Australian
Wildlife Health
Network

Chytridiomycosis – amphibian chytrid fungus in Australia
FACT SHEET

Introductory Statement

In Australia, the oldest record of *B. dendrobatidis* is from a museum frog specimen collected in south-east Queensland near Brisbane in 1978 (Department of the Environment and Heritage 2006a), which coincides with sudden frog declines in a number of species and two species extinctions in the region (Berger *et al.* 1998; Hines *et al.* 1999). Subsequent amphibian declines in central coastal Queensland (1985-86) and the Wet Tropics (1990-95) suggest that *B. dendrobatidis* spread north to its current northern limit at Big Tableland near Cooktown (Laurance *et al.* 1996; Berger *et al.* 1999a; Skerratt *et al.* in review). In southern Australia, the spread of *B. dendrobatidis* is poorly documented but its distribution extends down the entire east coast to Tasmania (first detected in 2004) (Obendorf & Dalton 2006; Pauza & Driessen 2008). Two separate foci occur in other states, one in southwest Western Australia, where the earliest record dates to 1985, and another around Adelaide in South Australia (earliest record 1995) (Berger et al. 2004, Murray et al. in prep.). The Northern Territory is currently considered amphibian chytrid free (Skerratt et al. 2008). 63 (29%) of Australia's 219 endemic frogs have positive records for infection. It is a pathogen capable of driving species to extinction.

Aetiology

Batrachochytrium dendrobatidis (Longcore et al. 1999), the Amphibian Chytrid Fungus.
Fungi, *phylum Chytridiomycota, Order Chytridiales.* Only member in genus.

Natural hosts

Amphibians (Berger *et al.* 1998). Currently found in two of the three extant amphibian orders: Anura (frogs and toads) and Caudata (salamanders and newts). Currently known from at least 233 anuran and 24 salamander species worldwide (Olson & Ronnenberg 2008) but this number will rise as search effort and reporting continues.

No sex-linked predisposition. No age-linked predisposition to infection, since tadpoles are commonly infected, but there is age-linked mortality. Adults and juveniles die from chytridiomycosis while tadpoles have not been reported to die from chytridiomycosis. Mortality in susceptible species is in general higher in metamorphs than adults.

World distribution

Known from all continents where amphibians occur. *Africa* - Botswana, Ghana, Kenya, Lesotho, Morocco, South Africa, Tanzania, Zambia; *Australia*; *Pacific* - New Zealand, Hawaii; *Central America* - Cost Rica, Cuba, Dominica, Honduras, Mexico, Panama, Puerto Rico; *North America* - Canada, USA; *South America* - Argentina, Brazil, Ecuador, Peru, Uruguay, Venezuela; *Europe* - Denmark, France, Germany, Hungary, Italy, Portugal, Spain, Switzerland, United Kingdom; *Asia* – Indonesia, Japan, Philippines.

Occurrences in Australia

Chytridiomycosis is listed on the OIE Wildlife Diseases List (World Organisation for Animal Health 2008). *Batrachochytrium dendrobatidis* is now endemic in Queensland, New South Wales, Australian Capital Territory, Victoria, Tasmania and Western Australia. Little is known about *B. dendrobatidis* in South Australia. Much of the continent is considered too hot and/or dry to sustain Bd and as such it is not endemic in all Australia. It has been found in wild amphibian populations on the east coast of Queensland and New South Wales on or between the Great Dividing Range and the coast, in the Australian Capital Territory, Victoria, Tasmania and in southwest Western Australia. Not found in Northern Territory to date (Table 1). Currently known from 63 amphibian species in 4 families (Hylidae, Myobatrachidae, Microhylidae, Bufonidae - introduced) (Table 2) (Murray et al. in prep.).

Table 1. Number of Australian amphibian species found with chytridiomycosis by state compared with total species of amphibians and total species tested in each state.

State	Species +ve for Bd	Species tested	Species	% of tested species +ve	% of all species +ve
ACT	1	2	18	50.0	5.6
NSW	20	25	84	80.0	23.8
NT	0	6	47	0.0	0.0
QLD	34	69	123	49.3	27.6
SA	4	6	27	66.7	14.8
TAS	2	4	10	100.0	40.0
VIC	4	8	33	50.0	12.1
WA	16	30	77	53.3	20.8
Australia	**63**	**115**	**219**	**54.8**	**28.8**

From Murray et al. (in prep.)

Table 2. Amphibian hosts (n=63) reported with chytridiomycosis in Australia

Family	Genus	Species	First reference
Bufonidae	*Rhinella*	*marina* (introduced; formerly *Bufo marinus*)	Berger (2001)
Hylidae	*Litoria*	*adelaidensis*	Aplin & Kirkpatrick (2000)
Hylidae	*Litoria*	*aurea*	Berger (2001)
Hylidae	*Litoria*	*barringtonensis*	Berger (2001)
Hylidae	*Litoria*	*booroolongensis*	Hunter unpub. data
Hylidae	*Litoria*	*burrowsi*	Obendorf & Nelson (2004)
Hylidae	*Litoria*	*caerulea*	Berger (2001)
Hylidae	*Litoria*	*chloris*	Berger (2001)
Hylidae	*Litoria*	*citropa*	Mahony (2000)
Hylidae	*Litoria*	*dayi (formerly Nyctimystes)*	Berger (2001)
Hylidae	*Litoria*	*ewingii*	Berger (2001)
Hylidae	*Litoria*	*fallax*	Kriger & Hero (2007)
Hylidae	*Litoria*	*genimaculata*	Berger (2001)
Hylidae	*Litoria*	*gracilenta*	Berger & Speare (2004)
Hylidae	*Litoria*	*infrafrenata*	Berger (2001)
Hylidae	*Litoria*	*jungguy*	Berger (2001)
Hylidae	*Litoria*	*latopalmata*	Kriger & Hero (2007)
Hylidae	*Litoria*	*lesueurii*	Berger (2001)
Hylidae	*Litoria*	*lorica*	Puschendorf et al (2009)
Hylidae	*Litoria*	*moorei*	Aplin & Kirkpatrick (2000)
Hylidae	*Litoria*	*nannotis*	Berger et al (1998)
Hylidae	*Litoria*	*nasuta*	Donovan et al (1999)

Hylidae	*Litoria*	*pearsoniana*	Berger (2001)
Hylidae	*Litoria*	*peronii*	Berger (2001)
Hylidae	*Litoria*	*phyllochroa*	Mahony (2000)
Hylidae	*Litoria*	*raniformis*	Norman & Waldman (2000)
Hylidae	*Litoria*	*rheocola*	Berger (2001)
Hylidae	*Litoria*	*spenceri*	Berger (2001)
Hylidae	*Litoria*	*tyleri*	Berger (2001)
Hylidae	*Litoria*	*verreauxii*	Berger (2001)
Hylidae	*Litoria*	*wilcoxii*	Berger (2001)
Hylidae	*Litoria*	*xanthomera*	Phillott & McDonald unpub. data
Limnodynastidae	*Adelotus*	*brevis*	Speare & Berger (2004)
Limnodynastidae	*Heleioporus*	*australiacus*	Berger (2001)
Limnodynastidae	*Heleioporus*	*barycragus*	Speare website
Limnodynastidae	*Heleioporus*	*eyrei*	Aplin & Kirkpatrick (2000)
Limnodynastidae	*Lechriodus*	*fletcheri*	Berger (2001)
Limnodynastidae	*Limnodynastes*	*dorsalis*	Aplin & Kirkpatrick (2000)
Limnodynastidae	*Limnodynastes*	*dumerilii*	Berger (2001)
Limnodynastidae	*Limnodynastes*	*peronii*	Berger (2001)
Limnodynastidae	*Limnodynastes*	*tasmaniensis*	Berger (2001)
Limnodynastidae	*Limnodynastes*	*terraereginae*	Berger (2001)
Limnodynastidae	*Neobatrachus*	*kunapalari*	Berger (2001)
Limnodynastidae	*Neobatrachus*	*pelobatoides*	Speare website
Microhylidae	*Cophixalus*	*ornatus*	Kriger (2006)*
Myobatrachidae	*Assa*	*darlingtoni*	Kriger & Hero (2007)*
Myobatrachidae	*Crinia*	*georgiana*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Crinia*	*glauerti*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Crinia*	*insignifera*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Crinia*	*pseudinsignifera*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Crinia*	*subinsignifera*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Crinia*	*tasmaniensis*	Pauza & Driessen (2008)
Myobatrachidae	*Geocrinia*	*rosea*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Geocrinia*	*vitellina*	Aplin & Kirkpatrick (2000)
Myobatrachidae	*Mixophyes*	*fasciolatus*	Berger (2001)
Myobatrachidae	*Mixophyes*	*fleayi*	Berger (2001)
Myobatrachidae	*Mixophyes*	*iteratus*	Mahony (2000)
Myobatrachidae	*Pseudophryne*	*corroboree*	Speare & Berger (2004)
Myobatrachidae	*Pseudophryne*	*pengilleyi*	Berger et al. (2004)
Myobatrachidae	*Taudactylus*	*acutirostris*	Berger (2001)
Myobatrachidae	*Taudactylus*	*eungellensis*	Retallick et al. (2004)
Myobatrachidae	*Uperoleia*	*fusca*	Kriger & Hero (2007)
Myobatrachidae	*Uperoleia*	*laevigata*	Berger (2001)

From Murray et al. (in review)

*Positive by qPCR from a single individual only. The specificity of the test used on these individuals may be less than 100%.

Epidemiology

Morbidity rate: When frogs show clinical signs, death usually follows within 2-3 days. Prevalence of infection in apparently aclinical frogs in infected populations in Australia can approach 100%.

Mortality rate: In captivity and in challenge and transmission experiments, some adult frogs are capable of surviving and clearing infections but mortality rates of up to 100% are common. Pathogenicity varies with host species, fungal strain, exposure dose and period, temperature and body size. High mortality or susceptibility to infection observed in the laboratory may not always occur in the wild and vice versa. Recently metamorphosed frogs appear most sensitive to the disease in some species. Infections of tadpoles are limited to their keratinized mouthparts and often appear to have no negative effects (implicating them as potential disease reservoirs), although some evidence suggests that infected tadpoles of some species may lose body condition and suffer reduced survival. Tadpole infections can be carried through metamorphosis and cause high metamorph and juvenile mortality. Presence and prevalence of Bd in the wild varies with species, life-stage, season, altitude and latitude and may be broadly governed by temperature.

- Incubation period: time to clinical signs and death usually ranging between 14 and 70 days post-exposure
- Transmission: Via zoospore, waterborne.
- Sources of agent:

  -Shedding of zoospores from infected skin. Zoospores leave host via discharge papillae projecting through surface of epithelial cell. Zoospores require water to survive, although a film is adequate.

  -Skin on frogs and mouthparts on tadpoles are the only infective tissues

  -Zoospore invades stratum corneum to infect new host

  -Frogs are subclinical for the majority of the duration of the infection. *B. dendrobatidis* is not an obligate parasite and can exist and grow in moisture in the laboratory. However, it is easily outcompeted by environmental microorganisms. Hence, frogs can be infected from water containing zoospores generated either from frogs or potentially from non-parasitic growth that might occur under certain conditions (has not been demonstrated to date).

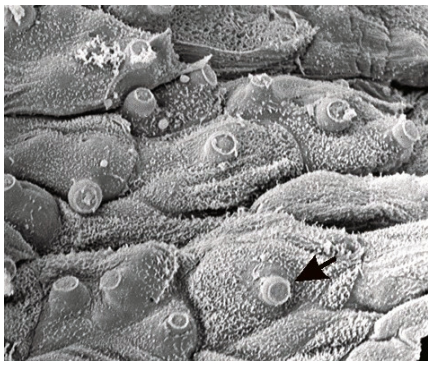
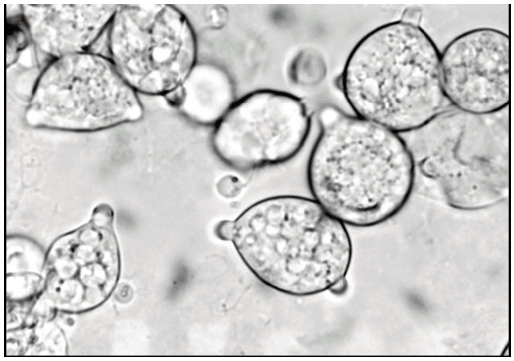
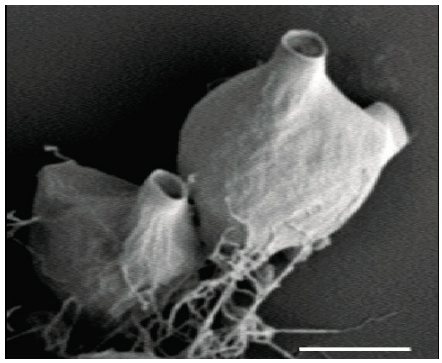


Image of infected skin from a scanning electron microscope. Fungal discharge tubes are protruding through the surface



B. dendrobatidis *in culture*



Scanning electron micrograph of zoosporangia with open discharge tubes, and rhizoids at the base (Images courtesy Lee Berger)

Clinical signs

In the majority of infected animals for most of the time, clinical signs are absent. The period of showing signs is typically short and limited to those amphibians that will mostly die. Central nervous system signs predominate; behavioural change, slow and uncoordinated movement, abnormal sitting posture, tetanic spasms, loss of righting reflex, paralysis. Skin changes in chytridiomycosis are typically microscopic and not detectable at the clinical level with any

degree of confidence although abnormal skin shedding occurs (skin shed more frequently and in smaller amounts) and erythema may be seen.

*Figure 1. Great barred frog (*Mixophyes fasciolatus*), a lethargic frog with shedding skin accumulating on the body (Courtesy Lee Burger).*

Diagnosis

Chytridiomycosis is diagnosed by detecting *B. dendrobatidis* in the skin of amphibians. This is done by 1) light microscopy, or 2) PCR.

- Light microscopy: Zoosporangia detected in stratum corneum. There are two routine tests: 1) Examination of skin slough with or without staining, or 2) examination of histological sections stained with haematoxylin and eosin (Berger *et al.* 1999b; Pessier *et al.* 1999), with silver stain (Green et al 2002), or with an immunoperoxidase stain using a polyclonal antibody against *B. dendrobatidis* (Berger *et al.* 2002).
- Molecular tests: The most common test is the real-time Taqman PCR which can quantify the amount of DNA in the sample (Boyle *et al.* 2004; Hyatt *et al.* 2007).

Clinical Pathology

No consistent pattern although electrolyte changes have been seen in blood in some species (Voyles *et al.* 2007).

Pathology

Gross lesions: In most cases nil. Occasional cases have increased sloughing of skin, but this is rarely detectable with the unaided eye.

Histology/ microbiology: For complete description see Berger et al (1999) available online at http://www.jcu.edu.au/school/phtm/PHTM/frogs/histo/chhisto.htm. Summary: Local hyperkeratosis of infected and adjacent cells with presence of sporangia inside cells. Epithelial cells in the layer beneath the superficial layer undergo dissolution, often leading to sloughing of the most superficial layer. Usually no associated inflammatory reaction in dermis. Sites of prediliction are the feet, hands and ventral surfaces, but in heavy infections other sites on the body are infected. There are no consistent lesions in other organs.

Differential diagnoses

Using histopathology: Other fungal infections of skin. Artefacts of skin capable of being confused with sporangia by inexperienced diagnosticians.

Laboratory diagnostic specimens

For histopathology: Skin of feet or toe tips are often adequate but whole frog for necropsy is best to aid diagnosis and rule out other diseases, Fixed in 10% buffered neutral formalin or 70% ethanol.
For PCR: Swab of skin from feet, hands and ventral body surface

Laboratory procedures

- Light microscope examination of pieces of stratum corneum, unstained or stained with congo red (Briggs & Burgin 2003) or Diff Quick.
- Histopathology: haematoxylin and eosin (Berger *et al.* 1999b; Pessier *et al.* 1999) or silver stain (Green et al 2002).
- PCR: Taqman real-time PCR. See Boyle et al (2004) and Hyatt et al. (2007).

Treatment

The terminal disease has not been successfully treated. The early phases of infection have been treated successfully. However, results are variable and 100% cure cannot at the moment be guaranteed. In many frogs, treatment will control and suppress the infection, but not eliminate it. Frogs remain infected but at a lower level. The infection will then build up over time and can result in death. However, some frogs will be cured by treatment.

At the current time no treatment is 100% effective for Australian amphibians. Chytridiomycosis can be suppressed in almost all cases, but cured in a smaller percentage. Some treatments have been successful overseas. Bathing in 0.01% itraconazole suspension for 5 minutes a day for 11 days was reported to successfully treat chytridiomycosis in *Dendrobates tinctorius* (Nichols & Lamirande 2000) and several other dendrobatid frogs (Forzan *et al.* 2008). 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). Raising the temperature of experimentally infected Red Eyed Tree Frog, *Litoria chloris*, a native Australian species, to 37℃ resulted in cure of chytridiomycosis. All members of a group of 10 experimentally infected *L. chloris* were cured after being held at 37℃ for 2 periods of 8 hours 24 hours apart (Woodhams *et al.* 2003). However, when this regime was used in other Australian species, it was not 100% successful. Topical treatment with chloramphenicol treated Bd effectively in 12 Archey's frogs in New Zealand (Bishop *et al.* 2009), but further testing is required to confirm the safety of chloramphenicol for other species.

Prevention and control

Temperature: *B. dendrobatidis* stops growth at ~28°C in vitro and continues growing slowly at 10°C (Piotrowski *et al.* 2004). B. dendrobatidis is highly susceptible to temperatures above 32°C. Cultures of *B. dendrobatidis* die at 32°C after 4 days and at 37°C after 4 hrs (Berger 2001; Johnson et al 2003). Life-history trade-offs that see increased zoospore production as sporangia maturation rate slows appears to maintain population growth at sub-optimal temperatures (Woodhams *et al.* 2008).

B. dendrobatidis can grow over a wide range of pH (4-9), but optimum is 6-7 (Piotrowski *et al.* 2004). The amphibian chytrid fungus is susceptible in vitro to many standard disinfectants (Table 3), but is not killed by sterilising ultraviolet light.

Table 3. Disinfection techniques that will kill 100% of Bd zoospores and zoosporangia. From Johnson et al (2003) and Webb et al. (2007). RH = relative humidity.

	Temp / concentration	Minimum time of exposure
Physical techniques		
Heat	60°C	1 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
Bleach	0.4%	3 min
Didecyl dimethyl ammonium chloride	$1x10^{-3}$	0.5 min
Benzalkonium chloride	1%	0.5 min
TriGene Virucidal Disinfectant Cleaner	0.1 ml l–1	1 min
F10 Super Concentrate Disinfectant	0.33 ml l–1	1 min
Betadine Antiseptic Liquid	100 ml l–1	1 min

TriGene is the most effective disinfectant yet to be found, and both TriGene and F10 are more effective than previously tested disinfectants (Webb *et al.* 2007). *B. dendrobatidis* will survive and grow in the external environment; it is not an obligate parasite. It can be grown on a range of keratin supplemented sterile media, including frog skin, snake skin and feather meal agars (Symonds *et al.* 2008), and it can survive in moist river sand and on bird feathers (Johnson & Speare 2005). Bare human skin has a fungicidal effect on B. dendrobatidis, but this killing effect is reduced by repeated washing with water and ethanol. Nitrile gloves kill B. dendrobatidis on contact, but washing in water decreases this effect. Latex and polyethylene gloves have no killing effect (Mendez *et al.* 2008).

Surveillance and management

Infection of amphibians with the amphibian chytrid fungus has been listed as a Key Threatening Process in Australia by the Commonwealth Department of Environment and Heritage and a Threat Abatement Plan (TAP) prepared (Department of the Environment and Heritage 2006b; Speare 2006). Surveillance for chytridiomycosis especially in currently chytrid-free zones is proposed in this TAP and sampling design has commenced (Skerratt *et al.* 2008; Murray *et al.* in review; Skerratt *et al.* in review).

Chytridiomycosis is not currently included in AUSVETPLAN. Threat Abatement Plan recommendation 1.1.3 proposes that a risk-based approach be used for chytridiomycosis using AUSVETPLAN as a model (Department of the Environment and Heritage 2006b). However, this has not yet progressed. Nation-wide mapping protocols and disease risk models have been developed and should serve as the basis for cost-sharing arrangements between states (Murray *et al.* in review; Skerratt *et al.* in review). Implementing this step remains a priority.

Risk analysis performed by Biosecurity Australia in "Quarantine requirements for the importation of amphibians or their eggs into zoological facilities" (Animal Biosecurity Policy Memorandum 2003/26) did not list chytridiomycosis as a risk since it is endemic in Australia. However, this disregards the risk of importation into chytrid free areas. Although chytridiomycosis is not specifically mentioned, the general hygiene strategies recommended will prevent the release of imported strains of *B. dendrobatidis* during the initial two years. After two years the amphibians can be released without testing for *B. dendrobatidis*. However, if being released into a chytrid free area, the same requirements imposed on Australian bred amphibians under the Threat Abatement Plan would apply.

Amphibian chytridiomycosis was listed on the OIE Wildlife Diseases List and has been declared an international notifiable disease (World Organisation for Animal Health 2008).

Australia now reports to OIE on its chytrid stautus via Aquatic Animal Health Committee (AAHC).

Statistics

The most complete dataset currently available on Chytrid in Australia is managed by the Amphibian Diseases Group, School of Public Health and Tropical Medicine, James Cook University. See also National Wildlife Health Surveillance Database (http://www.wildlifehealth.org.au/AWHN/home.aspx). NOTE: access to this dataset is restricted. If you would like access please contact awhn@zoo.nsw.gov.au.

Research

Key research questions:

1. What can be done to mitigate the impact of chytridiomycosis where it is endemic?
2. What can be done to prevent further spread of chytridiomycosis?

More detailed research questions that may help answer the above:

3. Why do infected amphibians die?
4. What areas in Australia are chytrid free?
5. Can *B. dendrobatidis* spread to and establish in these disease-free areas?
6. In populations where chytridiomycosis is endemic what determines the impact on the frog population?
7. Can resistance to infection or clinical disease caused by *B. dendrobatidis* be selected for?
8. Can acquired immunity protect amphibians?
9. Does *B. dendrobatidis* exist as a free-living organism in suitable habitats, particularly natural water bodies and moist substrate in the absence of amphibians?
10. Can detection of *B. dendrobatidis* in water bodies be used as a technique to map contaminated and chytrid-free areas?
11. How do environmental characteristics of natural water bodies (pH, pO_2, ion content, nitrate, organic content) and climate (e.g., temperature, rainfall) affect the biology and survival of *B. dendrobatidis*?
12. What density of zoospores in natural water bodies can infect susceptible species of amphibians and what is the role of natural water bodies in transmission?
13. Does the density of zoospores in natural water bodies correlate with intensity of infection of amphibian populations living in those water bodies, and with the level of clinical chytridiomycosis? Can the density of zoospores in natural water bodies or on amphibians be used to predict periods of high risk for amphibian populations?
14. How does *B. dendrobatidis* spread between water bodies and amphibian populations?
15. Are there non-amphibian vectors of *B. dendrobatidis*?
16. Can *B. dendrobatidis* be eradicated from ponds or small standing water bodies?
17. Can amphibian populations be treated or vaccinated?

Known research activities:

Testing of protocols for mapping regions with unknown chytrid status.
Investigation of pathogenicity and epidemiology including monitoring of amphibian populations that have survived initial epidemic inasion to look for evidence of recovery or ongoing impact and to decipher the host-pathogen relationship in the wild.
Assessing effectiveness of management options such as supplementing critically endangered populations by captive-breeding and reintroduction.
Determining whether innate immunity can be used to improve reintroduction success
Assessing the potential of selection for innate immunity in protecting amphibian populations
Testing of hygiene protocols used to reduce the risk of spread
Assessing the effectiveness of treatment regimes
Predictive climatic and environmental modelling for risk of impact and spread

Human health implications

Nil. *B. dendrobatidis* will not grow above 28°C and dies if held at 37°C for 4 hours. Homeotherms are thus considered unsuitable hosts.

Conclusions

For control at the national level we need to confirm whether chytridiomycosis is absent in areas predicted as unsuitable by species distribution models (e.g., the Northern Territory), whether there are any areas that are suitable that are currently free, the most effective strategies for monitoring these chytrid-free suitable areas and the best methods to prevent spread of chytridiomycosis to these areas. We need emergency response plans in case of spread to these areas and for species currently threatened with extinction. We need to determine whether chytridiomycosis can be eradicated from small contaminated water bodies and whether these can be kept disease free Understanding the medium- to long-term consequences of endemic chytridiomycosis for amphibians is critical for future management in the medium to long-term. We need research to enable us to better mitigate the effects of chytridiomycosis in the short to long-term in affected populations.

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Other information sources:

Speare R. Amphibian diseases home page.
http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm

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To provide feedback on this fact sheet

The Australian Wildlife Health Network would be very grateful for any feedback on this fact sheet. Please provide detailed comments or suggestions to rwoods@zoo.nsw.gov.au. We would also like to hear from you if you have a particular area of expertise and would like to produce a fact sheet (or sheets) for the network (or update current sheets). A small amount of funding is available to facilitate this.

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