Main preventative management strategies for the Chytrid fungus *Batrachochytrium dendrobatidis*

Improving Diagnostics and Knowledge of Epidemiology:

Diagnostics is an important part of any disease control management programme. If frogs develop symptoms characteristic of amphibian chytridiomycosis or there is a high mortality rate in two to three week old metamorphs they may be infected with *B. dendrobatidis*. However, diagnostic tests are required to confirm the presence of the fungus, and usually consist of histological examination (using a light microscope) of an unstained piece of skin (taken from fresh, fixed or frozen specimens) (Berger and Speare, 1998). To view a document outlining the histological identification of amphibian chytridiomycosis please see: Berger, Speare and Kent. 1999. Diagnosis of Chytridiomycosis in Amphibians by Histologic Examination.

Polyclonal antibodies were produced for diagnosing chytridiomycosis in amphibians. Antisera were produced that reacted strongly with all forms of *B. dendrobatidis*. Cross-reactivity occurred with (only) some fungi in the Chytridiomycota. It has been suggested that this immunoperoxidase stain is a useful screening method for *B. dendrobatidis* when combined with morphological investigation and symptom identification (Berger *et al.* 2002). The development of diagnostic tests such as enzyme-linked immunosorbent assays or in situ hybridisation are appropriate areas for future research (Daszak *et al.* 1999).

Infection by *B. dendrobatidis* does not always lead to mortality in amphibians. Individuals within a species vary in susceptibility to the infection, and some appear to recover from the infection. Because the susceptibility of different individuals and species varies and the cause of death by *B. dendrobatidis* is still largely unknown, research into the epidemiology of the disease is certain to benefit preventative management strategies. CSIRO is investigating the presence of toxic effects on amphibian's cells and organs infected with *B. dendrobatidis* to determine whether the cause of death is caused by fungal toxins (Davidson *et al*. 2003; Michigan Frog Survey, 2003).

Developing Trade and Quarantine Regulations:

Regulations regarding quarantine, testing, treatment and movement of amphibians need to be introduced on an international scale to prevent the proliferation of *B. dendrobatidis*. In 2001 the World Organisation for Animal Health (also known as *Office Internationale des Epizootes)* placed amphibian chytridiomycosis on the Wildlife Diseases List. This was in recognition of the risks involved in global transportation of amphibians and was the first time an amphibian disease had been listed (Berger *et al.* 1999; Johnson and Speare, 2003). To comply with the intentions of *Office Internationale des Epizootes* listing, amphibians, when moved between countries, should be placed in a different container on arrival; all water, soil, plants, and litter in contact with the amphibian during transport should be adequately disinfected by using techniques capable of killing *B. dendrobatidis*. Water in contact with amphibians should be regarded as contaminated for up to at least 7 weeks after

the last contact with the amphibian. All water, moist soil, and wet fomites (A fomite is defined as an inanimate object that serves to transmit an infectious agent from person to person) in contact with an amphibian imported into a country, or moved between locations within a country (eg: between captive amphibian colonies), should be regarded as infectious. Storage of infected materials for a period of time should not be used as a means of ensuring water is not contagious. All water and wet soil in contact with an amphibian should be disinfected before discharge into the wastewater system or the natural environment. Enclosures previously used by amphibians should be disinfected before introducing new amphibians. Any other wet objects that have been in contact with amphibians should either be disposed of or disinfected (Johnson and Speare, 2003).

If Africa is the source of *B. dendrobatidis* (a hypothesis that has been supported by recent research) identifying trade routes involved in its spread may aid prevention. Prime candidates for the international spread of the disease have been members of the Pipidae family (in particular, *Xenopus laevis* and *Hymenochirus curtipes* see in IUCN Red List of Threatened Species) as they are exported to North America and Europe. They are not killed by the fungus themselves, which makes them ideal natural carriers of the disease (Hey, 1986, in Weldon *et al*. 2002).

It is pertinent to note that the regulation of amphibian trading may not be sufficient in preventing all trade-linked spread of the disease. The movement of infected habitat material (such as water, mud or soil containing the fungus) in non-amphibian trade industries may also spread of the disease.

Raising Awareness:

National and international structures for the rapid dissemination of information between scientists, politicians, and the public may be crucial in combating the threat of globally emergent pathogens. However, large geographic areas (e.g., Africa and much of Asia) have not yet been surveyed for declining amphibian populations or for the occurrence of these pathogens. Raising awareness of this threat should be one of the highest priorities for the immediate future (Daszak *et al.* 1999). Amphibians carrying *B. dendrobatidis* have been detected in the pet trade in Europe, the USA (Johnson and Speare, 2003). Organisations associated with the pet trade and stakeholders should be targeted with the aim of reducing reservoirs of *B. dendrobatidis* (in captive amphibians and stopping release of captive amphibians into the wild. Amphibian owners should be informed about the risks of releasing captive amphibians into the wild. Pet shops should be persuaded to regularly quarantine amphibians and materials in contact with amphibians and observe quarantine procedures before accepting new amphibians.

Chemical:

The most effective products for field use were Path-XTM and the quaternary ammonium compound 128, which can be used at dilutions containing low levels of the active compound didecyl dimethyl ammonium chloride. Bleach, containing the active ingredient sodium hypochlorite, was effective at concentrations of 1% sodium

hypochlorite and above. Didecyl dimethyl ammonium chloride at a concentration greater than 0.0012% for 2 min, or sodium hypochlorite at a concentration greater than 1% for 1 min are effective treatment procedures (Johnson *et al.* 2003; M. L. Johnson *et al.* unpub. Data, in Johnson and Speare, 2003). Antifungals used for amphibians or fish may prove useful in treating *B. dendrobatidis*. Benzalkonium chloride is a disinfectant that has been used at 2 mg/l to successfully treat a similar superficial mycotic dermatitis in amphibians caused by *Basidiobolus ranarum*. The regime used experimentally was 30 minutes of bath treatment, on three alternate days. This was repeated in 8 days (i.e. 6 treatments in total). Oral itraconazole has also been used to treat *Basidiobolus ranarum* infections. Other possible antifungal treatments include oral Ketoconazole (10mg/kg once a day) or copper sulphate baths (500mg/l dip 2 minutes daily to effect) (Berger and Speare, 1998).

Treatment of a number of *Xenopus tropicalis* showing disease symptoms with commercial formalin/malachite green solution (at a dilution of 0.007 ml/L of tank water for 24 h) was curative when carried out once every two days (for four treatments) (Parker *et al.* 2002).

Physical:

One of the most effective strategies of disinfection is thermal sterilisation. *B. dendrobatidis* is sensitive to heat and 100% mortality is achieved by heating to 37°C for 4 hours, heating to 47°C for 30 mins) or heating to 60°C for 5 mins. Housing frogs (*Litoria chloris*) at an environmental temperature of 37°C for less than 16 hours can kill *B. dendrobatidis*. Sterilisation of soil, water or habitat material in contact with amphibians can be disinfected by heating to greater than 47°C for 30 min (Johnson and Speare, 2003; Johnson *et al.* 2003; M. L. Johnson *et al.* unpub. Data, in Johnson and Speare, 2003).

Appropriately applied thermal manipulations of amphibians and their enclosures may prove to be a safe and effective way of eliminating the fungus from captive amphibian populations and preventing spread of the pathogen when animals are translocated or released (Woodhams *et al.* 2003).