Frog Body Temperature Influences How the Chytrid Pathogen Grows and Reproduces

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Understanding the impacts of the chytrid fungus (*Batrachochytrium dendrobatidis*) on amphibians is inherently difficult because the life history of the pathogen depends on temperature (*1*). Subtle changes in body temperature that amphibian hosts experience in nature (*e.g.*, over hourly, daily or seasonal timescales) likely influence how the pathogen grows and reproduces (*2*). Unfortunately, we know relatively little about the thermal biology of amphibians, limiting our understanding of how temperature influences disease susceptibility. Recent advances in miniaturized tracking devices are now allowing us to better quantify the temperatures experienced by free-living amphibians in nature (Figure 1; reviewed by *3*). We have used this technology to record, in detail, the temperatures experienced by three sympatric species of rainforest frogs at both low and high elevations (Fig. 1; *2*). We then—for the first time—incubated the chytrid fungus in the laboratory under the mean temperatures experienced by each of these three frog species in the wild (*2*). Our aim was to test whether patterns of decline in nature were related to the relative temperatures experienced by each species, and whether exposure to these temperatures enhances rates of chytrid growth in culture.

Fig. 1: We used miniaturized tracking devices to follow individual rainforest frogs to better quantify their microhabitat use, including patterns of daily body temperature fluctuations at both low and high elevation sites. We captured frogs, fitted them with miniature radiotransmitters (left, top is a male *Litoria serrata* and left, middle is a male *L. nannotis*, both with radiotransmitters affixed around their waists). An automated receiver antenna (right, top) connected to a datalogger (right, bottom) recorded external body temperatures of frogs at 15-minute intervals during the day and night. Frog body temperatures collected using automated radiotelemetry are tightly correlated to the data obtained by placing pairs of physical models in locations used by frogs (left, bottom; fully described and explained in 3). Photographs: Elizabeth Roznik.

We re-created conditions emulating the mean thermal regimes of three rainforest frog species (*Litoria nannotis*, *L. rheocola* and *L. serrata*) from high- and low-elevation populations (*2*). These species are stream-associated rainforest specialists with broadly overlapping distributions in the Wet Tropics region of northern Queensland, Australia (*4*) and differ substantially in their ecology, behavior and microhabitat preferences (Fig. 2). The Waterfall frog *L. nannotis* is stream dwelling; both males and females spend much of the day sheltering behind waterfalls or wedged between rocks in the stream, and do not often venture far from streams (Fig. 2*; 5-7*). The Green-eyed treefrog *L. serrata* reproduces in streams, but can often be found in or on surrounding vegetation (Fig. 2*; 6, 8*). The Common mistfrog *L. rheocola* is intermediate in habitat use between *L. nannotis* and *L. serrata*, remaining near the stream and using both rocks and vegetation (Fig. 2*; 9, 10*).

The rainforest frogs *Litoria nannotis*, *L. rheocola* and *L. serrata* maintained thermal regimes within the optimal range for chytrid growth (15–25 °C) at both low- and high-elevation rainforest sites. The species that has recovered from *Bd*-related declines, *L. serrata*, maintained the warmest and most variable body temperatures that resulted in the slowest pathogen growth. The other two species, which have experienced more long-lasting declines, maintained cooler and less variable thermal regimes, resulting in faster pathogen growth. We also found that the chytrid fungus has a faster life cycle when maintained in the thermal regimes corresponding to high elevations than in those corresponding to low elevations, where frogs maintained warmer body temperatures. Many of these findings match ecological patterns documented in nature (*6-9*), which suggests that interspecific differences in the microhabitat preference of sympatric frogs can influence disease susceptibility (*2*).

The majority of what is known about the life history of the chytrid fungus, and most strategies to predict and mitigate its impacts, are based on the results of constant-temperature experiments (2). In our latest research, all of the thermal regimes we tested—based on real frog body temperatures in the wild—resulted in pathogen growth rates that were equivalent to, or faster than, rates expected from constant-temperature experiments (2). This demonstrates that the mean thermal regimes that frogs maintain, and the associated variation around the mean, can have important effects on the life cycle of the chytrid fungus. Fluctuating temperatures cause the pathogen to grow faster than at constant temperatures because of the asymmetrical nature of how *Bd* responds to temperature. When fluctuations in frog body temperature exceed the overall mean value for a given species, chytrid growth accelerates more rapidly than when temperatures drop below the mean by the same amount. This biological phenomenon results in growth rates in a fluctuating thermal environment that can be higher than expected based on the mean temperature of that environment (*2*, *11*).

The effects of host body temperature on the life history of the chytrid fungus can explain many of the broad ecological patterns of population declines in our focal species, via direct effects on pathogen fitness (*2*). Our study highlights an urgent need to study the microhabitat use and thermal biology of amphibians to better understand how these attributes influence disease susceptibility. Experiments testing the susceptibility of live amphibians to this pathogen will be more realistic when they can incorporate species-specific thermal regimes. This will improve our ability to predict not only the spatial and temporal spread of disease, but spatial and temporal susceptibility of hosts. Understanding the functional responses of the chytrid fungus to the conditions that are experienced by amphibian hosts is crucial for determining the ecological drivers of disease outbreaks and mitigating the impacts of disease.

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Figure 2: We studied three species of rainforest frogs in the Wet Tropics of northeastern Australia: (left, top) *Litoria serrata*, (middle) *L. rheocola* and (bottom) *L. nannotis*. These species span a gradient of habitat use ranging from relatively warm and dry (*Litoria serrata*) to cool and wet cool and wet (*L. nannotis*). The Green-eyed treefrog (*L. serrata)* is often found in or on vegetation surrounding the stream, and thus maintains relatively warm body temperatures. The Common mistfrog (*L. rheocola)* remains near the stream and uses both rocks and vegetation, which results in intermediate body temperatures. The waterfall frog (*L. nannotis)* spends much of the day sheltering behind waterfalls or wedged between rocks in the stream, and do not often venture far from streams at night, resulting in cool body conditions. Typical rainforest stream habitat is shown at right. Photographs: Elizabeth Roznik.

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