ECOLOGY

Global warming and amphibian losses

Arising from: J. A. Pounds *et al.* *Nature* **439,** 161–167 (2006)

Is global warming contributing to amphibian declines and extinctions by promoting outbreaks of the chytrid fungus *Batrachochytrium dendrobatidis*? Analysing patterns from the American tropics, Pounds *et al.*[1] envisage a process in which a single warm year triggers die-offs in a particular area (for instance, 1987 in the case of Monteverde, Costa Rica). However, we show here that populations of two frog species in the Australian tropics experienced increasing developmental instability, which is evidence of stress[2–4], at least two years before they showed chytrid-related declines. Because the working model of Pounds *et al.* is incomplete, their test of the climate-linked epidemic hypothesis could be inconclusive.

Biotic or abiotic agents may cause stress. During amphibian growth and development, disease or physical stressors can increase the asymmetry of limb lengths[3,4]. Pounds *et al.* propose a mechanism for chytrid outbreaks, in which global warming favours the pathogen directly by shifting microscale temperatures towards its optimum. Unusual climatic conditions, however, may also stress amphibians[5], potentially altering development and raising susceptibility to chytridiomycosis, as well as other diseases[6]. During an epidemic in New South Wales, Australia, in 1999, the chytrid was found in only seven of 32 moribund frogs (*Limnodynastes tasmaniensis*)[7].

To investigate stress, we analysed limb asymmetries in populations near Paluma, in northern Queensland, Australia, just before their decline, and in non-declining 'control' populations of the same species. We measured limb elements of 164 adult males, including 112 *Litoria nannotis* (38 'control' and 74 'pre-decline') and 52 *L. genimaculata* (23 'control' and 29 'pre-decline'). The frogs were obtained during 30 samplings at different times (12 'control', Fig. 1a; and 18 'pre-decline', Fig. 1b).

At Paluma between April and September 1991, *L. nannotis* collapsed and disappeared; *L. genimaculata* declined within 3 months afterwards but later recovered[8]. The controls, from 12 tropical wet forest sites, were obtained during nine years that preceded the die-offs at Paluma by an average of 16 years.

Using a blind protocol[9], we measured every frog three times. We then examined asymmetry in relation to body size, and, using analysis of variance, partitioned the variation representing the different kinds of asymmetry and measurement error[2,9]. To compare samples, we examined the total unsigned asymmetry per individual (the difference between the left and right forelimbs, plus the corresponding value for the hindlimbs).

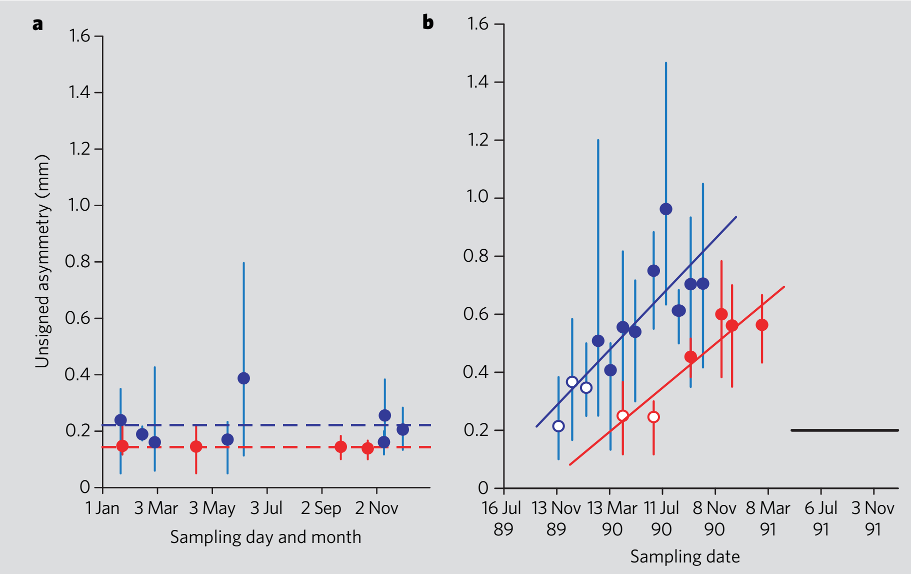

Figure 1 | Limb asymmetry in frog populations that soon declined, compared with non-declining 'control' populations. a, Asymmetry does not vary significantly among control samples of *Litoria nannotis* (blue) or *L. genimaculata* (red) (Kruskal-Wallis tests; $P > 0.165$). Sampling years are pooled. Circles are sample means; error bars are ranges of individual values ($3 \le n \le 9$). Dashed lines are pooled means. **b**, Asymmetry increases over time in pre-decline samples (symbols as in **a**). Filled circles are means that are significantly higher than the corresponding pooled controls (Mann–Whitney U-tests, Bonferroni-corrected for multiple comparisons; $P < 0.05$). Open circles are means that are not significantly higher than controls. Horizontal black bar indicates the time period of the local declines.

We found that asymmetry was greater in the pre-decline samples than in the controls (Fig. 1). It was not correlated with body size, which varied little. We found no directional asymmetry, and measurement error was small relative to unsigned asymmetry. At Paluma, the unsigned asymmetry increased over time, becoming significantly higher relative to the controls at 15–19 months before the declines. The frogs reach adult size in 1–2 years, so stress probably began to increase at least two years before these die-offs.

Irrespective of whether this increasing stress reflects disease or changes in the envi-

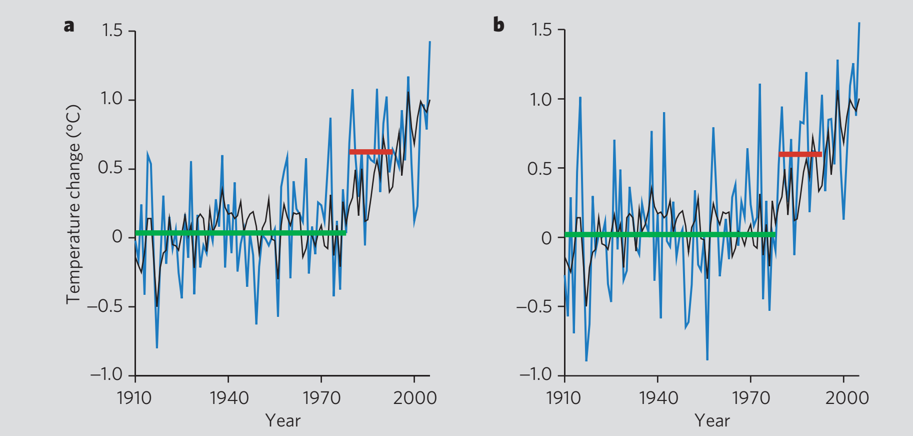

Figure 2 | Temperature changes and the period of major frog die-offs in Queensland, Australia. Annual mean surface temperature for global land areas (black line) is correlated with the corresponding mean (blue line) for **a**, Australia, and **b**, Queensland ($r \ge 0.71$, $P < 0.0001$). Values are departures from a baseline mean for 1910–39. For both regions, the mean for the period of die-offs (red bar) was about 0.6 °C above the baseline, whereas the mean for previous years (green bar) was not significantly increased. During 1988–91, just before the declines at Paluma, the means for these regions were 0.82 °C for Australia and 0.65 °C for Queensland. Data are from the Australian Bureau of Meteorology; details are available from the authors.

ronment, the chytrid was probably not the only agent involved. According to the extinction-wave hypothesis, which has long been controversial[10–12], this pathogen spreads gradually across large regions, causing population crashes within 4–6 months of invading a new site[13,14]. The association of die-offs with previous stress, however, runs counter to this model. It is possible that shifting conditions facilitated the chytrid's invasion, its irruption or its emergence from a saprophytic, quiescent or non-lethal parasitic state.

The data from Queensland cover too few independent die-offs to test for an association with climate change[10], but the period of the major declines[13,15] coincides with dramatic regional warming, which, on diverse spatial scales, mirrors the global trend (Fig. 2). It has been claimed[15] that shifts in temperature or precipitation can be ruled out as factors in these losses, but this claim is undermined by the choice of variables, stations and seasonal timeframes used in that particular study. The patterns from the Australian tropics agree with the climate-linked epidemic hypothesis[1,5,6,11].

Nevertheless, we question the analysis of Pounds *et al.*, which so far provides the only geographically broad test of this idea. Contrary to their working model[1], our Figs 1, 2 indicate that multiyear warm periods may be more important in amphibian declines than single warm years. By focusing on the latter, the authors' test could be inconclusive.

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The proximate cause of frog declines?

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Pounds *et al.*[1] argue that global warming contributes to amphibian declines by encouraging outbreaks of the chytrid fungus *Batrachochytrium dendrobatidis*. Although our findings agree with the climate-linked epidemic hypothesis[1–4], this pathogen is probably not the only proximate factor in such cases: in the Trasimeno Lake area of Umbria in central Italy, for example, the water frog *Rana lessonae* first declined in the late 1990s, yet chytridiomycosis was not observed until 2003 (refs 5, 6). Here we show that the chytrid was common there throughout 1999–2002, in a previously unknown form that did not cause disease. We therefore think that the focus by Pounds *et al.* on a single pathogen is hard to justify because the host–parasite ecology is at present so poorly understood.

Chytridiomycosis is thought to follow a simple progression, in which the zoospores encounter amphibian skin and quickly give rise to sporangia, which produce new zoospores[7]. The disease progresses as these reinfect the host. Neither resting spores nor saprobic forms of the chytrid have been reported, and even comparatively resistant hosts, once infected, are expected to harbour sporangia and zoospores. Evidence that the fungus can persist without these stages would suggest that the life cycle is not fully understood and that certain conditions may trigger a switch in modes.

During the European heatwave of 2003 (ref. 8), the water level in Trasimeno Lake fell by 183 cm and *R. lessonae* again declined, whereas the more stress-resistant *R. esculenta* remained comparatively stable[6]. (The latter is a hybrid of *R. lessonae* and *R. ridibunda* in this hybridogenetic complex[5,6].) Meanwhile, a parasitic mesomycetozoean protist that is prevalent in *R. lessonae* (*Amphibiocystidium ranae*)[5,9] spread over a larger area, and we observed chytridiomycosis for the first time — in 18% of the *R. lessonae* sampled[6].

The chytrid, however, was present long before this. During 1999–2002, we examined 400 water frogs in detail, using stereomicroscopy of epidermal surfaces, followed by light and electron microscopy of skin in histological preparations. We found no signs of chytridiomycosis: no sporangia, zoospores, or clinical symptoms[6]. Every year, though, we observed spherical, unicellular organisms, often with a thick wall, in 20–40% of the frogs sampled (Fig. 1). Usually attached to the skin surface, these microbes were confined to minute patches (80–120 μm across) that were easily missed in routine biopsies but appeared in stereomicroscopy as light-coloured pinpoints. Using the same diagnostic tests as before[6], we identified the organisms as a form of *B. dendrobatidis* (see Methods). Their size and other characteristics are suggestive of encysted zoospores: they may embody a resting spore, a saprobe or a parasitic form that is conditionally non-pathogenic.

These findings have several implications. They support the idea that climate change, perhaps combined with other aspects of environmental deterioration such as chemical pollution[10], contributes to amphibian

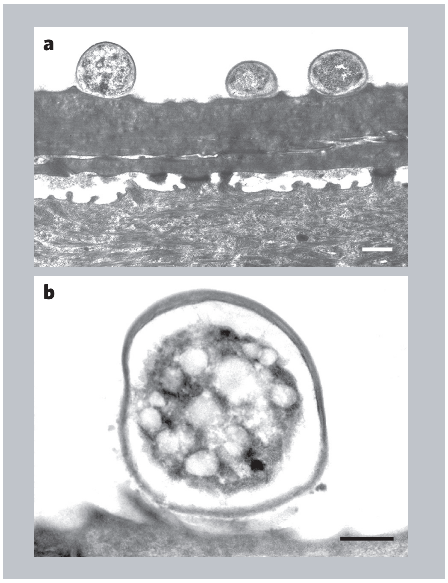

Figure 1 | A newly discovered form of the chytrid fungus *Batrachochytrium dendrobatidis* on the skin of the water frog *Rana lessonae*. a, Transmission electron micrograph of a cross-section of skin showing three of these unicellular organisms adhering to the epidermal surface (scale bar, 1 μm). Although usually attached in this way, they were occasionally intercalated in the superficial epidermis. **b,** Higher-magnification micrograph (scale bar, 0.5 μm) showing a thick cell wall. Sporangia, zoospores and other signs of chytridiomycosis were absent from our samples throughout the period 1999–2002, when the new form was common, but were readily observable in 2003.

declines[1–4,11]. They run counter to the idea[12] that the chytrid simply invades a locality and causes immediate die-offs. Just as this fungus can inhabit areas without causing declines[3,11,13], it may be present without causing disease. Moreover, characteristics of the new form suggest that it could persist in the environment long-term. If so, studying the chytrid largely on amphibian hosts might sketch an inaccurate picture of its spatiotemporal dynamics. This pathogen seems to have emerged recently, but the claim[12] that it is spreading gradually across large geographic regions is arguable[3].

It is also unclear whether climate-related amphibian declines are mediated mainly through outbreaks of the chytrid. This fungus may cause important mortality[12,14] but is not always implicated in such losses[2,11]. Nor is it the only parasite associated with die-offs[2,15]. The white tail tips and other symptoms of tadpoles (*Ptychohyla hypomykter*) that died by the thousands in Guatemala in 2002 (ref. 15) suggest an unidentified pathogen. Although some infections may be secondary, we found that the prevalence of *A. ranae* in 1999–2000, when the chytrid was present but chytridiomycosis was absent, was 49% in the declining *R. lessonae*, compared with 16% in the non-declining *R. esculenta*[5].

By causing stress to the animals, environmental change may raise their susceptibility to various pathogens, many of which are relatively unknown. The exclusive focus by Pounds *et al.* on the chytrid is therefore counterproductive to developing a broader understanding of host–parasite ecology and its role in amphibian losses.

Methods. The molecular analyses and immunohistochemical assays used to identify *B. dendrobatidis* in water frogs have been described[6]. To identify the new form, we examined sections of frog skin taken from the light-coloured pinpoints described in the text. For DNA sequencing, genomic DNA was extracted from these sections and amplified by PCR; for the indirect immunoperoxidase test, we incubated the sections with a polyclonal antibody against *B. dendrobatidis* (provided by A. D. Hyatt) that specifically labelled these unicellular organisms. Both tests demonstrated the presence of the chytrid.

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Pounds *et al.* reply

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Alford *et al.*[1] question the working model underlying our test[2] for a link between global warming and amphibian disappearances, and Di Rosa *et al.*[3] criticize our emphasis on a single proximate agent, the chytrid fungus *Batrachochytrium dendrobatidis*. Both teams report key pieces of the amphibian-decline puzzle and new evidence from different parts of the world that climate change is a factor in these losses. Here we show why our working model was appropriate and highlight the complexity of the imminent threat to species survival that results as global warming conspires with various other agents.

We agree with Alford *et al.* that multiyear warm periods are probably more important biologically than single warm years[1]. In the case of the harlequin frogs, *Atelopus*, this may explain why global warming, but not El Niño alone, loads the dice for extinction[2]. Replotting the data from our original Fig. 3a (ref. 2) shows that the warm year just before a disappearance typically followed a series of warm years (Fig. 1). As in Australia[1], raised temperatures characterize the overall period of declines. We found that about 80% of the die-offs corresponded to the warmer years within this period, indicating that the short-term climatic fluctuations probably acted together with the long-term change.

Other studies implicate extremes of climate and the chytrid fungus in these disappearances[4–6], but we agree that various pathogens could be important in such losses[1,3]. In Ecuador, *Telmatobius* frogs showed a high incidence of diseases other than chytridiomycosis during declines, coinciding with warm, dry years[7]. In Venezuela, *Atelopus* and *Nephelobates* frogs showed similar patterns, involving parasitic nematodes and acanthocephalans (J. Piñero and E.L.M., unpublished results)[6]. The rogues' gallery of amphibian pathogens that benefit from climate change is only just beginning to be assembled[3,6–9], and even the chytrid is poorly known[3]. Accordingly, our test of the climate-linked epidemic hypothesis assumed no particular disease or mechanism of outbreak[2].

The potential mechanism of chytridiomycosis outbreaks that we outlined afterwards[2,10] is one of various pathways of climate effects that need testing. These chains of events are not mutually exclusive, and their relative importance may vary in space and time[9]. Biotic links might range from a shift in microbial species interactions to a disruption of amphibian development[1–15]. Pathogens may not always be involved[12,15], and there may be other victims, such as reptiles[9,11,15]. We agree that shifts in temperature, precipitation, cloud cover, humidity or winds may be stressors that

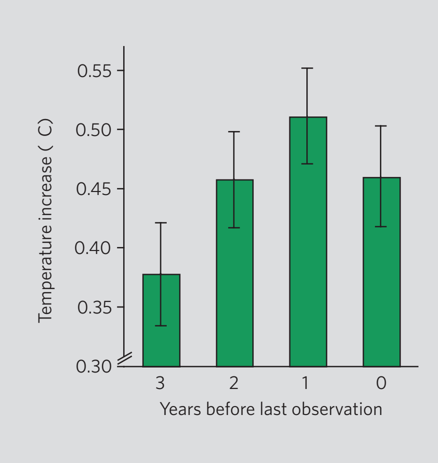

Figure 1 | Punctuated warm periods preceding the disappearances of harlequin frog species (*Atelopus*) in Central and South America. Each bar represents the mean surface temperature for the tropics (departure from a baseline mean for 1856–95) averaged for the set of disappearances ($n = 29$). The bars correspond to the years leading up to disappearances, with the 'zero' bar indicating the last year in which a species was observed. Error bars are 95% confidence intervals. Temperatures typically peaked just before disappearances, but were significantly increased in all of the years represented (see Pounds *et al.*[2] for details).

raise susceptibility to disease[1,3]. New evidence that global warming can degrade toads' body condition and survivorship is compelling in this regard[14]. However, the effects of climate change on host–parasite ecology, and on ecology in general, are manifold.

Global warming also conspires with other forms of environmental deterioration, such as landscape alteration, pollution and species invasions, to dim the prospects for amphibian survival[3,8–10]. For example, humans may have introduced the chytrid into new regions[13]. Because of the presence of exotic species, climate change is an even greater threat. It exerts its influence, not on primeval dioramas, but on present-day ecosystems. It is therefore pointless to debate whether its effects would be noteworthy today if it were acting alone, or whether an agent that it abets would be a problem without it. Likewise, it is fallacious to argue that species that weathered past climate change should survive the present rise in temperatures.

Alford *et al.*[1] and Di Rosa *et al.*[3] add to the mounting evidence that global warming contributes to the present amphibian crisis[1–15], and we reaffirm our test for such a link. The losses portend a planetary-scale mass extinction in the making[10]. Unless humanity takes immediate action to stabilize the climate, while also fighting biodiversity's other threats, a multitude of species is likely to vanish.

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