Recovering mountain yellow-legged frogs in the presence of a lethal fungal pathogen

Roland A. Knapp

Sierra Nevada Aquatic Research Laboratory, University of California, Mammoth Lakes, CA, 93546 & Earth Research Institute, University of California, Santa Barbara, CA, 93106-3060. roland.knapp@ucsb.edu

The mountain yellow-legged (MYL) frog is composed of the sister species *Rana muscosa* and *Rana sierrae* (Vredenburg et al. 2007, Byrne et al. 2023). These species are endemic to the highest mountain ranges in California (USA), with *R. sierrae* endemic to the northern and central Sierra Nevada and *R. muscosa* endemic to the southern Sierra Nevada and the adjacent Transverse Ranges. Both taxa are highly susceptible to infection by the recently-emerged amphibian chytrid fungus (*Batrachochytrium dendrobatidis*: "Bd"), and are emblematic of the global declines of amphibians caused by this pathogen (Scheele et al. 2019). Once the most common amphibian in many parts of its historical range (e.g., Grinnell and Storer 1924), during the past century this frog has disappeared from more than 90% of its historical range (Vredenburg et al. 2007). Due to the severity of its decline and the increasing probability of extinction, both species are now listed as "endangered" under the U.S. Endangered Species Act. In this box, I focus primarily on Sierra Nevada populations because of the unusually detailed understanding of frog-Bd dynamics in this area. The results I describe below are likely of direct relevance to MYL frog ecology and managwemwent in the Transverse Ranges. In the Sierra Nevada, the decline of MYL frogs was initiated by the introduction of non-native trout into the extensive historically-fishless region (Bradford 1989, Knapp and Matthews 2000) starting in the late 1800s. The arrival of Bd in the mid-1900s and its subsequent spread (Vredenburg et al. 2019) caused additional large-scale population extirpations (Rachowicz et al. 2006, Vredenburg et al. 2010). Given the current ubiquity of Bd in the Sierra Nevada, Bd represents a long-term alteration of invaded ecosystems that MYL frogs, and other amphibians susceptible to Bd infection, will need to overcome to re-occupy habitats following Bd-caused exirpations. However, given that most Bd-naive MYL frog populations are extirpated following Bd arrival, are there any mechanisms by which MYL frogs can reestablish populations in the presence of Bd? If so, can we utilize these mechanisms to facilitate recovery of MYL frogs across their historical range?

For gain insights into these important questions, my colleagues and I turned to MYL frog populations that are recovering naturally in the decades following Bd-caused declines (Knapp et al. 2016). Bd has been spreading across the Sierra Nevada since at least the 1970s, and its arrival in naive MYL frog populations is predicted by the distance between the population of interest and the nearest Bd outbreak documented in previous years (Zhou et al. 2015). As such, Bd spread between watersheds, typically separated by steep divides, often takes years and even decades, but once established in a watershed, Bd spreads quickly to all or nearly all MYL frog populations [estimated rate of spread is approximately 700 m per year, Briggs et al. (2010)]. The vectors responsible for Bd spread remain unknown, but the slow spread between drainages

and low annual probability of Bd arrival in a drainage argues against a vector with high dispersal capabilities (e.g., birds, humans). Instead, it suggests a relatively ineffective vector, as might result from passive dispersal in the air, or on aquatic insects capable of only short distance dispersal. The relatively rapid within-drainage spread is consistent with amphibians, including MYL frogs, serving as vectors. The arrival of Bd in a naive MYL frog population is characterized by either a failed invasion, or establishment and rapid increases in Bd load to very high, and often lethal, levels . Drivers of these divergent outcomes are unrelated to frog density and instead are predicted by environmental conditions, including summer temperature, winter severity, and the presence of pathogen reservoirs [Wilber et al. (2022)]. Although most MYL frog populations are extirpated following Bd epizootics (Vredenburg et al. 2010), a small fraction persist in an enzootic state, often for decades, at greatly reduced population sizes (Briggs et al. 2010). Some of these enzootic populations show sufficiently high adult survival and recruitment to allow slow and often episodic increases in adult abundance, and these recovering populations appear to persist over the long term.

Importantly, enzootic MYL frog populations show reduced susceptibility to Bd infection, as evidenced by low-to-moderate Bd infection intensity ("load"; Briggs et al. (2010); Knapp et al. (2011)) that is strikingly different from the high load characteristic of the epizootic state (Briggs et al. 2010). This reduced susceptibility is evident even in a tightly-controlled laboratory experiment (Knapp et al. 2016) in which frogs from naive and recovering populations were paired in identical tanks and exposed to Bd. The consistently lower loads developed by frogs from recovering populations indicates that host resistance against Bd infection is inherent in individuals, at least in part. This result also rules out the possibility that susceptibility is primarily an effect of factors that are external to individual frogs (e.g., environmental conditions). Resistance could result from several non-mutually exclusive mechanisms, including natural selection for more resistant genotypes (Savage and Zamudio 2016, Grogan et al. 2018a), acquired immunity (Grogan et al. 2018b), and/or inherent between-population differences that pre-date Bd exposure. Although all of these may underlie the observed resistance to some degree, frog evolution may be a particularly important mechanism because it is inherent in frogs and will be readily passed between generations. In contrast, acquired immunity needs to be developed by frogs in each generation and may result in lower survival due to the need for individuals to become infected, potentially at high levels, to develop robust immunity. Available evidence suggests that both frog evolution (Knapp et al. 2023) and acquired immunity (Grogan et al. 2018b) are involved in the Bd resistance of MYL frogs. Evidence of frog evolution in response to Bd comes from a recent study that used exome capture methods to compare the coding regions of frogs from replicate Bd-naive and adjacent recovering populations (Knapp et al. 2023). Frogs in recovering populations showed evidence of parallel evolution of numerous genomic regions, changes that in some cases are associated with genes that confer disease resistance in other animal taxa.

References

Bradford, D. F. 1989. Allotopic distribution of native frogs and introduced fishes in high Sierra Nevada lakes of California: implication of the negative effect of fish introductions. Copeia 1989:775–778.

Briggs, C. J., R. A. Knapp, and V. T. Vredenburg. 2010. Enzootic and epizootic dynamics of the chytrid fungal pathogen of amphibians. Proceedings of the National Academy of Sciences, USA 107:96959700.

Byrne, A. Q., A. P. Rothstein, L. L. Smith, H. Kania, R. A. Knapp, D. M. Boiano, C. J. Briggs, A. R. Backlin, R. N. Fisher, and E. B. Rosenblum. 2023. Revisiting conservation units for the endangered mountain yellow-legged frog species complex (rana muscosa, rana sierrae) using multiple genomic methods. Conservation Genetics.

Grinnell, J., and T. I. Storer. 1924. Animal Life in the Yosemite. University of California Press.

Grogan, L. F., S. D. Cashins, L. F. Skerratt, L. Berger, M. S. McFadden, P. Harlow, D. A. Hunter, B. C. Scheele, and J. Mulvenna. 2018a. Evolution of resistance to chytridiomycosis is associated with a robust early immune response. Molecular Ecology 27:919–934.

Grogan, L. F., J. Robert, L. Berger, L. F. Skerratt, B. C. Scheele, J. G. Castley, D. A. Newell, and H. I. McCallum. 2018b. Review of the amphibian immune response to chytridiomycosis, and future directions. Frontiers in Immunology 9:2536.

Knapp, R. A., C. J. Briggs, T. C. Smith, and J. R. Maurer. 2011. Nowhere to hide: Impact of a temperature-sensitive amphibian pathogen along an elevation gradient in the temperate zone. Ecosphere 2:art93.

Knapp, R. A., G. M. Fellers, P. M. Kleeman, D. A. W. Miller, V. T. Vredenburg, E. B. Rosenblum, and C. J. Briggs. 2016. Large-scale recovery of an endangered amphibian despite ongoing exposure to multiple stressors. Proceedings of the National Academy of Sciences USA 113:1188911894.

Knapp, R. A., and K. R. Matthews. 2000. Non-native fish introductions and the decline of the mountain yellow-legged frog from within protected areas. Conservation Biology 14:428–438.

Knapp, R. A., M. Q. Wilber, A. Q. Byrne, M. B. Joseph, T. C. Smith, A. P. Rothstein, R. L. Grasso, and E. B. Rosenblum. 2023. Reintroduction of resistant frogs facilitates landscape-scale recovery in the presence of a lethal fungal disease. bioRxiv.

Rachowicz, L. J., R. A. Knapp, J. A. Morgan, M. J. Stice, V. T. Vredenburg, J. M. Parker, and C. J. Briggs. 2006. Emerging infectious disease as a proximate cause of amphibian mass mortality. Ecology 87:1671–1683.

Savage, A. E., and K. R. Zamudio. 2016. Adaptive tolerance to a pathogenic fungus drives major histocompatibility complex evolution in natural amphibian populations. Proceedings of the Royal Society B: Biological Sciences 283:20153115.

Scheele, B. C., F. Pasmans, L. F. Skerratt, L. Berger, A. Martel, W. Beukema, A. A. Acevedo, P. A. Burrowes, T. Carvalho, A. Catenazzi, I. De la Riva, M. C. Fisher, S. V. Flechas, C. N. Foster, P. Frías-Álvarez, T. W. J. Garner, B. Gratwicke, J. M. Guayasamin, M. Hirschfeld, J. E. Kolby, T. A. Kosch, E. La Marca, D. B. Lindenmayer, K. R. Lips, A.

V. Longo, R. Maneyro, C. A. McDonald, J. Mendelson, P. Palacios-Rodriguez, G. Parra-Olea, C. L. Richards-Zawacki, M.-O. Rödel, S. M. Rovito, C. Soto-Azat, L. F. Toledo, J. Voyles, C. Weldon, S. M. Whitfield, M. Wilkinson, K. R. Zamudio, and S. Canessa. 2019. Amphibian fungal panzootic causes catastrophic and ongoing loss of biodiversity. Science 363:1459–1463.

Vredenburg, V. T., R. Bingham, R. Knapp, J. A. Morgan, C. Moritz, and D. Wake. 2007. Concordant molecular and phenotypic data delineate new taxonomy and conservation priorities for the endangered mountain yellow-legged frog. Journal of Zoology 271:361–374.

Vredenburg, V. T., R. A. Knapp, T. S. Tunstall, and C. J. Briggs. 2010. Dynamics of an emerging disease drive large-scale amphibian population extinctions. Proceedings of the National Academy of Sciences USA 107:9689–9694.

Vredenburg, V. T., S. V. McNally, H. Sulaeman, H. M. Butler, T. Yap, M. S. Koo, D. S. Schmeller, C. Dodge, T. Cheng, G. Lau, and C. J. Briggs. 2019. Pathogen invasion history elucidates contemporary host pathogen dynamics. PLOS ONE 14:e0219981.

Wilber, M. Q., R. A. Knapp, T. C. Smith, and C. J. Briggs. 2022. Host density has limited effects on pathogen invasion, disease-induced declines and within-host infection dynamics across a landscape of disease. Journal of Animal Ecology 91:2451–2464.

Zhou, H., T. Hanson, and R. Knapp. 2015. Marginal bayesian nonparametric model for time to disease arrival of threatened amphibian populations. Biometrics 71:11011110.