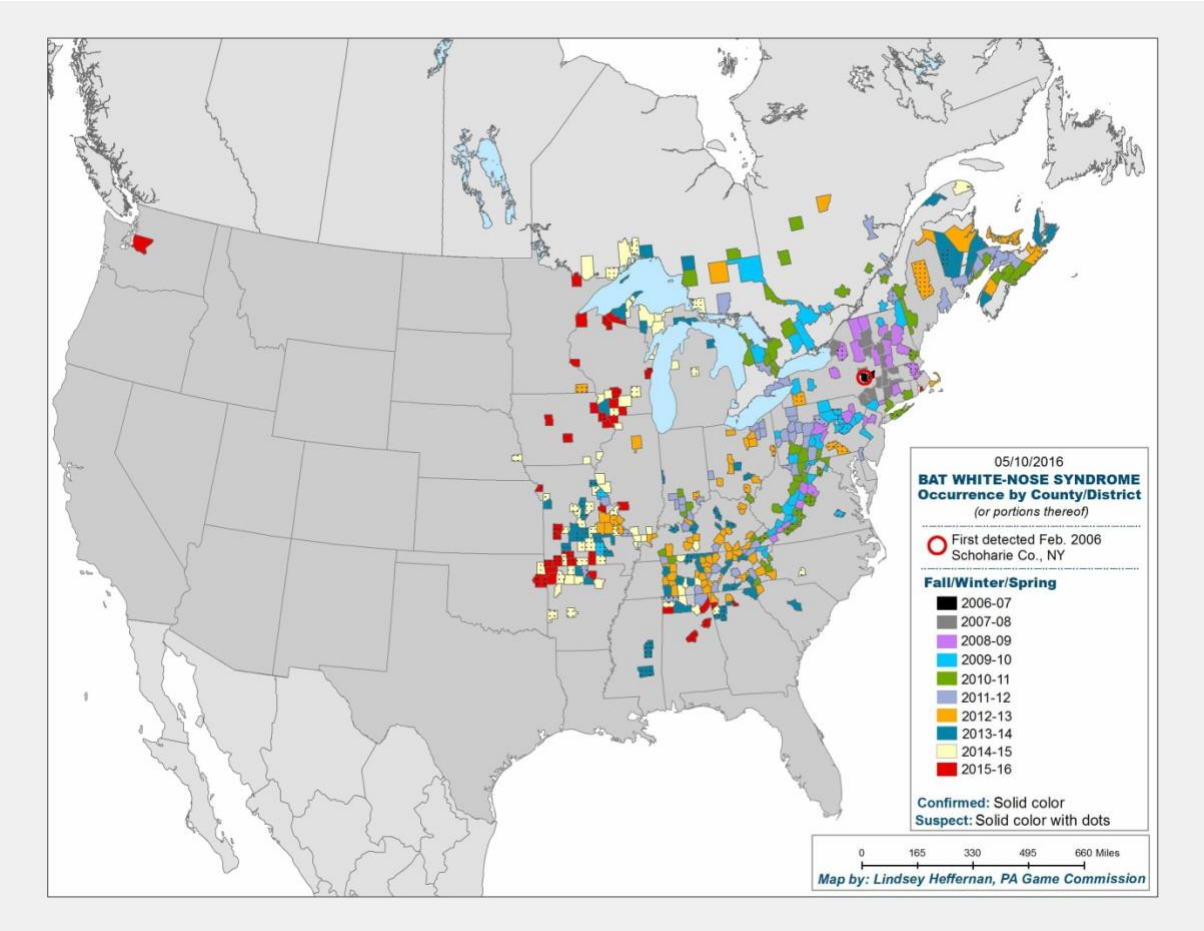

Figure 1. White-nose syndrome distribution in the USA and Canada as at 10 May 2016 (https://www.whitenosesyndrome.org/resource/updated-white-nose-syndrome-map-may-10-2016).

The disease was first identified in Canada in March 2010 in Ontario, and has since spread to Quebec, Nova Scotia, New Brunswick and Prince Edward Island. (https://www.whitenosesyndrome.org/about/where-is-it-now, http://www.promedmail.org).

In Europe, *P. destructans* has been found on bats with no evidence of fungal growth and also on bats with clinical signs of WNS in Austria, Belgium, Czech Republic, Denmark, Estonia, France, Germany, Great Britain, Hungary, Latvia, Netherlands, Poland, Romania, Russia, Slovakia, Slovenia, Switzerland, Turkey and Ukraine (Fig. 2). A survey of 367 bats in Sweden failed to find any evidence of the fungus (Ågren *et al.* 2012). There are no confirmed reports of *P. destructans* in Europe prior to 2008. However, anecdotal reports indicate the fungus may have been present as far back as the 1970s (Martinkova *et al.* 2010, Puechmaille *et al.* 2010, Wibbelt *et al.* 2010, Puechmaille *et al.* 2011a, Puechmaille *et al.* 2011b, Barlow *et al.* 2015).

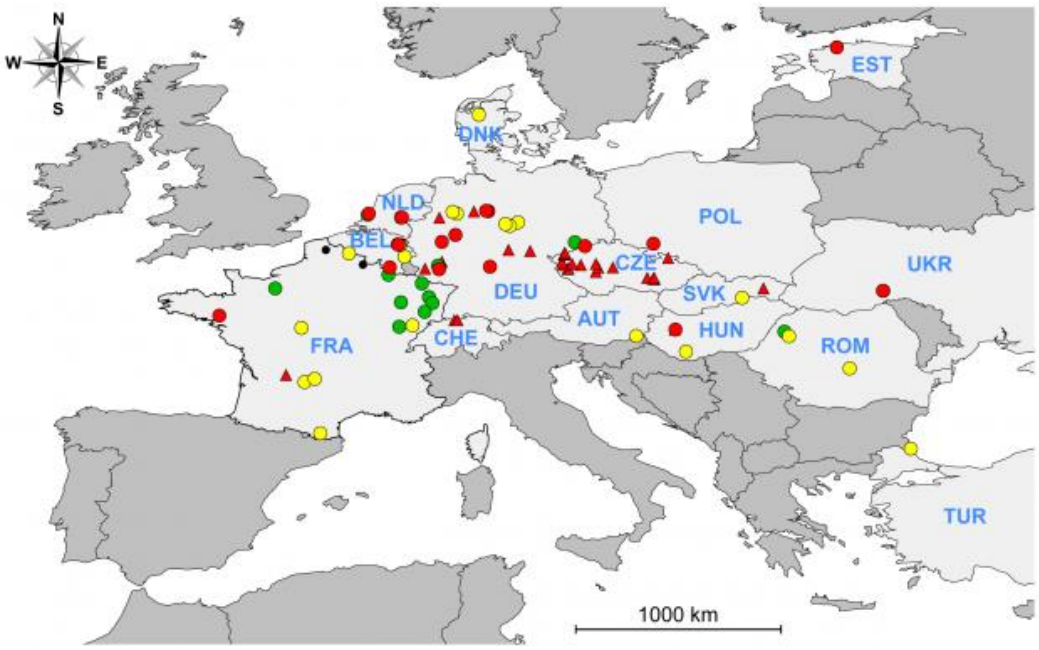

Figure 2. Distribution of confirmed and suspected records of *P. destructans* on hibernating bats in Europe, May 2011 (Great Britain, Latvia, Russia and Slovenia have since confirmed the presence of the fungus). Confirmed records of *P. destructans* in red, photographic evidence in yellow, visual reports in green (Zukal *et al.* 2016).

A survey conducted in north-eastern China (Shandong, Liaoning and Jilin provinces, and Beijing municipality) in 2014 and 2015 found *P. destructans* on six species of bats, and on nine of 12 cave surfaces sampled (Fig. 3) (Hoyt *et al.* 2016).

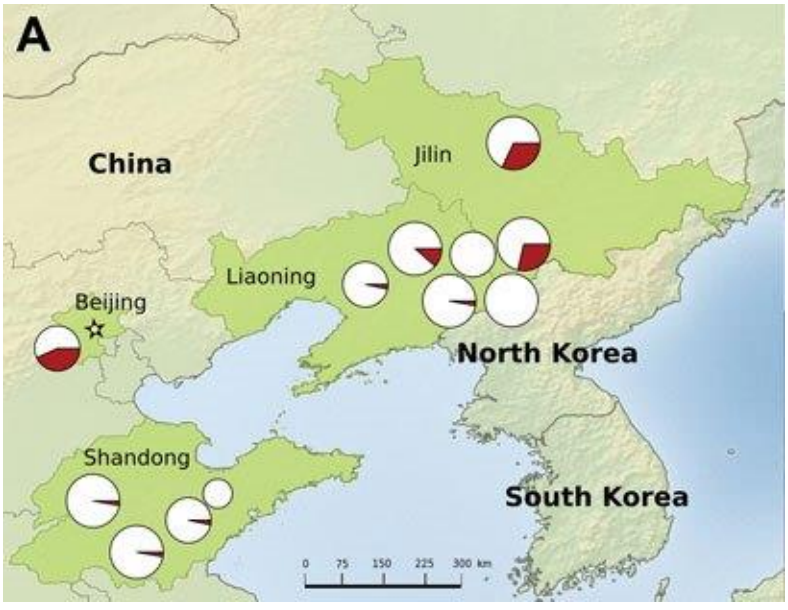

Figure 3. Distribution of *P. destructans* in cave environments, during the summer of 2014, in north-eastern China. Pie charts show the prevalence of *P. destructans* (in red), and the size of the pie graphs indicates the number of samples taken at each site (range 10-35) (Hoyt *et al.* 2016).

OCCURENCES IN AUSTRALIA

Pseudogymnoascus destructans has not been reported from Australia. The first recorded testing occurred in 2008 in NSW. Since then only a very small number of bats have been submitted for testing. Cases include an East-coast Free-tailed Bat (*Mormopterus norfolkensis*) and an unknown species of microbat from NSW, and horseshoe bats (*Rhinolophus* sp.), a Northern Free-tailed Bat (*Mormopterus lumsdenae*)[1] and a vespertilionid bat (*Scotorepens* sp. or *Vespadelus* sp.) from Queensland. There were fungi present but they were considered to be environmental overgrowths, not *P. destructans* (Barrett *et al.* 2014).

Swabs were collected in 2015 from 32 Southern Bent-winged Bats (*Miniopterus orianae bassanii*) trapped outside a cave in western Victoria and 61 Eastern Bent-winged Bats (*Miniopterus orianae oceanensis*) trapped outside a mine in the Yarra Valley, Victoria (unpublished data, Holz *et al.*). All tested negative by PCR for *P. destructans*. Twenty-three environmental samples collected from a mine in the Yarra Valley, and caves near Warrnambool, also tested negative (unpublished data, Holz *et al.*).

Based on current information, there are four possible scenarios: 1) *P. destructans* may be absent from Australia; 2) it may be present at a very low prevalence as it has only recently been introduced; 3) it may be present at a low prevalence but not associated with clinical signs or a population level effect (and therefore only mild consequences); 4) or it may be present in unmonitored bat populations. Further sampling is therefore required to attain a higher level of confidence that the fungus is not present.

FUNGUS PROPERTIES

The fungus is psychrophyllic, meaning it grows best at low ambient temperatures. *In vitro* studies have found that optimal temperatures for growth are between 12.5 and 15.8 °C, with cessation of growth above 20 °C. Typical *P. destructans* morphology is seen if the fungus is grown between 0 and 7 °C. Above 12 °C hyphae and conidia display atypical morphologies, with hyphae becoming thicker and diffusely septate, and conidia becoming pyriform to globoid in shape. Above 15 °C hyphae become markedly deformed, with a complete loss of characteristic hyphal structure and curved conidia above 18 °C. If grown between 7 and 12 °C colonies display a mixture of typical and atypical characteristics (Verant *et al.* 2012).

The fungus is slow growing. On Sabouraud dextrose agar and cornmeal agar, colony diameter, after 16 days, was 1.0 mm at 3 °C, 5 mm at 7 °C, and 8 mm at 14 °C. (Gargas *et al.* 2009).

P. destructans can survive exposure to 38 °C for at least three days but does not remain viable after eight days. It survives exposure to 30 °C for at least 15 days.

[1] Bat taxonomy follows Jackson and Groves (2015)

While the fungus grows best at humidity levels above 90% it is able to survive prolonged periods of low humidity. In one study, culture plates of *P. destructans* were incubated at 5 °C and 30 to 40% relative humidity for over five years. At the end of this period, conidia from the dehydrated plates were subcultured onto fresh media and incubated for 30 days at 10 °C. Fresh colonies grew on six of the nine plates (Hoyt *et al.* 2014).

In vitro P. destructans produces the enzymes β-glucosidase, N-acetyl-β-glucosaminidase, acid and alkaline phosphatases, esterase/esterase lipase/lipase, leucine and valine arylamidase, naphthol-AS-B1-phosphohydrolase, various proteinases, and urease. No enzymatic activity was indicated for endoglucanase, cysteine arylamidase, α-chymotrypsin, alpha/beta galactosidase, trypsin, β-glucoronidase, α-fucosidase, and α-mannosidase (Chaturvedi *et al.* 2010, Reynolds and Barton 2014). A range of peptidases are secreted by *P. destructans*. These proteolytic enzymes are capable of degrading collagen and likely facilitate tissue invasion (Field *et al.* 2015, O'Donoghue *et al.* 2015, Pannkuk *et al.* 2015).

In vitro studies have found that *P. destructans* is capable of germination and growth on freeze dried migratory locust (*Locusta migratoria*), fresh guppy (*Poecilia* sp.), dried Shiitake mushroom (*Lentinula edodes*) and demineralised Argentine red shrimp (*Pleoticus muelleri*) exoskeletons. The fungus demonstrated no keratinolytic activity. It was able to utilise multiple nitrogen sources (nitrate, nitrite, ammonium and L-asparagine) but this was pH dependent. Growth on nitrate was better at pH 5-7, growth on L-asparagine and ammonium was greatest at high pH and growth on nitrite only occurred at pH 7-8. *P. destructans* showed uniform growth between pH 5 and pH 11 and it was not inhibited by the presence of sulphur compounds (Raudabaugh and Miller 2013). While one study suggested that *P. destructans* had no chitinase or cellulase activity (Raudabaugh and Miller 2013) a more recent study indicated that chitinase and cellulase activity was present but at reduced levels compared with saprotrophic members of the genus (Reynolds and Barton 2014).

Based on these results *P. destructans* is capable of growth on a range of substrates including invertebrate exoskeletons, feathers, hair, skin, moist plant material, and guano. It prefers a neutral or alkaline cave environment and its growth is not inhibited by sulphur compounds that are found in some soils.

TRANSMISSION

Transmission of *P. destructans* has been shown to be by direct contact from infected bats to healthy bats and by direct contact between bats and the cave substrate. When infected bats were placed in cages 1.3 cm apart from uninfected bats transmission failed to occur. These findings imply that airborne transmission of *P. destructans* is unlikely (Lorch *et al.* 2011).

P. destructans can persist in the environment, even when bats are not present; making cave surfaces an important mode of transmission to bats. Viable *P. destructans* spores have been found on the walls of hibernation sites in Europe, while a US study detected *P. destructans* in sediment from two mines that had been closed to bats for one to two years (Puechmaille *et al.*

2011a, Lorch *et al.* 2013). A recent study also identified *P. destructans* in three out of 19 soil samples from hibernacula in states where WNS is known to occur (Massachusetts, Connecticut and New Hampshire). All five soil samples collected outside the region where WNS occurred at the time (Indiana, Kentucky, Minnesota, Mississippi and Wisconsin) were negative (Lindner *et al.* 2011). These findings were supported by a second study that found *P. destructans* in 17 out of 21 sediment samples collected from hibernacula within the known range of WNS, while all 28 sites sampled beyond the known range of the fungus were negative (Lorch *et al.* 2013).

Humans have been implicated in the spread of the disease as some caves frequented by people have WNS while nearby caves, not used by people, are not affected (Turner *et al.* 2011).

A recent study found evidence of *P. destructans* on bat mites of the genus *Spinturnix*. These mites were present on bats affected with WNS and the authors speculated that the mites could act as mechanical vectors transferring the fungus between bats (Lucan *et al.* 2016). It is possible that other ectoparasites, such as ticks and bat flies, could act in a similar manner.

EPIDEMIOLOGY

The current hypothesis is that *P. destructans* was imported into North America from Europe, possibly on shoes or equipment used in caves (Turner *et al.* 2011). This is partly based on its initial appearance at a single site followed by its radial spread from that index site. Evidence to support this hypothesis is provided by a study that examined 16 fungal isolates from New York State and Vermont and found them to be genetically identical, consistent with the clonal dispersal of a single genotype (Rajkumar *et al.* 2011). Two European studies sequenced 42 and 32 fungal samples, respectively. All 42 samples and 28 of the 32 samples were identical to the North American isolates. However, four of the 32 samples exhibited a different allele, leading the authors to speculate that the occurrence of *P. destructans* in Europe predated its presence in North America (Martinkova *et al.* 2010, Wibbelt *et al.* 2010).

Further support for this theory is provided by the absence of *P. destructans* from the environment of WNS negative areas and a study that infected Little Brown Bats with both the North American and European strains of *P. destructans*. Both groups of bats developed clinical signs of WNS, and increased frequency of arousal during hibernation (three to four times the control group), indicating that the European strain of *P. destructans* was as pathogenic as the North American strain and not an avirulent strain (Warnecke *et al.* 2012). However, it has been suggested that disease effects have not been as severe outside of North America at least partly because bats have some level of resistance to both infection and disease, as demonstrated by lower fungal loads and prevalence in European and Chinese bats (Zukal *et al.* 2016, Hoyt *et al* 2016b). The mechanism of this possible resistance has not been determined.

Temperatures in affected North American and European bat hibernacula range from 2 to 15 °C, permitting year round growth and maintenance of the fungus (Blehert *et al.* 2009, Puechmaille pers. comm.). Cave temperatures approximate the mean annual ambient temperature for the region they are in, with some variation depending on distance from the cave entrance and air flow through the cave (Smithson 1991). Therefore, if the mean annual temperature for an area is known the probable cave temperature in that region can be estimated (See Appendix III). More severely affected bats reside in areas with lower mean ambient temperatures and presumptive lower cave temperatures.

WNS is a seasonal disease with lesions first appearing in North American bats in late September, and mortalities starting in the middle of winter in January, approximately 120 days after the bats have entered hibernation. Mortality peaks in March and declines during April and May, before the disease disappears in June (Lorch *et al.* 2011). A more recent North American study found bat infection prevalence in early autumn to be between 5 and 50%. This increased to nearly 100% during winter and decreased to zero by late summer (Langwig *et al.* 2015).

A European study failed to find any infected bats between September and the end of January (early autumn to mid-winter). The first infected bats appeared in February (late winter) with numbers peaking in March (reaching a prevalence of 18-25% in 2007 and 28-55% in 2008) and declining again in April (mid spring) as bats began to leave their hibernacula. In stark contrast to the situation in North America infection rates were not associated with any increases in mortality (Puechmaille *et al.* 2011b).

The effect of WNS on bat colonies is related to colony size and behaviour. North American bat colonies were, on average, ten times larger than European ones before the introduction of WNS whereas, seven years after its introduction, North American colony sizes have decreased to the point where they are no longer significantly different from European ones (Frick *et al.* 2015).

A recent US study found that Northern Long-eared Bats and Tri-coloured Bats rarely form large clusters during hibernation, contact between individuals increasing as colony size increases, leading to density dependent fungal transmission and greater declines in larger colonies. In contrast Little Brown Bats and Indiana Bats form tight clusters during hibernation, irrespective of colony size, leading to equally severe population declines regardless of the number of bats present in a colony, thus putting these species at a greater risk of extinction. However, Little Brown Bats were observed to modify their behaviour after the arrival of WNS with more bats roosting individually or in smaller groups. Indiana Bats made minimal changes to their social behaviour post WNS, leaving them at greater risk of extinction (Langwig *et al.* 2012).

This study also found that Little Brown Bat population declines increased as cave temperature rose from 2 ºC to 9 ºC. However, Indiana Bat declines increased as cave humidity rose from 85% to 99%, but were unaffected by temperature, possibly because the

lower temperatures (1 ºC to 4 ºC) where Indiana Bats hibernate have less effect on fungal growth rates (Langwig *et al.* 2012).

European bats tend to hibernate in small clusters, rather than in large aggregations, which could partly explain the decreased effect of the fungus on these populations (Martinkova *et al.* 2010).

CLINICAL SIGNS

Many, but not all, affected bats have a grossly visible white fungal growth on muzzles, ears and wing membranes, which can lead to scarring and necrosis (Blehert *et al.* 2009).

On average North American bats wake every two to three weeks during winter (mean torpor length of 16 days for healthy Little Brown Bats), increasing their body temperature from close to ambient to 35 to 39 °C, and remaining active for one to two hours (38 to 180 minutes with an average of 78 minutes for Little Brown Bats) (Reeder *et al.* 2012). When affected by WNS, waking frequency for Little Brown Bats increased to approximately every eight days (Reeder *et al.* 2012) and resulted in abnormal behaviour such as increased time spent grooming to remove the fungus (22% of the total time aroused compared with 1% for uninfected bats) (Brownlee-Bouboulis and Reeder 2013) and flying during the day in temperatures at or below freezing, presumably searching for food and/or water (Reichard and Kunz 2009).

In healthy Little Brown Bats body fat increases from approximately 7% of total body mass (6 g) during summer to 27% of total mass (9 g), prior to hibernation. Because of the increased arousal frequency WNS-affected bats often have little or no identifiable fat stores and severely affected bats have a lower body mass index and lower blood glucose concentrations than unaffected ones (Warnecke *et al.* 2013). While periodic arousals account for 1.2% of the hibernation time budget, each arousal amounts to 5% of the winter energy budget (consuming approximately 108 mg of fat in a Little Brown Bat). Therefore, each additional arousal shortens the time a bat is able to hibernate by about nine days (Reeder *et al.* 2012).

Evaporative water loss through the wing membranes can account for as much as 99% of the total water loss in healthy hibernating bats. To help combat this bats tend to select roost areas of higher humidity, the more susceptible the species to evaporative water loss, the higher the humidity in their selected roost area. The three species most commonly diagnosed with WNS (Little Brown Bat, Northern Long-eared Bat and Tri-colored Bat) are also those, which seek out the most humid parts of caves in which to roost. Damage to the wing membrane leads to increased evaporative water and electrolyte (sodium and chloride) loss and dead bats are frequently dehydrated (increased haematocrit). A recent study involving experimentally infected Little Brown Bats found affected bats developed a respiratory acidosis and hyperkalaemia, and had double the energy expenditure of uninfected bats. It was proposed that increasing carbon dioxide levels, occurring as a result of increased production from an elevated metabolic rate associated with infection or decreased diffusion across the damaged

wing epithelium, could trigger hyperventilation, increased evaporative water loss, arousal from hibernation and depletion of fat stores (Cryan *et al.* 2010, Warnecke *et al.* 2012, Cryan *et al.* 2013, Warnecke *et al.* 2013, Verant *et al.* 2014).

Despite displaying similar symptoms and lesions as North American bats, European bats do not suffer similar levels of morbidity and mortality (Bandouchova *et al.* 2015). An affected Lesser Mouse-eared Bat from Hungary was recaptured five months later with no external signs of fungal infection and again six months after that with no visible signs of fungal growth, having presumably recovered from the infection (Wibbelt *et al.* 2010). Six Greater Mouse-eared Bats, out of a cave population of 1192, were found dead in poor condition in the Czech Republic. Two of the deaths were confirmed histologically to be associated with WNS (Pikula *et al.* 2012).

PATHOLOGY

Grossly, apart from the presence of white fungal material on the face and wings, affected bats may have patches of rough skin, pinpoint white foci on the muzzle, contraction of the wing membrane and a loss of pigmentation. Folded surfaces of severely affected wing membranes adhere to each other, tear easily, and appear to lose tone, tensile strength and elasticity (Cryan *et al.* 2010). If bats survive the infection they are capable of healing their wing membranes, which retain a variable amount of post-WNS scarring (Fuller *et al.* 2011).

Microscopically, Periodic Acid-Schiff positive non-pigmented fungal hyphae cover the epidermis leading to erosion and ulceration, even when fungal colonisation is not grossly evident. Destruction of the apocrine glands, hair follicles, sebaceous glands and blood vessels is common leading to wing membrane infarction distant to the fungal invasion. Hyphae are branching and septate with variable morphology ranging from parallel walls measuring 2 µm in diameter to irregular walls measuring 3-5 µm in diameter. When present the curved conidia are 2.5 µm in diameter and 7.5 µm long. Inflammation is minimal but, when present, consists predominantly of oedema and neutrophils within the connective tissue and is often associated with the presence of bacteria. Some affected bats have a mild to moderate, non-specific pneumonia with no evidence of fungal hyphae (Meteyer *et al.* 2009, Courtin *et al.* 2010, Cryan *et al.* 2010).

The body temperature of bats in torpor drops to within a few degrees of the ambient temperature (usually 2-10 °C) with a concomitant 96-98% drop in metabolic rate. This also leads to suppression of the immune system. Hence the minimal inflammatory response seen in affected hibernating bats. Despite this hibernating bats infected with *P. destructans* do mount a local acute inflammatory response consisting of the release of cytokines. However, leucocyte recruitment does not occur possibly because of the additional energy required to mount such a response (Field *et al.* 2015).

A neutrophil response does occur after the bat's body temperature has returned to euthermic levels when it emerges at the end of hibernation. If the bat has survived the hibernation

period, this immune response can be sufficient to clear the fungus from the bat's system (Meteyer *et al.* 2011). In some cases, however, the restoration of the immune response results in an exuberant mobilization of neutrophils to the sites of fungal infection, causing necrosis, oedema and the eventual death of the bat. This has been likened to a form of immune reconstitution inflammatory syndrome (IRIS), seen in HIV patients that have been treated with antiretroviral drugs while infected with another pathogen (Meteyer *et al.* 2012).

DIAGNOSIS

Although the appearance of white fungal material on the muzzle, ears and wings is suggestive of WNS, it is not pathognomonic as bats in the UK displayed similar lesions that were caused by *Rhizopus* sp. and *Paeciliomyces* sp. (Barlow *et al.* 2009). Histopathology is considered to be the 'gold standard' to confirm a diagnosis (Meteyer *et al.* 2009). Fungal culture may also be used to confirm the presence of *P. destructans*, which forms conidia identical to those seen in histologic sections (Gargas *et al.* 2009). A PCR test has been developed to detect fungus on bat wing tissue and in soil. A recent study found that this test had a 100% specificity and a 96% sensitivity when compared with histology, while culture had a sensitivity of 54%. (Lindner *et al.* 2011, Lorch *et al.* 2010).

Recently ultraviolet light (366-385 nm) was used to screen bats for WNS. Of 80 bats that were positive histologically 79 bats showed areas of orange yellow wing fluorescence when subjected to ultraviolet light. All 88 bats that did not fluoresce were also histologically negative for WNS (Turner *et al.* 2014).

TREATMENT

There is currently no treatment for WNS.

A recent study found that disease progression was unaffected by the topical application of apple cider vinegar, which has been reported to have some antifungal properties. However, when affected Little Brown Bats were warmed to between 18.3 ℃ and 23.9 ℃, administered lactated Ringer's (rehydration) solution subcutaneously and fed mealworms 25 out of 26 individuals recovered from the disease and were PCR negative for the fungus 70 days after being brought into captivity (Meteyer *et al.* 2011).

Certain volatile organic compounds, produced by bacteria, have fungistatic activity potentially giving them a role as chemical control agents. Decanal, 2-ethyl-1-hexanol, nonanal, benzothiazole, benzaldehyde and N,N-dimethyloctlamine all inhibited the growth of *P. destructans* in experimental studies (Cornelison *et al.* 2014a).

Pseudomonas spp. and *Rhodococcus rhodochrous* have also shown an ability to inhibit growth of *P. destructans* in *in vitro* experiments (Cornelison *et al.* 2014b, Hoyt *et al.* 2015). *Candida albicans* excretes trans, trans-farnesol, a bioactive sesquiterpene that has been

shown to kill several fungal species. *In vitro* studies have shown that *tt*-farnesol inhibits conidial germination and hyphal growth of *P. destructans* (Raudabaugh and Miller 2015).

Antifungal testing showed that *P. destructans* was susceptible to amphotericin B, ketoconazole, itraconazole, posaconazole and voriconazole. It was resistant to flucytosine, caspofungin, anidulafungin and micafungin and had dose dependent sensitivity to fluconazole (Chaturvedi *et al.* 2011). A separate study measured the *in vitro* release of terbinafine from an implant which could potentially be placed under the skin of bats with WNS. The mean amount of terbinafine released weekly during the 28 weeks of the study was 1.7 µg at 4ºC and 4.3 µg at 37 °C. While the MIC for *P. destructans* is not known these levels fall within the MIC for other fungi (Souza *et al.* 2012). This treatment has not been tested on bats.

PREVENTION AND CONTROL

Because of the possibility that humans might contribute to the spread of WNS, affected North American caves have been closed to human recreational use.

A complete list of decontamination procedures for people that visit caves or former mines can be found in the U.S. Fish and Wildlife Service Decontamination Documentation for Cavers (WNS Decontamination Supplement 1 of 2), Version 01.25.2011[2], or the U.S. Fish and Wildlife Service National White-Nose Syndrome Decontamination Protocol, Version 06.25.2012[3]. Briefly, recommended measures include wearing rubber boots that can be easily cleaned, removing all mud and debris from boots, clothing and equipment and placing them in a sealed plastic bag for later disinfection, and washing exposed skin (especially hands) with an anti-bacterial disinfectant. Clothing should be soaked in bleach and then washed with detergent using the hottest water cycle (the fungus is killed by exposure to 60ºC for 20 minutes or longer). Waterproof equipment should be cleaned with soapy water to remove dirt, then disinfect by immersing in bleach. Non-waterproof equipment should be cleaned with a soapy cloth and then disinfected with bleach. Ropes and harnesses should only be used in a single cave, or ideally not at all. The equipment must be decontaminated, but it is important to note that the repeated use of disinfectants may reduce its safety.

The documents mentioned above contain a list of disinfectants which have been shown to kill *P. destructans*. Included on this list are bleach, benzalkonium chloride, ammonium chloride, ethanol and triclosan (found in many antibacterial hand soaps). A separate study found that *P. destructans* was destroyed by exposure to 70% ethanol for a minimum of 24 hours or absolute ethanol for 30 minutes (Puechmaille *et al.* 2011c).

[2]https://www.whitenosesyndrome.org/sites/default/files/resource/wnsdecon_cavers_v012511_0.pdf

[3]https://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf

HAZARD IDENTIFICATION

The hazard is identified as white-nose syndrome (WNS) caused by the fungus *Pseudogymnoascus destructans*.

RISK QUESTION

This risk assessment considers the risk posed to Australian bats if white-nose syndrome (WNS) enters the country. The specific risk question addressed is:

What is the risk of WNS being introduced into Australia and causing significant impact on bat populations?

To answer the above question, the risk assessment follows the World Organisation for Animal Health (OIE) framework of release (or entry), exposure and consequence assessment (OIE Terrestrial Animal Health Code 2015). Specifically, it is divided into three key areas:

1. What is the risk that *P. destructans* will enter Australia?

2. What is the risk of a bat species in Australia being exposed to *P. destructans*, with the subsequent establishment and spread of the fungus throughout the country?

3. What is the risk of a bat species becoming impacted by *P. destructans* following exposure to the fungus?

RISK ASSESSMENT

Terminology related to the assessed level of risk

Entry refers to the probability of entry of *P. destructans* into Australia. Exposure refers to the probability of exposure of susceptible bat populations to *P. destructans* once it has entered Australia. Probability of occurrence refers to the likelihood that a bat population will be infected and develops WNS given the entry and exposure scores (Table 4). The consequence assessment estimates the magnitude of the potential biological, environmental and economic consequences associated with the entry, establishment or spread of *P. destructans* and the likelihood of its occurrence. Risk estimation summarises the results arising from the entry, exposure and consequence assessments.

For the purposes of this report, the categories below are defined here as the probability of an event happening at least once during a 10 year period. We have chosen a 10 year period as we believe, given the epidemiology of WNS, that it is a suitable time period in which to frame management and policy decisions.

Table 4. Categories used to assess probability of entry and exposure of *P. destructans* into Australia and Australian bat populations, respectively, and likelihood of overall chance of exposure to *P. destructans* in Australian bats (following the OIE Terrestrial Animal Health Code 2015).

		Probability of Occurrence	Middle of Range
I	Insignificant	$0 – 1 \times 10^{-6}$	-
R	Rare	$1 \times 10^{-6} – 0.01$	0.005
U	Unlikely	0.01 – 0.30	0.155
P	Possible	0.30 – 0.70	0.5
L	Likely	0.70 – 0.99	0.845
AC	Almost certain	0.99 – 1.00	0.995

Once a probability of entry and exposure for each possible pathway had been determined, we used the following matrix to determine the probability of occurrence:

Table 5. Table used to determine the probability of occurrence of WNS based on individual probabilities of Entry and Exposure to *P. destructans*. I – insignificant; Rare – rare; U – unlikely; P – possible; L – likely; AC – almost certain

		Entry					
		Insignificant	**Rare**	**Unlikely**	**Possible**	**Likely**	**Almost certain**
Exposure	**Insignificant**	I	I	I	I	I	I
	Rare	I	R	R	R	R	R
	Unlikely	I	R	U	U	U	U
	Possible	I	R	U	U	P	P
	Likely	I	R	U	P	L	L
	Almost Certain	I	R	U	P	L	AC

We used a combination of published literature and expert opinion (wildlife veterinarians; wildlife health researchers; veterinary epidemiologists; bat ecologists; ecological modellers) to determine the probability of each pathway. Where assumptions were made around an assessment, these are specified below.

LIKELIHOOD ASSESSMENT

The likelihood assessment is comprised of two phases: 1) entry into Australia, and 2) exposure of bats to the fungus predominantly through cavers or researchers taking the fungus as a fomite into a cave on infected equipment.

Entry assessment

In light of available information, and the absence of bats with symptoms of WNS, it seems likely that *P. destructans* is either not present in Australia, or present in caves not frequented by bats. However, because few people visit caves in winter (when WNS symptoms would be most obvious), few bat biologists currently work in bat caves, cavers may not recognise sick bats, dead bats on cave floors may be considered a normal occurrence or removed by scavengers, symptoms of WNS may not be obvious after a few days of death, bats can be affected but not necessarily display classical symptoms and there has been little real awareness of WNS in Australia, we acknowledge that *P. destructans* could be present in caves with bats but has hitherto gone undetected. For the purposes of this risk assessment, however, the assumption will be made that *P. destructans* is currently absent from Australia.

We have thus assessed individually each potential entry route for *P. destructans* into Australia (Entry), as well as the associated subsequent risk of exposure of bats (Exposure) (Table 6). Justifications for each assessment, as well as any assumptions made, are discussed subsequently.

1. **Airborne:** Insignificant. A study indicated that unaffected bats housed 1.3 cm apart from infected bats did not become infected (Lorch *et al.* 2011). Consequently the likelihood of the fungus travelling by air to Australia is deemed to be insignificant.

2. **Bat Borne:** Rare/Insignificant. The category "accidental" refers to inadvertent import of a live bat into the country, for example in a shipping container. Since 1999 30 bats have been intercepted at Australian border points as stowaways (Cassey pers. obs.). Figures for the attempted illegal importation of bats and bat tissues are not available. To date, only one study has provided evidence of a bat species migrating to or from Australia. Black Flying-foxes (*Pteropus alecto*) were observed to travel between northern Australia and Papua New Guinea (Breed *et al.* 2010). As the Black Flying-fox is not a cave dwelling bat and resides primarily in the tropics it is unlikely to act as a vector for the introduction of *P. destructans*. Bat tissues can be legally imported if fixed in alcohol. The fungus is destroyed by exposure to 70% ethanol for 24 hours or absolute ethanol for 30 minutes (Puechmaille *et al.* 2011c). Therefore, imported tissues are unlikely to contain viable fungus.

3. **Paratenic Host:** Rare (Domestic animals)/Insignificant (Wildlife). This category refers to an animal species, other than a bat, acting as a mechanical (not infected, but merely carrying the pathogen, e.g. on bird feathers) vector for the fungus. *P. destructans* has not been found on any groups of vertebrates other than bats. However, there is a knowledge gap here as no active surveillance of other species has been conducted.

   Human Mediated/Fomite

4. **Tourist/Traveller:** Insignificant/Likely. The risk of a tourist/traveller, who has not visited any caves (non-cave visitor), introducing *P. destructans* is insignificant. Show

cave visitors to Australia number between 500,000 and 1,000,000 annually (White pers. comm.). For a tourist/traveller who has visited caves (cave visitor) outside the known range of WNS the chances of transferring the fungus are unlikely but it could still occur as the actual range of *P. destructans* may be greater than the currently known range. However, if the tourist/traveller has visited caves in an endemic area it is likely that the fungus will be transferred as there is a greater chance of it being present on the person's footwear or clothing and tourist awareness of WNS is likely to be low.

5. **Cave Researcher:** Possible/Likely. Cave researchers frequently enter caves and move between caves. We have divided this category into separate types of cave researchers. Bat researchers from Australia are less likely to inadvertently introduce the fungus into Australia as this would involve a two stage process of visiting an infected cave overseas and then returning to enter an Australian cave with contaminated equipment. International bat researchers were deemed more likely to inadvertently bring the fungus into Australia as they would potentially have more exposure to the fungus if living and/or working in areas where the fungus is endemic. Other cave researchers (e.g. archaeologists, paleontologists, geologists, etc.) were also deemed more likely as they may be unaware of the situation of WNS overseas or in Australia.

6. **Caver:** Almost Certain. Cavers frequently enter caves and move between caves, but are less likely to have a scientific background than cave researchers or be aware of the biosecurity issues surrounding their movement and their equipment. The ropes and harnesses used during caving are difficult to disinfect. While exact figures are unavailable it is estimated that less than 100 cavers would enter Australia annually (N. White pers. comm.) The extent to which cavers are bringing in caving equipment that may contain *P. destructans* is unknown. However, if, conservatively, one caver enters Australia each year with contaminated equipment then ten cavers would enter over ten years, making it almost certain that *P. destructans* would be brought into the country. While the numbers appear low this is currently presumed to be how the fungus entered North America (Turner *et al.* 2011). Testing caving equipment as it enters the country would be informative but resource-heavy and difficult given the low number of cavers entering the country. An alternative approach would be to work with caving groups to gain more insight into the frequency of travel between Australian and overseas caves and decontamination activities. The information gained from this consultative process, should then be used to re-assess the necessity of any such testing.

7. **Cave Management:** Likely for international cave managers and guides, as they may visit local caves as part of professional development, exchange of management ideas or for personal interest. Australian cave managers may also visit overseas caves for the same reasons. Land owners with caves on their property, or those entering mines to provide infrastructure services (e.g. electrician), are deemed an insignificant probability of bringing the fungus to Australia, as they are unlikely to have visited WNS affected caves overseas.

Table 6. Likely modes of entry of *P. destructans* into Australia, and the risks associated with each one. I – insignificant; R – rare; U – unlikely; P – possible; L – likely; AC – almost certain

Mode of Entry				Entry	Exposure	Likelihood
Aerial - Wind				I	I	I
Bat Borne	Live	Accidental		R	I	I
	Live	Illegal Trade		R	I	I
	Live	Natural Migration		I	I	I
Bat Borne	Tissues	Illegal		I	I	I
	Tissues	Legal		I	I	I
Paratenic Host	Migratory Birds			I	I	I
	Imported	Wild		I	I	I
	Imported	Domestic		R	I	I
Human Mediated	Tourist/Traveller	Cave Visitor	Endemic Area Origin	L	R	R
	Tourist/Traveller	Cave Visitor	Non-Endemic Area Origin	U	R	R
	Tourist/Traveller	Non-Cave Visitor		I	I	I
	Cave Researcher	Bat Researcher	Local	P	P	U
	Cave Researcher	Bat Researcher	International	L	U	U
	Cave Researcher	Archaeology, etc.		L	U	U
	Cavers	Local		AC	L	L
	Cavers	International		AC	L	L
	Cave Management	Private	Owner	I	P	I
	Cave Management	Private	Guide	L	P	P
	Cave Management	Public	Park Officers	L	P	P
	Cave Management	Public	Infrastructure Services	I	P	I
	Mining Activity			R	I	I
	Mine Enthusiasts	Endemic Area Origin		L	R	R
	Mine Enthusiasts	Non-endemic Area Origin		U	R	R
	Wildlife Health Professional/Carer			P	R	R
	Bioterrorism			I	I	I

8. **Mining Related Activities:** Rare. The probability of mining equipment brought into Australia being contaminated with *P. destructans* appears possible, but would be rare.

9. **Mine Enthusiast (e.g. prospector):** Likely if coming from an endemic area due to entry into affected mines, but unlikely if coming from a non-endemic area.

10. **Bioterrorism:** Insignificant. As *P. destructans* is not a human or agricultural animal pathogen the likelihood of it being used in a bioterrorist attack is insignificant.

Exposure assessment

There is limited understanding of how bats become exposed to *P. destructans*, other than through direct contact with infected bats. However, given that the introduction to North American populations is thought to have been a result of human activity in caves, we have taken the conservative approach for this assessment of assuming that any introduction of the fungus into bat caves and/or mines inhabited by bats would be sufficient to result in exposure of resident bats.

1. **Airborne, Bat Borne, Paratenic Host:** Insignificant, for the reasons given above.

Human Mediated/Fomite

2. **Tourist/Traveller:** Rare/Insignificant for the non-cave visitor (including those not visiting caves in Australia). Rare for visitors who have visited caves as they would need to enter Australian caves wearing the same contaminated footwear and clothing, which would need to contain viable fungus, and if just a casual cave visitor, there may be long periods of time between their visits to caves.

3. **Cave Researcher:** Possible/Unlikely. If a local bat researcher was to inadvertently facilitate entry of the fungus into Australia, they would be more likely to expose local bat populations, as they would be likely to enter bat caves in Australia after arrival as part of their work. International bat researchers and other cave researchers would be less likely to expose local bat populations, as they would be less likely to visit bat caves after arrival in Australia, or if doing so would be more likely to be with an Australian bat researcher who would be aware of the potential risks. Nevertheless, after assessing both entry and exposure probability, the overall likelihood of cave researcher-associated exposure of Australian bats to the fungus was deemed possible for all types of researchers (based on the likelihood of this occurring at least once in a 10 year period).

4. **Caver.** Likely. Cavers are likely to transfer the fungus between caves, and less likely to be aware of the possible consequences.

5. **Cave Management.** Possible. Parks officers and guides regularly enter caves as part of their job, and could do this with their contaminated clothing/footwear and or equipment; but their knowledge of WNS could be variable. To improve awareness, guides could impart WNS information as part of their tour, as this would improve both the guides' and

the visitors' knowledge of the disease. If private land owners or infrastructure service providers did inadvertently bring the fungus into the country, they would be a possible risk of exposure if the caves they frequent include bats.

6. **Mining:** Insignificant/Rare. There are few operational mines that contain bats, thus it is unlikely that contaminated mining equipment would result in the exposure of bats.

7. **Wildlife Health Professional/Carer:** Rare. While they may come in contact with diseased bats they will have some awareness of the disease, are likely to adopt appropriate biosecurity measures when handling bats, and are not necessarily likely to enter Australian caves.

Probability of Occurrence

Our assessment suggests that the entry of *P. destructans* into Australia over the next ten years is Almost Certain, and subsequent exposure is Likely. Based on the combined entry and exposure likelihood matrix (Table 5), it is therefore Likely that Australian bats will be exposed to *P. destructans* at least once over the next ten years (Table 6). Higher risk situations for the introduction of *P. destructans* may also occur that may need to be managed, such as the International Congress of Speleology scheduled to take place in Sydney in July 2017, including a number of cave field trips for delegates (http://www.speleo2017.com/Excursions.html).

CONSEQUENCE ASSESSMENT

The consequences of introducing *P. destructans* to Australia can be divided into two major categories: economic and environmental. The economic consequences are flow-on effects from the environmental consequences, and this document therefore is primarily focused on the latter. However, potential economic consequences will be briefly summarised at the end of the consequence assessment.

The rating of environmental consequences is significantly guided by the consideration of a number of factors. These factors are likely to determine the impact of exposure to *P. destructan*s on bat populations, and thus directly affect the severity of its consequences. The questions to be considered are:

1. **Bat ecology/roosting location:** the importance of tree roosts versus cave roosts and the importance of cluster size.
2. **Susceptibility of Australian species**. *P. destructans* is fairly non-specific in the species that it infects. Bat genera that have been infected with *P. destructans* overseas are present in Australia.
3. **Australian climate.** Does the Australian climate sufficiently match North America given the temperature dependence of *P. destructans*? Is water and food available to Australian bats during the periods that they normally hibernate?

4. **Hibernation.** Do Australian bats have an equally long period of hibernation when compared with North American bats?
5. **Disease transmission and dispersal.** Do Australian bats cluster to the same degree as affected North American bats, thus facilitating spread of the fungus within colonies? Are bats likely to carry the fungus between roosts, resulting in spread of the fungus between colonies?

Factors affecting the consequence of exposure to *P. destructans*

The following factors have been considered in assessing the likely consequences of Australian insectivorous bats being exposed to *P. destructans.*

1. **Bat Roosting Location.** Cave (or Mine) v Tree. To date all major population declines and mortalities caused by *P. destructans* have occurred in cave-dwelling bats (Australian cave-dwelling bats are listed in Appendix I). While the fungus has been found on some tree-dwelling bats in the US, such as the Silver-haired Bat and the Eastern Red Bat, it was not associated with any clinical signs or mortality (Bernard *et al.* 2015). In southern Australia tree hole roosts used by bats are typically significantly warmer than nearby underground bat roosts used by the same species e.g. Large-footed Myotis (*Myotis macropus*) (Campbell *et al.* 2010), and so may not have the temperature regime suitable for *P. destructans.* The consequence is therefore deemed to be very minor, and the risk to tree dwelling bats is low.

2. **Phylogenetic Susceptibility.** It has been suggested that there may be a phylogenetic component to bat susceptibility with affected bat species coming from related families (Zukal *et al.* 2014). The majority of affected species belong to the *Myotis* genus, represented by only one species in Australia, the Large-footed Myotis. This genus however is the most widespread and speciose bat genus in the world and occurs primarily in temperate regions. Other genera that have tested positive for WNS and are found in Australia are *Rhinolophus*, the horseshoe bats, and *Miniopterus*, the bent-winged bats. *Chalinolobus* spp. (wattled bats and pied bats) and *Vespadelus* spp. are restricted to the southern hemisphere, so their susceptibility to infection is unknown.

   In a European study, one of 28 Lesser Horseshoe Bats sampled had clinical evidence of WNS. The other 27 tested negative for the fungus (Zukal *et al.* 2014). In a separate study all 14 Mediterranean Horseshoe Bats tested were positive for the fungus by PCR but only one had lesions of WNS. In the same study 29 out of 35 Lesser Horseshoe Bats tested positive and eight of these had clinical signs of WNS. Of 21 Common Bent-winged Bats tested, 20 were positive by PCR, while six had WNS lesions, confirmed histologically. Compared with other bat species the Common Bent-winged Bats and Mediterranean Horseshoe Bats carried a lower fungal load and suffered fewer WNS lesions compared with other species, possibly indicative of a reduced susceptibility to the fungus (Zukal *et al.* 2016).

In a recent WNS study, bats in North America were compared to bats at similar latitudes in China (Hoyt *et al.* 2016b). Sites were chosen that had similar ambient temperatures and colony sizes. *P. destructans* prevalence was significantly lower for the Chinese bats (51% compared with 95% for the North American bats). Fungal load was also lower for the Chinese bats, and only 28% of Chinese bats had skin lesions compared with 75% of North American bats. The authors speculated that the Chinese bats had a certain level of resistance to both *P. destructans* infection and the development of WNS (Hoyt *et al.* 2016b).

Based on these results, and the fact that Australian bats represent a population that is naïve with respect to *P. destructans*, the Large-footed Myotis, horseshoe bats and bent-winged bats must all be considered potentially susceptible to infection with *P. destructans* and the development of WNS. The susceptibility of the Chocolate Wattled Bat, Large-eared Pied Bat and Finlayson's Cave Bat is unknown, so they too should be considered potentially susceptible.

3. **Australian Climate.**

a) Cave temperatures: *Pseudogymnoascus destructans* ceases growth above 20 ºC and will not survive for prolonged periods above this temperature. An *in vitro* study demonstrated that *P. destructans* samples stored at 24 ºC would not germinate after eight months (Puechmaille *et al.* 2011c) and unpublished work indicated that *P. destructans* would not survive more than a few months at 20 ºC or above (Puechmaille pers. comm.). Therefore, the consequence of the fungus being introduced into a bat-inhabited cave that regularly exceeds 20 ºC is likely to be very minor.

While caves in tropical Australia may exceed 20 ºC, many caves in southern Australia may be below this temperature. Unfortunately temperature data for many of Australia's bat caves is lacking. Available data is provided in Appendix II, including both published and unpublished material. Some of these temperatures are above the upper range of *P. destructans*, others are within its temperature range part of the year and others are entirely within the potential temperature range. However, none of the caves in Australia are as cold as the most severely affected caves in North America.

Figure 4 is a depiction of the Australian latitudes, in relation to the latitudes in North America where *P. destructans* has been found. As there are no cave-dwelling bat species in Tasmania, we have used the latitude at the southern edge of Victoria to indicate the northern limit of the comparable zone in the US. The southern limit of the current spread of WNS in the US has been used to delimit the northern potential extent in Australia. It is apparent from this figure that Australia does not have cave bats in latitudes equivalent to the northern sections of North America where the impact of WNS has been most severe, and that Australian conditions are likely to be more similar to the southern extent of WNS in the US, where the impact has been less (Flory *et al.* 2012). Accordingly, the situation in these areas in the US will be most informative for the Australian risk

analysis. Bats that live in caves within the latitudes in Australia equivalent to where *P. destructans* has been recorded in the US are the Southern Bent-winged Bat, Eastern Bent-winged Bat, Eastern Horseshoe Bat, Large-footed Myotis, Chocolate Wattled Bat, Large-eared Pied Bat, and Finlayson's Cave Bat.

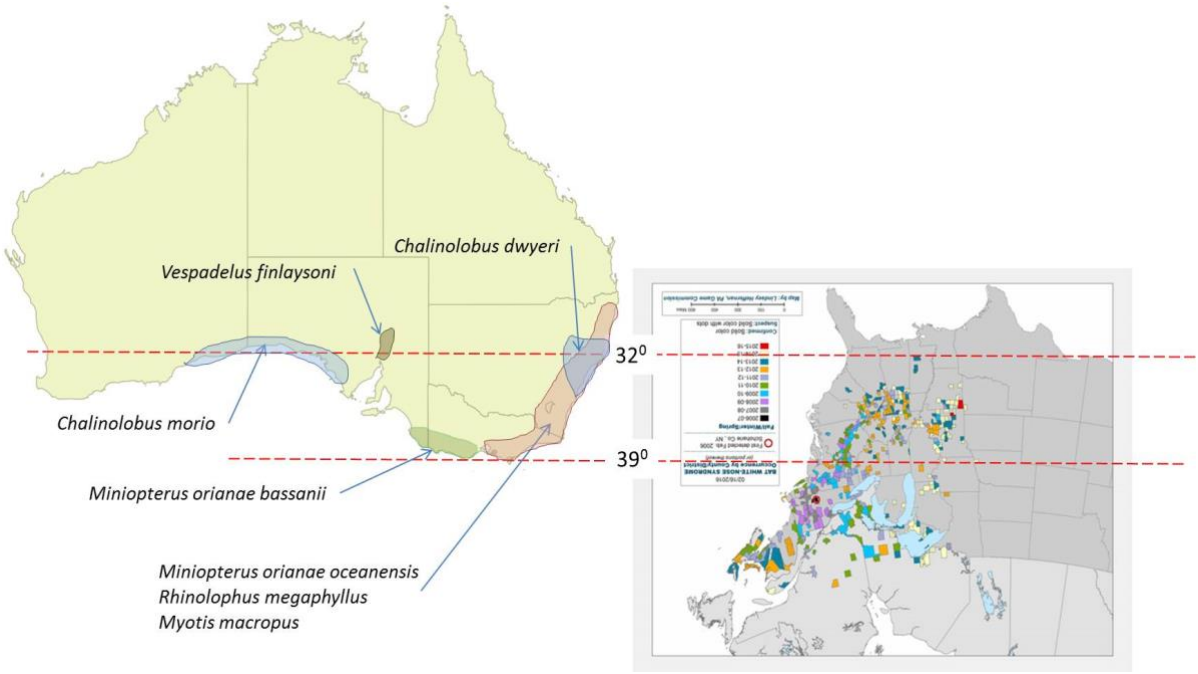

Figure 4. Australian latitudes, which correlate with the southern US latitudes where *P. destructans* has been found, and the Australian cave-dwelling bats that are found within these latitudes. The individual species distributions marked on the Australian map do not show the full extent of their distribution beyond the key latitude band.

b) Water and food availability. WNS has caused large scale mortalities in the regions of North America with extremely cold winters (Flory *et al.* 2012). This is mostly due to the fact that there is no food or free water available for hibernating bats roused due to infection with WNS. This would not be the case for Australian bats as, even in the middle of winter, waterbodies are not frozen. Average winter temperatures for US states that have reported bats infected with *P. destructans* is presented in Appendix III. While cold winter temperatures are a risk factor, they are not the complete picture, as some American states and European countries with cold temperatures have not reported large scale mortalities. Temperature averages alone clearly do not reflect local weather conditions. However, until recently, regions which do not regularly drop below freezing for extended periods have not experienced large scale mortalities (Flory *et al.* 2012). A possible exception to this situation was reported recently from Georgia, which has relatively mild winters (ProMed 2016). The report indicated the presence of *P. destructans* in 16 of 20 caves tested, with a large decline in bat numbers. However, unlike other severely affected

regions, dead bats were not found. There was just a decline in overall bat numbers, which was attributed to WNS.

Australian bats would not experience extended periods where ambient temperatures were below freezing, so there is likely to be sufficient available free water to replenish water loss. However, insufficient prey availability over winter could still be a constraint, even if the temperatures are not as extreme as in the north of North America, as temperatures regularly drop below 10 ºC, which is the approximate threshold for flying insect activity (Hoyt *et al.* 2016). Bats that are aroused from torpor multiple times over winter due to human disturbance can use up valuable fat reserves that then may be depleted prior to spring when insects become more available. This was reported to have occurred in the middle of last century when the movement of bat researchers in and out of caves during winter inadvertently resulted in mortalities (L. Lumsden, pers. comm.). Therefore, the consequences of *P. destructans* infection rousing bats repeatedly from torpor could be major.

4. **Hibernation.** WNS lesions and mortalities have only occurred during periods of hibernation. Therefore, for bats that do not hibernate, the consequence of exposure and risk are insignificant. However, all insectivorous bats in southern Australia, including the bent-winged bats, horseshoe bats, Large-footed Myotis and Chocolate Wattled Bat enter periods of torpor or hibernation. The depth of torpor (how low the body temperature gets) and duration of periods between arousal events during hibernation for southern cave-dwelling bats in Australia is not well-known but both are likely to be less than for bat species severely affected by WNS in the higher latitudes in the US. Nonetheless the duration of hibernation probably extends for around four months, enough time that the likely consequence of exposure to *P. destructans* remains major.

6. **Disease transmission and dispersal**
   a) Clustering. The roosting behaviour of bats and their propensity for clustering will affect the outcome for WNS affected bat colonies. Bats of susceptible species in cold areas suffer greater mortalities if they cluster together in large numbers. Some North American bat colonies (Little Brown Bat and Indiana Bat) tend to be extremely large with tens of thousands of bats clustered together in a relatively small area (Wibbelt *et al.* 2010). Other North American colonies (Northern Long-eared Bat and Tri-coloured Bat) and European bat colonies tend to be smaller with fewer numbers of bats clustering together (Martinkova *et al.* 2010).

   In Australia Large-footed Myotis colonies usually contain 10 to 15 individuals, potentially increasing to several hundred. Eastern Horseshoe Bat colonies usually contain five to 50 bats, except for the maternity colony at Nargun Cave which can contain as many as 5000 bats. However, horseshoe bats typically hang individually rather than clustering tightly together. Chocolate Wattled Bats cluster in groups from 10 to 1000. Bent-winged bats often occupy maternity sites in the tens of thousands but, outside the breeding season, it is more usual for individuals to roost in smaller groups, or even singly,

although clusters of a thousand or more are not uncommon (Van Dyck *et al.* 2013, DELWP 2013). In general, lower bat densities mean decreased fungal infection rates with a concomitant decrease in the likelihood of large scale mortalities occurring. However, this risk needs to be viewed in light of overall population numbers. While the absolute number of bats at risk may be lower for a rarer species, a single cluster of several thousand bats may represent a significant proportion of the total population (e.g. for Southern Bent-winged Bats). Based on cluster size alone, the consequences for Australian bats range from major to catastrophic.

b) Bat movement. Based on the temporal and geographical distribution of WNS in the US bats are capable of spreading the fungus from site to site and to each other (Turner *et al.* 2011). Therefore, bats using multiple caves or sharing caves with other species will facilitate the spread of the fungus. All Australian bats move between multiple caves, while Southern Bent-winged Bats roost with Eastern Bent-winged Bats and Large-footed Myotis in some caves, and Eastern Bent-winged Bats roost with Eastern Horseshoe Bats and Large-footed Myotis in eastern Victoria and NSW. Chocolate Wattled Bats, however, do not roost with any other cave-dwelling species. Therefore, the consequence is major.

In summary, based on the available information, for large scale mortalities to occur several criteria probably apply:

a) Bats need to roost and hibernate in caves that maintain a year round temperature below 20 ^{o}C.

b) Bats need to be susceptible to infection with *P. destructans* and the development of WNS, and

c) Bats need to cluster together in large numbers in regions with severe winters, which limit free water and prey availability, leading to dehydration and starvation as a result of repeated arousals from hibernation.

Rating of Environmental Consequences

The consequences of exposure to WNS in Australian bats have been assessed at two different levels: the single bat species level (irrespective of the conservation status of that species) and the Australian bat population level (taking into consideration the conservation status of affected species). The latter consequence rating was used to obtain an overall risk score for the entry of white nose syndrome into Australia. To describe the likely consequences of an exposure of Australian bats to *P. destructans* the definitions shown in Table 7 have been applied.

Table 7. Definition of categories used to assess the consequences of exposure to *Pseudogymnoascus destructans* at the single bat species- and the entire Australian bat population level. Consequences are based on actual occurrences, independent of monitoring.

	Single bat species	**Australian bat population**
Insignificant	Infection with no impact.	Infection with no impact
Very Minor	Infection with individual morbidities and/or mortalities but no measureable decline in population numbers.	Infection with individual morbidities and/or mortalities but no decline in population numbers.
Minor	Small population decline (<30%) in one cave	Small population decline (<30%) in one non-threatened species in one cave
Moderate	Small population decline in multiple caves, OR, moderate population decline (30-50%) in one cave.	Moderate population declines (30-50%) in more than one non-threatened species in one cave OR small population declines (<30%) in one threatened species
Major	Large scale mortality resulting in major population decline (50-80%).	Major population decline (50-80%) of one or more non-threatened species AND/OR moderate population decline (30-50%) of one threatened species
Catastrophic	Large scale mortality resulting in catastrophic population decline of >80% (including extinction)	Catastrophic population declines of >80%, including extinctions, of one or more species, with widespread ecological consequences

At the ecosystem level, the loss of cave-dwelling bat populations in southern Australia would also result in the loss of an invertebrate ecosystem dependent on the bat guano deposited on cave floors, including bat caves in the Naracoorte Caves World Heritage Area.

Australia has several bat species that fulfill some, but not all of the criteria (see previous section) thought to lead to large scale mortalities; importantly, they do not live in regions where the ambient temperature regularly drops below freezing. However, repeated or frequent rousing during torpor may nevertheless have significant impacts on Australian bats, as outlined above.

Based on the criteria discussed, we assigned a separate consequence score for each of the cave-dwelling bat species from southern Australia:

Table 8. Consequence score for each of the cave-dwelling bat species from southern Australia, based on the parameters affecting the spread and pathogenesis of white-nose syndrome, and the species' ecology and biology. None of the bats occur in regions where the temperatures are regularly below freezing.

Species	Consequence Score	Comment
Southern Bent-winged Bat (*Miniopterus orianae bassanii*)	Moderate	Critically endangered; large maternity colonies where individuals cluster tightly together, but lower hibernation density, although some over-wintering colonies can consist of thousands of bats that may represent a significant proportion of the total population, and any impact could be a tipping point for the conservation of this subspecies. The entire population occurs within the critical latitude band.
Eastern Bent-winged Bat (*Miniopterus orianae oceanensis*)	Minor	Large maternity colonies, with smaller colonies in winter. Individuals cluster tightly together throughout the year. About 80% of the entire population occurs outside the critical latitude band.
Eastern Horseshoe Bat (*Rhinolophus megaphyllus*)	Very Minor	Often in relatively small colonies, although thousands can roost together in maternity colonies. Individuals roost separately rather than in tight clusters. Share the same caves as Eastern Bent-winged Bats. About 80% of the entire population occurs outside the critical latitude band.
Chocolate Wattled Bat (*Chalinolobus morio*)	Very Minor	Medium colony size. A very small proportion of the entire population is cave-dwelling, but within the area where this species does roost in caves, the majority of the population uses caves.
Large-eared Pied Bat (*Chalinolobus dwyeri*)	Very Minor	Roosts in small shallow sandstone caves where temperatures are likely to vary seasonally. Small colony sizes. Occurs in the northern areas of the at risk range, and hence likely to be at lower risk. Over 75% of the entire population occurs outside the critical latitude band.
Large-footed Myotis (*Myotis macropus*)	Very Minor	Small colony size, with individuals roosting together in clusters. Shares caves with bent-winged bats. Over 90% of the entire population occurs outside the critical latitude band.

Finlayson's Cave Bat (*Vespadelus finlaysoni*)	Very Minor	Small colony sizes. Occurs in the northern areas of the at risk range, and hence likely to be at lower risk. Over 90% of the entire population occurs outside the critical latitude band.

The risk of WNS for Australia was then determined based on the likelihood and consequence of entry and dispersal of *P. destructans* into Australia for the Australian bat population overall, based on the definitions presented in Table 7, and the individual species assessments presented in Table 8. The consequences of exposure to *P. destructans* were deemed to be moderate for at least one threatened species, the Southern Bent-winged Bat (i.e. population declines of either <30% in multiple caves, or 30-50% in a single cave). The Southern Bent-winged Bat is critically endangered and exists entirely within the critical latitude band for WNS. **Based on the definitions for overall impact on the Australian bat population, this results in an overall consequence assessment of moderate – major.**

The following table (Table 9) presents the matrix used to determine overall risk of WNS in Australia.

Table 9. Table used to estimate the risk as a result of likelihood of entry and exposure (first column) and consequence (first header row).

	Insignificant	Very minor	Minor	Moderate	Major	Catastrophic
Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant	Insignificant
Rare	Insignificant	Very Low	Very Low	Low	Medium	High
Unlikely	Insignificant	Very Low	Low	Medium	High	Very High
Possible	Insignificant	Very Low	Low	Medium	High	Very High
Likely	Insignificant	Very Low	Low	High	High	Very High
Almost Certain	Insignificant	Low	Medium	High	Very High	Very High

The probability of occurrence that Australian bats will be exposed to *P. destructans* over the next ten years has been determined to be Likely (see above). **Based on an overall consequence assessment of moderate-major, the overall risk of WNS in Australia is thus assessed as high.**

Economic Consequences:

Economic consequences associated with the loss of populations or species of insectivorous bats would likely affect two areas: **agriculture and tourism.**

1. Agriculture. There is insufficient data to assess the consequences for agricultural productivity. However, bats consume an enormous number of insects (a colony of 150 Big Brown Bats has been estimated to eat 1.3 million pest insects each year in the US) and it has been suggested that in North America the loss of bats could lead to agricultural losses estimated at more than $3.7 billion/year due to the increased abundance of insect pests (Boyles *et al.* 2011).

The adult population of Southern Bent-winged Bats is estimated to consume nearly one quarter of a tonne of insects nightly over summer but how this translates to an economic service to agriculture has not been calculated. The likely consequence for agriculture is low, given the large foraging area for this species and therefore the dilution of impact, and that the main industries, namely viticulture, potato growing and forestry, have few insect pests likely to be significantly controlled by Southern Bent-winged Bats.

However, the potential increased numbers of insect pests will likely lead to increased use of pesticides, which could have further environmental and human health consequences.

2. Tourism. The Southern Bent-winged Bat maternity colony at Bat Cave in the Naracoorte Caves World Heritage Area is a main tourist feature of the park. There has been major infrastructure built (the Bat Observation Centre, linked to infrared and thermal cameras inside Bat Cave) to facilitate the bat interpretation program. There are current plans to build a new viewing platform and the installation of new state of the art interpretive elements. The loss of this colony would have a significant economic impact to the park.

CONCLUSIONS

- The confidence in the risk assessment would be significantly improved by an understanding of whether *P. destructans* is present in Australia. The conclusions of the risk assessment may be substantially changed if the pathogen is detected. An ongoing surveillance program will determine if the agent is present but not causing disease (and therefore of no concern) or detect it as soon as possible after introduction.
- The overall likelihood of *P. destructans* **entering** Australia on a fomite (e.g. tourist, caver, researcher), was assessed as being **Almost Certain** to occur at least once within the next 10 years.
- The overall likelihood of entry leading to **exposure** was assessed as being **Likely** to occur at least once within a 10 year period, leading to an **overall likelihood assessment of entry and exposure of Likely.**
- Our assessment of the consequences of an incursion of *P. destructans* into Australia relies on information about the many factors involved with the epidemiology and pathogenesis of WNS in other countries, some of which are not yet sufficiently known, and still unfolding. However, based on our best knowledge and expertise, we predict that there is a risk to southern Australian cave-dwelling bat populations from WNS, although the large scale mortalities seen in North America are unlikely due to the higher average temperatures experienced by Australian bats.
- Nevertheless, lower mortality rates associated with WNS may be significant for the survival of the Southern Bent-winged Bat which, as a result of other threatening factors, is already listed as Critically Endangered and whose entire population lies

within the low temperature zone. For other bat species whose distributions extend well outside this zone, low mortality rates may only have a significant impact on their respective southern populations.

- Assuming *P. destructans* is currently absent from Australia, there is sufficient information here to show that the logical next step in determining how Australia should respond to the risk is to examine management options in a structured way.

Appendix I

Australian Cave-dwelling Bats (Churchill 2008, Van Dyck *et al.* 2013)

Cave-dwelling Bats (Northern Australia)	**Cave-dwelling Bats (Southern Australia)**
Bare-backed Fruit Bat (*Dobsonia magna*)	Eastern Horseshoe Bat (*Rhinolophus megaphyllus*)
Ghost Bat (*Macroderma gigas*)	Eastern Bent-winged Bat (*Miniopterus orianae oceanensis*)
Eastern Horseshoe Bat (*Rhinolophus megaphyllus*)	Southern Bent-winged Bat (*Miniopterus orianae bassanii*)
Large-eared Horseshoe Bat (*Rhinolophus robertsi*)	Chocolate Wattled Bat (*Chalinolobus morio*)
Intermediate Horseshoe Bat (*Rhinolophus* sp.)	Large-eared Pied Bat (*Chalinolobus dwyeri*)
Dusky Leaf-nosed Bat (*Hipposideros ater*)	Large-footed Myotis (*Myotis macropus*)
Fawn Leaf-nosed Bat (*Hipposideros cervinus*)	Finlayson's Cave Bat (*Vespadelus finlaysoni*)
Diadem Leaf-nosed Bat (*Hipposideros diadema*)	Northern Cave Bat *(Vespadelus caurinus)*
Arnhem Leaf-nosed Bat (*Hipposideros inornatus*)	Yellow-lipped Cave Bat *(Vespadelus douglasorum)*
Semon's Leaf-nosed Bat (*Hipposideros semoni*)	Eastern Cave Bat *(Vespadelus troughtoni)*
Northern Leaf-nosed Bat (*Hipposideros stenotis*)	
Orange Leaf-nosed Bat (*Rhinonicteris aurantia*)	
Coastal Sheath-tailed Bat (*Taphozous australis*)	
Common Sheath-tailed Bat (*Taphozous georgianus*)	
Hill's Sheath-tailed Bat (*Taphozous hilli*)	
Troughton's Sheath-tailed Bat (*Taphozous troughtoni*)	
Little Bent-winged Bat (*Miniopterus australis*)	
Northern Bent-winged Bat (*Miniopterus orianae orianae*)	
Eastern Bent-winged Bat (*Miniopterus orianae oceanensis*)	
Large-footed Myotis (*Myotis macropus*)	
Northern Cave Bat (*Vespadelus caurinus*)	
Yellow-lipped Cave Bat (*Vespadelus douglasorum*)	
Finlayson's Cave Bat (*Vespadelus finlaysoni*)	
Eastern Cave Bat (*Vespadelus troughtoni*)	

Australian Tree Roosting Bats Found Within The Region That May Be Affected By White-nose Syndrome (See Figure 7) (Churchill 2008, Van Dyck *et al.* 2013)

Common Name	**Scientific Name (asterisked are tree and cave-dwelling)**
Yellow-bellied Sheath-tailed Bat	*Saccolaimus flaviventris*
East Coast Free-tailed Bat	*Mormopterus norfolkensis*
Eastern Free-tailed Bat	*Mormopterus ridei*
Inland Free-tailed Bat	*Mormopterus petersi*
South-western Free-tailed Bat	*Mormopterus kitcheneri*
South-eastern Free-tailed Bat	*Mormopterus planiceps*
White-striped Free-tailed Bat	*Austronomus australis*
Golden-tipped Bat	*Phoniscus papuensis*
South-eastern Long-eared Bat	*Nyctophilus corbeni*
Lesser Long-eared Bat	*Nyctophilus geoffroyi*
Gould's Long-eared Bat	*Nyctophilus gouldi*
Western Long-eared Bat	*Nyctophilus major*
Gould's Wattled Bat	*Chalinolobus gouldii*
Chocolate Wattled Bat	*Chalinolobus morio* *
Little Pied Bat	*Chalinolobus picatus*
Western False Pipistrelle	*Falsistrellus mackenziei*
Eastern False Pipistrelle	*Falsistrellus tasmaniensis*
Large-footed Myotis	*Myotis macropus* *
Greater Broad-nosed Bat	*Scoteanax rueppellii*
Inland Broad-nosed Bat	*Scotorepens balstoni*
Little Broad-nosed Bat	*Scotorepens greyii*
Eastern Broad-nosed Bat	*Scotorepens orion*
Inland Forest Bat	*Vespadelus baverstocki*
Large Forest Bat	*Vespadelus darlingtoni*
Southern Forest Bat	*Vespadelus regulus*
Little Forest Bat	*Vespadelus vulturnus*

Appendix II

Published Cave Temperature Data.

Temperatures in Thermocline Cave, near Braidwood, NSW ranged from 9.5 ℃ to 19.5 ℃ between September 1971 and December 1973 (Hall 1982). Temperature survey results published by Dwyer and Hamilton-Smith (1965) are presented in the following table:

Cave	Location	Section of Cave	Temp °C	Time of Year
Willi Willi	Kempsey, NSW	Main Chamber	15	Winter
			18.3	January
		Passage	15.6	Winter
			21.7	January
		Bat Cave	18.3	Winter
			27.8	January
Riverton	NSW/Qld Border	Two feet below the bats	20.5	Dec 1963
		Bat level	30.5	Dec 1963
			21.5-22.25	March 1963
			17.25	May 1962
Glen Lyon River	NSW/Qld Border	Three feet below the ceiling of the breeding chamber	18.75	7 Dec 1963
		Six inches below the bats	19.5-21.5	7 Dec 1963
Drum	Bungonia, NSW		13	Feb 1963
		Top of the shaft	12.5	14 Dec 1963
		Twenty feet below the bats	16.5	14 Dec 1963
Church	Wee Jasper, NSW	Breeding chamber entrance	23	18 Jan 1964
		Two and a half feet below the bats	23.75	18 Jan 1964
		Three to six inches below the bats	27.5-30	18 Jan 1964
		Within the group of bats	39.25	18 Jan 1964
Nargun	Nowa Nowa, Vic	Fifteen feet below the bats	16.1	15 Jan 1964
		Halfway up the large chamber	18.3	15 Jan 1964
Lake Gillear (Starlight)	Warrnambool Vic	First large chamber	15.25	12 Jan 1964
		Two feet below the ceiling of Recess 2	15.5	12 Jan 1964
Bat Cave	Naracoorte, SA	Breeding chamber	25.6	Oct 1956
			16.7	Winter
			21	Dec/Jan

Unpublished Cave Temperature Data (D. Mills pers. comm., T. Reardon pers. obs.).

Cave	Location	Temp °C	Humidity %	Time of Year
Cheitmore	280 km W of Sydney	8.9-13.5		Sep-Oct 2015
Mt. Fairy	40 km NE of Canberra	13.3-17.3		Mar-Apr 2015
		7.7-12.2		Sep-Oct 2015
Wyanbene	260 km SW of Sydney	8-10		Aug/Sep 2015
Bat Cave	Naracoorte, SA	4.5-22.2	47.0-91.5	Sep 2014
		13.6-25.3	40.7-91.3	Oct 2014
		20.5-22.2	91.2-96.2	Nov 2014
		21.1-22.5	92.2-96.1	Dec 2014
		21-23.1	92.1-97.6	Jan 2015
		20.9-22.2	95.4-98.0	Feb 2015
		20.6-21.5	96.3-97.2	Mar 2015
		20.9-21.5	96.5-97.3	Apr 2015
Nullarbor		13.8-20		

Unpublished Cave Temperature Data (A. Bush pers. comm., L. Lumsden pers. obs., P. Gray pers. comm.).

Cave	Location	Section of Cave	Temp °C	Humidity %	Time of Year
Starlight	Warrnambool, Vic	Ambient	1.1-38.6	17.1-100	Aug-Dec 2015
		Suspended from top of Chamber Two (Middle hole)	5.5-24.1	31.4-98.1	Aug-Dec 2015
		Suspended from top of Chamber Two (Small hole)	6.1-19.7	53.5-100	Aug-Dec 2015
		Suspended from top of Chamber One	4.1-26.1	20-100	Aug-Dec 2015
		Chamber One base	9.1-16.6	73.5-94.2	Sep-Dec 2015
		Chamber Two base	8.0-15.1	72.6-90.1	Sep-Dec 2015
		Ramp	9.1-17.1	63.8-95.8	Aug-Dec 2015
		Recess Two Camera Area	8.6-16.1	81.8-100	Sep-Dec 2015
		Recess Two Roosting Area	9.6-30.1	69.8-100	Sep-Dec 2015
		Sea Entrance Chamber	9.1-17.6	69.6-99.1	Aug-Dec 2015
		Suspended 10 m from top of Chamber One	15.67 ± 1.19	82.48 ± 4.97	Mar 2000
		Suspended 10 m from top of Chamber One	14.89 ± 1.84	81.73 ± 4.82	April 2000
		Suspended 10 m from top of Chamber One	15.19 ± 1.07	62.64 ± 3.74	June 2000
Grassmere	Warrnambool, Vic	Crack	11.1-14.6	96.1-100	Sep-Dec 2015
		Round	11.1-15.6	95.0-100	Sep-Dec 2015
Panmure	Warrnambool, Vic		8.6-13.1	98.1-100	Sep-Dec 2015
Mt. Porndon	180 km SW of Melbourne		8.6-13.1	98.1-100	Sep-Dec 2015

Unpublished Cave Temperature Data from Caves and Mines in Eastern Victoria (T. Mitchell pers. comm.).

Cave	Location	Date	Lowest Internal Temp °C	Entrance Ambient Temp °C
Nargun Cave	9 km SW of Nowa Nowa	13/08/1998	13	11.5
Nargun Cave	10 km SW of Nowa Nowa	4/05/1999	15	13.5
Mooresford Cave	9 km E of Buchan	24/09/1998	10.5	14
Mooresford Cave	9 km E of Buchan	28/12/1998	14	16
Mooresford Cave	9 km E of Buchan	3/08/1999	11.5	13.5
Anticline Cave	9 km NE of Buchan	22/07/1998	15.5	12
Camerons No 2 Cave	7 km SE of Nowa Nowa	21/07/1998	15.5	10.5
Wilsons Cave	3.5 km E of Buchan	10/02/1999	15	27
Genoa Bat Cleft	2.5 km SE of Genoa	23/07/1998	10	12
Mallacoota RAAF Transmission Room	6 km SW of Mallacoota	23/07/1998	10	12
GP-105 Cave	12 km SW of Mallacoota	13/02/2000	18	18
Wadsworth #2 Mine	5 km S of Club Terrace	18/05/1998	14	14
Wadsworth #2 Mine	5 km S of Club Terrace	6/05/1999	16	17
Bola Mine	11 km N of Club Terrace	21/07/1998	11	8
Bola Mine	11 km N of Club Terrace	18/10/1999	12	12
Accommodation Creek Copper Mine	22 km NW of Bonang	19/10/1999	11.5	12.5
MM2 Cave	38 km SE of Mitta Mitta	2/05/1999	11.5	10.5
Pioneer Mine Water Tunnel	Mitta Mitta township	1/05/1999	12.5	8
Bobby Burns Mine	4.5 km N of Mitta Mitta	30/04/1999	15.5	13
Lightning Creek Mine	14 km S of Mitta Mitta	30/04/1999	13	18
Cool Waters Mine	27 km W of Tambo Crossing	15/08/1998	12	5
Yahoo Mine	21 km W of Tambo Crossing	15/08/1998	16	11

Cont. **Cave**	**Location**	**Date**	**Lowest Internal Temp ºC**	**Entrance Ambient Temp ºC**
Stamping Mill Mine	16 km SW of Tambo Crossing	1/08/1998	9	14
Glenmaggie Water Tunnel	8.5 km N of Heyfield	4/07/1998	11.5	12
Jindivick Water Tunnel	7 km W of Neerim South	2/10/1999	13	11
Arch Rock (GP2)	9 km W of Walkerville	27/07/1998	10	Not recorded
Cape Liptrap Cave (GP3)	11 km SW of Walkerville	5/09/1998	13	15
Cape Schank Cave (GP4)	10 km W of Flinders	17/04/1999	16	15

Appendix III

Winter Temperature Data (in Degrees Celsius) for Selected USA States and Europe
States recording large bat mortalities (Turner *et al* 2011) are highlighted in bold. The average annual temperature for each state is also recorded, as this is a reasonable approximation of cave temperatures within the state (Smithson 1991).

USA State	Ave annual temp	Ave winter temp	Europe	Ave winter temp
Maine	5.0	-8.4	Estonia	-3.8
Minnesota	5.1	-10.9	Slovakia	-2.9
Vermont	**6.1**	**-7**	Latvia	-2.6
Wisconsin	6.2	-8.2	Ukraine	-2.2
New Hampshire	**6.6**	**-6.1**	Austria	-1.8
Michigan	6.9	-5.7	Poland	-1.8
New York	**7.4**	**-4.8**	Switzerland	-1.8
Iowa	8.8	-5.7	Czech Republic	-1.4
Massachusetts	**8.8**	**-2.6**	Romania	-1.4
Nebraska	9.3	-3.5	Slovenia	-0.6
Pennsylvania	**9.3**	**-2.0**	Hungary	0.1
Connecticut	**9.4**	**-1.9**	Germany	0.3
Ohio	10.4	-1.4	Denmark	0.8
Indiana	10.9	-1.4	Belgium	2.2
Illinois	**11.0**	**-2.1**	Netherlands	2.7
West Virginia	**11.0**	**0.4**	Turkey	3.6
New Jersey	11.5	0.6	Great Britain	4.2
Maryland	12.3	1.5	France	4.5
Missouri	12.5	0.2		
Virginia	**12.8**	**2.7**		
Delaware	12.9	2.3		
Kentucky	13.1	2.2		
Tennessee	14.2	3.9		
North Carolina	15.0	5.6		
Oklahoma	15.3	3.9		
Arkansas	15.8	5.3		
South Carolina	16.9	7.8		
Alabama	17.1	8.1		
Mississippi	17.4	8.2		
Georgia	17.5	8.8		

(https://www.currentresults.com/Weather/US/average-state-temperatures-in-winter.php, http://www.weatherbase.com/weather/country.php3?r=EUR)

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REFERENCES

Ågren EO, Nilsson S, Mattsson R and de Jong J. Initial surveillance of *Geomyces destructans* in Swedish bats and bat hibernacula. *61st Wildlife Diseases Association Annual International Meeting* 2012, Lyon, France.

Bandouchova H, Bartonicka T, Berkova H, Brichta J, Cerny J, Kovacova V, Kolarik M, Köllner B, Kulich P, Martinkova N, Rehak Z, Turner GG, Zukal J and Pikula J. *Pseudogymnoascus destructans*: evidence of virulent skin invasion for bats under natural conditions, Europe. *Transboundary and Emerging Diseases* 2015:62:1-5.

Barlow AM, Worledge L, Miller H, Drees KP, Wright P, Foster JT, Sobek C, Borman AM and Fraser M. First confirmation of *Pseudogymnoascus destructans* in British bats and hibernacula. *Veterinary Record* 2015:177(3):73.

Barlow A, Ford S, Green R, Morris C and Reaney S. Investigations into suspected white-nose syndrome in two bat species in Somerset. *Veterinary Record* 2009:481-482.

Barrett J, Bingham J, Eagles D, Bagnara A, Griffiths J, Valdeter S, Davies K, Cox-Witton K, Grillo T and Millers R. Infiltrative fungal dermatitis in an Australian free-tail bat – Do we have white-nose syndrome? *16th Australasian Bat Society Conference* 2014:42:11-12.

Bernard RF, Foster JT, Willcox EV, Parise KL and McCracken GF. Molecular detection of the causative agent of white-nose syndrome on Rafinesque's big-eared bats (*Corynorhinus rafinesquii*) and two species of migratory bats in the southeastern USA. *Journal of Wildlife Diseases* 2015:51:519-522.

Blehert DS, Hicks AC, Behr, M, Meteyer CU, Berlowski-Zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, Niver R, Okoniewski JC, Rudd RJ and Stone WB. Bat white-nose syndrome: an emerging fungal pathogen? *Science* 2009;323:227.

Boyles JG, Cryan PM, McCracken GF and Kunz TH. Economic importance of bats in agriculture. *Science* 2011:332:41-42.

Breed AC, Field HE, Smith CS, Edmonston and Meers J. Bats without borders: long-distance movements and implications for disease risk management. *Ecohealth* 2010:7:204-212.

Brownlee-Bouboulis SA and Reeder DM. White-nose syndrome-affected little brown myotis (*Myotis lucifugus*) increase grooming and other active behaviors during arousals from hibernation. *Journal of Wildlife Diseases* 2013;49:850-859.

Campbell S, Coulson G, and Lumsden LF. Divergent microclimates in artificial and natural roosts of the large-footed myotis (*Myotis macropus*). *Acta Chiropterologica* 2010:12:173-185.

Chaturvedi V, Springer DJ, Behr MJ, Ramani R, Li X, Peck MK, Ren P, Bopp DJ, Wood B, Samsonoff WA, Buchkoski CM, Hicks AC, Stone WB, Rudd RJ and Chaturvedi S. Morphological and molecular characterizations of psychrophilic fungus *Geomyces destructans* from New York bats with white-nose syndrome (WNS). *PLoS One* 2010:5(5):e10783. doi: 10.1371/journal.pone.0010783.

Chaturvedi S, Rajkumar SS, Li X, Hurteau GJ, Shtutman M and Chaturvedi V. Antifungal testing and high-throughput screening of compound library against *Geomyces destructans*, the etiologic agent of geomycosis (WNS) in bats. *PLoS ONE* 2011:6(3):e17032. doi: 10.1371/journal.pone.0017032.

Churchill S. *Australian Bats*. 2008. Allen and Unwin, Crows Nest.

Cornelison CT, Gabriel KT, Barlament C and Crow SA. Inhibition of *Pseudogymnoascus destructans* growth from conidia and mycelial extension by bacterially produced volatile organic compounds. *Mycopathologia* 2014:177:1-10.

Cornelison CT, Keel MK, Gabriel KT, Barlament CK, Tucker TA, Pierce GE and Crow SA. A preliminary report on the contact-independent antagonism of *Pseudogymnoascus destructans* by *Rhodococcus rhodochrous* strain DAP96253. *BMC Microbiology* 2014:14:246.

Courtin F, Stone WB, Risatti G, Gilbert K and Van Kruiningen HJ. Pathologic Findings and Liver Elements in Hibernating Bats with White-Nose Syndrome. *Vet Pathology* 2010:47(2):214-219.

Cryan P and Blehert D. European Observations of Fungi on Bats. 1st International Symposium on Bat Migration, Berlin, Germany – Jan 2009. Downloaded on 7 April 2010 from: www.caves.org/WNS/ICS%20Blehert%20and%20Cryan.ppt

Cryan PM, Meteyer CU, Boyles JG and Blehert DS. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biology* 2010:8:135. doi: 10.1186/1741-7007-8-135.

Cryan PM, Meteyer CU, Blehert DS, Lorch JM, Reeder DM, Turner GG, Webb J, Behr M, Verant M, Russell RE and Castle KT. Electrolyte depletion in white-nose syndrome bats. *Journal of Wildlife Diseases* 2013;49:398-402.

Department of Environment, Land, Water and Planning (DELWP). National recovery plan for the southern bent-wing bat *Miniopterus orianae bassanii.* 2013. Department of the Environment, Canberra.

Dwyer PD and Hamilton-Smith E. Breeding caves and maternity colonies of the bent-winged bat in south-eastern Australia. *Helictite* 1965:4:3-21.

Field KA, Johnson JS, Lilley TM, Reeder SM, Rogers EJ, Behr MJ and Reeder DM. The white-nose syndrome transcriptome: activation of anti-fungal host responses in wing tissue of hibernating little brown myotis. *PLoS Pathogens* 2015:11(10):e1005168. doi:10.1371/journal.ppat.1005168.

Flory AR, Kumar S, Stohlgren TJ, and Cryan PM. Environmental conditions associated with bat white-nose syndrome mortality in north-eastern United States. *Journal of Applied Ecology* 2012:49:680-689.

Frick WF, Puechmaille SJ, Hoyt JR, Nickel BA, Langwig KE, Foster JT, Barlow KE, Bartonicka T, Feller D, Haarsma AJ, Herzog C, Horacek I, van der Kooij J, Mulkens B, Petrov B, Reynolds R, Rodrigues L, Stihler CW, Turner GG and Kilpatrick AM. Disease alters macroecological patterns of North American bats. *Global Ecology and Biogeography* 2015:24:741-749.

Fuller NW, Reichard JD, Nabhan ML, Fellows SR, Pepin LC and Kunz TH. Free-ranging little brown myotis (*Myotis lucifugus*) heal from wing damage associated with white-nose syndrome. *EcoHealth* 2011:8:154-162.

Gargas A, Trest MT, Christensen M, Volk TJ and Blehert DS. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon* 2009:108:147-154.

Gray P and Lumsden L. The viability of Starlight Cave as a maternity site for the southern bent-winged bat (*Minopterus schreibersii bassanii*).

Hall LS. The effect of cave microclimate on winter roosting behavior in the bat, *Miniopterus schreibersii blepotis*. *Australian Journal of Ecology* 1982:7:129-136.

Hoyt JR, Langwig KE, Okoniewski J, Frick WF, Stone WB and Kilpatrick AM. Long-term persistence of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, in the absence of bats. *EcoHealth* 2014:12:330-333.

Hoyt JR, Cheng TL, Langwig KE, Hee MM, Frick WF and Kilpatrick AM. Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS ONE* 2015:10(4):e0121329. doi:10.1371/journal.pone.0121329.

Hoyt JR, Sun K, Parise KL, Lu G, Langwig KE, Jiang T, Yang S, Frick WF, Kilpatrick AM, Foster JT and Feng J. Widespread bat white-nose syndrome fungus, northeastern China. *Emerging Infectious Diseases* 2016:22:140-142.

Hoyt JR, Langwig KE, Sun K, Lu G, Parise KL, Jiang T, Frick WF, Foster JT, Feng J and Kilpatrick AM. Host persistence or extinction from emerging infectious disease: insights from white-nose syndrome in endemic and invading regions. *Proceedings of the Royal Society B* 2016:20152861. doi: 10.1098/rspb.2015.2861.

Jackson S and Groves C. 2015. Taxonomy of Australian Mammals, CSIRO Publishing, Clayton South.

Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH and Kilpatrick AM. Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. *Ecology Letters* 2012:15:1050-1057.

Langwig KE, Frick WF, Reynolds R, Parise KL, Drees KP, Hoyt JR, Cheng TL, Kunz TH, Foster JT and Kilpatrick AM. Host and pathogen ecology drive the seasonal dynamics of a fungal disease, white-nose syndrome. *Proceedings of the Royal Society B* 2015:282:20142335. doi: 10.1098/rspb.2014.2335.

Lindner DL, Gargas A, Lorch JM, Banik MT, Glaeser J, Kunz TH and Blehert DS. DNA-based detection of the fungal pathogen *Geomyces destructans* in soils from bat hibernacula. *Mycologia* 2011:103:241-246.

Lorch JM, Gargas A, Meteyer CU, Berlowski-Zier BM, Green DE, Shearn-Bochsler V, Thomas NJ and Blehert DS. Rapid polymerase chain reaction diagnosis of white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 2010:22:224–230.

Lorch JM, Meteyer CU, Behr MJ, Boyles JG, Cryan PM, Hicks AC, Ballmann AE, Coleman JTH, Redell DN, Reeder DM and Blehert DS. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* 2011:480(7377):376-378.

Lorch JM, Muller LK, Russell RE, O'Connor M, Lindner DL and Blehert DS. Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the eastern United States. *Applied and Environmental Microbiology* 2013:79(4):1293-1301.

Lucan RK, Bandouchova H, Bartonicka T, Pikula J, Zahradnikova A, Zukal J and Marinkova N. Ectoparasites may serve as vectors for the white-nose syndrome fungus. *Parasites & Vectors* 2016:9:16. doi: 10.1186/s13071-016-1302-2.

Martinkova N, Backor P, Bartonicka T, Blazkova P, Cerveny J, Falteisek L, Gaisler J, Hanzal V, Horacek D, Hubalek Z, Jahelkova H, Kolarik M, Korytar L, Kubatova A, Lehotska B,

Lehotsky R, Lucan RK, Majek O, Mateju J, Rehak Z, Safar J, Tajek P, Tkadlec E, Uhrin M, Wagner J, Weinfurtova D, Zima J, Zukal J and Horacek I. Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia. *PLoS ONE* 2010:5(11):e13853. doi:10.1371/journal.pone.0013853.

Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green E, Shearn-Bochsler V, Thomas NJ, Gargas A and Behr MJ. Histopathologic criteria to confirm white-nose syndrome in bats. *Journal of Veterinary Diagnostic Investigation* 2009:21:411-414.

Meteyer CU, Valent M, Kashmer J, Buckles EL, Lorch JM, Blehert DS, Lollar A, Berndt D, Wheeler E, White, CL and Ballmann AE. Recovery of little brown bats (*Myotis lucifugus*) from natural infection with *Geomyces destructans*, white-nose syndrome. *Journal of Wildlife Diseases* 2011:47:618-626.

Meteyer CU, Barber D and Mandl JN. Pathology in euthermic bats with white-nose syndrome suggests a natural manifestation of immune reconstitution inflammatory syndrome. *Virulence* 2012:3:583-588.

O'Donoghue AJ, Knudsen GM, Beekman C, Perry JA, Johnson AD, DeRisi JL, Craik CS and Bennett RJ. Destructin-1 is a collagen-degrading endopeptidase secreted by *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *Proceedings of the National Academy of Sciences* 2015:112(24):7478-7483.

OIE Terrestrial Animal Health Code. 2015. http://www.oie.int/index.php?id=169&L=0&htmfile=sommaire.htm.

Pannkuk EL, Risch TS and Savary BJ. Isolation and identification of an extracellular subtilisin-like serine protease secreted by the bat pathogen *Pseudogymnoascus destructans*. *PLoS One* 2015:10(3):e0120508. doi:10.1371/journal.pone.0120508.

Pikula J, Bandouchova H, Novotny L, Meteyer CU, Zukal J, Irwin NR, Zima J and Martinkova N. Histopathology confirms white-nose syndrome in bats in Europe. *Journal of Wildlife Diseases* 2012:48:207-211.

ProMed. White nose syndrome, bats – North America (Georgia). *www.promedmail.org* 2016: Archive Number 20160521.4236844.

Puechmaille SJ, Verdeyroux P, Fuller H, Gouilh MA, Bekaert M and Teeling EC. White-nose syndrome fungus (*Geomyces destructans*) in bats, France. *Emerging Infectious Diseases* 2010;16:290-293.

Puechmaille SJ, Wibbelt G, Korn V, Fuller H, Forget F, Mühldorfer K, Kurth A, Bogdanowicz W, Borel C, Bosch T, Cherezy T, Drebet M, Görföl T, Haarsma A, Herhaus F,

Hallart G, Hammer M, Jungmann C, Le Bris Y, Lutsar L, Masing M, Mulkens B, Passior K, Starrach M, Wojtaszewski A, Zöphel U and Teeling EC. Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality. *PLoS ONE* 2011:6(4):e19167. doi:10.1371/journal.pone.0019167.

Puechmaille SJ, Frick WF, Kunz TH, Racey PA, Voigt CC, Wibbelt G and Teeling EC. White-nose syndrome: is this emerging disease a threat to European bats? *Trends in Ecology and Evolution* 2011:26:570-576.

Puechmaille SJ, Fuller H and Teeling EC. Effect of sample preservation methods on the viability of *Geomyces destructans*, the fungus associated with white-nose syndrome in bats. *Acta Chiropterologica* 2011:13:217-221.

Rajkumar SS, Li X, Rudd RJ, Okoniewski JC, Xu J, Chaturvedi S and Chaturvedi V. Clonal genotype of *Geomyces destructans* among bats with white-nose syndrome, New York, USA. *Emerging Infectious Diseases* 2011:17:1273-1276.

Raudabaugh DB and Miller AN. Nutritional capability of and substrate suitability for *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome. *PLoS ONE* 2013:8(10):e78300. doi:10.1371/journal.pone.0078300.

Raudabaugh DB and Miller AN. Effect of trans, trans-farnesol on *Pseudogymnoascus destructans* and several closely related species. *Mycopathologia* 2015:180:325-332.

Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A, Britzke ER, Vodzak ME, Darling SR, Stihler CW, Hicks AC, Jacob R, Grieneisen LE, Brownlee SA, Muller LK and Blehert DS. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS ONE* 2012:7(6):e38920. doi:10.1371/journal.pone.0038920.

Reichard JD and Kunz TH. White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*). *Acta Chiropterologica* 2009:11:457-464.

Reynolds HT and Barton HA. Comparision of the white-nose syndrome agent *Pseudogymnoascus destructans* to cave-dwelling relatives suggests reduced saprotrophic enzyme activity. *PLoS ONE* 2014:9(1):e86437. doi:10.1371/journal.pone.0086437.

Smithson PA. Inter-relationships between cave and outside air temperatures. *Theoretical and Applied Climatology* 1991:44:65-73.

Souza MJ, Cairns T, Yarbrogh J and Cox SK. *In vitro* investigation of a terbinafine impregnated subcutaneous implant for veterinary use. *Journal of Drug Delivery* 2012:Article ID 436710. http://dx.doi.org/10.1155/2012/436710.

Turner GG, Reeder DM and Coleman JTH. A five year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future. *Bat Research News* 2011:52:13-27.

Turner GG, Meteyer CU, Barton H, Gumbs JF, Reeder DM, Overton B, Bandouchova H, Bartonicka T, Martinkova N, Pikula J, Zukal J and Blehert DS. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. *Journal of Wildlife Diseases* 2014:50:566-573.

Van Dyck S, Gynther I and Baker A. (eds.) *Field Companion to the Mammals of Australia.* 2013. New Holland Publishers, London, Sydney.

Verant ML, Boyles JG, Waldrep W, Wibbelt G and Blehert DS. Temperature-dependent growth of *Geomyces destructans*, the fungus that causes bat white-nose syndrome. *PLoS ONE* 2012:7(9):e46280. doi:10.1371/journal.pone.0046280.

Verant ML, Meteyer CU, Speakman JR, Cryan PM, Lorch JM and Blehert DS. White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat hose. *BMC Physiology* 2014:14:10. doi:10.1186/s12899-014-0010-4.

Warnecke L, Turner JM, Bollinger TK, Lorch JM, Misra V, Cryan PM, Wibbelt G, Blehert DS and Willis CKR. Inoculation of bats with European *Geomyces destructans* supports the novel pathogen hypothesis for the origin of white-nose syndrome. *Proceedings of the National Academy of Sciences* 2012:109(18):6999-7003.

Warnecke L, Turner JM, Bollinger TK, Misra V, Cryan PM, Blehert DS, Wibbelt G and Willis CKR. Pathophysiology of white-nose syndrome in bats: a mechanistic model linking wing damage to mortality. *Biology Letters* 2013:9:20130177. doi:10.1098/rsbl.2013.0177.

Wibbelt G, Kurth A, Hellmann D, Weishaar M, Barlow A, Veith M, Prüger J, Görföl, T, Grosche L, Bontadina F, Zöphel U, Seidl H, Cryan PM and Blehert DS. White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe. *Emerging Infectious Diseases* 2010;16:1237-1242.

Zukal J, Bandouchova H, Bartonicka T, Berkova H, Brack V, Brichta J, Dolinay M, Jaron KS, Kovacova V, Kovarik M, Martinkova N, Ondracek K, Rehak Z, Turner GG and Pikula J. White-nose syndrome fungus: a generalist pathogen of hibernating bats. *PLoS ONE* 2014:9(5):e97224. doi:10.1371/journal.pone.0097224.

Zukal J, Bandouchova H, Brichta J, Cmokova A, Jaron KS, Kolarik M, Kovacova V, Kubatovas A, Novakova A, Orlov O, Pikula J, Presetnik P, Suba J, Zahradnikova A and Martinkova N. White-nose syndrome without borders: *Pseudogymnoascus destructans* infection tolerated in Europe and Palearctic Asia but not in North America. *Scientific Reports* 2016:6:19829. doi:10.1038/srep19829.

GLOSSARY OF TERMS

Aetiologic	Causative.
Apocrine Gland	Sweat gland.
Avirulent	Not harmful in its effects.
Cellulase	An enzyme that breaks down cellulose, the main constituent of plant cell walls.
Chitinase	An enzyme that breaks down chitin, the main constituent of fungal cell walls and invertebrate exoskeletons.
Collagen	A structural protein that forms most of the connective tissue within the body.
Conidia	Asexual fungal spores.
Conidiophore	A fungal hypha that produces conidia.
Consequence Assessment:	The process of describing the relationship between specified exposures to a biological agent and the consequences of those exposures.
Cytokine	Proteins that are secreted by immune cells.
Entry Assessment	The process of describing the biological pathway(s) necessary to introduce pathogenic agents into a particular environment, and estimating the probability of that process occurring.
Exposure Assessment	The process of describing the biological pathway(s) necessary for exposure of animals to the pathogenic agents released from a given risk source, and estimating the probability of the exposure(s) occurring.
Fomite	Any object or substance capable of carrying infectious organisms, such as hair, clothing, etc.
Fungistatic	Inhibits the growth of fungi without killing them.
Haematocrit	The proportion of blood that is made up of cells.

Hazard	A biological, chemical or physical agent with the potential to cause an adverse health effect.
Hazard Identification	The process of identifying the pathogenic agents that could potentially be introduced.
Hyperkalemia	Elevated blood potassium.
Hyperventilation	Rapid breathing.
Hypha	Long branching filamentous fungal structure.
Inflammation	A process initiated by the body to protect it from harmful stimuli, by mobilizing immune cells.
Keratinolytic	Capable of breaking down keratin, the main protein found in skin, hair and feathers.
Leucocyte	White blood cell.
Morphology	The form and structure of an organism.
Necrosis	The death of body tissue.
Neutrophil	A type of white blood cell.
Oedema	Excess watery fluid collecting in the cavities or tissues of the body.
Pathogen	A biological agent that causes disease in its host.
Pathogenic	Capable of causing disease.
PCR	Polymerase Chain Reaction. Technology used to amplify a piece of DNA generating thousands to millions of copies of a particular DNA sequence. Used to amplify extremely small amounts of sample in order to detect a pathogen, if present.
Peptidase	An enzyme that breaks down protein.
Periodic Acid-Schiff	A stain used in pathology to highlight fungi.

Qualitative Risk Assessment	An assessment where the outputs on the likelihood of the outcome or the magnitude of the consequences are expressed in qualitative terms such as high, medium, low or negligible.
Respiratory Acidosis	When the lungs fail to remove all the carbon dioxide that the body produces, it builds up in the blood making it acidic.
Risk	The likelihood of the occurrence and the likely magnitude of the biological and economic consequences of an adverse event or effect to animal health.
Risk Assessment	The evaluation of the likelihood and the biological and economic consequences of the entry, establishment, and spread of a hazard.
Saprotrophic	Any organism, such as a fungus, that lives and feeds on dead organic matter.
Sebaceous Gland	A skin gland which secretes and oily substance.