

Chytridiomycosis: Population level consequences.

Current global declines in amphibian population are one of the most pressing environmental problems of the early 21st Century. Chytridiomycosis, the disease caused by a pathogenic fungus *Batrachochytrium dendrobatidis* has been identified as the cause of death of multiple amphibian species worldwide, including in the Americas, Europe and Australasia (Berger et al. 1998, Bradley et al. 2002, Bosch et al. 2001). Chytridiomycosis was first described in 1998 from dead amphibians in Australia and Panama where *B. dendrobatidis* was shown to be the only example of a chytrid parasitizing vertebrates.

According to Speare and Berger (2003) four patterns of response to the appearance of the amphibian chytrid by worldwide amphibian populations have been observed: (1) species extinction; (2) population extinction but species survival; (3) population decline followed by recovery; (4) no effect on the population. According to epidemiological models it is usually particularly rare for a pathogen to cause local population declines resulting in local host extinctions (Daszak et al, 1999). Virulent, microparasites such as *Batrachochytrium* have a short duration of infection and high death rates which usually reduces the host population density below a threshold value which is required to maintain transmission, resulting in pathogen extinction and recovery of the host population (Daszak et al, 1999). It is hypothesised that *B. dendrobatidis* may have developed a method to enable it to persist in reduced amphibian populations. The impact of chytridiomycosis may also be enhanced by the ecological characteristics of certain host species as well as the emergence of potential environmental Cofactors (Daszak et al, 1999). This report seeks to investigate these patterns of response; to try and discover what factors make some amphibian populations more susceptible than others to *Batrachochytrium dendrobatidis* infection.

It may be the case that environmental factors may have predisposed amphibian populations to the virulent pathogen and that Chytridiomycosis is only the proximate cause of the declines. A report by Pounds et al (2006) suggests that widespread amphibian extinctions from epidemic disease are driven by global warming. The paper analyses the timing of *Atelopus* species (Harlequin frog) extinction in the mountains of Costa Rica in relation to changes in sea surface and air

temperatures. 67% of the 110 species of harlequin frog endemic to the region have died out in the past 20 years due to Chytridiomycosis infection and the authors conclude that anthropogenic climate change encourages these outbreaks. There was a significant correlation (>99% confidence and less than one in a thousand chance that the correlation arose by chance) between these extinctions and years of unusually high temperatures. What is particularly enlightening about their study is that as well as offering a theory to explain the widespread losses they also provide a hypothesis to resolve the problematic 'climate-chytrid paradox'. The paradox lies in the climate-linked epidemic hypothesis which states that as temperatures rise pathogenic outbreaks may occur as many diseases are considered to become more lethal. *Batrachochytrium dendrobatidis* however, is a chytrid fungus with aquatic zoospores which becomes more pathogenic at lower temperatures between 17 and 25°C as well as in moist environments. So how can global warming with rising temperatures make *B. dendrobatidis* increasingly virulent? The authors suggest that enhanced cloud cover in the mountainous regions, (formed due to accelerated evaporation) reduces heat loss at night, shifting the temperature above the thermal minimum of *B. dendrobatidis*, whereby previously night temperatures may have been too cold for the chytrid to survive. Increased daytime cloudiness impedes solar radiation and shields the pathogen from excessive warmth and provides moist conditions. So a combination of day time cooling and night time warming may accelerate the disease. Furthermore it was noted that between 1,000 and 2,400 metres Atelopus species show higher rates of extinction than those living in the lowlands or only in the highest elevations. The authors suggest that these extreme sites provide thermal refuges with temperatures either being too high or too low for the chytrid. This is contrary to common belief which suggests that high land species are more prone to extinction as they have a smaller environmental range over which to survive. In this case Pounds et al suggested shifting temperatures were the ultimate trigger for the pathogenic fungus, however in other situations there may be other factors which could trigger an outbreak, including increased UV-B radiation, chemical pollution and stress.

It has been recognised that many declining amphibian species occupy high altitude rainforests with restricted geographic range, have smaller clutch sizes, aquatic larvae and spend a large proportion of time associated with streams (Berger, 1999). These populations are thought to be less able to recover from declines as well as inhabiting cool, moist environments that would support *B. dendrobatidis*. Lips et al (2006) report the rapid appearance of *B. dendrobatidis* in an upland neotropical riparian habitat around El Cope, Panama and the subsequent mass mortality across eight families of amphibian species. Initially Chytridiomycosis was not detected at the

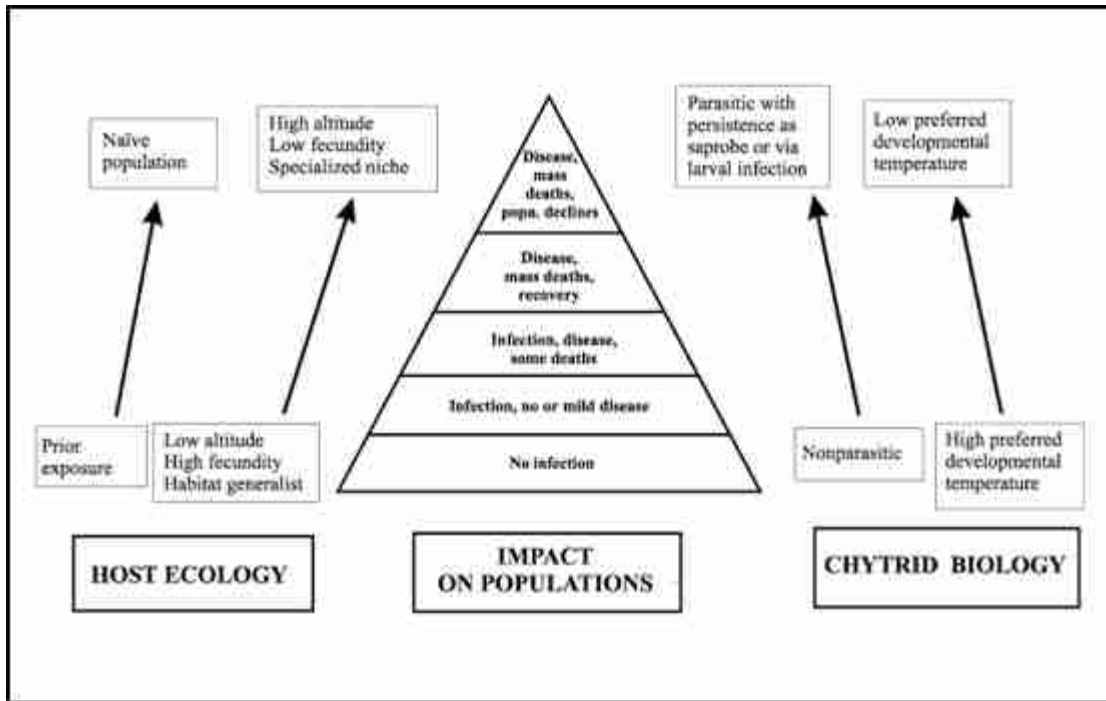
study site, after which 40 species were shown to be infected, demonstrating that the pathogen increased in prevalence between short periods of time. This suggests that *B. dendrobatidis* invaded the study region causing an epizootic. The authors suggest that the high species richness in the area provides a large pool of potential reservoir hosts that could lower the host threshold required for the pathogen to persist and drive rare or less resistant species to extinction. The die-off also occurred during the rainy season's peak, when many montane neotropical frogs gather near water bodies to breed, which facilitates the transmission of aquatic chytrid zoospores.

Lips et al suggested that some amphibian hosts could act as reservoir hosts implying that certain species may have a longer period of infectivity or even immunity to chytridiomycosis.

Hanselmann et al (2004) undertook histological studies of apparently healthy American Bullfrogs (*Rana Catesbeiana*) in the Venezuelan Andes. *B. dendrobatidis* was found to be present in 96% of the individuals examined and no mortality was observed. The authors suggest that bullfrogs are susceptible to infection by the pathogen but resistant to chytridiomycosis, making them reservoir hosts for other more susceptible amphibian species. They also report that chytridiomycosis has a negligible effect on bullfrog populations in Venezuela. The mechanism of immunity is unknown and the components of the immune system that are mobilised against *B. dendrobatidis* have not been determined. Amphibian abundance appears to be growing in certain areas where chytrid infection has become endemic, suggesting that resistance could be evolving. Retallick et al (2004) reported that populations of *T. eungellensis* may have stabilised following the evolution of resistance to this pathogen. The authors analysed toe tips of six species of frog in east-central Queensland, an area which had previously experienced catastrophic declines. The Gastric Brooding frog (*Rheobatrachus vitellinus*) became extinct while the study species the Eungella Torrent Frog (*Taudactylus eungellensis*) also disappeared only to be rediscovered in isolated locations. These remnant populations were found to persist with stable infections of *B. dendrobatidis*. This discovery allows us to discard the theory that *B. dendrobatidis* drove the population below the host threshold level and consequently became extinct itself, as a result the frog populations stabilised in the absence of the pathogen. Conversely, Retallick et al suggests that 'populations recovered or stabilised following evolution of resistance to the pathogen in the frogs or evolution of less-pathogenic strains of the fungus.' Interestingly there were also no seasonal fluctuations in levels of infection suggesting 'that *B. dendrobatidis* has become endemic and relatively stable in prevalence in these populations'.

As has already been mentioned in order for a virulent pathogen to drive a host species to extinction it requires a method of persistence to maintain transmission at low host densities, such as saprophytic ability. *Batrachochytrium dendrobatidis* has been cultured on tryptone agar without the addition of keratin or keratin derivatives (chytrid zoospores grow in the superficial keratinised layers of epithelium when infecting amphibian hosts) (Pessier et al. 1999, Longcore et al. 1999). The development of the fungus does not cease when the host dies, in the laboratory at least one generation of sporangia has been shown to develop on skin removed from dead frogs, and development has also occurred on boiled snakeskin (a source of keratin) (Longcore et al. 1999). It has been postulated that *B. dendrobatidis* might be able to live saprophytically on keratin (e.g. from decaying carcasses, shed skin and other sources) in nature, at least for short periods of time (Longcore et al. 1999, Daszak et al. 1999). This could be expected, as other members of the phylum Chytridiomycota are able to develop and reproduce as saprobes in the environment (Johnson and Speare. 2005, Daszak et al. 1999, Powell. 1993). *Batrachochytrium dendrobatidis* zoospores have been demonstrated to remain viable for 3 weeks in tap and deionised water and for 7 weeks in autoclaved pond water (Johnson and Speare. 2003). The longer survival time in pond water was thought to be due to the higher level of nutrients and nonliving organic substrate. Water or wet formites should, therefore, be regarded as potentially contaminated with *B. dendrobatidis* for at least 7 weeks after last contact with an infected amphibian and should be disinfected before being discharged back into the environment (Johnson and Speare. 2003). Johnson and Speare (2005) have recently shown that *B. dendrobatidis* can survive for up to 12 weeks in sterile, wet creek bed sand; however nothing was detected in damp potting mixture, apparently due to a pH difference. The pathogen survived for longer in sand compared to water, it was hypothesised that this is due to additional nutrients and substrate. It was suggested from these studies that moist soil is a critical factor in survival of the amphibian chytrid, risk however varies with soil type as soil types vary in pH and moisture contents.

In conclusion it is obvious that the range of disease outcomes in populations of amphibians affected by *B. dendrobatidis* is caused by a combination of factors that predispose some populations to declines. The diagram below demonstrates how these factors which can be categorised into either host ecological traits or parasite biology traits can combine to cause declines in a specific group of amphibian species.



Diagrammatic representation of the range of disease outcomes in populations of amphibians affected by a *Batrachochytrium*-like pathogen. (Daszak et al, 1999)

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