Box 13. 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 commonly develop severe symptoms of chytridiomycosis that often lead to mortality. As such, these taxa are emblematic of the global declines of amphibians caused by this pathogen (Figure 1) (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 US 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 ridgelines, 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* infection intensity (i.e., "load") on individual frogs to very high, and often lethal, levels (Figure 1 A). Drivers of these divergent outcomes are unrelated to MYL frog density

and instead are predicted by environmental conditions, including summer temperature, winter severity, and the presence of MYL tadpoles that serve as pathogen reservoirs (i.e., maintaining infection but not suffering Bd-induced mortality, 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).

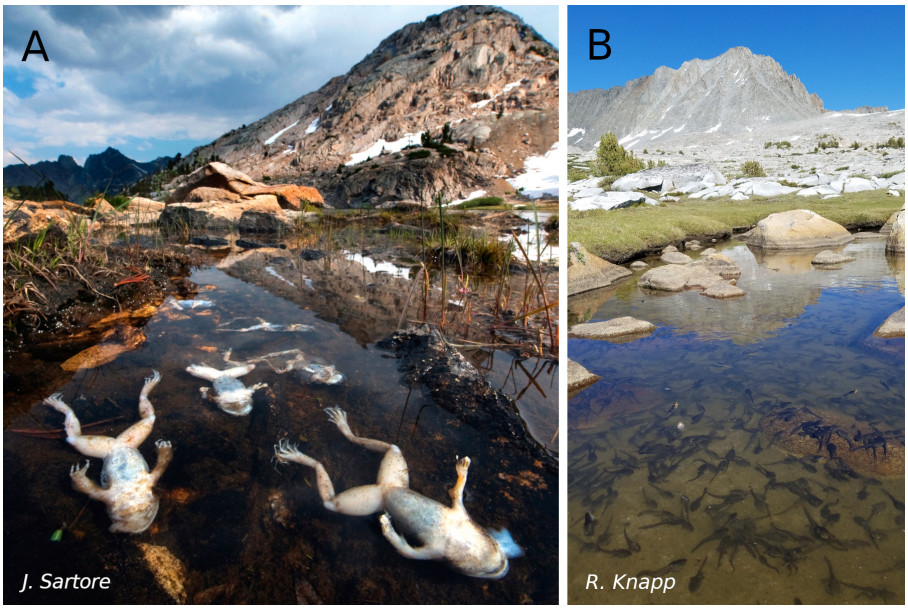

Figure 1: (A) Adult *R. muscosa* killed during a *Bd* epizootic that extirpated all populations in this watershed. (B) *R. sierrae* tadpoles in a population that recovered several decades following a *Bd*-caused decline to near-extirpation. Numerous other populations in this watershed have also recovered.

Across the hundreds of amphibian species globally that are impacted by *Bd* (Olson et al. 2021), 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* 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 was 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 translocation or reintroduction of frogs from these populations to expand the spatial scale of recovery expected from natural recovery alone.

To test the feasibility of reestablishing extirpated MYL frog populations despite the ongoing presence of *Bd*, we are conducting a large-scale translocation study in Yosemite National Park (Knapp et al. 2023). To date, we have translocated 24 cohorts of Bd-resistant adult frogs, collected from naturally recovering donor populations, to 12 sites. After translocation, 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 translocated frogs). Translocations 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 was highly variable across sites and was predicted primarily by site elevation (+), winter severity in the year following the tranlocation (-), and donor population. Frog survival was unrelated to *Bd* load at the time of the translocation, consistent with relatively high Bd resistance of frogs in naturally recovering populations. In addition, results from a stage-structured matrix model that included demographic and environmental stochasticity indicated that at least 50% of the translocated 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 headstart eggs and tadpoles collected from donor populations and rear them to adulthood (Box 19) in preparation for reintroduction. This has greatly increased the number of adult frogs available for reintroduction/translocation, allowing us to increase the number of frog reestablishment efforts and the size of frog cohorts used in these efforts. Also, this partnership has provided an opportunity to test whether exposing zoo-reared adult frogs to *Bd* prior to reintroduction to the wild ("immune priming") increases post-release survival (Figure B19-1).

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 translocation 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, translocations and 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.

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