CHYTRIDIOMYCOSIS IN AMPHIBIANS

CONTENTS

1. OVERVIEW OF CHYTRIDIOMYCOSIS

Catastrophic amphibian population crashes over the last 30 years have been caused by the spread of an emerging fungal disease, chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis*. The arrival of this pathogen in naïve populations has caused about 122 extinctions worldwide, and is the worst example of a disease impacting on biodiversity (Skerratt et al 2007- http://www.jcu.edu.au/school/phtm/PHTM/frogs/papers/skerratt-2007.pdf).

For reviews see:

Berger, L., Longcore, J., Speare, R., Hyatt, A., Skerratt, L.F. 2009. Fungal Diseases in Amphibians. In: H Heatwole and JW Wilkinson (eds). Amphibian Biology, Volume 8 Amphibian Decline: Disease, Parasites, Maladies, and Pollution. Surrey Beatty & Sons, NSW. Pp 2986-3052 http://eprints.jcu.edu.au/11302/

Fisher MC, Garner TW, Walker SF. 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annu Rev Microbiol. 63:291-310. doi: 10.1146/annurev.micro.091208.073435.

THE BD SEQUENCING PROJECT

The *Batrachochytrium dendrobatidis* sequencing project is part of the Broad Institute Fungal Genome Initiative. Its goal is to release an annotated assembly from 10X genome sequence coverage for *Batrachochytrium dendrobatidis* diploid strain JEL423, which was provided by Dr. Joyce Longcore at The University of Maine. This strain was isolated from a sick *Phyllomedusa lemur* frog in Panama. Genomic DNA from this strain was prepared by Tim James at Duke University.

http://www.broadinstitute.org/annotation/genome/batrachochytrium_dendrobatidis/MultiHome.html

2. DISTRIBUTION AND SPECIES INFECTED

GLOBAL

The global Bd mapping project maintains interactive maps and collates updated information and news at: http://www.bd-maps.net/

The database is published in: Olson DH, Aanensen DM, Ronnenberg KL, Powell CI, Walker SF, Bielby J, Garner TW, Weaver G, Bd Mapping Group, Fisher MC. 2013. Mapping the global emergence of *Batrachochytrium dendrobatidis*, the amphibian chytrid fungus. PLoS One 8(2):e56802. doi: 10.1371/journal.pone.0056802.

AUSTRALIA

A database of over 10,000 Australian records was collated and published in 2010:

Kris Murray, Richard Retallick, Keith R. McDonald, Diana Mendez, Ken Aplin, Peter Kirkpatrick, Lee Berger, David Hunter, Harry B. Hines, R. Campbell, Matthew Pauza, Michael Driessen, Richard Speare, Stephen J. Richards, Michael Mahony, Alastair Freeman, Andrea D. Phillott, Jean-Marc Hero, Kerry Kriger, Don Driscoll, Adam Felton, Robert Puschendorf, and Lee F. Skerratt. 2010. The distribution and host range of the pandemic disease chytridiomycosis in Australia, spanning surveys from 1956–2007. Ecology 91:1557.

The database is available online at http://esapubs.org/archive/ecol/E091/108/metadata.htm

The database is also available via interactive maps maintained by Atlas of Living Australia: http://collections.ala.org.au/public/showDataResource/dr642

SUMMARY OF SPREAD AND IMPACT WITHIN AUSTRALIA

B. dendrobatidis is not native to Australia. It probably arrived in southeast Queensland in the mid-1970s on imported specimens of the African clawed frog, *Xenopus laevis*. However, this is speculative. Our earliest record in Australia is December 1978 in the Connondale Ranges, west of Brisbane and our hypothesis is that the amphibian chytrid fungus escaped from captivity in Brisbane and spread centripetally. Once *B. dendrobatidis* invades an area, it stays. Epidemiological evidence indicates that the amphibian chytrid fungus spread north and south as an advancing front, colonising frogs and water bodies as the front passed. The rate of advance has been estimated as 100 km/year in coastal Queensland and 20-30 km/year in Central America.

From museum records the estimate of the earliest appearance of *B. dendrobatidis* in each zone has been obtained; east coast zone - December 1978, southwest zone - October 1985, and Adelaide zone - May 1996. With more extensive retrospective searching, we may be able to establish earlier arrival dates in each zone. We speculate that each zone was initiated by *B. dendrobatidis* escaping into the wild from an infected amphibian imported from an infected Australian zone or another country.

As the invasive front infected naive amphibian populations, the outcome was determined by interplay between pathogen, host and environment. Some species of amphibians were exquisitely sensitive to chytridiomycosis, occurred in habitats that favoured virulence and became extinct. Of these, *Taudactylus acutirostris*, was a species made extinct by *B. dendrobatidis*; the last population crashed as a result of chytridiomycosis in the upland rainforests of North Queensland in September 1993 and the last known specimen died from chytridiomycosis in captivity at the Melbourne Zoo in 1995.

The Southern Corroboree frog is currently Australia's most endangered frog, relying on intensive captive breeding until a solution is found to enable released frogs to survive.

http://www.corroboreefrog.com.au/corroboree-frog/corroboree-conservation

3. PROTOCOLS AND TECHNIQUES FOR WORKING WITH THE AMPHIBIAN CHYTRID FUNGUS

DIAGNOSTIC METHODS

DIAGNOSIS OF CHYTRIDIOMYCOSIS IN AMPHIBIANS BY HISTOLOGIC EXAMINATION

Berger, L., Speare, R., Kent, A. 1999. Diagnosis of chytridiomycosis of amphibians by histologic examination. Zoos' Print Journal 15:184-190.

Please see Attachment 1.

MOLECULAR DIAGNOSTIC PROTOCOLS FOR CHYTRIDIOMYCOSIS

A.D. Hyatt, D. G. Boyle, V. Olsen, D. B. Boyle, L. Berger, D. Obendorf, A. Dalton, K. Kriger, M. Hero, H. Hines, R. Phillott, R. Campbell, G. Marantelli, F. Gleason, A. Colling (2007) Diagnostic assays and sampling protocols for the detection of *Batrachochytrium dendrobatidis*. Dis Aquat Org 73:175–192.

http://www.jcu.edu.au/school/phtm/PHTM/frogs/papers/hyatt-2007.pdf

Garland S, Wood J, Skerratt LF. 2011. Comparison of sensitivity between real-time detection of a TaqMan assay for *Batrachochytrium dendrobatidis* and conventional detection. Dis Aquat Organ. 94:101-5. doi: 10.3354/dao02327. http://www.int-res.com/articles/dao_oa/d094p101.pdf

Skerratt LF, Mendez D, McDonald, KR, Garland S, Livingstone J, Berger L, Speare R. 2011. Validation of diagnostic tests in wildlife: the case of chytridiomycosis in wild amphibians. Journal of Herpetology, 45:444-450.

MANUAL OF DIAGNOSTIC TESTS FOR AQUATIC ANIMALS (OIE 2013)

The OIE Manual of Diagnostic Tests for Aquatic Animals describes standards for laboratory diagnostic tests. See Chapter 2.1.1 Infection with *Batrachochytrium dendrobatidis*

http://www.oie.int/en/international-standard-setting/aquatic-manual/access-online

PROTOCOLS FOR SURVEYING POPULATIONS

Skerratt LF, Berger L, Hines HB, McDonald KR, Mendez D, Speare R. 2008. Survey protocol for detecting chytridiomycosis in all Australian frog populations. Dis Aquat Organ. 80:85-94. doi: 10.3354/dao01923.
http://www.int-res.com/articles/dao_oa/d080p085.pdf

Skerratt LF, McDonald KR, Hines HB, Berger L, Mendez D, Phillott AD, Cashins SD, Murray KA, Speare R. 2010. Application of the survey protocol for chytridiomycosis to Queensland, Australia. Dis Aquat Organ. 92:117-29. doi: 10.3354/dao02272.

PROTOCOLS USED IN CULTURING *BATRACHOCHYTRIUM DENDROBATIDIS*

CULTURE METHODS FOR BD

Written by Rebecca Web. See Attachment 2.

ISOLATION OF *BATRACHOCHYTRIUM DENDROBATIDIS* FROM FROG SKIN

Written by Joyce Longcore. See attachment 3.

4. TREATMENT OF CHYTRIDIOMYCOSIS

Treatments include the following:

- Itraconazole (50- 100 mg/L) is commonly used as a 5-minute bath exposure daily for 10 days in frogs, but is toxic to some species. In tadpoles, 5-minute daily baths of 0.5–1.5 mg/L itraconazole were effective (Garner et al. 2009).
- Voriconazole spray (1.25 mg/L) daily for 7 days cured frogs and toads (Martel et al. 2011). Voriconazole appeared safe in tadpoles but was not tested in infected tadpoles.
- Terbinafine hydrochloride in alcohol (available over the counter as Lamisil AT [Novartis]) was used at 0.01% or 0.005% as a 5-minute soak for 5 days, and successfully treated six amphibian species (Bowerman et al. 2010).
- Chloramphenicol (20 mg/L) was used successfully as a shallow continuous bath for 28 days; frogs were removed to a dry tub for 1 hour per day for feeding (Young et al. 2012).
- Heating to 32 °C for 5 days was also effective in a heat-tolerant species (Retallick & Miera 2007).

Clinically infected animals may require supportive electrolytes to recover and be cured (Young et al. 2012). Amphibians under treatment should be held in simple tubs with minimal substrates that can be disinfected or changed at each treatment application.

PCR testing is necessary to confirm that treatment has cleared infection. The suggested protocol involves three PCR tests at least a week apart, starting the week after the last treatment (Berger et al. 2010).

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

Berger L, Speare R, Pessier A, Voyles J, Skerratt LF. 2010. Treatment of chytridiomycosis requires urgent clinical trials. Dis Aquat Organ. 92:165-74. doi: 10.3354/dao02238. Review.

5. CHYTRIDIOMYCOSIS: KEY THREATENING PROCESS AND THREAT ABATEMENT PLAN FOR AUSTRALIA

The Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) provides for the identification and listing of key threatening processes.

A threatening process is defined as a key threatening process if it threatens or may threaten the survival, abundance or evolutionary development of a native species or ecological community. For example, invasive species listed as key threatening processes are predation by the European red fox, feral rabbits or unmanaged goats.

A process can be listed as a key threatening process if it could:

- cause a native species or ecological community to become eligible for inclusion in a threatened list (other than the conservation dependent category); or
- cause an already listed threatened species or threatened ecological community to become more endangered; or
- adversely affect two or more listed threatened species or threatened ecological communities.

The assessment of a threatening process as a key threatening process is the first step to addressing the impact of a particular threat under Commonwealth law.

Extracted from: http://www.environment.gov.au/biodiversity/threatened/ktp.html

On 23 July 2002 the Minister for the Environment and Heritage accepted that infection with the amphibian chytrid resulting in chytridiomycosis was a key threatening process. The conclusions of the advice given to the Minister by the Threatened Species Scientific Committee were that:

> "The threatening process meets s188(4)(a), s188(4)(b) and s188(4)(c) of the EPBC Act. The threatening process could cause native amphibian species to become listed as threatened, could cause listed threatened species to become listed in another category representing a high degree of endangerment, and adversely affects at least 5 listed threatened amphibian species."

"A Threat Abatement Plan is considered to be a feasible, effective and efficient way to abate the process."

The 2006 Chytrid TAP is currently under review but has not yet been replaced. Please see Attachments 4a and 4b for the 2006 Chytrid TAP.

6. OTHER MANAGEMENT PLANS FOR CHYTRIDIOMYCOSIS

Tasmanian Chytrid Management Plan – http://www.dpiw.tas.gov.au/inter.nsf/Attachments/LJEM-8887K5/$FILE/Tasmanian%20Frog%20Management%20Plan.pdf

A Country-level Strategic Plan to Mitigate the Threat of Chytridiomycosis in Panama 2010 – 2015:

https://www.amphibians.org/wp-content/uploads/2013/03/Panama-Amphibian-Rescue-Strategic-Plan.pdf

Chytridiomycosis management plan for the lesser antilles region: minimising the risk of spread, and mitigating the effects, of amphibian chytridiomyosis.

http://www.mountainchicken.org/wp-content/uploads/2010/11/Chytridiomycosis-Management-Plan.pdf

The Disease Strategy Manual for *Batrachochytrium dendrobatidis* (emergency outbreak response plan) 2013 http://www.environment.gov.au/biodiversity/invasive/publications/pubs/chytrid-fungus-manual.pdf

RACE: Risk Assessment of Chytridiomycosis to European Amphibian Biodiversity http://www.bd-maps.eu/

Woodhams DC, Bosch J, Briggs CJ, Cashins S, Davis LR, Lauer A, Muths E, Puschendorf R, Schmidt BR, Sheafor B. 2011. Mitigating amphibian disease: strategies to maintain wild populations and control chytridiomycosis. Frontiers in Zoology 8:1-24. Free online at http://www.frontiersinzoology.com/content/8/1/8

Amphibian Conservation Action Plan (ACAP): www.amphibianark.org/ACAP.pdf

Murray KA., Retallick RWR, Puschendorf R, Skerratt LF, Rosauer D, McCallum H, Berger L, Speare R, and VanDerWal J. 2011. Assessing spatial patterns of disease risk to biodiversity: implications for the management of the amphibian pathogen, *Batrachochytrium dendrobatidis*. Journal of Applied Ecology, 48(1):163-173.

7. CONFERENCES AND NEWS

Please see our events page for a list of upcoming meetings, conferences and events.

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Berger, L., Speare, R., Kent, A. 1999. Diagnosis of chytridiomycosis of amphibians by histologic examination. Zoos' Print Journal 15:184-190.

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Fisher MC, Garner TW, Walker SF. 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annu Rev Microbiol. 63:291-310. doi: 10.1146/annurev.micro.091208.073435.

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Martel A, Van Rooij P, Vercauteren G, Baert K, Van Waeyenberghe L, Debacker P, Garner TWJ, Woeltjes T, Ducatelle R, Haesebrouck F, Pasmans F. 2010. Developing a Safe Antifungal Treatment Protocol to Eliminate *Batrachochytrium dendrobatidis* From Amphibians. Medical Mycology. DOI: 10.3109/13693786.2010.508185

Murray K, Retallick R, McDonald KR, Mendez D, Aplin K, Kirkpatrick P, Berger L, Hunter D, Hines HB, Campbell R, Pauza M, Driessen M, Speare R, Richards SJ, Mahony M, Freeman A, Phillott AD, Hero J-M, Kriger K, Driscoll D, Felton A, Puschendorf R, and Skerratt LF. 2010. The distribution and host range of the pandemic disease chytridiomycosis in Australia, spanning surveys from 1956–2007. Ecology 91:1557.

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Retallick RWR, Miera V. 2007. Strain differences in the amphibian chytrid *Batrachochytrium dendrobatidis* and non-permanent, sub-lethal effects of infection. Dis Aquat Org 75:201–207.

Skerrat LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of Chytridiomycosis Has Caused the Rapid Global Decline and Extinction of Frogs. EcoHealth DOI: 10.1007/s10393-007-0093-5

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Young S, Speare R, Berger L, Skerratt LF. 2012. Chloramphenicol with fluid and electrolyte therapy cures terminally ill green tree frogs (*Litoria caerulea*) with chytridiomycosis. J Zoo Wildl Med 43: 330−337.