

Prevalence of *Batrachochytrium dendrobatidis* in *Hyla arenicolor* in Washington County, Utah

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Introduction

Chytridiomycosis is a leading cause of worldwide amphibian decline. This often-lethal infection is caused by the fungus *Batrachochytrium dendrobatidis*, which grows within the skin of adult amphibians and the mouthparts of tadpoles. Some species have shown resistance to this disease, including the canyon tree frog (*Hyla arenicolor*). These frogs are found in the deserts of the southwestern United States and are frequently observed basking in the sun during the summer.

This unique behavior may be a protective factor against chytridiomycosis as *B. dendrobatidis* does not grow at temperatures above 30°C. The temperature extremes found in desert climates mean that these frogs must survive winter at temperatures that are conducive to chytrid growth. However, no mass die offs have been reported in this species, and *H. arenicolor* may resist infection through a combination of behavioral and immunological adaptations that respectively reduce their fungal load during the summer and prevent *B. dendrobatidis* from accumulating to fatal levels during the cooler months.

Methods

Adult *H. arenicolor* specimens were swabbed in various canyons on public land in Washington County Utah. Tadpoles were euthanized at these same locations and their mouths removed for analysis. Twelve adults were captured and cared for in the laboratory under conditions that were optimal for *B. dendrobatidis* growth. The laboratory frogs were swabbed two days in a row after six months. Protein was extracted from all swab and mouthpart samples and from a stock culture of *B. dendrobatidis*.

The extracted protein was transferred to an SDS-PAGE gel and analyzed using western blotting. Skin secretions were collected from the captive frogs using 0.01% epinephrine. Ampicillin was added to the secretion samples and to the epinephrine dilution following collection. *B. dendrobatidis* was streaked on two halves of 1% tryptone plates. A diffusion disc saturated with *H. arenicolor* skin secretion was placed on one half of each plate and a diffusion disc saturated in the epinephrine solution was placed on the other half.

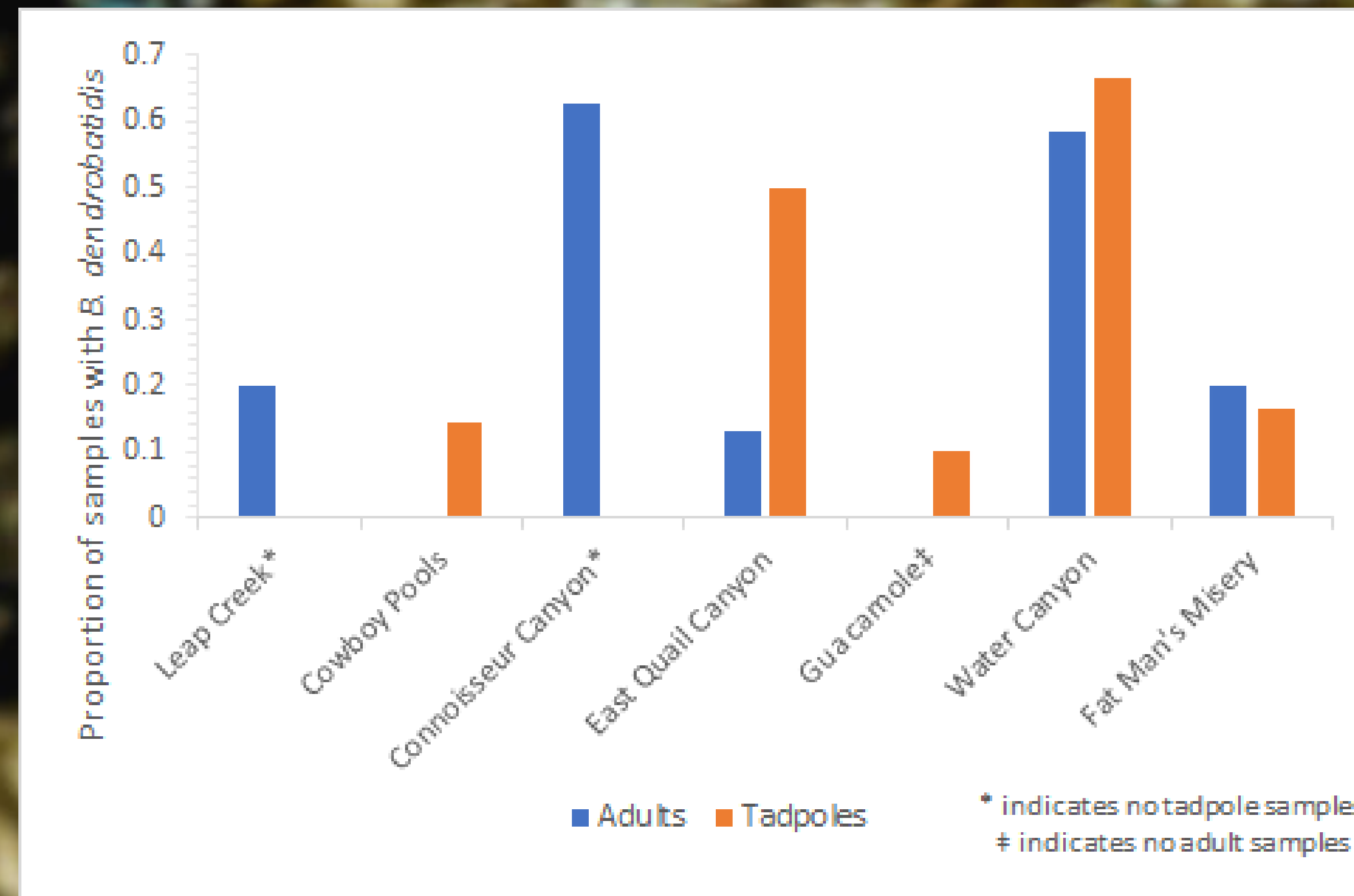


Figure 1: Results from field samples.

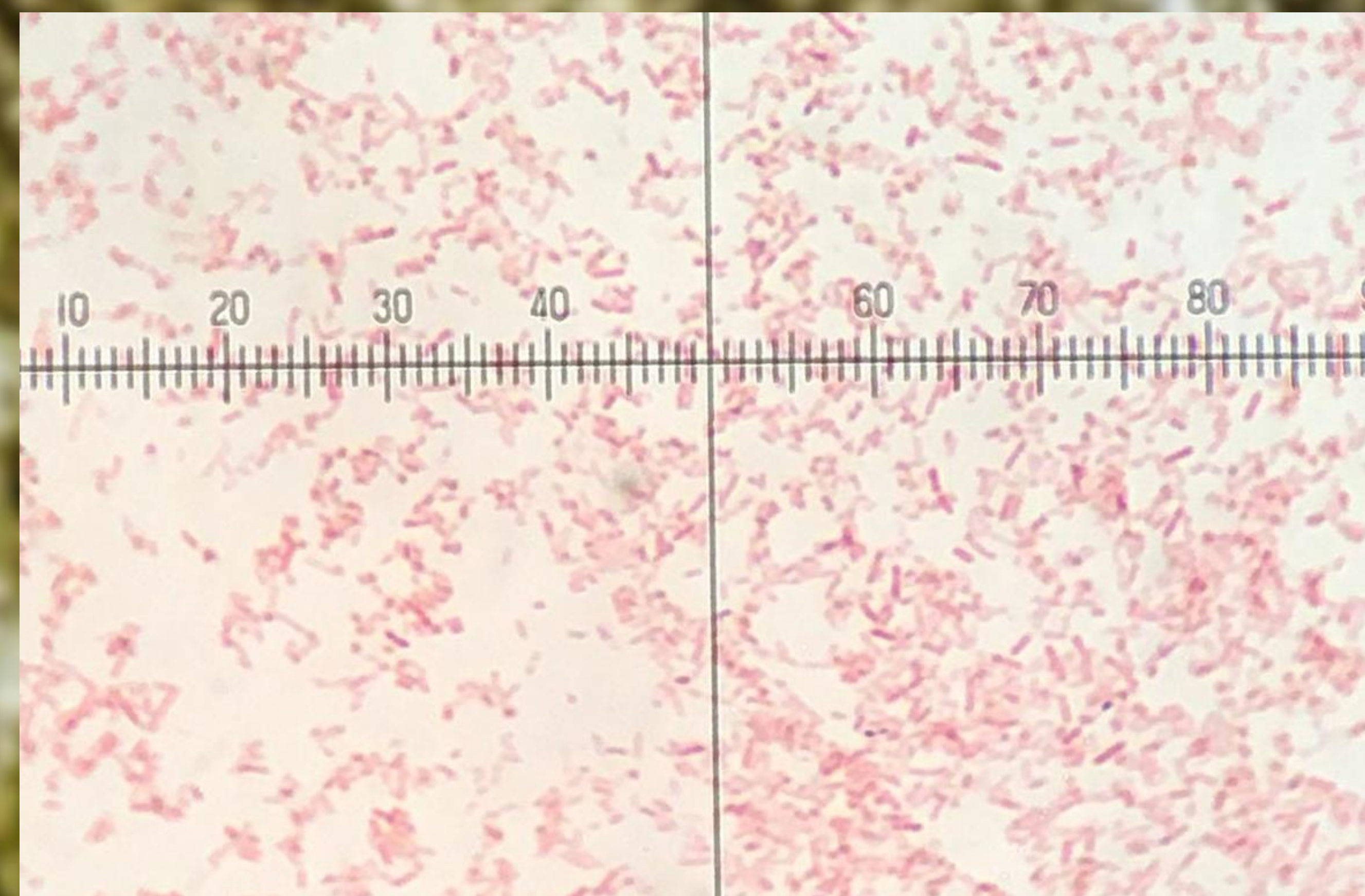
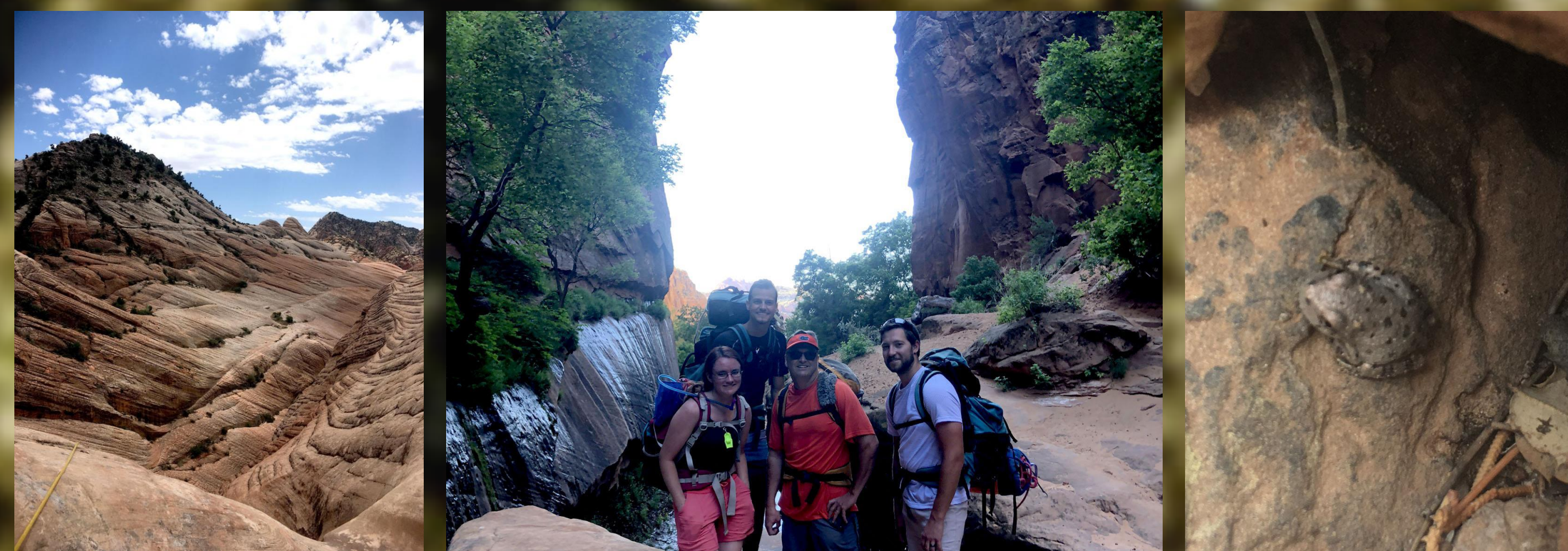


Figure 2: Gram-stain of *Salmonella* species from frog skin secretion sample.

Results

The sites with highest prevalence of chytrid in the collected samples were Connoisseur Canyon and Water Canyon. All captive specimens tested positive for chytrid on the swab from the first day, while only one second-day swab tested positive. Growth of chytrid on all plates was inhibited by an ampicillin-resistant gram-negative enteric bacterium that spread from the skin secretion discs.

Discussion

The high prevalence of chytrid in Water Canyon is likely correlated with the amount of traffic the canyon receives. Connoisseur Canyon is part of a BLM study area and may receive a higher volume of traffic than other sites. The field samples from several of the captive specimens were positive, and the spread of *B. dendrobatidis* in the laboratory can be traced to Leap Creek and Connoisseur Canyon.

The low prevalence compared to past years in previously studied areas may be indicative of low chytrid load in these frogs. In past years, PCR was used to amplify *B. dendrobatidis* DNA from field samples. It is possible that some of the negative results from the western blot would have been positive had PCR been used instead. The negative values for the second-day swabs of the captive frogs lends support to this possibility. While the bacteria found on all of the frogs inhibits *B. dendrobatidis* growth *in vitro*, further testing must be done to determine whether or not the bacteria helps *H. arenicolor* resist chytridiomycosis. The exact species of bacterium has not been determined, but it appears to be from the genus *Salmonella*.

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