THREAT ABATEMENT PLAN

BACKGROUND DOCUMENT

Infection of amphibians with chytrid fungus resulting in chytridiomycosis

Australian Government
Department of the Environment and Heritage

BACKGROUND DOCUMENT

for the

THREAT ABATEMENT PLAN

INFECTION OF AMPHIBIANS WITH CHYTRID FUNGUS RESULTING IN CHYTRIDIOMYCOSIS

Department of the Environment and Heritage

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Front cover photo: *Litoria genimaculata* (Green-eyed tree frog)
Sequential page photo: *Taudactylus eungellensis* (Eungella day frog)
Banner photo on chapter pages: Close up of the skin of *Litoria genimaculata* (Green-eyed tree frog)

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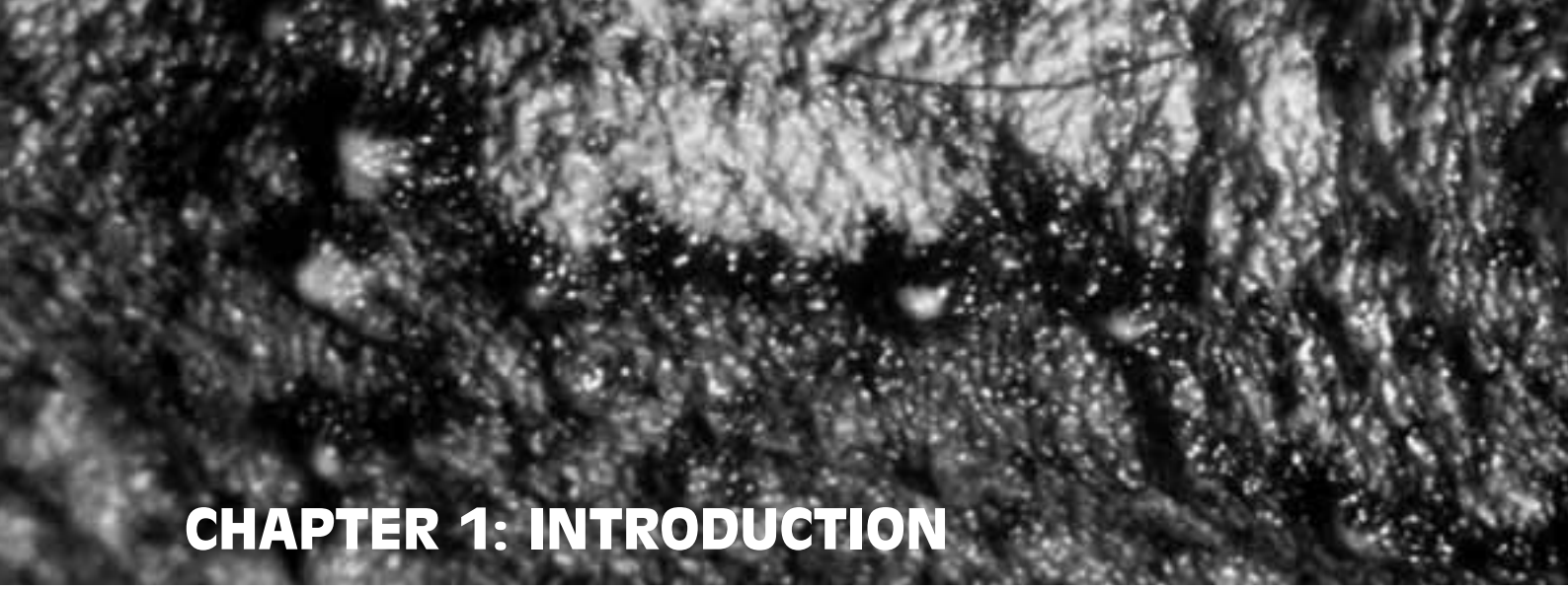


CHAPTER 1: INTRODUCTION

Australia's native amphibians are threatened by a pathogenic fungus, *Batrachochytrium dendrobatidis*, known either as the amphibian chytrid or the amphibian chytrid fungus, which causes the infection known as chytridiomycosis. This infection affects amphibians worldwide. It appears that the amphibian chytrid was introduced to southeast Queensland in the mid- to late-1970s, and subsequently spread to occupy a zone in eastern Australia from Big Tableland in north Queensland to Melbourne in Victoria. Three other zones of occurrence have been identified: southwestern Western Australia, Adelaide, and more recently Tasmania. A single record from the central Kimberley requires confirmation, and cannot be regarded as proven at this time. Other regions appear to be chytridiomycosis-free although intensity of searching has been too low in these other regions to provide convincing evidence of this status.

The effects of chytridiomycosis on amphibian populations, particularly those in upland eastern Australia, have been devastating with at least one population driven to extinction, and threatened species status of others worsened. Chytridiomycosis has now been identified in 52 per cent of threatened amphibian species. The level of the threat and its distribution could easily increase by movement of infected amphibians to chytridiomycosis-free areas and consquent escape of *B. dendrobatidis* into new wild amphibian populations. In addition, *B. dendrobatidis* can spread independently or with the assistance of amphibians.

'Infection of amphibians with chytrid fungus resulting in chytridiomycosis' is listed as a key threatening process under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). At the time of listing, the Australian Government Minister for the Environment and Heritage determined that having a threat abatement plan (TAP) was a feasible, effective and efficient way to abate the process, and directed a nationally coordinated TAP be prepared to guide management of the impact of the amphibian chytrid fungus on Australian amphibians. The TAP is intended to be brief and convenient to use, while this background document provides supporting information on a wide range of amphibian chytrid fungus issues such as its biology, population dynamics, its spread, diagnosis, impacts on biodiversity and management measures.

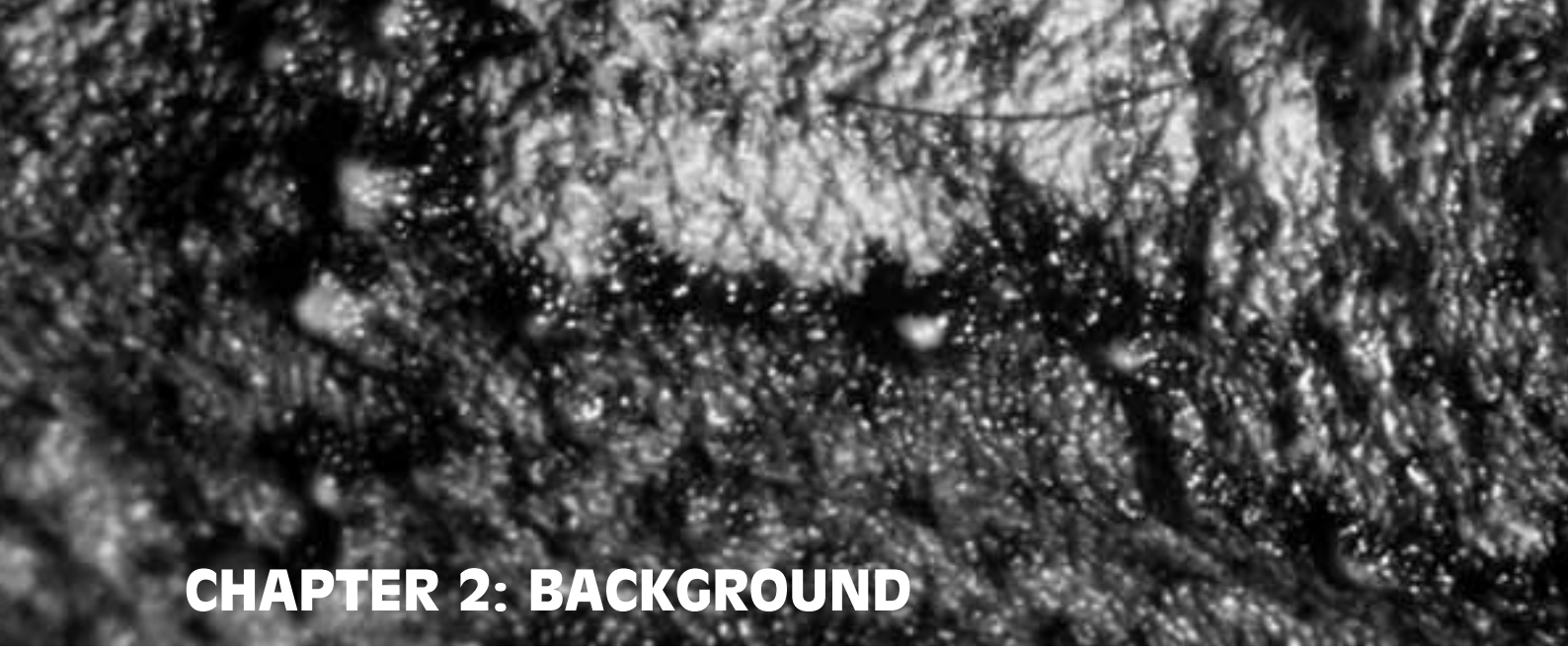
While eradication of *B. dendrobatidis* is not possible at present, well-developed management plans based on current knowledge can assist in restricting the impact and spread of known infestations of the amphibian chytrid and limit spread to new sites.

Reducing its impact will be achieved by implementing currently available strategies for controlling chytridiomycosis, providing for the development of new techniques, conducting a national survey to improve understanding of the extent of the pathogen, and improving understanding of the pathogen and its effects. Successful implementation of the plan will result in the stability of currently infected amphibian populations and the continuing chytridiomycosis-free status of amphibians in non-infected regions.

In addressing the conservation of species, the TAP provides decision support to species recovery plans and existing state and territory programmes. Action will also be taken to ensure that *B. dendrobatidis* does not become

established in important areas, particularly islands, that are currently free of the pathogen. In addition, there will be a focus on collecting and disseminating information to improve our understanding of control and mitigation methods and their effects on host populations, particularly in areas that are currently infected and in areas of manageable size newly identified as infected.

Implementation of the TAP will allow for consolidation and coordination of the process of managing the impact on native amphibian populations of infection with *B. dendrobatidis*. The main priority is to provide support for on-ground control programmes that are necessary for the recovery of threatened species and threatened amphibian communities. Control programmes will have to continue for some time and the costs of this will be considerable. The TAP therefore establishes a framework for allowing the best possible use of resources that are available for managing infection of amphibians with the amphibian chytrid fungus.



CHAPTER 2: BACKGROUND

2.1 Amphibian population declines and association with chytridiomycosis

Dramatic extinctions and declines in Australian frogs have occurred since the 1970's, mostly in protected mountainous areas. Of the 214 species of amphibians described from Australia, 27 are listed as threatened (15 as endangered, 12 as vulnerable) and 4 as extinct (DEH 2003). Chytridiomycosis was the cause of extinction of one species of threatened frog (Table C.2 Appendix C), suspected to have caused the extinction of the three other species, and currently is active in 14 of the 27 species listed as threatened (Table C.1 Appendix C). Of the remaining 13 species no adequate survey for chytridiomycosis appears to have been done. Although in many lowland and populated areas habitat loss, environmental degradation and introduced fish have reduced the distribution and abundance of amphibians, these factors are not associated with the disappearance of highland frogs. Declining frogs are from ten genera and most are from Queensland, NSW and Victoria. Lifestyle rather than taxonomy provides the common links between threatened species. They are mostly from high altitude areas (>300 m), have significantly smaller clutch sizes, occupy restricted geographic ranges, have aquatic tadpoles associated with streams, and many spend a large proportion of their time in or adjacent to streams (Williams and Hero 1998, McDonald and Alford 1999).

Amphibian populations often disappeared or declined so rapidly that initial events were unobserved, and studies of the cause of their decline are retrospective. While some species have become extinct, other species have recovered or have survived at reduced abundance. Initially a novel infectious agent was proposed as the cause of the declines (Laurance et al. 1996, 1997), but the cause was unknown. Chytridiomycosis was subsequently proposed as that novel disease (Berger et al. 1998).

The evidence now available about chytridiomycosis indicates that it was introduced to Australia and has spread through amphibian populations since the 1970's. Factors in the declines in protected areas of Queensland that are consistent with being caused by the introduction of chytridiomycosis, a waterborne infectious disease of high virulence to adults (but not tadpoles) of some species, and include:

- sudden, severe declines occurred over a few months at individual sites
- declines were asynchronous and spread as a front along the east coast of Australia; adults died while tadpoles survived and metamorphs died when they subsequently emerged
- no environmental changes were detected
- only stream-dwelling frogs disappeared *and*
- in one intensively monitored site, mass mortality was observed at the time of a significant population decline.

The mass mortality event occurred in 1993 at the montane rainforest location of Big Tableland in north Queensland (Fig 1). Sick and dying frogs were collected for pathological examination and diagnosed with chytridiomycosis (Berger et al. 1998). These frogs were of the endangered species *Taudactylus acutirostris*,

Litoria rheocola and *Litoria nannotis*. At the time of the declines, healthy tadpoles of T. acutirostris were collected to be raised in captivity at the Royal Melbourne Zoo, however those that metamorphosed died with chytridiomycosis (Banks and McCracken 2002). *T. acutirostris* has since been listed as extinct.

Declines in New South Wales and Victoria are also associated with chytridiomycosis although the best data are for Queensland.

Further work on the genetics and epidemiology of chytridiomycosis (see sections 2.3 and 2.5) supports the hypothesis that chytridiomycosis has been introduced and is likely the prime cause of these dramatic amphibian declines in highland areas, and possibly a contributing factor in declines of some lowland populations. The most likely explanation for the appearance of *B. dendrobatidis* as an emerging infectious disease of global significance is the escape of the pathogen from a host and locality with which it has evolved into novel host species and environments. The earliest case record is from 1938 from *Xenopus laevis* in South Africa and epidemiological data supports that *B. dendrobatidis* originated in Africa (Weldon et al. 2004). The first case detected in Australia occurred in 1978 in southeast Queensland, just before the populations of the southern gastric brooding frog *(Rheobatrachus silus)* and the southern dayfrog *(Taudactylus diurnus)* declined and disappeared (Speare and Berger 2003a).

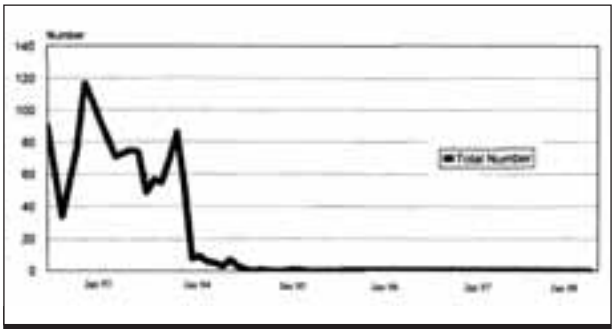


Figure 1. Total numbers of four frog species *(Litoria nannotis, Litoria rheocola, Taudactylus acutirostris and Nyctimystes dayi)* along a transect at Big Tableland. Frogs were collected dying from September 1993 to January 1994. The graph starts in June 1992 and ends at April 1998. The precipitous fall in numbers occurred between 18 August and 29 September 1993. (Reproduced from McDonald and Alford [1999]).

The current epidemiological model is that chytridiomycosis is an emerging infectious disease that has spread globally (Daszak et al. 1999, 2003). It has the potential to cause an epidemic wave of high mortality and morbidity when first introduced to a chytridiomycosis-free area, and then becomes established acting as an endemic pathogen interacting with the local amphibian hosts having pathological effects modified significantly by environment, and particularly by temperature.

2.2 Chytridiomycosis, the disease

Chytridiomycosis is due to infection with the amphibian chytrid fungus, *B. dendrobatidis*. The skin is the only organ invaded by the fungus. In a survey of ill and dead amphibians from eastern Australia between October 1993 and December 2000 chytridiomycosis was the most common disease in Australian amphibians and was the cause of death or morbidity for 133 (55.2%) of 241 free-living amphibians and for 66 (58.4%) of 113 captive amphibians (Berger et al. 2004).

Mortality rates of 100% occurred during natural outbreaks in captivity and in transmission experiments in captive frogs of susceptible species (Berger et al. 1998, Longcore et al. 1999, Berger 2001, Nichols et al. 2001). Incubation times during experimental exposures with susceptible species varied from 9 to 76 days, with most frogs dying between 18 and 48 days post-exposure. Few survived longer than 48 days. The time till death varied with fungal dose and fungal strain. However, infection is not fatal in all species of amphibians, and apparently healthy amphibians, such as bullfrogs *(Rana catesbeiana)*, may frequently carry light infections (Mazzoni 2000, Mazzoni et al. 2003, J. Longcore unpub data).

The clinical signs of chytridiomycosis are non-specific and the disease can not be diagnosed clinically. Laboratory testing is required for diagnosis. Clinical signs in Australian frogs with chytridiomycosis manifest in three ways: behaviour change, neurological signs, and skin lesions. The behavioural signs include lethargy, inappetence, and sitting unprotected during the day with hind legs slightly adducted (Berger et al. 1999). Frogs often change other normal behaviour — burrowing frogs are found uncovered, and arboreal frogs are seen sitting on the ground. Frogs in early stages of becoming symptomatic display some escape activity and have a fairly rapid righting reflex, but if turned over two or three times they rapidly become tired and respond slowly. Frogs become moribund within two to five days of showing clinical signs and soon die. Frogs found dead often appear to be sitting in normal postures as if they died instantaneously and remained in their final living posture. Others were also commonly found floating in water (Harry Hines and Gerry Marantelli, pers comm. 1997).

Skin lesions range from subtle to more obvious changes, and include darkening and patchy discolouration of skin, presence of excessive sloughed skin, erosions, and ulcerations less commonly (Fig 2). Some notable species variation in the clinical signs occurs. The rainforest frogs from Big Tableland, northern Queensland predominantly showed neurological signs; most commonly abnormal sitting posture with hind legs adducted, lethargy, and slow response to tactile stimuli (Berger et al. 1999). When handled, two of these species *(L. rheocola* and *L. nannotis)* became rigid and trembled with extension of the hindlimbs and flexion of the forelimbs. Many of the frogs appeared anaemic, with pale muscles and internal organs. In contrast, individuals of *Litoria caerulea* often become intensely hyperaemic on the belly, legs and feet. Moribund or dead adults of *Mixophyes fleayi* often have swollen thighs (Harry Hines, pers comm. 1997).

Although disease induced experimentally had a moderately long incubation period, frogs were active and ate normally until they suddenly showed behavioural changes and died within a few days (Berger 2001). Even frogs with heavy infections were able to appear and behave normally until some threshold was reached where signs of disease appeared. Frogs with severe clinical signs invariably die.

Most wild frogs found ill or dead with chytridiomycosis were in reasonable body condition with moderate or large fat bodies (Berger 2001). Many females were gravid. Gross pathology of internal organs was generally unremarkable. Congestion and reddening of organs occurred in *L. caerulea*.

In the skin of frogs with chytridiomycosis the zoosporangia occur most commonly in the layer of epidermis just below the surface (Fig 3). The keratinised layer is thickened with an irregular surface. Sloughing of the epidermis may occur with erosions and ulceration in severe cases. However, many frogs die with mild skin changes. There is usually no evidence of a cellular immune response in the skin.

Figure 2. *Mixophyes fasciolatus* in terminal phases of chytridiomycosis with depressed attitude, half closed eyes and accumulation of sloughed skin over the body (arrow head). [Fig 5.2 from Berger 2001] (Scale bar = 5 mm)

Changes in internal organs are non-specific. Neurological signs and death are probably due to absorption of a toxin secreted by *B. dendrobatidis*, but the mechanism has yet to be determined.

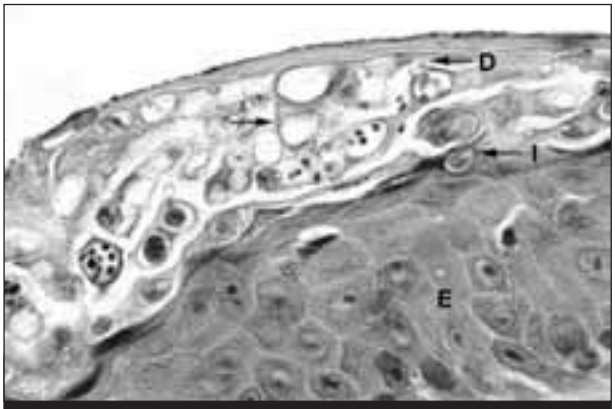


Figure 3. Section of skin from a heavily infected adult of *Litoria caerulea.* Note immature stage (I), zoosporangium with discharge tube (D) containing zoospores, and empty zoosporangium after zoospores have discharged (arrow). E = epidermis. [Fig 1 from Berger et al. 2000] (Scale: largest zoosporangia are 15 _m in diameter)

2.3 Taxonomy and life cycle of *Batrachochytrium dendrobatidis*

B. dendrobatidis is a fungus in the Phylum Chytridiomycota, Class Chytridiomycetes, Order Chytridiales having characteristics separating it from all other chytrid fungi (Longcore et al. 1999). *B. dendrobatidis* appears in two main forms; a spherical sessile zoosporangium (Figs 4–6), 10–40 µm in diameter, and a motile, flagellated zoospore, about 2 µm in diameter. The motile zoospore attaches to the substrate, develops rhizoids, and becomes a zoosporangium. Zoospores form within the zoosporangium and are released into the external environment via discharge tubes.

Other species within this phylum occur as free-living saprophytic fungi in water and soil or are parasitic, for example on plants, algae, nematodes and insects (Powell 1993). *B. dendrobatidis* is unique in the Chytridiomycota in that it invades the skin of amphibians. Zoosporangia grow in the superficial keratinised layers of the epithelium only. In frogs *B. dendrobatidis* can invade any keratinised epidermis, but sporangia are most commonly found on the feet

and ventral skin of the body and limbs. Discharge tubes penetrate through to the surface of the skin and allow zoospores to escape (Fig 6).

In culture *B. dendrobatidis* grows slowly at 6˚C, develops most rapidly at 23˚C but dies if kept at temperatures above 29˚C (Longcore 2000, Berger 2001, Johnson et al. 2003). In culture, the lifecycle takes about 4–5 days at room temperature (Longcore et al. 1999).

Electron microscopic studies of the zoospores from strains collected worldwide did not reveal any significant differences (Berger et al. 1998, Longcore et al. 1999). Genetic studies have also shown the population is homogeneous. Initially, the SSU-rDNA of an infected *L. caerulea* from Queensland was sequenced and compared with sequences in GenBank. This demonstrated that *B. dendrobatidis* is a chytrid related to *Chytridium confervae* (Berger et al. 1998). Initial molecular biological studies using sequencing of the ITS1 and ITS2 regions provided confusing data, not consistent with the epidemiological evidence (Morgan 1999). Multilocus sequence typing was then used to examine genetic diversity among fungal strains from North America (25 strains), Panama (3 strains), Australia (4 strains) and 3 strains isolated from frogs imported from Africa, and only five variable nucleotide positions were detected among 10 loci (5918 base pairs) (Morehouse et al. 2003). These results suggest that *B. dendrobatidis* is a widespread, recently emerged clone and support the epidemiological data showing that chytridiomycosis has been introduced into many countries from a common source.

2.4 Survival of *B. dendrobatidis* in the environment

Once amphibians in a location are found to be infected, *B. dendrobatidis* appears to persist in that location independently of the size of the amphibian population. For example, in the wet tropics in north Queensland individual amphibians of *L. nannotis* or *L. rheocola* that move up water bodies from the lowlands to above approximately 400 metres in altitude are unable to re-establish populations, and disappear as they did previously when their populations suddenly declined. In stable populations once an amphibian has been found with chytridiomycosis, subsequent infection with *B. dendrobatidis* is found in members of that population if the search effort is adequate (Aplin and Kirkpatrick 2000). A current hypothesis is that *B. dendrobatidis* does not depend on amphibians for its existence and either lives saprophytically or has alternative hosts (Speare and Berger 2002).

B. dendrobatidis can grow, but may not thrive, on many different nitrogen sources (Piotrowski et al. 2004) and is likely to be able to exist and proliferate as a saprobe in the environment (Longcore et al. 1999). *B. dendrobatidis* inoculated into sterile lake water remained infective for between 6 and 7 weeks (Johnson and Speare 2003). Optimal pH for growth is 6–7, but *B. dendrobatidis* can grow or survive over a wider range of acidities from 5 to 10 consistent with most water bodies (Piotrowski et al. 2004, Johnson and Speare 2005). Additional anecdotal findings also suggest it can survive and grow as a saprobe in the absence of frogs. For example, collection of moss from locations without frogs has apparently introduced *B. dendrobatidis* into captive collections (R. Puschendorf, unpub).

Zoospores of *B. dendrobatidis* are infective to frogs and tadpoles (Berger et al. 1999, Nichols et al. 2001). They can remain motile for over 24 hours (Berger 2001), with approximately 50% and 5% of zoospores motile after 18 h and 24 h respectively (Piotrowski et al. 2004). Zoospores are unwalled and require water for dispersal. Swimming distance of zoopores is small, less than 2 cm, suggesting that they are unable to actively swim long distances to find a host (Piotrowski et al. 2004). This is the most likely explanation for the clustering of sporangia on the skin of amphibians (Piotrowski et al. 2004).

Although zoospores tolerate a range of osmotic pressures, they die if transferred directly from distilled water to full strength TGhL broth (L. Berger, unpub). In culture *B. dendrobatidis* tolerates some salinity and zoospores will encyst and grow in 6.25 mg/ml NaCl but not in 12.5 mg/ml (Berger 2001).

Sporangia of *B. dendrobatidis* are relatively fragile. Resistant resting spores have not been found in histologically prepared slides of infected skin or during examination of fresh skin in the process of isolating the fungus from more than 80 amphibians (Longcore unpub data). Multi Locus Sequence Typing (MLST) studies indicate that B. dendrobatidis reproduces clonally, which supports the lack, or uncommon occurrence, of a sexually produced resting stage (Morehouse et al. 2003). A 50 mg/ml NaCl solution killed cultures in 5 min (Berger 2001, Johnson et al. 2003). Cultures of zoospores and zoosporangia were killed by drying (Johnson et al. 2003) and sporangia die at temperatures above 30˚C (Longcore et al. 1999, Berger 2001, Johnson et al. 2003).

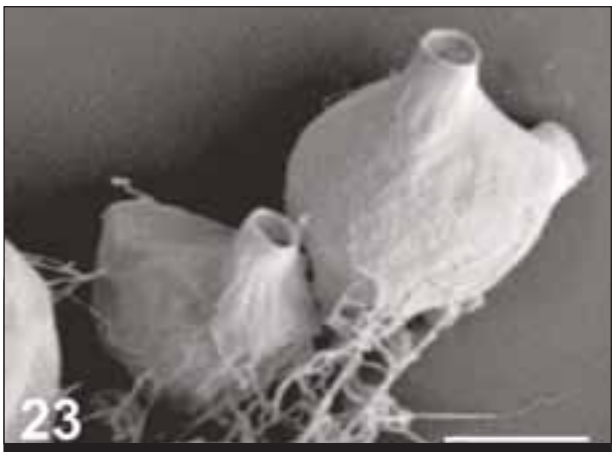


Figure 4. Zoosporangia of *Batrachochytrium dendrobatidis* growing in vitro. Note the roughly spherical shape and the open discharge tubes through which the zoospores escape. The zoosporangium on the right has two discharge tubes due to a wall internally which divides it into two. This is referred to as a colonial thallus. The fine fibres are rhizoids growing from the zoosporangia. [Fig 4.23 from Berger 2001] (Scale bar = 10 _m)

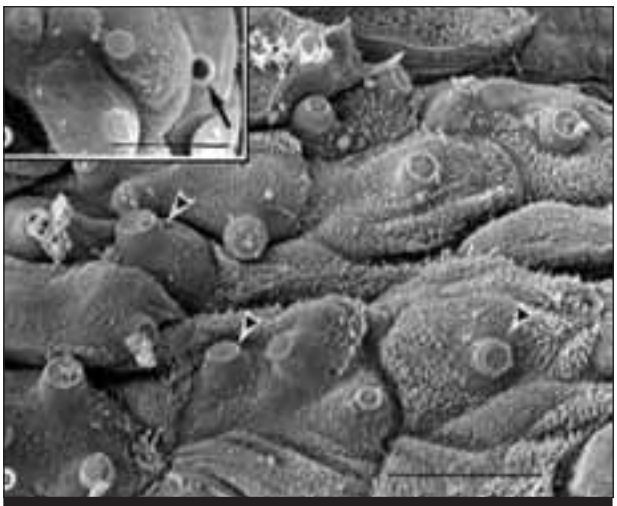


Figure 6. Surface of the epithelium of a *Litoria caerulea* with chytridiomycosis showing discharge tubes of zoosporangia of *B. dendrobatidis* emerging from the surface (arrowheads). Most plugs in the discharge tubes are still intact; in the inset one plug has disappeared (arrow) (Fig 2 SEM from Berger et al. [1998]) (Scale bar = 10 _m).

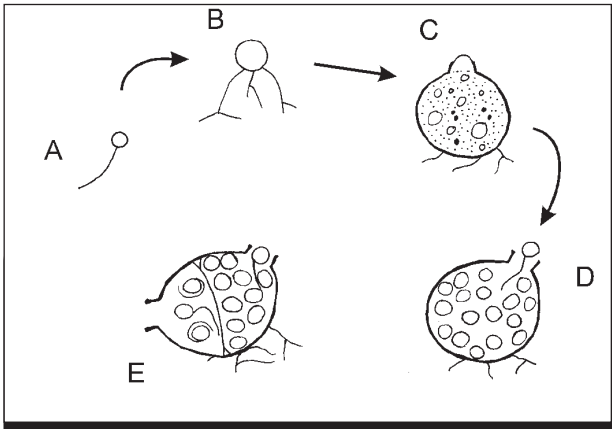


Figure 5. Diagram of the lifecycle of *B. dendrobatidis* in culture. After a period of motility, zoospores encyst, resorb their flagella and form germlings. Rhizoids appear from one or more areas. Sporangia grow larger and mature over 4–5 days. The sporangia become multinucleate by mitotic divisions and the entire contents cleave into zoospores while the discharge tubes form. The discharge tube is closed by a plug that absorbs water and dissolves when zoospores are ready to release. Some thalli develop colonially with thin septa dividing the contents into multiple sporangia each with their own discharge tube. A= zoospore, B = germling, C = immature sporangium, D = monocentric zoosporangium, E = colonial thallus [Fig 4.31 from Berger (2001)] (Not to scale).

2.5 Geographic and temporal occurrence of *B. dendrobatidis*

Chytridiomycosis has been recorded from Australia, New Zealand, Europe, Africa, and South, Central and North America, from a broad range of habitats (Berger et al. 1999, Mutschmann et al. 2000, Waldman et al. 2000, Bosch et al. 2001, Fellers et al. 2001, Waldman et al. 2001, Bradley et al. 2002, Bonaccorso et al. 2003. Surveys of wild African clawed frogs *(Xenopus spp.)* by Che Weldon and Louis Du Prez (Potchefstroom University) both in the wild and housed in museums in southern Africa have shown that *B. dendrobatidis* is widespread in southern Africa, appears to cause minimal clinical disease and has been present since at least 1938 (Weldon et al. 2002, Weldon et al. 2004), providing epidemiological evidence for the hypothesis proposed by Speare et al. (2001) that *B. dendrobatidis* originated in Africa.

In Australia *chytridiomycosis* has been found in amphibians from four geographic areas (Figure 7): a large eastern coast zone extending from Big Tableland near Cooktown in the north to Melbourne in the south, an Adelaide zone, a southwest zone which includes the whole of south west Western Australia to just north of Perth (Berger 2001, Speare and Berger 2003a, Berger et al. 2004), and just recently Tasmania (Obendorf 2005). In eastern Australia cases have occurred at high and low altitudes on or between the Great Dividing Range and the coast. These areas generally have lower maximum

temperatures than inland areas and include some of the wetter areas of Australia. To some extent this distribution also matches searching intensity. When comprehensive surveys were performed in Tasmania in 2004–5 after release of the draft TAP, positive specimens were found (Obendorf 2005). Surveys for chytridiomycosis in Cape York, in the Northern Territory and in the Ord region of Western Australia have been negative (Mendez, Speare, McDonald and Freeland unpub data). This suggests that heat and desiccation may limit the spread of chytridiomycosis, as suggested by studies on survival of the fungus (see section 2.4).

In Australia and New Zealand *B. dendrobatidis* appeared suddenly and then expanded its geographic range. Based on current records (Speare and Berger 2003a), the Australian epidemic appears to have commenced in southeast Queensland (SEQ) in the late 1970s (December 1978 is the earliest record) and extended north and south along the east coast. However, the appearance of commencing in SEQ may be an artifact of searching, since intensive monitoring and collecting of amphibians was occurring in this region; declines in northern NSW where monitoring was less intense may not have been detected. Chytridiomycosis in Western Australia appears to have commenced south of Perth in mid-1985 and subsequently spread in all directions, currently occurring over a wide area of southwest Western Australia (Aplin and Kirkpatrick 2000). The sudden appearance of *B. dendrobatidis* has been demonstrated in both locations of SEQ and southwest WA by retrospective examination of museum specimens, with 700 amphibians negative prior to the 1985 appearance in WA and 110 negative prior to the 1978 appearance in SEQ.

The earliest South Australian record is August 1995 in three specimens of *Limnodynastes tasmaniensis* from Campbelltown (Berger 2001). From that date chytridiomycosis was detected in four other species (see Table B.1 in Appendix B) from Adelaide and its environs.

One hundred and twenty amphibians tested from Northern Territory in 1999 were free of chytridiomycosis (Speare et al. 2001) and prompted a prohibition of imports of amphibians into Northern Territory by the Northern Territory National Parks and Wildlife Commission (Freeland 2000). From the Ord region of northwest Western Australia, 580 amphibians from 15 species tested negative using histopathology (R. Speare personal observation 2005).

Figure 7. Distribution of chytridiomycosis in Australia. From Retallick (2003). This map was generated prior to the discovery of chytridiomycosis in Tasmania (Obendorf 2005).

2.6 Seasonality and temperature effects

Low temperature tips the balance of the infective process in favour of the pathogen. In a survey of wild ill and dead amphibians the incidence of chytridiomycosis was higher in winter, with 53% of frogs from Queensland and New South Wales dying in July and August (Fig 8) (Berger 2001, Berger et al. 2004). Other diseases were detected mostly in spring and summer. The seasonal pattern has also been seen in southwest Western Australia for the motorbike frog, *Litoria moorei* (Aplin and Kirkpatrick 2000) and in north Queensland in wet tropics for *L. genimaculata, L. nannotis and L. rheocola* (McDonald et al. 2005) and at Eungella in Taudactylus eungellensis and Litoria lesueuri (Retallick et al. 2004). In experimental infections in the laboratory, lower temperatures enhanced the virulence of chytridiomycosis in *Mixophyes fasciolatus*. All frogs exposed to *B. dendrobatidis* at 17°C and 23°C died, whereas 50% of frogs exposed at 27°C survived (Berger 2001; Berger et al. 2004). Infections in survivors were eliminated by 98 days. Woodams et al. (2003) subsequently successfully eliminated *B. dendrobatidis* from experimentally infected Litoria chloris by placing them in an environmental temperature of 37°C for 16 hours. It is not known if the effect of temperature on disease and prevalence is predominantly due to direct results of temperature on growth of *B. dendrobatidis* or to a combination of reduced host immunity or metabolism and increased fungal growth at colder temperatures.

Although the increased mortality due to chytridiomycosis in colder months and at lower experimental temperatures generally supports the theory that amphibian populations at high altitude have disappeared due to lower temperatures and hence greater susceptibility to disease, it is difficult to explain how this effect is consistent over various latitudes. Multiple factors may be influencing the susceptibility of populations.

The sensitivity of *B. dendrobatidis* to temperature may limit its spread and it may not become established or affect frogs in locations where temperatures are consistently high.

During severe drought in 2001 no mass mortality events, as occurred in preceding years, were detected in Brisbane and environs (H. Hines, pers comm.)

A statistically significant decrease in prevalence of chytridiomycosis was detected over a five year period in *Litoria genimaculata* monitored in the wet tropics approximately five years after the population crashed (McDonald et al. 2005). This has been associated with recovery of these populations to pre-decline levels suggesting that if a species survives the initial epidemic wave, selection for innate resistance may naturally occur. Alternatively, the decrease in prevalence may be due to a lower than normal rainfall although additional evidence to support either hypothesis is not available. If selection for resistance did occur, it would have had to take place over 3–4 generations, and this is feasible since chytridiomycosis with its 100% mortality rate can exert a very high selective force. This highlights a need in infected populations to maintain a large number of individuals with sufficient genetic diversity to allow selection.

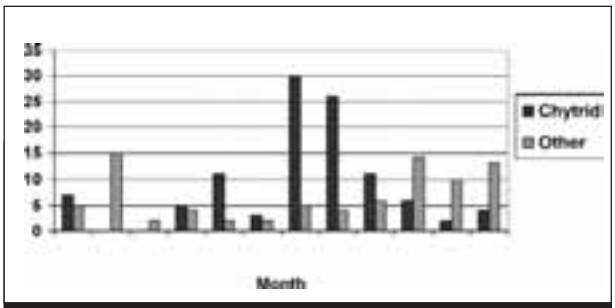


Figure 8. Total numbers of ill or dead wild frogs examined from Queensland and New South Wales per month, comparing frogs diagnosed with severe chytridiomycosis with frogs with other diseases. Includes frogs submitted from October 1993 to December 2000. [Fig 2 from Berger et al. 2004]

2.7 Effects on different species of amphibians

Chytridiomycosis has been found in 49 species of native amphibians in the wild in Australia (Table B.1 Appendix B). It has also been found in an additional three native species in captivity (Table B.1), and in two introduced amphibians, the cane toad *(Bufo marinus)* (wild and captive populations) and the axolotyl (captive). Of the five amphibian families in the wild in Australia, chytridiomycosis has been reported in three: Hylidae, Myobatrachidae and Bufonidae (Table B.3 Appendix B). The sampling effort on species in Microhylidae and Ranidae has been very low, so the lack of records may reflect this rather than a true absence. Globally, chytridiomycosis has been reported in amphibians from two orders in 19 families, 43 genera and 143 species (Table D.1 Appendix D). The amphibian chytrid appears capable of infecting any species of amphibian, but the intensity of infection and the pathological effects appear to be strongly dependent on the host species.

Declines have affected amphibian species to various extents and analysis of the ecology of declining species compared with non-declining species has shown that stream-dwelling species with small distributions that are less fecund are most likely to have declined (Williams and Hero 1998). However, within this general trend there are more complex interactions and apparently inherent differences in susceptibility operating. For example *L. genimaculata*, a species sympatric to the now extinct frog *T. acutirostris*, declined at the same site at O'Keefe Creek, Big Tableland at the time other species disappeared (McDonald and Alford 1999). However, it did not apparently suffer such a large mortality rate and the population has since recovered (McDonald et al. 2005). Healthy L. peronii have been found during chytrid outbreaks in other species (Berger 2001). Experimental data have now shown that the susceptibility of four species, *L. caerulea*, *L. chloris, M. fasciolatus* and *Limnodynastes tasmaniensis*, varies when inoculated under standard conditions (Ardipradja 2001). *B. marinus* appears to be a relatively resistant species (L. Berger, unpub).

2.8 Effects on tadpoles and eggs

While *B. dendrobatidis* may cause fatal disease in post-metamorphic amphibians, it does not appear to cause mortality in tadpoles and has not been found growing on eggs.

When groups of captive spawned tadpoles have become infected, tadpoles have appeared healthy but then close to 100% mortality has occurred within 3 weeks of metamorphosis (Berger et al. 1998).

On amphibians, *B. dendrobatidis* occurs only in keratinised epithelium. As tadpole skin is not generally keratinised, the pathogen has a restricted distribution on tadpoles, although they may remain infected for long periods (Berger 2001, Lamirande and Nichols 2002). In early tadpole stages *B. dendrobatidis* is found in the mouthparts as this is the only keratinised epithelium on the body. In later tadpole stages as the tail and feet become keratinised and the mouthparts are lost, zoosporangia begin to invade these sites (Berger 2001, Marantelli et al. 2004).

Tadpoles may be infected at Gosner stage 24–25 (Gosner 1960), usually the first stage after hatching in which keratinised teeth and jaw sheaths develop. A study in *M. fasciolatus* showed that *B. dendrobatidis* often occurred on all surfaces of the keratinised jaw sheaths, but were usually only present on the caudal aspect of tooth rows (Berger 2001, Marantelli et al. 2004). Heavier infections were seen on the jaw sheaths. Infection with *B. dendrobatidis* also extended caudally from the mouth a short way along the surfaces of the anterior buccal cavity. Infection around the mouth was lost when tadpole mouthparts were shed at stage 42.

The feet were first seen with a light infection with *B. dendrobatidis* at stage 42. As the tail resorbed, sporangia became established over the body by stage 45. The resorbing tails and tail stumps of infected frogs contained extremely heavy infections with *B. dendrobatidis* (Berger 2001, Marantelli et al. 2004).

Infection of embryonic stages appears unlikely, but the potential of *Batrachochytrium* to infect egg capsules needs to be investigated by experimental infection. These issues are important when assessing the risks of moving eggs or tadpoles, which is commonly done for conservation. For example, enhancement of tadpole survival in endangered frog species has been achieved by rearing tadpoles in captivity after collection of eggs from the wild (Hunter et al. 1999). Samples of southern corroboree frog *(Pseudophryne corroboree)* tadpoles raised in this way were sacrificed for testing before the remainder of the group were released. All 26 tadpoles tested from 3 release events were negative for *Batrachochytrium* by histology (Berger 2001).

B. dendrobatidis has been detected at high prevalence in free-living tadpoles. *B. dendrobatidis* occurred in 16 of 24 tadpoles of *Rana muscosa* in California (Fellers et al. 2001). All infected tadpoles had oral disc abnormalities, such as misshapen and missing tooth rows, loss of pigment and flattening of the epithelial cells. The tadpoles had large fat bodies and appeared healthy (Fellers et al. 2001). However, Rachowicz (2002) reports that loss of pigment occurs regularly in colder months without chytrid infections. Infections with *B. dendrobatidis* were found in the mouthparts of 13 of 15 wild tadpoles of *Mixophyes* sp. from eastern Queensland (Berger 2001). A sample of tadpoles of *Litoria verreauxii* and *Litoria peronii* were collected from Watagan State Forest NSW because they appeared slightly thinner in the abdomen. On examination, the jaw sheaths had partial missing keratin and some tooth rows were incomplete. They were diagnosed with chytrid infection at SPHTM (Anstis 2002 unpub.).

In experimental infections in blue-and-yellow poison dart frogs *(Dendrobates tinctorius)*, only recently metamorphed frogs died while sub-adults and adults became infected, but did not die (Lamirande and Nichols 2002). Similarly, mortalities in captive *B. marinus* occurred mostly in metamorphs, with few deaths in juveniles and none in adults kept in the same room (Berger 2001). In the wild in southeast Queensland, metamorphs are surviving in areas where 100% of tadpoles are infected, suggesting that infected individuals can survive through metamorphosis (Harry Hines, pers comm 2003). However, in many other species, such as *L. caerulea*, outbreaks in the wild cause deaths of adults as well as metamorphs.

2.9 Impact of chytridiomycosis on wild amphibian populations

Since the amphibian chytrid fungus is a highly virulent pathogen it has the potential to cause amphibian species to change threatened species status. Four patterns of response to the appearance of the amphibian chytrid have been seen; 1) species extinction, 2) extinction of local populations, but survival of the species, 3) population decline and recovery, 4) sporadic deaths. All populations in which *B. dendrobatidis* has been found and which have been intensively studied appear to show some impact, if at the minimum, occasional deaths at particular periods. The final balance between *B. dendrobatidis* and various frog species cannot be predicted, but the abundance of frogs may be permanently reduced.

The amphibian chytrid has now been associated with amphibian population declines in eastern Australia, New Zealand, USA, Panama, Ecuador, Venezuela and Spain. At least one Australian species, *T. acutirostris*, was made extinct by the amphibian chytrid. The last known captive specimen died in Melbourne Zoo from chytridiomycosis that had been acquired in the wild in North Queensland as a tadpole. A lone male was recorded in a tributary of the South Johnstone River in

November 1996 (Marshall 1998), but as no further sightings were made even after intensive searching, the species was declared extinct in 1999 (DEH 2003). A number of species currently listed as 'extinct' (DEH 2003), particularly the two species of gastric brooding frogs *(R. silus* and *Rheobatrachus vitellinus)* and the southern day frog *(T. diurnus)*, may well have been eliminated by the disease (Berger 2001). However the frogs had disappeared prior to any knowledge of chytridiomycosis and specimen numbers in museums were too small to enable statistically useful samples to be assessed. Other species which have undergone population declines with loss of upland populations had reduction in population numbers (Richards et al. 1993, McDonald and Alford 1999, Hines et al. 1999, Gillespie and Hines 1999, Osborne et al. 1999); or had sporadic deaths associated with chytridiomycosis (Speare 2000a).

2.9.1 Species extinction

Extinction is illustrated by the response of the sharp snouted dayfrog, *T. acutirostris*. The status of this frog changed from not listed, to endangered in 1992, to extinct in 1999. This frog occurred in Queensland only in a range that extended 310 km in upland wet tropics from Mt Graham to Big Tableland, just south of Cooktown (McDonald 1992). Populations were abundant in all locations, with 50–100 frogs per 100 metre transect being common (Richards et al. 1993). The first populations to disappear were in 1990. Populations then disappeared in a progressively northern direction, the final population at Big Tableland disappearing by early 1994. The decline of this population was precipitous, most frogs disappearing over 3 months and survivors dying over the next 6 months. Chytridiomycosis was found in wild *T. acutirostris* as well as being responsible for the death of adult frogs and metamorphs brought into captivity in late 1993. The last surviving captive *T. acutirostris* was a male that died from chytridiomycosis at Melbourne Zoo in 1995. Since the causative agent had not been identified at that time, the therapeutic measures adopted in captivity were ineffective. Although a report of a calling male was made in the field in November 1996 (Marshall 1998), the species was not found subsequently with intensive searching.

2.9.2 Local extinction, but survival of the species

Several examples are known where chytridiomycosis has caused local extinctions of populations. Good examples of this pattern are provided by the upland wet tropics species, *L. nannotis, L. rheocola* and *N. dayi*. These species followed the same pattern of population decline as *T. acutirostris*, with extinction of all upland populations. However, lowland populations on the same watercourses persisted. In the lowlands sporadic deaths occur, but population numbers appear to be stable. Experimental studies in upland wet tropics rainforest confirmed high rates of mortality in winter from chytridiomycosis in *L. rheocola* translocated from lowland sites. Frogs translocated in summer did not show a pattern of sudden high mortality and frogs at lowland sites showed some or no mortality due to chytridiomycosis in summer and a slight mortality in winter (Retallick and Dwyer 2000). The experimental studies mimicked the natural events with local extinctions at upland sites and sporadic deaths at lowland sites (Retallick 2002).

The spotted treefrog, *Litoria spenceri*, also showed a similar pattern when a stable population at Bogong Creek suddenly declined in 1996 and the last frog was seen in 1999 (Gillespie and Hines 1999; Gillespie and Marantelli 2000). Chytridiomycosis was a cause of death in frogs autopsied. The first appearance of the fungus in a retrospective survey of toe clips was in March 1996, the last day the frogs were seen in high numbers. In lowland populations of L. spenceri chytridiomycosis was present, but the population did not decline.

Chytridiomycosis is probably the cause of the disappearance of *Adelotus brevis* from upland areas of southeastern Queensland and northern New South Wales, and has resulted in listing of the New England Tableland population by New South Wales and could possibly result eventually in national listing of this species.

Species most likely to become newly listed as a result of chytridiomycosis are those that occupy habitat in currently chytridiomycosis-free zones. In particular frog communities in Cape York Peninsula and the Gulf Country in Queensland, northwest Western Australia, the Northern Territory and most of central Australia are potentially at risk as *B. dendrobatidis* expands from its current zones. The susceptibility of individual species is difficult to predict since we do not know what innate characteristics correlate with high mortality rates.

2.9.3 Population declines, but recovers

At Big Tableland, *L. genimaculata*, a species sympatric with *T. acutirostris, L. nannotis* and *L. rheocola*, suffered a sudden decline in numbers at the same time that *T. acutirostris* disappeared (Laurance et al. 1996, McDonald and Alford 1999, McDonald et al 2005). However, the population of *L. genimaculata* recovered to close to former numbers after five years. Occasional individuals of *L. genimaculata* are now found ill or dying from chytridiomycosis, but the prevalence declined significantly between 1998 and 2002, demonstrating

that *B. dendrobatidis* occurs in the environment, but apparently a balance between *L. genimaculata* and the pathogen has been arrived at (McDonald et al. 2005). As discussed in Section 2.6 this could have been due to selection for resistance or to a lower rainfall.

2.9.4 Sporadic deaths

A pattern of sporadic deaths due to chytridiomycosis is seen with many of the species infected by *B. dendrobatidis* in Australia (Speare and Berger 2003a). Once *B. dendrobatidis* has become established in a population of amphibians, sporadic death is to be expected as the minimum impact.

The amphibian chytrid may have a critical impact on any amphibian population that is infected. Depending on a complex interaction between the innate characteristics of individual species, the pathogen and the environment, including how the species is distributed across a range of environments, the outcome for a species can range from extinction of the species as the worst case scenario, to a stable population with sporadic deaths due to chytridiomycosis. However, the vulnerability of the apparently stable population to the effect of normal environmental variations and to abnormal environmental factors is probably increased.

2.10 Resistance to infection

Resistance to infection can be due to innate or acquired immunity. The innate response is a natural response to microorganisms and depends on the host recognising them as foreign. It is a response that is non-specific and not targeted against the particular pathogen. The acquired immune response is a highly specific response that uses antibodies and immune cells targeted against specific antigens of the pathogen. The acquired immune response relies on previous exposure to the antigens of the pathogen. The innate response is usually less effective at protecting the host than the acquired immune response. In chytridiomycosis the innate immune response mediated via phagocytic cells does not occur in most instances. The local epithelial cells respond to the presence of the amphibian chytrid, but the phagocytic and vascular components are missing.

Innate resistance to *B. dendrobatidis* appears to be present in some individuals and in some species (see section 2.7). As abundance of frogs in some areas is increasing despite the presence of *B. dendrobatidis*, this suggests resistance is evolving in these populations. However, the components of the immune system that are mobilised against *B. dendrobatidis* have not been determined. There is usually negligible cellular inflammatory response in the dermis of infected frogs. Antifungal peptides have been hypothesised to play a protective role.

Although peptides from amphibian skin have fungicidal activity against *B. dendrobatidis* in vitro (Berger 2001, Rollins-Smith et al. 2002), they obviously are not universally protective for frogs. For example *L. caerulea* secretes at least 40 peptides and this species is highly susceptible to chytridiomycosis. In the study of distribution of infection in severely infected *L. caerulea*, large numbers of sporangia occurred in all areas of ventral skin examined and very few or no sporangia occurred on two sites of dorsal skin. Large parotoid glands occur on dorsal skin behind the tympanum, and antifungal secretions from these glands or the smaller dispersed serous glands (which are more abundant in dorsal skin) may inhibit infection. However, sporangia were seen growing at the opening of ducts of serous glands, suggesting secretions are not effective in stopping infection (Berger 2001). There are major differences between frog species in the type and amounts of peptide produced.

Studies on whether immunity can be acquired in individual frogs have not been reported. However, when antifungal drugs or heat have been used to reduce infections to undetectable levels, but not eliminate infection, disease has been delayed but not cured except in one instance (Woodhams et al. 2003). Once treatment stops sporangia can multiply to pathogenic levels (G. Marantelli, unpub data). With many other diseases, a large reduction of pathogen burden enables the host to mount an effective immune response.

Recovery of amphibian populations possibly may be assisted by selection for genes that control innate resistance.

2.11 Transmission and spread of the pathogen

As *B. dendrobatidis* does not form a resting stage and does not survive drying, it appears most likely to be spread via water or by contact between frogs. The infective stage of *B. dendrobatidis* is the zoospore and transmission requires water. It can spread slowly over the landscape by natural methods, but movement long distances such as over oceans and across deserts is most likely due to human assisted translocation of infected amphibians or water contaminated with the amphibian chytrid.

B. dendrobatidis is highly infectious and even low experimental doses can result in fatal disease in susceptible species. Frogs can be experimentally infected by placing them in water containing zoospores. In one infection experiment an inoculating dose of 100 zoospores caused chytridiomycosis in all 3 *M. fasciolatus* while 10 zoospores did not cause disease in another 3 (Berger et al. 1999). Zoospores released from an infected amphibian enter the water and can potentially infect other amphibians in the same water. This has been demonstrated experimentally (Berger et al. 1998), and it is assumed that the same process occurs in the natural situation. However, the dynamics of infection in the wild has not been studied.

B. dendrobatidis appears to spread independently from infected foci into adjacent areas both along and between water bodies. In Queensland, Victoria and Western Australia the apparent rate of spread of chytridiomycosis has been approximately 100 km per year (Aplin and Kirkpatrick 2000, Speare 2000a,b). The mechanism of spread is unknown, but probably involves normal movement of individual infected amphibians, water bodies and surface water possibly during rain. *B. dendrobatidis* will grow on feathers and survive drying of feathers for short periods (Johnson and Speare 2005).

Since no resting stage of *B. dendrobatidis* has been found, the hypothesis is that new, distant zones are initiated by the escape of the amphibian chytrid from infected amphibians bought into that area. In the case of amphibians deliberately moved, infection could be initiated by release of the infected amphibian or by release of water containing zoospores (Johnson and Speare 2003). This hypothesis applies to new infections in countries and new infections in regions within countries. Movement of wet soil or other wet material could also potentially move *B. dendrobatidis*. However, there is no evidence as yet that *B. dendrobatidis* can survive outside water bodies. Currently, there is no test to detect the pathogen in the environment (see Section 2.12).

B. dendrobatidis is known to transmit in water via its zoospore (Berger et al. 1998; Longcore et al. 1999; Pessier et al. 1999; Nichols et al. 2001). In theory it may also transmit by contact (Ross Alford per com). New foci of infections are probably started by movement of infected frogs or contaminated water into a chytridiomycosis-free area. Frogs with chytridiomycosis have been detected in the scientific trade (Reed et al. 2000; Speare et al. 2001; Parker et al. 2002; Weldon et al. 2004), pet trade (Mutschmann et al. 2000; Speare et al. 2001), food trade (Mazzoni 2000, Mazzoni et al. 2003), zoo frogs (Nichols et al. 1998) and frogs accidentally translocated (Marantelli and Hobbs 2000). Administrative and public action can assist in reducing these methods of transmission.

Amphibians with chytridiomycosis are not uncommon in the amphibian pet trade. In Australia, 3 of 6 axolotls were infected when purchased in Townsville and Perth (Speare 2000a). In Germany over 200 amphibians in the pet trade have been found to have chytridiomycosis over a number of years (Mutschmann et al. 2000). Infected amphibians moved deliberately in the pet trade between or within countries are risks for bringing chytridiomycosis to new areas. Ensuring amphibians sold as pets or for collections are free of chytridiomycosis would be an effective management action.

Similarly, amphibians sold and moved for scientific studies can be a potential infection risk. *Xenopus laevis* and *X. tropicalis* caught in the wild in Africa and moved into scientific institutions in South Africa (Speare and Berger 2003b) and USA (Reed et al. 2000, Parker et al. 2002) respectively have been shown to be infected with chytridiomycosis. Within Australia the cane toad is the major amphibian sold for scientific purposes. Regulating the infection status of amphibians in the scientific trade within Australia and on imported animals would be a feasible management strategy.

Amphibians accidentally moved in agricultural produce also have the potential to move *B. dendrobatidis* long distances, either within or between countries. Native frogs rescued from produce in Melbourne have been found to be infected with *B. dendrobatidis* (Marantelli and Hobbs 2000). Work in banana farms in North Queensland showed that simple management practices can limit accidental translocation. The effective strategies consisted of raising the awareness of management and workers, safe removal of any amphibians found on bunches in the sheds by placing in a bucket with a lid for release in native habitat, and, after being filled with bananas, placing all cartons in chillers overnight to limit access of frogs prior to dispatch (McDonald, Speare and Mendez, unpublished). Frogs on bananas processed in bath systems generally leave the hands, enter the water of the bath and can be collected and placed in a bucket with a top. Frogs on bananas in wheel-based systems may not so readily leave the hands. A comparative study should be undertaken to determine the likelihood of frogs remaining with hands of bananas in both processing systems.

Since Australia has no food trade in live amphibians, this potential route for spread of the amphibian chytrid is not a risk.

Tadpoles often have a high prevalence of aclinical chytridiomycosis and should be regarded as a potential risk. Accidental movement of tadpoles with samples of aquatic plants in the aquarium or nursery trade has been noted (Deborah Pergolloti pers com 2004) and could potentially cause escape of *B. dendrobatidis* if the tadpoles, plants or water are released into natural or artificial water bodies.

Translocation and release of frogs for conservation purposes is also a potential risk for spread. A useful management strategy would be setting national guidelines for release of amphibians from captivity into the wild.

There is no evidence that scientists working with amphibians have transmitted chytridiomycosis. However, scientists working with amphibians are more likely to have closer contact with amphibians (and *B. dendrobatidis*) than other people using the catchment. Consequently, it is reasonable that scientists use hygiene protocols when handling frogs. Protocols to disinfect equipment used for handling frogs and for equipment immersed in water (see Section 3.3) can be regulated. The potential also exists to transmit the chytrid fungus between frogs in infected areas by handling. Hygiene practices to limit transmission can be incorporated into permits. People who interact with water should also be aware of the risk of coming into contact with the amphibian chytrid and use protocols to prevent water being moved between catchments.

The theory behind hygiene protocols is that handling should not increase the risk to individual amphibians above that provided by the background risk associated with living in the particular water body. Sterile or aseptic protocols are not required. Comprehensive protocols have been developed by several state parks and wildlife services. National guidelines could be proposed to unify these.

Chytridiomycosis once established at a geographic location appears to spread as an epidemic wave (Dasak et al. 1999, 2003). How this occurs is unknown, but it may be by movement of infected amphibians, spread of surface water, or other methods. Much more needs to be learnt about the natural history of *B. dendrobatidis* in the environment. The role played by human activities in transmission of the amphibian chytrid in already contaminated areas is unknown.

Determining the relative contributions of human assisted transmission and natural transmission is important in implementing policies and protocols. Policy and strategies based on better evidence can then be developed.

2.12 Diagnosis of chytridiomycosis

Chytridiomycosis can only be diagnosed by laboratory tests (Berger et al. 1998, Berger and Speare 1998, Berger et al. 1999, Berger et al. 2000, Hyatt et al. 2000, Hyatt 2003). The clinical signs of chytridiomycosis can be similar to those of other amphibian diseases, such as iridoviral infection and bacterial septicaemia (red leg) (Cunningham et al. 1996), so that laboratory tests are required to make a diagnosis. Also, healthy frogs may carry infections. The development of accurate tests that do not harm the amphibian is important for increasing the efficacy of quarantine measures.

The following diagnostic tests are available:

- Microscopy
  - Direct examination of skin scrapings
  - Histology — haematoxylin and eosin (H&E) or silver stain
  - Immunoperoxidase
- Enzyme Linked Immunosorbent Assay (ELISA)
- Polymerase Chain Reaction (PCR) — standard or real-time Taqman
- Culture

Most of the work to date has been done using microscopy to visualise sporangia (Table 1) (Berger et al. 2000, 2002, Berger 2001). Sensitivity of examination of unstained skin varies greatly with the experience of the operator. These routine tests have a high positive predictive value when used on diseased frogs which have heavy infections. However, healthy frogs typically have light infections and only small samples can be obtained without sacrificing the animal. The immunoperoxidase stain using polyclonal antibodies against *B. dendrobatidis* has greater sensitivity than the histological technique. Using the immunoperoxidase stain on toeclip samples, infections in 61.8% of lightly infected frogs were detected at 19 days post experimental exposure, while 52.7% were detected using H&E staining (Berger et al. 2002). Monoclonal and polyclonal antibodies have been produced for use in an ELISA, and a real time PCR test has been developed (Hyatt 2003). The ELISA detects heavily infected frogs only and is not a useful test for routine diagnosis (Hyatt 2003).

The realtime PCR is highly sensitive and can detect *B. dendrobatidis* within one week of experimental infection. The real time PCR appears likely to become the test of choice for healthy frogs (Hyatt 2003; Boyle et al. 2004). The PCR test can be done on frozen or ethanol-stored toe samples, or from saline in which frogs have been immersed. It is a quantitative test, giving an indication of the levels of infection, and can detect one zoopospore in a test sample (Boyle et al. 2004). A major advantage of the PCR test is that it does not involve removal of a toe or destruction of the amphibian (Hyatt 2003; Boyle et al. 2004). Frogs can be placed in a weak saline solution for 15 minutes, released and the sample tested or swabs can be rubbed over the skin to collect a sample. A standard PCR has also been developed (Annis et al; 2004), but there has been no comparison between the two techniques.

Isolation of strains and growth in culture is a specialised process requiring high-level skills (Longcore 2000). Bacterial contamination of cultures is a major problem. Sensitivity of isolation and culture is very low unless performed by an expert.

Table 1. Comparison of the characteristics of each diagnostic test based on light microscopy. All three methods of diagnosis are useful although they have various advantages and disadvantages. * = low, ** = medium, *** = high

Type of test	Skin scrapings	Histology	Immunostaining
Complexity and cost of preparation	*	**	***
Ease of interpretation	*	**	***
Sensitivity	*	**	***

The real time PCR test has not yet been used to detect the pathogen in environmental samples such as soil and water. The real time PCR test offers the sensitivity and specificity required to detect the low number of zoospores expected (Hyatt 2003). The Australian Animal Health Laboratory is currently working to develop such a test.

2.13 Interaction with other amphibian diseases

Ranaviruses that cause mass mortalities in free-living amphibians in America and Europe (Cunningham et al. 1996, Green et al. 2002) have not been detected in Australia. Australia has two described ranaviruses namely epizootic haematopoietic necrosis virus (Langdon et al. 1986) and the Bohle Iridovirus (BIV) (Speare and Smith 1992). Of these only BIV is known to infect and cause disease in amphibians. It is assumed that BIV is endemic, but its impact on amphibian populations is unknown. If introduced to Australia, the overseas ranaviruses may cause high mortality rates. This additional mortality may reduce the capacity of Australia's frog populations to recover from chytridiomycosis. Similarly the negative effects of other diseases currently in amphibians in Australia may be synergistic with chytridiomycosis. However, no data is available.

Many of the quarantine strategies that are suggested to reduce spread of chytridiomycosis will also reduce the risk of importing other diseases. However, ranaviruses are more resistant than *B. dendrobatidis* to physical and chemical disinfectants (Langdon 1989).

Monitoring amphibian disease nationally will allow an assessment of the impact of chytridiomycosis and rapid detection of other diseases that impact on amphibian populations. It took 20 years to detect chytridiomycosis from the apparent time of first serious impact until diagnosis. A national monitoring system for amphibian diseases would be expected to reduce this response time to weeks rather than decades.

CHAPTER 3: DEALING WITH THE PROBLEM

Interventions are possible in managing infection with the amphibian chytrid resulting in chytridiomycosis. The most proactive strategies fall into three categories:

1. extractive — remove species in whole or part to captivity and/or ensure the species' genes are preserved in a gene bank
2. enhancement — improve habitat to favour host, remove *B. dendrobatidis* from environment or decrease its pathogenicity to reduce mortality
3. introductive — restock populations from a captive nucleus or from other surviving population.

Other less innovative strategies are mainly directed towards preventing spread, particularly from infected to chytridiomycosis-free areas, but also between populations and individuals in chytrid-infected areas.

To manage the problem of chytridiomycosis a set of tools, skills and protocols are needed. Knowledge of the current chytrid status on a geographical and species basis is important, as well as an ongoing monitoring system that can detect changes, particularly in chytridiomycosis-free areas. Hence, mapping strategies, surveillance protocols and diagnostic tests are discussed in Sections 3.1 and 3.2. Active interventions that reduce transmission of *B. dendrobatidis* are disinfection of inanimate objects and treatment of potentially positive amphibians (Section 3.3 and 3.4). Strategies to tip the balance in favour of the host population are not well studied, but include habitat modification (Section 3.7), selection for resistance (Section 3.5) and restocking, preferably with resistant genotypes (Section 3.6). Strategies, such as use of antifungal agents, directed against the amphibian chytrid will be difficult to implement in remote areas, but may be useful in localised populations of high conservation value. However, these have to be developed and tested (Section 3.3).

For each threatened species, recovery actions to manage chytridiomycosis should include risk assessment based on best evidence as a feasible management strategy. To allow prompt implementation of recovery actions, effective monitoring and surveillance for chytridiomycosis is essential.

3.1 Monitoring and surveillance of populations

Monitoring and surveillance of populations provide information on disease outbreaks, as well as on distribution, prevalence, and incidence of *B. dendrobatidis*. Surveys can be one-off to determine if a population, and hence a water body, is infected with the amphibian chytrid, or they can be systematic and ongoing. Systematic ongoing surveys (surveillance) focused on key populations provide data on the epidemiology of chytridiomycosis over time, will assist in understanding when populations are vulnerable to other management practices, and the ideal times for management strategies to increase the population (e.g. reintroductions and translocations).

Studies to date on monitoring *B. dendrobatidis* in populations have been conducted by histological examination of sick or healthy frogs and tadpoles. Detection of sporangia on sick frogs is a sensitive method of detection. However, sick frogs are

generally not seen unless an outbreak is occurring. Healthy frogs can be tested by histologic examination of toe clip samples, and also by realtime PCR (see Section 2.12). However, healthy tadpoles often have a very high prevalence of infection, and testing tadpoles may be the most sensitive way to assess a population or location (Berger et al. 2000).

Culturing *B. dendrobatidis* from the environment is not likely to be achievable, due to the low concentrations of sporangia and the presence of many faster growing organisms. The development of PCR tests for DNA extracted from soil or water will allow environmental testing. Another method to detect infected sites may be the use of sentinel tadpoles that are placed within mesh bags in water bodies for a few weeks before returning to the laboratory for analysis.

Feasible survey protocols with sufficient power need to be designed for surveying amphibian populations to determine whether they are infected with chytrid or free of chytrid to a particular cutoff prevalence.

3.2 Defining chytridiomycosis-free status

3.2.1 Terminology

Currently two terms are used to define chytrid status of amphibian populations, water bodies and regions:

1. chytridiomycosis-contaminated or chytridiomycosis-positive; and
2. chytridiomycosis-free.

The first term is straightforward with status determined by a single positive record irrespective of how the record was derived. 'Chytridiomycosis-free' is less straightforward as searching effort and sampling technique must have sufficient power to be able to detect an expected level. An expected level must be defined as absolute certainty of absence in a population cannot be achieved unless the complete population is surveyed with a highly sensitive technique. One of the important tasks to be done is to determine what sampling protocols and intensity of survey is adequate. Three classifications of status can then be used:

1. chytridiomycosis-contaminated or chytridiomycosis-positive (population has a positive record);
2. chytridiomycosis-free (population has been adequately sampled and no positives detected); and
3. chytridiomycosis-unknown (population has not been adequately sampled).

'Chytridiomycosis-unknown' has not been used in this document. 'Chytridiomycosis-free' or 'currently chytridiomycosis-free' has been used for areas that are actually 'chytridiomycosis-unknown' as the concept of chytridiomycosis-free areas is essential for management and a timely response demands decisions based on available evidence even if imperfect.

3.2.2 Diagnostic tests for surveys

Chytridiomycosis can only be diagnosed by laboratory tests. The technique currently used for surveys is histological examination of toe clips (Berger et al. 2000). Valuable historical data can be obtained by examining archived amphibian specimens in museums and other collections using histology of toes or toe webbing. In Australia the technique has been used in two geographic areas, coastal Queensland and southwest Western Australia, to identify earliest records, December 1978 in southeast Queensland (Speare and Berger 2003a) and 1985 in southwest Western Australia (Aplin and Kirkpatrick 2000). In these surveys multiple species were used. A single genus, *Xenopus*, and four of its species were used by Weldon et al (2004) to survey southern Africa, and to obtain the earliest global record. A single species, *Atelopus cruciger*, was used in Venezuela to relate the first record of chytridiomycosis to just prior to the disappearance of this toad from Venezuela (Bonaccorso et al. 2003). Historical surveys of Australian species using histology should be performed where knowledge of the relationship of the arrival of the amphibian chytrid fungus to declines in the species would assist in risk assessment and preparation of frog recovery plans.

However, histology has a lower than ideal sensitivity, particularly in amphibians without clinical signs. The real time PCR test developed by AAHL has a greater sensitivity and can be done on an amphibian without removing a digit or sacrificing the animal (Hyatt 2003; Boyle et al. 2004). However, it has not yet been thoroughly tested in the field situation. Action to address this is recommended under Objective 3 of the threat abatement plan.

Laboratory based testing has the disadvantage that the result is delayed. If one is searching for populations with chytridiomycosis (the presence / absence approach noted in 3.2.3), once one positive frog is detected, no other frog needs to be surveyed. A test with high specificity is obviously needed to rely on one positive test. An ideal test would be one done in the field that gave a result rapidly. The researcher could then declare that site or population as infected with the amphibian

chytrid and reduce costs by not testing the full target quota of frogs. However, whilst this comment outlines an ideal situation it must be appreciated that in general 'field' assays do not have the same sensitivity and reproducibility as laboratory based tests. In addition the new diagnostic PCR assays incorporate rapid non-destructive sampling regimes and rapid turn around times in a nationally accredited laboratory. It is important that in any national survey data is collected and analysed in a manner whereby the results are not questionable. From this point of view the role of field testing will have to be carefully defined.

3.2.3 Expected prevalences in chronically infected populations

Using histological examination of digits, chronically infected populations of frogs showing no clinical disease appear to have prevalences averaged over the year of between 2%–10%. Prevalences from Australian surveys in chronically infected populations are far north Queensland (7%) (McDonald et al. 2005), central Queensland (>15%) (Retallick et al. 2004), corroboree frogs (3–30%) (Mendez, Hunter and Speare, pers obs), southwest Western Australia (1–20%) (Aplin and Kirkpatrick 2000) and from overseas — South Africa (3%) (Weldon et al. 2004).
It is important to realise that prevalence is related to incidence of infection and duration of infection.
A species that is highly susceptible to chytridiomycosis may have a low prevalence if infected members of the population die rapidly and hence duration of infection is short. This may be the situation with corroboree frogs as the size of the population is showing a steady reduction.
The prevalence peaks in winter and is lowest in summer (Aplin and Kirkpatrick 2000, Berger 2001, Retallick et al 2004, McDonald et al. 2005). However, collecting large numbers of samples in winter is not ideal for most species of amphibians as they are less abundant than in the warmer months of the year. A survey would have the greatest chance of detecting frogs with chytridiomycosis if performed in winter, but the lower numbers of frogs available to examine may decrease the usefulness.
A balance between numbers and prevalence needs to be determined for each area and amphibian population.

With a prevalence of 1%, the number of frogs that need to be examined to detect one frog with chytridiomycosis at a likelihood of 0.95 is 298 (DiGiacomo and Koepsell 1986). This is a presence / absence approach. It does not give an estimate of true prevalence, but, if positive, only indicates that chytridiomycosis is present, and, if all results are negative, what the maximum prevalence could be. Numbers needed to detect one frog at other prevalences are given in Table 2.

Table 2. Numbers of frogs that need to be sampled to detect one positive frog with a likelihood of 0.95 at a range of prevalences (DiGiacomo and Koepsell 1986).

Prevalence in population	Number needed to sample to detect one positive case
20%	13
10%	28
5%	58
4%	73
3%	98
2%	148
1%	298
0.5%	598
0.1%	2994

Obviously detecting prevalences of 1% and below requires an immense effort and may not be feasible. If the current prevalences are used as a guide for the prevalences expected in infected populations, a realistic cut-off level in surveys may be 2%, requiring a sample of 150 animals. If the number of frogs in a population are lower than this, only high prevalences can be excluded. However, if the whole population can be sampled, the statistical calculations do not apply.

These prevalence figures are those obtained using histological methods. If the real-time PCR is more sensitive, the "true" prevalences will be higher, and hence fewer frogs may need to be sampled.

Another option for stream-associated frogs may be to sample tadpoles as the prevalence of chytridiomycosis in tadpoles appears to be higher than in adults and juveniles (Pearl Symmonds pers comm). However, this needs to be evaluated (see actions under Objective 3 of the threat abatement plan).

3.2.4 Sampling of species or localities

Since *B. dendrobatidis* appears to be able to survive in the environment (see Section 2.4) and is usually found in multiple amphibian species at contaminated sites, a more cost-effective survey approach in detecting presence / absence in chytridiomycosis-free areas may be to combine results from all species at the same site. So that presence / absence results refers to site rather than species *per se*. Prevalence may vary with species within a site even if chytrid is at a constant level and can affect all species. Lumping all records may not be as informative as concentrating on individual species because of variability to chytrid infection between the species. However concentrating on single species has its

problems, as species may not be present in statistically useful sample sizes. Tests for detecting the amphibian chytrid in the environment are being developed by AAHL, but are not currently available.

3.2.5 Wide-scale survey protocols

To map the distribution of chytridiomycosis on a wide-scale, the sampling strategy needs to identify appropriate populations of amphibians to sample. The best species to sample are those already known to be susceptible to infection with the amphibian chytrid from other sites, particularly those species that typically have a high prevalence. A list of species in which chytridiomycosis has been detected is given in Appendix B Table B.1. The criteria to use in spacing sample sites are unknown.

3.2.6 Resources for national surveys

A national survey will require appropriate resources for collection of specimens and for laboratory testing. Any laboratory attempting to undertake the diagnostic assays will have to:

1. be ISO/NATA accredited;
2. have the appropriate equipment;
3. have the appropriately trained staff;
4. have the ability to process large numbers of samples; *and*
5. have the finances to pay for the reagents.

At present there is only one laboratory, AAHL, that can satisfy the first four of the five criteria. However, the number of expected submissions could not be handled by AAHL since its focus is performing other diagnostic assays of national importance. There is, therefore, a national requirement to establish further infrastructure within Australia to cater for the analyses of large numbers of samples that are not related to commercial livestock and aquaculture samples. A nationally accredited laboratory dedicated to testing for chytridiomycosis should be established.

This laboratory would be equipped with a robotic sample handling facility (for bar coding and sorting of samples), at least one real time (Taqman) PCR machine, a liquid handling robotic system and a qualified technician for one year. The cost of processing would be established to cover the cost of the technician, reagents and depreciation of equipment. Such a laboratory would be available for other 'environmental' emergencies and would function on a five year rotating (equipment) life cycle. Such a laboratory could be established at any scientific institute provided it met the basic criteria.

3.3 Disinfection

Effective disinfection protocols are essential to render equipment used with amphibians non-infectious. This has a major role in the field and in captive husbandry.

B. dendrobatidis is susceptible to a broad range of chemical and physical treatments (Berger 2001, Johnson et al. 2003). The most effective solutions for field use contained the quaternary ammonium compound, didecyl dimethyl ammonium chloride (DDAC), 0.1% dilution for 30 sec (Table 3). Sodium hypochlorite was effective at concentrations of 1% and above. Also effective was exposure to 70% ethanol, 1 mg/ml Virkon or 1 mg/ml benzalkonium chloride for 20 seconds. These chemicals can be used for disinfection in the laboratory, in amphibian husbandry, and in field work. For example, alcohol wipes can be used to disinfect scissors, calipers and other instruments between animals.

Cultures of *B. dendrobatidis* did not survive complete drying, but in practice persistence of water in droplets allows survival of the pathogen up to 3 hours after "drying" (Johnson et al. 2003). Heating to above 37˚C for four hours resulted in death of sporangia (Table 4). Ultraviolet light used routinely for killing bacteria, fungi and viruses was ineffective.

Table 3. Disinfection techniques that will kill 100% of zoospores and zoosporangia. From Johnson et al. (2003). RH = relative humidity.

	Temp / concentration	Minimum time of exposure
Physical techniques		
Heat	60˚C	5 min
Desiccation	25˚C RH 70%	3 hr
Disinfectants		
Ethanol	70%	0.5 min
Formaldehyde solution	1%	5 min
Vircon	0.1%	0.5 min
Sodium hypochlorite (bleach)	1% ≥	0.5 min
Didecyl dimethyl ammonium chloride	$1x10^{-3}$	0.5 min
Benzalkonium chloride	1%	0.5 min

Table 4. Times at which all sporangia were killed at different temperatures. From Berger (2001); Johnson et al. (2003).

Temperature	Time at which all sporangia were killed
100˚C	1 min
60˚C	5 min
47˚C	30 min
37˚C	4 hr
32˚C	96 hr
26˚C	No death
23˚C	No death

A combination of heating and drying is a safe method for disinfection for many objects, such as clothing and some equipment. Care is needed when using chemicals so that water bodies are not contaminated.

It is not yet known whether *B. dendrobatidis* grows well as a saprobe in the environment, although there is indirect evidence that it does. Johnson and Speare (2005) obtained long-lasting growth in sterile river sand. Much information is required on its ecology as a free-living organism — what substrates does it grow on, what is its temporal and spatial distribution in relation to climate and weather? Also, once an area becomes infected does it remain infected indefinitely, even in the absence of frogs? Research to increase our understanding of the natural history of *B. dendrobatidis* in the environment is an important priority.

Disinfection of large environmental sites is unlikely to be possible. Even if it were possible, the consequences of eradicating other fungi may be highly disruptive to the environment. In addition, if the surrounding areas are infected, then *B. dendrobatidis* is likely to return. However, it may be a useful strategy for smaller sites particularly in chytridiomycosis-free areas when a localised population is found to be newly infected. The drawbacks of local environmental damage due to the disinfectant may be justified if the pathogen could be eliminated and the chytridiomycosis-free status maintained. We do not know if elimination is possible and what technique could be used. Trialling of DDAC may be feasible as it is used as an agricultural fungicide. Being able to rapidly detect new incursions of the pathogen is essential in chytridiomycosis-free areas.

3.4 Treatment of chytridiomycosis

Effective treatments in adults and tadpoles are needed to prevent mortalities in captive programmes for threatened species, and will reduce the risks associated with movement of amphibians. As infected tadpoles survive and remain at sites after adults have died, a method of clearing them of infection would enable an emergency response (such as captive raising) to mortality and declines in threatened species.

Oral itraconazole was used successfully for treatment of a mycotic dermatitis that was diagnosed as due to *Basidiobolus ranarum in Bufo* in USA (Taylor et al. 1999), but was likely to be chytridiomycosis. The paper however gave few details on how cure was confirmed. Bathing in 0.01% itraconazole suspension for 5 minutes a day for 11 days was reported to successfully treat chytridiomycosis in *D. tinctorius* (Nichols and Lamirande 2000). A commercial solution of 25 ppm formalin and 0.10 mg/l malachite green was used for 24 hours every other day four times to successfully treat *Xenopus tropicalis* (Parker et al. 2002). Attempts to treat Australian frogs and tadpoles were not successful in eradicating *B. dendrobatidis* from all experimentally infected animals (Berger 2001; Marantelli et al. 2000).

Raising the temperature of experimentally infected *L. chloris* to 37˚C resulted in cure of asymptomatic chytridiomycosis (Woodhams et al. 2003). All members of a group of 10 experimentally infected L. chloris were cured after being held at 37˚C for 2 periods of 8 hours 24 hours apart. However, many species may not tolerate these high temperatures. Chytridiomycosis in captive Litoria spenceri was not well controlled using elevated temperature and bathing in terbinafine (Gerry Marantelli pers com 2004). The efficacy of high temperature as a treatment needs to be studied in other species and with larger numbers of frogs.

3.5 Selection for resistance

The possibility of selecting threatened species for innate resistance to chytridiomycosis should be critically assessed as a management strategy. This should be looked at in the field and in captivity. Increasing the number of animals and hence the genetic pool available for natural selection may be a feasible strategy for wild populations. Possibly using individuals that survive in infected areas for captive breeding and subsequent release may be a practical strategy.

3.6 Restocking threatened species

Restocking of threatened species through captive husbandry has a potentially important role to play in preventing populations from declining further. Restocking can be used to give a threatened species a 'window of opportunity' when faced with a mortality rate that will drive it to extinction. Restocking may buy time to allow the species to develop its own resistance

or to survive a particularly hazardous situation due to a confluence of adverse environmental, pathogen and host factors (McDonald et al. 2005). Unfortunately for most Australian frogs we know very little about their basic biology, even the common species. This makes restocking potentially an extremely expensive exercise, as much work has to be completed before commencing. At the time of this review only one attempt to reintroduce frogs in Australia had partial success. The project requires many steps to success including an understanding of basic ecological requirements of the species, successful husbandry techniques, successful reproduction in captivity, and successful reintroduction techniques. Modelling by Tenhumberg et al. (2004) predicts that a captive population is critical for the persistence of small populations.

Restocking should be undertaken before the final population is under threat. A good example of intervention occurring too late was the attempt with *Taudactylus acutirostris*. In 1993 specimens of adults and tadpoles were collected from the last remaining population at O'Keefe Creek, Big Tableland, and placed in three institutions. These were however already infected with the amphibian chytrid and as the population crashed in the field from chytridiomycosis, the captive specimens died from the same disease (Banks and McCracken 2002; Mahony and Dennis 1995). The last known frog died at Melbourne Zoo from chytridiomycosis in 1995 (Berger 2001), resulting in extinction of the species. If specimens from O'Keefe Creek had been placed in captivity, several years before, prior to arrival of *B. dendrobatidis* in this last population, extinction may have been avoided.

In Australia population augmentation through restocking has been used for the southern corroboree frog *(P. corroboree)*. This has involved collection of over-wintering eggs, raising of tadpoles in captivity to pre-metamorph stage and release of the advanced tadpoles back into the sites from where the eggs have been collected (Hunter et al. 1999). Mortality of tadpoles is lower in captivity resulting in more tadpoles entering metamorphosis (Hunter et al. 1999). This strategy appears to have played an important role in slowing the decline in this species as the release sites have higher numbers and densities of calling males than other sites (Gerry Marentelli pers comm). This project has been undertaken by the Amphibian Research Centre (ARC), Melbourne, and NSW Parks and Wildlife Service.

Captive breeding programmes of non-threatened species have been undertaken by ARC, Taronga Zoo, Melbourne Zoo, Lone Pine Koala Sanctuary, Sea World, but no threatened species appears to be currently bred. Part of the difficulty is that the knowledge on the specialised breeding habits of threatened species and how to breed them in captivity is minimal. State parks and wildlife services have given permits for captive breeding of related species, but there appear to be difficulties in obtaining permits to breed threatened species, particularly across state boundaries.

Captive breeding and husbandry offers great potential to preserve threatened species of a high likelihood of becoming extinct. Protocols to keep captive amphibians free of the amphibian chytrid are essential for restocking from captive raised amphibians to be successful (Marantelli 1999). Lynch (2001) has proposed protocols for use in zoological collections.

3.7 Habitat modification

There appears to be no information on whether modification of habitat used by a chytrid-infected population will tip the balance in favour of amphibians. However, observations in NSW that remnant populations of *L. aurea* and *Mixophyes balbus* with endemic chytridiomycosis, but stable numbers, have an association with water bodies that are either contaminated with heavy metals or contain water that is brackish (Ross Wellington pers comm. 2004). Confirming if heavy metals or salinity have a protective role is very important. Overall, a holistic approach with management strategies to increase the well-being of the amphibians, reduction in other stressors, and suitable modification of environment may result in lower mortality rates in susceptible species.

3.8 Management strategies

The degree of rigour required of hygiene protocols in the field and currently implemented in permit requirements is not based on evidence. Obtaining evidence would clarify how extensive these protocols should be. Protocols must be realistic. For high risk populations a higher level of rigour would be justified both from the perspective of wildlife managers and researchers.

Many of the recommendations made by the Getting the Jump on Amphibian Diseases Workshop (GJOADW) involved management strategies at the international, national, state and local levels (Speare 2001). TSSC (2002) recommended that for this TAP 'The first step in developing a threat abatement plan would be to review these recommendations and to incorporate those suitable relevant actions which are not already being implemented'. A number of these have been implemented in whole or part (see Appendix E) and the most significant are briefly dealt with below.

3.8.1 International level

In 2002 chytridiomycosis was placed on the Wildlife Diseases List by the World Organisation for Animal Health (OIE) as a disease of global significance (Recommendation 1.2). This was the first time amphibian diseases were listed. From this listing specific quarantine and disease testing conditions will have to be met for amphibians being moved between nations. The details of these conditions are currently under determination. Under this listing Australia will have a obligation not to export chytridiomycosis to any country, or to allow importation of chytridiomycosis, unless prescribed conditions are met (eg. for approved research purposes).

3.8.2 National level

National survey

Countries, including Australia, were advised to carry out a coordinated nation-wide survey to determine the chytrid status of the country and zones within the country (Recommendations 1.6, 1.12, 2.16). This has not been done for Australia.

Rapid detection of outbreaks and response

A system to detect new outbreaks was recommended and to be coupled with procedures to enable rapid response (Recommendation 2.6, 2.15). A surveillance system has been partly funded in Australia by the National Heritage Trust (Berger et al. 2004), but protocols for response have not been determined.

3.8.3 State level

Permit conditions

State governments were recommended to include measures to control amphibian diseases including chytridiomycosis in their permit requirements (Recommendations 2.7, 2.9). This has been implemented. These conditions on permits should be based on evidence. However, currently protocols have not been tested for effectiveness and states and territories must be willing to modify permit requirements as new evidence becomes available.

3.8.4 Community education and involvement

Recommendations of the GJOADW were made available on the internet at three sites on the Amphibian Diseases Home Page.

Researchers

A series of recommendations dealt with strategies to avoid transmission of chytridiomycosis by researchers in the field (Recommendations 2.8, 2.9, 3.23–3.35) and in the laboratory (Recommendations 2.13–2.16). Field researchers appear to be aware of the need for protocols to prevent their activities transmitting disease in amphibian populations. Most researchers appear to use some control strategies, but level of practical implementation is unknown. Laboratory research with chytridiomycosis appears to be done at the recommended level of PC2 (Recommendation 2.16). Awareness in the amphibian research community has been achieved through requirements being mandated by permits, biosafety requirements and word of mouth. However, other researchers working in water (fish biologists, hydrologists, entomologists, limnologists, botanists) have not been educated about the chytrid fungus and its spread.

Industry

Recommendations were made to reduce the number of amphibians accidentally translocated (Recommendation 3.1–3.5). Industry-specific educational material and a collaborative research project with banana industry at Tully was partly funded by National Heritage Trust. Other industries, including the tourism industry, have not been involved in strategies to decrease chytridiomycosis.

Public

Public education has been spearheaded mainly by community frog groups and World Wildlife Fund (WWF) Frogs using many media modes, including internet sites.

GLOSSARY AND ABBREVIATIONS

AAHL

CSIRO Australian Animal Health Laboratory (Geelong, Victoria)

ADG (SPHTM)

Amphibian Diseases Group (School of Public Health and Tropical Medicine, James Cook University, Townsville)

ARC

Amphibian Research Centre (Melbourne)

AQIS

Australian Quarantine and Inspection Service

AWHN

Australian Wildlife Health Network

Biocontainment

The procedures needed to ensure that infectious agents do not escape from laboratories or infected husbandry facilities.

Chytridiomycosis

The state of being infected with B. dendrobatidis. Amphibians can have chytridiomycosis without showing clinical signs (aclinical chytridiomycosis) or can show clinical signs (mild, severe) or death. The term was proposed by Berger et al (1998).

Cutaneous chytridiomycosis

Same as 'chytridiomycosis'

DAFF

Department of Agriculture, Fisheries and Forestry

DAPTF

Declining Amphibian Population Taskforce

DDAC

Didecyl dimethyl ammonium chloride

DEH

Department of the Environment and Heritage

ELISA

Enzyme Linked Immunosorbent Assay; a laboratory test developed to detect antigens of *B. dendrobatidis* by using an antibody detection system that binds to the specific antigen.

Emerging infectious disease

An infectious disease that has newly appeared or is increasing in incidence and geographic range.

Endemic

(Used in an epidemiological sense) Incidence of disease is largely stable in an area or population owing to the causative organism being present and sufficient naïve hosts being available to maintain infection.

Epidemic

An increase in the incidence of a disease in a population above the level that is "normal" or expected for that population.

Epidemiology

The study of disease in populations.

GJOADW
Getting the Jump on Amphibian Disease Workshop held in Cairns 26-30 August 2000

H&E
Haematoxylin and eosin; the usual staining technique used for histopathology.

Host specificity
The degree to which an infectious agent remains confined to one species of host or taxonomically related hosts. Low host specificity means that the infectious agent can infect many species of host, or species of host that are not closely related taxonomically.

Hyperaemic
Red colouration due to a greater amount of blood flowing in area.

Immunoperoxidase test
A diagnostic test for *B. dendrobatidis* using specific antibodies that bind to chytrid antigen in histological sections of amphibian skin. The bound antibodies are detected by the immunoperoxidase indicator system and stain *B. dendrobatidis* brown.

Incidence
The number of new cases of a disease occurring at a location in a defined period of time.

ISO
International Organisation for Standardisation

IRCEB
Integrated Research Challenges in Evolutionary Biology. Competitive funding programme in USA from National Science Foundation. A consortium based in Arizona had a grant to research chytridiomycosis and ranavirus.

ITS1 and ITS2
Internal Transcribed Spacer 1 and 2; conserved genes coding for segments of the ribosome.

KTP
Key threatening process

MLST
Multi locus sequence typing

Morbidity
Clinical disease

Mortality
Death

MSDS
Material Safety Data Sheet

NATA
National Association of Testing Authorities

NHT
Natural Heritage Trust

OIE
Office International des Épizooties or
World Organisation for Animal Health

Pathogenicity
The potential of a pathogen to cause disease

PC2
Physical Containment level 2 describes the minimum standard for construction and the general responsibilities and guidelines for safety in laboratories where micro-organisms are handled. The standard is defined in the Australian Standard AS/NZS 2243.3:2002 — Safety in Laboratories Part 3: Microbiological Aspects and Containment Facilities

PCR
Polymerase Chain Reaction: a diagnostic test using a molecular biological technique to manufacture additional DNA strands from small numbers of DNA strands in the original specimen

Positive predictive value
The probability that an amphibian actually is infected with *B. dendrobatidis* if it has tested positive

Prevalence
The percent of the population with the disease or condition of interest at a particular point in time

Real-time PCR
A PCR test that is able to quantify the amount of DNA present in the original sample

Resting phase
A stage in the life cycle of some chytrids which is resistant to dehydration. This stage does not appear to occur in *Batrachochytrium dendrobatidis*

Saprobe
Micro-organism that is capable of living and growing in the environment

Self-cure
The process in which a host cures itself of an infecting agent

Sensitivity
The probability of testing positive if chytridiomycosis is present

Specificity
The probability of testing negative if chytridiomycosis is truly absent

SSU-rDNA
Small subunit ribosomal DNA

Surveillance
The ongoing collection, collation, analysis and interpretation of disease specific data and dissemination to those who need to know to take steps to decrease the impact of the disease

TAP
Threat abatement plan

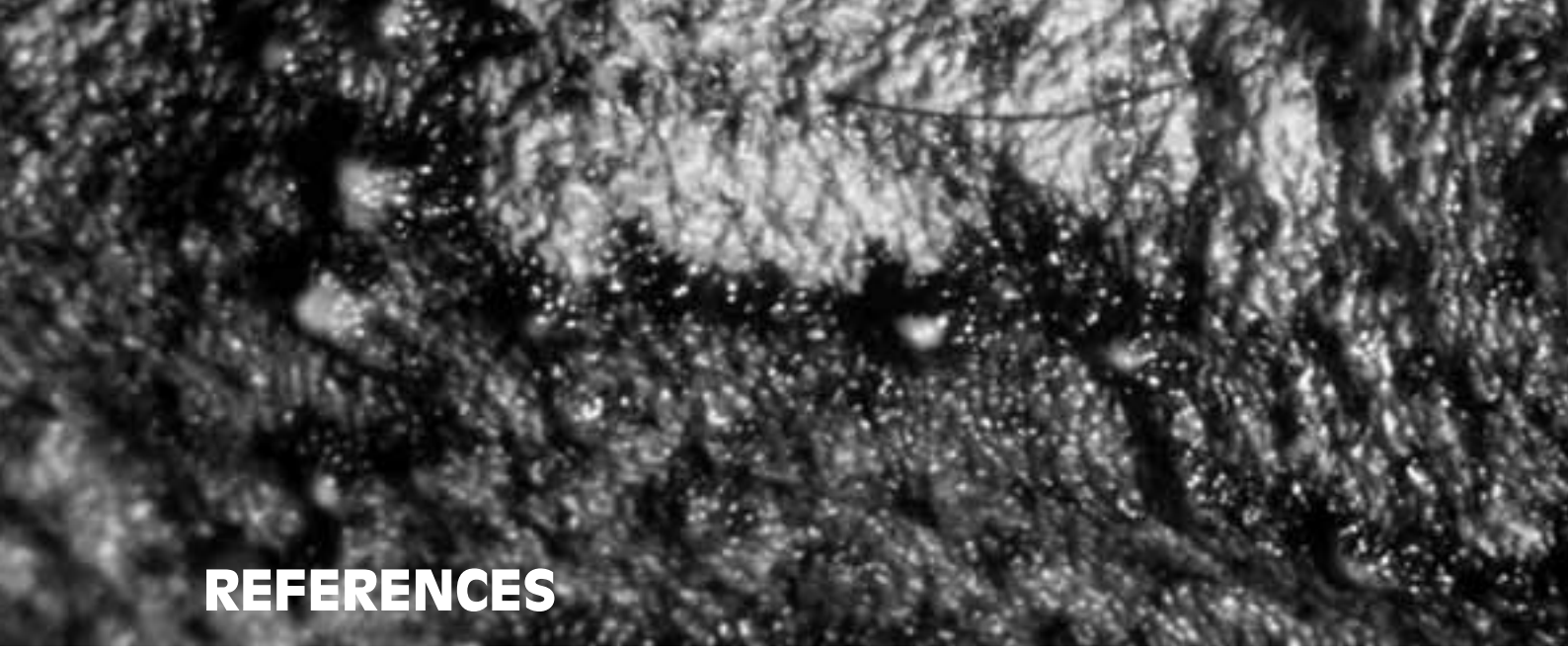
Transmissibility
The ability of a pathogen to transmit to a host or between hosts

TSSC
Threatened Species Scientific Committee

WWF(Aust)
World Wide Fund for Nature, Australia

Zoosporangium
The spherical structure of *B. dendrobatidis* found in epidermis and from which zoospores are released

Zoospore
The infectious stage of *B. dendrobatidis* that is motile in water



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APPENDIX A: THREAT ABATEMENT PLANS AND THE EPBC ACT

Relevant extracts from the *Environment Protection Biodiversity Conservation Act 1999* (EPBC Act) relating to the requirements for developing Threat Abatement Plans.

Section 271 Content of Threat Abatement Plans

(b) A threat abatement plan must provide for the research, management and other actions necessary to reduce the key threatening process concerned to an acceptable level in order to maximise the chances of the long-term survival in nature of native species and ecological communities affected by the process.

(c) In particular, a threat abatement plan must:

(a) state the objectives to be achieved; and

(b) state the criteria against which achievement of the objectives is to be measured; and

(c) specify the actions needed to achieve the objectives; and

(d) state the estimated duration and cost of the threat abatement process; and

(e) identify organisations or persons who will be involved in evaluating the performance of the threat abatement plan; and

(f) specify the major ecological matters (other than the species or communities threatened by the key threatening process that is the subject of the plan) that will be affected by the plan's implementation; and

(g) meet prescribed criteria (if any) and contain provisions of a prescribed kind (if any).

(d) In making a threat abatement plan, regard must be had to:

(a) the objects of this Act; and

(b) the most efficient and effective use of resources that are allocated for the conservation of species and ecological communities; and

(c) minimising any significant adverse social and economic impacts consistently with the principles of ecologically sustainable development; and

(d) meeting Australia's obligations under international agreements between Australia and one or more countries relevant to the species or ecological community threatened by the key threatening process that is the subject of the plan; and

(e) the role and interests of indigenous people in the conservation of Australia's biodiversity.

Section 274 Scientific Committee to Advise on Plans

(1) The Minister must obtain and consider the advice of the Scientific Committee on:

(a) the content of recovery and threat abatement plans; and

(b) the times within which, and the order in which, such plans should be made.

(2) In giving advice about a recovery plan, the Scientific Committee must take into account the following matters:

(a) the degree of threat to the survival in nature of the species or ecological community in question;

(b) the potential for the species or community to recover;

(c) the genetic distinctiveness of the species or community;

(d) the importance of the species or community to the ecosystem;

(e) the value to humanity of the species or community;

(f) the efficient and effective use of the resources allocated to the conservation of species and ecological communities.

(3) In giving advice about a threat abatement plan, the Scientific Committee must take into account the following matters:

(a) the degree of threat that the key threatening process in question poses to the survival in nature of species and ecological communities;

(b) the potential of species and ecological communities so threatened to recover;

(c) the efficient and effective use of the resources allocated to the conservation of species and ecological communities.

Section 279 Variation of Plans by the Minister

(1) The Minister may, at any time, review a recovery plan or threat abatement plan that has been made or adopted under this Subdivision and consider whether a variation of it is necessary.

(2) Each plan must be reviewed by the Minister at intervals not longer than 5 years.

(3) If the Minister considers that a variation of a plan is necessary, the Minister may, subject to subsections (4), (5), (6) and (7), vary the plan.

(4) The Minister must not vary a plan, unless the plan, as so varied, continues to meet the requirements of section 270 or 271, as the case requires.

(5) Before varying a plan, the Minister must obtain and consider advice from the Scientific Committee on the content of the variation.

(6) If the Minister has made a plan jointly with, or a dopted a plan that has been made by, a State or self-governing Territory, or an agency of a State or self-governing Territory, the Minister must seek the cooperation of that State or Territory, or that agency, with a view to varying the plan.

(7) Sections 275, 276 and 278 apply to the variation of a plan in the same way that those sections apply to the making of a recovery plan or threat abatement plan.

Environment Protection and Biodiversity Conservation Regulations 2000

Regulation 7.12 Content of Threat Abatement Plans

For paragraph 271 (2) (g) of the Act, a threat abatement plan must state:

(a) any of the following that may be adversely affected by the key threatening process concerned:

(i) listed threatened species or listed threatened ecological communities;

(ii) areas of habitat listed in the register of critical habitat kept under section 207A of the Act;

(iii) any other native species or ecological community that is likely to become threatened if the process continues; and

(b) in what areas the actions specified in the plan most need to be taken for threat abatement.

APPENDIX B: CHYTRIDIOMYCOSIS RECORDS FOR SPECIES OF AMPHIBIANS IN AUSTRALIA

Table B.1. Australian frog species and chytridiomycosis status of wild populations by state and nationally. For a particular species in a particular state, if one case of chytridiomycosis has been found, the species in that state is listed as infected (Inf). Occurrence of frog in state = Y. (For completeness, species which have been infected only in captivity (C-Inf) are listed, but not counted). Shaded cells = species does not occur.

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
Hylidae																		
Cyclorana	alboguttatata			Y		Y		Y										
	australis					Y		Y								Y		
	brevipes			Y				Y										
	cryptotis					Y										Y		
	cultripes			Y		Y		Y		Y						Y		
	longipes					Y										Y		
	maculosa					Y		Y										
	maini					Y		Y		Y						Y		
	manya							Y										
	novaehollandiae			Y				Y										
	platycephala			Y		Y		Y		Y						Y		
	vagita					Y										Y		
	verrucosus			Y				Y										
Litoria	adelaidensis															Y	INF	INF
	andiirrrmalin							Y										
	aurea[T]	Y		Y	INF									Y				INF
	barringtonensis*			Y	INF			Y	INF									INF
	bicolor					Y		Y								Y		
	booroolongensis			Y	INF													INF
	brevipalmata			Y				Y										
	burrowsae											Y						C-Inf
	caerulea	Y		Y	INF	Y		Y	INF	Y						Y		INF
	cavernicola															Y		
	castanea[T]	Y		Y														
	chloris			Y	INF			Y	INF									INF
	citropa			Y	INF													INF
	cooloolensis							Y										
	coplandi					Y		Y								Y		
	cyclorhynchus															Y		

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
Litoria	dahlii					Y		Y								Y		
	daviesae			Y										Y				
	dentata			Y				Y										
	electrica							Y										
	eucnemis																	
	ewingii			Y						Y	INF	Y	INF	Y				INF
	fallax			Y				Y										
	freycineti			Y				Y										
	genimaculata							Y	INF									INF
	gilleni					Y												
	gracilenta			Y				Y	INF									INF
	inermis					Y		Y								Y		
	infrafrenata							Y	INF									INF
	jervisiensis			Y														
	latopalmata	Y		Y				Y		Y								
	lesueuri	Y		Y	INF			Y	INF					Y	INF			INF
	littlejohni[T]			Y														
	longirostris							Y										
	lorica[T]							Y										
	meiriana					Y										Y		
	microbelos					Y		Y								Y		
	moorei															Y	INF	INF
	nannotis[T]							Y	INF									INF
	nasuta			Y		Y		Y	INF							Y		INF
	nigrofrenata							Y										
	nudidigitus			Y										Y				
	nyakalensis[T]							Y										
	olongburensis[T]			Y				Y										
	pallida					Y		Y								Y		
	paraewingi			Y										Y				
	pearsoniana			Y	INF			Y	INF									INF
	peronii	Y		Y	INF			Y		Y				Y				INF
	personata					Y												
	phyllochroa	Y		Y	INF													INF
	piperata[T]			Y														
	raniformis[T]	Y		Y						Y	INF	Y		Y				INF
	revelata			Y				Y										

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
Litoria	rheocola[T]							Y										INF
	rothi					Y		Y								Y		
	rubella			Y		Y		Y		Y						Y		
	spenceri[T]			Y										Y	INF			INF
	splendida					Y										Y		
	subglundulosa			Y				Y										
	tornieri					Y										Y		
	tyleri			Y				Y										C-Inf
	verreauxii	Y		Y	INF			Y						Y				INF
	v. alpina[T]			Y										Y				
	wotjulumensis					Y		Y								Y		
	xanthomera							Y										
Nyctimystes	dayi							Y	INF									INF
Microhylidae																		
Austrochaperina	adelphe					Y												
	fryi							Y										
	gracilipes							Y										
	pluvialis							Y										
	robusta							Y										
Cophixalus	bombiens							Y										
	concinnus							Y										
	crepitans							Y										
	exiguus							Y										
	hosmeri							Y										
	infacetus							Y										
	mcdonaldi							Y										
	monticola							Y										
	neglectus							Y										
Cophixalus	ornatus							Y										
	peninsularis							Y										
	saxatilis							Y										
	zweifeli							Y										
Myobactrachidae																		
Adelotus	brevis			Y	INF			Y	INF									INF
Arenophryne	rotunda															Y		
Assa	darlingtoni			Y				Y								Y		
Crinia	bilingua															Y		

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
Crinia	deserticola			Y		Y		Y		Y								
	georgiana															Y	INF	INF
	glauerti															Y	INF	INF
	insignifera															Y	INF	INF
	nimbus											Y						
	parinsignifera	Y		Y				Y		Y				Y				
	pseudinsignifera															Y	INF	INF
	remota					Y		Y										
	ripara									Y								
	signifera	Y		Y				Y		Y		Y		Y				
	sloanei			Y										Y				
	subinsignifera															Y		
	tasmaniensis											Y	INF					INF
	tinnula			Y				Y										
Geocrinia	alba[T]															Y		
	laevis									Y		Y		Y				
	leai															Y		
	lutea															Y		
	rosea															Y	INF	INF
	victoriana			Y										Y				
	vitellina[T]															Y	INF	INF
Heleioporus	albopunctatus															Y		
	australiacus[T]			Y	INF									Y				INF
	barycragus															Y	INF	INF
	eyrei															Y	INF	INF
	inornatus															Y		
	psammophilus															Y		
Kyarranus	kudagungan			Y				Y										
	loveridgei			Y				Y										
	sphagnicolis			Y				Y										
Lechriodus	fletcheri			Y				Y	INF									INF
Limnodynastes	convexiusculus					Y										Y		
	depressus					Y										Y		
	dorsalis															Y	INF	INF
	dumerili	Y		Y				Y	INF	Y	INF	Y	INF					INF
	fletcheri			Y				Y		Y				Y				
	interioris			Y										Y				

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
Limnodynastes	lignarius					Y												
	ornatus			Y		Y		Y								Y		
	peronii	Y?		Y				Y		Y		Y		Y				
	salmini			Y				Y										
	spenceri					Y		Y		Y						Y		
	tasmaniensis	Y		Y				Y		Y	INF	V	INF	Y				INF
	terraereginae			Y				Y										INF
Metacrinia	nichollsi																	
Mixophyes	balbus[T]			Y	INF									Y				INF
	fasciolatus			Y	INF			Y	INF									INF
	fleayi[T]			Y	INF			Y	INF									INF
	iteratus[T]			Y	INF			Y										INF
	schevilli							Y										
Myobatrachus	gouldii															Y		
Neobatrachus	albipes															Y		
	aquilonius					Y		Y								Y		
	centralis					Y		Y								Y		
	fulvus															Y		
	kunapalari															Y		
	pelobatoides															Y		C-Inf
	pictus			Y						Y				Y				
	sudelli	Y		Y				Y		Y				Y				
	sutor									Y						Y		
	wilsmorei					Y				Y						Y		
Notaden	bennettii			Y				Y										
	melanoscaphus					Y		Y								Y		
	nichollsi					Y		Y								Y		
	weigeli															Y		
Paracrinia	haswelli			Y										Y				
Philoria	frosti[T]													Y				
	kundagungan			Y				Y										
	loveridgei			Y				Y										
	sphagnicola			Y														
Pseudophryne	australis			Y														
	bibronii	Y		Y						Y				Y				
	coriacea			Y				Y										
	corroboree[T]			Y	INF													INF

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
Pseudophryne	covacevichae[T]							Y										
	dendyi	Y		Y										Y				
	douglasi															Y		
	guentheri															Y		
	major							Y										
	occidentalis									Y						Y		
	pengilleyi[T]	Y	INF	Y	INF													
	raveni							INF										
	semimarmorata									Y		Y		Y				
Rheobatrachus	silus[E]							Y										
	vitellinus[E]							Y										
Spicospina	flammocaerulea[T]															Y		
Taudactylus	acutirostris[E]							Y	INF									INF
	diurnus[E]							Y										
	eungellensis[T]							Y	INF									INF
	liemi							Y										
	pleioni[T]							Y										
	rheophilus[T]							Y										INF
Uperoleia	altissma							Y										
	arenicola					Y												
	aspera															Y		
Uperoleia	borealis					Y										Y		
	capitulata			Y				Y		Y								
	crassa															Y		
	fusca			Y				Y										
	glandulosa															Y		
	inundata					Y		Y										
	laevigata			Y				Y	INF					Y				INF
	lithomoda					Y		Y								Y		
	littlejohni							Y										
	marmorata															Y		
	martini			Y										Y				
	micromeles					Y										Y		
	mimula							Y										
	minima															Y		
	mjobergi															Y		
	orientalis					Y												

		ACT		NSW		NT		QLD		SA		TAS		VIC		WA		AUST
		OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	OCCUR	INFECT	INFECT
	rugosa			Y				Y										
	russelli															Y		
	talpa															Y		
	trachyderma					Y		Y								Y		
	tyleri			Y										Y				
Ranidae																		
Rana	daemeli					Y		Y										
Bufonidae																		
Bufo	marinus			Y		Y		Y	INF									INF
State	Number (%)	18	1 5.6%	84	16 19.0%	47	0	123	21 17.1%	27	4 14.8%	11	4 36.4%	33	2 6.1%	77	12 15.6%	50 22.8%

* *Litoria barringtonensis* has not been formally recognised. The Qld population at Kroombit is genetically different from *L. pearsoniana* and sometimes is given the title *barringtonensis*. The difficulty is determining if *barringtonensis* is a prior name for *L. pearsoniana*. (Keith McDonald pers com 2003).

T = Threatened species (endangered or vulnerable) (See Table C.1); E = Extinct species (See Table C.2).

Table B.2. Number of amphibian species reported with chytridiomycosis in the wild by state and nationally. The introduced cane toad (B. marinus) is counted in Queensland, New South Wales and Northern Territory. Chytridiomycosis in wild cane toads have been found in Queensland.

	Species	Infected	Percent
ACT	18	1	5.6%
NSW	85	20	23.5%
NT	48	0	0.0%
Qld	125	21	16.8%
SA	27	4	14.8%
Tas	11	4	36.4%
Vic	33	3	9.1%
WA	77	12	15.6%
Total species Australia-wide	219	50	22.8%

Table B.3. Number of species reported with chytridiomycosis in the wild by genera and family.

Genera	Species	Infected	Percent
Hylidae	**79**	**23**	**29.1%**
Cyclorana	13	0	0.0%
Litoria	65	22	33.8%
Nyctimystes	1	1	100.0%
Microhylidae	**18**	**0**	**0.0%**
Austrochaperina	6	0	0.0%
Cophixalus	13	0	0.0%
Myobatrachidae	**120**	**25**	**20.8%**
Adelotus	1	1	100.0%
Arenophryne	1	0	0.0%
Assa	1	0	0.0%
Crinia	15	5	33.3%
Geocrinia	7	2	28.6%
Heleioporus	6	3	50.0%
Kyarranus	3	0	0.0%
Lechriodus	1	1	100.0%
Limnodynastes	13	4	30.8%
Metacrinia	1	0	0.0%
Mixophyes	5	4	80.0%
Myobatrachus	1	0	0.0%
Neobatrachus	10	1	10.0%
Notaden	4	0	0.0%
Paracrinia	1	0	0.0%
Philoria	4	0	0.0%
Pseudophryne	13	2	15.4%
Rheobatrachus	2	0	0.0%
Spicospina	1	0	0.0%
Taudactylus	6	3	50.0%
Uperoleia	24	1	4.2%
Ranidae	**1**	**0**	**0.0%**
Rana	1	0	0.0%
Bufonidae	**1**	**1**	**100.0%**
Bufo	1	1	100.0%
Total	219	50	22.8%

APPENDIX C: THREATENED AMPHIBIAN SPECIES INFECTION STATUS

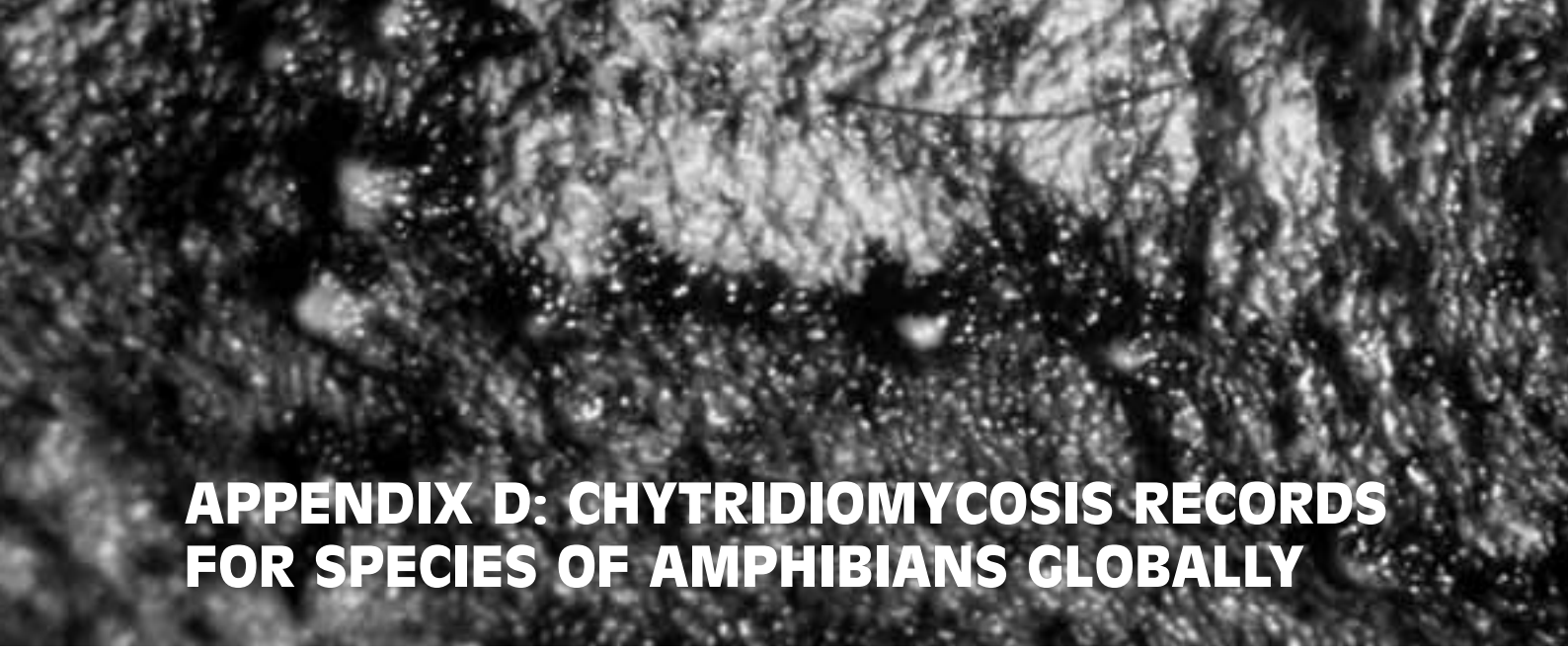
Table C.1. Species of threatened amphibians and their B. dendrobatidis infection status. Status from The Department of the Environment and Heritage (2003) web site: (www.deh.gov.au/cgi-bin/sprat/public/publicthreatenedlist.pl?wanted=fauna)

Scientific Name	Status re Infection with *B. dendrobatidis*	Reference
ENDANGERED SPECIES		
Geocrinia alba White-bellied frog	No records. In chytridiomycosis-positive zone.	Aplin and Kirkpatrick (2000)
Litoria castanea Yellow-spotted tree frog	No reports. Apparently no survey performed for chytridiomycosis. Disappeared in NSW about mid-1970s.	Mahoney (1999)
Litoria lorica Armoured mistfrog	No reports. No survey performed. Population declined in north Qld in 1991 when epidemic chytridiomycosis occurred.	McDonald and Alford (1999)
Litoria nannotis Waterfall frog	Upland populations disappeared in Qld with epidemic chytridiomycosis. Lowland populations have endemic chytridiomycosis.	Berger et al. (1998); McDonald et al. (2004)
Litoria nyakalensis Mountain mistfrog	No records. Upland populations disappeared in North Qld at same time as other frogs with epidemic chytridiomycosis.	McDonald and Alford (1999)
Litoria rheocola Common mistfrog	Upland populations disappeared in Qld with epidemic chytridiomycosis. Lowland populations have endemic chytridiomycosis.	Berger et al (1998); McDonald et al. (2004)
Litoria spenceri Spotted treefrog	Upland populations declined steadily over 20th century, but a precipitous decline of a population at Bogong Ck, Kosciuszko in 1996 was associated with chytridiomycosis. Victorian populations have endemic chytridiomycosis.	Berger et al. (1998); Gillespie and Hines (1999)
Mixophyes fleayi Fleay's frog	*B. dendrobatidis* endemic in SEQ and northern NSW populations.	Mahoney (2000); Speare and Berger (2003); Symmonds et al. (2003).
Mixophyes iteratus Southern barred frog	*B. dendrobatidis* is endemic in eastern NSW populations.	Mahoney (2000)
Nyctimystes dayi Lace-eyed tree frog	Upland populations disappeared in Qld with epidemic chytridiomycosis. Lowland populations have endemic chytridiomycosis.	Berger et al. (1998); McDonald and Alford (1999); McDonald et al. (in prep)
Philoria frosti Baw Baw frog	No reports. Survey of archived and extant specimens being planned.	Osborne et al. (1999)
Pseudophryne corroboree Southern corroboree frog	*B. dendrobatidis* endemic since 1991 and possibly major cause of population decline.	Speare and Berger (2003)
Spicospina flammocaerulea Sunset frog	No reports. Apparently no survey performed.	
Taudactylus eungellensis Eundella day frog	*B. dendrobatidis* detected as after declines; now endemic.	Speare and Berger (2003); Marshall (1998); Rettalick et al. (2004)
Taudactylus rheophilus Tinkling frog	No reports. Apparently only one specimen examined. Populations declined in 1989. In area endemic for *B. dendrobatidis*.	McDonald

VULNERABLE SPECIES		
Geocrinia vitellina Orange-bellied frog	*B. dendrobatidis* detected in survey of archived specimens.	Aplin and Kirkpatrick (2000)
Heelioporus australiacus Giant burrowing frog	*B. dendrobatidis* detected in survey of archived specimens.	Speare and Berger (2003)
Litoria aurea Green and gold bell frog	*B. dendrobatidis* endemic in NSW populations.	Mahony (2000); Speare and Berger (2003)
Litoria littejohni Littlejohn's tree frog	No reports. Apparently no survey performed.	Hines et al. (1999)
Litoria olongburensis Wallum sedge frog	No records. Apparently no survey for chytridiomycosis performed. Mainland sites endemic for B. dendrobatidis, but no positive records from limited surveys of Stradbroke Island. Nature of decline unclear. This species listed because of loss of habitat	Gillespie and Hines (1999)
Litoria piperata Peppered tree frog	No records. Apparently no survey performed. Occurs in region with endemic *B. dendrobatidis*.	Speare and Berger (2003)
Litoria raniformis Southern bell frog	*B. dendrobatidis* endemic in populations in Adelaide and environs.	Gillespie and Marantelli (2000)
Litoria verreauxii alpina Alpine tree frog	No records. Apparently no survey performed. In area endemic for *B. dendrobatidis*.	Mahony (2000)
Mixophyes balbus Southern barred frog	*B. dendrobatidis* endemic in populations in NSW.	
Pseudophryne covachevicae Magnificient brood frog	No records. No survey done.	
Pseudophryne pengilleyi Northern corroboree frog	*B. dendrobatidis* endemic since at least 1991.	Speare and Berger (2003)
Taudactylus pleione Kroombit tinker frog	No reports. No specimens examined. *B. dendrobatidis* endemic in region.	

Table C.2. Threatened species declared extinct in 2000 and their relationship to key threatening process of infection with *B. dendrobatidis* (DEH 2003).

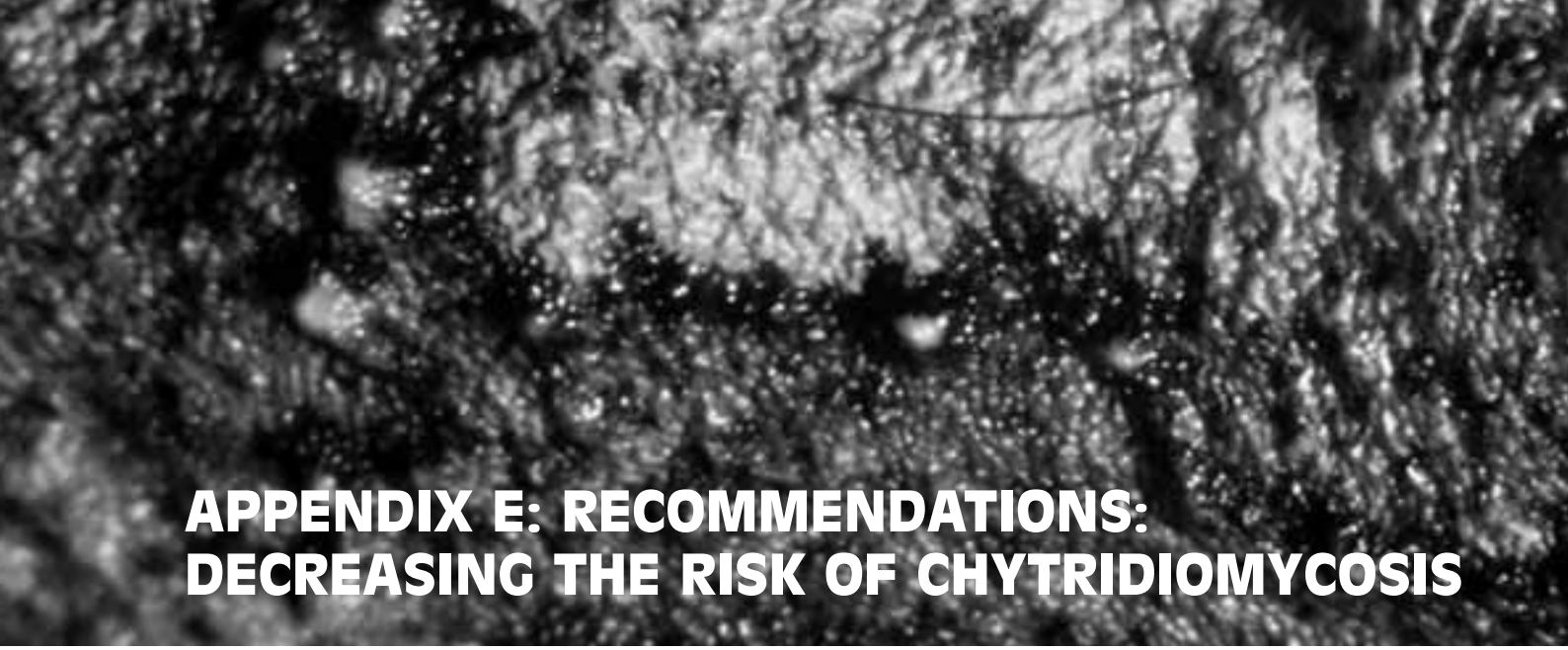
Scientific Name	Status re Infection with *B. dendrobatidis*	Reference
Rheobatrachus silus Southern gastric brooding frog	Pattern of decline consistent with epidemic chytridiomycosis. Chytridiomycosis not found in very small histological survey of toes of 4 museum specimens collected pre-decline.	Laurance et al (1996); Berger et al (1998)
Rheobatrachus vitellinus Northern gastric brooding frog	Pattern of decline consistent with epidemic chytridiomycosis. No survey of archived specimens.	Laurance et al (1996); Berger et al (1998)
Taudactylus acutirostris Sharp-snouted day frog	Last population at Big Tableland made extinct by epidemic chytridiomycosis that began in 1993.	Berger et al (1998); Berger et al (1999)
Taudactylus diurnus Southern day frog	Pattern of decline consistent with epidemic chytridiomycosis. Chytridiomycosis not found in survey (using direct smear of superficial epidermis) of 25 museum specimens collected pre-decline.	Laurance et al (1996); Berger et al (1998)



APPENDIX D: CHYTRIDIOMYCOSIS RECORDS FOR SPECIES OF AMPHIBIANS GLOBALLY

Table D.1. Summary of global records of chytridiomycosis in amphibians. This includes reports from Australia. Based on data from (Speare and Berger 2003).

FROGS and TOADS (Anura)			
	Families	**Genera**	**Species**
	Bombinatoridae	1	1
	Bufonidae	2	20
	Centrolenidae	1	2
	Dendrobatidae	2	11
	Discoglossidae	1	1
	Hylidae	8	37
	Leiopelmatidae	1	1
	Leptodactylidae	2	5
	Mantellidae	1	1
	Microhylidae	1	1
	Myobactrachidae	9	23
	Pipidae	3	7
	Ranidae	3	22
Subtotal	**13**	**35**	**132**
NEWTS and SALAMANDERS (Caudata)			
	Ambystomatidae	1	4
	Amphiumidae	1	1
	Plethodontidae	1	1
	Proteidae	2	2
	Salamandridae	2	2
	Sirenidae	1	1
Subtotal	**6**	8	11
TOTAL	**19**	**43**	**143**



APPENDIX E: RECOMMENDATIONS: DECREASING THE RISK OF CHYTRIDIOMYCOSIS

(Recommendations to decrease the risks of Chytridiomycosis from "Getting the Jump on Amphibian Diseases" Workshop Cairns August 2000)

In the Advice to the Minister on the application for listing (2002) the Threatened Species Scientific Committee (TSSC) recommended that the first step in developing a threat abatement plan would be to review the 110 recommendations made in the workshop "Developing Management Strategies to Control Amphibian Diseases: Decreasing the risks due to communicable diseases", held in August 2000. These recommendations are available at: http://www.jcu.edu.au/school/phtm/PHTM/frogs/gjoad.htm

TSSC recommended that those suitable relevant actions contained in the recommendations and not yet implemented be incorporated into the TAP. These 110 recommendations are presented in this Appendix together with comment on the current status of implementation, and an indication whether suitable relevant actions have been incorporated into the TAP.

Recommendation	Status re implementation at 31 October 2003
INTERNATIONAL ISSUES	
Recommendation 1.1 Diseases which may have serious implications for wild amphibians, with probable impacts on biodiversity, should be accepted as legitimate for restriction / control of movement of amphibians between nations.	Achieved. In 2002 the World Organisation for Animal Health (OIE) for the first time placed amphibian diseases on its Wildlife Diseases List. Conditions required for international movement of amphibians will follow from this listing.
Recommendation 1.2 Chytridiomycosis and ranaviral disease of amphibians should be placed on the Office International des Epizooties (OIE) List B with the consequent certification and testing requirements for export or import of amphibians.	Achieved. These 2 diseases have now been listed by OIE on the Wildlife Diseases List.
Recommendation 1.3 Declining Amphibian Population Task Force (DAPTF) should develop an international framework to support amphibian disease research.	DAPTF provides support for investigations in developing countries with amphibian declines.
Recommendation 1.4 Risk assessment should be carried out in all countries for chytridiomycosis and ranaviral diseases including determination of strains of pathogens and zones within countries.	Principle promoted. Assessments are in progress in a number of continents; Africa, Asia, Australasia, Europe, North and South America. Most limited in Asia; most well developed in Australia and New Zealand, North and South America. Incorporated for Australia in actions in TAP.
Recommendation 1.5 In particular risk assessment should be carried out for countries importing or exporting produce with a high risk of harbouring amphibians (e.g., bananas) or intending to import or export such produce.	Implemented for bananas from Philippines. Imput into Biosecurity Australia's Import Risk Assessment.
Recommendation 1.6 Countries and zones within countries should be classified as free or infected with chytridiomycosis by use of sampling protocols that have sufficient statistical strength to give defensible results.	Not yet implemented for any country except South Africa. National survey for Australia included as an action in TAP.
Recommendation 1.7 Protocols should be adopted to demonstrate that amphibians are free of chytridiomycosis and ranavirus before importation into or export from Australia.	AQIS guidelines does not impose the requirement to test for chytridiomycosis on imported amphibians since Australia is not chytrid-free (Biosecurity Australia 2003). This was prohibited under World Trade Organisation free-trade agreements. Freedom from foreign Ranaviruses must be demonstrated.
Recommendation 1.8 A precautionary approach should be adopted in classification of countries and zones: lacking evidence of freedom from chytridiomycosis using the recommended survey strategy, a country should be regarded as infected with B. dendrobatidis.	Promoted in principle.
Recommendation 1.9 All produce imported into Australia should be free of amphibians and importers must follow protocols at the point of packing of produce and during transit to achieve amphibian-free status.	Promoted in principle and adopted by Australian Banana Growers' Council. Argument used in case to Biosecurity Australia against importation of Philippine bananas.
Recommendation 1.10 The sensitivity and specificity of testing protocols for chytridiomycosis and ranaviral disease in live amphibians should be determined.	AAHL continues work on evaluating the sensitivity and specificity of diagnostic tests funded under IRCEB project.
Recommendation 1.11 Data on diagnostic tests and reagents for these should be freely shared between institutions within and between countries.	Implemented. AAHL and ADG(SPHTM) freely share expertise, and AAHL provides diagnostic antibodies for immunoperoxidase tests and DNA primers.
Recommendation 1.12 The global status of chytridiomycosis should be determined for all countries and zones within countries by conducting surveys using protocols that will give statistically defensible results.	Promoted. For Australia incorporated into actions for TAP.
Recommendation 1.13 A feasible sampling strategy at the country level should be designed to give a reliable indication of whether a country is free of *B. dendrobatidis*.	Protocol designed and implemented for South Africa by Weldon, du Prez and Speare. No protocol designed for Australia or other countries. Incorporated in action for TAP.

Recommendation	Status re implementation at 31 October 2003
Recommendation 1.14 IUCN should be asked to request that each nation carry out such a survey.	No action. Not relevant to TAP.
Recommendation 1.15 DAPTF should be requested to assist developing countries in carrying out their surveys.	No action. Not relevant to TAP.
Recommendation 1.16 Countries with diagnostic expertise in amphibian disease should make this expertise available to others, and reasonable costs for this should be supported in part or whole by these countries or non-government organisations.	Promoted in principle and in practice. ADG(SPHTM) and AAHL readily provide assistance to researchers and wildlife managers in developing countries and developed countries.
Recommendation 1.17 Risk assessment should be performed on the prevalence of *B. dendrobatidis* and ranaviruses in frogs imported deliberately or accidentally into countries.	This will be a requirement of actions flowing from listing of chytridiomycosis and ranavirus by OIE. Not relevant to TAP.
Recommendation 1.18 For Australia a survey of the species, number, destination and route of arrival of exotic amphibians should be carried out.	No action. Not relevant for TAP.
Recommendation 1.19 Imported amphibians should be examined using sensitive and specific diagnostic tests to determine the prevalence of *B. dendrobatidis* and ranaviruses.	Biosecurity Australia's ABPM 2003/26 (see Biosecurity Australia 2003) requires imported amphibians to be free of ranaviruses and herpesviruses. Chytridiomycosis was not listed since it is already in Australia.
Recommendation 1.20 Any frog found dead or ill on arrival at the point of importation should be examined for disease in a laboratory with the appropriate level of expertise.	Biosecurity Australia's 2003/26 requires imported amphibians to be pathologically examined if death occurs within 30 days.
Recommendation 1.21 A practical and effective treatment / disinfection protocol for administration to live amphibians should be developed to provide a chemical barrier at point of export and point of import.	Work has been carried out on this by ADG (SPHTM). However, a safe effective protocol has not been demonstrated as yet. Incorporated into actions for TAP.
Recommendation 1.22 A standard protocol should be developed for treating amphibians for chytridiomycosis upon arrival in Australia and at point of export from Australia as well as treating any amphibians prior to movement within countries.	Not achieved. Work has been carried out on this by ARC, AAHL, ADRG at JCU and Doug Woodhams. Most protocols reduce intensity of infection, but do not entirely eliminate it from the group. A safe effective protocol has not been demonstrated as yet although elevating temperature to 37˚C was demonstrated as effective by Woodhams et al (2003) in *L. chloris*. Incorporated into actions for TAP.
Recommendation 1.23 Individuals and organisations concerned with deliberate or accidental movement of amphibians between countries must be made aware of the risks of transporting chytridiomycosis, ranaviruses, and other pathogens between countries and the need to decrease those risks.	Promoted in principle and in publications.
Recommendation 1.24 These individuals and organisations must be informed of the testing and treatment protocols for amphibians needed to decrease risks of moving pathogens between countries and of any legislation, regulations or guidelines governing these.	No action. Not relevant to TAP.
NATIONAL ISSUES	
Recommendation 2.1 In Australia chytridiomycosis should be listed as a key threatening process.	Achieved.
Recommendation 2.2 A nomination should be prepared by the Core Working Group using this document and other data to list chytridiomycosis as a key threatening process in Australia.	Achieved. Submitted December 2000.
State Level	
Recommendation 2.3 Movement of amphibians between states should be subject to control on the basis of chytridiomycosis and ranaviral disease.	Promoted, but no action. Incorporated in actions for TAP.
Recommendation 2.4 Quarantine legislation should be established to limit the transmission of amphibian diseases between and within states.	No action. Incorporated in actions for TAP.
Recommendation 2.5 Quarantine areas or zones for chytridiomycosis should be established on the basis of disease presence and absence.	No action. Incorporated in actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Recommendation 2.6 Procedures to enable rapid response to disease outbreaks in amphibian populations should be established.	No action. Incorporated in actions for TAP.
Recommendation 2.7 Guidelines to minimise disease should be included in state policies for taking, keeping, trading and relocating amphibians, and these guidelines should be enforced.	Achieved. Most states have implemented these strategies. Incorporated in actions for TAP.
Recommendation 2.8 Strategies to minimise transmission of disease, particularly chytridiomycosis, should be implemented in all research programmes on amphibians.	Achieved. Permit requirements in most states require researchers to implement strategies to minimise transmission. Incorporated in actions for TAP.
Recommendation 2.9 These strategies should include conditions on permits.	Achieved. Incorporated in actions for TAP.
Recommendation 2.10 The public should be made aware of actions that may increase the risk of disease spread including actions that increase the number of infective stages in the environment and increase the geographic range of pathogens: 1. Artificial breeding of amphibians should be discouraged unless proscribed conditions are met. 2. Keeping of tadpoles in a location/region should be permitted only if tadpoles have been collected from that location/region. 3. Metamorphs resulting from tadpole raising should be returned to the wild at the same point of collection. 4. Adult amphibians should not be moved a distance greater than they would be expected to move unassisted. 5. If amphibians are to be moved in ways different from 2.10.2–2.10.4 above, they should be tested for chytridiomycosis and given complete treatment or disinfection protocols prior to release.	Most states have produced educational material for public. Groups and private individuals in Australia have established web sites containing educational material. Incorporated in actions for TAP.
Recommendation 2.11 High risk populations of amphibians should be identified and more stringent quarantine conditions applied to these populations.	No action. Incorporated into actions for TAP.
Recommendation 2.12 Representative members of all Australian species should be cryopreserved in such a manner as to allow cloning in the future or have gametes preserved to allow artificial breeding.	No organised action apart from Michael Mahoney's research work on cryopreservation. Incorporated into actions for TAP.
Recommendation 2.13 The knowledge and infrastructure on captive breeding of amphibians should be expanded, particularly with respect to species that are threatened or particularly vulnerable.	No concerted action. ARC continues some breeding programmes. Incorporated into actions for TAP.
Recommendation 2.14 Commercial breeders of amphibians must meet a standard that minimises the impact of disease in their activities and prevents the dissemination of chytridiomycosis and ranaviral disease. 1. This should be based on industry best practice, established by consultation between industry, government parks and wildlife departments and primary industries departments, and amphibian disease experts. 2. The standards should be implemented and monitored through industry self regulation. 3. Ongoing monitoring for disease should be an essential component of this. 4. Dead and terminal amphibians should be routinely submitted for pathological examination. 5. Premises should be accredited if they meet the set of standards, and this accreditation could be used in advertising material.	Protocol for husbandry collection produced by Michael Lynch, Melbourne Zoo and available at Amphibian Diseases Home Page (Lynch 2001). The major amphibian breeding centre in Australia, ARC, adopts high quality quarantine protocols. Other commercial amphibian producers have not been approached for engagement. Incorporated into actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Recommendation 2.15 A surveillance system for amphibian diseases should be maintained and adequately supported and should include: 1. Voluntary submissions of dead and live specimens by the public, wildlife managers and scientists 2. Compulsory submissions of dead specimens of threatened species, imported specimens, amphibians in industry, and specimens under permit. 3. Integration of the surveillance system with population monitoring projects.	Achieved. Surveillance system funded by NHT has been maintained at ADRG, JCU. Ongoing funding is needed to maintain surveillance. Incorporated in actions
Recommendation 2.16 A one-off national baseline survey for chytridiomycosis should be conducted as part of a national project designed to give a statistically meaningful baseline.	Not done. Incorporated in actions for TAP.
Recommendation 2.17 Data on surveillance should be collated, analysed, interpreted and disseminated to wildlife managers, researchers and public by an objective, authoritative, scientifically credible central group.	Data made available on the internet at the Amphibian Disease Home Page, but with minimal interpretation. More sophisticated system required. Incorporated in actions for TAP.
Recommendation 2.18 The level of expertise in amphibian disease diagnosis should be expanded by training of veterinarians and others.	Achieved. Diagnostic techniques for chytridiomycosis made available to all on WWW at Amphibian Disease Home Page. Information on other diseases also available at this site. AAHL plans training workshops in real-time PCR diagnosis.
Recommendation 2.19 The commercial amphibian industry should be encouraged to provide a source of specific pathogen free frogs for research, for routine toxicity testing and for pets in states where this is permitted.	Achieved. Discussions held with ARC and captive raised frogs for research supplied for a number of years. Incorporated in actions for TAP.
Recommendation 2.20 Environmental guidelines should include recommendations to minimise the impact of disease on amphibian populations by maximising the suitability of the environment. 1. General recommendations to give best habitat for amphibians 2. Specific habitat recommendations for amphibians appropriate to area 3. Included as part of land care packages 4. Local councils should be encouraged to reward landowners who comply by giving rate reductions.	Promoted in principle. Incorporated into actions for TAP.
Recommendation 2.21 Funding should be sought from: Recovery plans for endangered species Listing as a key threatening process Commercial sources	No coordinated action. Incorporated in actions for TAP.
Recommendation 2.22 Infrastructure, protocols and funding mechanisms should be established to enable rapid response in the event of amphibian disease outbreaks 1. The feasibility of adopting or integrating response strategies with Aquaplan should be investigated. 2. The resources, people, responsibilities, funding obligations and lines of command should be identified prior to a disease outbreak and be preapproved for rapid response.	No action. Incorporated in actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Research	
Recommendation 2.23 Standards for diagnostic tests for chytridiomycosis and ranaviruses in live amphibians should be established and their sensitivity, specificity and predictive values determined.	In progress. AAHL is evaluating these characteristics. Incorporated in actions for TAP.
Recommendation 2.24 Those laboratories and individuals with expertise to investigate and describe new diseases of amphibians including isolation and characterisation of novel pathogens should be identified: Australian Animal Health Laboratory, Australia National Wildlife Center, USA Reagent Park's Zoo, UK.	Additional labs should be added to list: ADRG at JCU, Veterinary Centre, Taronga Zoo, Uni of Potschrefstroom, South Africa.
Recommendation 2.25 The potential non-amphibian reservoirs of chytridiomycosis should be investigated.	Some limited research by ADG(SPHTM). Incorporated into actions for TAP.
Recommendation 2.26 The epidemiology of chytridiomycosis and ranaviral disease including the hypothesis of a novel agent being imported into countries and regions should be investigated.	Action by research groups in Australia, New Zealand, USA, Canada, Spain, Venezuela and South Africa. Partly incorporated into actions for TAP.
Recommendation 2.27 A standard experimental testing protocol should be defined to evaluate the susceptibility of species to chytridiomycosis: 1. Include laboratory-raised amphibian strain of known as a standard 2. Standard infection protocols with defined strains of *B. dendrobatidis* 3. Under standard environmental conditions	Initial work done by Lee Berger, Gerry Marantelli. Not yet rigorously standardised. Incorporated into actions for TAP.
Recommendation 2.28 The standard lab model should be used to evaluate the susceptibility of key amphibian species so that risk assessment for chytridiomycosis can be done for potentially threatened populations in countries and zones within countries.	Not done. Incorporated into actions for TAP.
Recommendation 2.29 Isolates of *B. dendrobatidis* and ranaviruses from around the globe should be collected into at least 2 secure centres. • In Australia this centre should be the Australian Animal Health Laboratory, Geelong, Victoria.	Achieved.
Recommendation 2.30 Disease work should collect data to enable better modelling for ranaviruses and chytridiomycosis. 1. Key factors include prevalence, incidence, pathogenicity, environmental influences, force of infection.	Early modeling work done for Australia by Richard Retallick, Hamish McCullum et al (2003).
Recommendation 2.31 Any analysis of susceptibility and ecological factors should take into account the phylogenetics of native frogs.	Unclear what is meant by this recommendation. Not relevant to TAP.
Recommendation 2.32 Immune response to chytridiomycosis and ranaviruses should be investigated particularly the role of immunosuppression in increasing susceptibility.	Work on this topic is being done in USA as part of IRCEB project. Not relevant to TAP.
Recommendation 2.33 The concept of strains (e.g., mutations, mutation rates and endemism) of *B. dendrobatidis* should to be investigated and data disseminated to assist in a global mapping survey.	Achieved. DNA work by Moorehouse et al (2002) showed all strains closely related. Work commencing in Africa by Weldon and Du Prez to look for greater degree of variation there. Not relevant to TAP.
Recommendation 2.34 The effect of temperature on the physiology and immunology of amphibians should be assessed.	Work in progress in USA as part of IRCEB project. Not relevant to TAP.

Recommendation	Status re implementation at 31 October 2003
Movement of amphibians in farm produce and other items	
Recommendation 3.1 Work should be done in collaboration with industry (banana, other fruit, vegetable, nursery industry) at local, state and national level to determine the critical control points where frogs enter product and where they can be most effectively removed.	Work done by McDonald et al (in preparation) with banana industry at Tully. Industry implemented changes. Incorporated into actions for TAP.
Recommendation 3.2 Educational campaigns for producers should be developed to: 1. highlight importance of worker training in frog spotting and removal before transport: • Poster for workers in packing sheds and other sites • Training videos for workers 2. communicate information on effective removal of frogs from produce to growers and packers: Additions to Farmcare and Freshcare manuals should be written to provide this information. Disseminate strategies using normal communication tools within industries including industry specific magazines and journals.	Posters developed and implemented for banana industry, presentations given at industry forums, articles published in banana industry magazine. Incorporated in actions for TAP.
Recommendation 3.3 Each industry should be assisted to set a defined standard for each product to meet "ecofriendly" criteria that minimise the number of frogs moved with produce.	Incomplete. Work done with banana industry only. Incorporated into actions for TAP.
Recommendation 3.4 The involvement of each industry in accidental translocation of amphibians should be quantified.	No action. Incorporated into actions for TAP.
Recommendation 3.5 For packing sheds a container should be designed and tested to facilitate the humane and effective removal of detected frogs from the shed environment.	Achieved for banana industry. Easily adapted to other industries. Incorporated in actions for TAP.
Recommendation 3.6 Exotic amphibians accidentally or illegally imported should be euthanased humanely and submitted to pathological examination in an institution with appropriate quarantine procedures.	Requirement of ABPM 2003/26. Not relevant to this TAP.
Recommendation 3.7 Native amphibians accidentally or illegally moved outside their native range should be placed in the pet trade in states where this is allowed after treatment / disinfection using a standard protocol.	No uniform protocol used nationally. Incorporated as action in TAP.
Recommendation 3.8 In states where placing translocated amphibians in the pet trade is an option, the infrastructure for collection, maintenance and allocation of these amphibians should to be supported and sustained.	No action. Incorporated as action in TAP.
Recommendation 3.9 Each region should assess the risk to their local amphibians in terms of accidentally translocated frogs, including their disease status and the current status of chytridiomycosis and ranaviral disease in amphibians in that region.	No coordinated action. Incorporated as action in TAP.
Recommendation 3.10 Community educational material should be developed to enable people to distinguish between local frogs and foreign frogs from overseas and from other areas in Australia.	No action. Incorporated as action in TAP.
Recommendation 3.11 The involvement of industries other than farm or nursery industries in accidentally moving amphibians should be investigated.	No action. Incorporated as action in TAP.
Recommendation 3.12 If some industries are found to pose a significant risk of accidentally moving amphibians, these industries should be encouraged to reduce or eliminate these risks.	Action with banana industry. Incorporated as action in TAP.

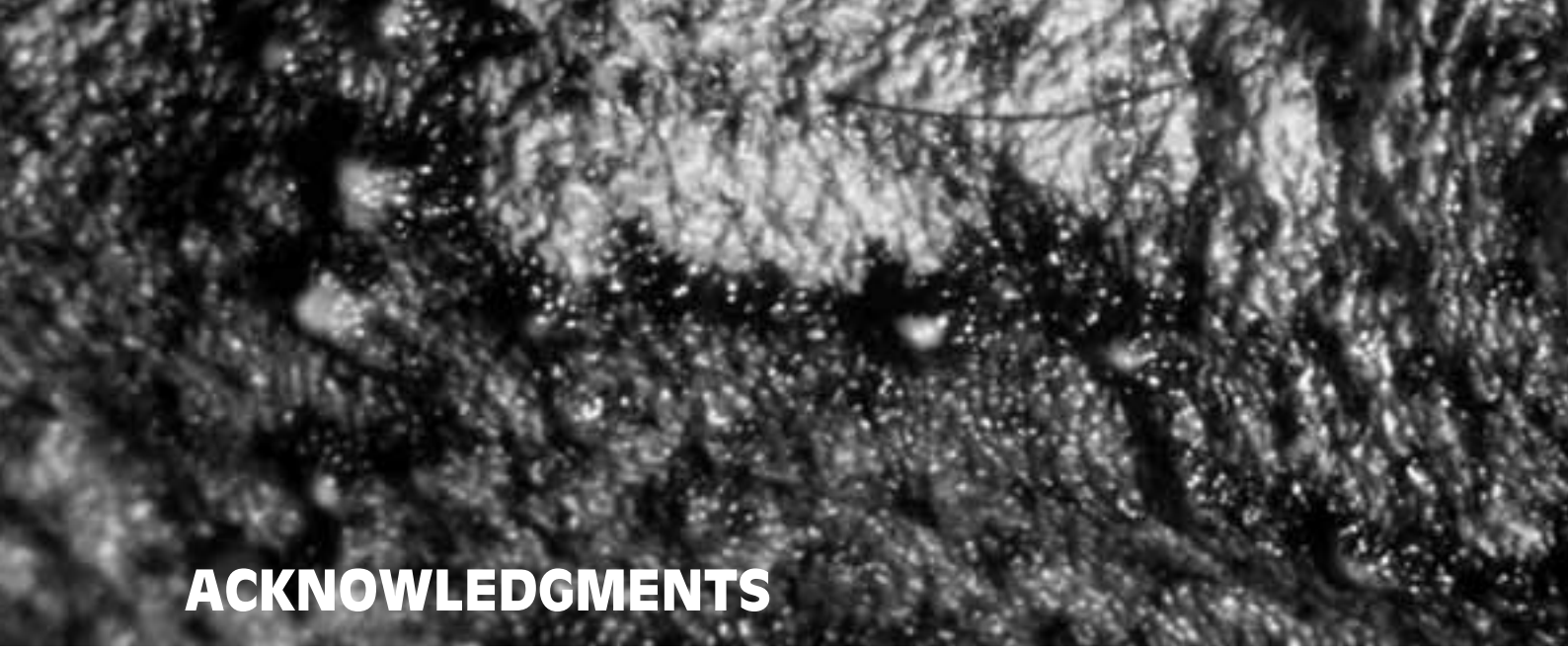
Recommendation	Status re implementation at 31 October 2003
Pathogens in the Laboratory	
Recommendation 3.13 Live amphibian pathogens, particularly the amphibian chytrid and ranaviruses, must be worked with only in a laboratory where facilities, protocols and training are of such a standard that pathogens can not escape into the wild.	Achieved. Generally implemented.
Recommendation 3.14 A higher level of safety for work with ranaviruses should be set than for *B. dendrobatidis*.	Both level PC2. Not now relevant.
Recommendation 3.15 For B. dendrobatidis and ranaviruses the safety level which laboratories have to meet to allow them to work with live agents is level 2 biosecurity.	Achieved. Incorporated in actions for TAP.
Recommendation 3.16 This information should be disseminated through the laboratory monitoring and ethics systems within universities and other research institutions.	No coordinated action. Incorporated in actions for TAP.
Amphibian Husbandry	
Recommendation 3.17 Standards should be established for four different amphibian husbandry situations: 1. native amphibian species only exposed to native amphibians 2. exotic amphibians and native amphibian species potentially exposed to exotic amphibians 3. threatened native amphibian species 4. large scale commercial production of exotic or native species.	Protocols developed for #2 by Michael Lynch (2001). Incorporated as actions in TAP.
Recommendation 3.18 For each situation guidelines should be developed on quarantine protocols within facilities to minimise the risk of introduction and transmission of pathogens within facilities: 1. native amphibian species only exposed to native amphibians, threatened and otherwise 2. exotic amphibians and native amphibian species potentially exposed to exotic amphibians 3. large scale commercial production of exotic or native species.	Protocols for #2 in 3.18 above. Incorporated as actions in TAP.
Recommendation 3.20 Industries should be primarily self-monitoring to ensure that their facilities and protocols confirm to appropriate standards.	Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.21 Animals that die in husbandry facilities should be pathologically examined to determine cause of death particularly for facilities dealing with endangered and threatened Australian frogs and with imported frogs.	Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.22 Protocols should be developed to lessen the risk of disease from native amphibians kept by members of the public as permitted under state regulations.	Achieved. Many state parks and wildlife services and frog organisations provide accurate information. Incorporated in actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Field Research	
Recommendation 3.23 Protocols should be developed to strike a balance between preventing disease transmission and allowing relevant research to be carried out.	Some criticisms by researchers that protocols are too rigorous. Incorporated in actions for TAP.
Recommendation 3.24 A hierarchy of risk should be developed by researchers using the conservation status of species at sites and the status of chytridiomycosis and ranaviral disease at sites.	No formal process undertaken. Incorporated in actions for TAP.
Recommendation 3.25 Work between sites should use this hierarchy of risk to 1) minimise the possibility of spread of pathogens and 2) minimise the potential severity of impact of a pathogen if it was introduced into a site.	Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.26 The sequence of work between sites should if feasible proceed from 1) sites of low prevalence and density of pathogens to sites of higher prevalence and density of pathogens (minimisation of potential for spread) and 2) from sites of high conservation status to sites of less conservation status (minimisation of impact on amphibian population).	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.27 For amphibian researchers working within river systems, separate transects should be regarded as separate sites and for isolated water bodies such as lakes, ponds and dams, separate water bodies should be regarded as separate sites.	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.28 Working within sites the recommendations for within populations should apply; working between sites the recommendations for between populations should apply	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.29 The standard to achieve between sites should be that researchers do not introduce pathogens into any site.	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.30 This standard should be maintained even if pathogens are already present at the site.	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.31 Disinfection procedures between sites should be carried out to kill all pathogens on personnel and their equipment: 1. Wash equipment in water to remove any visible organic debris. 2. Apply disinfecting solution for 1 minute. Sodium hypochlorite at 0.4% and 70% ethanol are 100% effective at killing • B. dendrobatidiszoosporangia and zoospores. 5% active chlorine for 1 min may be needed for ranaviruses. The disinfecting solution can be applied by spray, by • immersion or by wiping the surface of the item with the disinfecting solution. 3. Dry equipment if possible between sites. Drying alone for 3 hours will kill B. dendrobatidis, but not ranaviruses.	Included as requirement in state permits. Incorporated in actions for TAP.
Recommendation 3.32 If equipment cannot be disinfected between sites, it should be enclosed in watertight bags which should not be opened at other sites.	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.33 Items of clothing that cannot be easily disinfected between sites should be replaced at each new site.	Promoted in general. Unclear to what extent this is implemented. Incorporated in actions for TAP.
Recommendation 3.34 Amphibians should not be moved between sites.	Promoted in general. Unclear to what extent this is implemented. Included as requirement in state permits. Incorporated in actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Recommendation 3.35 The standard to aim for within an amphibian population should be the current risk that members of that population are exposed to naturally. Researchers do not have to adopt strategies that are designed to reduce risks below the natural baseline.	Promoted in general. Unclear if state permit authorities are adopting this standard. Incorporated in actions for TAP.
Recommendation 3.36 Handling techniques should be standardised so as not to increase the risk of transmission of pathogens between individual animals: 1. Gloves or plastic bags if used for handling should be used only on one amphibian unless sterilised adequately between uses or washed in the same water from which the amphibian population is collected. 2. Gloves and plastic bags can be sterilised in bleach (0.4%) and then washed in pathogen-free water or the same water occupied by the amphibian being collected. 3. Handling without protective covering is permitted if hands are washed thoroughly in the same water occupied by the next amphibian to be handled or by washing in pathogen-free water. If hand washing in suitable water is not possible between individuals, direct handling should not be done, and single use gloves or plastic bags should be used. 4. Water collected from natural water bodies should not be used for rehydrating or wetting amphibians not normally using that water.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.
Recommendation 3.37 Temporary housing or holding facilities for amphibians should not increase the risk of exposure above the natural baseline level for that population. 1. Each amphibian should be housed separately so that it does not have direct contact with others. 2. If containers that have been used to hold amphibians are to be reused, they should be sterilised by immersion in 5% bleach for at least 1 minute to kill ranaviruses and *B. dendrobatidis* before reuse. 3.To dispose of containers that have been used to hold amphibians, immerse containers in 5% bleach for at least 1 minute to kill ranaviruses and 0.4% bleach for 30 seconds to kill *B. dendrobatidis.* Containers are then sterile and can be disposed of by normal methods.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.
Recommendation 3.38 Protocols to prevent transmission of pathogens by non-invasive procedures such as weighing and measuring should be followed: 1. Implements should be physically cleaned of any secretions or body fomites between use 2. Implements should be sterilised by spraying or wiping with 70% alcohol or 0.4% bleach after cleaning and leaving the disinfectant on the surface for at least 30 seconds. 3. If possible, each specimen should be weighed in its own plastic bag without coming into contact with scales or measuring implements.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.
Recommendation 3.39 Standard protocols to prevent transmission of pathogens by surgical procedures such as toe clipping and implantation of devices should be followed: 1. instruments and other implements should be cleaned between individual amphibians 2. fluids and tissue physically removed from their surfaces 3. sterilised with 70% alcohol or 0.4% bleach for at least 30 seconds.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.
Recommendation 3.40 Researchers should incorporate measures to clean their hands and instruments into their procedures of surveying amphibians so that the protocols become routine and confusion about the state of sterilisation of instruments does not occur.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Recommendation 3.41 Amphibians with signs of illness are high risk in terms of pathogen transmission. Researchers should adopt protocols to minimise the risk of pathogens being subsequently transmitted to other frogs: 1. Ill amphibians should be captured using a glove or plastic bag, and placed in a temporary holding container, to be dealt with after all other amphibians have been examined 2. Any gloves or plastic bags used with ill amphibians should be disposed of and not sterilised and not reused. 3. Instruments used with ill amphibians should not be reused until adequately sterilised. This will include cleaning of secretions, blood and tissue, immersion in 70% ethanol or 0.4% bleach for 10 minutes, drying, coating with ethanol and immediate flaming or alternatively physical cleaning and subsequent autoclaving. 4. Ill amphibians should not be returned to the wild, but should be submitted for pathological examination.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.
Recommendation 3.42 Tadpoles cannot be treated as individuals in the survey situation, but each aggregate should be treated as a unit.	Promoted at Amphibian Disease Home Page. Techniques appear to be used by researchers. Incorporated in actions for TAP.
Recommendation 3.43 The effectiveness of practical disinfection techniques and procedures able to be used in the field should be determined.	In vitro work completed. Evaluation in field difficult. Incorporated in actions for TAP.
ROLE OF COMMUNITY	
Recommendation 4.1 Wildlife managers, researchers and community members should form a partnership to decrease risks of disease to amphibians.	Achieved. Incorporated in actions for TAP.
Recommendation 4.2 The community should be encouraged and assisted to be active in disease surveillance: • monitoring deaths • accurately recording clinical signs in ill amphibians • collecting ill and dead amphibians for pathological examination • assisting in surveys of road kill samples for chytridiomycosis.	Promoted at Amphibian Disease Home Page. Specimens submitted to ADRG at JCU and AAHL. Emails from public, wildlife managers and researchers responded to by ADG(SPHTM). Incorporated in actions for TAP.
Recommendation 4.3 Disease surveillance should be done under a structured and integrated system that includes the broad community (many eyes), voluntary frog groups (filtering information), amphibian pathologists (interpreting disease findings), information users (wildlife managers), with free-flow of information between all levels.	Partly achieved. Incorporated in actions for TAP.
Recommendation 4.4 The community should be encouraged and assisted to be active in caring for and "nurturing" ill amphibians: • retrieval of ill frogs from harm's way and bring into the care of skilled people • rehabilitation of frogs • repatriation and release of cured amphibians • developing good husbandry techniques • dissemination of information about techniques and diseases to other care groups, government and the scientific community	Achieved, but funding is mainly from members of public and corporations. Incorporated in actions for TAP.
Recommendation 4.5 The community should be encouraged and assisted to be active in monitoring of amphibian populations: • densities, movements, reproductive activity and success • surveillance for exotic amphibians.	No nationally coordinated approach. Unclear how widely this is implemented. Incorporated in actions for TAP.

Recommendation	Status re implementation at 31 October 2003
Recommendation 4.6 The community should be encouraged and assisted to be active in protection, improvement and creation of habitats for amphibians.	Achieved, but no nationally coordinated approach. Incorporated in actions for TAP.
Recommendation 4.7 Information on relevant diseases of amphibians should be disseminated to the community through a range of audiences (schools, general public, policy makers, herpetologists, disease specialists) with particular emphasis on the factors associated with amphibian population declines, the need to preserve Australian frog density and diversity, how to detect and control disease, and how to minimise risks associated with translocation of frogs.	Information available at Amphibian Diseases Home Page, WWF Australia Frogs programme and through frog groups and state parks and wildlife services. Incorporated in actions for TAP.
Recommendation 4.8 A range of mechanisms should be used to disseminate information to the community on amphibian diseases and their role in amphibian population declines; these mechanisms could include TV, printed media, internet, events, inclusion in school and tertiary curricula, workshops for educators, displays in zoos, museums and nature houses, and by CD-ROM.	Achieved, but no nationally coordinated approach. Incorporated in actions for TAP.
Recommendation 4.9 Funding to community organisations and others to support and assist the activities outlined in recommendations 4.1 to 4.8 should be provided by government, non-government organisations, commercial sources, and community donations.	Funding appears to be mainly through corporate donations and from members of the public. Incorporated in actions for TAP.



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Notes

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Australian Government

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