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OPINION PIECE

Responsible biosecurity and risk mitigation for laboratory research on emerging pathogens of amphibians

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ABSTRACT: The increasing study of emerging wildlife pathogens and a lack of policy or legislation regulating their translocation and use has heightened concerns about laboratory escape, species spillover, and subsequent epizootics among animal populations. Responsible self-regulation by research laboratories, in conjunction with institutional-level safeguards, has an important role in mitigating pathogen transmission and spillover, as well as potential interspecies pathogenesis. A model system in disease ecology that highlights these concerns and related amelioration efforts is research focused on amphibian emerging infectious diseases. Whereas laboratory escape of amphibian pathogens has not been reported and may be rare compared with introduction events from trade or human globalization, the threat that novel disease outbreaks with mass mortality effects pose to wild populations warrants thorough biosecurity measures to ensure containment and prevent spillover. Here, we present a case study of the laboratory biosecurity concerns for the emerging amphibian fungal pathogen *Batrachochytrium salamandrivorans*. We conclude that proactive biosecurity strategies are needed to integrate researcher and institutional oversight of aquatic wildlife pathogens generally, and we call for increased national and international policy and legislative enforcement. Furthermore, taking professional responsibility beyond current regulations is needed as development of legal guidance can be slow at national and international levels. We outline the need for annual laboratory risk assessments, comprehensive training for all laboratory personnel, and appropriate safeguards specific to pathogens under study. These strategies are critical for upholding the integrity and credibility of the scientific community and maintaining public support for research on wildlife diseases.

KEY WORDS: *Batrachochytrium salamandrivorans* · Biosecurity · Amphibians · Emerging diseases

1. INTRODUCTION

Emerging infectious diseases present increasingly complex biosecurity challenges (Cunningham et al. 2017, Kibenge 2019). These challenges are particularly evident in academic research focused on pathogens that do not pose an immediate threat to the health of humans, livestock, or wildlife with commercial value. Biosecurity standards for research on these pathogens are not typically regulated by uni-

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form protocols or restrictions at any level of institutional and governmental oversight, though such standards often exist (e.g. codes developed by the World Organization of Animal Health [OIE]). For example, at our institutions in the USA, Netherlands, and Germany, oversight of wildlife pathogens that pose no risk for humans, livestock, hunting, or fishing is lacking, except when experiments involve vertebrate animals. Rather, biosecurity practices may be set by members of the relevant research community acting as a self-regulating entity, or may be developed independently per research group outside a community network. As research communities and agencies responsible for wildlife management are often the first to take notice of obscure pathogens, these groups are often the first — and only — entities concerned with unintentional anthropogenic dispersal (Olson et al. 2021). Management of pathogen spillover at the ecological level is a growing area of research (Sokolow et al. 2019), but spillover from research laboratories is rarely addressed for wildlife pathogens. Although escape of non-agricultural wildlife disease-causing pathogens from research laboratories has rarely been documented, it is a risk that is eminently addressable, as we highlight here. For example, Shelley et al. (2013) make recommendations for speleologists to avoid spreading the fungal agent of bat white-nose syndrome, *Pseudogymnoascus destructans*, to new cave sites, and the United States Department of Agriculture Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine suggests containment guidelines for plant pathogenic bacteria such as mycoplasmas and spiroplasmas (APHIS 2010). The prevention of research-mediated pathogen spillover will require responsible self-regulation by research laboratories, in conjunction with better institutional safeguards (Gray et al. 2017), and improved, clear legislative policy. Such measures will be essential to uphold the integrity and credibility of the research community and safeguard ecologically valuable wildlife populations.

While it is possible for any party to unintentionally spread a pathogen associated with emerging disease (e.g. Walker et al. 2008), researchers intentionally translocate pathogens across vast distances in order to facilitate research using the best available expertise and resources. This transfer of study organisms is often necessary to address disparities in regional scientific capacity. On a global scale, we have seen how neglecting the study of wildlife pathogens, particularly potential zoonotic pathogens, can encumber responses when disease emerges (e.g. SARS-COV-2). However, translocation is accompanied by an elevated risk of introduction to wildlife in research areas outside the present range of occurrence of the disease. Research activities involving field surveillance similarly can promote unintentional pathogen introduction to immunologically naive populations through the use of equipment that is not properly decontaminated between locations (Olson et al. 2021). As these concerns expand as a function of increased study of emerging pathogens, we propose proactive strategies in both policy and protocol. We highlight current work in the amphibian research community addressing emerging pathogen biosecurity for the fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*). This focus is followed with generalized recommendations for those involved in research on emerging infectious diseases of wildlife. We conclude with an outlook towards implementation of these prescriptive and preventive recommendations, including policy development, education, and oversight.

2. CASE STUDY: *BATRACHOCHYTRIUM SALAMANDRIVORANS*

Bsal is a causal fungal pathogen of emerging chytridiomycosis in amphibians, particularly salamanders. It is thought to have originated in Asia (Laking et al. 2017, O'Hanlon et al. 2018, Yuan et al. 2018), and spread to Europe, where it has caused dramatic declines in fire salamanders *Salamandra salamandra* and may pose high risk to other species (Martel et al. 2013, 2014, Woodhams et al. 2018, Carter et al. 2020). This pathogen is of heightened interest in the amphibian disease research community, as it does not yet appear to have spread to North America, a global center for salamander biodiversity. Forestalling inadvertent translocation of this invasive pathogen species could protect several species of disease-susceptible amphibians from mass mortality episodes (Martel et al. 2014), with cascading effects on their communities (Grant et al. 2016, Gray et al. 2015).

Both research and commercially-traded animals may serve as pathogen reservoirs, facilitating spillover to native wildlife. For example, *Bsal* has been detected on captive amphibians (Cunningham et al. 2015, Sabino-Pinto et al. 2015, Fitzpatrick et al. 2018); it was likely introduced to Europe through the pet trade (Nguyen et al. 2017) and underwent subsequent range expansion to wild populations (Spitzen-van der Sluijs et al. 2016, Lötters et al. 2020, Martel et al. 2020). In 2015, trade regulation of *Bsal*-carrier amphibians was recognized as a pivotal step to forestall translocation to North America (Grant et al. 2016, Gray et al. 2015). In 2016, the US Fish and Wildlife Service (USFWS) listed 201 salamander species as 'injurious species' under the legal authority of the US Lacey Act to restrict their import into the USA and prevent the dispersal of *Bsal* in North America. Then in 2017, *Bsal* was listed as a World Organization for Animal Health (OIE) notifiable disease (OIE 2021). OIE member countries are guided to provide animal health certificates to accompany imports of domesticated animals that are potential hosts of OIE-listed pathogens and are supposed to report OIE-listed pathogen detections through the World Animal Health Information System (WAHIS; OIE 2021). However, pathogen-free shipment status is not required for wildlife in many countries, including the USA, and not enforced in others, such as trade within Europe and between most US states. Canada implemented a similar ban to that implemented in the USA, preventing importation of all caudates (live or dead) without permits (Canada Border Services Agency 2018), and the European Union (EU) recently passed legislation establishing a clean trade program, requiring health certificates, pathogen-free status, and quarantine for all salamanders imported into and among union countries (EU 2018; updated in EU Commission Decision 2021/361). These are critical steps to minimize the risk of *Bsal* invasion, and in fact USFWS injurious listings have been very effective at limiting entry of host organisms (Jewell & Fuller 2021). However, anurans are not covered by these restrictions and import of anurans into North America continues. Because some species of anurans can carry *Bsal* (Nguyen et al. 2017, Stegen et al. 2017), continued translocation of these species is a serious, unaddressed threat to North American salamander populations. These risks highlight the need for more stringent policies, regulations, and clean trade programs to reduce the potential for spillover to local ecosystems.

In addition to the inadvertent trade and movement of infected animals, pathogens can enter the environment from cultures used in disease research. Research-associated escape, though unlikely, would be particularly detrimental; besides compromising wild populations, researcher-mediated introduction of *Bsal* could result in public criticism of scientists, federal and state agencies, and non-government organizations involved in disease research. Under this scenario, continued support and funding for scientific research in amphibian disease ecology might be at risk.

To address concerns for vulnerable amphibian populations and research integrity, the amphibian disease community has already undertaken numerous initiatives for the amelioration of biosecurity risks associated with *Bsal* research. Beyond the policies restricting movement of amphibians described above, for *Bsal* researchers, advisory committees such as the North American *Bsal* Task Force can facilitate much-needed discussions and actions (www.salamanderfungus.org). Other considerations include the movement of equipment and even people between geographies with disparate disease endemicity (Canadian Herpetofauna Health Working Group 2017, Julian et al. 2020, Olson et al. 2021). Indeed, the implementation of basic containment procedures such as use of nitrile gloves can have order of magnitude effects on pathogen transmission risk (Gray et al. 2018, Thomas et al. 2020). For many other emerging infectious diseases of wildlife, this level of scrutiny of biosecurity risk is not in place.

Care must be taken in the transportation, receiving, and use of non-native pathogens. Before new partners begin research with pathogens such as *Bsal*, they consult with the North American *Bsal* Task Force (www.salamanderfungus.org/task-force/), who critically review their ability to safely contain the pathogen, assess the consequences of potential release for their area, and put in place proper safety measures *before* any research begins (see the Supplement at www.int-res.com/articles/suppl/d147p141_supp.pdf for a template biosecurity plan for oversight by institutional biosafety committees). These measures could be further developed and operationalized for independent researchers. Stringency of biosecurity protocols must match the risk of laboratory escape into regions outside the established pathogen range, similar to the contextualized conservation responses proposed by Langwig et al. (2015), and include risks posed by factors such as geography, host population susceptibility, and laboratory proximity to suitable habitat. Policies for research within the established pathogen range may not require the same rigor as for pre-arrival or an invasion front. For example, the use of locally-occurring genotypes of the related chytrid fungus *B. dendrobatidis* (*Bd*) in outdoor experimental mesocosms has been deemed an acceptable risk in areas where *Bd* chytridiomycosis is endemic and new outbreaks caused by inadvertent release would be unlikely (Parris & Beaudoin 2004, Hamilton et al. 2012, Hanlon et al. 2015). In this case, biosafety level 2 protocols may not be appropriate. Though perhaps the safest option, restricting research to regions where a pathogen is established may not be effective for developing quick and robust understanding across topics of pathogen biology, epidemiology, and disease management strategies.

While *Bsal* is typically managed under laboratory conditions consistent with a biosafety level 2 pathogen (Meechan & Potts 2020), this level of biosecurity does not address all concerns associated with *Bsal* or other wildlife pathogens in laboratory settings. Additional recommendations outlined here are given as examples of context-dependent actions researchers and managers can take to contain risk and secure research associated with potentially invasive pathogens. In particular, we emphasize planning ahead for laboratory emergencies and accidents, foot protection, quarterly autoclave spore tests, comprehensive training for all laboratory users, and annual risk assessments (Table 1). An example of biosecurity practices for use in compliance with institutional biosafety committee regulations is provided as Supplemental Material and additional laboratory protocols can be found here: www.chytrids.org/resources. Self-regulation among research communities is paramount, as policies and institutional oversight are slow to develop and are often reactive rather than proactive in dealing with biosecurity concerns.

3. GENERAL BIOSECURITY CONCERNS

The development of biosecurity protocols for emerging wildlife pathogens has been to date largely addressed by pathogen-specific focus groups. These groups have the unique ability to develop laboratory-specific biosecurity policies and procedures that can inform institutional oversight committees. Similar to institutional animal care and use committees (IACUCs) or institutional biosafety committees (IBCs), focus groups are often composed of scientists engaged in the work under consideration for biosecurity policy and procedural standard development. In amphibian research, recommendations from veterinary and taxon-specific societies and research collaboratives such as the *Bsal* Task Force (Grant et al.

Table 1. Biosecurity recommendations for *Batrachochytrium salamandrivorans* (*Bsal*) laboratory research supplemental to biosafety level 2 (BSL2) conditions (Meechan & Potts 2020)

Safeguards	Description
Transportation of *Bsal* to a new laboratory, and secure storage	Networking of *Bsal* research laboratories helps for oversight of biosecurity implementation. Movement of *Bsal* from a specific lab to a new lab for research should be recorded and follow standard procedures for pathogen transportation (Gray et al. 2017). Use of commercial package transportation companies with packaging guidance and tracking of biosecure materials is warranted. Storage recommendations are discussed in the Supplement (www.int-res.com/articles/suppl/d147p141_supp.pdf).
Personal Protective Equipment (PPE) and disinfection plan	Biosafety Level 2 PPE needed, including gloves and lab coats (eye protection not necessary), and a plan for spills on clothing that involves disinfection in the lab. Disinfectant chemicals prepared in advance of working with pathogens are described in the Supplement.
Foot protection	Shoe covers or room specific footwear will be worn, or decontaminating foot baths used upon exiting designated areas, particularly when working with aquatic animals exposed to pathogens. Floors will be regularly cleaned with disinfectants.
Separation of lab and field gear	Field equipment and sampling supplies (tubes, swabs, gloves, etc.) will be stored in a secure area outside the lab or any room containing pathogens to eliminate risk of accidental contamination or transport of pathogens to field sites, and to eliminate risk of DNA from the lab contaminating field samples, resulting in false positives.
Biosafety cabinet	Pathogen maintenance and culture work will be done in a Class II Biological Safety Cabinet. Culture work will be contained in an access controlled culture room.
Autoclave spore test	Monthly or quarterly spore tests will ensure autoclave function and appropriate sterilization of biohazardous waste.
Comprehensive training for all laboratory users and access control	All students and other users of the laboratory space will be trained in emergency decontamination and biosafety protocols and sign an acknowledgement upon completion of training for lab records. Access to animal care, pathogen culture, incubation, and storage will be controlled and limited to trained personnel.
Annual risk assessment	Lab users meet annually to discuss current protocols and determine possible improvements in safety training.

2016, North American *Bsa*l Task Force 2021) help guide appropriate safeguards against pathogen escape or inadvertent introduction. Working groups tasked with addressing biosecurity concerns also exist in other areas of emerging disease research, including viral hemorrhagic septicemia management plans in fish (Phelps et al. 2014), and *Ranavirus* surveillance strategies in amphibians, fish, and turtles (Gray et al. 2015, García-Díaz et al. 2017), which may inform strategies for other emerging diseases.

Although not a pathogen of aquatic organisms, biosecurity initiatives for white-nose syndrome (WNS) caused by *Pseudogymnoascus destructans* in bats are also noteworthy examples. The WNS National Management Plan, which focuses on the identification and development of biosecurity options, is a key component of these initiatives (Coleman et al. 2011). While WNS management has been actively instigated by research personnel, there is also substantial governmental intervention occurring through federally funded programs managed by USFWS and the US Geological Survey (USGS). Similarly, the US National Science Foundation has bolstered *Bsal* research in the USA, and the USGS has been involved in organizing planning workshops and in *Bsal* surveillance (Grant et al. 2016, 2017, Waddle et al. 2020). In addition, Black & Bartlett (2020) examine regulation of transboundary movement, particularly in aquatic environments, of potentially invasive alien species. These efforts provide guidance for pathogen biosecurity; however, biosecurity protocols largely remain the prerogative of institutions and the research community, and legislation for wildlife diseases can help to define policy.

In Germany, for example, the Committee of Biological Agents (ABAS, advisory body of the Federal Institute for Occupational Safety and Health [BAuA]) did a risk assessment on *Bsal* and classified it as risk group 1 (biological agents unlikely to cause disease in a (human) individual) in the TRBA 460 (Technical Rules for Biological Agents) which translates to biosafety level 1 (BSL1). The same applies for *Bd*. Thus, researchers should follow principles of good microbial practice. In general, the risk assessment considered the risk of infection for humans but not for wildlife. Because of this low biosafety level, the German Central Committee on Biological Safety did not provide a general recommendations statement for *Bsal*.

Federal or state level policy and legislative action can fill a gap in wildlife disease biosecurity to protect amphibians and other wildlife from diseases that impact their populations, which are not covered under existing laws regarding livestock or human diseases. Currently, for non-game species, federal agencies in North America and many US state agencies do not have the purview to manage or regulate wildlife pathogens directly. One potential direction, similar to the European Commission clean trade program, could be a comprehensive wildlife health bill that mirrors the Animal Health Protection Act (US Department of Agriculture), but for wildlife. Such legislation could give needed authority to the US Department of the Interior, which has oversight of invasive species, to (1) inspect and quarantine commercial wildlife trade shipments (to promote clean trade measures, e.g. through eDNA testing for pathogens in water from aquatic animal shipments), (2) manage pathogens and their vectors, and (3) enable declaration of and fund release for wildlife emergencies, as well as other actions to minimize the threat of wildlife pathogens.

To illustrate the various levels of regulatory oversight and guidance, we highlight 3 standard guidance categories of relevance to pathogen biosecurity, how such standards are implemented, and the general organizations involved in Table 2. We also include examples in each category. This overview should be helpful in thinking about policy decisions and the role of scientists in informing such decisions.

3.1. Biosecurity Training

Education and training about emerging pathogens and biosecurity is critical for implementing effective biosecurity protocols. Any education and training in this context should be regarded as multi-level and dependent on the involvement of personnel. The evolution of conceptual understanding in disease ecology will contribute to the continual development of biosecurity procedures and protocols. Task teams and working groups can take the lead on knowledge transfer to relevant agencies and personnel. Many universities have online training resources with general biosecurity instruction and modified training for discipline specific information. Examples of this service include the third-party Collaborative Institutional Training Program (CITI), internally developed programs through university Environmental Health and Safety (EHS) programs, and corporate education programs used in industry and private research (Braunschweiger & Goodman 2007). In addition to any online or lecture based training, hands-on or in-person training should also be part of any education for personnel

Table 2. Regulatory standards and suggestions concerning the biosafety of pathogens, animals, and their transport. Standards are given by type and location, where applicable

Biosecurity of pathogens in the laboratory	Biosecurity of animals used in infection experiments in a laboratory or experimental setting	Transport of pathogens and infected animals between laboratories
Modes of implementation:		
Funding mandates (e.g. NIH-funding requirements), material transfer agreements (MTAs; i.e. how a provided pathogenmay be used), institutional biosafety committees (IBCs), governmental agencies/departments	Funding mandates, institutional animal care and use committees (IACUCs), government agencies/departments, intergovernmental standards (European Union animal research license)	International treaties, bilateral agreements, UN, WTO legislation, MTAs, research groups, universities, government agencies/departments
Examples:		
https://osp.od.nih.gov/biotechnology/institutional-biosafety-committees/ (USA) https://www.agriculture.gov.au/sites/default/files/documents/pathogens-of-aquatic-animal-biosecurity-concern.pdf (Australia, Department of Agriculture, Water and the Environment) https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32016R0429 (Regulation (EU) 2016/429, European Parliament and the Council of the European Union) https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRBA/pdf/TRBA-460.pdf?__blob=publicationFile (Germany, Committee of Biological Agents, Federal Institute for Occupational Safety and Health)	https://www.centralecommissiedierproeven.nl/ (Netherlands) https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf (Appendix D; USA) https://olaw.nih.gov/resources/tutorial/iacuc.htm, https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/export/iregs-for-animal-product-exports/SA_International_Regulations/SA_By_Country/SA_M/CT_Product_mexico (specifically, Mexico Federal Animal Health Law, Article 89) https://ec.europa.eu/food/animals/animal-health/animal-health-law_en (EU Animal Health Law, April 2021)	https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2020-P.pdf (Appendix C; USA), https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-and-animal-product-import-information (USA) https://www.wto.org/english/thewto_e/coher_e/wto_oie_e.htm (World Trade Organization) https://www.nimh.nih.gov/research/research-conducted-at-nimh/collaborations-and-partnerships/material-transfer-agreements/material-transfer-agreements (National Institutes of Health, USA) https://eur-lex.europa.eu/legal-content/DE/TXT/?uri=CELEX%3A32021D0361 (European Commission implementing decision 2021/361)

working wherever emerging pathogen biosecurity is a concern. As per US Department of Agriculture APHIS guidelines, all personnel given access to laboratory spaces who may come into contact with restricted pathogens should be trained on appropriate security, PPE, and emergency cleanup procedures in the event of an accident or spill, regardless of their individual involvement in research. Although *Bsal* and other strictly wildlife pathogens are not regulated by APHIS, here we suggest implementing APHIS biosecurity strategies, and additional strategies, for *Bsal* and emerging amphibian pathogens (Table 1, Supplement).

Institutional oversight in the form of IBCs, for example, must uphold both regulated standards and responsible unregulated conditions in the mission for health and safety in research affecting wildlife and the environment. For those involved in the design, implementation, and oversight of personnel training programs, continual education in those research areas under their purview must be considered critical. At the university level, this includes administrative personnel such as IACUC veterinarians and board members, and institutional compliance officers. A disconnect often exists between researchers and oversight committees with respect to best practices for institutional policy development. In the case of emerging wildlife pathogens, task forces and society publications may be especially useful.

4. CONCLUSIONS

While research on wildlife pathogens can be critical for the conservation of biodiversity, it carries with it substantial risks. The case study of *Bsal* highlights concerns and associated risk amelioration strategies for research involving wildlife pathogens. Research or industry specific alterations and additions to standard biosecurity practices can be made where appropriate. These considerations should include the expertise and background of research personnel, as some protocols may be difficult to implement without corresponding expertise. In these instances, increased training and/or oversight should be undertaken. Where such oversight or training is not possible, alternative pathogen use strategies should be employed for proactive risk mitigation. Responsible and proactive development of research biosecurity protocols, including implementation of suggestions presented here, will allow for the continuation and creative independence of current research initiatives. We hope these recommendations will strengthen the important work of the wildlife disease research community.

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