ABSTRACT

Keeping White-nose syndrome out of Australia

YVONNE INGEME[1,2], PETER HOLZ[3]

White-nose syndrome (WNS) is an infectious fungal disease that has killed millions of cave dwelling insectivorous bats in North America since its appearance in 2006. It is caused by the fungus *Pseudogymnoascus destructans* that thrives below 15˚C and has been identified on bats in both Europe and China without causing population declines. The 'Qualitative Risk Assessment: WNS in Bats in Australia' has identified seven cave dwelling bats potentially at risk from contracting the disease and suggests that the most likely method of entry of *P. destructans* into Australia will be via infected surfaces such as clothing, footwear or equipment that has been used in affected caves. Implementation of strict hygiene and decontamination procedures is critical for keeping this disease from entering Australia. Any gear that has been taken into potentially infected caves and can't be treated using the appropriate decontamination procedures should not be brought into Australia. Caving clubs should establish standard procedures for responding to caving enquiries from overseas visitors highlighting the appropriate WNS hygiene protocol and potentially make gear available for use to prevent the risk of infected equipment entering Australia.

Keeping White-nose syndrome out of Australia

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White-nose syndrome

White-nose syndrome (WNS) is an infectious fungal disease that has killed over 6.7 million cave dwelling insectivorous bats in North America since its appearance in 2006 (WNS Response Team 2018). This estimate dates back to 2011, mass deaths have continued as the disease has continued to spread across North America (WNS Response Team 2018).

WNS is caused by the fungus *Pseudogymnoascus destructans (Pd)*, previously known as *Geomyces destructans*, which thrives at temperatures below 15˚C while growth ceases above 20˚C. It prefers high humidity (Warnecke et al. (2012) in Frick 2016). It grows on and adversely impacts hibernating cave dwelling bats while they are in torpor.

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Distribution

The disease has rapidly spread throughout USA and Canada, Figure 1. It occurs in Europe (Puechmaille et al. 2011) Figure 2, and has been confirmed as occurring in north eastern China and is believed to be widespread in eastern Asia (Hoyt *et al.* 2016), refer to Figure 3.

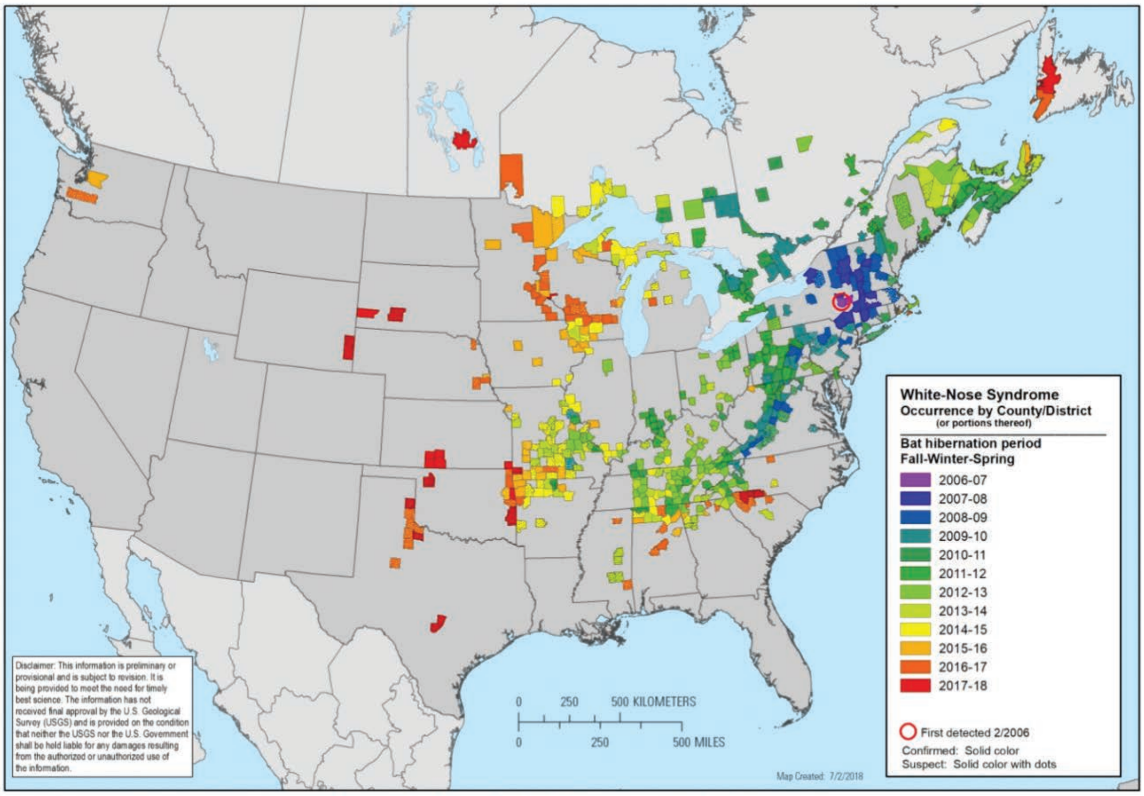

Figure 1. Distribution and annual spread of WNS in North America and Canada. Source: White-nose syndrome response team https://www.whitenosesyndrome.org/static-page/where-is-wns-now 20/12/2018

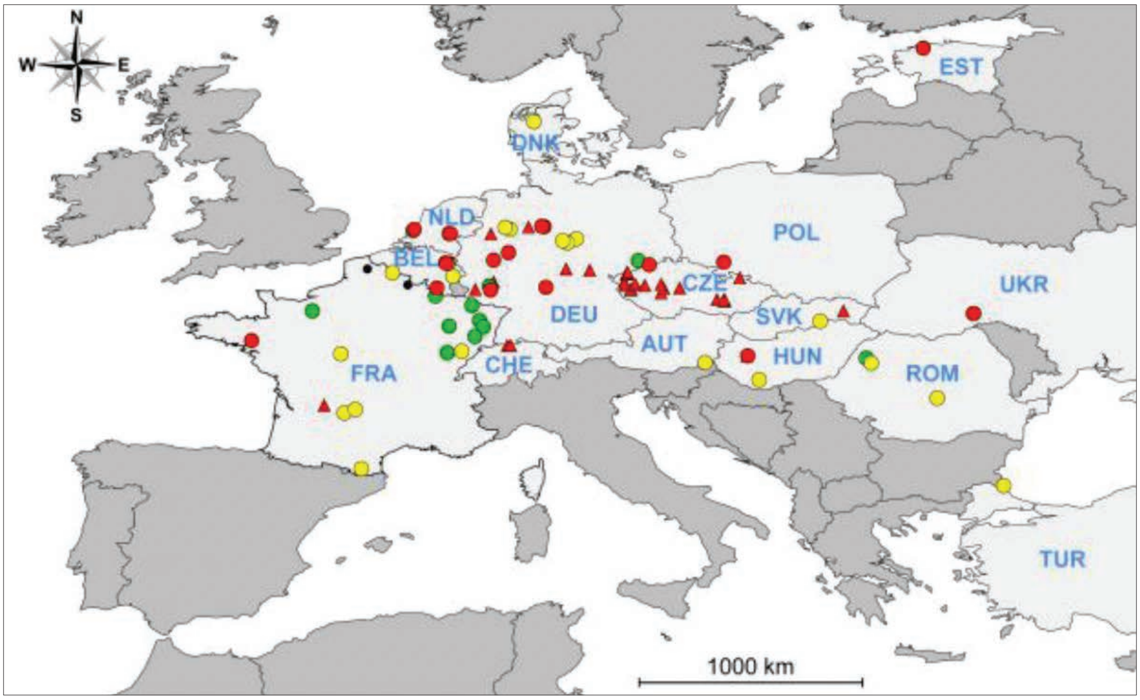

Figure 2. Distribution of confirmed and suspected records of *Pd* on hibernating bats in Europe, 2011 (Great Britain, Latvia, Russia and Slovenia have since confirmed the presence of the fungus). Confirmed records of *P. destructans* in red, photographic evidence in yellow, visual reports in green. Source: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

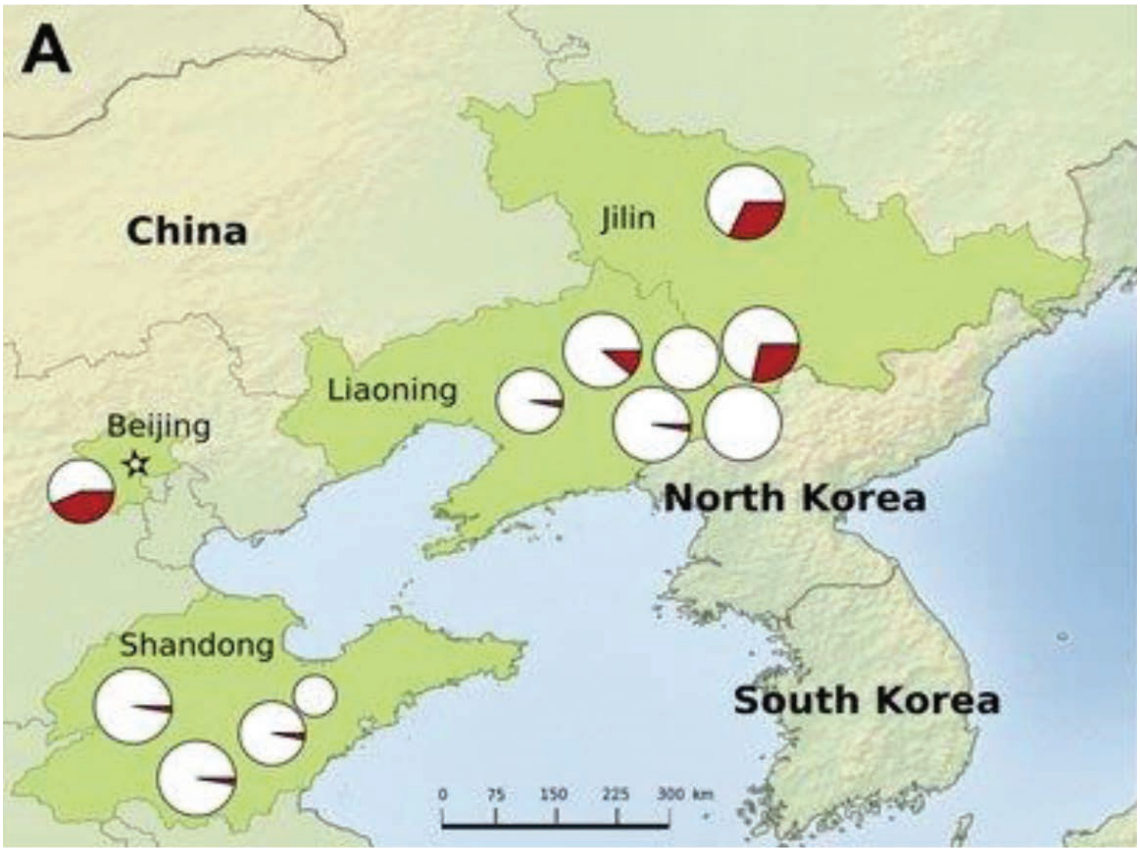

Figure 3. Distribution of *Pd* in cave environments, during the summer of 2014, in north-eastern China. Pie charts show the prevalence of *Pd* (in red) at 9 sites in northern China, and the size of the pie graphs indicates the number of samples taken at each site (range 10-35) Source: Holz *et al.* 2016.

Transmission

A photo taken by a caver in 2006 in Howes Cave, New York state, provides the first evidence of WNS in northern America (Turner and Reeder 2009 in Frick et al. 2016). Howes Cave is a popular tourist attraction with hundreds of thousands of visitors a year (Frick *et al.* 2016). The fungus is believed to have been introduced by someone with infected caving gear from Europe into North America (Puechmaille *et al*. 2011b; Leopardi *et al.* 2015 in Frick et al. 2016). Humans can spread the fungus from one hibernation cave to another by accidentally carrying the fungus on shoes, clothing, or gear (National Park Service 2019). Further transmission by humans was suspected within northern America even following extensive quarantine measures. Tourist visitation of show caves is also considered to be another means of spreading the disease widely through and outside a country and boot/shoe decontamination procedures and stations have been established at show caves, such as Mammoth Cave in Kentucky (National Park Service 2018) and throughout northern America (National Park Service 2017). Researchers are also a potential avenue to spread the disease and a comprehensive WNS decontamination protocol has been developed to minimise the risk of human-assisted transmission of WNS which applies to everyone who comes into contact with bats and/or their habitat (WNS Decontamination Team 2018).

The active on-going transmission of WNS is by bats through direct contact from infected bats to healthy bats (Lorch *et al*. 2011 in Frick *et al*. 2016) and direct contact between bats and the cave substrate. Larger movements of the disease across a country, as observed in northern America, may be through the transportation of infected hidden bats, e.g. within the awnings of camping equipment.

Pd has the ability to persist within the cave environment without bats (Puechmaille *et al*. 2011, Linder *et al*. 2011, Lorch *et al*. 2013 in Frick *et al*. 2016). The *Pd* spores are very long lived and there is a risk that the disease may be brought to Australia via a country that does not appear to have the disease physically expressed within bat populations. For example caves in New Zealand may have been contaminated by someone visiting the caves from Europe or America with contaminated gear. New Zealand bats largely roost in trees, only one species, Long-tailed bats, occasionally use caves, primarily roosting in small groups of less than a hundred (Sedgeley 2012) as such the disease may not necessarily appear evident, but potentially contaminated soil with Pd spores could then be brought to Australia and contaminate Australian caves and impact bat populations. Any cave environment with a climate that remains below 20˚C should be considered as a potential risk of supporting *Pd* and appropriate hygiene and decontamination procedures should be implemented.

Bat susceptibility

The susceptibility of a bat species to WNS is strongly influenced by the particular characteristics and behaviours of the bat species as well as the cave environment they choose to hibernate in. Bat behaviours known to increase their risk of contracting the disease, with potentially fatal consequences, include: tight clustering roosting behaviour; length of hibernation with longer hibernation periods presenting a greater risk (e.g. greater than 6 months); habitat preference e.g. cold caves with higher humidity; summer breeding swarming where large numbers of bats of different species and from different areas gather together. Where the above factors are at play, then the average weight of the bat also influences their ability to survive the disease with the lighter bats more at risk of a fatal outcome.

The cave environment strongly influences the growth rate of *Pd* with a habitat preference below 15˚C (Holz *et al*. 2016) and very high humidity (Warnecke et al. 2012).

Impact on hibernating bats

White-nose syndrome may adversely impact hibernating cave dwelling bats. Torpid bats dramatically drop their body temperature, metabolic rate and bodily functions resulting in a less active immune system (Meteyer *et al*. 2009 in Frick *et al*. 2016) thereby increasing their susceptibility to disease.

White-nose syndrome may lead to:

- Increased consumption of fat/energy reserves (infected bats used twice as much energy as healthy bats even during the early stages of WNS prior to behavioural changes (Verant, *et al*. 2014))
- Increased frequency and duration of arousals (Reeder *et al*. 2012)
- Damaged wing membrane increasing evaporation and water loss (Holz *et al*. 2016)
- Altered behaviour including spending more time grooming to remove the fungus and flying around looking for water and food when not available (Reichard and Kunz 2009 in Holz *et al*. 2016)
- Increased CO_2 levels in blood leading to respiratory acidosis and hyperkalaemia (high potassium in blood) (Verant *et al*. 2014) which can also cause heart failure
- Exhaustion of energy reserves and death (Verant *et al*. 2014)

Verant *et al*. (2014) researched the detailed physiological changes that occurred in WNS infected bats and have developed a multistage disease progression model, Figure 4, which enables a much better understanding of what is happening to the bats.

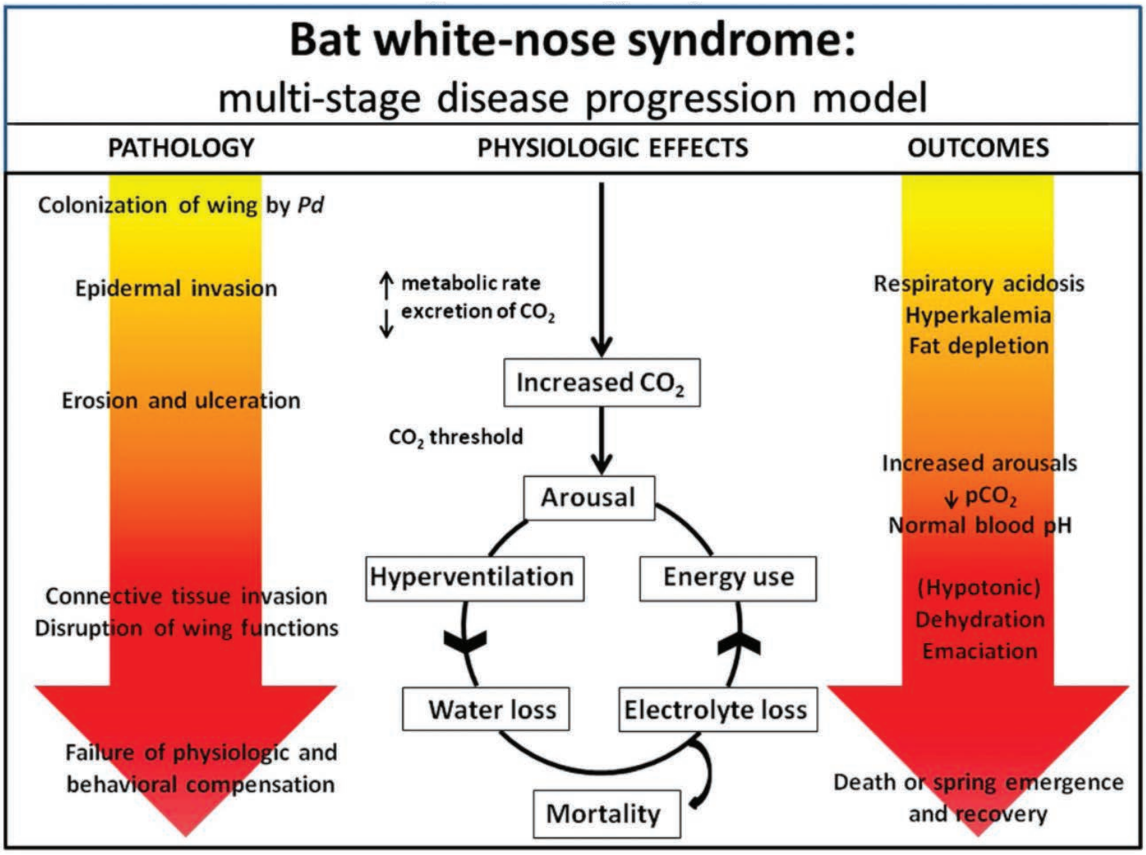

Figure 4. Disease progression model for bat white-nose syndrome (WNS). Verant *et al*. (2014) 'propose a mechanistic multi-stage disease model for WNS in a hibernating bat that encompasses current knowledge on the progression of fungal-induced wing pathology and physiologic sequelae leading to mortality from disease. Initial colonization and invasion of the wing epidermis by Pseudogymnoascus destructans (Pd) results in increased energy expenditure, chronic respiratory acidosis (elevated blood pCO_2 and bicarbonate), and hyperkalemia (elevated blood potassium). Erosion and ulceration of the epidermis stimulate increased frequencies of arousal from torpor, which remove excess CO_2 and normalize blood pH, but contribute to dehydration and depletion of fat reserves. As wing pathology becomes more extensive and severe, these effects are exacerbated by water and electrolyte loss across the epidermis (hypotonic dehydration), which stimulate more frequent arousals and create a positive feedback loop that ultimately leads to mortality when energy reserves and compensatory mechanisms become exhausted'. Source: Verant *et al*. 2014

Case study: Little Brown Bat — from common to threatened in a few years

This case study of Little Brown Bat (*Myotis lucifugus*) highlights how vulnerable a species can be to WNS. Little Brown Bat was a very common bat species numbering in the millions. It was so common no one paid any attention to it. Most of the pre-existing data available on the species was collected in an ad-hoc manner while collecting data on more threatened species of bats. Prior to WNS you would have considered the Little Brown Bat a pretty resilient species, utilising a wide variety of habitat types and overwintering in very cold humid caves rejected by most other bat species. The population has now been decimated by WNS with millions of bats dead (Figure 5) and now regarded as threatened in many places. A conservation status review of Little Brown Bat is being conducted in northern America to determine whether listing as federally endangered is warranted of this once common species (Frick *et al*. 2010 in Frick *et al*. 2016).

Unfortunately Little Brown Bats have a number of characteristics and behaviours that make them particularly susceptible to WNS with fatal consequences, these include:

- Very small bat (9g prior to torpor) (Reeder *et al*. 2012)
- Overall a very long winter torpor length (Sept to April or May depending on gender) (Langlois 2013)

Figure 5. Left: Healthy Little Brown Bat (Photo: www.batworlds.com) Centre: Little Brown Bat suffering from White-nose syndrome (Photo: Marvin Moriarty/USFWS). Right: Pile of dead Little Brown Bats killed by WNS on cave floor (Photo: A Hicks).

- Long torpor periods (average 16 days, this halves to approx 8 days with WNS) (Reeder *et al*. 2012)
- Tight clustering roosting behaviour (Wibbelt *et al*. 2010 in Holz *et al*. 2016)
- Summer breeding swarming (Humphrey and Cope 1976 in Turner *et al.* 2011
- Hibernates in very cold, humid caves (Frick *et al.* 2016, Langlois 2013)

These characteristics can be used as a benchmark for susceptibility and are a useful tool when looking at the potential vulnerability and susceptibility of other bat species to WNS.

Risk assessment

The 'Qualitative risk assessment: White-nose syndrome in bats in Australia' (Holz *et al.* 2016) has been completed. WNS is currently thought to be absent from Australia. Seven cave dwelling bat species in southern Australia are at risk from contracting the disease with potentially devastating consequences. The Southern Bent-wing Bat (*Miniopterus orianae bassanii*) was assessed as having a high risk and the Eastern Bent-wing Bat (*Miniopterus orianae oceanensis*) was assessed as having a medium risk, while the following species are regarded to be at low risk: Eastern Horseshoe Bat (*Rhinolophus megaphyllus*), Chocolate Wattled Bat (*Chalinolobus morio*), Large-eared Pied Bat (*Chalinolobus dwyeri*), Large-footed Myotis (*Myotis macropus*) and Finlayson's Cave Bat (*Vespadelus finlaysoni*) (Holz et al.2016).

The following map demonstrates the 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.

Holz *et al.* (2016) identify that both bent-wing bat 'subspecies that occur in southern Australia are likely naïve to *Pd*. and cluster in large groups, an activity associated with high mortality in the USA'. These species also occur in caves accessible to the public thereby providing a mechanism of infection. The other bats listed have stable populations and roost in smaller groups and therefore considered at lower risk (Holz *et al*. 2016).

Inadequate quarantine

The risk assessment has identified that the most likely method of entry of *Pd* into Australia will be via infected surfaces such as clothing, footwear or equipment that has been used in affected caves (eg by tourists, cavers, researchers) (Holz *et al.* 2016).

WNS is currently not a 'nationally notifiable disease' under Australian Quarantine Regulations and no specific quarantine or border security checks are in place for this disease. 'Cave visitation' is not a trigger question on the 'Australian Immigration Arrival Form'.

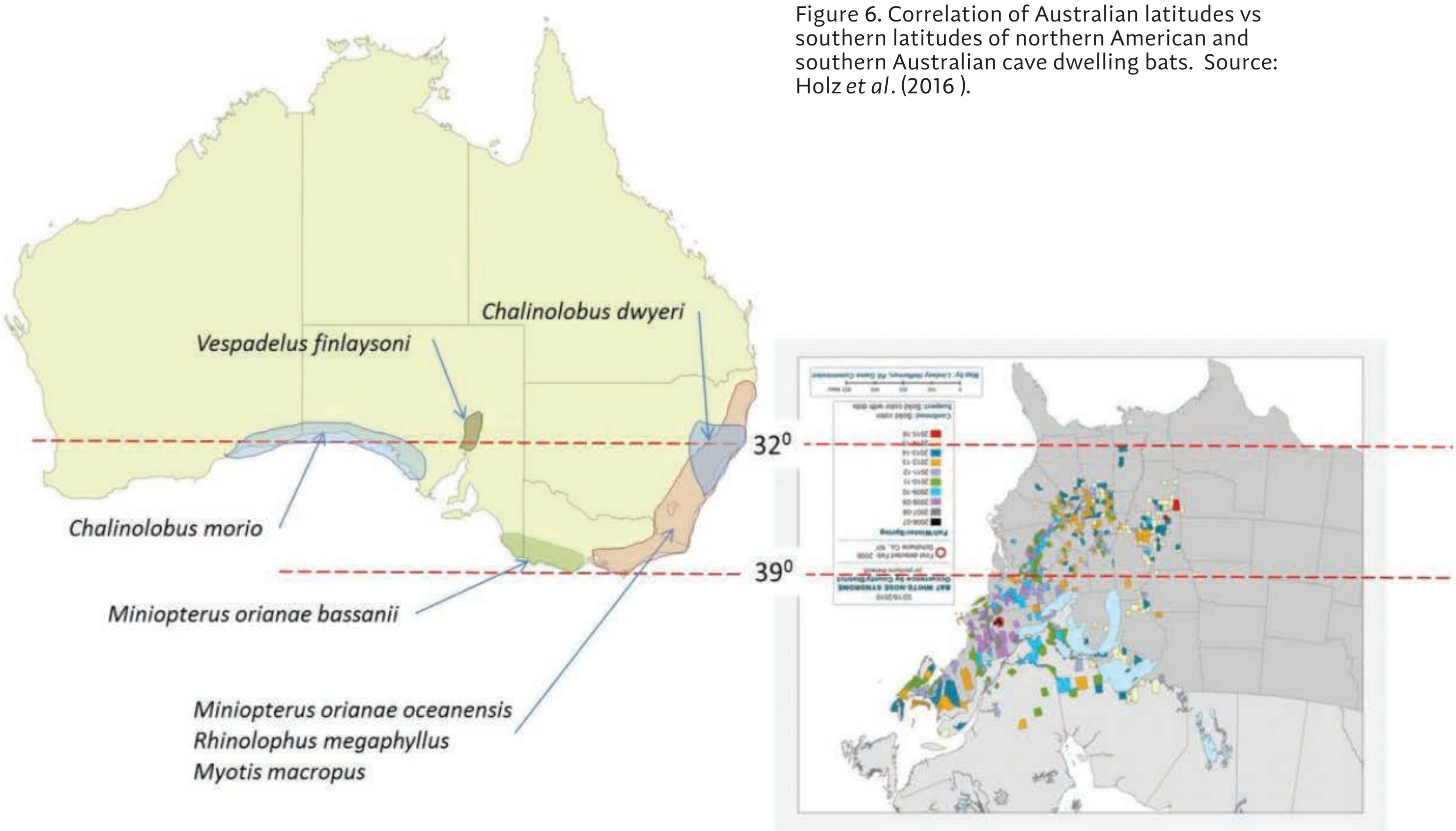

Figure 6. Correlation of Australian latitudes vs southern latitudes of northern American and southern Australian cave dwelling bats. Source: Holz *et al*. (2016).

No information is provided to incoming passengers about risks of contaminated gear or clothing from cave visitation.

Implementation of strict hygiene procedures is critical for keeping WNS from entering Australia. Cave gear hygiene needs to be 'caver driven'.

The safest way to keep the disease out of Australia is to not bring potentially contaminated gear into Australia. This can be achieved by not taking caving gear from Australia overseas and visitors to Australia not bringing potentially contaminated gear into Australia.

Therefore if you are considering travelling think —
INTERNATIONAL = LEAVE GEAR AT HOME

Decontamination

The WNS Decontamination Team (2018) recommends the following procedures to decontaminate caving gear and equipment.

DECONTAMINATION PROCEDURE FOR SUBMERSIBLES:

- Thoroughly clean caving gear by removing dirt and grime.
- Submersion in hot water maintaining a temperature of greater than 55 ˚C for a minimum of 20 minutes.

DECONTAMINATION PROCEDURE FOR NON SUBMERSIBLES:

- Non-submersibles require disinfection using 6% hydrogen peroxide spray or isopropanol disinfectant wipes, see list in Table 1.
- Boots should be scrubbed to remove all mud and dirt then sterilised using a suitable and appropriate chemical from the list in Table 1 below.

Any gear that has been taken into potentially infected caves and can't be treated using the appropriate decontamination procedures, should NOT be brought into Australia.

	Tested Applications & Products [3, 4, 5, 6, & 7]	Federal Reg No.:	Laboratory Results
Preferred Applications	Equipment Dedication	N/A	Clean according to manufacturer standards and dedicated to a site
	Submersion in Hot Water[4, 6, & 7]	N/A	Laboratory effectiveness demonstrated upon submersion in water with sustained temperature ≥ 55℃ *(131°F)* for 20 minutes.
Other Products	Ethanol (60% or greater)[4, 6, & 7]	CAS - 64-17-5	Laboratory effectiveness demonstrated upon exposure in solution for at least 1 minute.
	Isopropanol (60% or greater)[4, 6, & 7]	CAS - 67-63-0	
	Isopropyl Alcohol Wipes (70%)[4, 6, & 7]	CAS - 67-63-0	Laboratory effectiveness demonstrated immediately following contact and associated drying time.
	Hydrogen Peroxide Wipes (3%)[4, 6, & 7]	CAS - 7722-84-1	
	Rescue® (Fromerly Accel®) [4, 5, 6, & 7]	EPA - 74559-4	Laboratory effectiveness demonstrated when used in accordance with product label.
	Clorox® Bleach[3, 4, 5, 6, & 7]	EPA - 5813-100	
	Clorox® Wipes[4, 5, 6, & 7]	EPA - 5813-79	
	Clorox® Clean-Up Cleaner + Bleach[4, 5, 6, & 7]	EPA - 5813-21	
	Lysol® IC Quaternary Disinfectant Cleaner [3, 4, 5, 6, & 7]	EPA - 47371-129	

Table 1. Applications and products with demonstrated efficacy against *Pd* ([3]Shelley *et al.* 2011). Remember to consult equipment labels, registered product labels, and the appropriate Safety Data Sheet for regulations on safe and acceptable use. Source: WNS Decontamination Team (2018).

The US Environment Protection Authority has been testing various methods and disinfectants to determine effectiveness against *P. Destructans* (WNS Decontamination Team 2018), see Table 1.

How can cavers and caving clubs help?

- Consider WNS when travelling overseas and leave your gear at home to avoid contamination.
- Always use clean caving gear between caves, even in the same caving area, to avoid transferring any pathogens between caves in Australia.
- Think about purchasing caving gear that is easy to clean and decontaminate such as rubber boots rather than leather boots. Have multiple pairs of overalls to allow washing and drying between caves.
- Remember to **DECONTAMINATE!**

All Australian caving clubs should:

- Establish and implement a standard procedure for responding to caving enquiries from overseas visitors.
- Recommend to international cavers to leave their caving gear at home.
- Recommend members leave their gear at home when caving internationally.
- Provide information on WNS and decontamination procedures.

- Make gear available for use to prevent the risk of infected equipment entering Australia. (fund through grants).
- Establish and implement standard cave gear hygiene procedures to prevent the spread of any pathogens between caves.

Caving clubs can also help to fill critical knowledge gaps using minimal disturbance methods (eg climate data loggers, bat detectors, remote cameras)

- Identify bat caves and which species use them.
- Identify bat cave usage e.g. times of year etc.
- Monitor and record climatic conditions in those caves.
- Disease surveillance, recognise the signs and symptoms of WNS and report.

Wildlife Health Australia are currently trying to get *P. destructans* listed under Australian quarantine.

- The caving community could put pressure on the government demanding the disease be listed as a '**nationally notifiable disease'.**

What can ASF and ACKMA do?

- Update the Australian Speleological Federation (ASF) Minimal Impact Caving Code of Practice to include protocols and procedures around avoiding the spread of pathogens in general including WNS.
- ASF and Australasian Cave and Karst Management Association Inc. (ACKMA) can encourage all show caves to implement footware decontamination before visitors enter a cave.

Learn more about White nose syndrome by visiting https://wildlifehealthaustralia.com.au/ProgramsProjects/BatHealthFocusGroup.aspx

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