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Amphibian chytridiomycosis: strategies for captive management and conservation

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Dramatic declines and extinctions of amphibian species have occurred worldwide over the last three decades owing to the introduction of chytridiomycosis. This emerging infectious disease is caused by the chytrid fungus *Batrachochytrium dendrobatidis*, a virulent water-borne pathogen of many amphibian species. It has caused epidemic waves of high mortality as it spread through susceptible wild populations in Australia, North, Central and South America, and New Zealand, and is now endemic in surviving populations in these continents and in Europe and Africa. The prevalence of chytridiomycosis in the international amphibian trade is high and import of infected frogs into zoos has caused disease epidemics in established amphibian collections. Management of disease spread requires effective national and international quarantine and control strategies. Although *B. dendrobatidis* is susceptible to a range of commonly used disinfectants, there is no universally effective treatment regime for infected amphibians. Zoological institutions can play a key role in preventing pathogen spread between captive facilities, and in disease surveillance, captive-breeding and reintroduction programmes, to limit the impact of this formidable disease on wild amphibian populations.

Key-words: amphibian; *Batrachochytrium dendrobatidis*; captive management; chytridiomycosis; decline; disinfection; frog; quarantine.

INTRODUCTION

Over 30% of amphibian species are threatened and at least 43% are experiencing population declines worldwide (Stuart *et al.*, 2004). Since 1980, rapid declines have been reported in over 400 species, with just over half of these attributed to habitat degradation and to overexploitation. In at least 200 species declines were considered enigmatic, predominantly affecting stream-associated frogs in protected forests and tropical montane habitats in the Neotropics and Australia, where environmental problems were not detected

(Stuart *et al.*, 2004). Many of these declines have now been linked to the formidable emerging infectious disease chytridiomycosis, caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Berger *et al.*, 1998; Bosch *et al.*, 2001; Lips *et al.*, 2006; Schloegel *et al.*, 2006). Chytridiomycosis has been detected in at least 144 species, over-represented by four anuran families: Bufonidae, Hylidae, Myobatrachidae and Ranidae (Speare & Berger, 2005).

Chytridiomycosis has been recorded in wild amphibian populations in Australia, New Zealand, the Caribbean, Europe, Africa, and South, Central and North America (e.g. Berger *et al.*, 1998; Bosch *et al.*, 2001; Waldman *et al.*, 2001; Muths *et al.*, 2003; Hanselmann *et al.*, 2004; Weldon *et al.*, 2004; Lips *et al.*, 2006; Schloegel *et al.*, 2006). Epidemiological evidence supports the hypothesis that *B. dendrobatidis* originated in Africa and subsequently spread from that continent via the global trade in African clawed frog *Xenopus laevis* (Weldon *et al.*, 2004). In Australia, *B. dendrobatidis* has been associated with dramatic frog population declines and extinctions, particularly in the high-altitude rainforest areas of Queensland (Berger *et al.*, 1998; Schloegel *et al.*, 2006).

EFFECTS AND DETERMINANTS OF CHYTRIDIOMYCOSIS

Infection with *B. dendrobatidis* occurs through waterborne zoospores that invade the superficial keratinized epidermal layers of amphibian skin, causing hyperkeratosis,

sloughing and erosions of the epidermis, and occasional ulcerations, in post-metamorphic frogs (Berger *et al.*, 1998, 1999). Affected frogs may display discoloured and reddened skin, abnormal posture, lethargy, anorexia, delayed response to stimuli, loss of righting reflex, seizures and death (Nichols *et al.*, 1998, 2001; Berger *et al.*, 1999). The mechanisms by which the pathogen causes death are unknown, but may include toxin release or host osmoregulatory disruption secondary to skin damage (Berger *et al.*, 1998). *Batrachochytrium dendrobatidis* can be carried in the keratinized mouthparts of tadpoles, many of which exhibit oral abnormalities, particularly jaw sheath depigmentation (Berger *et al.*, 1999; Fellers *et al.*, 2001; Rachowicz & Vredenburg, 2004; Obendorf, 2005; Knapp & Morgan, 2006). Populations of wild tadpoles may have a high prevalence of infection (Fellers *et al.*, 2001; Knapp & Morgan, 2006). While tadpoles of most species studied do not die from infection, experiments have demonstrated a reduced growth rate and smaller size at metamorphosis (Parris, 2004). Interestingly, infection with *B. dendrobatidis* in Cope's gray tree frog *Hyla chrysoscelis* tadpoles caused slower development only when predators were present, demonstrating the cumulative effect of stressors (Parris & Beaudoin, 2004).

Morbidity and mortality rates in post-metamorphic amphibians vary greatly among species (Ardipradja, 2001; Nichols *et al.*, 2001; Woodhams *et al.*, 2003; Berger *et al.*, 2004; Carey *et al.*, 2006). Mortality rates of up to 100% have been recorded during experimental transmission and natural outbreaks of *B. dendrobatidis* in susceptible captive anuran species (Berger *et al.*, 1998, 2005; Ardipradja, 2001; Nichols *et al.*, 2001). The Common green tree frog *Litoria caerulea* and Great barred frog *Mixophyes fasciolatus* have been shown experimentally to be highly susceptible host species (Ardipradja, 2001). Incubation times are generally between 18 and 70 days, but may be shorter (Berger, 2001; Nichols *et al.*, 2001). Some species can survive infection, and apparently healthy amphibians may frequently carry light infec-

tions in the wild (Hanselmann *et al.*, 2004; Retallick *et al.*, 2004; McDonald *et al.*, 2005). Introduction of *B. dendrobatidis* to naïve susceptible populations has caused epidemics of high mortality. If populations survive, the pathogen persists and becomes endemic, with reduced mortality rates and, in some cases, recovery (McDonald *et al.*, 2005). This suggests that there is selection for host resistance against the disease.

Experimentally, the fungus has been shown to persist and survive in environmental samples, independent of its host, for varying periods (Johnson & Speare, 2003, 2005). Suspected mechanisms of spread of *B. dendrobatidis* include movement through water bodies and via surface water during precipitation, movement of individual infected amphibians and translocation on fomites and vectors, such as moist substrate or possibly birds (Speare *et al.*, 2001; Johnson & Speare, 2003, 2005). The role of alternative hosts in disease transmission is currently being investigated.

Survival and growth of the chytrid fungus is temperature dependent, the optimal range being 17–25 °C (Piotrowski *et al.*, 2004). *Batrachochytrium dendrobatidis* is highly sensitive to elevated temperatures, dying in 4 hours at 37 °C (Berger, 2001; Johnson *et al.*, 2003), and may be unable to persist outside the host when soil and water temperatures exceed 25 °C for an extended period of time. Host behaviour influences temperature regimes experienced by the pathogen, and basking to elevate host body temperature may play a curative role (Woodhams *et al.*, 2003). Prevalence of infection and mortality rates in wild populations increase during cooler months (Berger *et al.*, 2004; Retallick *et al.*, 2004; McDonald *et al.*, 2005; Kriger & Hero, 2007). Experimentally, lower temperatures enhanced pathogen virulence in *M. fasciolatus*, with 100% mortality in frogs maintained at 17–23 °C, but only 50% at 27 °C (Berger *et al.*, 2004). Woodhams *et al.* (2003) reported elimination of *B. dendrobatidis* by exposing infected Red-eyed tree frog *Litoria chloris* to 37 °C for two 8 hour periods.

DIAGNOSIS

Clinical signs of chytridiomycosis in post-metamorphic frogs manifest as abnormal behaviour, neurological signs and skin lesions (Berger *et al.*, 1998, 1999; Nichols *et al.*, 1998, 2001) (Plate 1). However, these signs are non-specific and the disease cannot be diagnosed clinically. Diagnosis of chytridiomycosis requires laboratory confirmation by routine histological examination of skin sections (e.g. toe clips), direct examination of unstained skin smears, immunohistochemical staining of skin sections or polymerase chain reaction (PCR) assay of skin swabs (Berger *et al.*, 1999, 2000, 2002; Berger, 2001; Boyle *et al.*, 2004) (Plates 2–5). Diagnostic samples can be collected from live, frozen or preserved frogs. While all tests can accurately detect infection in sick frogs with severe chytridiomycosis, PCR assay of skin swabs is recommended for screening healthy frogs owing to its increased sensitivity and non-invasive nature (Table 1). Live tadpoles can be tested by collecting mouthpart swabs for PCR assay (Obendorf, 2005; A. Hyatt, unpubl. data). Visual inspection of tadpole mouthparts may also be a reliable indicator of *B. dendrobatidis* infection (Fellers *et al.*, 2001; Rachowicz & Vredenburg, 2004).

MANAGEMENT OF CHYTRIDIOMYCOSIS

Disinfection

Effective disinfection protocols are essential for the management of chytridiomycosis in captive facilities and in the field. *Batrachochytrium dendrobatidis* is susceptible to a wide range of physical and chemical treatments (Johnson *et al.*, 2003; Webb *et al.*, 2007) (Table 2). It is highly sensitive to temperatures above 32 °C, with 100% mortality within 4 hours at 37 °C and within 30 minutes at 47 °C. Equipment can be disinfected by immersion in hot water (60 °C for ≥5 minutes). Desiccation is also effective, but requires extended periods to ensure that any water has evaporated completely. A combination of heating and drying is recommended for some objects, such as clothing and equipment.

The pathogen is susceptible to several chemical disinfectants, but concentration and time of exposure are important (Johnson *et al.*, 2003; Webb *et al.*, 2007). Recommended chemicals include the quaternary ammonium compound didecyl dimethyl ammonium chloride (DDAC), benzalkonium chloride, Virkon[®], F10SC Veterinary Disinfectant[®], TriGene[®], ethanol and sodium



Plate 1. Captive-bred Great barred frog *Mixophyes fasciolatus* metamorph with naturally acquired chytridiomycosis in the terminal stages of disease. Note the depressed attitude, half-closed eyes and accumulations of sloughed skin over the body. Scale bar = 0.5 cm. Reprinted from Berger (2001).

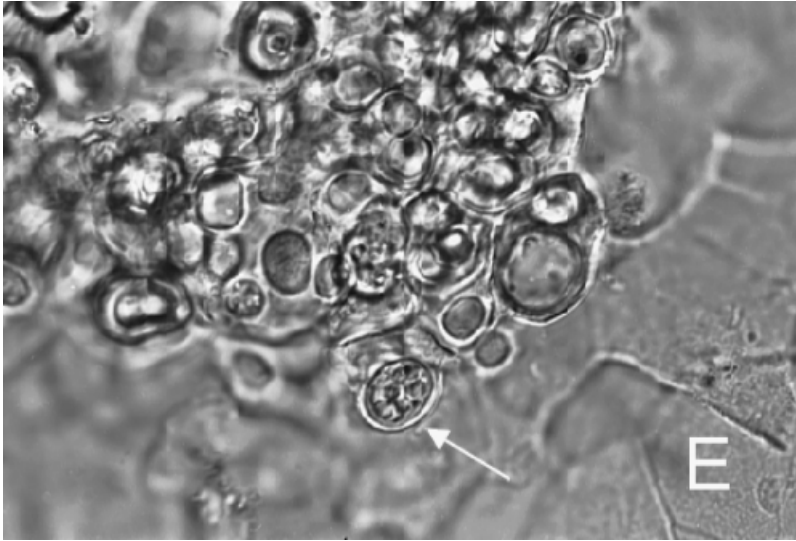


Plate 2. Unstained wet mount of shedding skin from a Common green tree frog *Litoria caerulea* infected with *Batrachochytrium dendrobatidis*. Note the refractile round and oval sporangia. Most sporangia are empty but one contains developing zoospores (arrow): E, epidermal cell; $\times 1000$ magnification. Reprinted from Berger (2001).

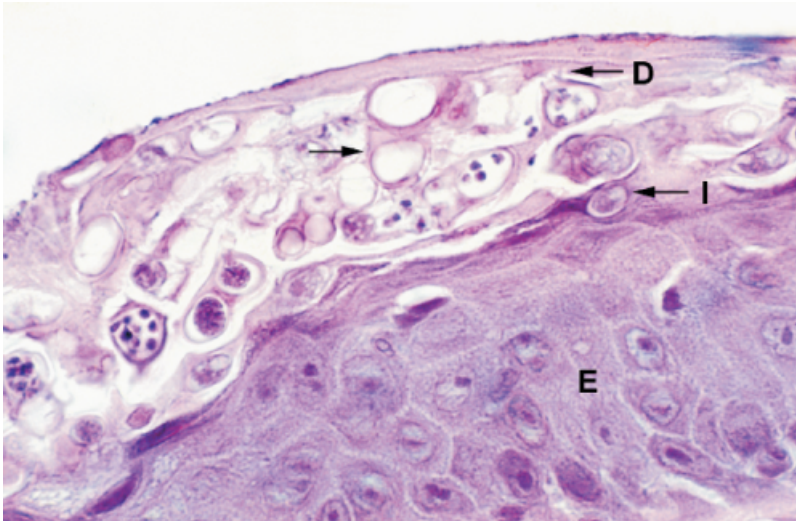


Plate 3. Histological section of skin from a Common green tree frog *Litoria caerulea* heavily infected with *Batrachochytrium dendrobatidis*. Note the homogenous immature stage (I), larger multinucleate stages, zoosporangium with discharge tube (D) containing zoospores, and empty zoosporangium after zoospores have discharged (arrow): E, epidermis. Stained with haematoxylin and eosin, $\times 1000$ magnification. Reprinted from Berger (2001).

hypochlorite (household bleach). These are particularly useful for disinfection of equipment in the field, but care must be taken

to prevent environmental contamination. Although sodium hypochlorite is an effective disinfectant, it may damage some equipment.

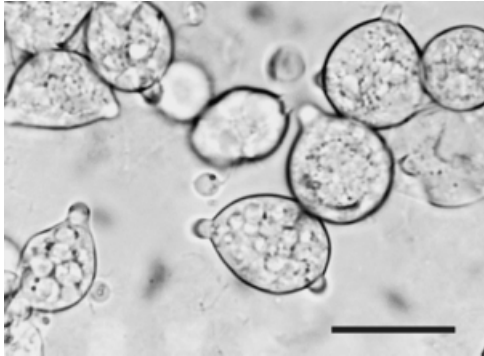


Plate 4. Live cultured *Batrachochytrium dendrobatidis* sporangia. The infective zoospores form internally and then escape by swimming out through the discharge tubes when the plugs dissolve. Scale bar = 20 μ m. Reprinted from Berger (2001).

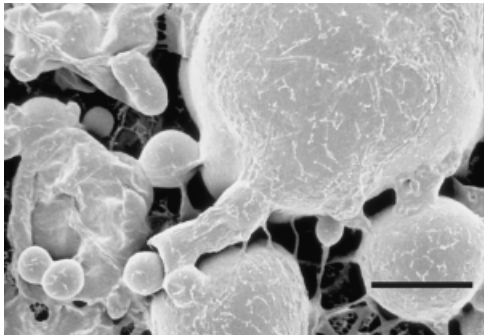


Plate 5. Scanning electron micrograph of a *Batrachochytrium dendrobatidis* zoosporangium on agar releasing a zoospore through a long discharge tube. Scale bar = 10 μ m. Reprinted from Berger (2001).

DIAGNOSTIC TEST	UNSTAINED SKIN SCRAPINGS/SMEARS	HISTOLOGY OF SKIN SECTIONS	IMMUNOSTAINING OF SKIN SECTIONS	PCR ASSAY OF SKIN SWABS
Complexity and cost of preparation	1+	2+	3+	4+
Resources required	1+	2+	3+	5+
Ease of interpretation	1+	2+	3+	5+
Sensitivity	2+	2+	3+	5+
Use for healthy frogs	Limited use	Limited use	Limited use	Very useful

Table 1. Comparison of the characteristics of diagnostic tests for amphibian chytridiomycosis. All four methods of diagnosis are useful, but each has various advantages and disadvantages. Note that frogs with clinically severe disease typically have very heavy *Batrachochytrium dendrobatidis* infections and highly sensitive tests are not required for diagnosis: 1+ = lowest, 5+ = highest; PCR, polymerase chain reaction. Adapted from Australian Government Department of the Environment and Heritage (2006) and Berger *et al.* (2007).

Treatment

Effective treatment protocols for chytridiomycosis are necessary to ensure success

of captive-breeding programmes for threatened species and to reduce risks associated with amphibian movements. Infected tadpoles survive and remain at sites after

DISINFECTANT	CONCENTRATION	EXPOSURE TIME
Ethanol	70%	1 minute
Virkon [®]	1 mg ml ⁻¹	1 minute
Benzalkonium chloride	1 mg ml ⁻¹	1 minute
Sodium hypochlorite	1%	1 minute
Didecyl dimethyl ammonium chloride	1:1000 dilution	30 seconds
F10SC Veterinary Disinfectant [®]	1:1000 dilution	1 minute
TriGene [®]	1:5000 dilution	1 minute
Complete drying	–	3 hours or more
Heat	60 °C	5 minutes

Table 2. Disinfection methods suitable for killing *Batrachochytrium dendrobatidis*, showing minimum effective concentrations and exposure times. From Johnson *et al.* (2003) and Webb *et al.* (2007).

adults have declined or disappeared following an outbreak of chytridiomycosis. Treatment of tadpoles collected from these sites would enable an emergency response to mortality and declines in threatened species through captive rearing of tadpoles and through reintroduction. Effective treatment would also prevent pathogen spread during translocation of amphibians between wild and captive populations (Australian Government Department of the Environment and Heritage, 2006). To date, no treatments have been consistently effective across species.

Itraconazole has been used successfully both orally (2–13 mg kg⁻¹ daily for 9–28 days) and via shallow immersion (0.01% suspension for 5 minutes daily for 11 days) to treat some adult amphibians (Taylor *et al.*, 1999; Nichols & Lamirande, 2000; Taylor, 2001). A commercial solution of malachite green (0.1 mg litre⁻¹) and formaldehyde (25 p.p.m.) (Formalite III[®]) effectively treated African clawed frog *Xenopus tropicalis* (Parker *et al.*, 2002), but malachite green can cause developmental deformities and is therefore not recommended for use in threatened species. Although temperature elevation to 37 °C was effective in treating *L. chloris* (Woodhams *et al.*, 2003), this treatment has not worked in other species (G. Marantelli, unpubl. data). Furthermore, some species may not tolerate these high temperatures. Itraconazole, fluconazole and F10SC Veterinary Disinfectant[®] baths for tadpoles were either ineffective or toxic (Marantelli *et al.*, 2000; B. McMeekin, unpubl. data). Treatment of water with

terbinafine hydrochloride (2–4 mg litre⁻¹ for 7 days) appears to be non-toxic to tadpoles and its efficacy in eliminating infection is currently being investigated (B. McMeekin, unpubl. data).

Quarantine

Routine quarantine procedures are critical for controlling chytridiomycosis in captivity, in the field and during translocations. Infected frogs may appear clinically normal and their transport internationally has been implicated in disease spread. There is a high prevalence of chytridiomycosis in the international amphibian trade including the pet trade (Berger *et al.*, 1999; Cunningham *et al.*, 2005), the scientific trade (e.g. Weldon *et al.*, 2004), the food trade (e.g. Mazzoni *et al.*, 2003; Hanselmann *et al.*, 2004), the ornamental trade (Daszak *et al.*, 1999) and the introduction of frogs into zoological collections (Nichols *et al.*, 1998, 2001; Pessier *et al.*, 1999; Banks & McCracken, 2002; Schloegel *et al.*, 2006). Infected frogs imported into zoos have caused epidemics of chytridiomycosis in established amphibian collections, but few of these cases have been published (Nichols *et al.*, 1998; Pessier *et al.*, 1999). This may reflect potential problems with quarantine and hygiene procedures within zoological collections.

Amphibians should be maintained in quarantine as individuals in separate containers for at least 2 months, whether moving between field sites, captive collections or

countries. During quarantine, individuals should be regularly examined for signs of disease and a thorough necropsy must be performed on any animals that die during the quarantine period (Daszak *et al.*, 2001; Lynch, 2001). Published protocols suggest temperature during quarantine should be maintained between 17 and 23 °C to increase the chance of infection becoming clinically apparent (Lynch, 2001). However, owing to the sensitivity of *B. dendrobatidis* to elevated temperatures and the high sensitivity of the PCR assay, the authors recommend holding heat-tolerant species at the maximum temperature they can tolerate, and collecting skin swabs for PCR assay on arrival and 7 weeks post-arrival.

Important hygiene practices include wearing disposable gloves and changing gloves between enclosures, disinfection of equipment between use, attending to animals in a consistent order starting with threatened species and those least likely to be infected, use of automated husbandry systems and disinfection of water used in enclosures before disposal (Marantelli & Berger, 2000; Lynch, 2001). Adhering to quarantine guidelines can prevent transmission of chytridiomycosis between frogs housed in close proximity, but even small numbers of zoospores are highly infectious and great care must be taken to prevent contamination of groups of animals with drops of water.

Hygiene protocols for fieldwork should be stringently followed, including disinfection of footwear and equipment between sites, the use of disposable gloves and plastic bags for handling frogs and disinfection of equipment between frogs. Adults should never be held together in the same container, and tadpoles for release should never be held with batches of tadpoles from other sites, even if they originate from a common water body (New South Wales National Parks and Wildlife Service, 2000; Speare *et al.*, 2004).

Control

'Infection of amphibians with chytrid fungus resulting in chytridiomycosis' was listed as a

'key threatening process' in Australia in July 2002 under the Environment Protection and Biodiversity Protection Act. This led to the development of a Threat Abatement Plan, which aims to minimize the impact of chytridiomycosis on amphibian populations through prevention of pathogen spread, recovery of at-risk threatened species, control of infection, education and coordination of management activities (Australian Government Department of the Environment and Heritage, 2006). Effective management of chytridiomycosis requires national surveillance to determine accurately disease distribution, protection of disease-free populations, rapid detection of and response to new outbreaks, restriction of amphibian movements, implementation of national hygiene and quarantine protocols, and routine monitoring of stock for chytridiomycosis.

Captive breeding can play a critical role in limiting the impact of chytridiomycosis on wild amphibian populations by providing supplemental numbers of threatened species for restocking and research (Australian Government Department of the Environment and Heritage, 2006). It may be possible to select for innate disease resistance by collecting tadpoles from declining populations, breeding from individuals that survive metamorphosis and restocking field sites with their progeny. Individuals must not be returned to their point of origin unless they can be shown to be disease-free, and diagnostic screening procedures must be implemented to ensure this. Augmenting remnant populations through restocking has been successfully used in Australia with Corroboree frog *Pseudophryne corroboree* and this strategy appears to have played an important role in slowing the decline of this Critically Endangered species (IUCN, 2006; G. Marantelli, unpubl. data). An intensive captive-breeding programme for the last remaining Wyoming toad *Bufo baxteri* population in Wyoming prevented extinction and efforts to reintroduce this species to the wild are under way (AmphibiaWeb, 2006). The success of this programme was largely a result of early recognition of the precarious status of wild populations, extensive collaborative captive-

breeding efforts involving government and zoological institutions, and habitat protection through land purchase. The urgent need for captive-breeding programmes for a number of other threatened species has been recognized but many of these programmes are in their infancy owing to limited captive-breeding success.

Restocking of threatened species may increase their chance of survival when high mortality rates threaten to cause extinction. This strategy can increase the time available for a species to develop disease resistance and maintain population numbers during adverse conditions that favour the pathogen. Captive-reared amphibians used for restocking must be kept disease-free, and restocking must be implemented before the final population of a species is under threat, or extinction may not be preventable (Australian Government Department of the Environment and Heritage, 2006). This is demonstrated by the case of Sharp-snouted day frog *Taudactylus acutirostris* in Australia, where frogs and tadpoles were transferred from the last remaining population into captivity. As the wild population crashed from chytridiomycosis, the captive specimens also died from the disease, resulting in extinction of the species (Banks & McCracken, 2002; Schloegel *et al.*, 2006; Australian Government Department of the Environment and Heritage, 2007).

Batrachochytrium dendrobatidis continues to spread through naïve populations in Panama, causing severe declines and threatening large numbers of frog species (Lips *et al.*, 2006). Emergency response to this situation has involved moving as many individuals as possible into captivity (B. Goodman, 'To stem widespread extinction, scientists airlift frogs in carry-on bags', *New York Times*, 6 June 2006, <http://www.nytimes.com/2006/06/06/science/06frog.html?ex=1307246400&en=7eadca40d157485d&ei=5088&partner=rssnyt&emc=rss>), but without established captive-breeding programmes and treatment and quarantine protocols, this strategy may not be successful in preventing extinctions. Until successful treatments are identified, prevention of pathogen spread, captive breed-

ing for restocking well in advance of disease outbreaks in the wild and captive rearing and breeding from tadpoles to select for innate resistance are the most important strategies to limit the impact of chytridiomycosis on wild populations.

FUTURE DIRECTIONS

Dedicated resources on a global scale must be made available in zoological and other institutions for captive breeding of threatened species and for emergency response to population declines. Researching the natural history and biology of these species in the wild and in captivity must be prioritized to enable captive-breeding success. To date, few captive-breeding programmes for threatened species have been successful owing to a lack of critical knowledge about the species. Captive-breeding programmes must be well-planned and implemented in advance of the threat to survival of a species, and there must be extensive collaboration between participating institutions worldwide. Although cryopreservation of amphibian semen has been achieved, the large size and thick capsules of eggs and embryos make successful freezing unlikely (Sargent & Mohun, 2005).

Further research is needed to determine effective treatments for tadpoles and adults, and whether selection for innate disease resistance is a feasible management strategy for both captive and wild populations. Surveillance of amphibian populations must be co-ordinated nationally and internationally to determine accurately the distribution of chytridiomycosis and to rapidly detect and respond to outbreaks (Australian Government Department of the Environment and Heritage, 2006). Increased efforts to educate the public should be undertaken to reduce disease transmission through human activity and through accidental translocation of infected amphibians and other materials. Zoological institutions can play an important role in supporting these activities, both internally and externally.

Finally, while zoos can play a key role in contributing to amphibian conservation,

maintaining threatened species in captivity must be recognized as only a short-term solution because of space limitations and the inability to maintain genetically viable populations indefinitely. Resources must be made available immediately to preserve and restore the natural habitats of amphibians. Removing other significant threats to amphibians such as habitat loss and degradation will maximize population sizes and may assist in survival and recovery from the impact of chytridiomycosis. Habitat protection will ultimately preserve whole ecosystems, not just individual threatened species, and should be a priority in the global response to chytridiomycosis.

For detailed information about chytridiomycosis, including diagnosis and management, see the Amphibian Disease Home Page: <http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm>

Some related amphibian conservation web sites include:

- AmphibiaWeb: <http://www.amphibiaweb.org>
- Declining Amphibian Populations Task Force: <http://www.open.ac.uk/daptf/index.htm>
- IUCN Global Amphibian Assessment: <http://www.globalamphibians.org>
- IUCN Species Survival Commission Conservation Breeding Specialist Group: <http://www.cbsg.org/amphibian/php>
- World Association of Zoos and Aquariums: <http://www.waza.org/conservation/campaigns21.php?view=campaigns&id=1>

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PRODUCTS MENTIONED IN THE TEXT

F10SC Veterinary Disinfectant[®]: broad-spectrum multi-purpose disinfectant and sanitizer, manufactured by Health and Hygiene Pty Ltd, PO Box 34, Sunninghill, 2157, South Africa.

Formalite III[®]: aquarium antimicrobial in-water treatment, manufactured by Aquatronics, PO Box 2457, Oxnard, CA 93034, USA.

TriGene[®]: broad-spectrum disinfectant concentrate, manufactured by MediChem International (Manufactur-

ing) Ltd, Unit 3, Stalham Business Centre, Rushenden Rd, Queenborough, Kent ME11 5HE, UK.

Virkon[®]: broad-spectrum disinfectant effective against viruses, bacteria and fungi, manufactured by Antec International Ltd, Sudbury, Suffolk CO10 2XD, UK.

DIAGNOSTIC SERVICES OFFERING CHYTRID PCR TESTING

Australian Animal Health Laboratory: CSIRO Division of Livestock Industries, 5 Portarlington Road, Geelong VIC 3220, Australia. Contact Alex Hyatt (alex.hyatt@csiro.au) before sample submission.

Institute of Zoology: Zoological Society of London, Regent's Park, London NW1 4RY, UK. Contact Matt Perkins (matthew.perkins@ioz.ac.uk) and Clyde Hutchinson (clyde.hutchinson@ioz.ac.uk) before sample submission.

Pisces Molecular: 5311 Western Avenue, Suite E, Boulder CO 80301, USA. Contact John Wood (jwood@pisces-molecular.com) before sample submission.

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