

Mitigating amphibian chytridiomycoses in nature

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Keywords: chytridiomycosis, mitigation, conservation strategy

Summary

Amphibians across the planet face the threat of population decline and extirpation caused by the disease chytridiomycosis. Despite consensus that the fungal pathogens responsible for the disease are conservation issues, strategies to mitigate their impacts in the natural world are, at best, nascent. Reducing risk associated with the movement of amphibians, non-amphibian vectors and other sources of infection remains the first line of defence and a primary objective when mitigating the threat of disease in wildlife. Amphibian-associated chytridiomycete fungi and chytridiomycosis are already widespread, though, and we therefore focus on discussing options for mitigating the threats once disease emergence has occurred in wild amphibian populations. All strategies have shortcomings that need to be overcome before implementation, including stronger efforts towards understanding and addressing ethical and legal considerations. Even if these issues can be dealt with, all currently available approaches, or those under discussion, are unlikely to yield the desired conservation outcome of disease mitigation. The decision process for establishing mitigation strategies requires integrated thinking that assesses disease mitigation options critically and embeds them within more comprehensive strategies for the conservation of amphibian populations, communities and ecosystems.

Introduction

We are confronting an expanding array of pathogenic fungi that cause extensive mortality, demographic decline, and extirpations in livestock, crop, and wildlife hosts¹. Developing strategies to limit the spread and impact of these pathogens is a priority that crosses the boundaries of politics, economics, science and health, and falls within the remit of the medical, veterinary, agricultural, and conservation sciences. Despite the increasing range of animal and plant taxa threatened by fungal pathogens, conservation science has not advanced disease mitigation in nature as a priority. This shortcoming has no better example than research on amphibian-associated chytridiomycete fungi. Our recognition of the threat posed by the global and regional emergences of the chytrid *Batrachochytrium dendrobatidis* (hereafter, *Bd*), has spurred significant advances in understanding the biology of the fungus and the dynamics of chytridiomycosis since the disease was first identified nearly twenty years ago². Similarly, we have gained important insights into the European emergence of another chytrid fungus, *Batrachochytrium salamandrivorans* (*Bsal*)³. Unfortunately, the development of field interventions for disease management has lagged far behind and managing amphibian health in nature remains a largely unexplored topic⁴⁻⁶. Because applied conservation always operates under enormous financial constraints, it is important to critically assess the viability of conservation strategies before significant investment, which has rarely been done for strategies for controlling chytridiomycosis in wild amphibians⁶⁻⁸. Here we assess some of the commonly proposed approaches to control the spread and impact of amphibian chytridiomycosis in the field. We assume that an ideal strategy will be; i) safe, legal, and ethical; ii) effective and reliable; iii) transferrable across host species, communities, and environments, iv) relatively simple to implement; and v) cost-effective.

Countering disease-driven amphibian declines should consist of a multifaceted approach adapted to the stages of pathogen emergence (pre-arrival, invasion front, epidemic, established)⁹. Current approaches include prevention and short term solutions (e.g. *ex situ* breeding programmes,

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3 cryopreservation) but long term, *in situ*, sustainable solutions are required if the goal of amphibian
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5 conservation is to be attained. This implies neutralizing the disease threat in wild populations.
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7 Although we do not discuss the prevention of pathogen introduction here in any detail, attempts to
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9 do this (e.g. via trade regulations, such as the recent establishment of restrictions on caudate
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11 amphibian trade in the USA in response to the emergence of *B. salamandrivorans*,
12 <https://federalregister.gov/a/2016-00452>) are probably the most effective disease mitigation
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14 measure available⁹⁻¹⁰. The international movement of amphibians plays a continuing role in
15
16 establishing and extending the distribution of amphibian-associated chytrids and other pathogens),
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18 but the control of chytridiomycosis and other purely wildlife diseases is largely overlooked in
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20 commercial trade^{3,11-13}. The World Organisation for Animal Health (OIE) is the international body that
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22 can regulate this, but even though its remit includes wildlife conservation it has a poor track record
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24 in doing so. *Batrachochytrium dendrobatidis* has been listed by the OIE but enforcement of
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26 chytridiomycosis control in the amphibian trade has not been implemented by OIE member states¹⁴.
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32 Here we review strategies for mitigating amphibian disease following pathogen emergence.
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34 These range from minimizing effects on host populations to pathogen eradication. Short term
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36 solutions have been discussed in detail or summarized elsewhere and these are considered vital in
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38 temporarily preserving amphibian populations at risk^{4,6,15,16}. For example, interventions with
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40 antifungals during an epidemic can alter infection dynamics and alleviate disease, but in the absence
41
42 of long term disease management *in situ*, any short term measure is unlikely to result in significant
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44 conservation success¹⁷. We focus on measures that offer the potential for long term
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46 chytridiomycosis management *in situ*. *Bd* currently infects hundreds of amphibian species on all
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48 continents where amphibians occur (Fig. 1)¹⁸. Amphibian infections with *Bd* predate the late 20th
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50 century identification of lethal chytridiomycosis, and global emergence of the lethal form of the
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52 disease at this time was widespread^{19,20}. Chytridiomycosis continues to emerge across four
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54 continents, precluding its elimination from widespread and complex infected host communities¹⁸.
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56 Instead of focussing on short term solutions, we examine a more pragmatic approach that strives for
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3 long-term, host-pathogen co-existence. An ambitious aim would be to preserve a maximum
4 proportion and diversity of amphibian species across as much of their distributions as possible. This
5 implies that conservation triage will be necessary, accepting the loss of individual populations and
6 even species^{21,22}. Indeed, culling of reservoir and superspreader hosts requires consideration (Fig. 2).
7 Irrespective, aims and methods will depend on local conservation priorities and should be defined by
8 local conservation managers²³.
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17 Amphibian chytridiomycosis treatments have been developed for captive populations, but
18 translating these to managing infections in wild amphibian populations and communities is not
19 straightforward. This is because amphibians affected by chytridiomycosis occupy terrestrial,
20 arboreal, aquatic, and subterranean habitats that can overlap in a single landscape. Host population
21 sizes fluctuate enormously, often exhibit highly dynamic spatial dispersion, and are frequently
22 undetectable for much of the year. Therefore, it is not surprising that the number of studies of
23 infection and disease in the wild, and those exploring management of infection in captivity, far
24 outstrip those on *in situ* intervention. We know of few published studies describing the outcomes of
25 attempted mitigation, and only two describing success. Four different strategies to mitigate the
26 impacts of chytridiomycosis in nature have been attempted and published:
27 translocation/reintroduction, augmentation of the host microbiome with probiotics, treatment of
28 individuals with antifungals, and a combination of antifungal treatment with chemical disinfection of
29 the environment^{16,17,24-26}.
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45 46 **Trialled and tested**

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48 Translocations/reintroductions often have strong appeal because they can promote the idea
49 that “something is being done”. They are erroneously perceived to be cost-effective, simple to
50 implement and transferrable. However, without a solid understanding of host-pathogen dynamics
51 and the biology of the host and pathogen in the landscape, translocations/reintroductions have little
52 probability of success. Several attempts have been made to repatriate amphibians affected by
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3 chytridiomycosis in Europe, North America, the Caribbean and Africa but none have led to
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5 successful, long-term amphibian re-establishment^{4,25,27,28} (but see 29 for evidence of short-term
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7 post-release survival). Although the majority of failures have been associated with the re-
8
9 emergence of lethal chytridiomycosis in the translocated/reintroduced species, the cause behind
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11 failure to re-establish in almost every case could not be attributed clearly^{25,27} (but see 11). This is
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13 important because lethal chytridiomycosis can be a secondary consequence of other threatening
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15 processes, which would mean conservation efforts focussed on the fungus could be
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17 misdirected^{27,28,30}. The inability to unambiguously identify cause demonstrates the relative
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19 immaturity of the science of amphibian reintroduction as a means of mitigating chytridiomycosis,
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21 falsifies the assumptions of simplicity and transferability and violates the requirement of threat
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23 mitigation before reintroduction³¹. It also calls for greater investment in pathological investigations
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25 in concert with post release field monitoring. Given our incomplete understanding of *Bd* dynamics
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27 and potential for the development of resistance to *Bd* in wild populations, the use of
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29 translocations/reintroductions as a research tool is perhaps more appropriate than as a mitigation
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31 strategy against *Bd*.
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37 A decade ago Harris and collaborators discovered that a subset of bacteria isolated from the
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39 skin of living amphibians has the ability to inhibit *Bd* growth *in vitro*³². Since then bacteria that inhibit
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41 *Bd* have been isolated from amphibians from across the Americas, Africa, Europe and Australia. Field
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43 studies of amphibian microbiomes indicate that the bacterial community on amphibian skin changes
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45 with amphibian life history stage, with fewer *Bd*-inhibitory species in later life stages, suggesting that
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47 targets for field intervention may be age-specific³³. An expanding research programme is underway
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49 to ascertain if resistance to or limitation of infection can be enhanced by augmenting amphibian skin
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51 microbiomes with inhibitory bacteria. Encouragingly, a limited, but successful, field trial has been
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53 published along with a strategy for the isolation and potential application of probiotics to augment
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55 skin microbiomes^{24,34}. This strategy outlines the advantages of bioaugmentation, including the use of
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3 local bacterial isolates, and describes the potential for environmental application of bacteria that will
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5 interact with an entire amphibian community³⁴.
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10 Several general issues need to be overcome before probiotics can be considered a viable
11 mitigation strategy. First, the potential risk probiotics pose to ecosystem and public health requires
12 assessment and the practicalities of probiotic development are also largely ^{unassessed}³⁵. For example,
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14 there is little available information regarding the relationship between chytrid growth inhibition *in*
15 *vitro* and effective inhibition of fungal growth or the development of disease *in vivo*. Experimental
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17 efforts using probiotics to control *Campylobacter* in poultry show that the relationship will likely not
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19 be straightforward and that some bacteria that are inhibitory are ineffective against pre-existing
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21 infections^{36,37}. Efficient and persistent host and environmental colonization needs to be established:
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23 amphibian skin microbiomes are dynamic and can be unstable and unpredictable, and bacterial
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25 community composition changes over the animal's lifetime³. Bioaugmentation requires a deeper
26
27 understanding of bacterial community assembly, stability and permeability, couched in the context
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29 of amphibian host community, the skin secretions produced by species members of the community
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31 and how these are in turn influenced by environmental heterogeneity^{38,39}. Probiotics should also
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33 exert their beneficial effect across *Bd* genotypes. It has already been documented that the ability to
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35 inhibit one isolate of *Bd* does not translate across different isolates of the globally pandemic
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37 lineage⁴⁰. Finally, a probiotic should show characteristics that render it suitable for mass production,
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39 including prolonged shelf life. As it stands, we have an unclear understanding of how interactions
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41 amongst all these factors will influence the development of effective probiotic therapies against
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43 chytridiomycosis. The research required to gain this understanding will likely to be less cost-
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45 effective, implementable and transferrable than that for chemical treatments (see below), and, if
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47 animal experiment requirements are extensive and not well-justified, ethically questionable.
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49 However, if candidate bacteria can be characterized that meet the required criteria, their application
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51 could be far more cost-effective, ethical, and less controversial than chemical treatment.
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3 Antifungals applied directly to susceptible hosts have proved ineffective as a long term
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5 strategy for *in situ* chytrid mitigation, as they afford no persistent benefits after treatment is
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7 stopped^{17,26}. However, in an isolated and structurally simple ecosystem containing a single
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9 amphibian host species, antifungal treatments of individuals combined with chemical treatment of
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11 the environment did eliminate *Bd* and clearance persisted across years²⁶. These findings suggest that
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13 the environmental application of fungicides may be a viable, cost-effective, simple to implement,
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15 and broadly transferrable strategy for controlling infection in some wild amphibian populations.
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17 Environmental treatment might not be applicable to many amphibian communities and species,
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19 however, and the environmental application of chemical pesticides has significant ecological, legal,
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21 and ethical ramifications. To be effective in the long-term, fungicides may have to be applied on a
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23 regular basis, much as they are in agricultural systems. Although any strategy that requires ongoing
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25 maintenance and has the potential for collateral impacts might seem untenable, decades of
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27 fungicide applications to food crops have had a significant and positive effect on global food yields⁴¹.
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29 The parallel suggests that in the face of the chytridiomycosis crisis environmental treatment with
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31 fungicides should be considered as a viable, long term management strategy for wild amphibians
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33 threatened by the disease. Very little effort has been expended in investigating existing chemical
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35 compounds that are effective against amphibian-associated chytrids or the development of chemical
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37 agents that specifically target chytrids, despite the evidence that some chemical pesticides mitigate
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39 infection in the aquatic environment without compromising amphibian development and larval
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41 survival⁴² (but see 43). Although the use of agricultural pesticides is greatly debated, the focal, short
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43 term application of antifungals targeted at a reduction of infection prevalence and infection load in
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45 specific cases of acute chytridiomycosis-driven amphibian die offs is worth exploring⁴⁴. The
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47 application of any such measure should be weighed against its potential negative impacts on
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49 biodiversity, ecosystem function, human health, and the potential for amphibian-associated chytrids
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51 to develop resistance to these treatments⁴⁵. Advances in our understanding of the virulence factors
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53 and cellular components key for chytrid reproduction, growth, and infectivity should inform the
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3 selection of compounds that exhibit multi-modal antifungal action and also guide the development
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5 of application strategies^{46,47}.
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8 **Horizon-scanning or wishful thinking?**

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11 Several mitigation strategies are gaining traction in the literature although they remain
12
13 untested in real world settings. Evidence is accumulating that at least some species are responding
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15 to the emergence of chytridiomycosis through natural selection on immunity^{48,49}. As a result, two
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17 arguments that incorporate selection into mitigation strategies are being promoted⁵⁰. The first is
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19 based on the idea that, given time, natural selection will operate on immunogenetic variation in
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21 amphibian populations. To enable this, amphibians need to persist in the face of the pathogen and
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23 translocation/repatriation have been proposed as methods to facilitate population persistence
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25 during the process of selection. The second strategy is to breed selectively for resistant or tolerant
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27 genotypes for release into the wild⁵¹. Both strategies seek to establish resistant or tolerant
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29 populations and are based on the assumption that amphibian host immune responses to chytrids
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31 can be selected for and that immune function will be protective in a wild setting.
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35 We can apply the points for and against translocations/reintroductions that we outlined
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37 above to the strategy of translocation/repatriation, compounded with the need to understand
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39 resistance and tolerance in captive populations before any release could be ethically undertaken.
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41 But what about selective breeding? We are aware of a single example where captive selection and
42
43 subsequent breeding created defined lines that exhibit variation in immunity in an amphibian: the
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45 genus *Xenopus*^{52,53}. The knowledge base on *Xenopus* captive breeding, cell biology, genetics, and
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47 immunity took decades to develop. Advances are being made in comparative immunogenetics that
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49 could conceivably guide breeding designs, but this is still a long way from understanding host-species
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51 immune responses to chytrids and exploring heritable variation of amphibian immunity with the goal
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53 of selective breeding⁵⁴. The elucidation of mechanisms underpinning resistance against *Bd* would
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55 greatly facilitate the development of resistance markers that could be used in marker-assisted
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3 selective breeding programmes. The chances of finding any such marker, or a set of markers, are
4 hampered by the context dependent interaction of *Bd* with the amphibian host⁵⁵. Establishing
5 captive colonies upon which selection can be imposed is a non-trivial task and requires extensive
6 investment and resources. Even if assisted selection does produce genotypes that have the ability to
7 resist or tolerate infection with chytrids, there is no guarantee that these abilities will function when
8 transferred to a natural setting. Research has repeatedly shown how environmental variation can
9 dictate the outcome of the amphibian host/chytrid pathogen interaction and the ability to mount
10 innate immune responses to *Bd* can be significantly impaired simply by modifying ambient
11 temperature^{30,55-58}. We do not dismiss the possibility that selection might provide conservation
12 benefits, only caution that the current knowledge base indicates significant research is still required
13 before natural and assisted selection can be applied widely to chytrid mitigation. If genetic
14 determinants of host-resistance are identified in multiple amphibian species and new technologies
15 for genetic manipulation prove amenable to immunogenetic modification of susceptible amphibian
16 species, the situation might change, but it will also open up new ethical issues for conservationists⁵⁹⁻
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61. Clearly, it is imperative to continue investigating the genetic basis of amphibian resistance and novel means by which it can be augmented.

At least three published studies have investigated whether frogs could be immunized against *Bd*. Systemic injections of killed *Bd* were ineffective at reducing the probability of infection or death^{62,63}. In contrast, increasing numbers of exposures to killed *Bd* or live *Bd* culture followed by clearance with antifungals was negatively correlated with strength of infection and positively correlated with survival following subsequent exposure to *Bd*⁶⁴. The authors themselves questioned how their findings might be applied in a conservation setting but noted the potential for priming hosts against infection prior to release to the wild. These findings are contradicted by Hudson et al., where repeated use of antifungals on naturally infected frogs generated no long term benefits once antifungal treatments ceased¹⁷. Perhaps more importantly, every immunization study to date has focussed on post-metamorphic animals and immunization of pre-metamorphic stages might not be

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3 possible as adaptive immunity is not available to pre-metamorphic stages⁶⁵ (but see 53).
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5 Amplification of infection is commonly associated with larval stages, with high rates of mortality
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7 occurring at metamorphic climax. Controlling infection in amphibian larvae will be a key factor in
8
9 mitigating impacts of chytridiomycosis because amphibian population growth rates are highly
10
11 sensitive to survival rates of postmetamorphic juveniles⁶⁶⁻⁶⁹.
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15 The ideal vaccine for *in situ* use should elicit a strong protective response across life stages
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17 and across species against a broad spectrum of relevant and virulent chytrid genotypes, be safe, and
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19 have both its production and administration feasible. Indeed, the research process should engage
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21 with the relevant authorities from the outset, as policy applicable to vaccinating free-living wildlife
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23 populations also requires development. So far, immunization experiments have been conducted
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25 with fairly straightforward and crude fungal preparations. Designing effective vaccines is a time- and
26
27 money-consuming undertaking, and for diseases in a range of species, fungal vaccines have proved
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29 far more difficult to develop than their bacterial and viral counterparts. To date, with few
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31 exceptions, potential vaccines against human fungal pathogens are still in preclinical stages of
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33 development and very few effective veterinary vaccines are available⁷⁰⁻⁷². Although vaccinations
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35 currently afford no clear contribution to chytridiomycosis mitigation in wild populations, continued
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37 research on vaccines will undoubtedly aid in our understanding of amphibian immunity and host-
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39 pathogen interactions, both topics essential for a variety of mitigation strategies including
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41 immunization, selection, and bioaugmentation.
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46 Manipulating environments to reduce infectivity or virulence of *Bd* is another strategy that
47
48 may hold promise. The principle behind this ecological, rather than evolutionary, approach underlies
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50 environmental treatments (e.g., see 26), but in practice is accomplished by exploiting environmental
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52 variations that reduce chytrid growth and zoospore density and does not require elimination of the
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54 pathogen from the environment. The concept follows the recognition that environmental variability
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56 can inhibit, as well as exacerbate, the impacts of chytridiomycosis, with evidence of reduced
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3 virulence even in highly susceptible host species^{6,73-76}. Refuges from disease, but not necessarily
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5 infection, could be created by altering habitats to reinforce environmental factors not conducive to
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7 *Bd* growth within the host or zoospore survival outside of it. Habitat management is already integral
8
9 to most amphibian conservation programmes and often involves repeated efforts to maintain useful
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11 habitats (e.g. 77), suggesting that environmental manipulations for the purposes of disease control
12
13 could have quick uptake by the conservation community, with both concepts and strategies readily
14
15 transferrable. Interventions could be chemical (e.g. altering salinity); physical (e.g., altering
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17 temperatures to not favour chytrid growth and reproduction), or biotic (e.g., promoting the
18
19 abundance of organisms that consume environmental zoospores)^{75,78-80}. These strategies will likely
20
21 focus, at least initially, on manipulating the aquatic environment, as environmental persistence of *Bd*
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23 in water is deemed essential for amphibian decline and extinction scenarios^{81,82}. Theory and
24
25 empirical evidence shows that conservation efforts targeting aquatic life stages that reduce disease-
26
27 driven losses of newly metamorphosed juveniles should improve recruitment and reduce or reverse
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29 the effects of disease-driven decline; additional population models addressing this topic are clearly
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31 needed^{15, 81,83}.

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36 Although environmental manipulations may create pockets of tolerance or resistance, they
37
38 offer limited opportunities for amphibians with broad geographic ranges and/or disproportionately
39
40 affected complex communities and habitats. As with environmental disinfection, even in simple
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42 settings environmental manipulations must be assessed for their impacts on biodiversity and other
43
44 ecosystem functions. As with translocations/reintroductions, host ecology must be well-understood
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46 before changes to the habitat are undertaken. For now, environmental manipulation might provide
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48 long-term refuges for focal species of high conservation concern, but offers no broad scope for
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50 chytridiomycosis mitigation.
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54 A focus on disease mitigation may not always be the best way forward because simpler
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56 actions might achieve the required results: improving habitat quality might enable losses from
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3 disease at one stage of the amphibian life cycle to be compensated for in gains at other life stages.
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5 For example, one might use pond draining to cull predators of amphibian larvae. As a consequence,
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7 tadpole survival might increase, leading to increased juvenile recruitment. Even if many juveniles still
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9 die of chytridiomycosis, this action might still facilitate population persistence. There is some
10
11 empirical evidence that this might work and existing theory of harvested and exploited populations
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13 might guide such a strategy^{5,84}.
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16 17 **Single strategies or a marriage of methods?**

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20 Clearly, we do not know how to manage amphibian diseases in the wild and yet
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22 conservation managers have to make decisions and manage populations. They cannot wait until we
23
24 understand amphibian-chytrid host-pathogen biology in great detail; a lack of action because of
25
26 imperfect information is a management decision⁸⁵. From our review, it is clear that a single strategy
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28 is unlikely to achieve the conservation outcome of disease mitigation. Each strategy has pros and
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30 cons but by combining methods strategically *in situ* mitigation is likely to have a greater likelihood of
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32 success. There are a number of tools to decide which management actions are best or most likely to
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34 succeed in the presence of uncertainty. Structured decision making and information analysis can be
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36 used to find a best management option and to define the direction of research most likely to
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38 illuminate critical uncertainties⁸⁶⁻⁸⁸. For example, structured decision making might identify
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40 important gaps in our understanding of chytrid epidemiology. These approaches have only recently
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42 been used in the context of chytrid mitigation^{7,23}. Converse et al. used such an approach to study the
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44 effects of translocations in a toad metapopulation and found that efforts to reduce disease spread
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46 had weak effects, selection for resistance would increase the number of sites occupied by toads and
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48 translocations would speed up species recovery⁷.
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53 Shortcomings of individual strategies outlined above may be compensated for by combining
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55 two or more strategies. In that sense, our outline of the major alternatives for *Bd* mitigation and the
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57 applicability and challenges of each forms a starting template that can inform decision-making
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3 processes. The science of decision making links management options to measurable objectives (e.g.
4 population persistence). Post-management monitoring then determines the outcome of
5 management actions against the objectives and is used to update models for the next round of
6 decision-making. This approach allows real-time assessment of the impact of management
7 alternatives so that management can be rapidly modified to improve outputs^{8,89}.

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14 For these approaches to work researchers investigating mitigation strategies have to engage
15 in the conservation management process and be willing to alter research programmes based on the
16 outputs of structured decision making and adaptive management exercises. Precedence for this can
17 be found in the literature on chytridiomycosis ecology, evolution and epidemiology and is
18 exemplified by the initial effort to identify chytridiomycosis as the cause of amphibian mass
19 mortality (Berger et al. 1998). Coordinating research and management efforts have already been
20 proposed for Australian amphibian species at risk from chytridiomycosis⁶. Joined-up efforts will
21 require field trials across a more extensive range of settings and amphibian communities than are
22 currently being attempted. It remains to be decided –the authors of this review disagree on this
23 point- at which stage of methods development sufficient knowledge has accumulated to justify field
24 trials.
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39 What must be considered at all stages of the conservation management process, however,
40 are the ethical and legal issues associated with whatever strategies are proposed or adopted.
41 Strategies that are illegal or unethical are inapplicable irrespective of their cost-and field-
42 effectiveness, reliability, transferability, or simplicity. Ethical issues may be identified at any scale.
43 Our example of conservation triage is a knotty ethical question: what is an acceptable format for
44 deciding which species to conserve and which to cull or allow to go extinct? Expending effort on the
45 mitigation of chytridiomycosis should also be subject to ethical consideration, as should any decision
46 to expend highly limited resources available for biodiversity conservation⁹⁰. Disease as a conservation
47 issue remains a novel concept for most policy-makers and conservation practitioners, so legal
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3 frameworks may have to be challenged and modified to account for responses to this new and
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5 growing threat to amphibian biodiversity. Ethical issues may be difficult to address, but failing to
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7 mitigate chytridiomycosis, a disease widely accepted as predominantly driven by human activities, is
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9 the least ethical option of all.
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11 12 **Conclusion**

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15 Despite decades of research into amphibian-chytrid host-pathogen biology, no effective
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17 method to reduce the impact of chytridiomycosis has emerged and been tested broadly in the field.
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19 A few case and proof-of-concept studies have produced mixed or limited success at best. A more
20
21 collaborative approach to chytrid mitigation research is necessary, one that should start with an
22
23 approach from the family of tools from decision sciences to define the most important research
24
25 questions. Such exercises to identify those questions should be conducted by interdisciplinary
26
27 research teams that are working with conservation managers and that can put research outputs into
28
29 the context of the overall conservation objectives. It is always uncertain how the findings of research
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31 undertaken away from the field setting will transfer to the real world, but it is clear from our review
32
33 that significant *ex situ* research efforts are required for all mitigation methods to ensure that the
34
35 results of field trials can be fully explained. A lack of *in situ* evidence from chytridiomycosis
36
37 mitigation efforts, however, indicates that field trials are not yet an objective in many research
38
39 programmes, despite invoking amphibian conservation as a potential consequence of research
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41 discoveries. Clearly, if we are to mitigate chytridiomycosis, research must be focussed on delivering
42
43 outputs that can be rapidly and critically assessed and, when warranted, implemented in field trials
44
45 as soon as possible.
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50 Authors' contributions. All authors reviewed and approved the final manuscript. Contributions were
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52 made by all authors to all components of the manuscript.
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56 Competing interests. We declare we have no competing interests.
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2
3 Funding. TWJG acknowledges generous funding provided by NERC (NE/K012509/1 and
4
5 NE/N009967/1) and the Morris Animal Foundation (D12ZO-002) and thanks the Royal Society for
6
7 hosting for the presentation that this manuscript was preliminarily based on. JB acknowledges
8
9 generous funding from the BBVA Foundation.
10

11
12 Acknowledgements. Disclaimer: Any use of trade, firm, or product names is for descriptive purposes
13
14 only and does not imply endorsement by the U.S. Government. This is contribution number 547 of
15
16 the USGS Amphibian Research and Monitoring Initiative (ARMI).
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For Review Only

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3 Figure 1. Examples of lethal chytridiomycosis from Latin America (a) and Europe (b). a) A *Craugastor*
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5 *underwoodi* dead and *in situ* *Craugastor* sp. killed by lethal chytridiomycosis in Monte Verde,
6 Costa Rica . The isolate derived from this animal in 2008 has served as the source of DNA for
7 qPCR positive controls for two of the authors to this day. B) An *Alytes obstetricans* again
8 dead and *in situ*, found in Peñalara Natural Park, Spain.
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16 Figure 2. The relative impact of culling and antifungal treatment in a simple, single species
17 population parameterised using data for the Mallorcan midwife toad⁹¹. (a) Culling of *Alytes*
18 tadpoles, undertaken at point m, results in pathogen elimination. Green line is adult
19 population size, red line is free-swimming zoospore density. (b-c) Population responses after
20 tadpole antifungal treatment and release (b) and culling (c), assuming maintenance of
21 infection in the adult population and keeping model parameters identical across models.
22 Mitigation is undertaken at point m. In (b), mitigation is unsuccessful due to increased host
23 density after antifungal-treated tadpoles are returned to the pond. In (c), pathogen
24 elimination is attributable to more persistent reduction in host density following culling.
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Fig 1a

206x137mm (300 x 300 DPI)

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Fig 1b

1083x812mm (72 x 72 DPI)

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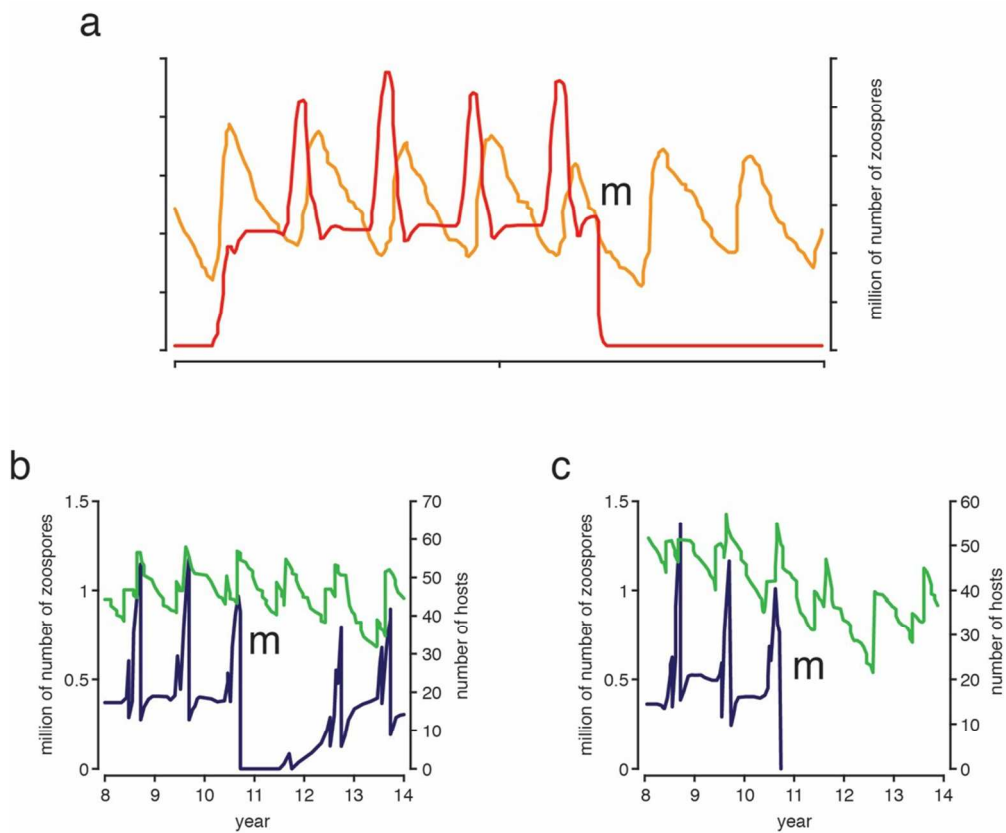


Fig 2

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