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Enhanced Between-Site Biosecurity to Minimize Herpetofaunal Disease-Causing Pathogen Transmission

There is increasing evidence for concern of the role humans may play in the transport and transmission of herpetofaunal pathogens. This is especially true for emerging infectious diseases caused by the fungal pathogens *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) which infect amphibians and iridoviruses of the genus *Ranavirus* (Rv) which infect amphibians and reptiles (*Bd* and *Bsal*: Fisher and Garner 2007; Schloegel et al. 2009; Fisher et al. 2012; Martel et al. 2013, 2014; Auliya et al. 2016; Nguyen et al. 2017; O'Hanlon et al. 2018; Rv: Picco and Collins 2008; Walker et al. 2008; Gray and Chinchar 2015). Preventing novel introductions of emerging infectious pathogens is of paramount importance (Gray et al. 2015; Grant et al. 2015, 2017), as once they gain a foothold, they can be "essentially unstoppable" (Fisher et al. 2012). To minimize anthropogenic influences on disease dynamics, biosecurity procedures and decision-support systems for biosecurity prioritization have been developed. In general, such procedures for herpetofaunal diseases have been framed relative to the stages of pathogen emergence (pre-arrival, invasion front, epidemic, and establishment: e.g., Garner et al. 2016; Grant et al. 2017) as well as the intertwining contexts of herpetological research, natural resource management activities, integrated biodiversity conservation practices, and the human dimension of transmission of novel pathogens, (e.g., Gray et al. 2018; More et al. 2018).

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Field-based biosecurity protocols generally aim to reduce the risk of human-mediated spread of disease-causing pathogens at specific sites (Gray et al. 2017). Such protocols address both within-site biosecurity to reduce disease-causing pathogen transmission among animals at the site (e.g., via prescribed animal capture and handling procedures; Gray et al. 2017) and between-site biosecurity to reduce pathogen transmission into or out of a site (Phillott et al. 2010; More et al. 2018). There may be gradients in biosecurity procedures applicable to meet different site contexts. In particular, Phillott et al. (2010) offered a risk calculator for standardized field hygiene decision making. However, there is no universal implementation of the most basic pathogen hygiene procedures such as disinfection of field gear between uses at different sites and forestalling the movement of water, fomites, or species among sites. This is especially true when field work is not focused on amphibians or reptiles and there is little awareness of herpetological pathogen transmission concerns. Given that such basics are not in broad use for all field work, addition of enhanced biosecurity measures under priority contexts may be more challenging to implement as more outreach may be needed to engage people and more time and effort could be required to implement enhanced procedures. For example, enhanced biosecurity may extend disinfection procedures to the public who venture into natural areas where prevention of emerging diseases is a high priority, or to large equipment used in habitat management or other human purposes such as fire prevention practices (NWCG 2017, 2020; Julian et al. 2020). For this reason, it is practical to have relatively simple processes for context-specific rapid risk assessments to lead to decisions for standard or enhanced biosecurity across diverse disciplines and types of field work.

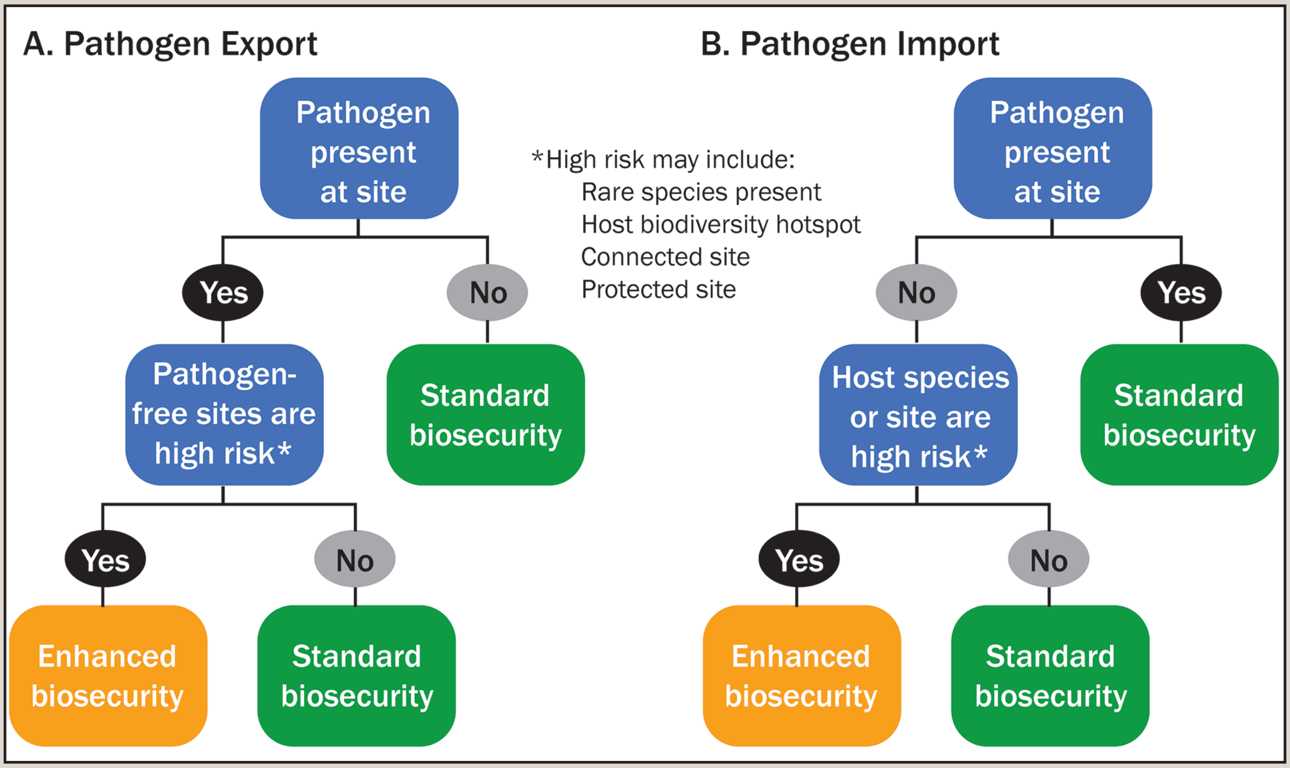

Fig. 1. Conceptual decision tree for enhanced and standard between-site biosecurity based on disease-causing pathogen occurrence and site risk determinations for pathogen export from the site (A) or import to the site (B). High-risk assignments can be multidimensional, with seven main contexts (Table 1).

Herein, we further describe biotic and abiotic factors that interact with field work to contribute to gradients in human-mediated herpetofaunal pathogen transmission (i.e., translocation) risk *between* sites. Using biotic and abiotic criteria, we identify site conditions that correspond to *high risk for pathogen import* [to a site] or *high risk for pathogen export* [from a site] for implementation of enhanced between-site biosecurity procedures to forestall human-mediated pathogen transmission. Our field-site criteria are based on seven contexts of the pathogen (occurrence, habitat), host(s) (occurrence, habitat, species richness), and geography (distance/topography, geopolitical land use) (Table 1). We do not provide an explicit decision tree because site contexts can be complex, and single contexts may be weighted heavily in some biosecurity decisions, warranting case-by-case decisions. A more conceptual decision tree (Fig. 1) about pathogen export or import can be more flexibly applied as site contexts vary. Our aim is to provide a rapid process to develop a qualitative narrative to support decisions for between-site herpetological disease biosecurity. We offer this rapid narrative approach to reinforce and expand upon elements of the more quantitative risk calculator provided by Phillott et al. (2010). In addition, although we use *Bd*, *Bsal*, and Rv as our main examples, our guidance is applicable to a variety of microparasites affecting herpetofauna health, including severe *Perkinsea* infections (SPI) of amphibians (e.g., Isidoro-Ayza et al. 2017), Snake Fungal Disease (ophidiomycosis; Baker et al. 2019), and Asian snake lungworms introduced to native USA snakes (pentastomes, Farrell et al. 2019).

Pathogen Contexts

Pathogen Occurrence.—The risk of herpetofaunal disease-causing pathogen transmission between field sites is related to pathogen occurrence patterns, and these can be considered relative to host infection, geographic area, and time period. Pathogen occurrence can most simply be characterized in a binary fashion, present or not, with biosecurity decisions made accordingly (Fig. 1). A binary occurrence designation can be applied to pathogen infection of or genomic detection on an individual host, a host population, a host species, or an assemblage of host species (e.g., taxonomic family or order rates of *Bd* infection; Olson and Ronnenberg 2014). It can be further applied based on geographic occurrence—a specific site or a broader geographic area including multiple sites (global *Bd* site summaries: Olson et al. 2013; USA watershed *Bd* occurrences: Olson and Ronnenberg 2014). With emerging technologies, it is now possible to detect pathogen DNA at a geographic location independent of host infection (e.g., via environmental DNA sampling: Chestnut et al. 2014). Further, a point in time (month, season, year) may be used to also refine a pathogen's occurrence pattern and associated risk(s) of transmission between sites.

Beyond a binary system of detection, occurrence patterns may be reported relative to prevalence, i.e., percent infected or frequency of infection, which similarly may be applied to various levels of biological or spatial organization as outlined above e.g., Olson et al. 2013; Olson and Ronnenberg 2014). Pathogen occurrence can also be reported relative to the intensity of host infection (Vredenburg et al. 2010).

Although all these pathogen contexts should be considered when assessing biosecurity priorities, each factor will likely be rife with unknown and uncertain variables. Herein, we focus on identifying binary site-level occurrence (detected or not) and associated risks, which may be the most common level of pathogen knowledge and highly relevant to forestalling site-to-site anthropogenic transmission.

Table 1. Seven field-site contexts of pathogen, host, and geographic conditions for development of a risk assessment narrative to support implementation of either standard or enhanced biosecurity procedures to forestall between-site human-mediated transmission of disease-causing pathogens. For simplicity, per context, two questions are posed regarding the risk of pathogen import to a field site or export out of a site. Examples are provided of select conditions that illustrate considerations for biosecurity decisions. *The use of continent in these examples is not intended to be a threshold condition but is relevant because some pathogens do not have global distributions.

Site Context	Is there a high risk for pathogen import?	Is there a high risk for pathogen export?	Priority	Enhanced Biosecurity needed and practical?
1. Pathogen occurrence		Example 1: A disease die-off is known from the site	High priority to not spread the pathogen further	Die-off may indicate caution is needed; enhanced biosecurity
2. Pathogen habitat		Example 2: Site has habitat for the pathogen, but pathogen is not known to occur on continent*	Low priority unless other contexts elevate	Enhanced biosecurity may not be needed or practical; examine other contexts
3. Host occurrence	Example 3: a rare host occurs at the site		Rare host conveys high priority	Enhanced biosecurity
4. Host habitat		Example 4: Site has habitat for host known not to become infected by pathogen, and pathogen not known on continent	Low priority unless other contexts elevate	Enhanced biosecurity may not be needed or practical; examine other contexts
5. Host species richness	Example 5: Site is a biodiversity hotspot with high species richness, and no known pathogens		High priority not to introduce pathogen to site; examine other contexts to see if relevant	Enhanced biosecurity may be warranted if a pathogen is likely to be transmitted to site
6. Site location, distance & topography considerations	Example 6: Headwater site of large watershed in montane landscape with unique species, no pathogen known at site but pathogen occurs lower in watershed		High priority not to spread pathogen there from lower elevations	Enhanced biosecurity could help retain pathogen-free headwater site
7. Land-use type	Example 7: Site is within a protected-area reserve for a rare amphibian species		Protected species areas are high priority	Enhanced biosecurity

TABLE 2. Considerations for standard and enhanced between-site field biosecurity procedures.

Biosecurity step	Standard procedures	Enhanced procedures
Planning at the office.	1. Generally review pathogen and host occurrences for awareness of wildlife health considerations. If appropriate, review permit procedures for site biosecurity requirements. 2. Define "site". Decontaminate between sites that are a predefined distance apart, in different watersheds, and/or if you drive between sites. This criterion should account for what is known about the host species home range, movement, etc. 3. Set protocols to follow in the field and train all staff that will be involved in field work. 4. Assemble gear; ensure all gear is clean and disinfected. 5. Review specifics and guidelines associated with any chemical used in decontamination. For more information, see Appendix II, Gray et al. (2017).	1. Review pathogen and host surveillance information for study area, pathogen habitat suitability, host conservation status, host species-specific and community metrics (species richness, pathogen susceptibility): are there known lower-risk or higher-risk sites to consider? 2. Define "site". Review abiotic considerations of site and study area (geographic metrics or land-use type): are there known lower- or higher-risk areas? 3. Set order of site visitation, if necessary. 4. Set protocols to follow in the field and train all staff that will be involved in field work. 5. Assemble gear; ensure all gear is clean and disinfected. Assemble vehicle biosecurity supplies; plan locations for field crew member, gear, and vehicle disinfection. 6. Review specifics and guidelines associated with any chemical used in decontamination. For more information, see Appendix II, Gray et al. (2017).
Staging at vehicle at site	1. Review gear and ensure all is clean and disinfected.	1. Upon arrival to the site, put on a disposable Tyvek® suit, followed by rubber boots and disposable gloves (e.g., nitrile). 2. Then, waders (if needed) specific to the site are placed over the suit. 3. If required for field-crew safety, heavy work gloves that are rubber (not latex) or another easily decontaminated material should be worn for handling animals.
Upon return to vehicle	1. Clean and disinfect all equipment. Or, clean and bag equipment for later disinfection. At a minimum, remove mud, dirt, etc. 2. Clean mud/debris from tires and outside of vehicle. 3. Properly store all equipment and samples. 4. Ensure adequate preparation to leave site and move to next site to minimize pathogen transmission.	1. Before leaving the contaminated site, all other equipment and surfaces (e.g., table) should be disinfected. Disposable gear, such as Tyvek suits and gloves, should be removed and placed in a biohazard container for appropriate disposal in an approved landfill or autoclaving and appropriate biohazard disposal. 2. Boots should be cleaned (mud and organic material removed) and disinfected using an appropriate disinfectant (i.e., Virkon-Aquatic) according to the label instructions (see Appendix 1 from Gray et al. 2017). 3. Boots and clothing should be bagged and the outside of the bag decontaminated (with disinfectant of choice at appropriate dose and duration) before being placed into vehicle. When back from the field, clothing and boots should be fully decontaminated. Options include a hot water bath (55°C) for 25 minutes; if this is not feasible, then at a minimum, clothes/soft gear should be machine washed on the hot cycle. People should also clean and disinfect themselves. 4. Before leaving a high-risk site the outside of a vehicle and tires should be cleaned (mud/organic material removed) and disinfected with appropriate disinfectant. a. If moving from a high-risk to a low-risk site, a different vehicle could be used. If this is not feasible, then the vehicle should be washed at a commercial car wash before entering different sites. b. Hard surfaces inside of the vehicle also should be disinfected with an appropriate disinfectant.
Review, Reassess, and Re-stage or Re-work protocols	1. Review how the protocols worked. If too complicated, perhaps simplify. If not adequate, then improve.	1. Review the field plan. 2. Revise if needed. For example, a changed site-visit order might be warranted if new information emerges (e.g., was a die-off encountered warranting a pause in field operations?).

High risk for pathogen import: Relative to pathogen occurrences and transmission risks, the import or introduction of a novel pathogen (species or strain) to areas where it had previously not occurred could be a high-risk event, especially for naïve hosts susceptible to the disease caused by that pathogen. Host contexts are considered further below. Such a scenario is of upmost importance and could be a dominant focus for application of enhanced biosecurity procedures relative to between-site transmission (Fig. 1). In this light for emerging pathogens, of particular significance is a "first detection" of a pathogen in a new geographic region or a new host species (e.g., Reeves 2008; Reshetnikov et al. 2014; Zhu et al. 2014; Arellano et al. 2017; Ghirardi et al. 2017; Moore et al. 2018), or identifying the likely leading edge of its spread (e.g., Lips et al. 2006). In contrast, if the pathogen is within its apparent native range where hosts are not susceptible to disease effects, this sort of site-import risk is not high.

High risk for pathogen export: Using pathogen occurrence as our initial metric for assessing transmission risk, if a live pathogen is known to occur at a site, then there is high risk for pathogen export. This would be of heightened concern if the export were to transmit the pathogen to other disease-free sites with hosts susceptible to disease, resulting in a decision for enhanced biosecurity (Fig. 1).

Pathogen Habitat.—Additionally, understanding where suitable conditions support pathogen survival, growth, and reproduction is necessary to understand or predict their occurrence and risk of import or export for a site. Adding complexity to this topic is that some pathogens have life stages both within and outside a host (e.g., *Bd*, *Bsal*, Rv, Ophidiomyces, Emydomyces), whereas pathogens such as Mycoplasma and *Herpesvirus* cannot survive for prolonged periods of time outside of their host. Thus, pathogen habitat conditions and the presence/absence of hosts could both constrain occurrence of certain pathogens.. For example, *Bd*, *Bsal*, and Rv are aquatic organisms reliant on hosts for life history functions for which laboratory experiments have described optimal growth at certain temperatures (Ariel et al. 2009; Longcore et al. 1999; Piotrowski et al. 2004; Martel et al. 2013; Brand et al. 2016).

Caution is needed in assigning pathogen habitat assessments conducted at landscape scales to site conditions due to between-site variation in several factors including: 1) microclimates; 2) seasonal changes in conditions; and 3) host population infection prevalence (Bradley et al. 2019). Downscaled assessments are prudent in order to base biosecurity procedures on habitat classifications. For example, although there may be relatively narrow windows of time where *Bd* thermal habitats are suitable for host infection at higher elevations and latitudes in north temperate zones, *Bd* has been documented from some of these areas, and some species in these areas are vulnerable to *Bd* chytridiomycosis (e.g., *Rana muscosa*, Sierra Nevada, California, USA; Vredenberg et al. 2010). Unlike the pathogen occurrence criterion above, pathogen habitat is not always binary but may occur across a range of conditions (e.g., seasonal occurrence patterns of herpeviruses in turtles: Kane et al. 2017; Lindemann et al. 2019). Nevertheless, if possible, on a case-by-case basis, it could be useful to impose a binary habitat or not-habitat condition to a site, for simplicity and while acknowledging uncertainty.

Importantly, emerging pathogens may be native to some regions of the world, but they are likely to be novel or invasive elsewhere, such that they have not yet reached a stable equilibrium across potentially suitable habitats. Hence, potential habitat that is not known to be occupied by a pathogen may still be relevant for biosecurity guidance. In addition, climate change projections provide an additional moving target for where pathogen habitat could survive in the future, as has been hypothesized with recent reports of greater Bd occurrences at north temperate latitudes (Xie et al. 2016). Keeping emerging pathogens out of potentially suitable habitat should be a high priority.

High risk for pathogen import: Simply, sites known to have suitable habitat for pathogen occurrence are higher risk for pathogen import. For *Bd*, *Bsal*, and Rv, these would be aquatic sites with host species that have optimal temperature conditions for pathogen growth at some time during the year (Ariel et al. 2009; Longcore et al. 1999; Piotrowski et al. 2004; Martel et al. 2013; Brand et al. 2016). Because *Bd*, *Bsal*, and Rv habitat may be described only in broad terms at this time, pathogen habitat as a sole criterion for risk assessment relative to implementation of enhanced biosecurity procedures is less compelling in comparison with other contexts such as pathogen occurrence, unless retention of pathogen-free host refuges is a priority.

High risk for pathogen export: Related to this previous scenario, if a first-detection of a pathogen occurs at a site in an area, then suitable habitat at an adjacent site should be considered as a potentially high risk for pathogen export during decisions for biosecurity measures.

Host Contexts

Occurrence of host species, host species habitat, and host-species richness at a site are additional contexts to consider for biosecurity decisions. Again, for simplicity as rapid assessments could be most practical, species and habitat occurrence could be considered binary contexts as above, with species or habitat either occurring or not. Site species richness is more complex as it could occur across a continuum from single-species sites to highly diverse multi-species community sites.

Host occurrences.—During assessments of pathogen transmission risks, three types of host species warrant consideration. First, hosts with conservation status of concern rise to the top of priority lists to reduce threat factors that could affect their persistence. Rare hosts known to be susceptible to diseases caused by pathogens, especially those hosts known to have lethal consequences of infection, are among the highest priorities for site-scale protective measures such as enhanced biosecurity procedures. Little-known host species may also be lumped with rare species as high priority for enhanced pathogen biosecurity if there are insufficient data to inform status rankings. Second, of lesser concern for species persistence reasons, but also high concern would be more common host species that are known to have lethal consequences of pathogen infection. Losses of common species may have community-level ramifications through their ecological functions or processes within their ecosystems. For example, metamorphosis of aquatic larvae to terrestrial habitats, and later return of terrestrial adults to aquatic areas for breeding, is an example of an organism providing reciprocal subsidies between distinct ecosystems, where aquatically derived nutrients and energy are transported to the land and terrestrially derived resources are transported to the water (Baxter et al. 2005). Such organisms can function as central cogs in food webs and energy cycles (carbon sequestration: Best and Welsh 2014; Semlitsch et al. 2014) in both water and on land. Third, host species that are potentially pathogen reservoirs, carriers, or even superspreaders are a concern. Characteristics of such hosts are their ability to become infected without lethal consequences and either being a relatively broad disperser among sites or in their role in promoting pathogen reproduction and release into the environment. Hence, reservoir hosts could promote pathogen transmission. In the US West, the Pacific Chorus Frog (*Hyliola regilla* = *Pseudacris regilla*) reproduces in aquatic habitats but is quite terrestrial after metamorphosis and can move from water source to water source. Reeder et al. (2012) identified it as a *Bd* reservoir species; due to its local abundance in many landscapes, it is a potential *Bd* superspreader. Both within and outside their native range, the American Bullfrog (*Rana catesbeiana*) also may be a superspreader. Ribeiro et al. (2019) reported that a bullfrog farm of about 1500 frogs had outflow water at a rate of 60,000 L/day with *Bd* zoospore concentration reaching about 50 million zoospores/L. This interaction with water flow is discussed below under geographic and landscape contexts, but here illustrates how a carrier species can be pivotal to transmit disease-causing pathogens to susceptible species. In summary, reducing novel pathogen import to sites with potential superspreaders is a priority to forestall such subsequent pathogen transmission.

High risk for pathogen import: Preventing pathogen import to sites with susceptible host species is a priority, with elevated prioritization for rare and little-known host species where their site persistence could be important for species conservation. There is also a concern for pathogen import to sites with common species that may provide key ecosystem services such as in food webs, energy flow across ecosystem boundaries, or carbon sequestration. Lastly, there is a higher risk of pathogen import to sites with host species known to act as carriers or superspreaders of pathogens.

High risk for pathogen export: Knowledge of a nearby site with a rare or little-known species or a critical refuge for such species could increase concerns for potential pathogen export. Similarly, knowledge of the presence of a potential carrier or superspreader host species at a neighboring site or a neighboring site with high host species richness could increase export concerns.

Host habitat.—With imperfect knowledge of host occurrences, suitable habitats with likely host occupancy should be considered for enhanced biosecurity guidance.

High risk for pathogen import: A high risk for pathogen import to a site could be considered when there is no information on host-site occurrence but the site conditions are suitable for and within the range of a rare or little-known host species, a more common host species with known key ecological functions, or a host species that is a likely pathogen carrier or superspreader. As this decision has an element of uncertainty, because only habitat is known and host occurrences are not known, it could be less compelling for implementation of enhanced biosecurity guidance than a decision based on known host occurrences. On a case-by-case basis, habitat likely occupied by some rare species may warrant considerations for enhanced biosecurity measures. See above for biosecurity decisions when only pathogen or pathogen habitat occurrences are considered as the host risk may be altered with pathogen occurrence knowledge.

High risk for pathogen export: If only host habitat suitability is known and neither host nor pathogen occupancy at the site is known, then it is prudent to consider the larger site context for biosecurity decisions. Is the area close to known sites of rare or little-known species or their refugia, or is it near a pathogen-free zone or at the edge of known pathogen occurrences? If so, there could be a high risk of potential pathogen export from the site. As this decision has an element of uncertainty, because only habitat is known and host occurrences are not known, it could be less compelling for implementation of enhanced biosecurity guidance than a decision based on known host occurrences. See above for biosecurity decisions when only pathogen or pathogen habitat occurrences are considered and see below for additional geographic contexts that may interact with habitat.

Host species richness.—Higher-risk sites for either import or export of a pathogen and thus application of enhanced between-site biosecurity may be those with higher species richness. Species composition warrants consideration relative to species-specific infection consequences and transmission risks. Are there rare species, species with key ecological functions, reservoir species or superspreaders at the site or at nearby sites? If one rare host within the community or area were vulnerable to disease, a high risk of pathogen import or export might be determined. The importance of protecting single species is growing as maintenance of biodiversity and the natural heritage of lands has become a foremost value for conservation efforts (Wilson 2016; Leopold et al. 2018). Several broad regions, such as the US Appalachia, the US Pacific Northwest, Central and South America, and Eastern Australia may have amphibian communities that could have species richness concerns elevating between-site biosecurity procedures. With widespread concerns for *Bd*-caused amphibian mortality, Australia's *Bd* Abatement Plan (ADEH 2006; Commonwealth of Australia 2016) incorporated several elements of enhanced between-site biosecurity to prevent *Bd* spread, including data updates to account for *Bd*-free areas for development of context-specific decisions.

High risk for pathogen import: Generally, areas with composite characteristics such as higher host species richness and host species with higher disease risk are high-risk sites for novel pathogen import.

High risk for pathogen export: Site-specific host community contexts would need to be evaluated relative to neighboring sites and the local region to assess pathogen export risk. As above, if there were host species with higher disease risk at neighboring sites or within the local region, then a higher risk for pathogen export could be relevant.

Geographic Contexts

Several geographic or geopolitical factors may affect risk of disease transmission between sites. Many of these contexts will not be binary categories resulting in either high or low risk of between-site transmission but will occur along a continuum. Also, geographic factors often interact with biotic factors and may not be stand-alone indicators of between-site pathogen transmission risk.

Distance and Topography.—First, between-site distance or topographic contexts may be important considerations for between-site pathogen transmission as some sites may be at higher likelihood of natural or human-mediated pathogen import or export due to their close proximity to other sites. For example, in geographic lowlands, plains, or river valleys there may be wetland clusters with relatively low overland distance between adjacent aquatic sites used as habitat by both aquatic-dependent hosts and pathogens. In the case of a first detection of disease in such a site cluster, it may be of paramount interest to heighten biosecurity to limit human-mediated pathogen spread across the cluster. In some topographic settings, there may be isolated habitat clusters, and it may be important to reduce transmission likelihood between clusters. In landscapes naïve to a pathogen causing an emerging infectious disease, it could be as important or more important to forestall transmission between distant sites than nearby sites. Inadvertent human-mediated transmission to more distant sites is likely less frequent, unless those sites are specifically visited in sequence by field crews or visitors for a targeted purpose. Remote sites without roads or trails may be most isolated, and potentially more important to address biosecurity to forestall inadvertent between-site pathogen transmission. Similarly, along linear stream networks, aquatic connectivity among nearby habitats within a stream reach could be greater than more distant habitats among stream reaches, and habitats within the same watershed would be closer than those among watersheds separated by topographic ridgelines. In pathogen-naïve landscapes, between-site pathogen transmission would be of greater concern among more distant stream habitats or across boundaries of distinct watersheds. An extreme perspective of distant or remote sites would be transmission of a pathogen to a novel region, state, country, or continent; this type of between-site transmission would be a very high risk for import, leading to narratives for the highest levels of biosecurity to forestall novel introduction of a disease-causing pathogen to potentially new host taxa.

High risk for pathogen import: Upon first detection of a novel pathogen to an area, there is a high risk for pathogen import to any other site, with enhanced biosecurity procedures a key consideration to quarantine the area of host infection. Distant or remote sites with potential hosts or host habitat but without pathogen occurrence are at very high risk for pathogen import, as transmission could introduce the pathogen to a novel region. Management actions including biosecurity to forestall import of novel pathogens to new geographic regions, especially with different vulnerable species or potentially susceptible rare species, are a high priority.

High risk for pathogen export: In response to a first-detection of a novel disease-causing pathogen at a specific site, it may be of paramount importance to manage for reduced transmission from that site to any nearby site, especially if an isolated single-site die-off is in process. Risk of pathogen export to more distant sites or to different watersheds could have more severe consequences; both warrant biosecurity management, with more distant sites and watersheds likely needing greater assurance of retaining pathogen-free status.

Geopolitical land-use type.—Second, geopolitically, land type may affect pathogen exposure potential, transmission risk, and biosecurity decisions. Land types include private versus public lands, urban versus rural vs remote lands, and land-use allocations ranging from highly managed lands (agricultural areas, industrial forests, mines, towns and cities) to protected natural areas and species refuges where species persistence is a priority. Each land type may need a case-by-case assessment relative to the pathogen(s) and host(s) in question, but some cross-cutting risk statements for import and export are possible. For example, land type and land-use allocation can be an indicator of human use, and human-mediated pathogen import or export risk is likely higher in areas that are more used by people. In *Bsal* risk models, US cities that were import centers were considered at higher risk of *Bsal* introduction due to pathogen transmission by amphibians within trade markets (Richgels et al. 2016). Also, when people are moving between land types (for work, restoration, recreation), inadvertent pathogen transmission risk may occur. Julian (2020) and NWCG (2017) addressed large equipment biosecurity when people were using equipment including vehicles in habitats occupied by invasive species, including invasive pathogens. Their scenarios include cases when people are moving habitat attributes (fomites) among sites (i.e., water for firefighting, soils for road building), as invasive species, including pathogens, could be moved at the same time. Retaining pathogen occurrences in a small area, potentially within single land types, and forestalling human-mediated transmission between land types could be a priority. Different land types often have different administrative jurisdictions, and it is optimal not to spread disease management across such boundaries, as not all administrative units will react similarly adding complex human dimensions to a biodiversity health issue.

Interaction of distance and topographic criteria with geopolitical boundaries may be important considerations, as well. Inadvertent human-mediated transmission to more distant remote sites with little land use is likely less frequent, unless, as mentioned above, those sites are specifically visited in sequence by field crews or visitors for a targeted purpose. Some lands are set aside as natural areas or for species protection priorities. These may include national parks, roadless areas, wilderness areas, or other types of habitat- or species-specific reserves. Maintaining the ecological integrity of such refugia may include priorities for forestalling transmission of novel amphibian pathogens and other invasive species. These would be higher-risk sites for human-mediated transmission of amphibian pathogens causing disease. At the other end of the spectrum, there are a variety of land types that are highly used by people. These include urban areas with higher human populations and consequent higher use of embedded natural areas, or rural areas with high agricultural use with crops or pastures. It may be impractical to manage for pathogen biosecurity within clusters of high-use areas, whereas between-cluster biosecurity may be more practically applied.

High risk for pathogen import: Risk of human-mediated transmission (import) of pathogens to new sites is higher where larger human population centers occur. It is a priority to address risk of pathogen import to sites across geopolitical jurisdictions such as different land ownerships, states, provinces, or nations. Pathogen import to natural areas and species' reserves may be especially high-risk scenarios warranting consideration of enhanced biosecurity. Merging geographic and geopolitical considerations, there is a greater consequence for pathogen import to remote wilderness areas or nature reserves where species persistence is a priority, and hence a high risk of import to those areas, with that risk increasing with isolation of such areas and distance from managed lands and population centers.

High risk for pathogen export: Conversely, there a high risk of pathogen export from managed lands and population centers due to more people moving in and out, especially those areas where inadvertent pathogen transmission may have gone unchecked. Likelihood of pathogen export could be related to both human use of areas and distance between host sites, with nearer sites with more use by people having a higher risk of between-site transmission. Use patterns and host or pathogen habitat suitability may have interactions, with some high-use areas having reduced habitat suitability. However, if hosts persist at suboptimal sites, they may be stressed or in another way more vulnerable to infections, although multiple interactions among habitat conditions, pathogen infections, and host species may be difficult to predict (review of experimental studies: Blaustein et al. 2018).

Integrating Pathogen, Host, and Geographic Contexts

As pathogen, host, and geographic contexts aggregate, their myriad interactions may become complex and difficult to evaluate for biosecurity decisions. The simple decision tree for enhanced biosecurity (Fig. 1) takes on more dimensions. Table 1 shows the seven contexts discussed above in a way that a yes/no question is posed about its importance for biosecurity decisions relative to site import or export of a pathogen. The cells can be populated with details of a situation warranting special consideration. Low-to-high priority (column 4, Table 1) may be assigned to each row's context, to summarize the row and carry weight in decision making. Although decisions for level of between-site biosecurity may be supported by information from any row (see single-context examples per row, Table 1), some facets of pathogen, host, or geographic contexts may be more important to consider in biosecurity decisions. In particular, as discussed below, enhanced biosecurity to forestall between-site pathogen transmission is especially warranted within the ranges of rare species with status of conservation concern (row 3, Table 1: host occurrence context) and in geographic areas set aside for ecological integrity including species-protection areas (row 7, Table 1: land-use type context). Some examples provided from a single context (each row, Table 1) may not be compelling for enhanced biosecurity decisions without consideration of other site contexts (other rows, Table 1; e.g., pathogen or host habitat contexts may need to be weighed together with other considerations). Although philosophically one could argue that enhanced biosecurity is prudent in all situations, practicality of a decision for enhanced or standard biosecurity may involve a balance of logistics or resources available to do biosecurity and the likelihood or consequence of human-mediated pathogen transmission. In that regard, known pathogens within a region with lethal effects on some hosts can be triggers for enhanced biosecurity, whereas absence of a realistic threat of disease could lead to relaxed measures. Having a narrative to support a decision can help provide an understanding for how decisions are made, and if contexts change, why biosecurity levels may similarly change.

Standard and Enhanced Biosecurity Guidance

Heightened awareness is emerging to prevent transmission of a broad spectrum of disease-causing pathogens during a variety of contemporary field practices. For example, chemical disinfection of water draws for wildfire management is conducted between watersheds in some areas of the US West (also with fungal pathogens of tree species under consideration for high-risk human-mediated transmission: USFS 2017; NWCG 2017). Further, elevated biosecurity is already in practice at some areas with novel pathogen detections (e.g., ADEH 2006; Commonwealth of Australia 2016; Bosch et al. 2015; Martel et al. 2020). Conceptual scenarios of first detections of novel diseases in an area have further advanced development of suites of response actions including between-site biosecurity measures (Hopkins et al. 2018; Bsal Task Force 2018; Canessa et al. 2020). More et al. (2018) broadly addressed concerns for translocation of fomites (e.g., substrates, clothing, other materials that may carry pathogens).

Herein, we consider standard between-site biosecurity protocols to include decontamination of field gear before use at a site and after use at a site (Table 2). Disinfection could occur at the site or elsewhere. General understanding of pathogen and host occurrences are useful to support biosecurity considerations by field crew members, but the seven contexts that we outline in Table 1 are not analyzed in depth once a decision for standard procedures is made and field work begins. For standard biosecurity, the "site" needs to be defined and could vary with project scope, pathogen risk assessments, or practicality issues. We describe enhanced biosecurity (Table 2) to include more detailed office assessments of pathogen, host, and geographic contexts, and more-strict procedures at the field site to prevent inadvertent between-site transfer of pathogens by people. For example, we recommend the use of disposable Tyvek® suits, gloves, and other easily decontaminated gear at higher-risk sites. The use of dedicated gear for high risk sites is another consideration (i.e., change gear between sites). In general, the standard procedures are lower effort and may be more practically applied routinely as a matter of course by private citizens or professional field workers (Table 2).

On a case-by-case basis, between-site decontamination efforts may be evaluated and vary between the extremes we have outlined for standard to enhanced guidance (Table 2). We consider approaches to working at a known high-risk (for import or export) pathogen-transmission site to include: 1) biosecurity planning at the office; 2) biosecurity staging at the vehicle on the road before entry to the field site; 3) biosecurity steps upon return to the vehicle; and 4) planning and re-staging before going to a new site. For clarity, whereas we focus on between-site biosecurity, within-site biosecurity considerations during animal capture procedures and animal processing are discussed by Gray et al. (2017). Chemical decontamination options and efficacies have been previously summarized (USFS 2017; Gray et al. 2017; More et al. 2018).

Enhanced between-site biosecurity includes assessment of the order of site visitation when sampling animals at multiple sites to help reduce likelihood of pathogen import or export. This may include working at pathogen-free sites first (More et al. 2018), rare-species sites first, or protected land-use types first. Using area maps with roads, waterways, pathogen and host-occurrence data, and geopolitical data, a daily and seasonal plan of field work can be charted. However, this order of operation is not always feasible. When a high-risk of transmission site must be sampled, either first or at all, considerations can be made to apply more stringent biosecurity procedures to minimize the risk of human-mediated spread of pathogens to subsequent sites.

Preplanning for vehicle and equipment decontamination procedures begins before the first high-risk site is visited (Julian et al. 2020). When enhanced biosecurity includes disinfection of vehicles or equipment due to their potential direct contact with pathogens, disinfection locations need to be identified and supplies assembled. If possible, different vehicles or gear could be used for high- and low-risk sites to reduce transmission likelihood, even with disinfection measures. At a minimum the outside of the vehicle (i.e., door handles, other areas of contact with potentially contaminated gear/objects), tires, and gear can be cleaned and sprayed with a disinfectant before leaving a site with pathogen occurrence and hence high risk of human transmission to the next site visited. It should be noted that, especially at known high-risk sites, the exterior of vehicles may be kept cleaner by avoiding driving through mud, water, or other on-site material(s) that may harbor the pathogen in question. If water or mud crossings cannot be avoided, it may be worth using a spray bottle with an appropriate disinfectant, according to product label (e.g., 2% Virkon-Aquatic), to decontaminate tires and the underside of the vehicle after crossing water or wet riparian areas and before moving into another aquatic environment. If the vehicle becomes contaminated with soil, water, or other material from the site, the entire vehicle can be washed in a commercial carwash after leaving a known high-risk site. This is necessary as many disinfectants (i.e., Virkon and bleach) are not chemically active in the presence of abundant organic material such as mud, which makes it necessary to ensure grossly visible contaminants are removed via washing prior to applying these biochemical cleaners. If the vehicle's interior may be contaminated, we recommend using seat covers that can be washed. Further, the interior of the vehicle should be cleaned and disinfected by vacuuming and spraying hard surfaces, especially those areas that come into direct contact with the animal, animal containers, or other potentially contaminated objects, thoroughly with 2% Virkon-Aquatic (or another disinfectant of choice, Gray et al. 2017). Julian et al. (2020) expand on herpetofauna disease disinfection guidelines for large equipment.

Ideally, a change of clothing or equipment between high-risk and low-risk sites can be made to reduce risk of inadvertent pathogen transmission. Sets of rubber boots, waders, and other gear can be designated for specific sites, but should still be cleaned and disinfected prior to leaving a site as well as when transported between sites. More et al. (2018) call attention to disinfection of exposed body parts of field-working personnel.

Conclusion

Preventing transmission of an emerging infectious disease to a novel area or species is akin to preventing invasion of a non-native species. Invasive species biosecurity protocols are especially comprehensive when the invasion outcome is costly relative to socioeconomic factors (e.g., effects on economically important native species, or human industry such as recreation) and ecological transformative effects (e.g., biotic homogenization). Similarly, for disease-causing pathogens, forestalling transmission to novel areas is especially critical when agricultural or ecological resources are at stake, as can be determined by scanning the lists of species warranting import restrictions per the World Organization of Animal Health (OIE 2020). With the recognition of the current mass extinction event ongoing globally (Stuart et al. 2004; Wake and Vredenburg 2008; Scheele et al. 2019), the integrity of native biodiversity is becoming of paramount importance as an ecosystem service warranting protection. For declining amphibian populations, diseases are among the top-identified threats (AmphibiaWeb 2017). Therefore, forestalling disease-causing pathogen transmission by human-mediated vectors has become a global imperative (Fisher et al. 2012; Phillott et al. 2010; More et al. 2018). Enhanced biosecurity protocols can help mitigate, or at least minimize, anthropogenic-mediated pathogen transmission between sites.

Enhanced decontamination and biosecurity procedures are especially relevant when visiting field sites known to be at high risk of pathogen transmission, either to that site (import) or out of that site (export). These high-risk sites are important to recognize because field work conducted at these sites could accelerate human-mediated translocation of pathogen(s). While preparing for fieldwork, pathogen occurrence and associated risk of transmission to new species or locations can be considered prior to entering the field. Evidence-based decision making in planning and implementing fieldwork using surveillance results can help reduce the anthropogenic spread of pathogens known to be detrimental to herpetofaunal populations. As researchers, field biologists, site managers, and herpetological enthusiasts, biosecurity stewardship can help ensure we are not vectors in spreading pathogens and contributing to biodiversity loss.

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Literature Cited

ADEH [Australia Department of the Environment and Heritage]. 2006. Threat abatement plan: Infection of amphibians with chytrid fungus resulting in chytridiomycosis. Commonwealth of Australia, Department of the Environment and Heritage, Canberra ACT. 21 pp.

AmphibiaWeb. 2017. Worldwide amphibian declines: What is the scope of the problem, what are the causes, and what can be done? https://amphibiaweb.org/declines/declines.html; Accessed 22 October 2018.

Arellano, M. L., M. A. Velasco, F. P. Kacoliris, A. M. Belasen, and T. Y. James. 2017. First record of *Batrachochytrium dendrobatidis* in *Pleurodema somuncurense*, a critically endangered species from Argentina. Herpetol. Rev. 48:68–69.

Ariel, E., N. Nocolajsen, M. B. Christophersen, R. Holopainen, H. Tapiovaara, and B. B. Jensen. 2009. Propagation and isolation of ranaviruses in cell culture. Aquaculture 294:159–164.

Auliya, M., J. Garcia-Moreno, B. R. Schmidt, D. S. Schmeller, M. S. Hoogmoed, M. C. Fisher, F. Pasmans, K. Henle, D. Bickford, and A. Martel. 2016. The global amphibian trade flows through Europe: the need for enforcing and improving legislation. Biodivers. Conserv. 25:2581–2595.

Baker, S. J., E. Haynes, M. Gramhofer, K. Stanford, S. Bailey, M. Christman, K. Conley, S. Frasca, Jr., R. J. Ossiboff, D. Lobato, and M. C. Allender. 2019. Case definition and diagnotistic testing for snake fungal disease. Herpetol. Rev. 50:279–285.

Baxter, C. V., K. D. Fausch, and W. C. Saunders. 2005. Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. Freshwater Biol. 50:201-2020.

Best, M. L., and H. H. Welsh, Jr. 2014. The trophic role of a forest salamander: Impacts on invertebrates, leaf litter retention, and the humification process. Ecosphere 5:Article 16.

Blaustein, A. R., J. Urbina, P. W. Snyder, E. Reynolds, T. Dang, J. T. Hoverman, B. Han, D. H. Olson, C. Searle, and N. M. Hambalek. 2018. Effects of emerging infectious diseases on amphibians: A review of experimental studies. Diversity 10:1–49.

Bosch, J., E. Sanchez-Tomé, A. Fernández-Loras, J. A. Oliver, M. C.

Fisher, and T.W. J. Garner. 2015. Successful elimination of a lethal wildlife infectious disease in nature. Biol. Lett. 11:20150874.

Bradley, P. W., M. D. Brawner, T. R. Raffel, J. R. Rohr, D. H. Olson, and A. R. Blaustein. 2019. Shifts in temperature influence how *Batrachochytrium dendrobatidis* infects amphibian larvae. PLoS ONE 14(9):e0222237.

Brand, M. D., R. D. Hill, R. Brenes, J. C. Chaney, R. P. Wilkes, L. Grayfer, D. L. Miller, and M. J. Gray. 2016. Water temperature affects susceptibility to Ranavirus. EcoHealth 13:350–359.

Bsal Task Force. 2018. *Batrachochytrium salamandrivorans* (Bsal) Rapid Response Template. 8 June 2018. http://www.salamanderfungus.org/: Strategic Plans & Annual Reports; Accessed 18 February 2020.

Canessa, S., A. Spitzen-van der Sluijs, T. Stark, P. J. Bishop, M. Bletz, C. J. Briggs, D. Daversa, M. J. Gray, R. A. Griffiths, R. N. Harris, X. A. Harrison, J. T. Hoverman, P. Jervis, E. L. Muths, D. H. Olson, C. L. Richards-Zawacki, J. Robert, G. M. Rosa, B. C. Scheele, B. R. Schmidt, and T. W. J. Garner. 2020. Conservation decisions under pressure: lessons from an exercise in rapid response to wildlife disease. Conserv. Sci. Pract. 2:e141. doi.org/10.1111/csp2.141

Chestnut, T., C. Anderson, R. Popa, A. R. Blaustein, M. Voytek, D. H. Olson, and J. Kirshtein. 2014. Heterogeneous occupancy and density estimates of the pathogenic fungus *Batrachochytrium dendrobatidis* in waters of North America. PLoS ONE 9:e106790.

Commonwealth of Australia. 2016. Threat Abatement Plan: For infection of amphibians with chytrid fungus resulting in chytridiomycosis. Department of the Environment and Energy, Commonwealth of Australia. www.deh.gov.au/biodiversity/threatened/publications/tap/chytrid/; accessed 27 August 2020.

Farrell, T. M., J. Agugliaro, H. D. S. Walden, J. F. X. Wellehan, A. L. Childress, and C. M. Lind. 2019. Spillover of pentastome parasites from invasive Burmese pythons (*Python bivittatus*) to pygmy rattlesnakes (*Sistrurus miliarius*), extending parasite range in Florida, USA. Herpetol. Rev. 50:73–76.

Fisher, M. C., and T. W. J. Garner. 2007. The relationship between the introduction of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. Fungal Biol. Rev. 21:2–9.

———, D. A. Henk, C. J. Briggs, J. S. Brownstein, L. C. Madoff, S. L. McCraw, and S. J. Gurr. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484:186–194.

Garner, T. W. J., B. R. Schmidt, A. Martel, F. Pasmans, E. Muths, A. A. Cunningham, C. Weldon, M. C. Fisher, and J. Bosch. 2016. Mitigating amphibian chytridiomycosis in nature. Phil. Trans. R. Soc. B 371:20160207.

Ghirardi, R., J. A. Lopez, E. A. Sanabria, L. B. Quiroga, and M. G. Levy. 2017. First report of *Batrachochytrium dendobatidis* in the warty toad, *Rhinella spinulosa* (Wiegmann, 1834), from the Argentinean Andean foothills. Herpetol. Rev. 48:64–65.

Grant, E. H. C., E. Muths, R. A. Katz, S. Canessa, M. J. Adams, J. R. Ballard, L. Berger, C. J. Briggs, J. Coleman, M. J. Gray, M. C. Harris, R. N. Harris, B. Hossack, K. P. Huyvaert, J. E. Kolby, K. R. Lips, R. E. Lovich, H. I. McCallum, J. R. I. Mendelson, P. Nanjappa, D. H. Olson, J. G. Powers, K. L. D. Richgels, R. E. Russell, B. R. Schmidt, A. Spitzen-van der Sluijs, M. K. Watry, D. C. Woodhams, and C. L. White. 2015. Salamander chytrid fungus (*Batrachochytrium salamandrivorans*) in the United States: developing research, monitoring, and management strategies. Open-File Report 2015-1233. 16 pp., http://dx.doi.org/10.3133/ofr20151233; accessed 22 November 2020.

———, ———, ———, ———, ———, ———, ———, ———, J. T. H. Coleman, M. J. Gray, M. C. Harris, R. N. Harris, B. Hossack, K. P. Huyvaert, J. Kolby, K. R. Lips, R. E. Lovich, H. I. McCallum, J. R. Mendelson III, P. Nanjappa, D. H. Olson, J. G. Powers, K. L. D. Richgels, R. E. Russell, B. R. Schmidt, A. Spitzen-van der Sluijs, M. K. Watry, D. C. Woodhams, and C. L. White. 2017. Using decision analysis to support proactive management of emerging infectious wildlife diseases. Front. Ecol. Environ. 15:214–221.

Gray, M. J., and V. G. Chinchar (eds.). 2015. Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates. Springer International Publishing, Switzerland. 246 pp. DOI 10.1007/978-3-319-13755-1_5.

———, A. L. J. Duffus, K. H. Haman, R. N. Harris, M. C. Allender, T. A. Thompson, M. R. Christman, A. Sacerdote-Velat, L. A. Sprague, J. M. Williams, and D. L. Miller. 2017. Pathogen surveillance in herpetofaunal populations: Guidance on study design, sample collection, biosecurity, and intervention strategies. Herpetol. Rev. 48:334–351.

———, J. P. Lewis, P. Nanjappa, B. Klocke, F. Pasmans, A. Martel, C. Stephen, G. Parra Olea, S. A. Smith, A. Sacerdote-Velat, M. Christman, J. Williams, and D. H. Olson. 2015. *Batrachochytrium salamandrivorans*: The North American response and a call for action. PLoS Pathogens 11:e1005251.

———, J. A. Spatz, E. D. Carter, C. M. Yarber, R. P. Wilkes, and D. L. Miller. 2018. Poor biosecurity could lead to disease outbreaks in animal populations. PLoS ONE 13:e0193243.

Hopkins, M. C., M. J. Adams, P. E. Super, D. H. Olson, C. R. Hickman, P. English, L. Sprague, I. B. Maska, A. B. Pennaz, and K. A. Ludwig. 2018. *Batrachochytrium salamandriovrans* (*Bsal*) in Appalachia—using scenario building to proactively prepare for a wildlife disease outbreak caused by an invasive amphibian chytrid fungus: U.S. Geological Survey Report 2018–1150. 31 pp. https://doi.org/10.3133/ofr20181150. Accessed 18 December 2018.

Isidoro-Ayza, M., J. M. Lorch, D. A. Grear, M. Winzeler, D. L. Calhoun, and W. J. Barichivich. 2017. Pathogenic lineage of Perkinsea associated with mass mortality of frogs across the United States. Sci. Rep. 7:10288.

Julian, J. T., P. F. P. Henry, J. M. Drasher, S. D. Jewell, K. Michell, K. J. Oxenrider, and S. A. Smith. 2020. Minimizing the spread of herpetofaunal pathogens in aquatic habitats by decontaminating construction equipment. Herpetol. Rev. 51:472–483.

Kane, L. P., M. C. Allender, G. Archer, K. Leister, M. Rzadkowska, K. Boers, M. Souza, and S. Cox. 2017. Pharmacokinetics of nebulized and subcutaneously implanted terbinafine in cottonmouths (*Agkistrodon piscivorus*). J. Vet. Pharmacol. Ther. 40:10.1111/jvp.12406.

Leopold, B. D., W. B. Kessler, and J. L. Cummins. 2018. North American Wildlife Policy and Law. Boone and Crocket Club, Missoula, MT, USA. 623 p.

Lindemann, D. M., M. C. Allender, D. Thompson, G. A. Glowacki, E. R. Newman, L. A. Adamovicz, and R. L. Smith. 2019. Epidemiology of emydoidea herpesvirus 1 in free-ranging Blanding's turtles (*Emydoidea blandingii*) from Illinois. J. Zoo Wildl. Med. 50:547–556.

Lips, K. R., F. Brem, R. Brenes, J. D. Reeve, R. A. Alford, J. Voyles, C. Carey, L. Livo, A. P. Pessier, and J. P. Collins. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. Proc. Nat. Acad. Sci. 103:3165–3170.

Longcore, J. E., A. P. Pessier, and D. K. Nichols. 1999. *Batrachochytrium dendrobatidis* gen. et sp. nov., a chytrid pathogenic to amphibians. Mycologia 91:219–227.

Martel, A., M. Blooi, C. Adriaensen, P. Van Rooij, W. Beukema, M. C. Fisher, R. A. Farrer, B. R. Schmidt, U. Tobler, K. Goka, K. R. Lips, C. Muletz, K. R. Zamudio, J. Bosch, S. Lötters, E. Wombwell, T. W. J. Garner, A. A. Cunningham, A. Spitzen-van der Sluijs, S. Salvidio, R. Ducatelle, K. Nishikawa, T. T. Nguyen, J. E. Kolby, I. Van Bocxlaer, F. Bossuyt and F. Pasmans. 2014. Recent introduction of a chytrid fungus endangers western Palearctic salamanders. Science 346:630–631.

———, A. Spitzen-van der Sluijs, M. Blooi, W. Bert, R. Ducatelle, M. C. Fisher, A. Woeltjes, W. Bosman, K. Chiers, F. Bossuyt, and F. Pasmans. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. Proc. Nat. Acad. Sci. 110:15325–15329.

———, M. Vila-Escale, D. Fernández-Giberteau, A. Martinez-Silvestre, S. Canessa, S. Van Praet, P. Pannon, K. Chiers, A. Ferran, M. Kelly, M. Picart, D. Piulats, Z. Li, V. Pagone, L. Pérez-Sorribes, C. Molina, A. Tarragó-Guarro, R. Velarde-Nieto, F. Carbonnell, E. Obon, D. Martínez-Martínez, D. Guinart, R. Casanovas, S. Carranza, and F. Pasmans. 2020. Integral chain management of wildlife diseases. Conserv.

Lett. 2020:e12707.

Moore, J. E., E. Parr, and S. M. Hanlon. 2018. First confirmed case of Ranavirus in turtles from Shelby County, western Tennessee, USA. Herpetol. Rev. 49:262–263.

More, S., M. A. Miranda, D. Bicout, A. Bøtner, A. Butterworth, P. Calistri, K. Depner, S. Edwards, B. Garin-Bastuji, M. Good, V. Michel, M. Raj, S. S. Nielsen, L. Sihvonen, H. Spoolder, J. A. Stegeman, H-H. Thulke, A. Velarde, P. Willeberg, CV. Winckler, V. Balá, A. Martel, K. Murray, C. Fabris, I. Munoz-Gajardo, A. Gogin, F. Verdonck, and C. G. Schmidt. 2018. Risk of survival, esablishment and spread of *Batrachochytrium salamandrivorans* (Bsal) in the EU. EFSA Journal 16:5259.

Nguyen, T. T., T. Van Nguyen, T. Ziegler, F. Pasmans, and A. Martel. 2017. Trade in wild anurans vectors the urodelan pathogen *Batrachochytrium salamandrivorans* into Europe. Amphibia-Reptilia 38:554–556.

NWCG [national Wildfire Coordinating Group]. 2017. Guide to preventing aquatic invasive species transport by wildland fire operations. Invasive species subcommittee, Equipment Technology Committee, National Wilfire Coordinating Group, United States. PMS 444. 64 p. https://www.nwcg.gov/sites/default/files/publications/pms444.pdf; accessed 26 August 2020.

———. 2020. Invasive species mitgation for ground resources. Invasive species subcommittee, Equipment Technology Committee, National Wilfire Coordinating Group, United States. Operations video. https://www.nwcg.gov/publications/training-courses/rt-130/operations/op819; accessed 26 August 2020.

OIE [World Organization for Animal Health]. 2020. OIE-listed diseases, infections and infestations in force in 2018. http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2018/; 20 February 2020.

O'Hanlon, S. J., A. Rieuz, R. A. Farrer, G. M. Rosa, B. Walman, A. Bataille, T. A. Kosch, K. A. Murray, B. Brankovics, M. Fumagalli, M. D. Martin, N. Wales, M. Alvarado-Rybak, K. A. Bates, L. Berger, S. Böll, L. Brookes, F. Clare, E. A. Courtois, A. A. Cunningham, T. M. Doherty-Bone, P. Ghosh, D. J. Gower, W. E. Hintz, J. Höglund, T. S. Jenkinson, C-F. Lin, A. Laurila, A. Loyau, A. Martel, S. Meurling, C. Miaud, P. Minting, F. Pasmans, D. S. Schmeller, B. R. Schmidt, J. M. G. Shelton, L. F. Skerratt, F. Smith, C. Soto-Azat, M. Spagnoletti, G. Tessa, L. F. Toledo, A. Valenzuela-Sánchez, R. Verster, J. Vörös, R. J. Webb, C. Wierzbicki, E. Wombwell, K. R. Zamudio, D. M. Aanensen, T. Y. James, M.T. P. Gilbert, C. Weldon, J. Bosch, F. Balloux, T. W. J. Garner, and M. C. Fisher. 2018. Recent Asian origin of chytrid fungi causing global amphibian declines. Science 360:621–627.

Olson, D. H., D. M. Aanensen, K. L. Ronnenberg, C. I. Powell, S. F. Walker, J. Bielby, T. W. J. Garner, G. Weaver, the Bd Mapping Group, and M. C. Fisher. 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS ONE 8:e56802.

———, and K. L. Ronnenberg. 2014. Global Bd mapping project: 2014 update. FrogLog 111:17–21.

Phillott, A. D., R. Speare, H. B. Hines, L. F. Skerratt, E. Meyer, K. R. McDonald, S. D. Cashins, D. Mendez, and L. Berger. 2010. Minimising exposure of amphibians to pathogens during field studies. Dis. Aquat. Org. 92:175–185.

Picco, A. M., and J. P. Collins. 2008. Amphibian commerce as a likely source of pathogen pollution. Conserv. Biol. 22:1582–1589.

Piotrowski, J. S., S. L. Annis, and J. E. Longcore. 2004. Physiology of *Batrachochytrium dendrobatidis*, a chytrid pathogen of amphibians. Mycologia 96:9–15.

Reeder, N. M. M., A. P. Pessier, and V. T. Vredenburg. 2012. A reservoir species for the emerging amphibian pathogen *Batrachochytrium dendrobatidis* thrives in a landscape decimated by disease. PLoS ONE 7: e33567.

Reeves, M. K. 2008. *Batrachochytrium dendrobatidis* in wood frogs (*Rana sylvatica*) from three national wildlife refuges in Alaska, USA. Herpetol. Rev. 39:68–70.

Reshetnikov, A. N., T. Chestnut, J. L. Brunner, K. Charles, E. E. Nebergall, and D. H. Olson. 2014. Detection of the emerging amphibian pathogens *Batrachochytrium dendrobatidis* and ranavirus in Russia. Dis. Aquat. Org. 110:235–240.

Ribeiro, L. P., T. Carvalho, C. G. Becker, T. S. Jenkinson, D. da Silva Leite, T. Y. James, S. E. Greenspan, and L. F. Toledo. 2019. Bullfrog farms release virulent zoospores of the frog-killing fungus into the natural environment. Sci. Rep. 9:13422.

Richgels, K. L., R. E. Russell, M. J. Adams, C. L. White, and E. H. Grant. 2016. Spatial variation in risk and consequence of B*atrachochytrium salamandrivorans* introduction in the USA. Open Sci. 3:150616.

Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho, A. Catenazzi, I. De la Riva, M. C. Fisher, S. V. Flechas, C. N. Foster, P. Frías-Álvarez, T. W. J. Garner, B. Gratwicke, J. M. Guayasamin, M. Hirschfeld, J. E. Kolby, T. A. Kosch, E. La Marca, D. B. Lindenmayer, K. R. Lips, A. V. Longo, R. Maneyro, C. A. McDonald, J. Mendelson, III, P. Palacios-Rodriguez, G. Parra-Olea, C. L. Richards-Zawacki, M-O. Rödel, S. M. Rovito, C. Soto-Axat, L. F. Toledo, J. Voyles, C. Weldon, W. M. Whitfield, M. Wilkinson, K. R. Zamudio, and S. Canessa. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459–1463.

Schloegel, L. M., A. M. Picco, A. M. Kilpatrick, A. J. Davies, A. D. Hyatt, and P. Daszak. 2009. Magnitude of the US trade in amphibians and presence of *Batrachochytrium dendrobatidi*s and ranavirus infection in imported North American bullfrogs (*Rana catesbeiana*). Biol. Conserv. 142:1420–1426.

Semlitsch, R. D., K. M. O'Donnell, and F. R. Thompson III. 2014. Abundance, biomass production, nutrient content, and the possible role of terrestrial salamanders in Missouri Ozark forest ecosystems. Can. J. Zool. 92:997–1004.

Stuart, S. N., J. S. Chanson, N. A. Cox, B. E. Young, A. S. L. Rodrigues, D. L. Fischman, and R. W. Waller. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306:1783–1786.

USFS (United States Forest Service). 2017. Guide to preventing aquatic invasive species transport by wildland fire operations. National Wildfire Coordination Group Publication PMS 444. Available at: https://www.nwcg.gov/sites/ default/files/publications/pms444.pdf.

Vredenburg, V. T, R. A. Knapp, T. S. Tunstall, and C. J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proc. Nat. Acad. Sci. 107:9689–9694.

Wake, D. B., and V. T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proc. Nat. Acad. Sci. 105:11466–11473.

Walker, S. F., J. Bosch, T. Y. James, A. P. Litvintseva, J. A. O. Valls, S. Piña, G. García, G. A. Rosa, A. A. Cunningham, S. Hole, R. Griffiths, and M. C. Fisher. 2008. Invasive pathogens threaten species recovery programs. Curr. Biol. 18:PR853–R854.

Wilson, E. O. 2016. Half-Earth: Our Planet's Fight for Life. Liveright, New York, New York. 272 pp.

Xie, G. Y., D. H. Olson, and A. R. Blaustein. 2016. Projecting the global distribution of the emerging amphibian fungal pathogen, *Batrachochytrium dendrobatidis*, based on IPCC climate futures. PLoS ONE 11:e0160746.

Zhu, W., F. Xu, C. Bai, X. Liu, S. Wang, X. Gao, S. Yan, X. Li, Z. Liu, and Y. Li. 2014. A survey for Batrachochytrium salamandrivorans in Chinese amphibians. Current Zoology 60:729–735.