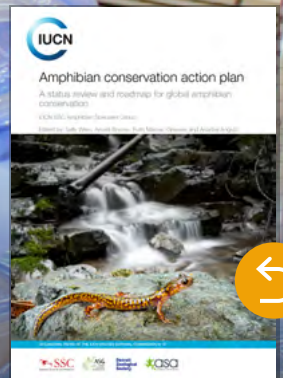


Chapter 6



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Chapter 6

Infectious diseases: Advances and challenges in addressing emerging pathogens

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Abstract

Emerging infectious diseases are major threats to amphibian biodiversity. Significant advances in our understanding of these diseases have been made with respect to the pathogens themselves, how amphibian hosts respond to and defend against pathogens, and the environmental conditions that can influence the course of disease. Here, we review recent advances in our understanding of infectious diseases of amphibians related to these three components – pathogen, host, and environment, and identify information gaps as research priorities. In particular, we highlight current diagnostic tools, we focus on ecological dimensions with relevance to development effective management strategies and review current proposed intervention strategies. We also discuss human dimensions of amphibian diseases with a focus on management and policy actions that can confront these threats and potentially minimise disease-driven declines at local and global scales.

Introduction

Pathogens and parasites including viruses, bacteria, protozoa, fungi, helminths and arthropods infect amphibians (Densmore & Green, 2007). Our knowledge of amphibian diseases and how to diagnose and treat them has improved dramatically in recent years, in part due to efforts of pathologists and veterinarians working with captive zoo collections (Wright & Whitaker, 2001), and work of molecular biologists and ecologists (e.g. Byrne

et al., 2017; Grogan et al. 2018; Rebollar et al., 2016). Infectious diseases are a natural part of any functioning ecosystem, and may fluctuate in natural cycles, leading to constraints between transmission and virulence fuelled by natural selection (Boots & Sasaki, 2003). Pathogens do not generally make their hosts go extinct, because that would also result in extinction of the pathogen, but exceptions may occur (de Castro & Bolker, 2005).

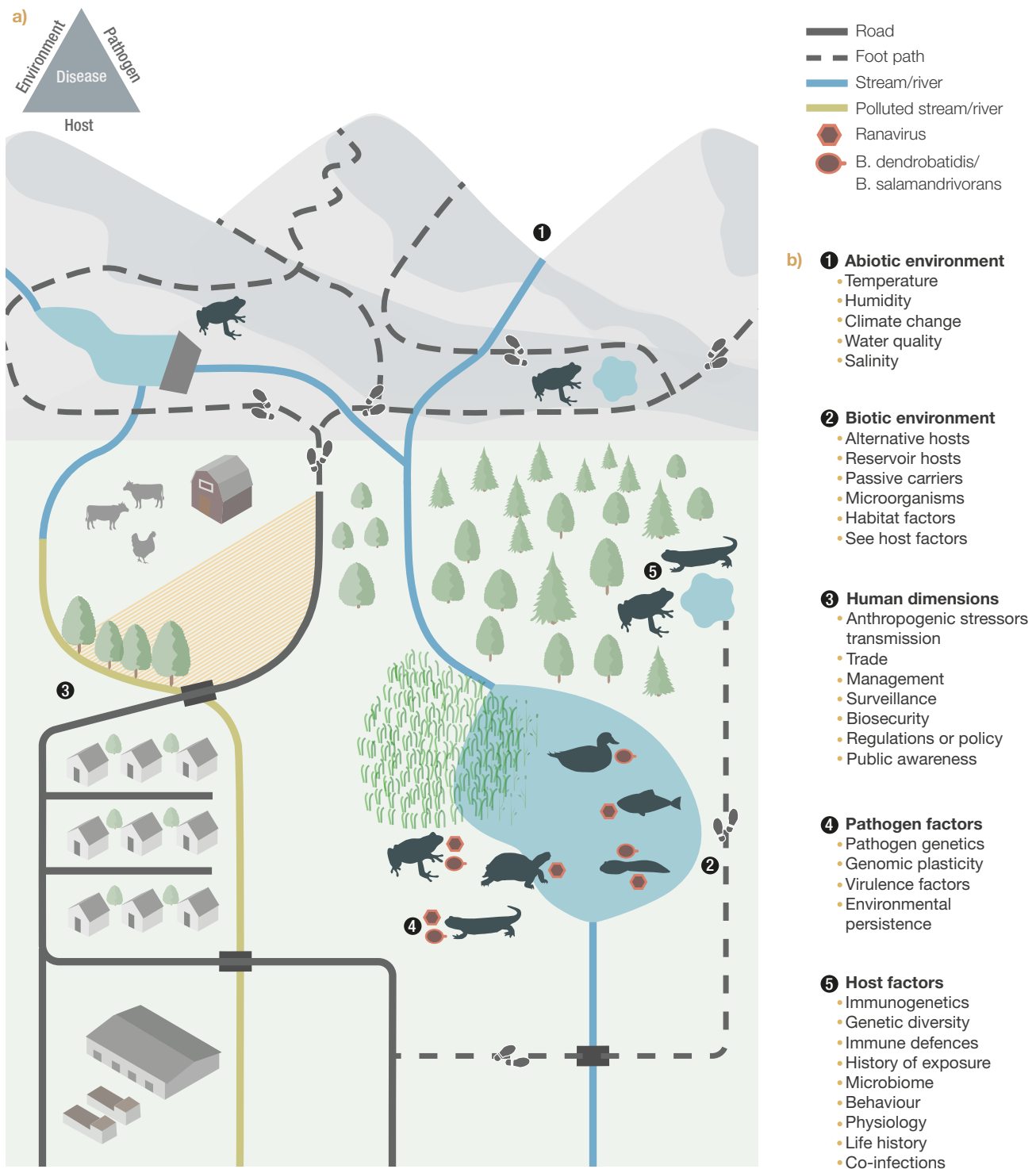


Figure 6.1: Schematic representation of specific elements of the disease triangle **a)**. Disease may develop where conducive factors of the environment (abiotic, biotic, human dimensions), pathogen, and host overlap **b)**. Source: Inspired by Fisher & Garner (2020).

Emerging wildlife diseases are usually caused by introduced pathogens or parasites that spread to areas inhabited by naive hosts that do not have natural defences and thus leading to population declines (Langwig et al., 2015). Chytridiomycosis-related declines have led to the disappearance of

many amphibians worldwide, primarily in places that have no evolutionary history with the disease, although the exact number of species affected remains controversial (Lambert et al., 2020; Scheele et al., 2019a). Indeed, proving disease-induced declines is a challenging task; simply surveying for a pathogen

or disease in a declining population is not sufficient to infer causality (Pessier, 2017). The gold standard for demonstrating disease-related declines involves collecting population data prior to emergence, estimating disease prevalence, observing disease signs and population effects or mortality, isolating the pathogen, and fulfilling Koch's postulates (Martel et al., 2013). These steps require substantial resources not normally devoted to wildlife taxa and may partly explain why it took so long for diseases to be connected to 'enigmatic amphibian declines' (Collins, 2010; Collins & Crump, 2009).

Many factors influence the course of disease related to the pathogen, the host and the environment (Figure 6.1). We consider recent advances in our understanding of infectious amphibian diseases related to these three components as well as human dimensions (Figure 6.1). We identify information gaps as research priorities for the revised *Amphibian Conservation Action Plan*.

Status update

Pathogen

A pathogen is a microscopic infectious viral, bacterial or fungal agent that causes disease in a host, and various macroscopic parasites such as helminths,

protozoa and arthropods also cause well-known diseases and illnesses in amphibians (Densmore & Green, 2007). General veterinary approaches have been developed for diagnosing and treating various amphibian diseases (Densmore & Green, 2007; Wright & Whitaker, 2001), but much attention has focused on the ecology of diseases and context-dependent responses to emerging diseases (Langwig et al., 2015).

• Emerging amphibian diseases

In the last 15 years, understanding of emerging amphibian pathogens has grown immensely (Table 6.1). Ranavirus emergence in naive amphibian populations has been associated with steep amphibian population declines of multiple species in Europe (Price et al., 2014; Teacher, Cunningham & Garner, 2010), and South America (Ruggeri et al., 2019). Whereas ranaviruses have been documented globally, their population-level impacts in many places have not yet been adequately assessed (Brunner et al., 2021; Duffus & Cunningham, 2010; Duffus et al., 2015). Three ranavirus species are known to affect amphibians, *Ambystoma tigrinum virus* (ATV), *Common midwife toad virus* (CMTV) and *Frog virus 3* (FV3) (Chinchar et al., 2017), but FV3 and CMTV are known to recombine as chimeric ranaviruses that have

Table 6.1: Known emerging amphibian infectious pathogens, and their characteristics

| Emerging Infectious Pathogens | Type | Competent hosts | Known Distribution |
|--|----------|---|--|
| <i>Ranavirus</i> | Virus | Amphibians, Reptiles, Fish | Global |
| <i>Batrachochytrium dendrobatidis</i> | Fungus | Amphibians, mortality associated with disease concentrated in the Americas and Oceania | Global |
| <i>Batrachochytrium salamandrivorans</i> | Fungus | Primarily salamanders, with alternate amphibian hosts | Asia (Native range) Europe (Invasive) |
| <i>Perkinsea</i> | Protist | Amphibians, primarily tadpoles | US, Europe, Mesoamerica |
| <i>Elizabethkingia miricola</i> | Bacteria | Anurans, additional concern as it is a zoonotic pathogen (mostly in captive and frog farm settings) | China, Europe, Madagascar, possibly global |

increased virulence and pose a large threat to wild populations (Peace et al., 2019; Vilaca et al., 2019). *Batrachochytrium dendrobatidis* (Bd) was described in 1999 (Longcore, Pessier & Nichols, 1999), but in 2013 a new *Batrachochytrium* species was reported, *B. salamandrivorans* (Bsal) (Martel et al., 2013). Bd has a global distribution (James et al., 2015; Olson et al., 2013; Olson et al., 2021a), and Bsal has a restricted distribution in Asia, where it originates; it is also found in Europe where it is invasive and lethally spreading in European amphibians, particularly fire salamanders (Beukema et al., 2018; Lötters & Vences, 2020; Spitzen-van der Sluijs et al., 2016). In the US, a pathogenic protist causes severe Perkinsea infections resulting in mortality of tadpoles, a potential third emerging infectious disease of amphibians (Isidoro-Ayza et al., 2017). *Elizabethkingia miricola* is an example of a recently discovered emerging bacterial disease in amphibians. This zoonotic pathogen can also affect humans and causes meningitis-like symptoms and mass die-offs in Chinese spiny frogs (*Quasipaa spinosa*) farmed for food (Hu et al., 2017; Lei et al., 2019).

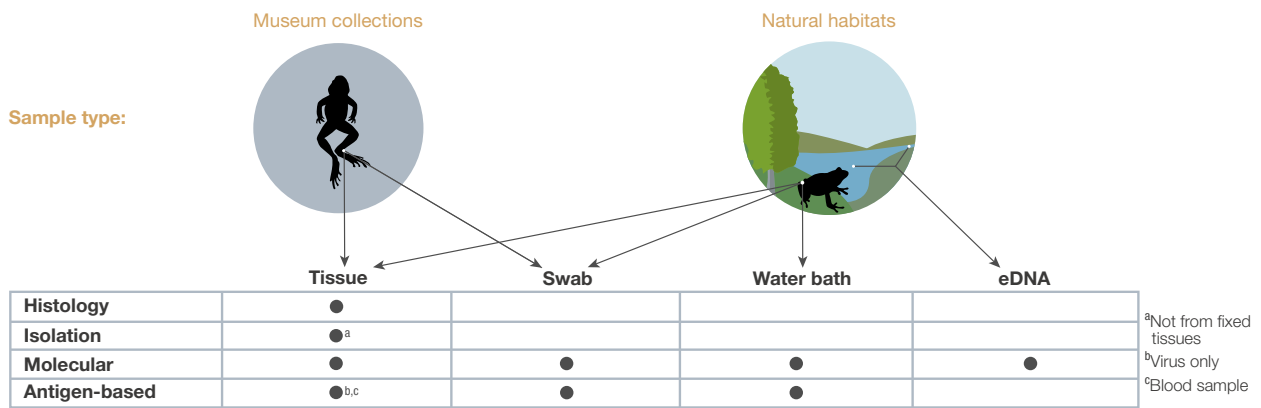
- **Diagnostics and monitoring**

Amphibian pathologists have established a growing body of diagnostic knowledge that has improved our ability to evaluate disease signs and attribute them to causative agents that may have historically been dismissed as “Red Leg Disease” (Forzan et al., 2017; Pessier, 2017). The fact that severe Perkinsea infections were only recently discovered as a source of amphibian mortality in the US is a lesson to retain disciplined vigilance when examining new amphibian mortality events and declines. Multiple tools are available for detecting pathogens, confirming infection and diagnosing disease. Histology and microscopy remain the primary tool of pathologists forensically examining contemporary specimens, especially when preservation techniques limit use and consistency of DNA-based diagnostic tools, but advances in isolation techniques, molecular methods and DNA sequencing have expanded our understanding

of amphibian pathogens, including lineage/strain distribution, genetic variation and virulence factors (Figure 6.2). However, documenting a pathogen is not necessarily indicative that it causes disease and decline (Russell et al., 2019). To better understand if a host is susceptible, tolerant or resistant, infection must be linked to longer-term clinical disease outcomes, such as death, persisting with infections, or clearing infections (Figure 6.3).

- **Disease origins and virulence**

The genomics revolution has advanced our understanding of the origins of amphibian pathogens, the multitude of pathogen genotypes, and virulence factors that make these pathogens deadly. Evidence suggests both Bd and Bsal originate in Asia - work that has been facilitated by improved isolation methods and genome sequencing (O’Hanlon et al., 2018). Our understanding of Bd has moved beyond seeing it as a singular pathogen to an understanding of a complex matrix of genotypes, some of which are endemic and others pandemic lineages that vary in virulence (Belasen et al., 2022a; Byrne et al., 2019; Jenkinson et al., 2016; Rosenblum et al., 2013). Bd genotypes have been cultured from hotspots, and whole genome sequences of globally distributed strains are identified: Bd GPL (Global Panzootic Lineage), Bd CAPE (Africa and Europe), Bd ASIA 1 (Asia), Bd Asia 2/ BRAZIL and Bd ASIA-3 (O’Hanlon et al., 2018; Bryne et al., 2019). Most cultured Bd isolates belong to the Bd GPL lineage (Fisher et al., 2018), and that has led to strengthening of our knowledge about GPL impacts on amphibians. In Bd infection ‘cold-spots’ (e.g. Asia and Africa), where prevalence can be high, but infection intensity low (Mutnale et al., 2018; Sreedharan et al., 2023), the probability of obtaining pathogen cultures is poor, limiting our capacity to adequately understand the emergence and epidemiology of chytridiomycosis globally. Enzootic genotypes may be dominant in such regions and hybridisation of enzootic hypovirulent and panzootic hypervirulent Bd strains can result in genotypes that show high virulence on



● Techniques ● Advantages ● Limitations * Test are not validated

Histological examination

- Hematoxylin/Eosin Stain (all pathogens)¹
- Immunohistochemistry (all pathogens)²
- In-situ hybridization assay (Bd/Bsal)³
- Confirms true infection and disease
- Can concurrently see histopathology
- Detect co-infection
- Low-moderate sensitivity⁴
- Specialized personnel required
- Can be expensive
- Invasive or lethal sample
- Time-consuming

Isolation

- Tail/toe clip (Bd/Bsal)⁵
- Internal organ, tail/toe clip*(Rv)⁶
- Diagnoses active infection
- More detailed studies possible from obtained culture (genomics⁷, experiments, etc.)
- Tissue required from living animal (Bd/Bsal)
- Time-consuming
- High failure rate
- Contamination prone

Molecular examination

- Traditional PCR⁸
- Nested PCR⁹
- Quantitative PCR¹⁰
- Digital droplet PCR¹¹
- Fluidigm assay¹²
- Metagenomic sequencing
- High sensitivity – detects low level infections⁵
- Quantitative (selected techniques)
- High specificity (Bd/Bsal/Rv specific assays)
- Genotype specific detection possible¹³
- Relatively quick and inexpensive
- Can be done from non-invasive samples
- Widely accessible (tPCR, nested qPCR)
- Only confirms presence of DNA, not infection or disease
- Risk of DNA contamination or PCR inhibition possible
- Can be expensive (Taqman, fluidigm)
- Fluidigm assay required high quality DNA and does work with low infection levels
- eDNA detection cannot confirm which species in community are infected

Antigen-based

- Antigen-capture ELISA (Rv only¹⁴)
- Lateral flow assay (*Batrachochytrium*)¹⁵
- Quick and cheaper than molecular methods
- High sensitivity and specificity
- Very quick
- Only works from blood samples
- Poor sensitivity and specificity
- Expensive

Figure 6.2: Diagnostic tools for amphibian pathogens and disease. Diagnostic screening techniques can be applied to museum specimens, field-caught individuals as well as environmental substrates (e.g. water). Histological, isolation, molecular and antigen-based tools are available, each with their own set of advantages and disadvantages. Histological examination is still the only method capable of diagnosing clinical infection and disease, but has from low to moderate sensitivity and is costly. Isolation of pathogens can be difficult, but is essential for developing a deeper understanding of pathogens, their ecology, physiology and behaviour. Molecular tools offer non-invasive sampling methods and high sensitivity for detecting genetic material of pathogens, but quantitative PCR (qPCR) based methods don't come without important caveats. There can be wide variation in quantification according to laboratory methods making direct comparisons across studies difficult. This variation may be attributed to using different standard cultures/strains of Bd, different qPCR cycling parameters and molecular techniques, and different DNA extraction techniques (Bletz, Rebollar & Harris, 2015; Brannelly et al., 2020). There has been an attempt to standardise across studies using Bd intergenic transcribed spacer (ITS) copy number standards (Longo et al., 2013; Rebollar et al., 2017); however, with variation in ITS within the fungal genomes the biological meaning can be skewed (e.g. 1000 ITS copies could be equivalent to 2 zoospores if copy number is 500, or 200 zoospores if the copy number is 5). It is additionally important to understand the detection limits of molecular techniques like qPCR. Low and inconsistent qPCR positives may be false positives, and mutations in the ITS region at the Taqman probe binding site in certain regions, such as Asia, can lead to false negatives (Mutnale et al., 2018). This caveat is also true for qPCR methods used to detect ranaviral DNA (Wynne et al., 2020). Development of the lateral-flow assay by Dillon et al. (2017) shows some promise; however, this assay lacks specificity (it cross-detects related fungi) and sensitivity (it fails to detect low loads). Source: ¹Berger et al. 1999, Forzan et al. 2017; ²Olsen et al. 2004, Jerret et al. 2015; ³Ossiboff et al. 2019, Forzan et al. 2019; ⁴Kruger et al. 2006; ⁵Cook et al. 2018; ⁶Granoff et al. 1965, Balseiro et al. 2009; ⁷Rosenblum et al. 2008, O'Hanlon et al. 2018; ⁸Annis et al. 2004; ⁹Goka et al. 2009; ¹⁰Boyle et al. 2004, Bloi et al. 2013, Standish et al. 2018; ¹¹Pinheiro et al. 2012; ¹²Byrne et al. 2019; ¹³Ghosh et al. 2020; ¹⁴Kim et al. 2015; ¹⁵Dillon et al. 2017.

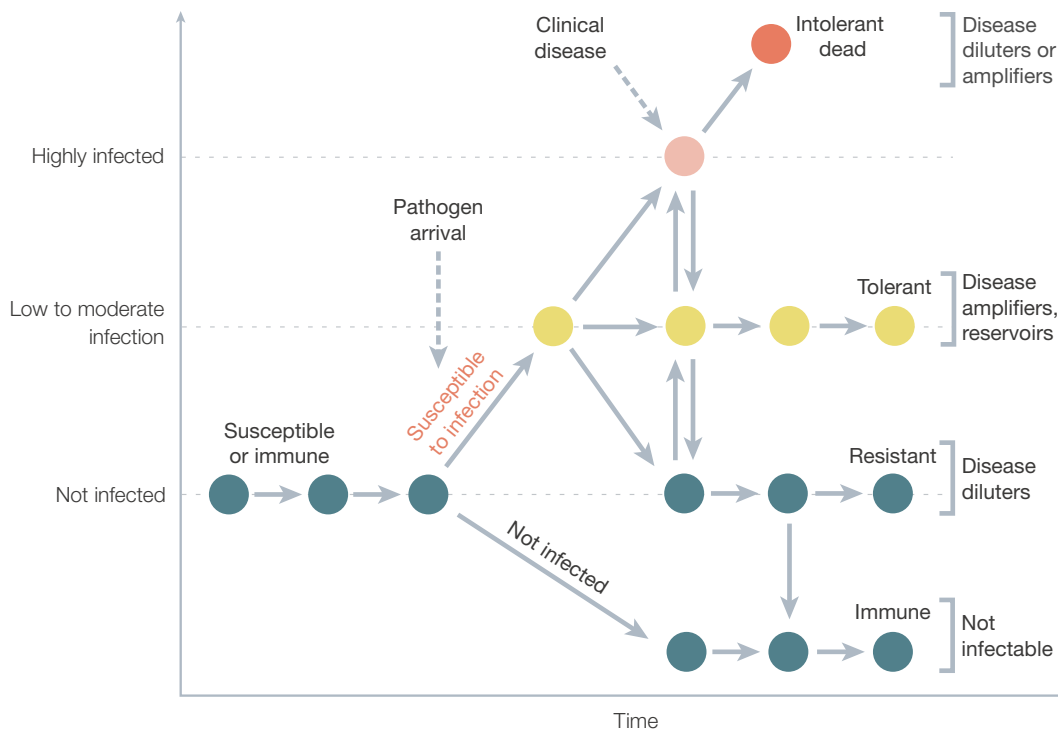


Figure 6.3: Diagrammatic representation of outcomes for amphibian hosts when exposed to a potential pathogen. **Immune** refers to individuals that cannot be infected. **Susceptible** refers to individuals that can become infected. **Resistant** refers to individuals that, once infected, exhibit resistance mechanisms that lower or eliminate the infection. **Tolerant** represents individuals that can survive infection and build up high infection loads with little negative impact. **Intolerant** refers to hosts that exhibit clinical disease and can ultimately succumb to infection (i.e. experience disease-induced mortality). These host states are not necessarily static across host species, populations, or individuals, and can vary with endogenous and exogenous factors.

native hosts (Greenspan et al., 2018). Similarly, recombination of ranaviruses can result in changes in virulence (Peace et al., 2019; Vilaca et al., 2019), while bacteria frequently evolve antibiotic resistance, reducing our ability to treat host infections (Lei et al., 2019). Lineage-specific diagnostics as well as genomic tools that don't require culturing can help fill this gap (Ghosh et al., 2021). The ability to genotype Bd from swab DNA has given the field an invaluable technique to understanding global Bd lineage distribution (Byrne et al., 2017).

Cultured isolates, experimental infection trials and -omics techniques have also expanded our understanding of virulence factors and mechanisms that may induce disease. Genomic and transcriptomic comparisons of Bd/Bsal as well as enzootic and panzootic Bd show us signatures of virulence including metalloproteases, serine proteases and

crinkle-like proteins (Ellison et al., 2017; Farrer et al., 2017; McDonald et al., 2020). Further understanding about these pathogens will emerge as culturing efforts and genomic techniques for Bd and Bsal are intensified globally (Fisher et al., 2018).

- **Future steps & recommendations**

Significant gaps in our knowledge of these pathogens remain. Greater understanding of hot and cold spots for pathogen presence and disease can give us a lens into what environmental conditions, host properties, and interactions between these allow amphibians to survive these diseases in nature and in turn guide management for susceptible populations. Currently, there is no rapid, field-ready test for prominent amphibian pathogens. Such diagnostic tools could rapidly improve our understanding of

pathogen distributions and fill rapid-detection needs, while genomic innovations like high-throughput sequences can continue to push the bounds of pathogen ecology and dynamics globally.

Host

Once a pathogen infects a host, the host may survive through resistance mechanisms (e.g. the host mounts an effective immune response that reduces pathogen burden and clears infection), or through tolerance (i.e. negative impacts are minimised while the pathogen continues replicating and spreading (Figure 6.3)). However, if pathogen burdens increase to a level resulting in clinical disease, the burden to the host is magnified and may result in death if infection is not reduced or treated (Figure 6.3). Reviews are available for the patho-physiology of Bd (Baitchman & Pessier, 2013), Bsal (Martel et al., 2013), and ranaviruses (Miller et al., 2015).

- **Host range & susceptibility**

Host range differs between Bd, Bsal, and ranavirus. Bd can infect all three amphibian orders (Olson et al., 2021a); Bsal is known to infect anurans and caudates while disease primarily occurs in salamanders (Martel et al., 2014; Stegen et al., 2017); and ranaviruses infect amphibians, reptiles and fishes (Brenes et al., 2014; Duffus et al., 2015). Experimental infection of hosts can advance our understanding of host-pathogen-environment dynamics (Blaustein et al., 2018).

Host life stage also affects infection and disease progression. For Bd, larvae are typically tolerant, while recent metamorphs and juveniles experience higher mortality (Belasen et al., 2022a, Böll et al., 2012; Garner et al., 2009; Russell et al., 2010). Adults vary widely in susceptibility, tolerance, and resistance (Figure 6.3). Bsal chytridiomycosis has only been documented in post-metamorphic amphibians to date. For ranavirus, larvae tend to be particularly vulnerable to disease and mortality (Duffus, Nichols & Garner, 2014; Hoverman et

al., 2012), but adults of some species also show disease signs (Duffus, Nichols & Garner, 2013).

There is wide variation in disease outcomes across host populations, space, and time (Bradley et al., 2015; Briggs, Knapp & Vredenburg, 2010; Savage et al., 2011; Searle et al., 2011). Host susceptibility can shift over time as with Bd in South America (Becker et al., 2016; Carvalho, Becker & Toledo, 2017; von May et al., 2018) and Panama (Voyles et al., 2018). Although Bd has been implicated in a number of species extinctions, several populations that were formerly believed extirpated have been “rediscovered” by scientists (Abarca et al., 2010; Chaves et al., 2014; García-Rodríguez et al., 2012; González-Maya et al., 2018; Jaynes et al., 2022; Newell, Goldingay & Brooks, 2013; Puschendorf et al., 2013; Rodríguez-Contreras et al., 2008; Toledo et al., 2023; Whitfield et al., 2017). These cases present opportunities to understand what contributes to population recovery and mobilise this knowledge for conservation and management.

Differential disease outcomes over space and time may be related to host and ecological factors that mediate host susceptibility to infection and disease. Individual host factors include host defence mechanisms (e.g. innate and acquired immunity), and defence resulting associated microbiomes. Ecological factors include biotic factors (e.g. dilution effects, reservoir species, super-shedders), and abiotic conditions that impact host ecology and physiology. Understanding how these factors mediate host susceptibility is important for disease management and conservation.

- **Host defence mechanisms**

Innate and acquired immunity play a role in amphibian pathogen infections, varying across host species and environments. Innate immune mechanisms comprise the first line of defence against infections and show general efficacy for a variety of pathogens (Conlon, 2011; Rollins-Smith, 2009; Smith et al., 2018). Acquired or

adaptive immune mechanisms, such as the major histocompatibility complex (MHC) pathway and T and B cells, provide a more specific pathogen response and are linked to both host genotype and exposure history. However, Bd can sometimes inhibit immune responses, limiting amphibians' ability to mount a robust adaptive response to Bd (Fites et al., 2014). MHC Class I molecules are hypothesised to mainly be associated with immune responses to ranaviruses (Teacher, Garner & Nichols, 2009; Wang et al., 2017). MHC II immuno-genotype has been associated with susceptibility to Bd (Bataille et al., 2015; Belasen et al., 2022b; Kosch et al., 2016; Savage & Zamudio, 2011), ranavirus (Savage et al., 2019), and other potentially pathogenic microbes (Belasen et al., 2019). While immunity in amphibian larvae is less well-studied, tadpoles are known to have less functionally developed immune systems accompanied by immunosuppression through metamorphosis, while MHC expression expands greatly post-metamorphosis (Grogan et al., 2018).

Advances in molecular technologies, including high-throughput sequencing and transcriptomics, have deepened our understanding of cellular defence mechanisms and immune variation within and among host species (Zamudio, McDonald & Belasen, 2020). Common Bd response mechanisms include skin repair (Ellison et al., 2014a; Eskew et al., 2018; Poorten & Rosenblum, 2016) and innate and acquired immune activation (Ellison et al., 2014b; Ellison et al., 2017; McDonald et al., 2020). Recent studies have found that animals that down-regulated immune genes tolerated Bd infections better, and highly susceptible individuals significantly upregulated immune responses (Savage et al., 2020). These results suggest that immunopathology is a component of Bd susceptibility. Amphibian immune response reviews are available (chytridiomycosis: Grogan et al., 2018, 2020; ranaviruses: Grayfer et al., 2015).

Improvements in our understanding of amphibian immunity have applications for disease management and mitigation, for example, selective breeding for genetically resistant or

tolerant individuals, or development of vaccines that prime immune responses (Table 6.2). Vaccines against Bd have shown mixed success (e.g. Stice & Briggs, 2010), perhaps because Bd-produced toxins inhibit amphibian adaptive immune response (Fites et al., 2013). Ranavirus vaccine trials, however, have shown promising results (Chen et al., 2018; Zhou et al., 2017).

- **Host-associated microbiomes**

Host defences also include resident symbiotic bacteria, fungi and other micro-eukaryotes living on/in hosts, collectively called the host-associated microbiome. Mounting evidence suggests these communities play a role in disease dynamics (Jiménez & Sommer, 2016). High-throughput sequencing has enabled characterisations of microbial communities of diverse amphibians, enhancing our ability to understand the protective role these communities play (Kueneman et al., 2019). Thousands of bacteria have been cultured from amphibian skin and tested for inhibition against Bd and Bsal pathogens in vitro (Bletz et al., 2017; Woodhams et al., 2015). Bd and Bsal may induce shifts in the microbiome (Bates et al., 2019; Bletz et al., 2018; Jani & Briggs, 2014), and microbiome composition may predict disease susceptibility (Becker et al., 2015). Populations with higher proportions of frogs with Bd-inhibiting skin bacteria may persist through Bd emergence (Lam et al., 2010; Woodhams et al., 2007). Recent studies have explored the “mycobiome” (Kearns et al., 2017) as well as the full microeukaryotic community (Kueneman et al., 2016b), and how these communities interact with bacteria (Belasen et al., 2021). Skin and gut bacterial microbiomes have also been associated with ranavirus susceptibility in laboratory and field studies (Harrison et al., 2019; Warne, Kirschman & Zeglin, 2019). Modulating host immunity through probiotic bioaugmentation of hosts or their environments has been proposed as a disease mitigation strategy to capitalise on the role of these microbial communities (Table 6.2, reviewed in Bletz (2013) and Rebolgar et al. (2016)).

Table 6.2: Overview of amphibian disease mitigation interventions targeting amphibian hosts

| Intervention | Evidence | In situ examples |
|--|--|--|
| Treatment of the host directly with antifungals, antibiotics, or deworming agents | Treating hosts directly for the pathogen are widely used in veterinary medicine (Baitchman & Pessier, 2013; Wright & Whitaker, 2001), but they are mostly applicable in controlled settings and do not prevent reinfection. | Cascades frogs treated with itraconazole and released back into natural ponds showed reduced Bd pathogen burden and increased over-winter survival (Hardy et al., 2015). Treatment of mountain chickens for Bd using itraconazole without environmental pathogen reduction had only short-term benefits (Hudson et al., 2016a). |
| Treatment of host and translocation to disease-free refuge, or disinfection of environment prior to reintroduction | Disinfectants can be applied directly to the environment with varying environmental impacts (Lammens, Martel & Pasmans, 2021; von Rütte et al., 2009). | Successful at controlling Bd on the island of Mallorca, with limited Mallorcan midwife toads and limited habitat (Garner et al., 2016). An attempt to create a Bd-free population of Archey's frogs through translocation in New Zealand was unsuccessful (Linhoff et al., 2021). |
| Translocation of individuals with resistant genotypes | Recovering amphibian populations that have evolved resistance or tolerance to disease could serve as founders for low-cost reintroductions to historical sites (Mendelson, Whitfield & Sredl, 2019). Genetic markers may be difficult to identify as resistance traits may be associated with reduced gene expression (Savage et al., 2020). | Knapp et al. (2023) showed that translocations of mountain yellow legged frogs collected from recovering populations can allow population re-establishment in the face of ongoing Bd infection. |
| Selective breeding for resilience traits | The effectiveness of skin mucus secretions of frogs that survived a Bd epizootic became more inhibitory, providing evidence of natural selection that has the potential to be applied to captive populations (Scheele et al., 2014; Voyles et al., 2018). | Not attempted yet, due to high technical requirements, multigenerational timelines, and a need to better understand resistance phenotypes, mechanisms or genetic markers. If clear resistance-associated genes are identified, genetic engineering for resistance may be a further possibility as has been explored in American chestnuts (Newhouse et al., 2014). |
| Density reduction of hosts to reduce disease transmission | Contact rates were reduced in low density groups of newts, suggesting reduced density may reduce Bsal transmission and spread (Malagon et al., 2020). However, a field experiment found that Bd was effectively transmitted between tadpoles regardless of density (Rachowicz & Briggs, 2007). | Translocation of limited numbers of mountain yellow-legged frog tadpoles to create new low-density populations was unsuccessful at preventing outbreaks (Woodhams et al., 2011). It seems unlikely that deliberately reducing healthy threatened amphibian populations to reduce disease risk would be justified by experimental evidence. |

| Intervention | Evidence | In situ examples |
|--|---|--|
| Increase population buffering capacity through head starting, captive-releases | Demographically, increasing recruitment rates compensates for disease-related mortality (Lampo, Senaris & Garcia, 2017; Muths, Scherer & Pilliod, 2011; Scheele et al., 2014). | Populations of wild corroborree frogs declining due to Bd have been supplemented from captive populations and raised in predator-free enclosures to help sustain wild populations (Campbell et al., 1999; Linhoff et al., 2021). Head starting has helped to avoid extinctions and grow populations of agile frogs in the UK and northern leopard frogs in Canada (Linhoff et al., 2021), but has been unsuccessful at re-establishing breeding populations of Wyoming toads (Polasik et al., 2016). It is likely that success or failure of these efforts will be highly context-specific, and more studies are needed. |
| Augmenting protective skin microbes using probiotics | Probiotics aim to boost host immunity in the mucosal environment through the addition of locally occurring, Bd-protective skin microbes to amphibians (Bletz et al., 2013). Experimental trials have given mixed results, some have been successful or partly successful (Bletz et al., 2018; Harris et al., 2009; Kueneman et al., 2016a; Muletz et al., 2012), and others have been ineffective (Becker et al., 2011; Becker et al., 2015; Woodhams et al., 2012). One study that genetically modified a core skin microbe to produce antifungal metabolites did not confer disease protection (Becker et al., 2021). | One in situ field trial that augmented mountain yellow-legged frogs with <i>Janthinobacterium lividum</i> was associated with reduced Bd pathogen loads and improved survival after one season, but the population did not persist in the long term (Vredenburg, Briggs & Harris, 2011). Our understanding of the role of skin microbiomes and immune function is not yet developed enough to reliably manipulate microbiomes to impart a desired function, and further research is needed to understand the relationship between host, pathogen and microbiome. |
| Vaccines | Effective ranavirus vaccines have been developed and used in Chinese giant salamanders (Chen et al., 2018; Zhou et al., 2017). Vaccinations for Bd, however, have been ineffective, or only weakly improve the ability to combat infection (Cashins et al., 2013; McMahon et al., 2014; Stice & Briggs, 2010), but recent attempts in Vegas Valley leopard frogs have demonstrated improved effectiveness of previous exposure conferring improved survival (Waddle et al., 2021). | Ranavirus vaccines have not been used in the field but have high potential, especially with long-lived species like giant salamanders. Whether effective Bd and Bsal vaccines can be developed remains unknown. Improved survivorship of adult mountain yellow legged frogs treated with itraconazole to clear Bd infections has been attributed to a possible acquired immune response from the initial infection (Knapp et al., 2022). California red-legged frogs that were experimentally exposed and cleared from Bd prior to release, did not have reduced Bd loads or prevalence compared to controls (Adams et al., 2022). |

- **Impacts of pathogen co-occurrence and co-infections**

In the wild, multi-pathogen-parasite landscapes occur, including co-infections of ranavirus, Bd, and Bsal (Lötters et al., 2018; Warne et al., 2016; Whitfield et al., 2013). Where pathogens co-occur they can affect different subsets of the amphibian community. For example, ranavirus may have greater impacts at lower elevations while Bd has impacts at higher elevations (Rosa et al., 2017). Mortality and sublethal effects can be exacerbated by co-infections in some cases (Longo, Fleischer & Lips, 2019; McDonald et al., 2020); however, one recent study has suggested initial infection with low virulence Bd genotypes can shift Bsal infection dynamics (Greener et al., 2020).

- **Community-level factors**

The biotic community can play a major role in determining disease outcomes, with important implications for disease management. As hosts vary in their susceptibility to the same pathogens, host community composition can determine whether a disease is enzootic or epizootic. For instance, with many immune or resistant hosts, the community may experience a *dilution effect*, whereby disease is kept to low, enzootic levels. Alternatively, if many reservoir (i.e. highly tolerant) hosts or super-shedders are present, higher pathogen burdens may build up, resulting in negative impacts on susceptible hosts. Introduced African clawed frogs and American bullfrogs, and US-native Pacific chorus frogs are considered reservoir hosts for Bd (Reeder, Pessier & Vredenburg, 2012), whereas various anuran and urodelan hosts, such as midwife toads and alpine newts, can be reservoir hosts for Bsal (Stegen et al., 2017). Non-amphibian hosts, such as crayfish or waterfowl, have been suggested for Bd and Bsal; however, evidence remains mixed (Betancourt-Roman, O'Neil & James, 2016; McMahon et al., 2013; Van Rooij et al., 2015). For ranavirus, non-amphibian hosts are well-documented, including fish and turtles (Duffus et al., 2015).

- **Future steps & recommendations**

Over the last 15 years of research on amphibian disease hosts, there has been an increased understanding of the need to move beyond correlating pathogen presence with decline; rather, it is necessary to associate pathogen presence with disease, and in turn disease with decline. Given high levels of intraspecific and interspecific variability in disease outcomes, broad predictive markers for susceptibility are needed. These may include genetic markers, mucosome components that combat skin pathogens, proportion of the microbiome that is inhibitory against pathogens, or other measurable factors. Development of predictive assays will require additional comparative research and validation studies. Further understanding of factors associated with populations experiencing recovery as well as “cold spots” where disease outbreaks do not occur can advance development of targeted management methods.

Further, basic biological studies are lacking to provide context to correlational and experimental patterns. Studies of cellular responses to infection would enhance understanding of immune markers or responses most relevant to surviving pathogen infection. An improved understanding of the roles of non-bacterial microbes in amphibian microbiomes may clarify impacts of microbiome variation over species, space, and time, and of employing probiotic treatments in nature. Given that co-infections can exacerbate disease outcomes, understanding interactions of the widespread, well-studied pathogens featured in this review with more poorly studied pathogens and parasites will likely be important in effectively managing amphibian health broadly.

Environment

Environmental factors affect disease transmission and host-disease dynamics. Significant advances in understanding host-pathogen interactions with both abiotic and biotic environmental factors have been made in recent years.

- **Abiotic factors**

Abiotic factors such as temperature, water, and altitude help explain spatiotemporal variability in amphibian pathogen occurrence (Brunner et al., 2015; Olson et al., 2013; see also Table 6.3). For example, reported localities of fatal chytridiomycosis are scarce, concentrated mainly in tropical regions of the Americas and Australia (Scheele et al., 2019a), and most ranaviral disease die-offs have been in temperate regions during warmer seasons (Price et al., 2019). Predicting disease impacts on amphibian populations, however, is challenging due to several interacting contexts (Blaustein et al., 2018). Furthermore, amphibian pathogens are a moving target, as amphibian trade (food, pets) spreads pathogens with panzootic potential (O'Hanlon et al., 2018) and climatic shifts may trigger new epizootic outbreaks (See Chapters 3 and 7).

Temperature also can affect pathogen life history traits. Optimal in vitro temperature range for *Bd* is 17–25°C (Piotrowski, Annis & Longcore, 2004), for *Bsal* is 10–15°C (Martel et al., 2013), and for ranavirus is 20–28°C (Ariel et al., 2009). Experimental exposures of *Bd* strains to various thermal regimes in vitro showed that warmer temperatures may increase zoospore production within the host, but decrease zoospore viability in aquatic environments (Woodhams et al., 2008; Woodhams et al., 2012). Hence, *Bd* could have higher impact on populations under thermal conditions that are suboptimal for pathogen replication, if propagules remain viable outside their host for longer periods (Voyles, Rosenblum & Berger, 2011; Voyles et al., 2012). Models have shown free *Bd* zoospore persistence in the environment is a major determinant of the fate of host populations (Dodding et al., 2013; Louca, Lampo & Doebeli, 2014; Mitchell et al., 2008). For ranavirus, a greater pathogenicity at warmer temperatures appears to be related to a faster viral replication (Brand et al., 2016).

Temperature effects on host immune systems are less clear. During host hibernation, the immune response involved in *Bd* clearance is impaired

(Rollins-Smith, 2020), and hosts may be less effective at resisting disease after cold pulses (Greenspan et al., 2017a; Greenspan et al., 2017b). Higher rates of *Bd* clearance in warmer environments have been attributed to increased amphibian skin sloughing, a mechanism that lowers infection burdens (Grogan et al., 2018), but repeated exposure to extreme heat also causes a corticosterone response characteristic of chronic stress that could suppress amphibian physiological endocrine sensitivity to pathogenic diseases (Narayan & Hero, 2014). Temperature variability itself affects amphibian immune responses; further investigation is needed (Raffel et al., 2006).

Chytridiomycosis tends to have greater impact and higher infection prevalence on highland populations in cooler habitats (Catenazzi, Lehr & Vredenburg, 2014; Scheele et al., 2019a; Woodhams & Alford, 2005). Warmer habitats have been proposed as thermal refuges where frogs are more likely to coexist with the fungus because *Bd* tends to grow sub-optimally (Puschendorf et al., 2009; Zumbado-Ulate et al., 2014). Post-epizootic population recoveries have been more frequent in lowland than upland locations, supporting this hypothesis (Grogan et al., 2016; Lampo, Senaris & Garcia, 2017; Phillott et al., 2013). Hosts are not always constrained passively to ambient conditions; if hosts can raise their body temperature by spending more time in microhabitats where temperature exceeds the pathogen's optimum, amphibians can alter their infection risk (Richards-Zawacki, 2010; Rowley & Alford, 2013). However, recurring findings of conflicting correlations between prevalence, outbreaks, and climatic conditions (Ron, 2005) led to an examination of the effects of climatic conditions in terms of differential performance of the pathogen and its host relative to their thermal optima, an idea referred to as the thermal mismatch hypotheses (Cohen et al., 2017; Nowakowski et al., 2016). Consequently, infection risk in ectotherms may change as the difference between host and pathogen environmental tolerances (i.e. tolerance mismatch) increases. Infection risk is expected to decrease, for example, if hosts can access thermal niche spaces suboptimal for *Bd* (Nowakowski et

al., 2016). Conversely, infection risk could increase if available temperatures shift away from host optimums (Cohen et al., 2019).

Humidity and water availability also play a role in amphibian disease dynamics. *Bd* has severely impacted populations associated with perennial waters (Scheele et al., 2019a), but hydrological regimes also can affect other pathogen-host dynamics. *Batrachochytrium* fungi do not tolerate desiccation and water availability or humidity is fundamental for effective transmission, but *Bd* transmission can increase during driest months when adults congregate near water sources (LaBumbard, Shepack & Catenazzi, 2020; Piovita-Scott et al., 2011; Ruggeri et al., 2015). Also, *Batrachochytrium* fungi may persist 1-7 months in sediment or lake water (Johnson & Speare, 2003; Martel et al., 2013; Stegen et al., 2017) and ranavirus can survive for >30 days in sediments (Munro et al., 2016; Nazir, Spengler & Marschang, 2012). Hence, pathogens can persist after their hosts have been removed from their habitats. Models suggest that one of the most important mechanisms promoting *Bd* establishment and driving host populations to extinctions is its capacity to survive outside its host in water or humid substrates (Doddington et al., 2013; Louca, Lampo & Doebeli, 2014; Mitchell et al., 2008). Spatial distribution and *Bd* zoospore life expectancy in the environment is becoming more apparent at some US amphibian breeding sites (Chestnut et al., 2014), but dynamics in tropical stream environments and the relationship to environmental factors remains a knowledge gap. Recent development of eDNA sampling techniques will hopefully expand zoospore detectability across microhabitats for *Bd* (Hauck et al., 2019; Walker et al., 2007). For ranavirus, however, it should be noted that titres collected from eDNA samples may not correspond with infection levels in the amphibian hosts at the same site (Kaganer et al., 2021).

Extreme climatic events also can impact fecundity, recruitment and survival of uninfected amphibians, undermining the ability of populations to offset disease-induced mortality and possibly tipping infection outcome from coexistence to extinction.

Extended droughts can lead to breeding failure, and reduce post-metamorphic survival and adult recruitment (Cayueta et al., 2016; Richter et al., 2003). Yet, post-epizootic recovery of remnant populations from several regions where *Bd* is highly pathogenic has been linked to a high recruitment of healthy adults (Lampo, Senaris & Garcia, 2017; Muths, Scherer & Pilliod, 2011; Scheele et al., 2015). Similarly, in amphibian populations challenged by ranavirus, recruitment success was better explained by hydroperiod length than viral presence or other contaminants (Smalling et al., 2019). This suggests that population resilience to disease-associated impact is highly dependent on climatic conditions, and climate plays an important role in the probability of post-epizootic recovery.

Identifying conditions in which amphibian populations can coexist with infection opens a promising avenue for long-term conservation of wild populations threatened by chytridiomycosis (Hettyey et al., 2019). Although several interventions are proposed that modify temperature, hydrological regimes or water quality, manipulate host microbial communities, or use micro-predators as biocontrol agents for reducing pathogen survival (Table 6.3), field tests have lagged.

- **Biotic factors**

Amphibian pathogens are part of complex aquatic communities, with natural predators and parasites. Community-level biotic factors can lead to density- and trait-mediated trophic cascades in the broader aquatic ecosystem, inclusive of other microbiota (Harjoe et al., 2022). Some aquatic predators of chytrid zoospores are water fleas (Cladocera), copepods (Copepoda), and seed shrimp (Ostracoda) (Woodhams et al., 2011). Higher abundances of protozoans and microscopic metazoans reduced *Bd* zoospores amounts at amphibian breeding sites in the Pyrenees (Schmeller et al., 2014). Zoospore viability inversely correlated with *Bd* infection prevalence, suggesting that *Bd* predatory microfauna affected *Bd*-host dynamics (Schmeller et al., 2014). Mesocosm experiments using *Daphnia*

Table 6.3: Potential disease interventions that manipulate environmental factors

| Intervention | Evidence | In situ examples |
|---|--|--|
| Prune overhanging vegetation to increase terrestrial or aquatic temperatures | Frogs that select habitats with higher temperatures reduce their Bd infections (Richards-Zawacki, 2010; Rowley & Alford, 2013). Canopy modification to create warmer microclimates is postulated as a tool to permit coexistence with the pathogen (Scheele et al., 2019b). Bd prevalence declines associated with cyclone-canopy disturbance in Australia supports this hypothesis (Roznik et al., 2015). | Riparian tree canopies in Australia were trimmed to reduce the suitability of the habitat for Bd at spotted tree frog release sites (Scheele et al., 2014), but the canopy pruning was discontinued (B.C. Scheele, personal communication). |
| Translocations to environmental refugia | Release captive-bred animals in warmer parts of their range that may act as environmental refugia or disease-free refuges (Scheele et al., 2014). Timing of releases to coincide with low Bd prevalence may also influence post-release success. | A translocation of yellow-legged frogs to colder, higher elevations postulated to limit Bd in frogs, but did not work (Knapp et al., 2011). |
| Artificial heating stations | Natural thermal springs act as Bd refugia for frogs (Savage et al., 2011), and provision of artificial heating stations in situ are postulated as a mitigation tool (Hettyey et al., 2019). | The Mountain Chicken Recovery Program is conducting release trials using artificially heated pools as one Bd-mitigation strategy (Dagano, 2018). |
| Add fungicides or salts to ponds to reduce pathogen loads | Adding salt to experimental ponds reduced Bd transmission between infected and uninfected animals (Clulow et al., 2018). Addition of commercially available fungicides to mesocosms reduced Bd prevalence and load, but also affected tadpole growth rates (Geiger & Schmidt, 2013; Hanlon, Kerby & Parris, 2012). | Addition of salt to ponds where captive-bred green and bell frog tadpoles were released improved survival and reduced Bd prevalence (Stockwell et al., 2014). A multi-year study in Mallorca found that pond drying, environmental disinfection, and fungicidal treatment of resident midwife toads eliminated Bd for at least 2 yrs post mitigation efforts (Bosch et al., 2015). |
| Increase population buffering capacity through habitat improvements or predator removal | This strategy aims to improve habitat, or optimise hydroperiods to increase recruitment in order to compensate for disease-related losses (Scheele et al., 2014). Ideally habitat improvement will occur proactively while populations are still resilient (Sterrett et al., 2019). | Construction of additional breeding ponds for Puerto Rican crested toads have been partly successful and increased the number of populations of this threatened species (Linhoff et al., 2021). Creation of habitats that excluded fish helped increase green and gold bell frogs even in the presence of Bd (Beranek et al., 2021). |
| Microbial bioaugmentation of substrate | Experimental augmentation of soil with bacteria that produce antifungal metabolites prevented Bd colonisation of amphibian skin (Muletz et al., 2012). | Not tested yet |
| Micropredator augmentation | Zooplankton as a micropredators of Bd, and experimentally reduce Bd zoospores and transmission of Bd to tadpoles (Schmeller et al., 2014). | Not tested yet |

further corroborated the idea that microfauna can reduce *Bd* zoospore counts in lentic habitats (Buck, Truong & Blaustein, 2011; Hamilton, Richardson & Anholt, 2012). *Bsal* is similarly affected by aquatic micropredators, but the existence of an encysted zoospore stage makes them less vulnerable (Stegen et al., 2017).

Ranaviruses have cross-taxonomic host boundaries (Brenes et al., 2014; Duffus et al., 2008; Schock et al., 2008), and are further transmitted through scavenging, direct contact, and contact with contaminated water (Blaustein et al., 2018). Host predation can reduce ranavirus infection rates because predators tend to attack individuals who are weak or have altered avoidance behaviours; some pathogens including ranaviruses can alter tadpole behaviour and result in greater predation of infected individuals, leading to 'healthier but smaller herds' (DeBlieux & Hoverman, 2019).

- **Future steps & recommendations**

While correlations between some environmental factors and mechanisms governing the infection dynamics are now well established, predicting and mitigating the impact of infections on amphibian populations continues to be a challenge. The relative contributions of mechanisms of transmission and disease tolerance in promoting pathogen-host coexistence appear to be context-dependent and field data are often scarce. Also, the role of biotic interactions in the infection outcome remains poorly understood. Future investigation and management of amphibian diseases will need to consider the context-dependence of interactions and address the complexities arising from multispecies and multiscale interactions. Context modelling can be useful for a rapid assessment of effective strategies, given the urge of mitigating amphibian diseases.

Human dimensions

Human dimensions in amphibian diseases are multifaceted including knowledge discovery through research and monitoring, inadvertent pathogen

transmission, and direct conservation, management and policy actions (Olson & Pilliod, 2021).

- **Trade**

International and national policies focus on reducing human-mediated transmission. For example, the recently proposed Asian origin of *Bd* and *Bsal* has raised concerns for risk of international transmission within trade markets (Carvalho, Becker & Toledo, 2017; Nguyen et al., 2017; O'Hanlon et al., 2018). In 2008, chytridiomycosis was added to the OIE's list of notifiable diseases due to increasing evidence of *Bd* spread through live amphibian trade. Both chytrid fungi and ranavirus are now OIE listed as notifiable diseases (OIE, 2020; Schloegel et al., 2009). In 2018, a motion was passed by the Convention on Biological Diversity (CBD) for member states to adopt measures to reduce risk of invasive alien species moving unintentionally in pathways associated with trade of live organisms (CBD, 2018).

Clean trade is a priority for immediate action across wildlife species due to rapidly increasing pathogen concerns for both wildlife and potential spill over to humans (Fisher et al., 2018; Kolby, 2020). Research advances in rapid and cost-effective pathogen detection and procedures for biosecure captive-animal handling in trade markets are increasing the feasibility of taking measures to reduce risk of spreading diseases (e.g. Brunner et al., 2019; Gray et al., 2018). However, a web of regulatory authorities with vague authority and overlapping regulations makes it challenging to make progress in effecting policy changes, and is compounded by a lack of funding, capacity and regulatory backing that has slowed progress in developing clean-trade markets (see [Chapter 7](#) for more information on policy efforts).

Recognising the role of trade in spreading diseases is important but getting ahead of the problem and preventing spread is likely the most cost-effective action. *Bsal* is one example of a pathogen known only to occur in parts of Europe, with a likely east

Asian origin (Martel et al., 2014). Scientists called for action to prevent its spread to North America which is home to exceptional salamander species richness that is naive to this pathogen (Gray et al., 2015). In June 2015, a US Geological Survey workshop in Colorado, USA convened to form a Bsal Task Force with eight working groups to address response and control, surveillance and monitoring, diagnostics, communication and outreach, clean trade, research and decision science, and data management (North American Bsal Task Force, 2022). These emphasis areas each help to get ahead of disease impacts.

- **Surveillance and monitoring**

In particular, pathogen surveillance in both captive and wild animals has been needed to understand geographic and taxonomic patterns of disease occurrence, the potential scope of trade effects, and the direction of biosecurity needs. However, surveillance and monitoring to date has been primarily focused in North America, Europe and Australia, while many amphibian-rich regions lack capacity for widespread monitoring (although see National Monitoring Initiative in Madagascar (Bletz et al., 2015; Weldon et al., 2013)). With severe documented Bd impacts, Australia was one of the first countries to establish survey protocols for national surveillance (Skerratt et al., 2008). Bsal detection in captive amphibians was reported in Europe (Fitzpatrick et al., 2018; Sabino-Pinto et al., 2018), but no Bsal detections were reported in captive samples in North America (Klocke et al., 2017), which can greatly inform usefulness of biosecurity policies such as a trade moratorium. Trade restrictions continue to be warranted as Bsal surveillance in North America has failed to detect it to date (Waddle et al., 2020). Surveillance of both Bd and ranavirus has accelerated rapidly in the last decade, supporting cross-jurisdiction concerns for amphibian disease threats. Global Bd and ranavirus community open-access databases are available with recent website updates. Worldwide, Bd has been detected in 1,375 of 2,525 (55%) species sampled, from 93 of 134 (69%) countries (Olson et al., 2021a; database:

amphibiandisease.org). Metadata analyses using these data have aided understanding of disease threats and host-pathogen-environment associations. Ranavirus surveillance reports are dominated by amphibians (63 genera; vs. 27 fish and 34 reptile genera) in North America and Europe, with a history of detections related to mortality events, some of which were in farm production settings (Brunner et al., 2021; see database: brunnerlab.shinyapps.io/GRRS_Interactive/).

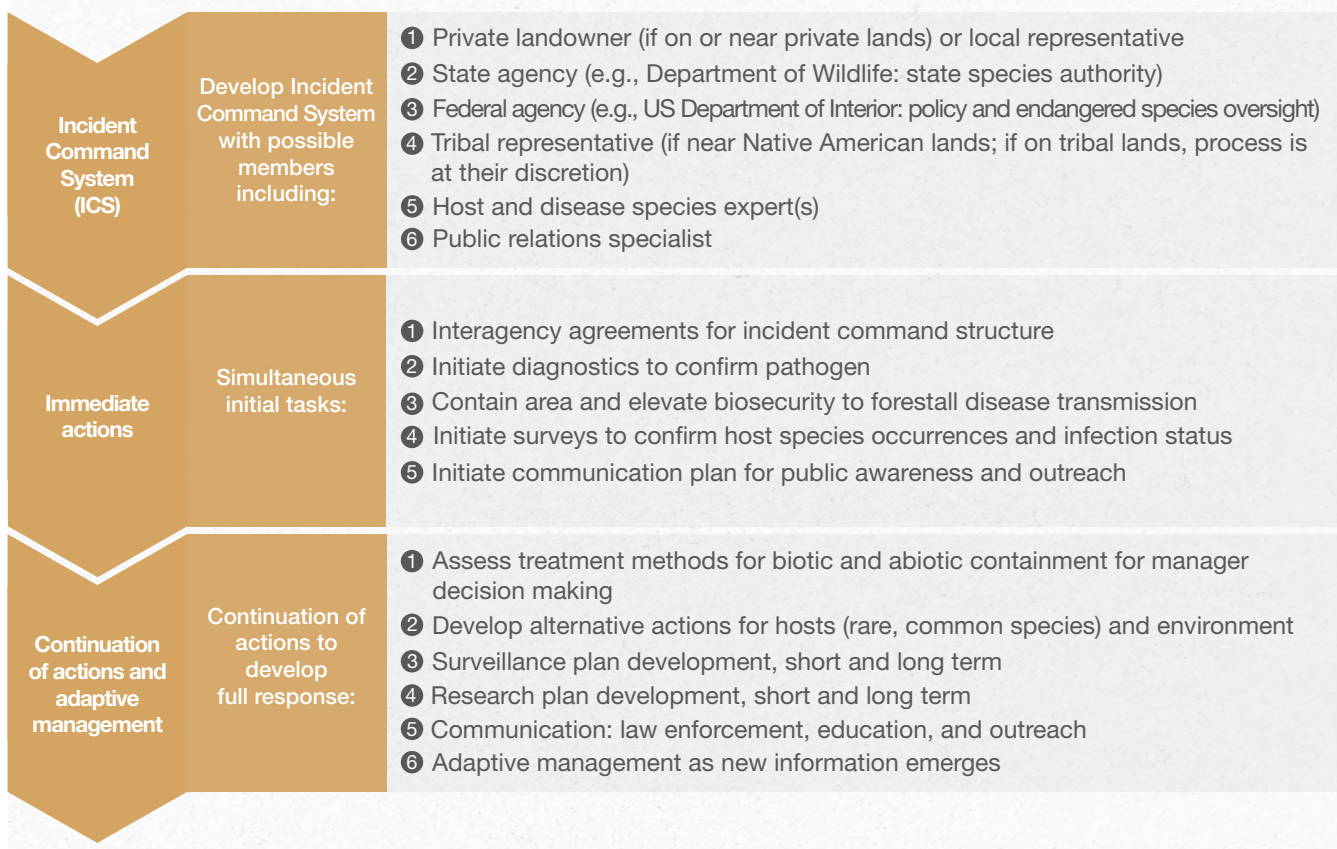
- **Decision science & proactive planning**

Decision science is a developing discipline to facilitate manager and policy maker decision-making processes. Importantly, decision science can aid in identifying models that can inform management decisions, e.g. in predicting outcomes of alternative actions in preparing for and initiating responses to disease outbreaks (e.g. Canessa et al., 2018; DiRenzo & Campbell Grant, 2019; Hopkins, 2018). Proactive planning can be further aided by the development of Incident Command Systems (Box 6.1). An Incident Command System is a standardised approach to the command, control, and coordination of response providing a common hierarchy within which responders from all stakeholders can be effective.

Hopkins (2018) showcased the importance of development of a USA incident command system through scenario planning exercises. This work highlighted differing trajectories of amphibian die-off responses due to land ownership (US National Park System lands, US Forest Service lands, and neighbouring tribal lands), and stall points in responses due to national, state, and local permissions required for actions such as implementing ground-disturbing activities or chemical applications in field settings. The North American Bsal Task Force response and control working group has also outlined a Response Plan Template including an outline of ICS (salamanderfungus.org). Importantly, these systems can and should be defined proactively at local, regional and national scales to expedite effective response and management actions.

Box 6.1: Incident Command System for rapid disease response

An Incident Command System (ICS) is a disaster management system that has been applied to emergency response situations such as for human hazards including wildfire, hurricanes, earthquakes, chemical spills, and search-and-rescue operations, invasive species and disease outbreaks. Development of An Incident Command System (ICS) for amphibian disease outbreaks can facilitate an effective response through immediate and cascading follow-up actions, including assembly of a command team, biosecurity implementation, survey and diagnostics, development of effective response actions, and active communication with stakeholders. Hopkins (2018) described an Incident Command System for responses to amphibian die-off scenarios from hypothetical outbreaks of chytridiomycosis due the chytrid fungus *Batrachochytrium salamandrivorans* (Bsal) in the salamander biodiversity hotspot of the Appalachian Mountains in the eastern United States.



- **Disease control strategies**

Biosecurity protocols outline basic steps to reduce amphibian pathogen transmission in both captive (Brunner, 2020; Gray et al., 2015; Pessier & Mendelson, 2017) and field situations (Gray et al., 2018; Phillott et al., 2010). Biosecurity measures range from between-site hygiene measures to prevent pathogen transmission in field situations (Health et al., 2018; Julian et al., 2020; Olson et al., 2021b), to between-individual precautions (Cashins, Alford & Skerratt, 2008; Greer et al.,

2009), while stringent quarantine and disinfection measures can prevent disease outbreaks in both captive and field situations (Pessier & Mendelson, 2017). Australia has developed national guidelines for intra- and inter-state implementation of hygiene protocols to prevent Bd spread (Commonwealth of Australia, 2016).

Disease management strategies beyond biosecurity protocols have developed considerably in the last two decades. Garner et al. (2016) and Thomas et al. (2019) reviewed alternative

strategies in the toolbox of approaches to mitigate pathogen outbreaks, many of which are in active research-and-development at this time, including: habitat modification, chemical treatments, vaccines, probiotics (Tables 6.2 and 6.3, see also Smith & Sutherland (2014) for evidence of effectiveness for disease control and biosecurity practices).

AmphibianArk (www.amphibianark.org) was created in 2006 to carry out ex situ components of the IUCN SSC Amphibian Specialist Group's *Amphibian Conservation Action Plan* (ACAP). Its vision was to leverage existing captive husbandry resources in zoos and aquaria around the world to meaningful ex situ conservation efforts, and it has made great strides in training staff and building capacity, conducting prioritisation and providing funding to support ex situ amphibian conservation efforts globally (McGregor Reid & Zippel, 2008). It now spans more than 60 organisations in 28 countries working to conserve 115 anuran species (Gratwicke & Murphy, 2016; Harding, Griffiths & Pavajeau, 2016). Whereas captive breeding efforts do not directly mitigate the threats, and have had mixed success (Harding, Griffiths & Pavajeau, 2016), they have created numerous opportunities to conduct integrated research (Hudson et al., 2016b; Lewis et al., 2019; Skerratt et al., 2016). Linhoff et al. (2021) provided guidelines for amphibian reintroductions and translocations, the final step in many ex situ efforts (See Chapters 11 and 14 for more information on these topics).

- **Community engagement**

Lastly, engaging people and communities is a necessary component of mitigating disease spread. Although this takes many forms, important factors in this sociological component include:

- 1) Accelerated scientist networking and collaborations to increase the global pace and scope of research and surveillance.
- 2) Mobilising funding to build capacity for an effective response.

- 3) Developing conservation partnerships to address common disease management goals.
- 4) Developing a communication strategy to increase targeted communication with defined audiences including the public, environmental groups, and policy makers, natural resource managers and disease specialists.

The Herp-Disease-Alert-System (HDAS; herp_disease_alert@parcplace.org) implemented by PARC (Partners in Amphibian and Reptile Conservation) in North America is an example of a public-management networking system gaining success for rapid disease responses that routes information to the correct authority for follow-up action. The Human Dimension may be the greatest challenge yet to mitigate amphibian disease threats, as the feral dynamics of the Anthropocene are all-encompassing, affecting multiple biodiversity threat factors (Tsing et al., 2020).

In summary, we recommend the following actions:

Surveillance

- » Build global capacity to conduct routine diagnostic testing and examination of amphibians for both known and novel emerging diseases.
- » Heightened vigilance for sick and dead amphibians, especially given symptom similarity between endemic and novel disease threats.
- » Develop inexpensive, sensitive and accurate field-tests.

Research

- » Move beyond correlating pathogen presence with decline; focus on causation.
- » Develop broad predictive markers or indicators for susceptibility to prioritise species conservation actions.

- » Understand why some species and populations are recovering as well as how disease “cold spots” persist.
- » Study cellular responses to infection to enhance understanding of immune function in relation to susceptibility.
- » Study pathogen interactions with the microbiome, host immune system, and poorly-studied pathogens and parasites.
- » Identify environmental conditions in which amphibian populations can coexist with pathogens.

Management & Policy

- » Continue to examine and develop novel ways to mitigate diseases both in captive settings and in the wild, particularly in field settings.
- » Facilitate cross-disciplinary connections between land managers, decision scientists, and ecologists to facilitate management decisions rooted in sound science.
- » Use adaptive management frameworks to make detailed observations, learn and improve management interventions in a field context.
- » Engage relevant land management agencies and government authorities in amphibian, and more broadly, wildlife disease issues.

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Japanese giant salamander (*Andrias japonicus*), classified as Vulnerable on the IUCN Red List. © Brian Gratwicke



Crocodile newt (*Tylotriton* sp.) being swabbed as part of a screening survey of captive salamanders in the USA for Bsal (Klocke et al 2017). Over three quarters of *Tylotriton* species are classified as threatened with extinction on the Red List. © Brian Gratwicke