Biological Conservation 195 (2016) 37–45

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

In-situ itraconazole treatment improves survival rate during an amphibian chytridiomycosis epidemic

Michael A. Hudson [a,b,c,*], Richard P. Young [c,d], Javier Lopez [e], Lloyd Martin [f], Calvin Fenton [f], Rachel McCrea [g], Richard A. Griffiths [b], Sarah-Louise Adams [c], Gerard Gray [f], Gerardo Garcia [e], Andrew A. Cunningham [a]

[a] Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK
[b] Durrell Institute of Conservation and Ecology, School of Anthropology and Conservation, University of Kent, Canterbury, Kent CT2 7NR, UK
[c] Durrell Wildlife Conservation Trust, Les Augres Manor, Trinity, Jersey, Channel Islands, UK
[d] Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot, Berkshire SL5 7PY, UK
[e] Chester Zoo, Cedar House, Caughall Road, Upton by Chester, Chester CH2 1LH, UK
[f] Montserrat Department of Environment, Montserrat, West Indies
[g] National Centre for Statistical Ecology, School of Mathematics, Statistics and Actuarial Science, University of Kent, Canterbury, Kent CT2 7NF, UK

ARTICLE INFO

Article history:
Received 13 October 2015
Received in revised form 30 November 2015
Accepted 28 December 2015
Available online xxxx

Keywords:
In-situ treatment
Amphibian declines
Batrachochytrium dendrobatidis
Chytridiomycosis
Itraconazole
Antifungal

ABSTRACT

The emerging infectious disease, amphibian chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (Bd), threatens hundreds of amphibian species globally. In the absence of field-based mitigation methods, the Amphibian Conservation Action Plan advocates captive assurance programmes to prevent extinction from this infectious disease. Unfortunately, with the cooperation of the entire global zoo community, the International Union for the Conservation of Nature Amphibian Ark estimates only 50 species could be saved. Clearly, if catastrophic losses are to be averted, alternative mitigation techniques need to be developed. There has been an absence of trialling laboratory proven interventions for chytridiomycosis in field settings, which must change in order to allow informed management decisions for highly threatened amphibian populations. We tested the in-situ treatment of individual mountain chicken frogs (*Leptodactylus fallax*) using the antifungal drug, itraconazole. Multi-state mark–recapture analysis showed increased probability of survival and loss of Bd infection for treated frogs compared to untreated animals. There was evidence of a prophylactic effect of treatment as, during the treatment period, infection probability was lower for treated animals than untreated animals. Whilst long term, post-treatment increase in survival was not observed, a deterministic population model estimated antifungal treatment would extend time to extinction of the population from 49 to 124 weeks, an approximated 60% increase. In-situ treatment of individuals could, therefore, be a useful short-term measure to augment other conservation actions for amphibian species threatened by chytridiomycosis or to facilitate population survival during periods of high disease risk.

1. Introduction

Emerging infectious diseases are a growing threat to both humans and biodiversity globally (Daszak et al., 2000; Morens and Fauci, 2013). Three main strategies exist for the management of wildlife disease: prevention of introduction, mitigation of impact, and eradication (Wobeser, 2002). Globalisation, with its increased rate and volume of trade and travel, means preventing the introduction of novel diseases is increasingly difficult (Marano et al., 2007). Whilst neutralisation of threats has long been considered a pre-requisite for successful wildlife conservation (Caughley, 1994), the emergence of threats which cannot be negated pose a difficult challenge to conservation managers. One example is amphibian chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd), which is implicated in the rapid decline or extinction of over 200 amphibian species globally (Skerrat et al., 2007), and has been described as "the worst infectious disease ever recorded among vertebrates in terms of the number of species impacted, and it's propensity to drive them to extinction" (Amphibian Conservation Summit, 2005). This rapid global loss of amphibians is likely to have major implications for the environment (Whiles et al., 2006).

In the absence of in-situ mitigation for amphibian chytridiomycosis (Woodhams et al., 2011; Joseph et al., 2013), the Amphibian Conservation Action Plan advocates the creation of Bd-free captive populations for eventual release as a key conservation strategy (Gascon et al., 2007).

Abbreviations: Bd, *Batrachochytrium dendrobatidis*; CJS, Cormack–Jolly–Seber; CMR, capture–mark–recapture; DNA, deoxyribonucleic acid; GE, genome equivalent; IT, itraconazole treatment; NBC, non-bath control; PCR, polymerase chain reaction; PIT, passive integrated transponder; SWC, stream water control.

* Corresponding author at: Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK.
E-mail address: Michael.Hudson@ioz.ac.uk (M.A. Hudson).

http://dx.doi.org/10.1016/j.biocon.2015.12.041
0006-3207/

Currently, conservation practitioners rely on such captive assurance programmes to prevent species extinctions (Mendelson et al., 2006), but this is only a short to medium term solution and Amphibian Ark estimates that only around 50 species can be saved in this way (Zippel et al., 2011). Even so, zoos are currently failing to prioritise species that are likely to require captive breeding programmes to prevent their extinction (Dawson et al., 2015). There is, therefore, an urgent need to change the research focus from the treatment of captive animals to in-situ mitigation (Scheele et al., 2014; Harding et al., 2015).

A range of potential in-situ interventions to mitigate the impacts of chytridiomycosis have been suggested, but so far these remain largely untested in the field (Berger and Skerrat, 2012; Scheele et al., 2014). These include habitat manipulation to inhibit Bd (Scheele et al., 2014), reintroduction after selection for resistance in captivity (Venesky et al., 2014), and in-situ use of antifungal treatments (Berger and Skerrat, 2012). Some antifungal drugs, including itraconazole, are effective in the treatment of Bd infection in captivity, but only following multiple daily applications (e.g. Forzan et al., 2008; Tamukai et al., 2011; Jones et al., 2012; Georoff et al., 2013; Brannelly et al., 2015). In addition to being effective, the application of itraconazole is relatively easy, being via immersion in an aqueous solution — albeit that repeated administration is required for successful treatment (Nichols and Lamirande, 2000). Whilst there have been some reported side-effects in certain species (Brannelly et al., 2012; Brannelly, 2014) and life stages (Garner et al., 2009; Woodhams et al., 2012), itraconazole is considered to be the treatment of choice for amphibian chytridiomycosis (Holden et al., 2014). Reducing the dose from 0.01% for 11 days to 0.0025% for 5 days has been shown to reduce side effects whilst maintaining efficacy (Brannelly, 2014). Bosch et al. (2015) described the eradication of Bd from the wild Mallorcan midwife toad (*Alytes muletensis*) tadpoles by treating them with itraconazole in captivity and returning them to the wild following chemical disinfection of their breeding ponds and surrounding rocks. As other amphibians and vegetation were absent from the disinfected sites, and as these were rock pools containing little organic matter (which rapidly inactivates most disinfectants), this technique is unlikely to be transferable to many other species or locations.

In-situ treatment regimens provide challenges in field settings due to, for example, large target population sizes, low capture rates the potential of reinfection and the need for a continuous supply of labour. As a result, previous studies have treated individuals with itraconazole in captivity prior to re-release rather than treating them in-situ (e.g. Hardy et al., 2015). Environmental persistence of Bd zoospores (Johnson and Speare, 2003, 2005) and the possible presence of infected sympatric amphibians (Daszak et al., 1999) mean animals treated in-situ would likely be exposed to Bd both throughout and after the treatment period, increasing the likelihood of their extirpation (Retallick et al., 2004; Mitchell et al., 2008). Antifungal treatment in a field setting, however, might enable treated animals to persist by lowering their Bd infection load until the initial epidemic has passed (Briggs et al., 2010; Vredenburg et al., 2010). There is some evidence that animals surviving the epidemic phase persist by tolerating subsequent lower levels and frequencies of infection (Retallick et al., 2004; Briggs et al., 2010). Also, repeated infection and clearance of Bd might allow the development of resistance in some species (McMahon et al., 2014).

The Caribbean is a global hotspot of amphibian endemism, with 99% of the 197 species being endemic (Fong et al., 2015), and it has the highest proportion (84%) of threatened amphibians within a region (Stuart et al., 2008). One species, the mountain chicken frog (*Leptodactylus fallax*), has suffered a precipitous decline due to chytridiomycosis (Magin, 2003; Fa et al., 2010; Mountain Chicken Recovery Programme, 2014). *L. fallax* is classified as Critically Endangered on the IUCN Red List of Threatened Species (Fa et al., 2010) and is restricted to only Dominica and Montserrat in the Lesser Antilles. A 2005 survey found no evidence of Bd in amphibians on Montserrat (Garcia et al., 2007), but in January 2009 *L. fallax* mortality due to chytridiomycosis was first discovered on Montserrat and this was rapidly followed by epidemic mortality across the island (Mountain Chicken Recovery Programme, 2014). The characteristically rapid rates of chytridiomycosis-driven declines (Lips et al., 2006), such as those observed in *L. fallax*, limit the time available to react effectively. Interventions that can reduce rates of decline can be valuable for providing extra time to implement further conservation actions.

In this study we report the use of itraconazole treatment in a field setting in an attempt to mitigate the impact of epidemic chytridiomycosis. We assess whether in-situ antifungal treatment is a feasible and effective method for improving the survival of a critically endangered species undergoing a precipitous decline due to epidemic chytridiomycosis. *L. fallax* is an ideal species to use as a model for such in-situ treatment as it is a large territorial animal with predictable behaviours, making it relatively easy to detect and individually identify. Also, the species has been studied for over ten years on Montserrat, so there is a great deal of knowledge about its distribution, abundance and behaviour, and field sites were already established (Garcia et al., 2007; Martin et al., 2007). On Montserrat the presence of a sympatric amphibian fauna of species (*Eleutherodactylus johnstonei* and *Rhinella marina*) able to carry Bd renders an in-situ treatment study realistic for extrapolation to other species and regions where sympatric amphibians act as Bd reservoirs. Effective treatment of chytridiomycosis in captive *L. fallax* using itraconazole has shown the drug to be safe for this species (authors' unpublished observations). Finally, *L. fallax* has a voracious appetite and requires large enclosures in captivity, therefore it is difficult and expensive to hold a large enough captive population for a viable, long-term conservation breeding programme.

2. Materials and methods

2.1. Study site

Montserrat is a U.K. overseas territory in the Eastern Caribbean (16.45°N, 62.15°W). The centre of the island comprises an active volcano which has been erupting regularly since 1995. As a consequence *L. fallax* is restricted to a circa 17 km^2 mountainous area; the Centre Hills region which is typified by montane rainforest and deep valleys (or ghauts — Fig. 1) (Young, 2008).

The field site (Fairy Walk) is a forested relatively-shallow-sloped ghaut of approximately 1 km^2 on the eastern flank of the Centre Hills at an approximate elevation of 250 m asl. Prior to 2009, Fairy Walk was home to the highest known population density of *L. fallax* on Montserrat (Young, 2008) and, at the commencement of this study, it contained the last remaining intact population following the emergence of chytridiomycosis on the island in 2009.

2.2. Study design

The field experiment took place between August 2009 and January 2010. We visited Fairy Walk three times a week for 24 weeks and surveyed a predefined 800 m transect along the stream (Fig. 1) at a slow walking pace in a team of five. On each occasion the team caught all *L. fallax* seen within 5 m of the transect and recovered any dead animals. We individually marked all captured frogs using a passive integrated transponder (PIT) (11 mm × 2 mm, ID-100A Microtransponder, Trovan Ltd.), which we subcutaneously implanted in the dorsum where retention rates are maximal (Blomquist et al., 2008). We skin-swabbed each frog for Bd on every capture using a rayon-tipped swab (MW 100-100, Medical Wire and Equipment Co.) three times across each of the following sites: ventral abdomen, ventral thighs and calves, and plantar surfaces of both hind-feet. We assigned frogs to one of three groups during the study: itraconazole treatment (IT), stream water control (SWC), and non-bath control (NBC). On each capture, after skin-swabbing, we immersed each animal in the IT group for 5 min in a