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silent streams

Although we now know why frogs are dying, their future is far from assured. **Judith Maunders** traces international efforts to understand the devastating chytrid fungus.

The common mist frog (*Litoria nannotis*).

Australia's rainforests, riverbanks and creeks are much quieter these days: the frogs that once inhabited them are gone. In the past 30 years, 27 frog species (13%) have become threatened, 14 species have declined, and eight have disappeared.

The cause of this disaster has been something of a mystery. Environmental factors such as chemical pollution, climate change, deforestation and increased UV radiation were thought to be contributing to the decline. But these factors could not account for the loss of whole frog populations in ecologically pristine areas, such as Australia's montane tropical rainforests.

Four years ago, scientists from CSIRO and other national and international research organisations finally identified the culprit: a killer fungus. Scores of frogs worldwide have been infected, and scientists face an uphill battle against a foe about which they know little.

The story begins as far back as the late 1970s, when anecdotal evidence suggested that frog populations had started to decline. It wasn't until 1989 however, that associate professor Rick Speare, a veterinarian and medical doctor from James Cook University, found a possible cause. That year, he isolated a virus from an ornate burrowing frog (*Limnodynastes ornatus*) in northern Australia.

Speare took the virus to Dr Alex Hyatt at CSIRO's Australian Animal Health Laboratory (AAHL) at Geelong in Victoria. Hyatt, an electron microscopist with expertise in identifying new and emerging pathogens, determined it was an iridovirus, a pathogen that can infect amphibians, reptiles and fish. It was the first time such a virus had been seen in an Australian amphibian.

Four years later, Speare joined a Queensland Parks and Wildlife team monitoring frogs at O'Keefe Creek in Big Tableland, north Queensland, where a number of dead or sick sharp-snouted day frogs (*Taudactylus acutirostris*) were found. Some of the apparently healthy frogs were placed in captivity at James Cook University, Melbourne Zoo and Taronga Zoo, but they soon died, despite researchers' efforts.

Three months later, all the sharp-snouted day frogs, waterfall frogs (*Litoria nannotis)* and common mist frogs (*L. rheocola)* were gone from O'Keefe Creek, and the sharp-snouted day frog is now considered extinct.

While the loss of the frogs was devastating, frog bodies were now available for autopsy. A team headed by Speare quickly ruled out bacterial septicemia, but they could not find a definite cause. There were no obvious signs of disease and interestingly, no virus could be isolated.

Then in 1995, a young PhD student named Lee Berger joined the team and was based with Hyatt at AAHL. Berger, a qualified veterinarian with an interest in wildlife diseases, began examining the organs and tissues of the Big Tableland specimens using light and electron microscopy.

Despite many months work, Berger found no viruses. But she did observe a strange organism in the skin of the dead frogs and decided to investigate further.

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taxonomy, and Dr Louise Goggin at the CSIRO Centre for Research into Introduced Marine Pests (CRIMP) in Hobart.

Daszak looked at the organism under the electron microscope and Goggin analysed its genetic sequence. Both suggested it was a type of fungus, a chytrid fungus.

This was not the answer the researchers had expected. Other types of chytrid fungi had been observed on plants and insects, but they had never been found on vertebrates. The chytrid fungus has recently been discovered in other areas of the world, including Central America, Europe and New Zealand.

Today, 46 different species of Australian frogs, including seven threatened species, have been found infected with the fungus. Scientists believe the fungus was a factor in the extinction of six Australian frog species and the extinction of Panama's golden toad (*Bufo periglenes*). Globally, almost 100 species have been found infected with this pathogen. This killer fungus is now known as *Batrachochytrium dendrobatidis*.

Fighting back

Berger says research has shown that the fungus invades the keratin layer of the frog's skin. Tadpoles only have keratin in their mouths, so while they may carry the fungus, they do not succumb to it. But once they metamorphose into a frog, the

Berger took infected frog skin and filtered one batch, to remove any viruses but retain larger pathogens. The filtered skin was then added to the water of a group of frogs. Unfiltered skin was added to a second group, and a third group of untreated frogs acted as a control.

The filtered skin and control frogs remained healthy, while the frogs treated with unfiltered skin all died. This indicated that the skin organism was pathogenic and a likely factor in the amphibian decline, but its identity remained unclear.

Tracking down the killer was frustrating for Berger at first. 'Everyone I consulted had a different opinion about what it was,' she says.

Using her international contacts, Berger finally tracked down Dr Peter Daszak, a UK researcher with expertise in parasite

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A tadpole of the spotted burrowing frog (*Heleiporus albopunctatus*). Tadpoles can carry the chytrid fungus, but do not succumb to it until they metamorphose into frogs.

fungus can take hold and kill the animal. Exactly how it kills frogs is still unknown, but scientists are at least buoyed by the discovery of the fungus.

'Now we know what we are dealing with we can focus the research and attempt to do something about it,' Berger says.

Hyatt agrees. 'We now have the option to manage. If you can manage effectively, you can minimise transmission of the fungus through the environment,' he says.

Researchers across Australia and overseas, are now working to learn more about the fungus. Berger has been testing anti-fungal drugs on cultured fungi in the laboratory. The promising treatments are then tested on fungus-infected tadpoles and frogs at the Amphibian Research Centre in Coburg, Victoria.

Hyatt is developing diagnostic tests to detect the fungus. The aim is to transfer this technology to a range of laboratories, aiding in the management of the fungus in Australia and overseas. Samples are being collected from frogs across Australia to determine the distribution of the fungus. These are tested at James Cook University under Speare's direction.

Worryingly, the fungus continues to cause declines in more and more amphibian populations. The fungus has recently been found in populations of Australia's most endangered frog, the southern corroboree (*Pseudophyryne corroboree*).

This strikingly coloured frog gains it name from the aboriginal word for 'gathering' or 'meeting'. For tens of thousands of years, corroboree frogs have gathered in their hundreds on Mount Kosciusko, in the Snowy Mountains, for their summer breeding season. Now the largest gathering the frogs can muster is just 30 calling males.

Gerry Marantelli's team at the Amphibian Research Centre has reared and released these frogs during the past four years. As part of a new program, the team will soon attempt captive breeding of this species. He believes that without assistance, these frogs will be extinct in a few years.

'We will never be able to save frogs such as the southern corroboree unless we know much more about the fungus and its effects on frogs. Understanding how the fungus threatens is a key step in reversing the trend of frog declines,' he says.

The fungus has decimated many of the world's frog populations. Scientists hope that international collaboration will be able to overcome the disease and provide a lifeline to our frogs. Without their efforts there will be no corroborees atop Mount Kosciusko or anywhere else.

'There is still a lot of work to do to understand the impact of the disease in the wild,' Berger says.'The situation is still dynamic and the final balance between the frog fungus and the various amphibian species cannot be predicted. It is likely frog populations will continue to decline and further species may disappear.'

For further information about the chytrid fungus, try these web addresses: CSIRO Information Sheet 'Researching frog fungus': http://www.csiro.au/frogfungus, Amphibian Diseases Homepage: http://www.jcu.edu.au/school/phtm/PHTM/frogs/ampdis.htm.

Abstract: Globally, about 100 species of frogs have been infected with a killer fungus whose identity, until recently, remained a mystery. More than 30 years of research by CSIRO and other national and international collaborators was finally rewarded however, when the chytrid fungus (*Batrachochytrium dendrobatidis*) was identified. Further research showed that the fungus invades the keratin layer of the frog's skin, but exactly how it kills frogs is unknown. Scientists are now looking at management strategies, trialling anti-fungal drugs and developing a diagnostic kit, in an attempt to halt further declines in frog populations.

Keywords: frogs, species decline, chytrid fungus, fungal diseases, *Batrachochytrium dendrobatidis.*

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The ornate burrowing frog (*Limnodynastes ornatus*).