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Behaviour of hibernating little brown bats experimentally inoculated with the pathogen that causes white-nose syndrome

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Pathogens can affect host behaviour in ways that influence disease transmission as well as survival and fitness for both host and pathogen. Hibernating bats with white-nose syndrome (WNS) show a number of unusual behaviours including increased frequency of arousal from torpor, altered roosting behaviour and premature emergence. However, mechanisms underlying these patterns are not understood, and the behaviour of bats with WNS has not been examined systematically. Three hypotheses could explain increased arousal frequency. Bats may arouse to (1) groom in response to skin infection, (2) drink to offset dehydration or (3) increase activity, possibly in an attempt to access resources, avoid a source of infection or limit the risk of infecting relatives. We tested these hypotheses with captive little brown bats, *Myotis lucifugus*, inoculated with *Pseudogymnoascus destructans*, the fungus that causes WNS. In contrast to predictions of all three hypotheses, bats inoculated with the fungus tended to be less active than controls during arousals from torpor and did not increase grooming or visits to the water source in their enclosures. However, bats showed a dramatic reduction in clustering behaviour as infection progressed. Reduced activity and clustering could represent adaptive, maladaptive or pathological responses. Reduced activity could be an energy-saving mechanism or a pathological consequence of infection while reduced clustering could have beneficial or detrimental consequences for transmission, energetics, water balance and survival. Our results highlight the need for studies of host behaviour to understand dynamics of wildlife infectious diseases.

Emerging wildlife diseases can threaten populations and have large ecological and economic impacts (Boyles, Cryan, McCracken, & Kunz, 2011; Daszak, Cunningham, & Hyatt, 2000; Kilpatrick, Briggs, & Daszak, 2010). Many wildlife diseases are associated with behavioural changes in their hosts (Bos, Lefèvre, Jensen, & d'Ettorre, 2011; Weary, Huzzey, & von Keyserlingk, 2009) that can be adaptive if they improve host survival or reduce transmission to genetically related individuals (Hart, 1988; Loehle, 1995; Moore, 2002; Rueppell, Hayworth, & Ross, 2010). For example, caribou relocate during parasite outbreaks to avoid becoming infected (Downes, Theberge, & Smith, 1986) and infected individuals of some colonial insects self-isolate in the presence of disease to reduce transmission to relatives (Bos et al., 2011; Moore, 2002; Rueppell et al., 2010). Alternatively, changes in host behaviour can be maladaptive if they reduce host survival. For instance, behavioural isolation of infected individuals can increase their risk of predation (Hart, 1988; Loehle, 1995) and, in some cases, pathogens appear to actively manipulate host behaviour to increase predation risk and complete their own life cycle (Libersat, Delgado, & Gal, 2009; Møller, 1993). Understanding behavioural changes of hosts following disease emergence can shed light on mechanisms underlying disease processes and pathogen transmission, and potentially aid management and conservation efforts (Hawley & Altizer, 2010).

Since it was the first photo-documented in Howes Cave in New York State in 2006, white-nose syndrome (WNS) has spread to 22 U.S. states and five Canadian provinces (Blehert et al., 2009; U.S. Geological Survey, 2012). WNS affects seven bat species (Reeder & Turner, 2008; U.S. Fish and Wildlife Service, 2012b) and causes mortality rates as high as 99% in some hibernacula, with millions of bats killed to date and regional extinctions predicted (Frick et al., 2010; U.S. Fish and Wildlife Service, 2012a). The disease is caused by the fungus *Pseudogymnoascus destructans* (*Pd*; formerly

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Geomyces destructans; Minnis & Lindner, 2013) that damages skin by invading and eroding the epidermis, connective tissue and glands (Gargas, Trest, Christensen, Volk, & Blehert, 2009; Lorch et al., 2011; Meteyer et al., 2009; Warnecke et al., 2012). The fungus infects bats throughout Europe but without the mass mortality observed in North America (Martínková et al., 2010; Puechmaille et al., 2011; Wibbelt et al., 2010). This suggests that European bats may exhibit characteristics that aid their survival, or that they evolved behavioural or physiological mechanisms of resistance to *Pd* (Puechmaille et al., 2011; Wibbelt et al., 2010). The hypothesis that *Pd* is an invasive pathogen to North America is supported by experimental evidence demonstrating that North American bats are similarly susceptible to *Pd* from Europe and North America (Warnecke et al., 2012), and genetic evidence demonstrating that isolates of North American *Pd* represent a single, clonal genotype that is not found outside of the known range in North America (Lorch et al., 2012; Rajkumar et al., 2011).

Pseudogymnoascus destructans is cold adapted and infects bats during hibernation. Infection is associated with a number of changes in behaviour. Normal hibernation consists of repeated bouts of torpor (during which body temperature and metabolic rate are dramatically reduced) interspersed with brief periodic arousals to normothermic body temperature that occur on average between every 2–3 weeks in healthy bats (Jonasson & Willis, 2012; Warnecke et al., 2012). One of the most pronounced behavioural changes in bats infected with *Pd* is an increase in the frequency of periodic arousals as infection progresses, resulting in premature depletion of fat stores (Boyles & Willis, 2010; Reeder et al., 2012; Warnecke et al., 2012). Bats with WNS are also often found roosting closer to the entrances of hibernacula than normal (McAlpine, Vanderwolf, Forbes, & Malloch, 2011) and emerging from hibernacula prematurely during winter (Cryan, Meteyer, Boyles, & Blehert, 2010). In addition to affecting arousal frequency and emergence, WNS may also influence social behaviour and clustering. Two of the species most affected by WNS, little brown bats, *Myotis lucifugus*, and Indiana bats, *Myotis sodalis*, typically cluster in groups during hibernation (Brack & Twente, 1985; Clawson, LaVal, LaVal, & Claire, 1980). However, since WNS was first detected, the proportion of individuals of these species observed roosting individually has increased (Langwig et al., 2012). This suggests that either WNS is selecting for bats that tend to roost individually, or infection leads to a behavioural change in the tendency of individual bats to cluster (Langwig et al., 2012). Such a behavioural change could be adaptive if it reduces disease transmission (Langwig et al., 2012) or maladaptive if it increases water loss or energetic costs (Boyles, Storm, & Brack, 2008).

The trigger for increased arousal frequency in bats with WNS is still not understood. However, this pattern could be explained by three nonmutually exclusive hypotheses. The first hypothesis suggests that, even though infection with *Pd* causes little inflammation (Gargas et al., 2009; Meteyer et al., 2009), irritation due to fungal invasion and damage to the epidermis could trigger arousal. This hypothesis predicts that infected bats should increase grooming during arousals. Second, wing damage due to fungal infection could increase cutaneous evaporative water loss, loss of fluids and dehydration, forcing bats to arouse more frequently (Cryan et al., 2010; Cryan et al., 2013; Warnecke et al., 2013; Willis, Menzies, Boyles, & Wojciechowski, 2011). This predicts that infected bats should drink more than normal during arousals to replenish water stores and should devote a greater proportion of their time to drinking. Third, bats may arouse more frequently as they become increasingly motivated to leave the hibernaculum or as a consequence of a mechanism to limit pathogen transmission. This hypothesis is consistent with a number of possible scenarios. Increased activity could reflect the energetic or hygric consequences of infection and increasing demand for food or water as fat stores decline or bats become dehydrated. Increased activity could also reflect a behavioural tendency for individual bats to reduce their own risk of pathogen exposure by moving away from unhealthy roostmates, as occurs in other wildlife species (Downes et al., 1986). On the other hand, if bats tend to cluster with relatives during hibernation, increased activity or self-isolation by badly infected individuals could be a mechanism to avoid infecting kin (Bos et al., 2011; Rueppell et al., 2010). If the reduced rate of clustering observed in WNS-affected hibernacula represents a behavioural change rather than selection for solitary bats, this could reflect attempts by individuals to minimize pathogen exposure or transmission to roostmates (Bos et al., 2011; Langwig et al., 2012; Rueppell et al., 2010). Therefore this hypothesis also predicts that infected bats should show heightened activity during arousals and reduced clustering as a mechanism to slow pathogen transmission (Langwig et al., 2012).

Despite the apparent behavioural changes associated with WNS, and the potential for behavioural studies to shed light on disease processes (Moore, 2002), so far only one study has quantified behavioural observations of hibernating bats infected with *Pd* (Brownlee-Bouboulis & Reeder, 2013). This partly reflects the fact that behaviour of free-ranging bats is extremely difficult to observe during hibernation. Bats are highly sensitive to disturbance and hibernacula are often remote and usually too large for effective video recording of the behaviours of individuals. Although some factors experienced in the wild by hibernating bats cannot be simulated in captivity (e.g. freedom to leave the hibernaculum, size/volume of a typical hibernaculum), many conditions can be replicated and, under the right environmental conditions, some bat species, including those susceptible to WNS, hibernate normally in captivity (e.g. Warnecke et al., 2012; Warnecke et al., 2013). Moreover, in the field, all individuals in an affected hibernaculum must be assumed to have been exposed to *Pd*. As a result, comparisons between WNS-affected and unaffected bats may be confounded by site-specific characteristics that do not reflect infection (e.g. microclimate, volume of the hibernacula, possible genotypic and phenotypic difference between bats in different sites) and effects of infection on behaviour cannot be isolated from effects of 'site'. In captivity, environmental variables like microclimate as well as the origin of the bats can be readily controlled. Thus, while results must be interpreted carefully, captive studies of behaviour have strong potential to shed light on disease mechanisms in WNS.

To better understand behavioural changes associated with WNS, and to test hypotheses about mechanisms underlying the disease, we analysed infrared video recordings of captive bats experimentally inoculated with *Pd* by Warnecke et al. (2012). We quantified a range of behaviours associated with each of the hypotheses explaining increased arousal frequency and tested the hypothesis that observations of reduced clustering by bats in affected hibernacula reflect behavioural change rather than natural selection by the disease for solitary bats. Given recent physiological evidence for the dehydration hypothesis (Cryan et al., 2010; Cryan et al., 2013; Warnecke et al., 2013; Willis et al., 2011), we predicted that bats would increase visits to the water source in their enclosure. Bats with WNS show little to no inflammation during hibernation (Meteyer et al., 2009), so we did not expect to observe more grooming by infected bats. We did predict an increase in activity during arousals, consistent with the hypothesis that bats become motivated to access resources and/or move away from roostmates as the disease progresses. In keeping with the hypothesis that reduced clustering represents a behavioural change by individuals, rather than selection (Langwig et al., 2012), we also predicted a reduction in clustering throughout hibernation.