**Boulder County Parks and Open Space Small Grants Program Final Report**

# Distribution of an invasive species and an amphibian pathogen in the Front Range of Colorado

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## Project Summary:

The overarching goal of this study was to **understand the differing roles that Colorado amphibians may play in influencing dynamics of the amphibian pathogen *Batrachochytrium dendrobatidis*** (hereafter referred to as *Bd*) **in Front Range wetlands**.

I focused a large portion of my sampling on collecting *Bd* swabs from North American bullfrogs (*Lithobates catesbeianus*), in order to determine if this species is acting as a reservoir for the pathogen *Bd* in the Colorado Front Range. Bullfrogs often do not suffer from pathology when infected with *Bd* (Daszak et al. 2004) and have been shown to have a high prevalence of infection in some systems (Garner et al. 2006, Schloegel et al. 2010), suggesting this species may be a reservoir for this pathogen. However, these studies have examined pathogen dynamics at bullfrog farms and in recently introduced populations, where population densities may be artificially higher than they would be in more natural settings. Therefore, in this study I sought to clarify patterns of *Bd* prevalence in bullfrog populations that have been established for decades, as is the case in the Colorado Front Range.

Additionally, defining and identifying reservoirs of infection can be a challenge, as the literature lacks a clear consensus about what constitutes a disease reservoir. Ashford (1997) describes a reservoir of infection as: "any system in which a parasite survives indefinitely," and more specifically states that animal disease reservoirs often meet the following characteristics: 1) have a high prevalence of infection with the pathogen of interest, 2) are capable of transmitting infection to other species, and 3) have an increased duration of infection (Ashford 1997). In the field portion of this project I sought to more accurately determine the role of bullfrogs as a reservoir by clarifying the first two of these criteria. At all wetlands where bullfrogs were present, I collected swab samples to determine prevalence of the pathogen in bullfrog populations. Additionally, I collected samples from amphibians at wetlands with co-occurring populations of bullfrog and native amphibians as well as at wetlands where only native amphibians were present.

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The purpose was to determine if *Bd* prevalence is higher in native amphibian populations that co-occur with bullfrogs in comparison to those populations that are not found co-occurring with bullfrogs.

Additionally, previous research in Colorado has suggested that the Western Chorus frog (*Pseudacris triseriata*) may also act as a reservoir or transport vector for *Bd* (McKenzie, unpublished data). In systems in California, members of the genus *Pseduacris* as well as salamanders in the genus *Ambystoma* are both hypothesized to be potential reservoirs for *Bd* due to their high prevalence of infection (Padgett-Flohr & Hopkins 2009). In my study I collected samples from *Pseudacris triseriata* and *Ambystoma tigrinum*, in order to help clarify prevalence of this pathogen in these other potential reservoir species.

Finally, Northern Leopard frogs (*Lithobates pipiens*) are known to be in decline across much of their range, including the Colorado Front Range (Johnson et al. 2011). Thus, I also sought to determine the presence of this species at all wetlands visited.

### Accomplishments to date:

Over the course of the summer of 2011 I, along with two undergraduate assistants, visited 37 wetlands located on Boulder County Open Space property (see attached spreadsheet). Wetlands were sampled using a standardized technique. Upon arriving to each wetland, we conducted a visual encounter survey (VES), in which we walked the perimeter of each wetland and noted the presence of any amphibians and other vertebrate seen. We also kept a tally of all adult/metamorphic amphibians observed during the VES. Following the VES we conducted standardized dip-net sweeps. We conducted sweeps by pulling the net rapidly through the water in a 1.5 m line perpendicular to the shore. A total of 10 sweeps were conducted around the perimeter of each wetland. Finally, we conducted 3 seine net sweeps 3-10 m in length (depending on wetland), to further verify the presence of larval amphibians. We identified all amphibians captured in the seine net and dip net to species, and then immediately released them back into the water. Metamorphic or adult amphibians were detained, swabbed with a sterile cotton tipped-swab, and then immediately released. At wetlands with large populations of metamorphic amphibians, targeted searching was conducted for 30 min - 1 hour to obtain additional individuals for swabbing. We attempted to obtain swab samples from at least 25 individuals of each species in order to obtain prevalence information. However, at some wetlands this was not possible, and thus we swabbed as many individuals as we were able to catch in the targeted 1-hour search time.

Eight of the 37 wetlands that we visited were not present at the time of sampling (i.e. were dry- in grey on the attached maps). Of the remaining 29 wetlands, 24 had the presence of amphibians. I found Northern leopard frogs (*Lithobates pipiens*) at 3 of these wetlands (Middle Mayhoffer, Hodgson Harris Reservoir, Unknown Wetland 2 North), but breeding Northern leopard frog populations at only one wetland (Middle Mayhoffer). Woodhouse's toads (*Bufo woodhousii*) were found at 6 wetlands, Western Chorus frogs (*Pseudacris triseriata*) were found at 10 wetlands, Tiger salamanders (*Ambystoma tigrinum*) were found at 6 wetlands, and North American bullfrogs (*Lithobates*

*catesbeianus*) were found at 11 wetlands. I found co-occurring populations of bullfrogs and native amphibians at 4 BCPOS wetlands. See the attached table and maps for a more detailed summary of localities of all wetlands visited, species found, and swab samples collected from amphibians on BCPOS properties.

I collected *Bd* swab samples from individuals from 14 of the wetlands visited. These samples are currently stored in the freezer in the McKenzie lab at the University of Colorado, Boulder. I will extract DNA from these samples using Qiagen DNeasy DNA extraction kits, and then run these samples with quantitative real-time PCR (qPCR) according to the protocol outlined in Boyle et al. 2004 on equipment available in the Ecology and Evolution Department at the University of Colorado. By utilizing qPCR I will be able to determine not only the presence or absence of *Bd* on the collected swab samples, but also determine the relative amount of *Bd c*ollected on positive swabs. This will provide an estimate of infection load of the different amphibians sampled. DNA extraction of a small subset of these samples has already begun, and I am currently working on optimizing the qPCR protocol. As soon as this protocol is optimized I will begin qPCR on all samples. Due to the high cost and labor of DNA extraction and qPCR analysis, I will focus on running only swab samples from wetlands where I was able to collect samples from a large number of individuals (i.e. >20 individuals). Previous work in Colorado suggests that at least 20 individuals need to be sampled in order to consistently detect *Bd* in a population and provide prevalence information (McKenzie, pers. comm.).

**Future Directions**

Once I have extracted and run qPCR on my samples, I can utilize this information to identify wetlands that have high prevalence of *Bd* infection, and can determine the spatial distribution of these wetlands across the landscape. Furthermore, this information will help to determine the prevalence of *Bd* in differing populations of amphibians, and identify potential reservoir species for this pathogen in Colorado Front Range wetlands. The DNA extraction and qPCR analysis is labor intensive, and will hopefully be completed by February or early March of 2012. As soon as these analyses are completed, I will provide BCPOS a document outlining *Bd* prevalence rates in the different amphibian species found at all wetlands sampled in this study.

The information provided by this study will help to identify particular wetlands or wetland systems with high instances of *Bd* infection, which may help to direct management, especially when considering species of concern (i.e. Northern Leopard frogs). Additionally, this work will determine if native amphibians that co-occur with bullfrogs have a higher prevalence of *Bd* infection. If this is the case, it may highlight the importance of reducing contact between invasive bullfrogs and susceptible native amphibians. Additionally, the results of this study may suggest that bullfrogs do not influence disease dynamics in native amphibians, which would suggest other areas (i.e. habitat restoration) that may be more important for amphibian conservation on BCPOS properties.

Finally, I anticipate that 1-2 publications will result from this work conducted on BCPOS property. Boulder County Open Space will be acknowledged in any of these

publications, as well as any conference presentations and posters that arise from this research.

## Literature Cited

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