



SYMPOSIUM

Conservation Physiology and Conservation Pathogens: White-Nose Syndrome and Integrative Biology for Host–Pathogen Systems

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From the symposium “Physiology in Changing Landscapes: An Integrative Perspective for Conservation Biology” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2015 at West Palm Beach, Florida.

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Synopsis Conservation physiology aims to apply an understanding of physiological mechanisms to management of imperiled species, populations, or ecosystems. One challenge for physiologists hoping to apply their expertise to conservation is connecting the mechanisms we study, often in the laboratory, with the vital rates of populations in the wild. There is growing appreciation that infectious pathogens can threaten populations and species, and represent an important issue for conservation. Conservation physiology has much to offer in terms of addressing the threat posed to some host species by infectious pathogens. At the same time, the well-developed theoretical framework of disease ecology could provide a model to help advance the application of physiology to a range of other conservation issues. Here, I use white-nose syndrome (WNS) in hibernating North American bats as an example of a conservation problem for which integrative physiological research has been a critical part of research and management. The response to WNS highlights the importance of a well-developed theoretical framework for the application of conservation physiology to a particular threat. I review what is known about physiological mechanisms associated with mortality from WNS and emphasize the value of combining a strong theoretical background with integrative physiological studies in order to connect physiological mechanisms with population processes and thereby maximize the potential benefits of conservation physiology.

Introduction

Conservation physiology is an emerging discipline that aims to quantify physiological responses of organisms to human-caused changes in the environment and inform management actions by incorporating information from integrative, whole-organism biology (Wikelski and Cooke 2006; Cooke et al. 2013). Ultimately, the objective is to link cause and effect at the level of the whole-organism (i.e., the scale of traditional physiological studies) with responses to anthropogenic threats and conservation actions at the levels of populations and ecological communities (i.e., the scale of most research on conservation and management) (Cooke et al. 2013; Cristine et al. 2014). Although it has not always been explicitly labeled as conservation physiology, this approach has been applied to many issues in wildlife conservation, including the effects of human activities on physiological stress (i.e., studies of glucocorticoid stress

hormones), the influence of habitat quality and/or changing climate on the energy balance of free-living wildlife, the role of diet and nutritional status in reproduction, and the direct and indirect effects of chemical spills, or other pollutants, on an individual’s physiology, survival, or reproduction (reviewed by Cooke et al. 2013; Cristine et al. 2014).

Conservation physiology is, by nature, integrative and inter-disciplinary (Cooke et al. 2013), which creates opportunities but also presents challenges. One considerable challenge for physiologists hoping to apply their research to the many conservation problems facing the modern world is the all-important connection between physiological mechanisms and populations (e.g., rates of survival and reproduction as they affect growth of populations) (Cristine et al. 2014). While many studies have identified the physiological consequences of human activities that might be predicted to influence population processes for

threatened wildlife, relatively few have established these links directly and fewer still have tested physiologically-based interventions that could allow managers to manipulate population growth. Clearly-articulated eco-physiological theory, and predictive models that connect organismal level mechanisms to population processes, could enhance our potential to make these connections by helping prioritize research questions and hypotheses.

Infectious diseases of wildlife are of increasing conservation concern as global travel and trade affect the dispersal of pathogens, and environmental change affects susceptibility of hosts (Daszak et al. 2000; Jones et al. 2008). There is growing realization that what I term “conservation pathogens” (i.e., pathogens causing, or contributing to, dramatic declines in the sizes of host populations) can cause extinctions of species and loss of biodiversity. There is also a growing appreciation that pathogens of wildlife are relevant to human public health (Daszak et al. 2000). For example, most recent emerging infectious diseases of humans (e.g., Severe Acute Respiratory Syndrome (SARS), Ebola, Hendra virus, Nipah virus) appear to have arisen when human encroachment forced wildlife into contact with people or livestock (Daszak et al. 2000). Despite economic, public health, and conservation implications, however, we have few data about most pathogens of wildlife. Chytridiomycosis in amphibians is perhaps one of the best-known recent examples of a conservation pathogen causing catastrophic declines of wildlife and extinctions of species. Indeed, the fungal pathogen *Batrachochytrium dendrobatidis* is credited with the largest disease-induced loss of biodiversity in history (Fisher et al. 2009; Kilpatrick et al. 2010; Voyles et al. 2011). More recently, white-nose syndrome (WNS) in bats, caused by another fungus, *Pseudogymnoascus destructans*, has led to similarly catastrophic declines in populations of bats throughout eastern North America with extirpations predicted (Bleher et al. 2009; Frick et al. 2010, 2015; Langwig et al. 2012). Fungal pathogens appear to be on the rise worldwide (Fisher et al. 2012) but other pathogens and parasites have had dramatic implications for conservation. Avian malaria has caused declines of many native Hawaiian bird species (e.g., van Riper et al. 1986). An unusual facial tumor disease, caused by infectious cancer cells, has resulted in large population declines of the Tasmanian devil (*Sarcophilus harrisi*) (e.g., McCallum 2008). Sylvatic plague, caused by the bacterium *Yersinia pestis*, and canine distemper, caused by a paramyxovirus have both complicated captive rearing and re-introduction programs for endangered

black-footed ferrets (*Mustela nigripes*) in North America (e.g., Thorne and Williams 1988; Rocke et al. 2008).

One of the challenges for understanding and potentially mitigating conservation pathogens is that they often lead to such rapid population declines that large impacts have occurred before disease pathophysiology, host–pathogen dynamics, or even the basic biology of healthy, pre-disease host populations, are well understood (Voyles et al. 2014; Langwig et al. 2015a). WNS in hibernating bats of eastern North America is one such example. Until large die-offs of bats were observed in hibernacula, our understanding of hibernation physiology and ecology for healthy, pre-disease bats was limited. Since then, one priority has been to catch up on our understanding of healthy bats while also characterizing the mechanisms underlying the die-offs (White-Nose Syndrome Science Strategy Meeting 2008). An integrative approach, incorporating whole-animal physiological and behavioral studies has helped advance our understanding of the physiology of healthy bats alongside the pathogenesis and population impacts of WNS. Although WNS is still certainly a long way from being “solved”, aspects of the research response to this disease have been relatively effective (Voyles et al. 2014). Indeed, the response to WNS could serve as a model to help integrative biologists and conservation physiologists address other conservation challenges in part because the well-developed theory of disease ecology can guide physiological research.

Although most researchers studying consequences of WNS for hosts’ physiology have not referred to the theory of disease ecology explicitly (although, see Foley et al. 2011; Johnson et al. 2014), many questions about the disease are relevant to this well-developed theoretical framework. For example, a growing body of research about WNS could be useful in the context of Anderson’s and May’s (Anderson and May 1979; May and Anderson 1979) seminal models quantifying impacts of pathogens and parasites on hosts’ population processes. Their conceptual framework, and the large body of research built upon it, is well known to disease ecologists but likely less familiar to researchers working in integrative biology or conservation physiology. Anderson’s and May’s (Anderson and May 1979; May and Anderson 1979) classic SIR microparasite model (i.e., for microbial parasites that complete their life cycle inside hosts) conceptualizes hosts’ population dynamics in terms of susceptible (S), infected (I), and (R) resistant/recovered population pools (or SIS if survivors of the pathogen acquire

no immunity). Individuals transition from S to I as a function of transmission rate of the pathogen, and from I to R as a function of the rate of survival from infection. Each population pool has a birth rate and a death rate and these rates drive overall population dynamics (Anderson and May 1979). The innovation of this simple framework was to consider a host's population size as a dynamic variable and this way of thinking has been foundational for modern research in disease ecology. One could argue that the most important advances in our understanding of WNS to date have been those that might help parameterize this kind of model.

Here I use our preliminary understanding of the pathogenesis of WNS in hibernating bats to demonstrate the potential value of a conservation physiology approach for understanding and potentially mitigating a conservation pathogen. I argue that the strong theoretical foundation of disease ecology provides a useful framework for prioritizing hypotheses and designing field and laboratory research. This kind of “theory to physiology to populations” approach could be usefully applied to other conservation issues to help connect organism-level integrative biology with population processes of interest to managers. I first review what is known about physiological mechanisms, pathogenesis, and hosts' behavior in relation to WNS. I then suggest ways that these findings could be used to inform models of the impacts of pathogens in the context of May and Anderson's (1979) classic disease ecology framework, with suggestions for critical questions that could be addressed using a conservation physiology approach. A growing body of excellent primarily field-based research in disease ecology has shed important new light on transmission of *P. destructans* and the impacts of this pathogen on populations and communities (e.g., Langwig et al. 2012, 2015b; Hoyt et al. 2014; Reynolds et al. 2015; Frick et al. 2015). This work is reviewed elsewhere (Frick et al. Forthcoming 2015) so here I focus primarily on studies of the host's physiology to highlight the potential contribution of conservation physiology to the management of conservation pathogens.

Physiology and pathogenesis of WNS

During the winter of 2007 large numbers of emaciated, dead, and dying bats were found in and around several bat hibernacula in New York state (Frick et al. Forthcoming 2015). White fungal growth was observed on the exposed skin of many of these animals leading to the name WNS but it took several years of integrative laboratory and field studies to

establish a cause–effect relationship between fungal infection, disease, and mortality (Frick et al. Forthcoming 2015). Fungal pathogens rarely cause direct mortality in mammals so, initially, many alternative explanations for dead and dying bats were considered, if not favored (White-Nose Syndrome Science Strategy Meeting 2008). These included the possibility that the fungus was a secondary consequence of immune suppression due to pollutants, deficiencies in the pre-hibernation diet, or viral/bacterial co-infections, among other possibilities (White-Nose Syndrome Science Strategy Meeting 2008). Pathology indicated that the cold-tolerant fungus *P. destructans* was infecting exposed skin of the faces, ears, and wings of hibernating bats, causing lesions (i.e., cup-shaped erosions now considered diagnostic for WNS), eroding the epidermis, and invading hair follicles and glandular tissue (Bleher et al. 2009; Gargas et al. 2009; Meteyer et al. 2009). In a landmark laboratory study, Lorch et al. (2011) demonstrated that inoculation with *P. destructans* conidia, as well as physical (but not aerosol) contact among naturally infected bats, caused the diagnostic WNS lesions in naïve bats. However, it was still not clear why cutaneous infection caused emaciation and mortality and it was in this area that consideration of hibernation ecophysiology became especially useful.

Like all mammalian hibernators, bats spend the majority of their time during hibernation in energy-saving bouts of deep torpor but they spend most of their winter energy reserves on periodic rewarming (Geiser 2004, 2013). Every few weeks, bats must rewarm to normothermic body temperatures, presumably to restore homeostatic functions that cannot occur while they are cold (e.g., Jonasson and Willis 2012; Czenze and Willis 2015). Bats may arouse prematurely and expend extra energy if disturbed, for example by human visitors to hibernacula (Speakman et al. 1991; Thomas 1995) and one early hypothesis was that infection with *P. destructans* could disrupt arousal patterns in a similar way. Boyles and Willis (2010) provided support for this hypothesis using an energetic model showing that increasing frequency or duration of arousal (but not pre-hibernation body condition as predicted by several hypotheses implicating *P. destructans* as a secondary consequence of some other conservation issue) could replicate the timing of mortality observed in the wild. Subsequent inoculation experiments in the laboratory (Warnecke et al. 2012) and observations in the field (Reeder et al. 2012) comparing arousal patterns of infected and un-infected little brown bats (*Myotis lucifugus*), using skin temperature dataloggers, showed pronounced increases in the frequency of arousal as

WNS progressed in bats. In the laboratory this increase was associated with a dramatic depletion of fat reserves and mortality beginning between about day 80–90 post-inoculation confirming that infection with *P. destructans*, alone, was the cause of die-offs of bats observed in the wild (Warnecke et al. 2012). That inoculation experiment also showed that an isolate of *P. destructans* originally collected from European bats caused disease in North American little brown bats that was at least as, if not more severe than, disease caused by isolates of the fungus from North America. This was consistent with the novel pathogen hypothesis (Rachowicz et al. 2005) suggesting that *P. destructans* was accidentally introduced to North America from Europe or Asia. Indeed, it is now clear that the fungus is widespread on bats throughout Europe and perhaps Asia but with no evidence of mass mortality (e.g., Wibbelt et al. 2010; Puechmaille et al. 2011; Pikula et al. 2012).

Collectively, these findings helped focus attention on *P. destructans* as the underlying cause of bats' die-offs and highlighted hibernation energetics as a possible lynchpin of mortality or survival. It still remains unclear, though, how a cutaneous fungal infection disrupts energy balance during hibernation and affects torpor/arousal cycles. Given that water loss during torpor is one potential trigger for arousals during hibernation (Thomas and Geiser 1997), Cryan et al. (2010) proposed the dehydration hypothesis that damage to the skin following infection leads to the loss of fluid across highly vascularized, physiologically-active wing tissue. A modeling exercise connecting water loss to torpor/arousal patterns supported the plausibility of this hypothesis (Willis et al. 2011) and experimental comparisons of blood chemistry for WNS-affected and unaffected bats was also consistent, at least for bats with advanced infections (Cryan et al. 2013; Warnecke et al. 2013). Bats with severe WNS exhibited hypotonic dehydration (based on reduced concentrations of plasma sodium and chloride), hypovolemia (based on dramatically elevated hematocrit levels), and altered acid-base balance (possibly associated with reduced perfusion of damaged tissue) (Cryan et al. 2013; Warnecke et al. 2013). Warnecke et al. (2013) used these findings to propose a mechanistic model linking wing damage from WNS to physiological processes that could lead to mortality. Recent studies have begun to test predictions of this model, as well as predictions based on the original hypothesis of Cryan et al. (2010) (e.g., Carey and Boyles 2015) but, for most bats, the exact cause of death from WNS is still not clear.

Hypotonic dehydration appears to play a role in WNS for bats with advanced infections but recent work suggests that physiological consequences of early-stage disease are very different. Verant et al. (2014) used doubly-labeled water and measurements of blood chemistry to study mild to moderate infections and found little evidence of increased water loss. However, they made the important discovery that energy expenditure, estimated from changes in ratios of lean mass to fat mass over the course of their experiment, was twice as high for infected bats, even before any increase in frequency of arousal could be detected. This means that torpid and/or normothermic metabolic rates must have increased for infected bats and suggests that increased frequency of arousal in late-stage infection is not the sole explanation for exhausted reserves of energy. Verant et al. (2014) found no difference in skin temperatures of infected bats versus controls and proposed several non-exclusive hypotheses for the increased use of fat by infected individuals. These included higher costs of thermoregulation if infection inhibits relatively inexpensive vasomotor mechanisms of thermoregulation and, instead, requires bats to use more expensive mechanisms to thermoregulate, increased use of fat for production of metabolic water to compensate for increased water loss resulting from infection, or some other energetic cost associated with invasion by *P. destructans*. Verant et al. (2014) did not suggest what these other costs might be, but genes associated with the innate immune response appear to be upregulated for infected little brown bats (Rapin et al. 2014), and little brown bats with WNS exhibit altered immune function (Moore et al. 2011, 2013). This immune response, even if ineffective for combatting WNS in the most affected species, would increase the expenditure of energy (Moore et al. 2011, 2013; Rapin et al. 2014). While considerable progress has been made in understanding the physiological consequences of infection, and the factors that ultimately impact rates of mortality, we still do not understand precisely what kills bats with WNS.

Behavior and population impacts of WNS

The physiological studies described above have helped reveal important information about disease mechanisms and additional work on behavior and ecology has helped connect these mechanisms to impacts on populations. Field surveillance based on quantitative polymerase chain reaction (qPCR) analysis of wing swabs has detected 11 species of bats

carrying *P. destructans* (or at least carrying *P. destructans* DNA) but rates of mortality vary widely among species from no apparent disease or mortality to severe infection, dramatic mortality, and possible extirpation of populations (e.g., Langwig et al. 2012; Frick et al. 2015). Characteristic infections (i.e., cup-shaped erosions caused by fungal invasion) have been observed on seven bat species, and dramatic declines of the most affected of these have been quantified based, both on counts of bats remaining in hibernacula during winter (e.g., Frick et al. 2010; Wilder et al. 2011; Langwig et al. 2015b) and acoustic data during the active season (e.g., Brooks 2011; Dzal et al. 2011; Ford et al. 2011). Little brown, tri-colored (*Perimyotis subflavus*) and northern long-eared bats (*Myotis septentrionalis*) are among the most severely impacted (Langwig et al. 2012) and all three species are now formally listed as federally endangered in Canada (Environment Canada 2015) with northern long-eared bats listed as threatened in the United States (United States Fish and Wildlife Service 2015). The latter species is perhaps the most adversely affected, having been effectively extirpated from up to 69% of hibernacula in the northeastern United States where it formerly occurred (Frick et al. 2015).

A number of excellent recent studies have taken a disease-ecology approach to important questions about transmission and invasion dynamics of *P. destructans*, and noted differences in the density-dependence of transmission and impacts among various species (e.g., Langwig et al. 2012, 2015b; Frick et al. 2015). These studies are reviewed by Frick et al. (Forthcoming 2015) but aspects of these papers are especially relevant in the context of conservation physiology because they connect physiological and behavioral mechanisms, and environmental variation, to impacts on populations. Langwig et al. (2012) found that counts of free-ranging little brown bats declined the most in relatively warm hibernacula following the emergence of WNS while counts of Indiana bats (*M. sodalis*) declined the most in hibernacula with the highest relative humidity. Similarly, in the laboratory Johnson et al. (2014) found that WNS had the most pronounced effect on captive bats maintained under relatively warm conditions near the upper limit of the growth range for *P. destructans* (Verant et al. 2012). In terms of the influence of humidity on hibernation physiology of bats, absolute humidity, not relative humidity, is the most relevant parameter (Kurta 2014) so these relationships are tricky to interpret because of the dependence of water-vapor pressure on temperature (i.e., absolute humidity and temperature cannot be

teased apart in these studies). Nevertheless, these papers highlight the potential importance of environmental variation in temperature and/or humidity as drivers of variation in survival. More work is needed to understand if this influence results from environmental effects on the hibernation physiology of bats, the growth of *P. destructans*, or both. Impacts of WNS could be greater in warm conditions because high temperatures increase torpid metabolic rates of bats cause more frequent arousals and increase energy expenditure (Geiser 2004, 2013). On the other hand, warm conditions also increase the rate of fungal growth in the laboratory, up to a limit of about 15°C (Verant et al. 2012). Higher absolute humidity resulting from higher temperatures could also favor more rapid fungal growth, although effects of humidity on growth of *P. destructans* have yet to be assessed experimentally.

Studies of behavioral responses of bats to *P. destructans* have also been important for connecting physiological mechanisms to population processes. Langwig et al. (2012) reported that a dramatically larger proportion of free-ranging little brown bats were roosting solitarily in their hibernacula after the arrival of WNS and suggested that this could reflect a behavioral change resulting from infection. Wilcox et al. (2014) provided support for this hypothesis using infrared video to quantify the behavior of little brown bats during arousals throughout an inoculation study. They found that bats stopped clustering and spread out in their hibernacula as infection progressed, suggesting that the pattern observed for free-ranging bats by Langwig et al. (2012) did not reflect selection against clustering behavior by WNS but, rather, a behavioral response of individuals to infection. This behavioral response could have important implications for the transmission and severity of disease. Even for bats that are already infected, minimizing subsequent contacts with other infected individuals could be beneficial if repeated contacts increase points of infection on an individual's skin (Frick et al. Forthcoming 2015). On the other hand, reduced clustering could be detrimental for energy balance if it negatively impacts thermoregulatory capability or, given the links between water loss and clustering behavior observed for other species of bats (e.g., Boratyński et al. 2015), further increases in evaporative water loss (Wilcox et al. 2014; Frick et al. Forthcoming 2015).

In addition to reduced clustering, Wilcox et al. (2014) found that experimentally infected little brown bats failed to compensate for physiological dehydration by drinking more frequently during

arousals. Indeed, bats reduced overall activity (i.e., movement around the cage, grooming) during arousals compared with sham-inoculated controls. When combined with less clustering, reduced activity by infected bats is consistent with a general pattern of behavior known as “sickness behavior” which is exhibited by many species during pathogenic infections (Hart 1988; Adelman and Martin 2009). Sickness behavior refers to a coordinated response to infection that is not simply an absence of healthy behavior but an adaptive pattern of lethargy and reduced sociality to save energy for re-allocation to immune responses (Hart 1988; Adelman and Martin 2009). This behavioral pattern is thought to be mediated by the innate immune system and, in bats with WNS, could be associated with the increased expression of innate immune genes observed by Rabin et al. (2014). For bats, reduced behavioral activity could help save energy, given that infection with *P. destructans* increases the frequency of arousal in late-stage infections (Reeder et al. 2012; Warnecke et al. 2012) as well as torpid or normothermic energy expenditure in early-stage disease (Verant et al. 2014). Compared with sham-inoculated controls, experimentally-infected bats also appear more sensitive to disturbance by arousals and activity of their roost-mates in the hibernaculum (Turner et al. 2015). Self-isolation and reduced clustering could be energetically beneficial if they reduce an individual’s potential to be disturbed by sick colony-mates (Turner et al. 2015). Sickness behavior could also reduce the risk of transmission. As noted above, reduced clustering could prevent infected bats from acquiring additional points of infection on their skin from other infected bats. Given the likely importance of substrates in the hibernaculum as environmental reservoirs for *P. destructans* (e.g., Hoyt et al. 2014; Reynolds et al. 2015), less activity during arousals could also reduce the chance for additional exposures from those substrates. Behavioral changes resulting from infection could also have implications for our ability to enumerate bats and quantify declines of populations. Most ecological studies of the impacts of WNS to date have relied on counts of bats in hibernacula (e.g., Langwig et al. 2012, 2015b; Frick et al. 2015) but if bats isolate themselves and potentially roost in less obvious locations after infection, we could overestimate rates of decline. Behavioral and physiological studies of individual bats, perhaps using radio-telemetry inside hibernacula, combined with more work to enumerate bats during summer (particularly for species which do not roost in buildings like northern long-eared bats) are needed to help determine whether

behavioural changes resulting from infection with *P. destructans* are influencing our estimates of bat population sizes.

From physiology to populations to management

Although they may not have been explicitly framed in these terms, the findings about physiological mechanisms of WNS described above could be useful for understanding population processes when considered in terms of the conceptual frameworks of disease ecology pioneered by May and Anderson (1979). Most fundamentally, the early studies of WNS defined the conservation issue as a host–pathogen problem (Blehert et al. 2010; Lorch et al. 2011; Warnecke et al. 2012) and, therefore, pointed to disease ecology as the framework for subsequent study. Since then, studies in conservation physiology have helped inform how physiological mechanisms might translate into rates of mortality versus survival (i.e., whether individuals in the “infected” pool of the population die or instead transition to the “recovered” pool). This approach also has great potential for prioritizing future research. For example, if mortality from WNS reflects energy balance, and starvation is the most common cause of death, will bats that accumulate larger pre-hibernation reserves of fat exhibit higher survival rates? Particularly for females, which are capital breeders and depend on fat stored over the winter to initiate pregnancy before food is available in the spring, hibernation physiology and energetics could strongly influence reproduction and survival. Willis and Wilcox (2014) suggested that resistance to the lipostat hormone leptin during the fattening phase in autumn could serve as a potential physiological (and likely heritable, see below) mechanism of phenotypic variation leading to variation in mortality from WNS. For healthy bats, patterns of torpor and the expenditure of energy during hibernation vary among individual bats but may be repeatable within individuals (Matheson et al. 2010; Menzies 2014). One important question for connecting hosts’ physiology to population processes is whether individual, sex, or age-specific differences in the tendency to express torpor during hibernation (e.g., Jonasson and Willis 2011, 2012; Menzies 2014) have implications for survival from WNS. Female bats have been predicted to exhibit reduced impacts because they accumulate larger fat reserves than do males in the fall and use these fat reserves more slowly during winter (Jonasson and Willis 2011). Counter to this hypothesis, however, Johnson et al. (2014) found

that in the laboratory females were more severely impacted than males by infection with *P. destructans*. This discrepancy highlights the need to understand how variation in energy balance affects survival and also highlights the value of experimental conservation physiology studies, like that performed by Johnson et al. (2014), toward informing models of the impacts of disease.

One of the most important criteria for prioritizing management responses to WNS is determining whether populations can evolve mechanisms of resistance to, or tolerance of, infection with *P. destructans* on their own. To date, several field studies have shown that declines in counts of hibernating bats in many sites are ameliorating and that counts of some species appear to be increasing slowly, albeit from a post-WNS baseline that is vastly smaller than pre-WNS numbers (Langwig et al. 2012; Frick et al. 2015). One possible explanation for this pattern is evolutionary rescue via heritable phenotypic variation in traits that might provide protection from WNS (e.g., Maslo and Fefferman 2015). The breeder's equation quantifies evolutionary change of a trait in a population as the product of heritability of the trait (the proportion of the trait's variance attributable to additive genetic effects) and the selection differential (the association between values of the trait and reproductive fitness) (Falconer and McKay 1996). Clearly, WNS-affected species of bats are passing through a tremendous survival bottleneck and, therefore, could be subject to extremely high selection differentials for physiological, behavioral, and/or immune traits that might lead to resistance or tolerance of WNS (e.g., Willis and Wilcox 2014; Maslo and Fefferman 2015). Quantifying selection differentials and determining heritability of candidate traits should be an urgent priority, particularly since the value of this approach for conservation has been illustrated for other hibernators. For example, traits associated with energy balance during hibernation (i.e., the same kinds of traits that could be critical for surviving WNS) are heritable in Columbian ground squirrels (*Urocitellus columbianus*) and are potentially evolving in response to climatic change (Lane et al. 2011, 2012). For ground squirrels multi-generation pedigrees have been used to quantify heritability because pups can often be directly observed with their mothers at burrow entrances, and paternity by local males can be assigned using molecular methods. Such pedigrees, based on known parent-offspring relationships, will be much more difficult to obtain for bats because of their lower reproductive rates, larger home-ranges, the long temporal delay between mating and parturition (which

complicates paternity assignment), and the difficulty of directly observing parent-offspring interactions by bats sharing inaccessible roost sites with both relatives and non-relatives. However, advances in molecular approaches based on analyses of large numbers of single nucleotide polymorphisms (i.e., SNPs) can help generate such pedigrees for species that are difficult to observe directly and hold great promise for assessing questions about the heritability of traits which might help bats survive WNS (Jones and Ardren 2003; Pemberton 2008).

A conservation physiology approach combining laboratory studies of physiological traits with fieldwork on large, marked populations of bats for which phenotypic traits have been measured, and genetic samples collected, could help advance this priority. To prepare for the arrival of WNS in central Canada, we have marked thousands of little brown bats and hundreds of northern long-eared bats with passive integrated transponders (PIT tags) at known hibernacula since 2008 (Norquay and Willis 2014). We have also measured a range of phenotypic traits (pre-hibernation body condition, hibernation phenology based on PIT-tag detection by remote antennas at entrances to 10 hibernacula, personality traits such as activity and sociability which could affect transmission) and obtained tissue samples for subsequent genetic analyses. The hope is that, once WNS arrives, we will be able to combine heritability analyses of phenotypic traits with physiological data on hibernation energetics with data on survival, to address critical questions about potential evolutionary response of bats to WNS.

Arguably, solving the breeder's equation for WNS should be our most important priority because it will help us decide on actions for management. In particular, it will help determine whether we should focus resources on interventions to treat the disease during winter, or focus on protecting and enhancing summer habitats that might improve reproductive rates of survivors and facilitate the evolution of traits promoting host survival (e.g., Kilpatrick 2006). Indeed, if bats can evolve resistance to, and/or tolerance of, WNS then a range of management interventions that might, at first, seem highly attractive, could have counter-productive effects. For example, if differential survival and heritable phenotypic variation in particular traits can lead to the evolution of resistance or tolerance in bats, the kinds of treatments that have received widespread attention in the popular media, such as modification of the microclimate of existing hibernacula (e.g., Boyles and Willis 2010) or the application of chemical/biological control agents (e.g., Cornelison et al. 2014; Hoyt et al. 2015),

could be counter-productive to the long-term viability of populations, species, or communities of bats. If these interventions were implemented on a large scale, they might reduce selection differentials exerted on the host by the pathogen and could theoretically reduce rates of evolution of traits important for resistance or tolerance. Given the complexity of *P. destructans* as a multi-host pathogen, treatments leading to these kinds of effects in one or a few of the affected species could have unintended consequences for other species and for assemblages of bats on the whole. Perhaps even more insidious, treatments that are only partially (or even highly) effective could favor the evolution of resistance in the fungus itself, in much the same way that misuse of antibiotics in medicine and agriculture has led to the well-known problem of antibiotic resistance (e.g., Neu 1992). In many hibernacula, a large proportion of bats may roost in locations that are impossible for us to access, and many bat hibernacula on the landscape are unknown. Therefore, treatments may reach only a subset of host individuals in a given population, generating selection pressure favoring resistance in the fungus affecting those hosts. If these hosts then spread treatment-resistant *P. destructans* to new sites, including hibernacula that have yet to be discovered, this could render these additional sites more difficult to treat in the future. In short, our attempts to mitigate this crisis in conservation could easily make things worse. However, conservation physiology has great potential to help address these kinds of questions. An integrative physiology approach can help test the potential benefits of microclimate manipulation, or the application of chemical or biological interventions, in controlled settings. At the same time, conservation physiology studies aimed at quantifying the evolutionary potential of different North American bat species in the face of WNS could help us decide whether to focus on treatments targeting the disease in winter or alternatives such as summer habitat protection to enhance reproduction by survivors.

The importance of resolving this issue has been illustrated by recent controversy in the United States surrounding the potential listing of northern long-eared bats as an endangered species. Some politicians and representatives of industry have argued that an endangered listing, and the associated restrictions on industrial activities that might protect forest habitats, are un-warranted and, instead, management should focus entirely on “curing” the disease rather than protecting habitat (National Law Review 2015; Rodriguez 2015). However, if there is potential for an evolved response to WNS then, arguably, the most important priority for management should be

the protection of habitats that are likely to favor spring survival, recovery, and reproduction of previously infected individuals (e.g., high-quality roosting and foraging habitats of northern long-eared bats in forests). A conservation physiology approach, aimed at determining the potential effectiveness of a range of interventions, quantifying the evolutionary potential of populations of hosts, and quantifying the importance of certain habitats for survival and reproduction, before and after WNS, has great potential to help inform this important debate. Importantly, a framework like Anderson’s and May’s (Anderson and May 1979; May and Anderson 1979) model of pathogen impacts on host populations provides guidance on the physiology to population connections that must be addressed to understand long-term impacts of WNS and the costs and benefits of potential interventions to address the disease.

Conclusions

It is challenging to think in terms of “success” when millions of animals have been killed by WNS so far and solutions still seem like a distant possibility. Nevertheless, considerable progress has been made in the 8 years since the first die-offs of bats were observed and this progress has occurred more quickly than for similar conservation pathogens (Voyles et al. 2014). Obviously a critical first step in the WNS response was to identify the actual cause of the decline in order to prioritize research efforts. This helped ensure that the right body of theory (i.e., the framework of disease ecology) could be applied to research and management. In this article I have focused primarily on physiological studies of hosts. However, understanding pathogen physiology and the influence of the environment on host–pathogen interactions also are critical, as evidenced by important recent work combining empirical measurements of the growth of *P. destructans* with a sophisticated SIS model of the impacts of the pathogen (Reynolds et al. 2015). This kind of modeling that incorporates an understanding of the variation in the physiology of the host and/or the pathogen is a promising area for subsequent work because it connects empirical physiological data on mechanisms with population processes.

For integrative biologists that would like to apply their skills in physiology to important conservation problems, well-developed and clearly articulated theory will help connect the mechanisms we study to the population processes that we hope to manage. For some conservation issues, as in disease ecology, this theory will already be well developed. For others

more work may be needed to develop models that can connect physiological mechanisms to population processes. Nevertheless, developing and testing this kind of theory will help advance the discipline of conservation physiology and improve the potential of integrative biologists to apply their skills and expertise to the wide range of conservation issues facing modern society.

Acknowledgments

I thank the organizers of the Conservation Physiology symposium at SICB 2015 for their kind invitation to participate, as well as Brock Fenton, Quinn Webber, and two anonymous reviewers for helpful suggestions on the article. I am also grateful to past and present members of the University of Winnipeg Bat Laboratory for discussions in the University Club and The Goodwill which led to the thinking behind this manuscript.

Funding

Work on WNS in my laboratory was funded by the United States Fish and Wildlife Service, the Natural Sciences and Engineering Research Council (Canada), The Manitoba Hydro Forest Enhancement Program, the K.M. Molson Foundation, and the Species at Risk Research Fund of Ontario.

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