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 and adjacent Nevada (USA), with *R. sierrae* found in the northern and central Sierra Nevada and *R. muscosa* found in 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 known localities (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 on the Sierra Nevada portion of the range because of the unusually detailed understanding of causes of decline and of frog-Bd dynamics that we have developed for populations in this area. In the Sierra Nevada, the decline of MYL frogs was initiated by the introduction of non-native predatory trout into the extensive historically-fishless region starting in the late 1800s (Bradford 1989, Knapp and Matthews 2000). Decades later, the arrival of Bd and its subsequent spread (Vredenburg et al. 2019) caused additional large-scale population extirpations (Rachowicz et al. 2006, Vredenburg et al. 2010).

Bd has been spreading across the Sierra Nevada since at least the 1970s, and its arrival in naive MYL frog populations is best predicted by the distance to the nearest previous Bd outbreak site, resulting in localized wave-like spread (Zhou et al. 2015). However, spread is slower and less predictable between watersheds than within watersheds. Watersheds are typically separated by steep divides, and spread across watershed boundaries often takes years or even decades. In contrast, once established in one MYL frog population in a watershed, Bd spreads quickly to all or nearly all MYL frog populations in that watershed (estimated rate of spread is approximately 700 m per year, Briggs et al. 2010). The arrival of Bd in a naive MYL frog population is characterized by either a failed invasion, or establishment and rapid increase in Bd load to very high, and often lethal, levels (Figure 1 A). 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 despite ongoing Bd infection, 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 (Knapp et al. 2016, Figure 1 B).

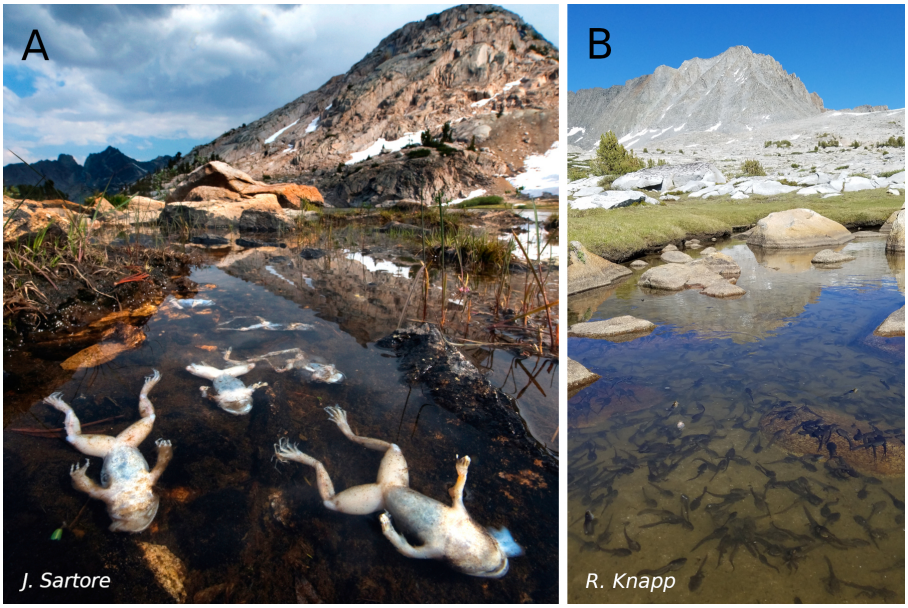

Figure 1: (A) Adult *R. muscosa* killed during a Bd epizootic that extirpated all populations in this watershed. (B) *R. sierrae* tadpoles in a population that recovered several decades following a Bd-caused decline to near-extirpation. Numerous other populations in this watershed have also recovered.

Across the hundreds of amphibian species globally that are impacted by Bd, the natural recovery of MYL frogs following Bd-caused declines is a rare positive outcome, and raises two key questions. First, what mechanisms allow the recovery of MYL frogs despite the ongoing presence of Bd? Second, can we utilize these mechanisms to accelerate recovery of MYL frogs across their historical range? Detailed study of naturally recovering populations has provided several important insights into these questions. Importantly, enzootic MYL frog populations show reduced susceptibility to Bd infection, as evidenced by low-to-moderate Bd infection intensity ("load") on adult frogs that is strikingly different from the high loads characteristic of the epizootic state (Briggs et al. 2010, Knapp et al. 2011). This reduced susceptibility is evident even in a tightly-controlled laboratory experiment in which frogs from naive and recovering populations were paired in identical tanks and exposed to Bd (Knapp et al. 2016). Collectively, these results indicate that reduced susceptibility is primarily the result of disease resistance inherent in individuals and not an effect of external factors (e.g., environmental conditions). Resistance could result from several mechanisms, including natural selection for more

resistant genotypes (Savage and Zamudio 2016, Grogan et al. 2018a) and/or acquired immunity (Grogan et al. 2018b). Although both of these mechanisms need detailed investigation, a recent study suggests that MYL frogs have evolved increased resistance following Bd exposure. Comparison of genomes of frogs from replicate Bd-naive and adjacent recovering populations provided evidence of parallel evolution of numerous genomic regions in recovering populations, including some regions that are associated with genes that confer disease resistance in other animal taxa (Knapp et al. 2023).

The evolution of disease resistance is likely a primary factor allowing MYL frog populations to recover in the presence of Bd. Although this natural recovery is a critically important step toward range-wide reestablishment of MYL frog populations, the spatial scale of this recovery will be greatly limited by anthropogenic habitat fragmentation and isolation. MYL frog habitats in the Sierra Nevada are naturally fragmented due to high topographic relief and the frogs' relatively limited dispersal ability. However, these habitats are now substantially more fragmented and isolated because of the widespread presence of introduced trout in aquatic habitats, including frog dispersal corridors (i.e., streams), and the relatively small number of recovering MYL frog populations across the landscape. As such, natural recovery of MYL frogs across their historical range will likely only occur in fishless aquatic habitats located in the immediate vicinity of naturally-recovering populations. However, the presence of recovering MYL frog populations containing large numbers of Bd resistant individuals suggests the possibility of using reintroduction of frogs from these populations to expand the spatial scale of recovery expected from natural recovery alone.

To test the feasibility of reintroduction of resistant frogs to reestablish MYL frog populations in the presence of Bd, we are conducting a large-scale reintroduction study in Yosemite National Park (Knapp et al. 2023). To date, we have reintroduced 24 cohorts of resistant adult frogs, collected from naturally-recovering donor populations, to 12 sites. Post-reintroduction populations are assessed using capture-mark-recapture and visual encounter surveys, allowing us to describe reproductive output (i.e., number of tadpoles and juveniles), and estimate survival of adult frogs, adult population size, and recruitment of new adults (i.e., progeny of reintroduced frogs). Reintroductions resulted in the establishment of reproducing MYL frog populations at most recipient sites despite the ongoing presence of Bd. Bd loads changed relatively little following frog translocation from donor to recipient sites, consistent with Bd load being influenced primarily by frog resistance and not external factors such as habitat characteristics. Frog survival is highly variable across sites, and is predicted primarily by site elevation (+), winter severity in the year following the reintroduction (-), and donor population. Frog survival is unrelated to Bd load at the time of the reintroduction, consistent with relatively high frog resistance against Bd infection. In addition, results from a stage-structured matrix model that included demographic and environmental stochasticity indicate that at least 50% of the reintroduced populations have a high probability of population growth and long-term viability (Knapp et al. 2023).

The presence of robust donor populations remains an important limitation on the number of translocations we can conduct. To partially overcome this limitation, we are partnering with

the San Francisco and Oakland Zoos to head-start eggs and tadpoles collected from donor populations and rear them to adulthood (reference to Zoo Box). This has greatly increased the number of adult frogs available for reintroduction, allowing us to increase the number of reintroductions and the size of reintroduced cohorts. This partnership has also provided an opportunity to test whether exposing zoo-reared adult frogs to Bd prior to reintroduction ("immune priming") increases survival in the wild.

Recovering MYL frogs across their extensive and remote historical range is a daunting prospect, but the observed natural recovery of some populations and our success in reestablishing populations via reintroduction of resistant individuals are hopeful indications that recovery of MYL frogs is achievable. Although the remarkable ubiquity and abundance that once characterized their populations is likely an unrealistic recovery target, reintroductions of resistant frogs to the many suitable but unoccupied habitats in coming years and decades will at least ensure that MYL frogs are once again a common feature of the Sierra Nevada landscape. Hopefully, the methods we've developed to recover MYL frogs will also prove useful in recovering at least some of the many other Bd-impacted amphibians around that world.

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 107:9695–9700.

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 113:11889–11894.

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 71:1101–1110.