

**Results of Amphibian Resurveys in
Sequoia-Kings Canyon National Park**

2004-2005

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Background

The decline of amphibians worldwide has received greatly increased attention during the past decade (Stuart et al. 2004). Research conducted in California's Sierra Nevada indicates that at least two amphibian species (mountain yellow-legged frog: *Rana muscosa*; Yosemite toad: *Bufo canorus*) have suffered serious declines during the past century, with the decline of *R. muscosa* being particularly precipitous (Bradford et al. 1994, Drost and Fellers 1996, Jennings 1996). Because of the severity of these population declines, listing of *R. muscosa* under the U.S. Endangered Species Act was recently found to be "warranted" (Federal Register 2003). Numerous separate studies indicate the importance of introduced fish, the fungal disease *Batrachochytrium dendrobatidis* (Bd), and airborne pesticides in causing these declines (Bradford 1989, Knapp and Matthews 2000, Davidson 2004, Vredenburg 2004, Rachowicz et al. 2006).

In an effort to provide the first site-specific description of the distribution of *R. muscosa* throughout Sequoia-Kings Canyon National Park (SEKI), during the summers of 1997, 2000, 2001, and 2002, my field crews and I conducted amphibian, fish, and habitat surveys of all lentic sites in this park ($n = 3639$). To determine the trajectories of these populations following initial surveys, in 2004 and 2005 we resurveyed nearly all sites that contained *R. muscosa* at the time of the initial survey. During resurvey visits, we conducted visual encounter surveys for amphibians, reptiles, and fish. If *R. muscosa* was present, we conducted inspections of tadpole mouthparts (mouthpart depigmentation is an accurate indicator of chytridiomycosis; Knapp and Morgan 2006). In early 2005, we finalized the protocol for a molecular assay to detect the presence/absence of Bd from skin swabs. Therefore, in Summer 2005, we also collected skin swabs from 846 *R. muscosa* post-metamorphs and tadpoles from a total of 77 sites.

Results and Discussion

During the summers of 2004 and 2005, we surveyed 547 and 463 sites, respectively. These resurveyed sites all contained *R. muscosa* during initial surveys. The number of sites surveyed in 2005 is lower than in 2004 because of weather constraints we experienced in 2005. The very large snow pack of the 2004-2005 winter kept SEKI survey sites frozen and therefore unsurveyable until early August and sites began refreezing again in mid-September after an unseasonably cold period. Of the sites that were surveyed in 2004 but not in 2005, the majority were in the Monarch Divide area.

Extinction rate of R. muscosa populations

In 2004, of the 547 sites surveyed, 343 still contained *R. muscosa* (63%). Similarly, of the 463 sites surveyed in 2005, 299 still contained *R. muscosa* (64%). Of the 447 sites that were surveyed in both years, we failed to detect *R. muscosa* in either year at 187. Therefore, at least 42% of sites occupied by *R. muscosa* during the initial survey have apparently gone extinct only 3-8 years later. One explanation for this high extinction rate is that *R. muscosa* populations exist in an extinction-recolonization balance, where the number of sites going extinct is approximately balanced by the number of sites being recolonized by *R. muscosa*. This does not appear to be the case, however, as the recolonization rate of sites in SEKI by *R. muscosa* appears to be less than 2% per year (Knapp, unpublished data). In addition, recolonization events rarely result in the long-term establishment of new *R. muscosa* populations. Instead, these populations have a high likelihood of going extinct soon after recolonization.

Numerous previous studies have provided evidence that nonnative trout are an important cause of the decline of *R. muscosa* (Bradford 1989, Bradford et al. 1998, Knapp and Matthews 2000, Knapp et al. 2003, Vredenburg 2004, Knapp 2005). However, the direct effects of introduced trout are likely to have occurred within 1-2 decades of trout stocking, and since the initial introduction of trout into the study lakes generally occurred more than 50 years ago, trout likely play a relatively minor role in the extinctions observed during our study. Instead, Bd appears to be a more important proximate cause of the declines we observed in SEKI during the last several years.

B. dendrobatidis as a proximate cause of R. muscosa extinctions

Of the 306 sites where we were able to capture and inspect tadpole mouthparts on at least one occasion, 132 (43%) showed patterns of mouthpart depigmentation suggestive of chytridiomycosis (linear length of upper jaw sheath <100% pigmented; Knapp and Morgan 2006). In addition, the distribution of Bd in SEKI is closely correlated with the distribution of *R. muscosa* die-off and extinction sites. Since 2002, we have documented this association between Bd presence and *R. muscosa* decline in numerous basins, including Hitchcock, Laurel, Coyote, Upper Kern, Milestone, Pinchot, Sixty Lake, Amphitheatre, Observation, and Barrett Lakes. In some cases, Bd outbreaks resulted in the extinction of all *R. muscosa* populations (Hitchcock, Laurel, Pinchot; Rachowicz et al. 2006), while in other cases the number of *R. muscosa* populations and population sizes were greatly reduced but complete extinction has not yet occurred (Monarch Divide, Amphitheatre, Observation). In fact, in at least two basins recent surveys suggest that *R. muscosa* populations may be slowly increasing again following Bd-related population crashes (Figure 1). These apparent population increases are occurring despite very high prevalence of Bd in *R. muscosa* at these sites. These characteristics are very similar to those at sites elsewhere in the Sierra Nevada where *R. muscosa* populations have recovered following Bd-related population crashes. Additional resurveys of these sites in Sequoia-Kings Canyon National Park in future years will be necessary to determine whether these populations are persisting with Bd or eventually go extinct.

Based on PCR assays conducted on skin swabs collected in 2005, *B. dendrobatidis* was detected at 42 *R. muscosa* populations (55%) and not detected at 35 *R. muscosa* populations (45%; Figure 2). Data from the Sixty Lake Basin, a basin currently experiencing a major Bd-related die-off of *R. muscosa*, are not included in these results because they were collected by Vance Vredenburg as part of a separate project. Data presented in Figure 2 largely confirms results on *B. dendrobatidis* distribution based on inspections of tadpole mouthparts. Most notably, *B. dendrobatidis* is widespread in *R. muscosa* populations across SEKI. The only *R. muscosa*-containing basins in SEKI where we have not yet detected the presence of Bd are Rambaud, Dusy, Glacier Creek, Center, Forester). In addition, PCR assays indicated that the two major *R. muscosa* die-offs detected during the 2005 surveys (Barrett Lakes, Coyote) were both associated with *B. dendrobatidis*.

Two interesting results arose from the PCR assays that tadpole mouthpart surveys had failed to detect. First, we detected Bd in samples in the Upper LeConte Basin, the site of current fish eradication efforts. The Bd status of this site is somewhat questionable, because PCR assays of swabs collected by the Knapp field crews at this site indicated very low Bd values (suggestive of light Bd infections on sampled frogs). Assays were rerun on all samples, and all come up Bd-negative. However, a recent assays run on swabs collected by the Boiano field crews also come up Bd-positive. Determining the Bd status of this basin will require additional sampling in 2006,

but currently-available data suggests that the *R. muscosa* in this basin may be showing the early stages of Bd infection. The second interesting result from the PCR assays were several Bd-positive samples from Wanda Lake in Evolution Valley. This site is adjacent to a basin that experienced a Bd outbreak in 1997, but we have never detected Bd at Wanda Lake until 2005. Bd values from samples collected in 2005 were low, but suggestive of the early stages of a Bd outbreak.

Conclusions

The emergence of Bd as a major factor in the decline of *R. muscosa* drastically increases the difficulty of halting the decline of this increasingly imperiled species. Fish removal efforts in SEKI have resulted in huge increases in *R. muscosa* population densities (Vredenburg 2004, Boiano, unpublished data), but the emergence of Bd suggests that recovery by *R. muscosa* at these sites may be temporary, with recovering populations eventually succumbing to Bd. Although the intensive research currently being conducted on Bd in the Sierra Nevada and throughout the world will likely provide important new insights into the dynamics of this emerging amphibian disease in coming years, at the present time it seems prudent to continue using fish removals to restore as many *R. muscosa* populations as possible. Larger *R. muscosa* populations may have a higher likelihood of surviving a Bd outbreak than small populations. In addition, fish removal efforts benefit a wide diversity of native taxa, including zooplankton, benthic macroinvertebrates, and birds (Knapp et al. 2001, Knapp et al. 2005).

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Figures

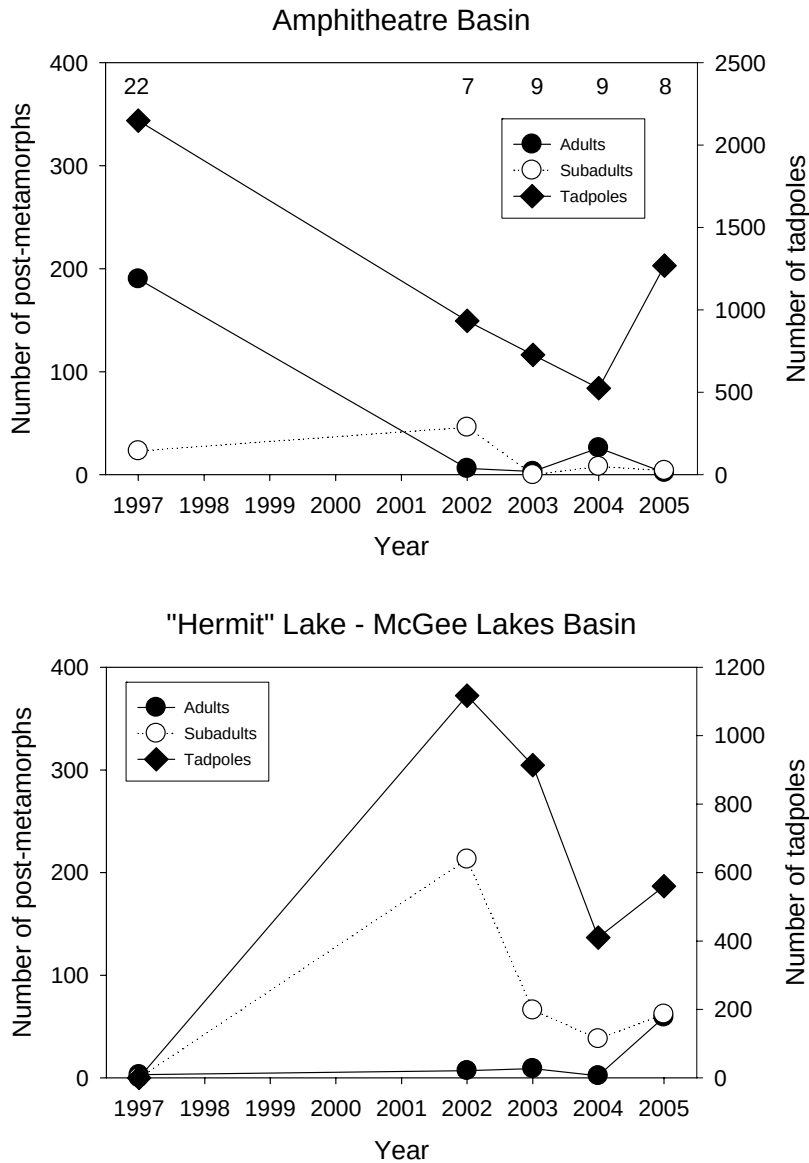


Figure 1. Numbers of *R. muscosa* tadpoles in two basins in Sequoia-Kings Canyon National Park following population crashes associated with outbreaks of *B. dendrobatidis*. In Amphitheatre Basin (upper graph), the *B. dendrobatidis* outbreak occurred sometime between 1997 and 2001. Numbers across the top of this graph indicate the number of sites in the basin harboring *R. muscosa*. In the McGee Lakes Basin (lower graph), *R. muscosa* only persists at a single site ("Hermit" Lake). This basin experienced a *B. dendrobatidis* outbreak sometime prior to 1997. *R. muscosa* in both basins show what may be the early signs of population recovery.

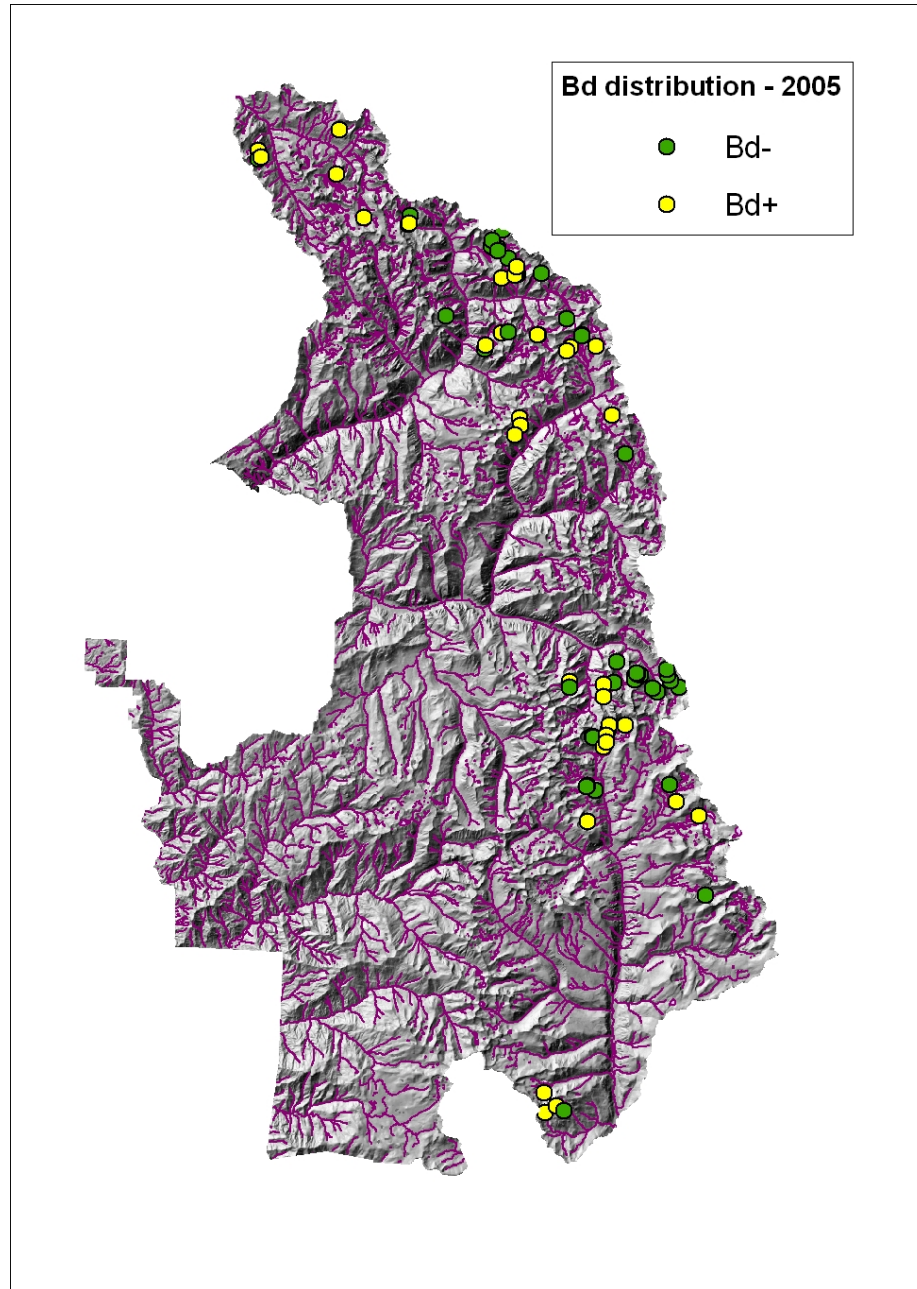


Figure 2. Distribution of *B. dendrobatidis* across 77 *R. muscosa* populations sampled in 2005. *B. dendrobatidis* presence/absence is based on PCR assay of skin swabs (1-51 per *R. muscosa* population).