

Chytridiomycosis in New Zealand frogs

Worldwide amphibian decline

Populations of many frogs and toads around the world have undergone severe declines or have disappeared entirely since the late 1980s. While habitat destruction or other anthropogenic causes may explain many local cases, patterns are confusing. Amphibians have been declining more dramatically in tropical rainforests, for example, than in industrial regions of North America or Europe. In some areas virtually all frogs have been wiped out; yet elsewhere few if any species appear to have been affected. Even in the midst of mass die-offs, particular species survive unscathed. What most perplexes scientists is that precipitous declines have occurred almost simultaneously in remote pristine localities around the world⁽¹⁾.

Population declines are sometimes associated with identifiable bacterial, fungal, or viral pathogens⁽²⁾. Numerous reports suggest that natural populations of frogs and toads can succumb to common bacteria, such as *Aeromonas hydrophila*, that are normally harmless. Possibly individuals become vulnerable because they suffer immunosuppression induced by sublethal environmental stresses⁽³⁾. Empirical field observations suggest multifactorial causes for declines in amphibian populations⁽¹⁾.

Three years ago, a newly discovered chytrid fungus, *Batrachochytrium dendrobatidis* (phylum Chytridiomycota), was found infecting keratinised tissues of dying wild frogs in Australia and Central America⁽⁴⁾⁽⁵⁾. Most chytrid fungi are saprophytic and none had been known to parasitise vertebrates. Yet experimental infections have demonstrated the lethal pathogenic potential of *B dendrobatidis* to healthy frogs⁽⁶⁾⁽⁷⁾. Wild frogs infected by chytrid fungus have been found in Spain, Uruguay, Ecuador and the western USA just in the last year. Many consider chytridiomycosis a new emerging infectious disease that may be driving global amphibian decline and species extinctions⁽²⁾.

New Zealand frogs

New Zealand's native frogs (genus *Leiopelma*) are of special scientific significance because they closely resemble the earliest frogs that evolved 200 million years ago. Although they previously formed an abundant and widespread fauna, the three species now surviving are restricted to limited, fragmented ranges in the northern half of the North Island and on three predator-free islands in Cook Strait⁽⁸⁾. Their decline has been attributed to predation, competition, and habitat alienation following Polynesian settlement and the introduction of the kiore (*Rattus exulans*)⁽⁹⁾. In recent times, populations have been thought to be stable although apparent declines in the Coromandel Peninsula over the past several years may be associated with unusual weather patterns. Native frogs tend to be cryptic and silent, so censuses can be difficult.

New Zealanders are more likely to be familiar with three species of Australian frogs (genus *Litoria*), which have become widely

Chytridiomycosis was diagnosed in dead and dying southern bell frogs (*Litoria raniformis*) at an ephemeral pond in Christchurch over summer 1999-2000, the first such record in New Zealand. Subsequent fieldwork demonstrated its spread within and possibly beyond Canterbury. The implications of isolation of this emerging pathogen in New Zealand for the conservation management of native frog species may be serious.

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dispersed since they were introduced more than 100 years ago⁽¹⁰⁾. Until recently, populations had been thriving in New Zealand even as two of the species have been undergoing serious declines in their Australian homeland. Indeed *Litoria raniformis*, known commonly as the southern bell frog, has been classified as endangered and faces imminent extinction in its native range. Recent observations in New Zealand suggest that these frogs may soon face a similar fate here.

Epizootic in wild frogs

Disappearances of amphibian populations are becoming ominously common, but rarely have sick or dying frogs been found in the wild. Hence in most cases the causes of decline remain speculative.

In November and December 1999, within a two-week period, University of Canterbury zoologists found 16 sick and dying southern bell frogs in an ephemeral pond at Godley Head near Christchurch⁽¹¹⁾. Sick frogs, although normal on first appearance, showed unusual posture, failed to flee upon approach during daylight hours, and subsequently had difficulty righting themselves. All but one of the sick frogs collected from the pond died within days of being placed into laboratory quarantine. A single individual survived, appeared to recover fully, and lived in our laboratory for more than a year.

Five frogs that died were fixed in 10% formalin or 70% ethanol and a range of tissues including skin were embedded in paraffin, sectioned at 5 microns and stained with haematoxylin and eosin or by the periodic acid Schiff technique or with a silver stain. Selected blocks of skin were deparaffinised, postfixed and re-embedded in epon resin, and sections cut at 1 micron were stained with alcian blue. Further ultrathin sections were stained in osmium and examined with a transmission electron microscope.

The only gross change observed in some frogs was retention and accumulation of skin sloughs. No lesions were observed in sections of heart, lung, kidney, liver, or alimentary tract. Organisms resembling the chytrid fungus *B dendrobatidis* were observed in histological sections of skin from each of five frogs that died (Figure 1). Double-blind analyses of a sixth control frog, collected

from the same pond but showing no symptoms, failed to reveal any evidence of infection by chytrids.

Delayed impact

Notably, despite intensive monitoring of the population until the pond dried in March 2000, no further evidence of morbidity or mortality was found in adult frogs. Although 15 frogs apparently succumbed to infection by chytrid fungus, many hundreds of adult frogs resident in the pond appeared healthy and showed no behavioural abnormalities.

However, as tadpoles in the pond approached metamorphosis they began to develop pathological symptoms including distended bodies, skin ulceration, internal bleeding and various developmental abnormalities. Tadpoles that had been taken home and put into outdoor ponds by members of the public developed the same symptoms immediately preceding metamorphosis. Large numbers of post-metamorphic frogs died, and these were diagnosed with chytridiomycosis by phase-contrast and light microscopy.

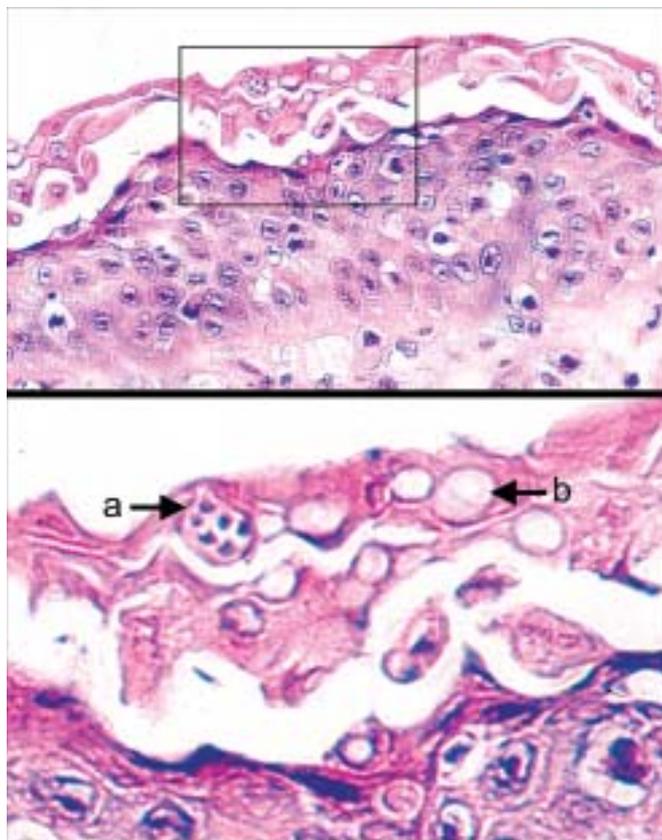


Figure 1. Transverse section of frog skin showing epidermis and loosely adherent stratum corneum layer. Full and empty chytrid zoosporangia are embedded in this layer. Below, enlarged: (a) A full zoosporangium containing zoospores. The zoosporangium has developed a discharge papilla to the outer surface in preparation for release of zoospores. (b) A zoosporangium after release of zoospores.

During summer 2000-2001, only six adult frogs returned to the pond. Even though most adults appeared unaffected during the short epizootic, the ensuing population crash suggests that chytridiomycosis prevented adults from successfully overwintering.



Sign on a pond

Concurrently, tadpoles and adults with behavioural and morphological symptoms indicative of infection were identified in other nearby ponds.

Transmission

Chytrid fungus infects keratinised tissues, but tadpoles lack such tissues except in their mouthparts. Symptoms manifest themselves only as epidermal tissues become keratinised at metamorphosis. Our observations demonstrate that tadpoles can carry and transmit the disease⁽²⁾. Translocation of apparently healthy tadpoles by members of the public and by the pet trade has spread chytridiomycosis to new localities in New Zealand.

Even if such translocations could be prevented, most natural dispersal in frog populations occurs among juveniles, and we found massive mortality among this age class. Healthy 'carrier' adults also might be able to transmit the disease when they disperse. Possibly transmission can occur on boots, clothes, or field gear of human visitors to ponds. Waterfowl also might facilitate the spread of chytrid fungus.

Source of incursion

Global amphibian declines may be due to a newly emerged pathogenic strain of chytrid fungus that has spread rapidly. If this is true, the recency of its origin leads to the prediction that genetic differentiation among strains isolated from infected frogs on different continents should be minimal.

University of Canterbury researchers are comparing sequences of *ssrRNA* of the chytrid strain isolated from infected Christchurch frogs with those of strains that have been cultured from frogs overseas. Preliminary results suggest that the New Zealand strain is almost identical to that initially identified in the USA from captive frogs, but varies by up to 3% from the Australian strain. Although further work is needed, the results suggest that New Zealand chytrids are recent arrivals but perhaps not from Australia.

Some workers believe that patterns of population declines in Australia and Central America resemble 'extinction waves' typical of epizootics involving highly virulent agents that spread by global trade in exotic aquarium fishes⁽¹²⁾. Most stock imported into New Zealand comes via Singapore but it may be sourced around the world. Although some pet shops in New Zealand operate their own on-site licensed quarantine facilities, it is difficult even for veterinary experts to diagnose chytridiomycosis and infected animals are likely to escape detection.

Pet trade

The Godley Head pond serves as a major source of tadpoles and frogs for the pet trade. Chytrid fungus might have been introduced into the pond by commercial collectors who had unknowingly come into contact with it from imported stock. Moreover, tadpoles

and frogs are shipped around the country for commercial sale. They may be carrying chytrid fungus but show no symptoms until after they are liberated by owners who tire of them.

Of special concern is the possibility that chytridiomycosis might be transferred from *Litoria* frogs to our protected native frogs.

Commercial trade in frogs makes this scenario much more likely.

For example, if Canterbury frogs were released in the Waitakeres, our only native stream-living frog, *Leiopelma hochstetteri*, might rapidly fall victim to chytridiomycosis.

At an international level, *Litoria* frogs are regularly exported, without regulation, from New Zealand frog farms to the United States and other countries.

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Dr Lee Berger of the Australian Animal Health Laboratory provided advice, re-examined sections and performed immunoperoxidase staining to confirm the diagnosis. Pam Slack and Graeme Bull prepared sections for light and transmission electron microscopy.

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Merits of a slaughterhouse surveillance programme used in the eradication of Aujeszky's disease

The North Island slaughterhouse survey continued for nearly 11 years and was based on testing 'chopper' pigs (mainly culled breeding pigs and some overweight baconers) slaughtered at several North Island abattoirs. At the abattoirs, blood samples were collected on to filter paper discs as described previously⁽²⁾ and sent to the MAF Animal Health Reference Laboratory at Wallaceville for testing. All samples were identified according to the abattoir at which they were collected and the owner of the piggery.

The development of a database of samples tested has been described previously⁽³⁾. Results of all tests were entered on the database. The name of the pig owner was compared with the names and addresses on the database and if no record existed for a particular herd, then a new entry was made.

The North Island slaughterhouse surveillance programme, sponsored by the New Zealand Pork Industry Board, commenced in 1988 as part of the Aujeszky's disease eradication campaign and was completed in 1999 when eradication from New Zealand was confirmed⁽¹⁾. The programme played a major role in detecting infected herds. Aspects of the slaughterhouse survey are described here.

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Survey design

The locations of the abattoirs that slaughter chopper pigs in the North Island are shown in the figure. At the commencement of the survey, 10 abattoirs submitted samples and later three new premises were involved. During the survey, three premises closed down and two premises ceased slaughtering chopper pigs.