FIRST DO NO HARM: RECOGNIZING AND MITIGATING THE RISK OF DISEASE INTRODUCTION ASSOCIATED WITH CHELONIAN HEAD-STARTING INITIATIVES

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Abstract.—Head-starting is an integral component of many chelonian conservation initiatives. However, the release of captive-raised individuals into wild populations carries the inherent risk of transmitting disease-causing microorganisms between the two populations, possibly with devastating consequences. Therefore, an essential component of any head-starting initiative is a preventive medicine program to identify and mitigate risks associated with disease transmission, and should include quarantine of captive animals as well as thorough health assessments of both captive and wild populations. Despite such efforts it is impossible to eliminate the risk of disease transmission, so prior to initiating head-starting projects a thorough risk assessment should be performed to ensure that the benefits of the project outweigh this risk.

Key Words.—chelonian; conservation; head-starting; infectious disease; quarantine; tortoise; turtle

Because humans have been directly and indirectly involved in the decline of many wild chelonian populations, it is understandable that we feel obligated to undo the damage we’ve done. Such efforts include head-starting, the practice of raising hatchlings in captivity to a size that will decrease predation potential and presumably increase the chance of reproductive success (Griffith 1989; Heppell 1996; Mortimer 1995). This is a long established practice that has been the topic of much debate due, in large part, to the inherent risk of disease transmission between head-started animals and wild populations (Dodd Jr 1991; Gilmartin 1993; Griffith 1993; Wolff 1993; Jacobson 1993, 1994; Bloxam and Tonge 1995; Alberts 1998; Seigel 2000; IUCN 2012). Additionally, introducing captive-raised individuals into wild populations could have detrimental genetic effects by mixing divergent gene pools or artificially selecting for maladaptive survival traits that are favored in captive populations (e.g. tractability, bright coloration, fecundity) (Reinert 1991). The purpose of this article is to discuss the risk of infectious disease transmission, in either direction, between captive-raised animals and native fauna (conspecific and sympatric species), and ways to mitigate these risks. This is by no means a novel concept; there are many references that promote thorough risk assessment and health screening of released animals as well as native populations, and more comprehensive overviews of specific disease risks are available (Cook 1993; Jacobson 1993; Spalding 1993; Woodford 1993; Brown 1999; Flanagan 2000). Furthermore, conservation organizations and scientific working groups have established procedures to address the risk of disease transmission during head-starting projects, including general recommendations for risk assessment, quarantine, and disease monitoring (AZA 1992; IUCN 2012) as well as guidelines, recommendations and protocols for quarantine, disease investigation, and health screening of wildlife and captive animals destined for release (Cook 1993; Munson 1993; Spalding 1993; Mikota 1996). Nevertheless, the practice of assessing and mitigating the risk of
disease introduction is not routinely integrated into chelonian head-starting programs (Brown 1999; Seigel 2000), and therefore the topic warrants continued discussion and promotion, considering the potential devastation to wild populations that could ensue following the inadvertent introduction of pathogenic microorganisms. To underscore this point: a review of ten chelonian head-starting projects performed over the past 15-20 years revealed that eight failed to mention pre- or post-release health screening of captive or wild populations in the methods section (Wood 1997; Mitrus 2005; Roosenburg 2009; Frederick 2009; Spinks 2003; Van Leuven 2004; Vander Haegen 2009; Kuhns 2010); two acknowledged the disease risk and alluded to minimal health screening with or without testing, but provided no description of their specific methods (Herlands 1999; Bell 2005). These findings are consistent with others’ conclusions that health assessment data are not given adequate attention before captive animals are released into wild populations (McDougal 2000).

Great care must be taken when trying to address species decline or extirpation not to make matters worse by inadvertently introducing infectious disease-causing organisms into target populations or other species within the ecosystem. As Auguste François Chomel first proclaimed, “primum non nocere; first do no harm.” This saying, which long ago became a principle precept in medical ethics, serves as a reminder that it is often better to refrain from intervention if the intervention itself may lead to greater harm to the patient, and at the very least, one must weigh the risks against the potential benefits in order to determine if the risk is warranted. In the case of head-starting programs, this principle can be applied at the population level of both target and sympatric species within the release area. Conversely, released animals may be susceptible to and succumb to pathogens carried by native conspecifics or sympatric species, especially if they have not been previously exposed and had an opportunity to develop immunity. Populations that have suffered significant decline or fragmentation are much less likely to recover from disease outbreaks than healthy populations. The fact that such populations are the targets of most head-starting programs makes the consequences of introducing disease inherently more severe. (Flanagan 2000)

Concern regarding the introduction of infectious disease into wild populations in the manner described above is underscored by a number of documented epizootics in both captive and wild reptiles and amphibians involving several pathogens (Jacobson 1993, 1994): Iridovirus mortality in tadpoles (Wolf 1968), Entamoeba invadens (Jacobson 1986), Cryptosporidium (Brownstein 1977), multiple herpesviruses (Jacobson 1993, 2012), ophidian paramyxovirus (Jacobson 1993, 1994, 1992; Kolesnikovas 2006), ranaviruses (Allender 2013; Berger 1998; Chinchar 2002), and Batrachochytrium dendrobatidis (Daszak 2003; Kiersecker 2004). More to the point, some epizootics are believed to have resulted from the release of infected captive animals, as is the case with the mycoplasmal upper respiratory tract disease (URTD) epizootic in wild Desert Tortoises (Gopherus agassizii) in the western Mojave Desert (Knowles 1989; Jacobson 1991; Jacobson 1995; Brown 1999), or invasion of non-native species into ecosystems containing vulnerable species, exemplified by worldwide epidemics of chytridiomycosis in amphibians (Daugherty 2013). An emerging risk to chelonian populations is disease associated with viruses of the genus Ranavirus, specifically Frog Virus 3-like virus (FV3). Disease outbreaks associated with morbidity and mortality have been reported across the eastern United States in chelonians (particularly Eastern Box Turtles), and the IUCN has listed Ranavirus as a suspected cause of decline (Allender 2013). While disease epidemics can be catastrophic in wild populations, they need not reach catastrophic proportions to severely impact the survival of a declining species. Such sub-catastrophic effects of disease may include decreased survival and reproduction, increased
susceptibility to predation, or increased susceptibility to environmental stress, all of which can have significant long term effects on population survival (Ballou 1993).

Risk assessment is a crucial aspect of any head-starting program. Before releasing animals, one should first conduct a thorough and thoughtful analysis of risks and benefits to the target population as well as other animal populations within the area of release. Ideally, risk assessment and mitigation requires the following information and resources: incidence, distribution, and risks of disease in captive populations; incidence, distribution, and risks of disease in wild populations; quarantine systems that will totally prevent disease transmission; and detection and monitoring systems that will identify disease without error (Wolff 1993). Mathematical models are often used to facilitate risk assessment by quantifying the effects of risk factors such as disease, and have been eloquently discussed in the context of conservation efforts (Ballou 1993; Wolff 1993). Systems that categorized disease threat based on relative risk further aid risk-based decision making to focus resources on investigating and managing diseases that pose a high risk (Munson 1993).

If head-starting programs are to be used as successful conservation tools, then animals must be intensely scrutinized and guidelines developed for both selecting and determining which animals should be bred and which captive-reared animals should be returned to the wild (Beck 1993; Cook 1993; Gilmartin 1993; Jacobson 1994). In doing so, one should not only consider screening for pathogens that have detrimental effects on the target species (e.g. Mycoplasma sp., Ranavirus, Herpesvirus, Papillomavirus), but also on sympatric taxa. For example, the fungus *Batrachochytrium dendrobatidis* has had a devastating impact on susceptible amphibian populations worldwide. While this is generally considered to be exclusively an amphibian disease (Berger 1998), reptiles have recently been documented to harbor this pathogen asymptomatically in a natural environment (Kilburn et al. 2011). Consider the scenario in which captive raised turtles or tortoises are inadvertently infected with *B. dendrobatidis* while in captivity, establish asymptomatic infections, and are then released into an ecosystem in which susceptible amphibians live. Overlap in habitat use between many chelonian species and amphibians within their ecosystem may increase the risk of such interspecies transmission occurring. The result could very well be decline or extirpation of an entire population (Berger 1998). If possible, the same level of scrutiny should be used to determine the presence of disease in the wild populations into which animals are to be released. Head-starting has been called a halfway technology when turtles are released without first addressing the original cause(s) of the population decline or extirpation (Frazer 1992). Therefore, it should go without saying that releasing head-started animals into a population decimated by overt infectious disease would likely amount to sending hundreds or thousands of additional animals to meet the same fate, with no appreciable benefit to the target population. The ethical considerations of such an endeavor should be sufficiently discouraging, but what about releasing animals into a population that has endemic, subclinical disease? Introducing naive animals into a population with endemic disease could put the introduced animals at risk for clinical disease with associated morbidity and possible mortality, and possibly serve as an additional reservoir in which to amplify pathogens. In other words, host immune status often influences an organism’s pathogenicity, so it is important to consider whether the head-started animals are likely to cope with new pathogens encountered at release sites (IUCN 2012). Furthermore, a number of factors associated with captivity and release into a new environment, (e.g. nutrition, stress, injury) can impact an animal’s immune function and increase its susceptibility to disease, increase the pathogenicity of a particular organism, or reactivate latent infection, thereby increasing both the risk of wild animals
acquiring infections from released animals and, conversely, of released animals acquiring infections from wild animals. Compounding this risk is the fact that some pathogens cause minimal to no clinical disease in the natural host species, but may cause severe, often fatal disease in aberrant hosts that may occupy the same ecosystem. This pattern of host-specific pathogenicity is typical of a number of pathogens, perhaps most notably the alpha herpesviruses (Jacobson 2012). Because disease-free populations don’t occur naturally, it is important to not only have an understanding of what infectious organisms are present in native populations at the proposed release site, but also to reasonably surmise what, if any, clinical effects they will have on introduced animals. This requires a thorough working knowledge of the target species, its significant pathogens, and the immunology, pathophysiology and epidemiology of resulting disease. Unfortunately, this information is rarely available for reptile pathogens and their hosts. Nevertheless, enlisting the assistance of a veterinarian with reptile experience can add considerable value to a head-starting project. (Ballou 1993; Cook 1993; Mikota 1996; Woodford 2009).

Both wild and captive animals can acquire infectious agents horizontally (direct contact, fomite or vector-borne transmission) or vertically (passed “in utero”), and should never be considered free of infectious organisms (Jacobson 1993). However, perhaps due to limited opportunities to transmit infection under normal behavioral and environmental conditions, relatively few infectious diseases are associated with naturally occurring outbreaks that result in significant morbidity or mortality in chelonian populations. Conversely, under captive conditions the spread of infectious organisms is facilitated by the close proximity of individuals, shared water sources, higher environmental levels of normally nonpathogenic organisms, or by suboptimal environmental conditions predisposing animals to infection (Flanagan 2000). Quarantine of animals destined for release is a key component of mitigating the risk of disease transmission. This involves strict physical isolation of the animals from other captive and wild animals for the entire course of their captivity to avoid direct contact with potentially infected animals, their excretions, and contaminated caging materials. Furthermore, it involves procedures to minimize the risk of fomite transmission, such as strict sanitation and disinfection practices; dedicated equipment for each quarantine group; room/facility entry orders, moving from “clean” to “dirty” if such designations can be applied; strategically placed footbaths to disinfect footwear; and personal protective equipment such as gowns/coveralls, gloves, and dedicated boots or shoe covers while working with quarantined animals. Two basic types of quarantine include passive quarantine, in which animals are merely isolated and observed for clinical signs of disease, and active quarantine, which involves physical examination and diagnostic testing to detect disease. The latter is necessary to detect subclinical disease, and therefore preferred prior to release of animals into the wild. The duration of quarantine, as well as specific quarantine and disease surveillance protocols, depend on the goals and assumptions of the program as well as sociobiological considerations and available resources (Beck 1993).

Animals exhibiting clinical signs of illness should not be released or used for breeding to produce animals to be released, and those that have recovered from clinical illness may or may not be suitable for such purposes, depending on the specific etiologic agent and whether or not it is present in wild populations near the release site. In the case of many infectious diseases shedding of organisms may persist beyond the resolution of clinical signs, or triggers such as stress, which is undoubtedly associated with transport and release, may reactivate latent infection. It is also important to note that even if a pathogen does not cause clinical disease in captive animals, this does not mean that it cannot cause disease in wild animals of the same or different species. The converse is also true. Therefore, in addition to gen-
eral health screening procedures such as physical examination, fecal examination (flotation and direct smear) for parasites, serum biochemical and hematologic analysis, pre-release health screening of chelonians should involve testing for a panel of known agents based on the likelihood of occurrence in both captive and wild populations (e.g. *Mycoplasma* sp., Ranavirus, Herpesvirus, Papillomavirus, Chytrid fungus). This testing typically utilizes serologic techniques (e.g. ELISA) to detect a humoral immune response to a foreign protein (antigen), indicating exposure to a specific pathogen, or molecular techniques (e.g. polymerase chain reaction-PCR) to detect the nucleic acid of selected pathogens in a sampled tissue, indicating active infection. While a single serologic assay only indicates exposure to a pathogen (not necessarily active infection), paired samples demonstrating a rising titer can indicate recent infection. Further, distinguishing antibody class can provide information on the time that has past since exposure, with IgM appearing early in the course of infection and IgY developing weeks later. However, time sequence data is lacking in reptiles (Jacobson 2002). A limitation of specific testing methods is that testing is generally limited to known agents for which diagnostic reagents (e.g. antibodies or PCR primers) are available. Therefore, despite performing general health screens such as fecal parasitology and diagnostic blood work, and testing both captive and wild populations for a panel of known infectious agents, it is still possible to inadvertently introduce infectious disease into wild populations of the target species or species sharing the same ecosystem. In addition to carefully considering and selecting health-screening methods, care must be taken in interpreting results, as false positives and false negatives may occur as a product of sensitivity and specificity respectively. For example, cross reactivity between closely related antigens may result in false positive results and adversely affect decisions (Jacobson 2012). Ideally, a diagnostic assay will have both high sensitivity (minimizing the risk of false negative results) and high specificity (minimizing the risk of false positive results). However, very rarely are either 100%, so verifying results is an important step in disease surveillance and investigation. Once the infection status of the animals to be released has been verified, then decisions must be made regarding their suitability for release. Because completely pathogen-free animals are unlikely, the presence of infectious agents does not necessarily preclude an animal’s release into the wild. A number of factors must be considered, such as the presence and prevalence of an identified pathogen in wild populations in ecosystem into which head-started animals are to be released, the pathogenicity of the agent in both the target species as well as sympatric species at the release site, and whether or not pre-release treatment and eradication of the infectious agent is a feasible option. As a general rule, if evidence of disease is present in both populations at relatively similar incidences the project can proceed. If any identifiable disease is absent from or more prevalent in one population, then animals should not be released (Flanagan 2000).

Some people believe that, because novel diseases can be introduced into susceptible populations despite taking precautions such as the use of quarantine or surveillance procedures, the risk is high enough to preclude the release of captive animals into new or historic habitats (Seal 1992; Wolff 1993). Many others feel that head-starting programs are integral components of successful conservation efforts, as evidenced by the numerous projects that have been undertaken or are ongoing (Soorae 2011). Regardless, if head-starting is to be part of a conservation initiative, then it is of utmost importance to do everything possible to mitigate the risk of introducing disease to the target population, or to other animals sharing the same ecosystem. This involves quarantine and thorough health assessment of both to-be-released and native populations. Impediments to these efforts include budgetary constraints, limited resources, limited availability of diagnostic reagents (e.g. specific antibodies and PCR...
primers), limitations of diagnostic assays, and gaps in our collective knowledge of pathogens that may impact the populations we are trying to save. At a minimum, strict quarantine practices should be implemented and ongoing health assessments during the captive period should be performed, including measurements of weight and length, behavioral evaluations, thorough physical examination, parasitological examination, hematology, and serum biochemistry analysis (Flanagan 2000). Necropsy of diseased animals or those animals euthanized due to illness can also provide valuable information that could contribute to risk assessment. As project resources and diagnostic modalities allow, molecular and/or serological testing for specific agents should be added to this regimen. Selection of agents should be based on risk assessment and knowledge of target and sympatric species in the area of release. Projects involving fieldwork with *Gopherus* spp. tortoises provide good examples of thorough health assessment, implementation of molecular and serological diagnostics, and procedures to minimize disease transmission between study animals (Berry 2001; Berish 2010; Jacobson 2012; Jacobson and Berry 2012). The populations into which head-started animals are to be released should be tested in the same manner as captive animals and results between the two populations compared. As stated previously, only animals with compatible microbiological and health status should be released. However, despite our best efforts to do so, the risk of introducing disease to native or released populations with potentially devastating effects can never be completely eliminated, so the likely benefits of these actions must always outweigh the risk.

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