A NEW FUNGAL DISEASE ASSOCIATED WITH AMPHIBIAN POPULATION DECLINES: RECENT RESEARCH PUT INTO PERSPECTIVE

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There have been intense scientific, public and political interest in amphibian population declines. Despite some controversy (Pechmann & Wilbur, 1994; Blaustein, 1994), there is solid evidence that recent, marked declines have occurred in Australia (Richards, McDonald & Alford, 1993), North America (Wake, 1991; Drost & Fellers, 1996) and Central America (Pounds & Crump 1994; Lips, 1998). The situation in the UK is less clear – however populations of some species have clearly declined (Beebee *et al.*, 1990; Cunningham, 1993). Whilst some of the global declines have been linked to habitat loss, the causes of many remain a mystery. Hypotheses include adverse weather, increased UV irradiation, introduction of or colonisation by predators, acid rain, pollution and disease; however, many of these remain unproven. Some researchers have proposed a single global cause (see Wake, 1991), but this has not been demonstrated and current scientific opinion is that multiple casual factors are responsible (Halliday, 1998).

Perhaps the most alarming population crashes have occurred in the pristine, tropical rainforests of Australia and Central America. Here, amphibian populations have been falling rapidly (some say "catastrophically"), in remote, protected regions which were thought exempt from human influence (eg. agriculture, deforestation, pollution). These declines have been dramatic, often resulting in complete loss of amphibians from large swathes of habitat (Lips, 1998; Richards *et al.*, 1993; Mahony, 1996). Associated with these declines are the rapid disappearance and presumed extinction (see Bulletin Nos. 44, 46 & 55) of the Golden Toad of Costa Rica (rumours are currently circulating that this species has been rediscovered) and the almost certain extinction of 2 species of Gastric Brooding Frog in Australia. It is thought that as many as seven amphibian species endemic to Eastern Queensland have become extinct during the last decade (Laurance, 1996). Although disease has been suspected as a cause of the Australian die-offs (Laurance, McDonald & Speare, 1996; Laurance, McDonald & Speare, 1997), difficulties in obtaining fresh carcasses from these remote, tropical regions (where bodies are rapidly scavenged or decay) mean that only a few bodies have become available for post mortem examination. Despite these problems, an Australian Government-funded team lead by Drs Rick Speare (James Cook University, Queensland), Lee Berger and Alex Hyatt (CSIRO, Melbourne) collaborating with UK and US scientists, recently reported a major breakthrough in investigating the causes of these declines.

Their research implicates a new fungal disease ("chytridiomycosis") as the cause of death of amphibians from Australia and Costa Rica (Berger *et al.*, 1998). The real significance of this work is that the *same* fungal pathogen appears to have caused mass mortalities on two continents in areas where significant, documented declines have occurred. This "pandemic" has for the first time provided a casual link between two large, widely separated population declines in pristine rain forest habitats (Halliday,

1998; Kaiser, 1998; Daszak & Cunningham, 1998). Importantly, these are areas where UV irradiation has not increased significantly, thus discounting the most widely accepted alternative causes of declines. The causative agent of the disease is a new species and genus of chytrid – a primitive class of fungi which normally inhabit ponds, lakes or moist soil and don't produce the branching hyphae characteristic of higher fungi. Chytrids are small (development is intracellular and "spores" are usually less than 30 microns or about the size of 2-3 red blood cells), ubiquitous and normally live on substrates such as cellulose-based detritus, chitin and keratin, where they degrade these complex molecules. They are known parasites of diatoms, other fungi and insects. However this is the first reported case of a chytrid parasitising a vertebrate.

The chytrid infecting amphibians invades only the superficial keratinised cell layer of the epidermis, where it presumably degrades the keratin in the cell. This layer thickens to four or five times its normal thickness and becomes filled with the developing parasites. It is thought that the cause of death is either directly due to the formation of this thick waterproof barrier blocking supplementary respiration and/or osmoregulation through the skin or may be due to fungal toxins released during infection. An experimental infection of captive-bred adult frogs has demonstrated that the disease is highly pathogenic, with infected animals succumbing after 10-18 days. It appears that tadpoles, which lack keratin in the skin, become infected only in their keratinised mouthparts and don't succumb to the lethal effects of the disease. This probably doesn't significantly lower the death rate in the wild, since tadpoles with infected mouthparts would metamorphose into an environment where the pathogen is already present and able to infect their now-keratinised epidermis, possibly leading to death.

Research has only just begun on this new disease, but using the epidemiological, pathological and experimental evidence available, preliminary answers can be given to some of the most basic questions:

How does the organism pass between individuals and populations?

Chytrid fungi produce motile single-celled stages (zoospores) which swim through the aqueous environment. Zoospores occur in the amphibian chytrid and this organism probably moves between individuals in the water of streams and/or when animals make contact with each other. This might explain why the most severely affected amphibian populations are riparian (river-dwelling) species.

Where did the disease originate?

The epidemic was first noted in Australia in 1993 and found in Costa Rica in 1997. From a study of archival tissues at the National Zoo, Washington, USA (Pessier *et al.*, in press), we know that the disease was present in captive collections from at least 1988. Indeed, it may have been seen by earlier pathologists, but not correctly identified (gross lesions are not always apparent). So far it isn't possible to say for definite whether the disease was always present in the tropical rain forests of Australia and Central America. However, this seems unlikely, since the pattern of the die offs (multiple species affected, high mortality rates, "wave-like" spread etc.) suggest that the disease has recently been introduced to these pristine areas (Berger, Speare & Hyatt, 1998). Work is currently underway to test this hypothesis.

Could this disease be involved in mortality of UK amphibians?

Extensive, recent research on Common Frog (*Rana temporaria*) mortality incidents by Andrew Cunningham at the Institute of Zoology, Regent's Park suggests that chytridiomycosis is not a factor in these deaths. This work has involved investigation of well over 200 sites of frog mortality in the UK, and no evidence of this disease has so far been uncovered. Andrew Cunningham's work has demonstrated two distinct virus-associated disease syndromes associated with cases of mass mortality in Common Frogs (Cunningham *et al.*, 1993; 1996).

Are captive collections of tropical amphibians at risk from this new disease?

The list of wild amphibians found to be infected with chytridiomycosis includes some kept commonly as pets in the UK: the White's Tree Frog, *Litoria caerulea*, The Cane Toad, *Bufo marinus*, and members of the brightly-coloured genus *Atelopus*, the Harlequin Frogs of Central America. In a paper to be published in January 1999, Drs. Allan Pessier and Don Nichols of the National Zoo, Washington, USA report an outbreak of chytridiomycosis in longterm captive Dart Frogs (*Dendrobates* spp), White's Tree Frogs and the Ornate Horned Frog (*Ceratophrys ornata*). It may be that recently imported amphibians could bring the pathogen into collections, but the extent of it's occurrence in captivity (and the wild) are not very well known.

What should be done if dead amphibians are found in the UK?

Incidents of large numbers of wild amphibians found dead in the UK should be reported to the Frog Mortality Project. This group was set up to investigate the occurrence and causes of frog mortality in the UK and can be contacted at: FMP, Triton House, Bramfield, Halesworth, Suffolk IP19 9AE, Tel: 01986-784-518.

Unfortunately, no groups are able to investigate dead or dying captive amphibians and these cases should be taken to an interested vet for pathological examination.

For further information on amphibian population declines and for making donations to assist research investigating their causes, contact the Declining Amphibian Populations Task Force (DAPTF). The DAPTF is a network of experts set up in 1990 by the IUCN to investigate relationships between amphibian declines, chemical contaminants, climate change and disease. The address to contact the taskforce or make donations is: Tim Halliday, DAPTF International Director, Biology Department, The Open University, Walton Hall, Milton Keynes, MK7 6AA, UK.

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