

**Disease Strategy**  
**Chytridiomycosis**  
**(Infection with *Batrachochytrium*  
*dendrobatidis*)**  
**Version 1, 2012**

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# Preface

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This disease strategy is for the control and eradication of Chytridiomycosis/*Batrachochytrium dendrobatidis*. It is one action among 68 actions in a national plan to help abate the key threatening process of chytridiomycosis (Australian Government 2006). The action is number 1.1.3: "Prepare a model action plan (written along the lines of AusVetPlan – <http://www.aahc.com.au/ausvetplan/>) for chytridiomycosis – free populations based on a risk management approach, setting out the steps of a coordinated response if infection with chytridiomycosis is detected. The model action plan will be based on a risk management approach using quantitative risk analysis where possible and will be able to be modified to become area-specific or population-specific. The plan could be implemented in the face of new outbreaks in chytridiomycosis-free areas or in chytridiomycosis-free populations. Individual jurisdictions can modify the model action plan as a preventative strategy or at least have it available as the framework for a response plan if needed. This will help ensure national consistency in responses to any new outbreaks. For threatened species, the action plan should inform relevant species recovery plans. Infrastructure, protocols, responsibilities and funding sources should be identified in this action plan, using the approach used in AusVetPlan. To protect areas that are chytridiomycosis-free, an underlying principle should be that amphibians with chytridiomycosis are not transported into chytridiomycosis-free areas. Actions to reduce transmission into chytridiomycosis-free areas should aim for reduction of risk at source, and prevention of dissemination of *B. dendrobatidis* at destination. "

The national threat abatement plan which addresses five objectives of disease threat abatement: 1. reducing spread, 2. reducing impact, 3. research and monitoring, 4. informing and 5. coordinating is available at <http://www.environment.gov.au/biodiversity/threatened/publications/tap/chytrid.html>). It is currently undergoing a 5 year review. There are a number of recommendations in this disease strategy that could be adopted by this review such as a list of species to which this strategy could apply as well as a list of endangered species that have declined due to chytridiomycosis which would benefit from adopting some aspects of this strategy. Ideally this should be done with the relevant State and Territory environmental agencies and stakeholders (see section 3.4 Funding and compensation arrangements) to ensure adoption of the strategy by amphibian managers.

Disease strategy manuals are response manuals and do not include information about other aspects of controlling disease such as preventing the introduction of disease into Australia.

For example, the Australian Quarantine and Inspection Service (AQIS) provides quarantine inspection for international passengers, cargo, mail, animals, plants and animal or plant products arriving in Australia, and inspection and certification for a range of agricultural products exported from Australia. Quarantine controls at Australia's borders minimise the risk of entry of exotic pests and diseases, thereby protecting Australia's favourable human, animal and plant health status.

Information on current import conditions can be found at the AQIS ICON website.<sup>1</sup>

This strategy sets out the disease control principles for use in an emergency incident caused by Chytridiomycosis/*Batrachochytrium dendrobatidis* in Australia. Chytridiomycosis was introduced into Australia at least by 1978 and is thought to have caused amphibian declines and extinctions in 1979 (Skerratt et al 2007, 2011b, Murray et al 2010). A disease investigation began in 1993 and the novel disease chytridiomycosis was found to be the cause of widespread amphibian declines and extinctions (Laurance et al 1996, Berger et al 1998). Now the disease is widespread throughout most of its preferred range and there are only a few uninfected populations where chytridiomycosis may have an impact on conservation (Murray et al 2010, 2011a, 2011d).

The trigger for implementing this disease strategy should be uninfected amphibian populations predicted to be at risk of decline from chytridiomycosis. Protocols for surveying populations and predictive tools for risk of decline are available (Skerratt et al 2008, Murray et al 2011a) and have been used to inform use of this disease strategy in some regions (Pauza et al 2010, Skerratt et al 2010a).

In addition, there are several species that have undergone dramatic decline due to chytridiomycosis and survive as a small remnant population of less than a 1000 individuals that have not had an emergency response such as the armoured mist frog, *Litoria lorica* (Puschendorf et al 2011). These species could benefit from undertaking components of this strategy.

Chytridiomycosis/*Batrachochytrium dendrobatidis* is listed as a notifiable disease in *Australia's National List of Reportable Diseases of Aquatic Animals*<sup>2</sup> and by the World Organisation for Animal Health (OIE, formerly Office International des Epizooties) in the *Aquatic Animal Health Code*.<sup>3</sup>

This first edition of this manual was prepared by Lee Berger and Lee F. Skerratt, James Cook University. The authors were responsible for drafting the strategy, in consultation with a wide range of stakeholders throughout Australia. However, the policies expressed in this version do not necessarily reflect the views of the authors. The authors would like to thank Rupert Woods, Tiggy Grillo, Alison Oberg, Katrina Daniels, Murray Evans, David Hunter, Renate Velzeboer and Alistair Herfort for their contributions. Contributions made by others not mentioned here are also gratefully acknowledged.

The format of this manual was adapted from similar manuals. The format and content have been kept as similar as possible to these documents, in order to enable animal health professionals trained in emergency animal disease procedures to work efficiently with this document in the event of an emergency.

The manual has been reviewed and approved by the following representatives of government and industry:

**Government**

**Industry**

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<sup>1</sup> <http://www.aqis.gov.au/icon32/asp/homecontent.asp>

<sup>2</sup> <http://www.daff.gov.au/animal-plant-health/aquatic/reporting/reportable-diseases>

<sup>3</sup> [http://www.oie.int/eng/normes/fcode/fcode2006\\_back/en\\_sommaire.htm](http://www.oie.int/eng/normes/fcode/fcode2006_back/en_sommaire.htm)

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# Contents

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Preface .....	2
1 Nature of the disease .....	7
1.1 Aetiology .....	7
1.2 Susceptible species .....	8
1.3 World distribution.....	8
1.4 Diagnosis of infection with <i>Batrachochytrium dendrobatidis</i> .....	10
1.4.1 Field methods: clinical signs and gross pathology .....	10
1.4.2 Laboratory methods.....	11
1.4.3 Confirmation of diagnosis.....	13
1.4.4 Differential diagnosis.....	14
1.5 Resistance and immunity .....	14
1.6 Epidemiology.....	15
1.6.1 Incubation period .....	15
1.6.2 Persistence of the pathogen .....	15
1.6.3 Modes of transmission.....	16
1.6.4 Factors influencing transmission and expression of disease.....	16
1.7 Impact.....	17
2 Principles of control and eradication .....	18
2.1 Introduction .....	18
2.2 Methods to prevent spread and eliminate pathogens .....	19
2.2.1 Quarantine and movement controls.....	19
2.2.2 Tracing .....	21
2.2.3 Surveillance.....	21
2.2.4 Treatment of infected host species.....	22
2.2.5 Treatment of host products and by-products.....	23

2.2.6	Destruction of hosts .....	23
2.2.7	Disposal of hosts.....	23
2.2.8	Decontamination .....	23
2.2.9	Vaccination.....	24
2.2.10	Vector control.....	24
2.3	Environmental considerations .....	25
2.4	Sentinel animals and restocking measures.....	25
2.5	Public Awareness.....	25
2.6	Feasibility of control or eradication of <i>Batrachochytrium dendrobatidis</i> in Australia .	26
2.6.1	Response Option 1: [eg Eradication] .....	26
2.6.2	Response Option 2: [eg Containment, control and zoning] .....	27
2.6.3	Response Option 3: [eg Control and mitigation of disease].....	27
2.6.4	Trade and industry considerations.....	28
3	Preferred Australian response options .....	30
3.1	Overall policy for Chytridiomycosis .....	30
3.2	Response Options.....	31
3.2.1	Option 1 - Eradication.....	31
3.2.2	Option 2 - Containment, control and zoning.....	32
3.2.3	Option 3 - Control and mitigation of disease .....	32
3.3	Criteria for proof of freedom.....	33
3.4	Funding and compensation.....	34
Appendix 1	OIE <i>Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals</i> .....	36
Appendix 2	Approval of chemicals for use in Australia .....	37
	Minor use permit system .....	37
	Emergency use permits .....	37
References	.....	38

# 1 Nature of the disease

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Chytridiomycosis is the most significant disease to affect vertebrate biodiversity and has caused the decline and extinction of several hundred amphibian species globally (Skerratt et al 2007). In Australia it has caused the extinction of at least four species, probably 7, and the dramatic decline of at least 10 more (Australian Government 2006, Murray et al 2010). The four species listed as extinct are from Queensland: *Rheobatrachus silus* (Southern Gastric-brooding Frog, last seen 1981), *Rheobatrachus vitellinus* (Northern Gastric-brooding Frog, 1985), *Taudactylus acutirostris* (Sharp-snouted Day Frog, 1997), and *Taudactylus diurnus* (Southern Day Frog, 1979). Many persisting species remain at lower abundance and smaller distributions than pre-disease, some are continuing to decline and significant mortality from chytridiomycosis is ongoing even decades after introduction (McDonald and Alford 1999, Murray et al 2009, Hunter et al 2010).

Chytridiomycosis is caused by the amphibian chytrid fungus *Batrachochytrium dendrobatidis* and affects many species of amphibians. It is a disease of keratinised epithelia such as the skin of frogs and mouthparts of tadpoles. The disease first appeared in Australia in the 1970s and is now widespread (Murray et al 2010) but there appear to be suitable habitats where the fungus is still absent (Murray et al 2011d). Chytridiomycosis is recognised as a Key Threatening Process to biodiversity under the EPBC Act by the Australian Government (Australian Government 2006, Skerratt et al 2007).

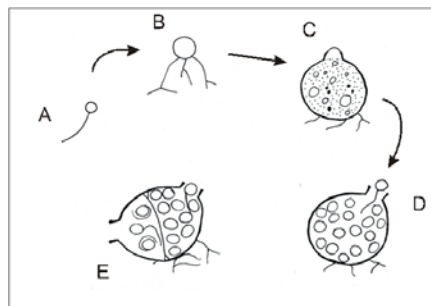
Currently there are no proven methods to control the disease in the wild. For currently endangered frog species, emergency measures are needed to increase population sizes through captive assurance colonies. As *B. dendrobatidis* is now widely distributed in Australia, control efforts should be aimed at protecting uninfected areas - this is the opposite focus to standard emergency responses. Naive areas exist containing endemic frogs that are at high risk. As strains vary in virulence, reducing the risk of spread between infected areas is also important. Research to improve mitigation of the impact of the disease in infected wild populations is urgently required.

## 1.1 Aetiology

*Batrachochytrium dendrobatidis* is an aquatic fungus in the phylum Chytridiomycota. The spherical sporangia occur within superficial cells of the keratinised epidermis of amphibians (Berger et al 1998, Longcore et al 1999). The transmissible aquatic flagellated zoospore stage is released via discharge tubes into the environment, and lives for about a day before encysting. The life cycle takes about 4-5 days at 23°C. There appears to be a pandemic lineage of high virulence which is present in Australia (Berger et al 2005b, Garland et al 2011a, Farrer et al 2011). Isolates of this lineage show slight variation in virulence. Genetic data suggests that this strain is highly virulent due to a previous recombination event (James et al 2009, Farrer et al 2011). The parent lineages have not been identified but other less virulent lineages do exist (Goka et al 2009, Farrer et al 2011). The pandemic lineage has lost heterozygosity as it has spread (Velo-Antón et al 2012). A commonly used

diagnostic real time qPCR test used in Australia detects the pandemic lineage but may not detect other lineages (Boyle et al 2004, Goka et al 2009). Preventing further spread and mixing of strains may reduce the risks of additional virulent strains appearing.

**Figure 1. Life cycle of *Batrachochytrium dendrobatidis*. A = waterborne, flagellated zoospore, B = germling with rhizoids, C = immature sporangium, D = mature zoosporangium with open discharge tube, E = colonial growth form of mature zoosporangium. (from Berger et al 2005a)**



## 1.2 Susceptible species

Most amphibians appear to be susceptible to infection. Many suffer severe morbidity and mortality under laboratory conditions (Berger et al 2009), but not all innately susceptible species have declined in the wild. Life history of the host and the nature of the environment determine likelihood of exposure to and virulence of the pathogen as *B. dendrobatidis* is susceptible to desiccation and extreme cold and heat and amphibians are poikilothermic (Berger et al 1998, 2004, Piotrowski et al 2004, Murray et al 2011b). Therefore, only approximately half of Australia's amphibian species have been shown or predicted to be infected (Murray et al 2010, Murray and Skerratt 2012). Infection with *B. dendrobatidis* has been recorded from 63 frog species in Australia. All infected species belong to the Hylidae, Limnodynastidae, and Myobatrachidae, except for the introduced cane toad of the family Bufonidae and one PCR positive individual of a species from the Microhylidae. A list of species reported to be infected are included in Murray et al (2010). Some of these species are resistant to mortality and serve as disease reservoirs and carriers. The number of species that occur in areas that are, or are predicted to be, severely impacted by the disease is further reduced to approximately a quarter of species (Murray et al 2011a,b). These are generally species that are associated with cool aquatic environments especially streams or ponds in habitats sheltered from climatic extremes such as wet forests. *Batrachochytrium dendrobatidis* is not zoonotic (Mendez et al 2008).

## 1.3 World distribution

The highly virulent lineage of *B. dendrobatidis* causing chytridiomycosis is widespread throughout the world although some countries remain free (Skerratt et



al 2007, Berger et al 2009, global *B. dendrobatidis* website: <http://www.bd-maps.net/>).

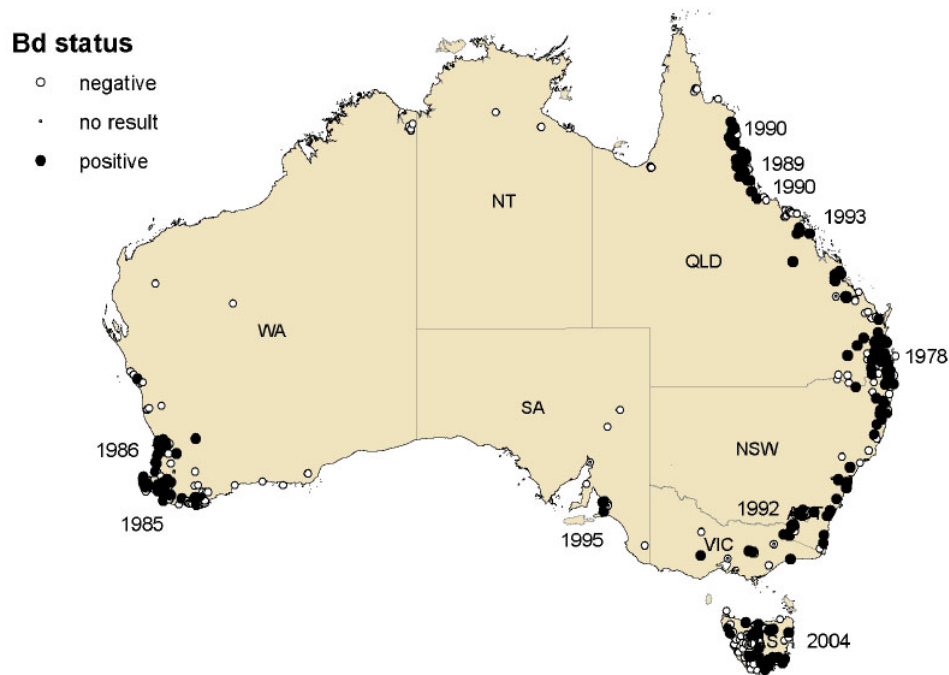
In Australia, distribution models show *B. dendrobatidis* has spread to most regions of suitable environment except for the World Heritage Area in southwest Tasmania and the Iron Range on Cape York (Murray et al 2011d). There are some pockets of free areas within infected regions due to the isolated nature of some amphibian populations.

“Chytridiomycosis has been found in all Australian states and the Australian Capital Territory, but not in the Northern Territory. Currently it appears to be confined to the relatively cool and wet areas of Australia, such as along the Great Dividing Range and adjacent coastal areas in the eastern mainland states of Queensland, New South Wales, and Victoria, eastern and central Tasmania, southern South Australia, and southwestern Western Australia (Figure 1). Results from testing archived museum specimens indicate *B. dendrobatidis* may have been introduced into Australia via the port of Brisbane around 1978 and spread northward and southward. It did not appear to arrive in Western Australia until 1985. The earliest records from South Australia and Tasmania are from 1995 and 2004, respectively, although since archival studies from these states have not been completed the date of arrival is unknown. Negative surveys show that the disease does not currently occur in some areas that appear to be environmentally suitable, including Cape York Peninsula in Queensland and most of the World Heritage Area in western Tasmania” (Murray et al 2010). The incidence is high in infected populations and varies seasonally. A few populations in NSW and Victoria are currently free of infection.

Isolates of the pandemic lineage in Australia show variation in virulence and genotype (Berger et al 2005b, Garland et al. 2011a) but their distribution is unknown.

General hygiene and biosecurity practices aim to mitigate the spread of chytridiomycosis into naive populations and limit the impact on populations already infected (Phyllott et al 2010, Murray et al 2011b). State environmental agencies such as NSW OEH and Queensland DERM require scientific permit holders to adopt recommended hygiene protocols. A participatory biosecurity program is being conducted by NRM South, Tasmanian DPIPWE and JCU to control the spread of chytridiomycosis into the Tasmanian World Heritage Area.

Figure 2. Map of the distribution of chytridiomycosis with dates of first detection. (from Murray et al 2010)



#### 1.4 Diagnosis of infection with *Batrachochytrium dendrobatidis*

Diagnostic tests are needed to detect infection in frogs and tadpoles. The preferred tests are the standard and real-time PCR tests which are rapid, have good sensitivity and can be used in all situations. The real-time test is more expensive but provides data on intensity of infection. PCR can be done on non-invasive swab samples.

Microscopy is only highly accurate in sick animals, and is useful when facilities and skills for microscopy are more easily accessed than PCR. Microscopy includes histology, wet preparations and immunostaining and requires pieces of whole or shed skin.

Histological examination of all organs by a pathologist is important if ill or dead frogs are found, as part of general disease surveillance for other endemic or emerging diseases.

Details on various specific tests for *B. dendrobatidis* are presented below.

##### 1.4.1 Field methods: clinical signs and gross pathology

Clinical signs of chytridiomycosis in juvenile and adult frogs may include erythema of ventral surfaces, abnormal posture such as splayed limbs, depression, slow righting reflex, abnormal skin shedding and ulceration and tetanic spasms upon handling (Berger et al 2009). None of these signs are specific for chytridiomycosis. They generally occur in the terminal stages of disease and correlate with heavy infections, severe skin pathology and loss of plasma electrolytes due to disturbance of epidermal ion transport (Voyles et al 2009). Therefore most of the course of infection remains subclinical.

In tadpoles infection causes mouthpart abnormalities including loss of dark tooth rows which can lead to emaciation (Cashins 2009). Adults and tadpoles may recover fully from severe disease with antifungal and supportive treatment and show no signs of lesions afterwards (Young et al in press).

#### **1.4.2 Laboratory methods**

Technical details of diagnostic methods are available in the online OIE Manual of Diagnostic Tests for Aquatic Animals 2011 (Chapter 2.1.1 Infection with *Batrachochytrium dendrobatidis* [http://www.oie.int/fileadmin/Home/eng/Health\\_standards/aahm/2010/2.01.01\\_BATRACHO.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.01.01_BATRACHO.pdf)).

#### **Sample submission**

For PCR testing the most common diagnostic sample is an air dried swab from tadpole mouthparts or adult ventral surfaces.

Other sampling methods are available such as using air dried filters but are less efficient (Hyatt et al 2007). These methods may be required under certain circumstances such as when testing water bodies or when handling amphibians is contraindicated.

Swabs or filters can be stored for at least 18 months when frozen or at 23 °C and shipped at ambient air temperatures without loss of analytical sensitivity (Hyatt et al 2007). There is, however, some slight loss of analytical sensitivity due to DNA degradation at air temperatures of 45 °C (Van Sluys et al 2008). Diagnostic PCR tests for swabs and filters are available at CSIRO AAHL<sup>4</sup>, JCU ABC<sup>5</sup> and Tasmanian DPIPWE<sup>6</sup>.

For histopathology, toe clips, ventral skin biopsies, whole tadpoles or frogs with ventral midline incisions can be fixed in 10% neutral buffered formalin or 70% alcohol and submitted to veterinary pathology laboratories.

If sick or dead frogs are found, they should be necropsied or preserved and submitted for pathology testing (Rose 2007).

#### **Microscopy**

Microscopy has high specificity and, when used on diseased frogs with heavy infections, high sensitivity. Microscopy is not recommended for surveys of healthy wild frogs as sensitivity is low (~25%) (Skerratt et al 2011a).

#### ***Examination of Wet Skin Preparations***

Samples can be obtained from fresh or fixed tissue for diagnostic testing. Shedding stratum corneum, whole skin, or excised tadpole mouthparts are spread out flat or squashed on a slide with a drop of water, covered by a cover-slip and examined under a light microscope (Berger et al 2009). The round to oval, refractile

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<sup>6</sup> Tasmanian Department of Primary Industries, Parks, Water and Environment, Mt Pleasant Laboratory, Ph: 03 6336 5216, Email: specimenreception@dpipwe.tas.gov.au

intracellular sporangia occur in clumps. Old empty sporangia are the most prevalent stage in shedding skin, although sporangia containing zoospores are commonly found. Discharge tubes usually point perpendicularly to the skin's surface and thus appear as small circles, which can be difficult to discern. The observation of internal septa within sporangia, a characteristic of *Batrachochytrium dendrobatidis* infection, increases confidence in the diagnosis. Epidermal cell nuclei are of similar size to sporangia but can be differentiated by their irregular, indistinct membranes and flat, granular, grey appearance.

Stains have been used to highlight sporangia such as 0.01% Congo red stain (20 mins) or a 1:1 mixture of cotton blue (Parker ink) and 10% aqueous KOH. These may increase ease of detection, however, a comparison of their sensitivity with unstained skin has not been published.

### ***Histology***

Cross sections of ventral abdominal or thigh skin, digits, whole feet or tadpole mouthparts can be examined with H&E staining (Berger et al 2009). In the *stratum corneum* the sporangia appear spherical or oval (5 to 13  $\mu\text{m}$ ) with a smooth eosinophilic wall. Discharge papillae are occasionally seen and project towards the skin surface. The contents of the zoosporangia vary with three developmental stages; (1) immature sporangia have a central basophilic homogenous mass, (2) zoosporangia become multinucleate and then the cytoplasm divides to form zoospores - 4 to 10 may be visible, and (3), after zoospore release the empty zoosporangia remain. In some empty colonial stages, thin septa are visible dividing the sporangium into internal compartments.

Focal hyperkeratosis and erosions are common in infected areas. Irregular thickening of the epidermis (hyperplasia) may be present. There may be necrotic changes in the basal epidermal cells. Extensive sloughing of the hyperkeratotic layer can leave the epidermis with few organisms. To confirm suspect cases, sporangia can be highlighted using special fungal stains, such as periodic acid-Schiff (PAS) or silver stains.

Where there is doubt about a histological diagnosis, samples can be referred to Karrie Rose (krose@zoo.nsw.gov.au) at Australian Registry of Wildlife Health or Lee Berger (Lee.Berger@jcu.edu.au) at James Cook University or veterinary pathologists with an interest in chytridiomycosis.

### ***Immunohistochemistry***

Polyclonal antibodies against *B. dendrobatidis* can be used in a robust immunoperoxidase test for easier detection and slightly increased sensitivity (Berger et al 2002, Hyatt et al 2007, Skerratt et al 2011a). The immunoperoxidase stain is useful for confirming suspect infections seen on H&E sections, and is more specific than special fungal stains. The stain is available at CSIRO AAHL and JCU, or AAHL can supply the antibodies to laboratories for in-house testing.

### ***Culture methods***

As *B. dendrobatidis* is slow-growing compared with microorganism contaminants, culture is difficult and is not used for diagnosis (Longcore et al 1999, Berger et al 2009). To obtain isolates, small pieces (<1 mm) of fresh infected skin or tadpole mouthparts are wiped on nutrient agar in an attempt to remove other microbes,

then placed on a clean plate and incubated at 17 - 23C. Tryptone or TGH (Tryptone, Gelatine Hydrolysate, Lactose) agar with antibiotics (penicillin /streptomycin) are routinely used.

### **Preparation for parasite identification**

Identification can be performed by recognition of characteristic morphology on microscopy using wet preparations or histology. The presence of discharge tubes, internal septae and zoospores, as well as size, clustering and epidermal location of zoosporangia are diagnostic features.

Some strain genotyping methods can be done using skin swabs (Garland et al 2011a) but others require pure isolates (James et al 2009, Farrer et al 2011). A standard method for differentiating strains is not yet available.

### **Molecular Techniques**

PCR is the preferred test for diagnosis of chytridiomycosis and can be used in all situations. Tests can be completed in a few hours. See the online OIE Manual of Diagnostic Tests for Aquatic Animals 2011 for details of the real-time PCR method ([http://www.oie.int/fileadmin/Home/eng/Health\\_standards/aahm/2010/2.01.01\\_BATRACHO.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.01.01_BATRACHO.pdf)).

There are two diagnostic PCR tests which detect *B. dendrobatidis* DNA - conventional PCR (Annis et al 2004) and a quantitative real time PCR (Boyle et al 2004, Garland et al 2010). Only the Boyle et al (2004) test has been rigorously validated in the laboratory and in the field (Hyatt et al 2007, Skerratt et al 2011a). The Boyle et al (2004) test detects the pandemic lineage but may not detect other lineages (Goka et al 2004). The real time qPCR test enables more accurate quantification but conventional PCR is almost as sensitive, requires less costly equipment and is cheaper (Garland et al 2011b). In five species of frogs at different times of the year at six locations in the Wet Tropics of northern Queensland the sensitivity and specificity of the Boyle et al (2004) qPCR test and histology were estimated using prior estimates from previous laboratory studies and Bayesian methods to incorporate field data to obtain posterior estimates. The qPCR test was almost three times as sensitive 72.9% (62.7-82.2%) than histology 26.5% (19.9-33.9%) but was less specific 94.2% (89.3-98.6%) than histology 99.5% (98.4-100%), which was likely caused by laboratory contamination (Skerratt et al 2011a). Following OIE protocols and the methods suggested in Skerratt et al (2011a) is likely to reduce laboratory contamination to <1%. Monitoring of the negative control success rate of the qPCR test is potentially a good indicator of specificity achieved in each laboratory.

#### **1.4.3 Confirmation of infection**

Confirmation of clinically severe chytridiomycosis requires observation of typical clinical signs and a moderate to heavy burden of *B. dendrobatidis* as detected by microscopy or PCR. A pathogenic burden varies between species but approximately 10,000 zoospore equivalents on PCR has been reported from ill frogs of some species (Kinney et al 2011). Occasionally, histology of clinical cases reveals severe skin pathology with erosions and ulcerations but only a light infection remaining. Lack of significant pathology in internal organs is typical.

Subclinical infection can be confirmed by PCR although specificity will be very slightly improved if histology is also done (Skerratt et al 2011a). Conducting both diagnostic tests is useful in some situations, such as confirming the index case in a naive area. Alternatively, positive PCR results from more than one individual would be sufficient to confirm infection in an area or species.

#### **1.4.4 Differential diagnosis**

There are potentially many unknown diseases with signs similar to those found with chytridiomycosis. This is likely due to the non-specific clinical signs associated with chytridiomycosis and the paucity of knowledge of amphibian diseases. If ill or dead frogs are found, pathological investigations are essential as part of general disease surveillance for all diseases.

Accumulation of small pieces of shedding skin is highly suggestive of chytridiomycosis, however, lethargy, abnormal posture and erythema are typical in sick frogs and may be the only signs seen with chytridiomycosis.

The two major diseases that can present similarly are ranaviral disease and bacterial septicemia “redleg” (Hemingway et al 2009). Ranaviral disease is listed as notifiable by the OIE (Schloegel et al 2010) and causes mass mortality events overseas, however, despite also being listed nationally as reportable, ranavirus species in Australia have not been associated with disease outbreaks in wild frogs. Clinical signs of ranaviral disease may include reddening of skin, dermal ulcers, ascites, and haemorrhage. Diagnosis is with histopathology and virus PCR or cell culture.

Bacterial septicemia is a disease of captive frogs and can present with reddening of skin and ascites. As bacteria are commonly cultured from sick frogs with any disease, detection of bacteria in organs by histology, as well as tests to rule out other infections, are needed to confirm bacterial septicemia as the prime aetiology.

## **1.5 Resistance and immunity**

Amphibians vary in their innate immunity to chytridiomycosis both within and among populations and species (Berger et al 2009, Woodhams et al 2007, Savage and Zamudio 2011). Resistant species include some of the major pest amphibians e.g. cane toads, bullfrogs and African clawed frogs. Some resistant species are healthy carriers whereas others may clear infection rapidly. Juveniles followed by adults and then tadpoles are the life stages most susceptible to morbidity from chytridiomycosis (Berger et al 2009).

Within a species it has been shown that Major Histocompatibility Complex heterozygosity is associated with improved survival (Savage and Zamudio 2011). Antimicrobial peptides from skin glands may help to control infection (Rollins-Smith et al 2011). Other mechanisms of innate immunity and natural selection for immunity have not been clearly demonstrated. *Batrachochytrium dendrobatidis* appears to be immunosuppressive (Kindermann et al 2012, Rosenblum et al 2009, Rollins-Smith et al 2011) and may prevent an adaptive immune response controlling the intensity of infection in some life stages and species (Dr Sam Young JCU 2011 et al unpublished, Dr Scott Cashins JCU 2011 et al unpublished).

Adaptive immunity has not been demonstrated in Australian species. Prior experimental infection did not protect frogs (*Litoria booroolongensis*) from chytridiomycosis during a second exposure (Dr Scott Cashins JCU et al unpublished 2011). However, some adaptive immunity appears present in some species of tadpoles and frogs under certain conditions (Murphy et al 2011, Cashins 2009). A vaccine is unlikely to be available in the near future given the difficulties in overcoming the immunosuppressive effects of *B. dendrobatidis*.

## 1.6 Epidemiology

Chytridiomycosis mostly affects amphibian species associated with permanent water such as streams, moist bogs or soaks and ponds (Kriger and Hero 2007b, Skerratt et al 2008, 2010a, Murray et al 2011a, Murray and Skerratt 2012). The disease is strongly mitigated by high temperatures (Piotrowski et al 2004, Berger et al 2004, Skerratt et al 2007) and hence is more severe in moderate climates such as winter in north eastern Australia, spring in central eastern Australia and early summer in southern Australia (Berger et al 2004, Murray et al 2009, Kriger and Hero 2007a, Aziz et al 2011, Skerratt et al 2010b, Dr David Hunter NSW OEH 2011 et al unpublished). Higher prevalence and disease outbreaks occur seasonally – winter outbreaks occur in eastern Queensland precipitated by moderate temperatures (Berger et al 2004, McDonald et al 2005). Chytridiomycosis is unlikely to occur in arid regions based on predictive modelling and surveys although there may be small isolated wet habitats where it could occur (Murray et al 2010, Skerratt et al 2010a, Simpkins et al 2010).

### 1.6.1 Incubation period

Incubation periods have been reported between 10 days and several months in the laboratory and the field, but vary greatly depending on the strain and dose of the pathogen as well as host and environmental factors (Berger et al 2005b, 2009, Murray et al 2009). Individuals may lose or gain infection repeatedly over time depending on environmental conditions, but may still eventually die from the disease (Murray et al 2009). Frogs deteriorate rapidly after the onset of clinical signs and become moribund and die within a few days (Voyles et al 2009).

### 1.6.2 Persistence of the pathogen

*Batrachochytrium dendrobatidis* is a relatively fragile fungus and is susceptible to desiccation, heat and most disinfectants (Table 1) (Phillott et al 2010, Webb et al 2007, 2012). A resistant resting stage has not been found. It is not an obligate parasite but appears to be easily outcompeted by other microbes in the environment (James 2007). For this reason the main environmental life stage appears to be the aquatic transmissible zoospore which has a limited life span of a few days (Berger et al 2009, Skerratt et al 2010b). However, under sterile laboratory conditions it has been shown to grow for weeks on moist soil (Johnson and Speare 2003), and the possibility for extended survival away from the host has not been ruled out.

**Table 1. Disinfectants effective against *B. dendrobatidis* (Phillott et al 2010). No disinfectant has yet been proven for use against *B. dendrobatidis* in water-bodies or other environments.**

Application	Disinfectant	Concentration	Time
Disinfecting surgical equipment and other instruments (e.g. scales, callipers)	Benzalkonium chloride	2 mg ml <sup>-1</sup>	1 minute
	Ethanol	70%	1 minute
Disinfecting collection equipment and containers	Sodium hypochlorite	1%	1 minute
	Path X or Quaternary ammonium compound 128	1 in 500 dilution	0.5 minutes
	Trigene	1 in 5000 dilution	1 minute
	F10	1 in 1500 dilution	1 minute

### **1.6.3 Modes of transmission**

Disease transmission occurs via the aquatic zoospore that invades skin and grows into the parasitic zoosporangium. Zoosporangia develop for about 4 days then release new zoospores into the environment via the discharge tube (Berger et al 2004). Transmission occurs indirectly within water bodies and potentially via moist substrates and by direct contact with hosts or shedding skin (Berger et al 2009, Rowley et al 2007). Amphibian eggs have not been found to be infected but potentially could be contaminated on their surface with *B. dendrobatidis*.

The disease is readily spread within Australia by movement of infected hosts through the amphibian pet trade or accidental movement via trade of produce (e.g. fruit, building supplies) that harbour amphibians. Thousands of frogs are moved long distances each year in Australia as stow-aways in produce (Marantelli and Hobbs 2000).

Water and moist substrates in contact with infected hosts are potential fomites. Water birds (Garmyn et al 2012) and insects (Cashins 2009) have been suggested as possible vectors but survival of the fungus on live animals has not been demonstrated.

### **1.6.4 Factors influencing transmission and expression of disease**

Moderate ambient air temperatures can stimulate disease outbreaks in captivity and in the wild (Berger et al 2009). This correlates with optimal growth of *B. dendrobatidis* in vitro at temperatures around 17-23°C (Piotrowski et al 2004). Temperature dependent pathogen growth models based on in vitro experiments are better at explaining variation in intensity of infection in the wild than combinations of ambient air temperature variables (Dr Kris Murray, JCU 2012). The moderating effects of habitat on climate and the use of microhabitats by amphibians are important determinants of temperatures experienced by amphibians and hence infection intensity (Dr Robert Puschendorf, JCU 2009).



Increased density of frogs also appears to play a role in promoting disease expression in captivity (Dr Scott Cashins, JCU 2012) and possibly in the wild (Briggs et al 2010).

Experimentally increasing the salinity of natural water bodies has resulted in reduced prevalence in green and gold bell frogs (Dr Michelle Stockwell, Uni Newcastle 2011, White 2006, Stockwell et al 2012).

For relatively resistant species (e.g. cane toads) age is a key determinant and 100% of captive metamorphs may die while adults appear unaffected (Berger et al 2009). In tadpoles, life history is a determinant of exposure with relatively long lived torrent adapted tadpoles having high prevalence and intensity of infection due to their behaviour of attaching and feeding on stream substrates contaminated with *B. dendrobatidis* (Cashins, 2009).

## 1.7 Impact

Chytridiomycosis is the most significant disease to affect vertebrate biodiversity and has caused the decline and extinction of several hundred amphibian species globally (Skerratt et al 2007). In Australia it has caused the extinction of at least four species, probably 7, and the dramatic decline of at least 10 more (Australian Government 2006, Murray et al 2010). Many persisting species remain at lower abundance and smaller distributions than pre-disease, some are continuing to decline and significant mortality from chytridiomycosis is ongoing even decades after introduction (McDonald and Alford 1999, Murray et al 2009, Hunter et al 2010). Some critically endangered species now have very small populations and need intervention to become secure. Naive areas containing endemic frogs are also at risk.

Chytridiomycosis is a key threatening process to amphibians (Australian Government 2006). It has become endemic through most of the favourable climates and habitats in Australia for the disease. Within these areas it can usually be found at >5% prevalence, and disease outbreaks may occur seasonally. Species at critically low population levels or within uninfected sites may require immediate management to prevent loss of biodiversity. The next priority for management of *B. dendrobatidis* is species that have declined due to chytridiomycosis but are persisting at sustainable population levels.

## 2 Principles of control and eradication

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### 2.1 Introduction

This section provides background information to enable the choice of the most appropriate response option to an outbreak of chytridiomycosis in Australia.

As evidence is lacking for the efficacy of control measures against an outbreak of chytridiomycosis, recommendations will evolve as results of research and management are reported. All interventions should be planned as scientific trials.

A national list is not available for species and populations at risk of an outbreak and would assist in prioritising species and populations for implementing this disease strategy to mitigate outbreaks. This list should be based on freedom from chytridiomycosis, and those species predicted to be at risk of decline from chytridiomycosis and a subsequent change in their conservation status (Skerratt et al 2010a, Murray et al 2011a, Murray and Skerratt 2012). There are species and populations in south west Tasmania and southern NSW that are currently free of chytridiomycosis and at risk of decline (Pauza et al 2010, Murray et al 2011a). Some aspects of this strategy are being implemented such as containment, control and zoning in Tasmania (Phillips et al 2010) and NSW and control and mitigation of the disease in NSW. Currently, there is only limited implementation of aspects of this strategy in Queensland. Lack of or limited resources, capacity and knowledge appear to be reasons for failure to implement some aspects of disease control outlined here. It is hoped that this disease strategy will help address some of these issues.

The trigger for implementing this disease strategy should be freedom from chytridiomycosis and prediction of risk of decline of amphibians from chytridiomycosis. Protocols for surveying populations and predictive tools for risk of decline are available (Skerratt et al 2008, Murray et al 2011a) and have been used to inform use of this disease strategy in some regions (Pauza et al 2010, Skerratt et al 2010a). Due to limited resources and awareness of risk, disease responses often occur after disease has spread and outbreaks are underway. This is not recommended as it may result in suboptimal mitigation of outbreaks.

As *B. dendrobatidis* is now widely distributed in Australia, any control and eradication efforts should be aimed at protecting uninfected areas and decreasing the impact of the disease during new outbreaks. This disease strategy is also aimed at infected endangered amphibian populations that are continuing to decline or have not recovered from declines caused by ongoing chytridiomycosis outbreaks. Reducing the risk of spread of different strains between areas is also important and aspects of this disease strategy are relevant.

There are three potential control and eradication options:

1. Eradication: Eradication of chytridiomycosis is not feasible nationally, or generally at a state or territory level, as the disease is widespread among many wild amphibian species (Australian Government 2006). It is possible that the disease could be eradicated from small areas under some circumstances, such as by excluding amphibians or treating individuals if few remain alive after an outbreak.

This has not been attempted to date in Australia, and would require intensive efforts with uncertain results. An attempt to eradicate the fungus from a population of midwife toads in ephemeral ponds in Mallorca failed (Lubick 2010).

2. Containment, control and zoning: Reducing risk of spread into naive areas is a high priority but will only work for mitigating anthropogenic spread of the disease via amphibian or fomite movement. The success of biosecurity measures in mitigating the risk of anthropogenic spread has not been assessed. Control of natural spread will only be applicable to small areas and would require barriers to amphibian movement such as fences. Off shore islands and isolated habitats have natural barriers to disease spread where containment could be successfully achieved. Currently the only large uninfected areas predicted to be suitable for *B. dendrobatidis* establishment are in Tasmania and Cape York (Murray et al 2011d). Both areas contain endemic frog species that are predicted to be susceptible to decline (Murray et al 2011a).

Even in infected areas, control measures are important to prevent incursions of new strains, which may have higher virulence (see Section 1.1). This is a high priority for habitats containing endangered frog species.

3. Control and mitigation of impact of disease to amphibian populations:
  - Includes measures to directly mitigate disease impact in the field and indirectly through;
  - treating individuals and transferring them to captivity in order to establish captive disease free assurance colonies, and;
  - improving population abundance of endangered species via reintroductions and translocation.

The latter two options have been used successfully as a last resort to prevent extinctions (Mendelson et al 2006, Gagliardo et al 2008). These intensive options are essential for critically endangered species and are currently underway for some Australian frog species at various scales.

Current research topics for direct mitigation of disease impact include vaccination and treatment; however these are still experimental and unproven. Current research topics for indirect mitigation are assisted selection for innate immunity, control of reservoir species, habitat modification and bio-augmentation (Woodhams et al 2011).

Some infected highly susceptible populations can persist under suboptimal environmental conditions for chytridiomycosis (Pushendorf et al 2011).

## **2.2 Methods to prevent spread and eliminate pathogens**

### **2.2.1 Quarantine and movement controls**

As *B. dendrobatidis* is now widely distributed in Australia, any quarantine efforts should be aimed at the uninfected areas. This is the opposite focus to standard emergency responses where the restricted area is the infected zone. The following quarantine and movement restrictions should be implemented to protect high priority uninfected areas from incursions from adjacent infected areas. A

corresponding surveillance and monitoring program for *B. dendrobatidis* would be required to support the zoning policy and monitor effectiveness of quarantine.

### ***Establishment of Quarantine areas***

Establishment of specified areas (see the emergency animal disease response plan Enterprise Manuals for more details), including:

- *Declared area* – includes restricted area and control area
- *Restricted area* – area around the infected area
- *Control area* – a buffer between the restricted area and free areas
- *Free area* – uninfected area in favourable habitat<sup>7</sup>

In the declaration of quarantine areas, the following factors need to be taken into account:

- Conservation status of species
- Presence and movement of reservoir amphibian species
- Visitation to site – e.g. hikers, forestry
- Natural barriers to spread, such as arid zones, large saline water bodies, location in relation to infected area

The following groups must be considered when implementing response options:

- Hikers, fishermen etc
- Researchers
- Forestry
- Construction – e.g. roads

### ***Movement Controls***

Movement controls include:

- Stop movement of live host species into control areas (natural and anthropogenic).
- Restrict access of humans and other animals that may carry *B. dendrobatidis*, such as livestock.
- Ensure disinfection of all equipment if taken into the zone.

Implementation of bans and restrictions will be a dynamic process, determined by the location and extent of the disease outbreak and whether the aim is to eradicate the disease agent or to control its spread. Some restrictions may be impractical or unnecessary but others will be of critical importance to eradication or control.

The feasibility of restrictions and bans and extent to which these are able to be enforced will depend on the location of infection, the location and type of enterprises affected and the control response option chosen.

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<sup>7</sup> An uninfected area in an unfavourable habitat is not a priority for quarantine

## *Zoning*

Principles of zoning for infected and non-infected zones in Australia are outlined in the AQUAPLAN Zoning Policy Guidelines<sup>8</sup> and in the OIE *Aquatic Animal Health Code* (OIE 2011).

To protect uninfected areas of habitat suitable for *B. dendrobatidis*, such as areas where it hasn't yet spread to and any areas where it may be eradicated in the future, a zoning policy specific for *B. dendrobatidis* may be necessary to protect uninfected areas. Zones would be based on the distribution of *B. dendrobatidis* surrounding the free area, susceptible host species and of any vector species present within the free area (if appropriate), the geographical and hydrological characteristics of water bodies and landform, and predictions of the most likely method of spread of infection into the free area. Zoning may rely on the identification of biogeographic barriers.

### **2.2.2 Tracing**

Tracing is not applicable for chytridiomycosis in Australia as the disease is widespread and few uninfected areas remain.

### **2.2.3 Surveillance**

#### **General Surveillance**

Information on general requirements for surveillance for recognition of freedom from infection is provided in the online OIE *Manual of Diagnostic Tests for Aquatic Animals* (2011). The manual also provides specific information on diagnosis for surveillance for *B. dendrobatidis*.

Surveillance is necessary to:

- Define the extent of the infection
- Detect new outbreaks in currently uninfected, or locations of unknown disease status
- Monitor the impact of the disease on frog populations
- Establish restricted and control areas to which quarantine and movement restrictions are applied
- Establish infected and non-infected areas/zones for a zoning program and
- Monitor the progress and success of a control strategy

A surveillance protocol is available for chytridiomycosis in Australian frogs (Skerratt et al 2008, 2010). The impact of the disease requires regular monitoring in endangered species.

Surveillance of subclinical animals involves swabbing frogs or tadpoles for PCR testing. The surveillance protocol (Skerratt et al 2008, free online) discusses choice of season, species and life stage in order to maximise detection probabilities, which varies with location. PCR testing 60 individuals per population, using cluster sampling, is recommended for detecting positive locations and declaring a population free of chytridiomycosis based on a minimum apparent prevalence of 5%. This sampling strategy has been shown to work where chytridiomycosis is

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<sup>8</sup> <http://www.daff.gov.au/aquaticanimalhealth>

distributed throughout a water body as occurs in watersheds with connected streams regardless of the potential bias associated with non-random sampling (Skerratt et al 2010a). The required sample size will increase if chytridiomycosis is not distributed throughout a water shed, for example if infected animals are clustered in specific ponds (Skerratt et al 2010a).

Chytridiomycosis is by far the most common disease of frogs in Australia (Berger et al 2004). As frogs are small and cryptic, sick frogs and even mass mortality events can be easily overlooked without dedicated searches. However, testing clinically affected animals encountered opportunistically is an efficient method of surveillance. Ill or dead animals can be collected whole for diagnosis at the laboratory. Microscopy and PCR are both accurate in clinical cases, but histology is needed to diagnose other diseases.

### **Targeted Surveillance for Uninfected Quarantine Zones**

The edges of uninfected areas are a high priority for intensive surveillance and include the World Heritage Area in Tasmania.

Similar methods can be used to maximize the chance of detecting incursions of *B. dendrobatidis* into previously uninfected zones. However, frequent survey efforts should focus on locations near potential entry pathways - for example areas where people arrive, amphibian dispersal corridors, water ways, and gravel roads (Pauza et al 2010).

#### **2.2.4 Treatment of infected host species**

Several topical treatments are available for subclinically infected hosts but few species have been assessed in clinical trials; further research is needed to validate and optimize treatments (Berger et al 2010).

- Itraconazole (100 mg/l) is commonly used as a 5 minute bath exposure daily for 10 days in frogs, but is toxic to some species. In tadpoles, 0.5 – 1.5 mg/L itraconazole 5 minute baths daily were effective (Garner et al 2009).
- Voriconazole spray (1.25 mg/l) daily for 7 days cured frogs and toads (Martel et al 2011). Voriconazole appeared safe in tadpoles but was not tested in infected tadpoles.
- Terbinafine hydrochloride in alcohol (available over the counter as Lamisil AT [Novartis]) was used at 0.01% or 0.005% as a 5 minute soak for 5 days, and successfully treated 6 amphibian species (Bowerman et al 2010).
- Chloramphenicol (20 mg/l) was used successfully as a shallow continuous bath for 28 days, and frogs were removed to a dry tub for 1 hour/day for feeding (Young et al in press).
- Heating to 32 °C for 5 days was also effective in a heat tolerant species (Retallick and Miera 2007).

Clinically infected animals may require supportive electrolytes to recover and be cured (Young et al in press). Amphibians under treatment should be held in simple tubs with minimal substrates that can be disinfected or changed at each treatment application.

It is necessary to confirm treatment has cleared infection by PCR testing. The suggested protocol involves 3 PCR tests at least a week apart, starting the week after last treatment (Berger et al 2010). Proof of freedom from the disease should follow the principles outlined by Skerratt et al (2008) and the OIE *Aquatic Animal Health Code* (OIE 2011).

To increase knowledge of efficacy and safety of treatments, results should be reported and where possible treatments should be conducted as experimental controlled trials (Berger et al 2010).

### **2.2.5 Treatment of host products and by-products**

Fresh amphibian products are rarely available in Australia. However, heating is a recommended treatment for host products as *B. dendrobatidis* dies at 60°C within 5 minutes (Johnson et al 2003). Freezing is also likely to kill the fungus (Boyle et al 2003).

### **2.2.6 Destruction of hosts**

Amphibians can be killed humanely via several methods (Rose 2007). The preferred method is a bath of anaesthetic overdose, such as Aqui-S or MS-222. Frogs should be kept in the bath for 10 minutes after respiration stops. Aqui-S (Isoeugenol, ADS-I) is used at 15-25 mg/L water. MS-222 (Tricaine methane sulfonate, Biolab) is used at 0.2-0.5 g/L water for tadpoles and 0.5- 2 g/L for frogs. MS-222 is acidic and should be buffered with 1 part MS-222 to 2 parts sodium bicarbonate. Other effective anaesthetics include isoflurane, halothane and benzocaine. Striking an amphibian on the head to rapidly destroy the brain is also acceptable. Decapitation or placing in a freezer may cause pain and are unacceptable methods.

Any chemicals used must be approved for that use by the Australian Pesticide and Veterinary Medicines Authority (APVMA) – refer to Appendix 2. In addition, any chemical that is used directly or indirectly for the control of an animal disease is governed in its use by relevant ‘control of use’ legislation in each state and territory. The relevant state or territory authority (in most cases this is the veterinary registrar within the relevant state department of primary industry or agriculture) should also be consulted for advice prior to the use of the chemical.

### **2.2.7 Disposal of hosts**

Not likely to be applicable as most carcasses are scavenged or decompose before being found (Wobeser et al 1994, Berger et al 1998). Any carcasses that remain are likely to be collected whole for necropsy and diagnostic testing. Burial on site (away from water), or incineration are viable options if required. *B. dendrobatidis* is not expected to survive long in a carcass as it is killed by bacterial overgrowth.

### **2.2.8 Decontamination**

#### **Captivity**

In captive collections, decontamination is needed for aquarium tubs and equipment such as tadpole nets before re-use. Detailed quarantine and husbandry protocols are available online (Pessier and Mendelson 2010, Murray et al 2011).

#### **Field**

In the field care is needed not to 1) increase transmission between animals at the site and 2) spread disease between sites. A detailed risk assessment and appropriate hygiene protocols to mitigate the risks associated with amphibian field work have been published (Phillott et al 2010, Murray et al 2011). For example, when handling each frog a new pair of gloves must be worn and separate containers are used for holding animals. When moving to a new site, boots and equipment such as nets may need to be cleaned and disinfected depending on the level of risk of spread.

Cleaning and disinfection for other activities in wilderness areas (that include for example machinery, boats and movement of soil) are described in “Keeping it clean: A Tasmanian field hygiene manual to prevent the spread of freshwater pests and pathogens” by Allan and Gartenstein (2010), available online at [www.dpiw.tas.gov.au/internnsf/Publications/LBUN-8896DT?open](http://www.dpiw.tas.gov.au/internnsf/Publications/LBUN-8896DT?open).

Due to differences in the use(s) of infected sites, disinfection protocols may need to be determined on an individual basis involving the site manager, the state/territory CVO and/or Director of the relevant state/territory department. The protocol should take into consideration the factors outlined above in Section 1.6, in particular:

- The type of habitat or enterprise (eg water body type, remoteness, conservation status of species, zoo, breeder, water source etc);
- Proximity of the site to other waterways;
- Current disinfection protocols;
- Workplace safety concerns;
- Environmental impact of the disinfectant protocol;
- Legislative requirements (occupational health and safety, environmental protection, chemical use);
- Availability of approved, appropriate and effective disinfectants.

See the emergency animal disease response plan Operational Procedures Manuals for decontamination for details of decontamination methods and their indicators.

### **2.2.9 Vaccination**

No vaccines are currently available for *B. dendrobatidis*, despite recent research efforts. It appears production of a vaccine is unlikely in the near future due to the immunosuppressive effect of infection (section 1.5).

### **2.2.10 Vector control**

Movement and release of contaminated water, for example from aquariums housing infected animals, is a risk for spread (Berger et al 2009, Skerratt et al 2010b). Theoretically anything contaminated with infected sloughed skin or aquatic zoospores of *B. dendrobatidis* such as herpetological equipment, dirty boots, water birds and flying aquatic insects could be potential vectors (Phillott et al 2010, Cashins 2010, Skerratt et al 2010b, Garmyn et al 2012). The amphibian chytrid fungus does not survive drying (Johnson et al 2004), thus mechanical vectors must remain wet.



## 2.3 Environmental considerations

Chemicals used in field situations are generally used in such small amounts that safe disposal is readily achieved with no environmental effects. However, disinfection should occur away from water bodies as disinfectants (including quaternary ammonium compounds, bleach and Path X) are toxic to aquatic organisms including invertebrates and tadpoles. Disinfectants should be carried out of environmentally important areas for disposal (Allan and Gartenstein 2010). Schmidt et al (2009) recommended Virkon S as a safer disinfectant for field use as it is less toxic to tadpoles and zooplankton.

See the emergency animal disease response plan Procedures Manuals for details of decontamination methods or “Keeping it clean: A Tasmanian field hygiene manual to prevent the spread of freshwater pests and pathogens” by Allan and Gartenstein (2010).

## 2.4 Sentinel animals and restocking measures

The main environmental life stage appears to be the aquatic transmissible zoospore which has a limited life span of a few days (Berger et al 2009, Skerratt et al 2010b). Although the fungus can grow for weeks on sterile moist soil (Johnson et al 2003), it is unknown how long it can survive in the environment. As it is difficult to detect in environmental samples, there is potential to use sentinel animals to assess infection status of an area. Ideally a readily infected but tolerant species, native to the area would be used. However, there are large logistical, ethical and licensing problems in securing and keeping sentinel frogs that make it unlikely to succeed. A previous attempt to maintain frogs long term in outdoor mesocosms in remote habitats for research purposes had to overcome major difficulties in providing live food and preventing frog escape (Retallick and Dwyer 2000).

Restocking has had success where chytridiomycosis is either no longer present or now occurs at low levels due to low amphibian densities (Briggs et al 2010, Dr David Hunter NSWDECC 2012 et al unpublished). Outbreaks can reoccur if chytridiomycosis is reintroduced or if amphibian densities achieve high levels again. Reintroduction does not appear to work where reservoir hosts remain at reasonable density. Research is underway to improve reintroduction success and includes habitat modification (e.g. increased salinity) and selection for resistance. Resistant populations would ensure the long term survival of species without the need for ongoing mitigation. As remnant frog populations have been found in drier and hotter areas of their range that are less suitable for the fungus (Puschendorf et al 2011), identifying such areas and habitats for restocking is a priority.

## 2.5 Public Awareness

A public awareness campaign emphasising education, surveillance and cooperation from industry and the community is essential. The public should be informed that:

- Tadpoles and frogs must not be moved between habitats as this is a risk for disease spread.

- If mass mortalities are observed, notify state environment department.
- Those involved in domestic amphibian pet trade need to adhere to quarantine and testing recommendations. A manual for captive quarantine in conservation programs (Pessier and Mendelson 2010) is available online, and a simplified version should be developed for the pet trade.
- Public should purchase amphibians from certified chytrid free dealers, and not release their pets into the environment under any circumstances.
- In trade of produce (such as fruit) where amphibians can be inadvertently moved, risk of spreading the disease is high and reducing amphibian stowaways is important.
- Research into chytridiomycosis in order to minimise the decline of frog species, populations and their distribution, is fundamental to conserving biodiversity.

## **2.6 Feasibility of control or eradication of Chytridiomycosis/*Batrachochytrium dendrobatidis* in Australia**

The feasibility of controlling an outbreak of chytridiomycosis depends on the nature and location of the outbreak and the management strategy adopted. Essentially, as outlined in Section 2.1 there are 3 response options:

- 1) Eradication
- 2) Containment, control and zoning
- 3) Control and mitigation of disease

### **2.6.1 Response Option 1: Eradication**

Eradication has not been achieved for chytridiomycosis despite limited attempts overseas (Lubick 2010). Further effort is worth attempting but eradication is not considered an option unless:

- the population is closed or semi-closed (ie. isolated) and all the population can be cured via treatment; or
- amphibians have been eliminated for restocking of uninfected individuals.

Disinfection of the water body may involve desiccation or application of antifungal compounds. It is suspected, but unproven, that leaving a site devoid of hosts for an extended time will eliminate the fungus which does not have a resistant resting phase and may not grow in the environment. Duration of treatment after cure or time until restocking will depend on the time that it takes for environmental reservoirs to senesce, which is currently unknown.

The feasibility of eradication cannot be judged until further efforts are made. The intensive effort required would be worth attempting to enable critically endangered

species to establish self-sustainable wild populations. However, developing techniques to mitigate the impact of *B. dendrobatidis* in infected populations (section 2.6.3) appears likely to have broader application compared with eradication.

### **2.6.2 Response Option 2: Containment, control and zoning**

Risk of anthropogenic spread of chytridiomycosis to naive populations, and between infected regions, may be mitigated through containment, control and zoning. This is a priority for isolated populations such as those on islands or in habitats where natural spread is unlikely to, or could not, occur. In moist wilderness areas with abundant wildlife, attempts to stop natural spread appear to be unlikely to succeed.

Control of anthropogenic spread involves restricting access to sites and the use of stringent hygiene protocols on equipment and clothing (Allan and Gartenstein 2010, Phillott et al 2010, Murray et al 2011). Hygiene protocols for use in natural environments have been developed which are feasible and recommendations vary with the probability and consequences of spread. However, the efficacy of reducing the risk of spread by focussing on humans has not been assessed. The implementation of bans and restrictions will be a dynamic process, determined by the location and extent of the uninfected area to be kept free of infection. Some restrictions may be impractical or unnecessary, but others will be of critical importance to control.

### **2.6.3 Response Option 3: Control and mitigation**

#### **In field mitigation of disease impact**

Currently there are no proven methods to control the disease in the wild. It is important to investigate a broad range of potential solutions. Experimental ideas include control of reservoir species, treatment and vaccination of hosts, disinfection of water bodies, bio-augmentation of hosts with inhibitory bacteria; and habitat modification, such as increased salinity (Woodhams et al 2011). As infected frog populations can thrive in naturally suboptimal habitats for the fungus (Puschendorf et al 2011), eradication of disease is not necessary for successful mitigation and any method that limits the impact of the disease is worthy of investigation.

#### **Improving population abundance of endangered species via captive assurance colonies, translocation or reintroductions**

Some populations and species have been saved from extinction or severe loss of genetic diversity via movement into biosecure captive facilities. Collection may involve 1) uninfected frogs before arrival of *B. dendrobatidis* at a site, 2) treating frogs from an exposed site, or 3) taking uninfected eggs. An alternative aim to establishing long-term captive assurance colonies may be to rear wild-caught eggs or tadpoles in captivity with associated higher survival rates and then release into the wild to maximise recruitment potential. Direct translocation to suitable sites is also possible and requires fewer resources than maintaining captive colonies once suitable sites have been identified. Ideas that are currently being researched for reducing mortality rates in reintroduced or translocated frogs where chytridiomycosis is still present include selection for resistant offspring. This could ensure the long term survival of species without the need for ongoing mitigation.

Establishing captive insurance colonies is expensive but has been used successfully to save species that are extinct in the wild and for successful reintroduction back into the wild. As each species has different requirements, problems with husbandry are common (Shaw et al 2012) but can be overcome if interventions occur early allowing time for optimising husbandry. Use of surrogate species to experimentally test optimal husbandry conditions may be necessary if few individuals of an endangered species remain. Determining optimal husbandry conditions and treatment regimes for chytridiomycosis and establishing captive assurance colonies are more likely and easily achieved prior to the occurrence of outbreaks.

Cryopreservation of amphibian sperm is also an option for storing diverse genetic material (Browne et al 2002), but methods for preserving amphibian eggs are yet to be developed.

#### **2.6.4 Trade and industry considerations**

In Australia, wild amphibians are typically collected for research, zoos and conservation programs. The pet industry involves frog breeders and domestic trade. Exotic frogs are rarely allowed to be imported into Australia and are restricted to biosecure facilities such as zoos or research institutes due to the risk of introduction of other significant amphibian pathogens, such the ranaviruses.

Trade regulations must be considered as part of a response strategy. State and Territory environment department permits may be required to allow amphibians from declared areas to be collected and moved.

##### **Export Markets**

Some countries may have import conditions in place related to chytridiomycosis, such as requiring imports to be certified free of *B. dendrobatidis* (OIE Aquatic Code). The Australian Quarantine and Inspection Service is responsible for the health certification of all exports and should be contacted for further information ([export@aqis.gov.au](mailto:export@aqis.gov.au)).

##### **Domestic Markets**

In Australia, there is no accreditation process for dealers/collectors/license holders, to reduce sale and movement of infected frogs. A cautious approach is therefore required for the collection of exposed or partially exposed wild amphibians for domestic use on the basis of animal health concerns. Decisions regarding the use of wild amphibians for domestic use should depend on their conservation and infection status and the conservation response strategy implemented.

Premises for holding amphibians should be biologically secure to prevent escape of animals or *B. dendrobatidis* - for example in discharged waste water. Monitoring infection status of collections by PCR is important. Quarantine guidelines for captive collections (Murray et al 2011) are available at <http://www.environment.gov.au/biodiversity/invasive/publications/pubs/frog-s-hygiene-protocols.doc>.

In trade of various products/produce where amphibians are inadvertently moved, such as on bunches of bananas or other boxes of fruit, methods should be

implemented to 1) reduce the number of stowaways, and 2) enable collection of frogs on delivery. This will require education of people most likely to find these stowaways.

## 3 Preferred Australian response options

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### 3.1 Overall policy for chytridiomycosis/*Batrachochytrium dendrobatidis*

A rapid and vigorous emergency response is currently justified for endangered species and endemic species in naïve habitats at risk of introduction and population decline. In other instances, it will be important to monitor population impacts.

For species that are experiencing the ongoing effects of a chytridiomycosis outbreak such as continual decline (such as the critically endangered southern corroboree frog) or failure to recover (such as the critically endangered armoured mist frog) then aspects of this disease strategy may be useful to mitigate the effects of chytridiomycosis. Species which have declined due to chytridiomycosis but which persist at larger sustainable population levels, are the next priority for management.

1) Eradication should be considered for saving small populations of endangered frogs but is not generally feasible unless the population is isolated and all the population can be cured via treatment, or amphibians have been eliminated for restocking of uninfected individuals. The site must have physical boundaries to prevent entry of infected amphibians and waterborne zoospores.

2) Containment, control and zoning is a priority for isolated naïve populations such as those on islands or in habitats where natural spread is unlikely to or could not occur. Reducing the risk of spreading strains between infected areas is also important. Control of anthropogenic spread involves restricting access and use of stringent hygiene protocols.

3) To control and mitigate the impact of chytridiomycosis, establishing captive assurance colonies is currently the only proven method to stop extinction or severe loss of genetic diversity in the wild, but requires an intensive and specialised effort. All frog species that are at risk of decline from chytridiomycosis require development of husbandry methods and captive colonies and facilities to reduce their risk of extinction. There are a number of endangered species that have declined due to chytridiomycosis that do not have captive assurance colonies such as the armoured mist frog (*Litoria lorica*). Their conservation status could be improved through adoption of some of the aspects of this strategy such as establishing captive assurance colonies, translocating to suboptimal habitats for chytridiomycosis and reintroducing resistant individuals into the wild.

All these options involve a combination of disease management methods, which may include quarantine and movement controls, and surveillance.

Interventions should be conducted scientifically with controls, and reported widely to scientists and managers in environmental departments, to provide evidence needed urgently for improved management.

The Director of the relevant state or territory department and/or the CVO in the state or territory in which the outbreak occurs will be responsible for developing an emergency animal response plan (EAD Response Plan). This plan will be submitted to the appropriate Consultative Committee on Emergency Animal Diseases (CCEAD), who will provide advice on the technical soundness of the plan and its consistency with the relevant emergency animal disease response plan.

Directors of the relevant state or territory department and/or CVO's will implement the disease control measures as agreed in the EAD Response Plan and in accordance with relevant legislation. They will make ongoing decisions on follow up disease response measures in consultation with CCEAD. The detailed response measures adopted will be determined using the principles of control and eradication (see Section 2), epidemiological information about the outbreak and the financial feasibility of the option.

For information on the responsibilities of the other state or territory disease control headquarters and local disease control centres, see the relevant emergency animal disease response plan manuals for Control Centres Management.

## 3.2 Response Options

The specific aim of the intervention regarding chytridiomycosis will greatly influence selection of the most suitable response option.

### 3.2.1 Option 1 – Eradication

Eradication could be considered for saving small populations of endangered frogs but is not generally feasible unless:

- 1) the population is closed or semi-closed (ie. isolated) and all the population can be cured via treatment; or
- 2) amphibians have been eliminated for restocking of uninfected individuals.

To prevent reinfection from surrounding zones, sites must be chosen that have physical boundaries to prevent entry of infected amphibians and waterborne zoospores.

An eradication plan should include the following activities:

- Quarantine and movement controls must be stringently enforced to stop movement of amphibians, water, and any potential vectors. Restrictions must apply to movement into the uninfected area of anything capable of transmitting *B. dendrobatidis*.
- All amphibians must be collected and removed for treatment – currently this involves daily antifungals for 1 week, then confirmation of cure by weekly PCR testing over 3 weeks.
- Disinfection of the water body may involve desiccation, application of antifungal compounds or keeping it free of amphibian hosts for an extended period.

- Time until restocking will depend on the time that it takes for environmental reservoirs to senesce, which is currently unknown. Suggest restocking on a small scale initially, potentially with free-living or captive frogs acting as sentinel animals to be tested and monitored for at least 3 months, depending on the season.

And/or:

### **3.2.2 Option 2 – Containment, control and zoning**

Risk of anthropogenic spread of chytridiomycosis to naive populations could be mitigated through containment, control and zoning. This is a priority for isolated populations such as those on islands or in habitats where natural spread is unlikely to or could not occur. Control of anthropogenic spread involves restricting access and use of stringent hygiene protocols (Allan and Gartenstein 2010, Phillott et al 2010, Murray et al 2011c) before arrival and during any work carried out on site. Actions may include:

- Wash-down stations for hikers entering *B. dendrobatidis* free zones
- Closed access for vehicles and boats
- Restricted permits for research activities
- Ensuring disinfection for forestry and fire fighting activities
- Surveillance and mapping to define edges of the naive zone

And/or:

### **3.2.3 Option 3 – Control and mitigation of disease**

Control and mitigation acts to prevent extinction of critically endangered populations by reducing the impact of chytridiomycosis and supporting reproduction and recruitment.

#### **In field mitigation of disease impact**

Currently there are no proven methods to control the disease in the wild. Experimental ideas include control of reservoir species, treatment and vaccination of hosts, disinfection of water bodies, release of avirulent strains of *B. dendrobatidis*, bio-augmentation with inhibitory bacteria; and habitat modification, such as increased salinity or increasing temperatures by reducing canopy cover (Woodhams et al 2011). As chytridiomycosis appears to be host density dependent (Briggs et al 2010), removal of resistant frog species that are acting as reservoir hosts is a promising option. Infected highly susceptible frog populations may persist if they occur in naturally suboptimal habitats for the amphibian chytrid fungus (Puschendorf et al 2011).

#### **Improving population abundance of endangered species via captive assurance colonies, translocation or reintroductions**

Some populations and species have been saved from extinction or severe loss of genetic diversity via movement into biosecure captive facilities and treatments to effect cures, if necessary (Mendelson et al 2006, Woodhams et al 2011). Treatment



is not needed if uninfected frogs are collected before introduction of chytridiomycosis into the population, or if eggs are collected (which have not been found infected with *B. dendrobatidis*).

Establishing captive assurance colonies is currently the only proven method to stop extinction or severe loss of genetic diversity, but requires an intensive and specialised effort. Eggs, tadpoles or adults can be collected and treated if necessary. As wild tadpoles undergo high mortality rates due to predation, one approach is to raise them in captivity and then release into the wild before or after metamorphosis to maximise recruitment to the adult life stage. Captive breeding is the aim for self-sustaining captive populations but is difficult for some species and may take many years to achieve.

Husbandry differs between species and experimental trials on captive conditions may be needed to optimize health of captive animals (Murray et al 2011b). Similarly the efficacy and safety of treatment for chytridiomycosis may need to be assessed (Berger et al 2010). For critically endangered species a similar surrogate species can be used for these experiments. As establishing husbandry takes time, it is urgent to identify at risk species long before populations are decimated by introduction of chytridiomycosis. Protocols detailing hygiene necessary for healthy captive populations are available (Murray et al 2011b).

State environmental departments should coordinate establishment of captive colonies and zoos are typically approached for husbandry expertise, human resources and donation of facilities. In addition to providing husbandry expertise, zoos are particularly important for ex-situ conservation programs where resource commitment over an extended period is required. Moreover, for species threatened with imminent extinction in the wild, it is necessary to replicate colonies across multiple institutions as a risk management strategy.

Uncertainty remains regarding optimal reintroduction protocols, which may involve release of various life stages (Dr David Hunter, NSWDECC 2010). In many cases simply releasing numerous individuals to the wild has not worked as they die from chytridiomycosis (Stockwell et al 2008). Suggested methods include release of tadpoles to *B. dendrobatidis* free tubs, release to *B. dendrobatidis* free sites or sites less favourable for chytridiomycosis. Within the foreseeable future, establishing self-sustaining wild populations of susceptible species may require translocation to areas identified as 'climate refugia'. Direct translocation to such sites is also possible and requires fewer resources than maintaining captive colonies. Selection for resistant offspring to reduce mortality rates from chytridiomycosis and enable recovery of populations in order to ensure their long term survival is currently being investigated.

Cryopreservation of amphibian sperm is feasible and should be considered for storing genetic diversity (Browne et al 2002).

### **3.3 Criteria for proof of freedom**

Criteria for proof of freedom are given in the OIE *Aquatic Animal Health Code* (2011). Large regions within Australia are predicted to be unfavourable for survival of *B. dendrobatidis* and frogs populations therein will remain uninfected. It

is possible that favourable wet microclimates in arid areas may allow chytridiomycosis to occur if introduced.

### 3.4 Funding and compensation

There is currently no cost sharing agreement in place for managing chytridiomycosis, and for conserving wildlife in general. Most of the costs are covered by federal and state/territory government with some ad hoc philanthropic contributions. Currently industry that directly benefits from biodiversity such as tourism is not levied. Within government, environmental departments are responsible for preserving biodiversity but do not currently have the funds required to safeguard all species. One major obstacle is that the value of a species is difficult to estimate and often relies on public sentiment. For this reason funding is skewed to charismatic fauna such as koalas and Tasmanian devils which are funded at levels 10,000 times greater than much more critically endangered species such as the armoured mistfrog, *Litoria lorica*, where a few hundred individuals remain.

Another issue for environmental departments is lack of capacity to manage disease. Most governmental veterinary and medical expertise required for managing disease is located within agricultural and health governmental departments. There are currently few cost, expertise or capacity sharing agreements among governmental departments to manage diseases affecting biodiversity.

In 2008, the OIE listed chytridiomycosis/infection with *B. dendrobatidis* as a notifiable disease in the OIE Aquatic Animal Health Code. In Australia, the state and territory CVOs are responsible for collecting and reporting on the quarterly occurrence of *B. dendrobatidis* in amphibians to the Office of the Australian Chief Veterinary Officer which reports twice yearly to OIE on any change in Australia's disease status including spread of the disease into new areas. CVO's must rely on government laboratories for definitive diagnoses as the basis for reporting to the OIE. Currently there are limited resources allocated to state and territory government laboratories for detecting disease in amphibians and reporting generally comes to CVO's via universities and other research institutes. As a consequence the quality and validity of amphibian disease reporting is not necessarily consistent with Australia's reporting standards to the OIE.

There is currently no compensation mechanism for the loss of biodiversity due to the introduction of chytridiomycosis into Australia. The mechanism of introduction is unknown and requires research but could have been through the amphibian pet trade in the 1970's. Biosecurity Australia within the Australian Government Department of Agriculture, Forestry and Fisheries and its equivalent back in the 1970s were responsible for ensuring Australia's amphibians were protected from exotic diseases. Unfortunately disease risk assessments in those days and even sometimes today do not include uncertainty and as chytridiomycosis was unknown at the time, infected animals were allowed to be imported.

Various contributions to research and some management actions within Australia have been by institutions and funding agencies, currently including but not

limited to the Australian Government Department of Sustainability, Environment, Water, Population and Communities, James Cook University, Amphibian Research Centre, Queensland National Parks and Wildlife Service, New South Wales Office of Environment and Heritage, Tasmanian Department of Primary Industries, Parks, Water and Environment, Australian Capital Territory Government, Australian Research Council, Taronga Conservation Society, Tidbinbilla Nature Reserve, Healesville Sanctuary, Currumbin Wildlife Sanctuary, Australian Wildlife Health Network, Australian Registry of Wildlife Health, Australian Biosecurity Cooperative Research Centre, Newcastle University, Royal Melbourne Zoo, Morris Animal Foundation, Australian Government Department of Agriculture, Fisheries and Forestry, and the United States Fish and Wildlife Service.

An example of a successful multi-institute collaboration involves the corroboree frogs. The ACT government has built and funded facilities for the captive assurance population, including use of some additional funding from previous Commonwealth grants (through NHT and Caring for our Country). ACT government has also funded field work research projects and supports the Amphibian Research Centre captive facility in Melbourne. Healesville Sanctuary contributes to the project by breeding northern corroboree frogs. The ACT and NSW governments maintain close contact with the recovery programs, facilitated by membership of the corroboree frog recovery team.

Most of the current efforts involve collaborations, often between government, universities and zoos. Overall co-ordination of these efforts by various groups requires a national network, as recommended by the Threat Abatement Plan for Chytridiomycosis.

## Appendix 1 OIE Aquatic Animal Health Code and Manual of Diagnostic Tests for Aquatic Animals

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### OIE Aquatic Code

The objective of the OIE *Aquatic Animal Health Code* (OIE 2011) is to prevent the spread of aquatic animal diseases, while facilitating international trade in aquatic animals and aquatic animal products. This annually updated volume is a reference document for use by veterinary departments, import and export services, epidemiologists and all those involved in international trade of aquatic animals and their products.

The current edition of the OIE Aquatic Code (14<sup>th</sup> edition) was published in 2011 and is available on the OIE website at:

<http://www.oie.int/international-standard-setting/aquatic-code/access-online/>  
(Accessed on 29 May 2012)

The following chapter is relevant to this manual:

Chapter 8.1. Infection with *Batrachochytrium dendrobatidis* available at:

[http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre\\_1.8.1.htm](http://www.oie.int/index.php?id=171&L=0&htmfile=chapitre_1.8.1.htm)

### OIE Aquatic Manual

The purpose of the OIE *Manual of Diagnostic Tests for Aquatic Animals* (OIE 2011) is to contribute to the international harmonisation of methods for the surveillance and control of the most important aquatic animal diseases. Standards are described for laboratory diagnostic tests and the production and control of biological products (principally vaccines) for veterinary use across the globe.

The current edition of the OIE Aquatic Manual was published online in 2011 and is available on the OIE website at:

[http://www.oie.int/eng/normes/fmanual/A\\_summry.htm](http://www.oie.int/eng/normes/fmanual/A_summry.htm)  
(Accessed on 17 April 2012)

The following chapter is relevant to this manual:

Chapter 2.1.1 Infection with *Batrachochytrium dendrobatidis*  
[http://www.oie.int/fileadmin/Home/eng/Health\\_standards/aahm/2010/2.01.01\\_BATRACHO.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/2010/2.01.01_BATRACHO.pdf)

### Further information

Further information about the OIE Aquatic Code and Aquatic Manual is available on the OIE website at:

[http://www.oie.int/eng/normes/en\\_acode.htm](http://www.oie.int/eng/normes/en_acode.htm)  
(Accessed on 17 April 2011)

## Appendix 2 Approval of chemicals for use in Australia

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The Australian Pesticides and Veterinary Medicines Authority (APVMA) evaluates, registers and regulates agricultural and veterinary chemicals. Before an antibiotic or vaccine can enter the Australian market, it must go through the APVMA's rigorous assessment process to ensure that it meets high standards of safety and effectiveness. (In addition, an import permit is required from the Australian Quarantine and Inspection Service if a product containing biological material is to be sourced from overseas.)

Detailed data about the product and its proposed use pattern must be submitted to the APVMA with the application for registration or permits. Since the assessment process is so detailed, the evaluation may take some time to complete.

### **Minor use permit system**

The minor use permit (MUP) system is a temporary approval system for the use of drugs and chemicals. The system was devised by the APVMA for Australia, and allows the restricted use of a limited amount of a drug or chemical in a specified species when inadequate data are available to satisfy APVMA requirements for registration. Conditions are applied to the permit, which often include the collection of data related to the use of the product. The MUP system aims to enable restricted use of a drug or chemical until sufficient data are available to enable full registration.

For example, the APVMA may set a temporary withholding period with a wide margin of safety for a MUP. This withholding period may have been extrapolated from data relating to the use of the product in other species. In such cases, a condition of the MUP will be the collection of residue testing data. Results from the data are assessed by the APVMA (usually after 12 months – the duration of most permits) and used to more accurately set a withholding period for the product.

### **Emergency use permits**

The APVMA has a permit system for the emergency use of a product that is either unregistered in Australia or registered for use in a different species or for a different use pattern. The APVMA will verify with the appropriate state and territory coordinators that the emergency is genuine.

For further details or permit application forms, visit the APVMA website.<sup>9</sup>

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<sup>9</sup> <http://www.apvma.gov.au>

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