1 **Chytridiomycosis, amphibian extinctions, and lessons for the prevention of future**
2 **panzootics**

3 Running head: Lessons for the prevention of future panzootics.

4 Word Count: 1,804

5
6 **ABSTRACT**

7 The human-mediated transport of infected amphibians is the most plausible driver for the
8 intercontinental spread of chytridiomycosis, a recently emerged infectious disease
9 responsible for amphibian population declines and extinctions on multiple continents.
10 Chytridiomycosis is now globally ubiquitous, and it cannot be eradicated from affected
11 sites. Its rapid spread both within and between continents provides a valuable lesson on
12 preventing future panzootics and subsequent erosion of biodiversity, not only of
13 amphibians, but of a wide array of taxa: the continued inter-continental trade and
14 transport of animals will inevitably lead to the spread of novel pathogens, followed by
15 numerous extinctions. Herein we define and discuss three levels of amphibian disease
16 management: (1) post-exposure prophylactic measures that are curative in nature and
17 applicable only in a small number of situations; (2) pre-exposure prophylactic measures
18 that reduce disease threat in the short-term; and (3) preventive measures that remove the
19 threat altogether. Preventive measures include a virtually complete ban on all
20 unnecessary long-distance trade & transport of amphibians, and are the only method of
21 protecting amphibians from disease-induced declines and extinctions over the long-term.
22 Legislation to prevent the emergence of new diseases is urgently required to protect
23 global amphibian biodiversity.

24
25 **FORUM**

26 The human-mediated transport of infected amphibians is the most plausible driver for the
27 intercontinental spread of chytridiomycosis (Mazzoni et al. 2003; Weldon et al. 2004;
28 Garner et al. 2006; Fisher and Garner 2007; Skerratt et al. 2007), a recently emerged
29 infectious disease responsible for amphibian population declines and extinctions on
30 multiple continents (Berger et al. 1998; Bosch et al. 2001; Lips et al. 2006; Rachowicz et
31 al. 2006). The chytrid fungus *Batrachochytrium dendrobatidis* (causative agent of
32 chytridiomycosis) currently infects at least 297 amphibian species and occurs in at least
33 39 countries spanning six continents (Figure 1; Supporting Online Information Tables 1
34 & 2). It is widespread throughout Australia, Europe, Africa and the Americas (Garner et
35 al. 2005; Ouellet et al. 2005; Lips et al. 2006; Goldberg et al. 2007; Kriger et al. 2007;
36 Pearl et al. 2007). *Batrachochytrium dendrobatidis* has been detected as far north as
37 Alaska and as far south as Tasmania, as high as 5,348m a.s.l. (Seimon et al. 2007) and as
38 low as nearly sea level (Kriger et al. 2007). The fungus was detected across the altitudinal
39 gradient in all five studies that have examined its altitudinal distribution (Retallick et al.
40 2004; McDonald et al. 2005; Woodhams and Alford 2005; Puschendorf et al. 2006;

Kruger and Hero 2008) and bioclimatic modeling predicts it could occupy a significant portion of both the eastern and western hemispheres (Ron 2005).

The apparent ubiquity of *B. dendrobatidis*, combined with the rapidity with which the fungus spreads within continents (Lips et al. 2006; Lips et al. 2008) and the high number of opportunities for its human-mediated transport between continents (Fisher and Garner 2007), suggests that, barring drastic and immediate amphibian trade restrictions, *B. dendrobatidis* is likely to invade any currently unoccupied areas of its bioclimatic envelope. As approximately one-third of the world's 6,350 amphibian species are already threatened with extinction (Stuart et al. 2004), preventing the further spread of chytridiomycosis and other infectious diseases represents one of the scientific community's most urgent challenges.

Herein we define and discuss three levels of amphibian disease management: (1) post-exposure prophylactic measures, which include curing infected captive individuals, eradicating disease from infected wild populations, and facilitating evolved resistance; (2) pre-exposure prophylactic measures such as routine diagnostic testing and quarantine of apparently healthy individuals destined for translocation, which are intended to reduce the likelihood of disease transfer; and (3) preventive measures that remove the threat of disease altogether, such as banning the translocation of amphibians.

Post-exposure prophylactic measures

Post-exposure disease prophylaxis is currently a viable option for only a very small proportion of the world's amphibians, namely those in captivity whose diseases can be cured by heat treatments or chemicals (Parker et al. 2002; Woodhams et al. 2003). There are no published accounts of studies that have successfully facilitated amphibians' evolved resistance to diseases, and it can be assumed that no such protocols will be imminently developed that can protect the world's amphibian populations in the short-term. This is due to (1) the slow reproductive rate, and therefore evolutionary adaptivity of most amphibians; (2) our current lack of knowledge regarding amphibian husbandry (Lee et al. 2006), and (3) potential negative effects associated with the captive-breeding of amphibians over multiple generations (Kraaijeveld-Smit et al. 2006).

It is not currently feasible to eradicate *B. dendrobatidis* from infected sites (DEH 2005) and it is unlikely that suitable methods will be developed prior to the fungus' initial epidemic wave wreaking havoc on any remaining naïve and susceptible amphibian populations. Even if suitable eradication techniques were developed, the rapidity with which *B. dendrobatidis* causes population declines at sites in which it is newly introduced (Lips et al. 2006; Rachowicz et al. 2006) suggests that only a small proportion of the world's susceptible amphibians could be protected: the vast majority of the world's amphibians live in tropical countries (Duellman 1999) where there are few herpetologists available to conduct regular disease monitoring, and even the best available disease detection techniques have a time-lag prior to *B. dendrobatidis* being detectable in

81 infected amphibians (Hyatt et al. 2007). Thus in most cases, the majority of the damage
82 would be done before researchers would know to implement eradication protocols. In
83 these tropical countries, most population declines due to chytridiomycosis are predicted
84 to occur in moist areas (Ron 2005; Kriger et al. 2007) where both amphibians and *B.*
85 *dendrobatidis* are likely to persist away from water bodies, making eradication even more
86 difficult. Further, eradication methods would likely provide only a short-term solution, as
87 the fungus would inevitably re-colonize any areas from which it had been temporarily
88 extirpated.

89 The direct management of *B. dendrobatidis* in infected amphibian populations is
90 thus futile in all but a few isolated cases, such as with endangered populations living on
91 small islands that have only a few aquatic breeding sites (e.g. *Leiopelma hamiltoni* or
92 *Alytes muletensis*). For such populations, the currently available management option
93 would be to manually catch and then cure every infected individual, and repeat this
94 process either indefinitely or until a suitable eradication method is developed. Thus while
95 continued research into eradication methods is important and may be necessary for
96 protecting some critically endangered species, current (and quite possibly future)
97 management options for the vast majority of the world's infected amphibian populations
98 are restricted to the mitigation of all other threats to the populations (i.e. habitat
99 destruction, invasive species, over-harvesting, loss of genetic variation, climate change
100 and pollutants).

101 As post-exposure prophylactic measures are unlikely to protect more than a small
102 proportion of the world's amphibians, the optimal method of directly managing *B.*
103 *dendrobatidis* is to prevent it from reaching new isolated locations where transport of
104 proposed disease vectors is limited (e.g. Madagascar and Pacific Islands), and to prevent
105 the transfer of new strains to currently affected sites, so as to facilitate amphibian
106 populations' natural adaptation to the fungus (Berger et al. 2005; Kriger and Hero 2006).
107 Protecting remaining uninfected amphibian populations from *B. dendrobatidis* (and
108 perhaps more importantly future diseases) will require drastic and immediate restrictions
109 on the domestic and global trade and transport of amphibians, on a scale unlike any that
110 have been implemented in the past. Appropriate restrictions would affect the pet, bait,
111 food, zoo and laboratory trades, all of which have been implicated as driving the global
112 spread of chytridiomycosis (Aplin and Kirkpatrick 1999; Pessier et al. 1999; Parker et al.
113 2002; Garner et al. 2006).

114 115 **Pre-exposure prophylactic measures**

116 The goal of a pre-exposure prophylactic measure is to reduce the likelihood of disease
117 transfer in scenarios where infection is likely to be encountered. Such measures include
118 quarantines and subsequent diagnostic tests to confirm infection-free status, batch-testing
119 shipments, certifying farms as disease-free, and other similar bio-security precautions.
120 These actions are necessary when translocations must occur, as is the case for zoos and

laboratories, both of which can contribute to disease spread (Pessier et al. 1999; Parker et al. 2002), yet perform important conservation services such as captive-breeding and scientific research. As zoos and laboratories are part of the scientific community and are thus amenable to laws that benefit amphibian conservation, a reduction in trade and the enactment of appropriate quarantine and testing procedures by these sectors could likely be implemented with relative ease (Young et al. 2007). To further reduce the threat of disease transfer, captive-breeding should take place in the amphibian species' native geographical region whenever possible, and laboratories should conduct their research on either native species, or locally bred exotics.

The amphibian food trade is important in many developing countries where amphibians serve as a traditional source of protein. In these locations, amphibians that are consumed tend to be harvested locally and thus are unlikely to be significant sources of disease transfer. Such countries therefore have little reason to restrict domestic sales of amphibian species, assuming they are harvested in a sustainable manner. Conversely, most developed countries have no shortage of alternative food sources, and the amphibians they eat are seldom harvested locally. France, for instance, banned commercial frog farming in 1977 and now relies primarily on imports from southeast Asia. In such scenarios, countries should be required to either restrict their imports to dead amphibians, or to submit all imported amphibians to both quarantine periods and comprehensive disease testing and prophylactic treatment, the costs of which would be paid for by the importers and consumers. Similar restrictions should be placed on the domestic food trade if the species in question is being transported outside of its natural range.

We wish to make it clear however that it only takes one diseased individual to initiate an epizootic. Pre-exposure prophylactic measures are an important improvement over current laws, but are unlikely to protect biodiversity in the long-term: diseased individuals would inevitably pass undetected, as existent diagnostic tests are seldom perfect and many diseases have yet to be discovered. Thus any unnecessary long-distance transfer of live amphibians, including those in a strictly regulated food trade, should eventually be phased out.

Preventive measures

Preventing disease spread into naïve amphibian populations can only be accomplished by removing the source of the problem: the translocation of infected amphibians. Unlike zoos and laboratories, whose conservation services render the translocation of amphibians an occasional necessity, the amphibian pet and bait trades are for the large part disposable, that is, they are unnecessary, serving little benefit to society. Their nearly-complete dismantling would benefit amphibian populations not only by eliminating a primary source of disease transfer, but also by simultaneously reducing the over-harvesting of wild amphibian populations, which is largely unregulated in many

parts of the world (Li and Wilcove 2005; Schlaepfer et al. 2005). At a minimum, these trades should be restricted to local sales of captive-bred individuals.

Though restricting the pet, bait and food trades may have some initial negative economic impact, these actions may save perhaps hundreds of species from extinction, and thus will significantly reduce conservation and remediation costs (currently estimated at US\$82 million per year: Gascon et al. 2007) in the long run. The free ecosystem services and benefits to human health that amphibians provide (Tyler et al. 2007) give further economic incentive that compensates for any initial economic losses. Australia successfully banned virtually all amphibian sales outside of zoo and research settings, yet remains one of the wealthiest countries in the world. There is little reason to believe that other nations would suffer undue losses were they to follow Australia's example.

Conclusions

In terms of its impact on biodiversity, chytridiomycosis may be the worst disease in recorded history (Skerratt et al. 2007). That the disease cannot be managed at affected sites should serve as a valuable lesson for the prevention of future panzootics: the continued inter-continental trade and transport of animals (be they amphibians or other taxa) will inevitably lead to the spread of novel pathogens, followed by numerous extinctions. To protect global amphibian biodiversity, we must now focus on preventing future panzootics. The immediate implementation and enforcement of appropriate biosecurity measures, including a virtually complete ban on all unnecessary long-distance trade & transport of live amphibians, should be the highest priority for the scientific and legislative communities.

REFERENCES

- Aplin K, and Kirkpatrick P (1999). Progress report on investigations into chytrid fungal outbreak in Western Australia. Western Australia Museum, Perth.
- Berger L, Marantelli G, Skerratt LF, and Speare R (2005). Virulence of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* varies with the strain. *Diseases of Aquatic Organisms* **68**:47-50.
- Berger L, Speare R, Daszak P, Green DE, Cunningham AA, Goggin CL, et al. (1998). Chytridiomycosis causes amphibian mortality associated with population declines in the rain forests of Australia and Central America. *Proceedings of the National Academy of Science, USA* **95**:9031-9036.
- Bosch J, Martínez-Solano I, and García-París M (2001). Evidence of a chytrid fungus infection involved in the decline of the common midwife toad (*Alytes obstetricans*) in protected areas of central Spain. *Biological Conservation* **97**:331-337.
- DEH (2005). Threat Abatement Plan for infection of amphibians with chytrid fungus resulting in chytridiomycosis. Department of Environment and Heritage, Canberra.
- Duellman WE (1999). *Patterns of Distribution of Amphibians: A Global Perspective*. The Johns Hopkins University Press, Baltimore.

- Fisher MC, and Garner TWJ (2007). The relationship between the emergence of *Batrachochytrium dendrobatidis*, the international trade in amphibians and introduced amphibian species. *Fungal Biology Reviews* **21**:2-9.
- Garner TWJ, Perkins MW, Govindarajulu P, Seglie D, Walker S, Cunningham AA, et al. (2006). The emerging pathogen *Batrachochytrium dendrobatidis* globally infects introduced populations of the North American bullfrog, *Rana catesbeiana*. *Biology Letters* **2**:455-459.
- Garner TWJ, Walker S, Bosch J, Hyatt AD, Cunningham AA, and Fisher MC (2005). Chytrid fungus in Europe. *Emerging Infectious Diseases* **11**:1639-1641.
- Gascon C, Collins JP, Moore RD, Church DR, McKay JE, and Mendelson III (eds) JR (2007). *Amphibian conservation action plan*. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, UK. 64pp.
- Goldberg TL, Readle AM, and Lee MH (2007). Chytrid fungus in frogs from an equatorial African montane forest in western Uganda. *Journal of Wildlife Diseases* **43**:521-524.
- Hyatt AD, Boyle DG, Olsen V, Boyle DB, Berger L, Obendorf D, et al. (2007). Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. *Diseases of Aquatic Organisms* **73**:175-192.
- Kraaijeveld-Smit FJL, Griffiths RA, Moore RD, and Beebee TJC (2006). Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. **43**:360-365.
- Kruger KM, and Hero J-M (2006). Survivorship in wild frogs infected with chytridiomycosis. *EcoHealth* **3**:171-177.
- Kruger KM, and Hero J-M (2008). Altitudinal distribution of chytrid (*Batrachochytrium dendrobatidis*) infection in subtropical Australian frogs. *Austral Ecology* **in press**.
- Kruger KM, Pereoglou F, and Hero J-M (2007). Latitudinal variation in the prevalence and intensity of chytrid (*Batrachochytrium dendrobatidis*) infection in eastern Australia. *Conservation Biology* **21**:1280-1290.
- Lee S, Zippel K, Ramos L, and Searle J (2006). Captive-breeding programme for the Kihansi spray toad *Nectophrynoides asperginis* at the Wildlife Conservation Society, Bronx, New York. *International Zoo Yearbook* **40**:241-253.
- Li YM, and Wilcove DS (2005). Threats to vertebrate species in China and the United States. *Bioscience* **55**:147-153.
- Lips KR, Brem F, Brenes R, Reeve JD, Alford RA, Voyles J, et al. (2006). Emerging infectious disease and the loss of biodiversity in a neotropical amphibian community. *Proceedings of the National Academy of Science* **103**:3165-3170.
- Lips KR, Diffendorfer J, Mendelson III JR, and Sears MW (2008). Riding the wave: reconciling the roles of disease and climate change in amphibian declines. *PLoS Biology* **6**:441-454.
- Mazzoni R, Cunningham AC, Daszak P, Apolo A, Perdomo E, and Speranza G (2003). Emerging pathogen of wild amphibians in frogs (*Rana catesbeiana*) farmed for international trade. *Emerging Infectious Diseases* **9**:995-998.
- McDonald KR, Mendez D, Muller R, Freeman AB, and Speare R (2005). Decline in the prevalence of chytridiomycosis in frog populations in North Queensland, Australia. *Pacific Conservation Biology* **11**:114-120.

- Ouellet M, Mikaelian I, Pauli BD, Rodrigue J, and Green DM (2005). Historical evidence for widespread chytrid infection in North American amphibian populations. *Conservation Biology* **19**:1431-1440.
- Parker JM, Mikaelian I, Hahn N, and Diggs HE (2002). Clinical diagnosis and treatment of epidermal chytridiomycosis in African clawed frogs (*Xenopus tropicalis*). *Comparative Medicine* **52**:265-268.
- Pearl CA, Bull EL, Green DE, Bowerman J, Adams MJ, Hyatt A, et al. (2007). Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in the Pacific Northwest. *Journal of Herpetology* **41**:145-149.
- Pessier AP, Nichols DK, Longcore JE, and Fuller MS (1999). Cutaneous chytridiomycosis in poison dart frogs (*Dendrobates* spp.) and White's tree frog (*Litoria caerulea*). *Journal of Veterinary Diagnostic Investigation* **11**:194-199.
- Puschendorf R, Bolanos F, and Chaves G (2006). The amphibian chytrid fungus along an altitudinal transect before the first reported declines in Costa Rica. *Biological Conservation* **132**:136-142.
- Rachowicz LJ, Knapp RA, Morgan JAT, Stice MJ, Vredenburg VT, Parker JM, et al. (2006). Emerging infectious disease as a proximate cause of amphibian mass mortality. *Ecology* **87**:1671-1683.
- Retallick R, McCallum H, and Speare R (2004). Endemic infection of the amphibian chytrid fungus in a frog community post-decline. *PLoS Biology* **2**:1-7.
- Ron SR (2005). Predicting the distribution of the amphibian pathogen *Batrachochytrium dendrobatidis* in the new world. *Biotropica* **37**:209-221.
- Schlaepfer MA, Hoover C, and Dodd CK, Jr. (2005). Challenges in evaluating the impact of the trade in amphibians and reptiles on wild populations. *Bioscience* **55**:256-264.
- Seimon TA, Seimon A, Daszak P, Halloy SRP, Schloegel LM, Aguilar CA, et al. (2007). Upward range extension of Andean anurans and chytridiomycosis to extreme elevations in response to tropical deglaciation. *Global Change Biology* **13**:288-299.
- Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, et al. (2007). Spread of chytridiomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* **4**:125-134.
- Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fischman DL, et al. (2004). Status and trends of amphibian declines and extinctions worldwide. *Science* **306**:1783-1786.
- Tyler MJ, Wassersug R, and Smith B (2007). How frogs and humans interact: influences beyond habitat destruction, epidemics and global warming. *Applied Herpetology* **4**:1-18.
- Weldon C, du Preez LH, Muller R, Hyatt AD, and Speare R (2004). Origin of the amphibian chytrid fungus. *Emerging Infectious Diseases* **10**:2100-2105.
- Woodhams DC, and Alford RA (2005). Ecology of chytridiomycosis in rainforest stream frog assemblages of tropical Queensland. *Conservation Biology* **19**:1449-1459.
- Woodhams DC, Alford RA, and Marantelli G (2003). Emerging disease of amphibians cured by elevated body temperature. *Diseases of Aquatic Organisms* **55**:65-67.
- Young S, Berger L, and Speare R (2007). Amphibian chytridiomycosis: strategies for captive management and conservation. *Int. Zoo. Yb.* **41**:1-11.

295
296