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Article

Balancing the Costs of Wildlife Research with the Benefits of Understanding a Panzootic Disease, White-Nose Syndrome

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Abstract

Additional ethical issues surrounding wildlife research compared with biomedical research include consideration of the harm of research to the ecosystem as a whole and the benefits of conservation to the same species of animals under study. Research on white-nose syndrome in bats provides a case study to apply these considerations to determine whether research that harms ecosystems under crisis is justified. By expanding well-established guidelines for animal and human subjects research, we demonstrate that this research can be considered highly justified. Studies must minimize the amount of harm to the ecosystem while maximizing the knowledge gained. However, the likelihood of direct application of the results of the research for conservation should not necessarily take priority over other considerations, particularly when the entire context of the ecologic disaster is poorly understood. Since the emergence of white-nose syndrome, researchers have made great strides in understanding this panzootic disease and are now in a position to utilize this knowledge to mitigate this wildlife crisis.

Key words: animal collection; animal use; bioethics; Chiroptera; white-nose syndrome; wildlife research

History and Spread of White-Nose Syndrome

White-nose syndrome (WNS) is an emerging infectious disease of hibernating bats that is causing one of the most precipitous declines of wild mammals ever recorded. Since its emergence in North America in 2006, millions of bats are estimated to have died (US Fish and Wildlife Service 2012), resulting in an overall 90% decrease in the abundance of bats in many affected North American hibernacula and the predicted regional or range-wide extinction of at least two North American species of bat (Frick et al. 2010, 2015; Thogmartin et al. 2012, 2013). WNS was first documented by wildlife researchers in New York state, has since spread extensively, and continues to be documented at new locations (Figure 1; see updates at www.whitenosesyndrome.org; last accessed August 8, 2015).

When WNS was first discovered, the root cause was unclear. Several species of cave-hibernating bats were affected; signs included emerging from hibernation early, death with little to no remaining fat stores, and white fungal growth on the muzzle, wings, and ears. Fungal pathogens are rare and are usually opportunistic infections that rely on a weakened immune response. For this reason, early researchers studying WNS first had to document whether other infections or environmental stressors were leading to fungal infection or whether WNS was caused by the fungus. Researchers identified the newly described cold-loving fungus (*Pseudogymnoascus destructans, Pd*) (Gargas et al. 2009;

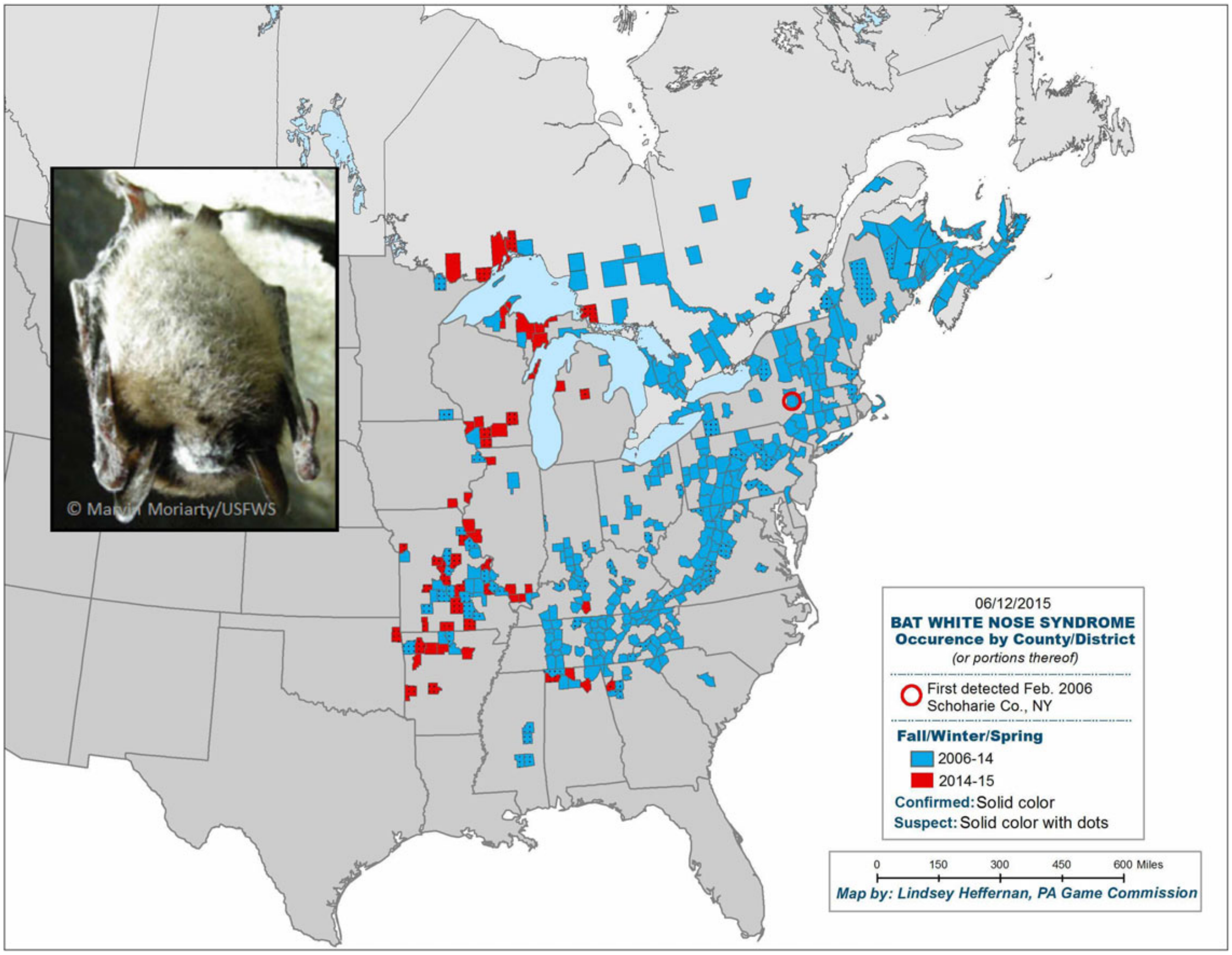

Figure 1 The current and historical distribution of white-nose syndrome (WNS) in North America, by county (as of June 12, 2015). Inset shows a little brown myotis (*Myotis lucifugus*) with typical signs of *Pseudogymnoascus destructans* (*Pd*) infection.

Minnis and Lindner 2013) as the causative agent by demonstrating Koch's postulates (Lorch et al. 2011; Warnecke et al. 2012) in studies that required healthy bats to be collected from the wild, brought into captivity, and infected with the pathogen. *Pd* fungal hyphae invade the epidermis and dermis of hibernating bats (Blehert et al. 2009; Courtin et al. 2010; Meteyer et al. 2009), leading to a series of physiologic changes that result in mortality for some bats (Cryan et al. 2010, 2013; Reeder et al. 2012; Verant et al. 2014; Warnecke et al. 2012). DNA from *Pd* has been identified on 12 North American species of bat from six genera; seven of these species have been documented with skin lesions diagnostic of WNS (see www.whitenosesyndrome.org for current data; last accessed August 8, 2015). *Pd* DNA and skin lesions characteristic of WNS have also been found in multiple bat species throughout Europe (Martínková et al. 2010; Pikula et al. 2011; Puechmaille et al. 2010; Puechmaille, Frick, et al. 2011; Puechmaille, Wibbelt, et al. 2011; Sachanowicz et al. 2014; Wibbelt et al. 2010; Zukal et al. 2014). As expected given the great host breadth of *Pd* (Zukal et al. 2014), infected bat species are variably affected by WNS. In North America, some species exhibit significant mortality, whereas others are relatively resilient (Frank et al. 2014; Langwig et al. 2012; Turner et al. 2011). In Europe there are no reports of mortality despite widespread presence of *Pd* and growth of the fungus on bats that histologically resembles that found on North American bats (Martínková et al. 2010; Puechmaille, Wibbelt, et al. 2011).

Anthropogenic spread appears to have played a key role in WNS. Bats exhibiting signs of WNS were first documented at a commercial tourism cave, Howe Caverns (the likely epicenter) in upstate New York in 2006 (Reichard and Kunz 2009; Turner et al. 2011). The difference in the manifestation of WNS in North America versus Europe is largely explained by the hypothesis that *Pd* is a novel pathogen introduced anthropogenically from Europe to which European but not North American bats are adapted. This hypothesis is supported by results from experimental inoculations and genetic analyses with both European and North American *Pd* isolates (Leopardi et al. 2015; Warnecke et al. 2012).

The Dynamics of WNS

When WNS was first determined to be caused by a fungal pathogen, a number of critical questions arose: Could the spread of the pathogen be stopped or slowed? What is causing mortality in infected bats? Could some resistant bat species or individuals recover? To address these questions, a variety of projects have been conducted, ranging from monitoring of caves and populations to examining broader questions about bat and fungal physiology. A number of research projects have also focused on identifying mechanisms to control the pathogen or to treat the illness. For these to be successful in mitigating WNS, a better understanding of the context in which *Pd* causes mortality is needed. This context is provided by the classic disease triangle, in which WNS emerges from the interaction of pathogen, susceptible host, and optimal environment; *Pd* is the highly virulent pathogen, North American temperate, insectivorous, hibernating bats are the susceptible hosts, and their hibernacula (cold and humid caves and mines) provide the environment conducive to pathogen proliferation.

Understanding the basic biology of bats was on a relatively strong foundation before the emergence of WNS (e.g., see Kunz and Fenton 2003). However, *Pd* exploits an area of bat physiology that was not as well understood: hibernation. Insectivorous bats at northern temperate latitudes, such as those affected by WNS, cope with limited food availability in the winter by increasing fat deposition in autumn and subsequently hibernating (Humphrey and Cope 1976; Racey and Speakman 1987; Studier and O'Farrell 1972). Bats balance energy during hibernation through a variety of physiologic and behavioral mechanisms, including adopting a torpid body temperature at or near ambient temperature (and thus lowering metabolic rate, heart rate, respiration, etc.), selection of favorable temperature and humidity microclimates within the hibernacula, clustering with other bats, and the display of optimal thermoregulatory patterns, including periodic arousals from torpor (Boyles and Brack 2009; Boyles et al. 2007, 2008; Humphries et al. 2002). Hibernation is but one part of the annual life cycle for these bats, as illustrated in Figure 2, and understanding this cycle is critical to understanding the dynamics of WNS. The fat stored by bats prior to the winter energetic bottleneck is critical not only for fueling the periodic arousals from torpor that occur in hibernation (Geiser 2004; Jonasson and Willis 2012; Reeder et al. 2012; Thomas et al. 1990) but also for enabling the spring migration and, for females, early pregnancy (Jonasson and Willis 2011). In the context of this annual cycle, it is important to note that the fungal infection does not persist on bats during the summer (Langwig et al. 2014) and that bats will be exposed to infection only during times when they return to cold hibernacula.

The little brown myotis was once the most common bat in North America and thus most heavily studied previous to WNS. As one of the most highly affected species, with population declines of up to 91% in affected areas (Frick et al. 2010, 2015; Turner et al. 2011), it has been the subject of much of the WNS research to date. Because of their abundance (at least in areas not yet affected by WNS), removal of enough individuals to study in captivity does not come at a high cost to the ecosystem. This is not true of some other species of bats that were either endangered or threatened prior to WNS (e.g., Indiana myotis [*Myotis sodalist*] and gray myotis [*Myotis grisescens*]) or have become so as a result of WNS (northern long-eared myotis [*Myotis septentrionalis*]). In the little brown myotis, WNS is associated with rapid body fat depletion (Blehert et al. 2009; Courtin et al. 2010; Meteyer et al. 2009; Moore et al. 2011; Storm and Boyles 2011; Warnecke et al. 2012), altered thermoregulation leading to increased frequency in arousals from torpor (Reeder et al. 2012; Warnecke et al. 2012), behavioral changes during interbout arousals (Brownlee-Bouboulis and Reeder 2013; Johnson et al. 2014; Wilcox et al. 2014), altered blood physiology (Verant et al. 2014), and wing damage that persists after hibernation (Francl et al. 2011; Fuller et al. 2011; Meteyer et al. 2012; Reichard and Kunz 2009). These studies that have examined the effects of *Pd* infection on either free-ranging or captive bats have been essential for defining the ways that WNS causes mortality in susceptible bats.

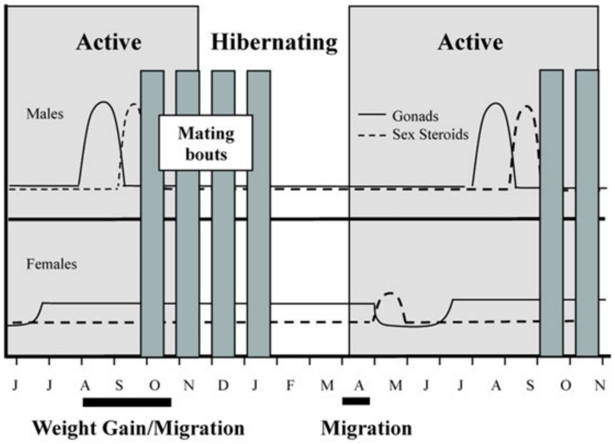

Figure 2 The annual, dissociated reproductive and life history pattern of little brown myotis (*Myotis lucifugus*), which both hibernates and migrates in North America. Modified from Gustafson (1979), Mendonça et al. (1996), Oxberry (1979), Rowlands and Weir (1984), and Wimsatt (1969).

Both individual- and species-specific physiologic responses to *Pd* infection may play a role in susceptibility. Understanding these variable responses will allow us to predict which of the 14 North American bat species not yet affected by WNS are at greater risk and how quickly they may succumb to the disease. For example, Willis and colleagues (2011) demonstrated that the highly susceptible little brown myotis exhibits higher rates of evaporative water loss than the European Natterer's myotis (*Myotis natteri*), presumably making it more susceptible to dehydration and thus potentially to adverse effects of *Pd* growth. Processes such as evaporative water loss and thermoregulation are intimately tied to conditions in the environment, which must also be considered. Both caves and mines can presumably act as reservoirs of *Pd* (Blehert et al. 2011; Lindner et al. 2011; Puechmaille, Wibbelt, et al. 2011), and *Pd* conidia that persist in the environment likely can infect or reinfect bats in subsequent years. An important question that remains unanswered is how transmission between bats and the environment occurs.

In the case of WNS, it is clear that pathogen–host–environment interactions converge to create the perfect storm. This conceptual disease triangle framework can help us understand how some species are less or even not at all affected whereas others are severely affected by *Pd*. For example, we know that species that are relatively larger, such as Virginia big-eared bats (*Corynorhinus townsendii virginianus*) and big brown bats, hibernate for shorter periods of time and typically select colder roost sites within the hibernacula (Kunz and Martin 1982; Kurta and Baker 1990; Reeder and Moore 2013). These characteristics—shorter total time in torpor and colder roost microclimate (and hence body temperature)—should confer an advantage against *Pd* because they provide less time for fungal growth and a less-than-optimal growth temperature. Other species, such as the little brown myotis, engage in behaviors that may increase their susceptibility to WNS, including hibernating for a longer period than the larger species, hibernating at temperatures more conducive to *Pd* growth, and clustering, which likely increases disease transmission (Langwig et al. 2012). Species differences in the physiologic (including immunologic) response to *Pd* infection also may explain differential susceptibility, although Johnson and colleagues (2015) demonstrated that species differences in antibodies against *Pd* did not explain survivorship differences. Together, these studies that compare different species of bats demonstrate that it is valuable to conduct research on multiple species of bats, even though that will increase the cost to the ecosystem.

Studies of WNS with captive bats have been essential for understanding this crisis and will be critical for evaluating potential WNS solutions. Captive studies have been particularly valuable for determining the role of environmental factors in influencing WNS because they can be carefully controlled. It is then important to confirm that observations made in captivity are also found in the wild. For example, Johnson and colleagues (2014), by means of captive *Pd*-inoculated little brown myotis, and Grieneisen and colleagues (2015), by means of naturally infected little brown myotis housed in captivity, both showed a protective effect of colder hibernation temperature on survival of *Pd* infection; Langwig and colleagues (2012) found a similar effect in free-ranging bats. These environmental conditions can then be incorporated into models to predict the spread of WNS (Flory et al. 2012, Hallam and Federico 2012). Understanding the interactions between the pathogen and each bat species within their variable hibernacula will require studying bats in both captivity and the wild.

Wildlife Research for the Sake of Saving Wildlife – Considerations for Animal Care and Use

Studying WNS requires collecting (and sometimes harming or euthanizing) individual organisms, causing disturbances to local populations by entering their habitats and potentially disrupting ecosystems in which they are embedded. Such harms must be weighed against both the potential knowledge gained and its practical consequences. In this section, we raise (and begin to address from a broadly utilitarian ethical perspective) some questions that should be kept in mind by both investigators and institutional animal care and use committees (IACUCs) working with wild populations.

Field Work on Ecosystems in Crisis

Traditionally, IACUCs approach justifying the use of nonhuman animals in research by considering the Three Rs: replacement, reduction, and refinement (Russell and Burch 1959). In wildlife animal research, as for all animal research, investigators must provide justification that the benefit in knowledge gained is worth the costs and that there is no way to obtain the same benefit with lower costs. Costs to the individual organisms being studied are obvious, but researchers studying free-ranging animals must also take into account harms to larger populations (e.g., colonies or species) and to networks of organisms (e.g., ecosystems).

At first glance, one might question the expansion of ethical concern to these supraorganismic levels. Neither ecosystems nor species are sentient—despite sometimes being composed of sentient organisms—and do not obviously have interests (Sandler 2012). On the other hand, some environmental ethicists invest greater value in these entities (Leopold 1949; Rolston 1985). Whether we see individual organisms or ensembles of organisms as the primary bearers of value, it does seem plausible that ecosystemic harms should be taken into account when considering field research. For even if one denies that ecosystems possess inherent worth, their health is crucial to that of individual organisms that comprise them.

Recently, Curzer and colleagues (2013) argued that we should extend the list of Rs considered by investigators and IACUCs in the context of wildlife research to incorporate consideration of ecologic harms. Whether we extend our stock of principles or simply consider also the ecologic dimensions of the traditional principles, the result is similar; the important point is that the full scope of the actual and potential harms and benefits be taken into account. Particularly in the case of field research, investigators must avoid thinking narrowly about risks posed to the study population, as incursions into wilderness areas will inevitably affect many other species—for example, by disrupting their normal behaviors or by introducing invasive species. Good decontamination practice is especially critical when studying diseased free-ranging animals where anthropogenic spread of wildlife disease is a risk. Proper cleaning and decontamination of field equipment should be routine after each outing but is particularly critical when the same equipment is used in multiple sites (guidelines specific for studying bats are available at www.whitenosesyndrome.org/topics/decontamination; last accessed August 8, 2015). More general guidelines are needed to prevent great ecologic harm by anthropogenic spread of unidentified zoonotic diseases and invasive species.

Just as risks to ecosystems must be considered, knowledge that puts us in a good position to better conserve already threatened species or ecosystems can be seen as a benefit with the potential to justify harms to individual organisms. This seems especially compelling in cases where the organisms being studied are members of the group under threat. In the case of WNS research, the primary aim is to mitigate an environmental catastrophe (likely caused, at least in part, by humans) and thus directly benefit the species under threat. We might think of this as a nonhuman analogue of Article 20 of the Declaration of Helsinki (World Medical Association 2013), which states that "research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research." For example, the use of little brown myotis to demonstrate that WNS is caused by *Pd* infection (Lorch et al. 2011) clearly benefits conservation efforts to preserve this species. In these circumstances, researchers are well-positioned to address a common criticism of research on nonhuman animals: that it is an unethical expression of speciesism (Singer 1977).

Although perhaps a reasonable source of justification, a similar principle—that the scientific use of individual animals may be justified only by benefits that redound to their species—does not seem plausible as a constraint. For one, it would entail the cessation of almost all animal research, as much of this research is conducted solely for human benefit. More important, it takes too simplistic a view on how costs and benefits are balanced between individual organisms, species, and ecosystems.

Here we should consider a nuanced version of the replacement principle, which bids us to ask whether it is possible to conduct the study in a more robust ecosystem. As WNS has spread across North America, bat researchers have had to consider whether to conduct each study on naive populations, populations in crisis, or remnant populations. Remnant populations are clearly the most vulnerable, and the knowledge gained from a study needs to be very large to justify significant ecosystem costs for these bats. Naive populations, typically at least 10 times larger than remnant populations (Frick et al. 2015; Turner et al. 2011), are more robust (although increasingly rare, as WNS spreads), but extra care must be taken to prevent anthropogenic spread of the disease. Investigators must also consider whether studies that use a less vulnerable species could provide the same benefit with lower ecosystem costs.

Consider an example: WNS threatens certain bat species more seriously than others. The Indiana myotis or the northern long-eared myotis are especially vulnerable and already endangered or threatened. Collecting individuals of these species represents a greater risk to that species than does collection of the more abundant little brown myotis. In this case, studying one species's response to WNS is justified by the benefit it brings a related but more vulnerable species, even if not all species respond to the disease equally. A utilitarian might characterize this as achieving a greater balance of happiness rather than a mere net increase of happiness (Norcross 2007, 653).

What is the moral relevance of the relatedness of species? This is a vexed question. Once again, much research on animals is conducted without an expectation that the species being studied (much less the individual animals) will benefit. One of the challenges for IACUCs is to weigh the significance of the potential knowledge and positive practical consequences to be gained against the harms incurred by the research. Phylogenetic relatedness of species or similarities in life history traits may, in some cases, be relevant to increasing the chances that a potential benefit would be shared across many species. The use of little brown myotis to understand WNS makes more sense than the use of, say, big brown bats, as the latter appear to be less affected by WNS and one might argue that we stand to learn less. On the other hand, comparative studies that include both of these species may be even more valuable because the conclusions may be applied to an even greater number of bat species. Even if the primary motivation in studying little brown myotis is to halt the precipitous decline in populations of northern long-eared myotis, there is at least a reasonable chance for the knowledge gained to benefit the little brown myotis.

The benefit from a study may also apply to the ecosystem as a whole. For studies on free-ranging animals that are part of an ecosystem in crisis, it should be straightforward to demonstrate benefit from ecologic studies. Even if a species was well studied prior to the crisis (which is often not the case), comparative work to determine how the changing environment is affecting this species is informative. What is more difficult to determine is whether the same knowledge could be gained with a lower impact on the ecosystem. Here we come to the (nuanced) reduction principle: Is it possible to reduce the impact of the research on the ecosystem? Studies that involve removing animals from the wild, either by placing them in captivity or by terminal sampling, must consider the minimum number needed, as is done for all animal research. In the context of the ecosystem, further harm reduction can also be considered by altering such factors as the season for collection (for example, by waiting until after weaning) or the sex of the collected animals. It is often possible to further reduce the impact of ecologic research by careful selection of a study site. This consideration requires investigators to consult with local wildlife personnel and should be a normal part of the permitting process (Paul and Sikes 2013).

Finally, consider the refinement principle: Is it possible to reduce the harm or increase the benefit of the study? In addition to the factors that can be refined to minimize the amount of harm to the animals in the study, field work should consider refinements that can increase the amount of knowledge gained without any additional cost to the ecosystem. This can easily be accomplished in two ways: combining studies and saving all biological samples. Combining multiple studies at the same field site can significantly reduce the ecosystem harm by minimizing disturbance. It is for this reason that field research stations have been very successful in fostering collaborations among investigators. For studying ecosystems in crisis, however, suitable field stations may not already exist, and, again, investigators should consult with local permitting authorities. These state and provincial wildlife biologists should be authorized to direct applicants to combine studies with another investigator that is already studying the same population if the two studies are compatible with each other. By saving all biological specimens, investigators can increase the benefit of the study without any additional cost to the ecosystem and minimal additional cost to the individual animal. For nonterminal studies, this typically involves recording as much data as possible and marking the animals (e.g., applying wing bands in bats) while they are handled. In addition, it could now include noninvasive sampling of the skin by swabbing and preservation of any fecal samples available. For studies that will be collecting terminal samples, the potential benefits of the study can be increased with no additional harm by preserving all biological specimens appropriately and making them available to other researchers. In addition to any tissue samples collected directly for the study, we recommend that at least one organ be preserved in an RNA-stabilizing agent and that the remaining carcass be preserved by fixation in 90% ethanol for eventual archiving in a natural history repository.

Captive Studies

Any investigator who plans on removing animals from the wild to study them in captivity must address each of the above concerns about ecosystem harm. In addition, housing and caring for wild animals in captivity or under semi-captive conditions presents several additional problems for investigators and IACUCs to consider.

First, captivity is a significant stressor for most wild animals. Investigators should consider whether the stress of captivity might adversely affect the study and compromise the results. Although there might not be an alternative way to obtain the same information, studies should be performed in such a way as to minimize the impact of captivity on the results. This can be accomplished with carefully designed controls and providing ample time for acclimatization to captivity and handling. Although methods will depend upon study objectives, hibernating bats removed from natural hibernacula and brought into captivity should generally be placed in hibernation conditions right away to minimize stress. If bats are captured outside of the hibernation season, they will need to learn to self-feed on a laboratory diet and to adjust to handling, which may take several weeks.

Second, housing conditions should be as similar as possible to wild conditions. Guidelines for laboratory animals (e.g., *The Guide* by the National Research Council, 2011) are not necessarily appropriate for housing of wild-caught animals (Sikes and Paul 2013). For studies that house mammals, fish, reptiles, or birds, it is more appropriate to consult taxon-specific guidelines provided by American Society of Mammalogists (Sikes et al. 2011), the American Society of Ichthyologists and Herpetologists (www.asih.org/publications; last accessed August 8, 2015), and the Ornithological Council (www.nmnh.si.edu/BIRDNET/guide/index.html; last accessed August 8, 2015). These guidelines, when properly consulted, should relieve the IACUC from the necessity of special approval for housing conditions as exceptions when they fall outside the descriptions for laboratory animals in *The Guide*. For example, bats housed in hibernation chambers in captivity require significantly less room than active season bats (as they can be placed in small cages; Brownlee-Bouboulis and Reeder 2013) and require very high relative humidity (> 90%), which exceeds normal humidity limits for laboratory animals.

Third, animal care programs should be designed in consultation with rehabilitation or zoo experts or other researchers with specific taxonomic experience. In addition to the required consultation with an attending veterinarian, those caring for wild-caught animals should consider the methods used by experts with experience handling related species of animals under similar conditions. Each investigator should be expected to document whether taxon-specific guidelines exist, and if they do, they should be followed (Sikes et al. 2012). If such guidelines are not found, then investigators should document how they determined that the animal husbandry plan is the most appropriate for the species being studied. This description should include consultation with veterinarians or other experts with experience caring for similar species of animals under similar conditions in captivity.

Conclusions

From an ethical perspective, research on animals that has a goal to better understand that species and/or the ecosystems in which that species lives can be considered highly justified, especially when an ecologic disaster such as WNS is threatened. As these crises occur, wildlife researchers need to respond by, first, doing no harm, and second, taking careful steps to understand the crisis—ideally in ways that can reasonably be thought to enable their mitigation. Only by understanding an ecologic crisis in the context of the ecosystem can we provide conservationists with the tools needed to mitigate the threat and avoid wasting scant resources.

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