Title: Interventions for reducing extinction risk in chytridiomycosis-threatened amphibians

Running title: Reducing extinction risk in amphibians

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Word count: 6000

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Key words: amphibian decline, *Batrachochytrium dendrobatidis,* chytrid, disease management, emerging infectious disease, frog, wildlife management

Abstract

Wildlife diseases pose an increasing threat to biodiversity and are a major management challenge. A striking example of this threat is the emergence of chytridiomycosis. Despite diagnosis of chytridiomycosis as an important driver of global amphibian declines 15 years ago, researchers are yet to devise effective large-scale management responses other than biosecurity measures to mitigate disease spread and the establishment of disease free captive assurance colonies prior to or during disease outbreaks. Here we focus on the development of management actions that can be implemented after an epidemic in surviving populations. We develop a conceptual framework with clear interventions to guide experimental management and applied research aiming to prevent further extinctions of amphibian species that are threatened by chytridiomycosis. Within our framework there are two management streams; 1) reducing *Batrachochytrium dendrobatidis* (the fungus that causes chytridiomycosis) in the environment or on amphibians, and 2) increasing population capacity to persist despite increased mortality from disease. The latter stream emphasises that mitigation does not necessarily need to focus on reducing disease associated mortality. We propose promising management actions that can be implemented and trialled based on current knowledge including habitat manipulation, antifungal treatments, animal translocation, bioaugmentation, head starting and selection for resistance. Case studies where these strategies are being implemented will demonstrate their potential to save critically endangered species.

Introduction

In a globalizing world, emerging infectious diseases are a growing threat to biodiversity (Daszak et al. 2000; Fisher et al. 2012) and can have a rapid and widespread impact on wildlife, driving species to extinction (Berger et al. 1998; Joseph et al. 2013). Despite the rise of disease as a key conservation challenge, the management of wildlife diseases affecting biodiversity, especially non-mammals, remains in its infancy (Joseph et al. 2013).

Chytridiomycosis, caused by the pathogenic skin fungus *Batrachochytrium dendrobatidis* (hereafter Bd), has devastated amphibian communities globally and is considered the worst recorded wildlife disease (Berger et al. 1998; Skerratt et al. 2007). Infection with Bd has been detected in 516 of 1240 (42%) amphibian species sampled (Olson et al. 2013) and a conservative estimate suggests that spread of chytridiomycosis caused severe declines or extinction of over 200 species (Skerratt et al. 2007). Amphibians are a functionally important group and their loss is likely to have major ramifications throughout ecosystems (Whiles et al. 2006).

Although experimental management strategies are underway (Woodhams et al. 2011), there are few studies on the in-situ management of species threatened by chytridiomycosis (Zippel et al. 2011; Joseph et al. 2013). To date, amphibian disease management has generally targeted mitigating disease spread and securing captive assurance colonies rather than restoring populations after an epidemic. Existing literature is largely directed towards policy makers, regional managers and researchers rather than on ground wildlife managers (see Australian Government Department of the Environment and Heritage 2006; Mendelson et al. 2006; Skerratt et al. 2008; Murray et al. 2011; Woodhams et al. 2011; Berger & Skerratt 2012). We provide a framework to guide management including experimental strategies that directly target reducing chytridiomycosis in host populations as well as strategies to improve population buffering capacity against disease-induced mortality which is only briefly covered in previous disease management recommendations (Australian Government Department of the

Environment and Heritage 2006; Woodhams et al. 2011). We summarise new and updated strategies aimed at mitigating the impact of chytridiomycosis to assist wildlife managers to select interventions.

Although populations of some species that declined have recovered (Newell et al. 2013), other species remain at low abundance or continue to decline and face increased risk of extinction (Hunter et al. 2010; Vredenburg et al. 2010). One of the main reasons for this elevated extinction risk is on-going mortality and restricted recruitment caused by endemic chytridiomycosis (Murray et al. 2009; Muths et al. 2011; Phillott et al. 2013). In addition, many remnant populations have limited connectivity, occur in sub-optimal habitat and are likely to have increased vulnerability to stochastic events and other threatening processes (Murray et al. 2009; Puschendorf et al. 2011).

A huge research effort over the last decade has resulted in Bd becoming one of the most studied wildlife pathogens. The ecology and pathogenesis of chytridiomycosis is relatively well understood, Bd distribution has been mapped and modelled, high risk species have been identified, biosecurity protocols have been implemented, captive assurance colonies have been established and antifungal treatments and disinfectants (including chemical, physical and biological treatments) have been developed for implementation in controlled environments (e.g. Murray et al. 2011; Woodhams et al. 2011). However, a major gap remains in translating research into post-epidemic, in-situ management actions and it is crucial that we overcome a fear of in-field interventions and use existing knowledge to trial novel solutions such as those suggested below (Berger & Skerratt 2012).

In this article we: 1) Define short- and long-term goals for the management of species threatened by chytridiomycosis to provide greater clarity for setting conservation objectives. 2) Provide a framework that divides management actions into two streams based on whether strategies a) target reducing Bd in the environment or on the host, or b) strategies that aim to increase population capacity to buffer against Bd associated mortality. Within each stream, management strategies are classified into three action classes based on whether strategies are implemented in-situ, involve amphibian introductions or are ex-situ. 3) We then provide a scientific underpinning for novel management strategies that hold considerable promise including habitat manipulation, in-situ antifungal treatment, animal translocations, bioaugmentation, head starting and ex-situ selection for resistance, highlighting examples where researchers are implementing these strategies in conjunction with conservation agencies. Given the limited application of interventions to date, we hope that highlighting techniques currently being trialled will inform and stimulate the development and implementation of conservation strategies for Bd-threatened species.

Timeframes defining the scope of management objectives

A key challenge to managing species affected by chytridiomycosis is the difficulty of developing long-term solutions. To move forward we have divided this challenge into two separate goals based on timeframes; 1) the short-term goal of establishing robust holding populations of Bd-threatened species in response to immediate threats in the wild (Table 1) and 2), the long-term goal of establishing self-sustaining, wild populations. Because these goals operate on different timeframes they often require different approaches and techniques. Intensive and expensive options are acceptable as short-term emergency measures while long-term sustainable measures need to be more cost effective, recognising that species may remain reliant on conservation management, to various degrees, into the future.

Here we focus on developing actions that can be implemented immediately to achieve the first goal of securing populations that have experienced major declines. Predicting and mitigating disease spread, and "trigger points" for intervening when chytridiomycosis does spread have been addressed elsewhere (DPIPWE 2010; Murray et al. 2011; Berger & Skerratt 2012). It is important that robust holding populations of chytridiomycosis-threatened species are secured both in captivity and in the wild to facilitate the establishment of self-sustaining wild populations. While long-term solutions remain elusive, achieving short-term goals will provide a platform for research into long-term goals such as natural or assisted evolution of resistance and behavioural modification (e.g. Richards-Zawacki 2010; Savage & Zamudio 2011; Venesky et al. 2012).

Managing Bd-threatened species

Our conceptual framework (Table 1) provides a summary of different management options to help managers identify appropriate conservation actions. We identify two management streams; (1) reducing Bd in the environment or on the host and; (2) increasing population buffering capacity against Bd-induced mortality, emphasising that intervention need not focus directly on reducing disease. Within these two streams, we recognise three action classes; environmental manipulation, amphibian introductions and ex-situ conservation (Table 1). Thus far, the management of Bd-threatened species has focused on the third action class, establishment of ex-situ captive colonies (Mendelson et al. 2006; Zippel et al. 2011). This is a critical first stage and the only option for some species. However, where possible we propose that this should be combined with techniques to maintain species in-situ to reduce costs, avoid negative consequences associated with captive breeding (e.g. reduced fitness Araki et al. 2007) and facilitate the natural evolution of host resistance. This is where environmental manipulation and introductions can contribute.

Environmental manipulation

Manipulation to reduce Bd

In remnant populations of Bd-threatened species, environmental manipulation can be implemented to decrease infection rates and/or burdens and hence improve host survival. Environmental manipulation is an in-situ method that has been successfully used to combat wildlife diseases and can be implemented across a wide range of scales (Wobeser 2007). For example, decreased shading and improved drainage of nesting sites minimised avian cholera, and creating artificial watering points lowered harmful trematode infections in moose (Wobeser 2002). Environmental and biological factors can exert a strong influence on infectious diseases and therefore manipulating environmental conditions can influence disease development (Wobeser 2007). The thermal preference of Bd is relatively well understood, with optimal growth between 17°C and 25°C (Piotrowski et al. 2004;

Stevenson et al. 2013). Either side of this range (5ºC -17ºC and 25ºC - 28ºC), growth is slow, the fungus dies when temperature is above 30ºC (Piotrowski et al. 2004) and mortality is rapid at higher temperatures (4 hrs. at 37ºC) (Johnson et al. 2003). Bd is not tolerant to desiccation and is killed within one hour of drying (Johnson et al. 2003). Field studies and models are consistent with these results and suggest that factors affecting Bd growth (particularly temperatures above 25ºC during the month prior to sampling), are key limiting factors for chytridiomycosis dynamics (Richards-Zawacki 2010; Murray et al. 2013; Rowley & Alford 2013). Furthermore, high climatic variability, especially unusually low temperatures, increases the impact of chytridiomycosis (Rohr et al. 2013).

Warm water (>30ºC) provides an important refuge from Bd for aquatic amphibians (Forrest & Schlaepfer 2011; Savage et al. 2011). Because over-hanging vegetation lowers the water temperature of amphibian breeding ponds (Freidenburg & Skelly 2004), the strategic removal of patches of vegetation, particularly over shallow, near-shore locations is likely to create warm water refuges for infected individuals (Geiger et al. 2011). Field evidence suggests that decreased shading of ponds is linked to lower Bd infection intensities (Raffel et al. 2010; Heard et al. 2013). Water temperature may also be increased through the creation of near-shore, shallow water areas that warm up rapidly, or by changing substrate colour or texture. For example, *Bufo americanus* tadpoles can aggregate in shallow, warm water pockets adjacent to scrap sheet metal in breeding ponds (Beiswenger 1977).

Environmental manipulation may also be used to increase temperature in terrestrial habitats. Many riverine species bask to raise body temperature and increasing the amount of solar radiation reaching basking sites through vegetation removal could clear or reduce infection (Fig. 1). In the highly susceptible species *Litoria lorica*, Puschendorf et al. (2011) hypothesised that short-term exposure to warm rock temperatures along a sunny stream section may be facilitating population persistence with endemic Bd. This is supported by a follow-up study showing that exposing Bd cultures to 33ºC for just one hour significantly reduced fungal growth (Daskin et al. 2011).

In situations where habitat modification is unsuitable, artificial heat sources on land or in water could provide refuges for infected individuals to reduce or clear infection. This strategy has been suggested for protecting bat populations in North America threatened by White Nose Syndrome (Boyles & Willis 2010). Artificial heat sources provide opportunities for individuals to maintain preferred body temperatures, which are often higher than ambient air temperatures, and are likely to be particularly effective for species that display behavioural fever (Richards-Zawacki 2010; Murphy et al. 2011).

Developing chemical treatments for environmental application is an area of important research, with salt and several agricultural products able to clear or reduce Bd infections under laboratory conditions (Hanlon et al. 2012; Stockwell et al. 2012; McMahon et al. 2013). For example, thiophanate-methyl, a widely used, broad-spectrum fungicide cleared infection in tadpoles when applied six days post experimental inoculation, but tadpoles grew larger than controls suggesting side effects may occur (Hanlon et al. 2012). Similarly, the addition of salt to pond environments is a promising strategy for inhibiting Bd growth, however it may also have negative effects (Woodhams et al. 2011; Stockwell et al. 2012; Heard et al. 2013). Recently, Geiger and Schmidt (2013) used General Tonic® (acriflavin/methylene blue) to reduce Bd in captivity and further research is underway to evaluate the effectiveness of pond applications. Therefore, while use of chemicals in natural habitats holds promise, it is important to determine concentrations and rates of application and assess potential negative side effects.

Bioaugmentation could help maintain threatened populations and facilitate successful reintroductions (Woodhams et al. 2011; Joseph et al. 2013). Bioaugmentation involves inoculating amphibian hosts or habitats with microbes that produce metabolites that inhibit Bd growth and survival (reviewed in Bletz et al. 2013). Locally occurring microbes are most appropriate and Bletz et al.(2013) provide methods to identify suitable microbes that both inhibit Bd and persist on target hosts. As soil provides an important reservoir for beneficial microbes (Loudon et al. 2013) which can be transmitted to amphibians (Muletz et al. 2012), environmental application appears feasible. As with other interventions, research to improve understanding is needed while concurrently assessing field applications.

Manipulation to increase population buffering capacity

An alternative approach to directly reducing Bd pressure in disease-threatened amphibian populations is to minimise other sources of mortality. Amphibian populations can tolerate adult mortality from Bd when recruitment is sufficiently high (Muths et al. 2011; Tobler et al. 2012; Phillott et al. 2013). Habitat loss and degradation are key threatening processes for many amphibian species (Stuart et al. 2004) and it is crucial to protect habitat for species threatened by chytridiomycosis. Introduced species can also increase juvenile and adult mortality and their exclusion can increase population size (e.g. Vredenburg 2004). However, increased population densities following the removal of introduced species will theoretically increase Bd transmission and this risk should be considered against potential benefits (Briggs et al. 2010). Finally, in many amphibian populations climatic extremes are a major source of mortality (Shoo et al. 2011). To minimise drought-induced recruitment failure, amphibian breeding habitats can be manipulated to increase hydroperiod length, while adult mortality can be reduced through the creation of moist refuges (see Shoo et al. 2011). When manipulating habitat, it is important to consider the relative effects of different sources of mortality because there may be trade-offs between improved survivorship and enhanced habitat suitability for Bd (Murray et al. 2011).

Amphibian introductions

Introductions to environments unfavourable for Bd

When Bd cannot be controlled in-situ, translocations can be used to move animals into environments unfavourable to Bd growth, or into Bd-free locations. Animal translocation can mitigate infectious disease in mammals (Wobeser 2002), but remains untested for combating chytridiomycosis. We propose the translocation of animals into environmental refugia within or near to their former range. Refugia must have suitable habitat characteristics (Hoegh-Guldberg et al. 2008) and either occur within the physiological stress limits of the target species or be manipulated to remain within those limits. Refugia can be identified through a combination of Bd field sampling and distribution modelling (Puschendorf et al. 2009; Puschendorf et al. 2013). In general, refugia are most likely to occur at lower elevations where environmental temperatures exceed the optimum for Bd growth or in drier areas (Fig. 1). However, other factors, such as the absence of disease reservoir species may be equally important in some circumstances (Joseph et al. 2013). Translocations can have unintended consequences, and potential benefits and risks require careful evaluation (see McLachlan et al. 2007; Hoegh-Guldberg et al. 2008). Importantly, it is crucial to follow biosecurity protocols to mitigate the risk of disease spread and subsequent outbreaks (Australian Government Department of the Environment and Heritage 2006; Zippel et al. 2011).

Introductions to increase population buffering capacity

It may be possible to counteract the population impacts of increased mortality caused by Bd by adding captive bred individuals to wild populations. Two strategies that build on traditional reintroduction approaches are head starting and population augmentation (Fig. 2). Head starting involves raising wild harvested individuals, typically eggs or tadpoles, through to an optimal age for release thus enabling survival through periods of naturally high mortality (e.g. due to predation) or high Bd-induced mortality or Bd exposure. To devise effective head starting strategies for each species, it is crucial to know which life history stage has highest exposure to Bd or undergoes mortality from chytridiomycosis. For example, in upland rainforest streams in Central America chytridiomycosis causes much higher mortality during metamorphosis than in adults (Kolby et al. 2010). To enhance survival, late development stage tadpoles will be brought into captivity (Fig. 2), cleared of infection, and maintained through metamorphosis, then released as young adults back at their capture site. Head starting has an important benefit over ex-situ breeding programs; individuals for reintroduction can be produced quickly, removing the challenges and failures associated with captive breeding in species with diverse reproductive and husbandry requirements. Therefore, in systems where Bd is endemic but adults continue to produce offspring, head starting eggs or tadpoles could contribute to population survival.

When recipient sites are unavailable, and habitat manipulation is not suitable, creating new habitat for translocated animals is likely to be useful, with human-created ponds already providing important refuges for chytridiomycosis-threatened amphibians (Heard et al. 2013). Benefits of habitat creation include a high level of control of environmental conditions and avoiding impacts on natural habitat for non-target species. A variety of habitats should be created (Lesbarreres et al. 2010) that include warm environments where individuals can reduce or clear Bd infection. Created habitat should be designed to minimise the impacts of other threats such as fish predation or drought-induced recruitment failure (Shoo et al. 2011), because increased recruitment may compensate for chytridiomycosis-induced mortality (c.f. Muths et al. 2011).

Ex-situ conservation

Selection for resistance

For species relying on captive colonies to survive, maintaining the genetic diversity of founding individuals through generations in captivity is important because this diversity cannot be regained. However, selecting for increased disease resistance could facilitate population persistence with Bd infection, leading to sustainable populations (see Venesky et al. 2012; Venesky et al. 2013 for discussion on selection for increased disease resistance and tolerance). A population of *Mixophyes fleayi* recovered naturally due to increased adult longevity suggesting, in this species, disease resistance was evolving (Newell et al. 2013). Direct selection for disease resistance in captivity involves exposing frogs to Bd and breeding from survivors or from those that survive for longer – these can be treated with antifungals to avoid mortality (Venesky et al. 2012). Alternatively genetic markers for disease resistance such as MHC (Savage & Zamudio 2011) might be used to identify resistant individuals for breeding. In addition, breeding stock should be updated with potentially resistant individuals currently surviving in the wild, under natural selection. Similarly, selection for increased reproductive capacity may enable some populations to persist by offsetting chytridiomycosis induced adult mortality (Muths et al. 2011; Phillott et al. 2013). Selection pressure should be moderate to avoid inbreeding depression for other traits by occasional outbreeding with less resistant or reproductive individuals (Frankham et al. 2011). Finally, in all ex-situ operations it is important to develop treatments to clear Bd infection for use in emergency situations in the case of a breach in biosecurity and an outbreak of chytridiomycosis in the captive colony.

Chemical and heat treatment

Antifungal compounds and heat treatment can be used to reduce or clear Bd infection (Woodhams et al. 2011). Itraconazole is the most commonly used chemical treatment and can clear infection in a range of species (Baitchman & Pessier 2013). Voriconazole (Martel et al. 2011), chloramphenicol (Young et al. 2012) and terbinafine hydrochloride (Bowerman et al. 2010) can also clear infection in various species, providing alternatives to itraconazole. Species-specific optimisation is needed for chemical treatments as itraconazole use has been associated with toxicity in tadpoles and adults (Baitchman & Pessier 2013) and may lead to increased infection rates after subsequent Bd exposure (Cashins et al. 2013). Heat treatment offers an inexpensive alternative to chemical treatments (Chatfield & Richards-Zawacki 2011). Exposure to temperatures between 27°C and 37°C has cleared infection in a variety of species (Geiger et al. 2011; Woodhams et al. 2011; Baitchman & Pessier 2013), although was ineffective in other species (Woodhams et al. 2012). Chemical and heat treatments should be trialled on a small number of individuals to confirm effectiveness and safety for each species. Baitchman and Pessier (2013) provide a detailed review, including dosage rates and exposure times, for chemical and heat treatments. In populations with predictable seasonal die-offs we suggest collecting and holding amphibians for short course treatment during times of peak burdens to improve survival. Although reducing burdens may increase survival during die-offs, failure to clear infection enables the development of drug resistance by pathogens.

What strategy to use?

Assessing which management strategies are most suitable for a given species depends on a detailed understanding of Bd dynamics and species ecology. Interventions against Bd should target amphibian life history stages most impacted by disease or at high risk of Bd exposure. Ecological surveys are needed to identify outbreaks, ongoing declines, and prioritize high risk populations (Skerratt et al. 2008; Murray et al. 2011). We provide an example illustrating how a multifaceted response can be developed to target specific life history stages from the two management streams and three action classes (Table 1, Fig. 3). For most species, a variety of approaches implemented at different spatial scales will be necessary (Fig. 3), such as head starting at sites where the environment has been manipulated to decrease Bd suitability. Given the lack of proven effective strategies, all interventions should be implemented within an experimental framework. To optimise progress, research aimed at understanding the mechanisms underlying interventions should occur concurrently with their application.

Conclusion

Preserving habitat is not enough to mitigate the effects of novel diseases, which require direct intervention to protect species. As more amphibian extinctions are expected in the next decade (Bletz et al. 2013), the consequences of not acting are likely to be more severe than conducting experimental management, such as translocations into natural or created refugia. We suggest trialling relatively simple, locally adapted strategies rather than waiting for the invention of a broadly applicable "silver bullet" solution to chytridiomycosis.

Developing strategies to secure chytridiomycosis-threatened species is an achievable challenge and will enable the longer-term goal of species recovery. Managers and conservation biologists in government, universities, zoos and conservation groups must collaborate closely to identify and undertake research focused on achieving this objective (Mendelson et al. 2006). Coordination of ex-situ responses under the Amphibian Ark umbrella provides a promising example of collaboration

(Zippel et al. 2011). We hope that managers and researchers investigate the ideas presented here and develop other complementary strategies. It is imperative that we act now using existing knowledge to establish in- and ex-situ populations of Bd-threatened amphibian species. Failure to do so will only add to the immense tragedy chytridiomycosis has bestowed on the world's amphibian fauna.

Acknowledgements

We thank R. Speare for helpful discussions on chytridiomycosis management. Comments from two anonymous referees greatly improved the article.

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Table 1. A framework for action to maintain populations of Bd-threatened amphibians.

Short-term goal: To secure populations of Bd*-threatened amphibians (both in captivity and in the wild)		
	Reduce Bd in the environment or on hosts	*Increase population buffering capacity*
Environmental manipulation	manipulate habitat (shallow warm water for tadpoles, decrease shading to create open basking sites for adults and metamorphs) artificial heat sources (all life stages) exclude Bd reservoir host species (Woodhams et al. 2011; McCallum 2012) introduce Bd inhibitors (salts, fungicides) (Woodhams et al. 2011; Stockwell et al. 2012) bioaugmentation with commensal bacteria (Muletz et al. 2012; Bletz et al. 2013) alter water flow or pond drying regime	minimise human impacts (e.g. hunting, collection, habitat degradation) manage other threatening processes (e.g. invasive species, sympatric competition, predation) prevent introductions and reducing impacts of other diseases (e.g. Ranaviruses) modify habitat to minimise mortality from climatic extremes (Shoo et al. 2011)
Amphibian introductions	identify environmental refugia where Bd is absent (mountain tops, small islands) or refugia where environmental suitability for Bd is low (lower elevation, drier habitat) and translocate avoid recipient sites with Bd reservoir host species identify life-stage(s) where Bd is threatening population viability and temporarily bring individuals into captivity to clear infection and return to the wild (chemical or heat treatment)	head start wild or captive bred progeny to minimise natural mortality from predators, competition and insufficient hydroperiod length population augmentation from captive bred progeny create new habitat with a high buffering capacity against climate variability and other species-specific threats and translocate
Ex-situ conservation	treatments to clear Bd infection (e.g. chemical and physical treatments) (Woodhams et al. 2011; Baitchman & Pessier 2013) selection for resistance or other traits in captive colonies	establish ex-situ populations in biosecure facilities (Mendelson et al. 2006; Zippel et al. 2011) biobanking of genetic resources (Kouba et al. 2013)

* *Batrachochytrium dendrobatidis*

Figure 1. Introductions and environmental manipulation for spotted tree frog conservation in Australia. In Kosciuszko National Park, the critically endangered *Litoria spenceri* is restricted to one stream (stream 1) and Bd is endemic in the population. Because of the high likelihood of extirpation a captive assurance population was initiated and now provides offspring for experimental release into the wild. In addition to considering reintroductions after habitat modification at the source site, broad-scale surveys identified another suitable recipient stream (stream 2) and temperature loggers were set up at representative *L. spenceri* basking sites on both steams (**A**). At the end of January over-hanging vegetation was pruned from half of the locations on stream 1, and this increased the temperature at basking sites (**A**). However, locations on stream 2 were considerably hotter despite being at a similar elevation and latitude, indicating that stream 2 more frequently experienced conditions unsuitable for Bd growth. Furthermore, surveys revealed an absence of any reservoir frog species at stream 2 and a series of waterfalls that exclude invasive fish that prey on tadpoles – making stream 2 an ideal recipient site.

Figure 2. Examples of head starting and population augmentation. **(A)** In Cusuco National Park, Honduras, larval and metamorphic amphibians of three critically endangered species (*Plectrohyla dasypus* (pictured)*, P. exquisita,* and *Duellmanohyla soralia*) will be collected and treated for Bd infection. They will be held in captivity until they have attained about half adult length, and then released to their collection sites at this more resistant life-stage. **(B)** Animals will be maintained at Lancetilla Botanical Gardens, Honduras, within isolated amphibian rooms modelled after those at Omaha's Henry Doorly Zoo, with rigorous biosecurity. **(C)** Since 2009 in Kosciuszko National Park, Australia, captive and wild bred eggs from the critically endangered *Pseudophryne corroboree* have been placed in artificial ponds embedded in natural breeding habitat. Major benefits include reduced chytridiomycosis prevalence in metamorphs due to minimal contact with a co-occurring Bd reservoir host species and eliminating mortality from premature pond drying (D. Hunter unpublished results).

(D) A recently metamorphosed *Pseud. corroboree* emerging from one of the artificial ponds pictured in C.

Figure 3. Proposed timeline for management actions for the southern corroboree frog based on an understanding of environmental factors and life history characteristics. Weather conditions over a 20 month period at a *Pseudophryne corroboree* breeding site in Kosciuszko National Park, Australia revealed optimum conditions for Bd growth occur during late spring and summer. To increase population persistence a combination of actions from both management streams could be implemented, including environmental manipulation and frog introductions (see Table 1). Head starting and population augmentation can only be undertaken at specific times of the year coinciding with the species life-cycle, whereas habitat manipulation could be implemented year round.