

# **Rocky Mountain Tailed Frog (*Ascaphus montanus*) and *Batrachochytrium dendrobatidis* (Bd) Survey using environmental DNA (eDNA)**

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## **Introduction**

A phase one survey for Rocky Mountain Tailed Frog (*Ascaphus montanus*) and *Batrachochytrium dendrobatidis* (Bd) Survey using environmental DNA (eDNA) occurred in the Wallowa Valley Rangers District and Hells Canyon National Recreation Area in streams with habitat characteristics associated with Rocky Mountain tailed frog. The project objectives were to establish presence of Rocky Mountain tailed frogs and Bd within the Wallowa Valley Ranger District and Hells Canyon National Recreation Area using environmental DNA (eDNA).

## **Background to the project**

Rocky Mountain tailed frogs (*Ascaphus montanus*) are known to occur in the Wallowa Mountains, including Hells Canyon National Recreation Area. The habitat of this species is cold, fast-moving permanent streams (Bull and Wales 2001). The slope gradient and the amount of boulders, and cobble and fines in the stream are the stream characteristics that are most important for successful populations (Bull and Carter 1996). This species has a non-migratory sedentary life style. Bull and Carter (1996) found that there was no difference in the number of larvae or adults in streams with or without timber harvest. Any management activity along streams that increases sedimentation can be detrimental to Rocky Mountain tailed frogs (Bull and Wales 2001).

The Rocky Mountain Tailed Frog and Bd Survey will indicate the presence of both species in streams with characteristics of Rocky Mountain tailed frog habitat. This technique is cost efficient because it will indicate the presence of both species from one sample, entail less field work, and is non-invasive to sensitive species such as Rocky Mountain tailed frogs. This survey will confirm Rocky Mountain tailed frogs and Bd occurrence in stream reaches, species distribution, and Rocky Mountain tailed frog habitat over a large area in northeastern Oregon. Environmental DNA does not predict abundance, age, or gender of a species. Further research is needed to perfect a protocol for abundance, age and gender of a species using eDNA.

## **Methods**

The first phase was a pilot project to make sure all the eDNA protocols were working by verifying the presence of Rocky Mountain tailed frogs that were present in seven streams during the Bull and Carter 1996 study and the 2009 Puderbaugh Area Amphibian Survey. Three 5-liter samples were collected from each of four streams, one 5-liter sample from one stream, one 5-

liter and two 2-liter samples from one stream (same collection site), one 4-liter sample from the improvement canal, and no samples from one stream because it was dry.

Collection: All collections occurred in September 2013 to maximize detection of *Bd* and Rocky Mountain tailed frogs during low water flows. Samples were collected in the stream as follows: 1.) above tributaries, 2.) in the center of the confluence of tributaries of the stream, and 3.) below tributaries before the confluence of the stream with another stream or river. Each sample was filtered using a flow-through filter with a peristaltic hand pump and a 0.45  $\mu\text{m}$  cellulose nitrate filter paper. Filters were preserved in a 2 mL tube with 95% ethanol, marked with sample number and stream name. All date, collection time, water temperature, ambient temperature, and GPS data were recorded on data sheets during field work and then input into an Excel spreadsheet for analysis.

The eDNA extraction and analysis using species-specific primers to detect Rocky Mountain tailed frogs and *Bd* occurred in the Fish and Wildlife Resources laboratory at the University of Idaho in Moscow, Idaho using quantitative polymerase chain reaction (PCR) protocols for each species. Quantitative PCR uses an extra probe that matches exactly to indicate species presence.

## Results

### *Bd* eDNA

The *Batrachochytrium dendrobatidis* eDNA qualitative assessment did not detect *Bd* in any stream for this pilot survey. Potential reasons for no detection of eDNA include: no *Bd* was present in any of the streams or the concentration of eDNA was too low to detect.

### Rocky Mountain Tailed Frogs

eDNA from Rocky Mountain tailed frogs was detected at all collection sites in six streams and one canal. eDNA concentrations varied from 0.025 to 25.054 picograms (Table 1). Two of the samples with the lowest concentrations of DNA were assessed again for verification. Samples with low eDNA concentrations were from collections with only 2 or 3 liters of filtered stream water. At the collection site with the lowest concentration, equipment failure limited the number of liters filtered. At the other collection site two samples were taken because of the high concentration of debris in the stream water. Only two liters were filtered for each of these samples because the filter became plugged with debris. The highest concentrations of eDNA were from four of the streams at the highest elevation levels above all tributaries (Table 1). The highest concentration from one stream was at the mid elevation level in the middle of all tributaries (Table 1).

Table 1. Rocky Mountain tailed frog (RMTF) and *Bd* pilot survey results.

Stream	Collection Number	Dates	Elevation (m)	Stream Temperature	RMTF eDNA	<i>Bd</i> eDNA
Improvement Canal	1	9/23/2013	1845	4.0° C	0.025	0
Salt Creek	2	9/23/2013	1874	4.0° C	0.124	0
Deer Creek	1	9/11/2013	1401	9.4° C	0.157	0
Deer Creek	2	9/11/2013	1381	12.8° C	<b>0.476*</b>	0
Deer Creek	3	9/11/2013	1285	13.9° C	0.203	0
Lick Creek	1	9/12/2013	2016	11.0° C	<b>8.867</b>	0
Lick Creek	2	9/12/2013	1686	15.0° C	1.245	0
Lick Creek	3	9/19/2013	1615	5.0° C	2.089	0
Mud Spring Creek	1	9/18/2013	1776	7.0° C	<b>25.054</b>	0
Mud Spring Creek	2	9/19/2013	1759	4.5° C	2.452	0
Mud Spring Creek	3	9/19/2013	1699	7.3° C	2.969	0
Gumboot Creek	1	9/24/2013	1434	8.2° C	<b>1.673</b>	0
Gumboot Creek	2	9/24/2013	1248	9.7° C	0.276	0
Gumboot Creek	3	9/24/2013	1203	10.0° C	0.243	0
Grouse Creek	1	9/18/2013	1714	8.0° C	<b>1.646</b>	0
Grouse Creek <sup>1</sup>	3a	9/26/2013	921	9.3° C	0.029	0
Grouse Creek	3b	-	-	-	0.025	0

\*Bold are highest concentrations of eDNA collected for that stream.

<sup>1</sup>Grouse Creek 3a and 3b are the same collection site with two samples 2L each.

### Discussion (including lessons learned and potential next steps)

One of the difficulties was stream access due to hiking distance to collection locations from streams at high elevations in areas that were closed to motorized vehicles. We had an early snow in September that prevented access and then went on furlough October 1st. By the time furlough was over, access was not possible because gates were closed. This increased the 2-3 mile hike into two of the streams by 2-4 miles. This was also a dry year and one stream was dry by August. A second lesson learned was that I needed to know the stream characteristics to know the best location to collect samples for each stream. I found dry beds once I got to the collection sites in a couple of the streams. Or in one case realizing the improvement canal divided the stream and the lower portion was either murky water with algae covering the cobble and dry at the lower half. The map at times showed stream locations where there were none. I realized the stream was not as long as indicated on the map once we had hiked up to the point where the collection location was marked on the map. Thirdly was the consideration of where to take the sample at the collection site. It was found that cleaner collections came from water cascading over rocks than water just below the rocks. It was also found that some streams had more pristine conditions than others, which made filtering the sample much easier. The fourth lesson learned is that one sample from each stream will indicate presence or absence in a stream, but three water samples

could indicate where on the stream tailed frogs are located (elevation level or how far upstream) based on eDNA concentration. So, the amount of information desired for the stream would determine the number of samples collected from each stream. Additionally, in the course of collecting water samples, the embeddedness of the cobbles should be measured at each collection location. And a final lesson learned is to start the survey by the middle of August to allow more collection time and still have low enough water flows to result in eDNA data for Bd and Rocky Mountain tailed frogs.

The lack of detection of *Bd* may not mean that there is no *Bd* present; it may mean that the concentrations were too low to detect. A different assessment may detect *Bd* or a warmer time of year may result in detection. Additional surveys are needed to refine the collection method for *Bd*.

All collections sites resulted in detection of Rocky Mountain tailed frogs. The highest concentration of eDNA collected at various sites from a stream could indicate not only the presence but also the location of Rocky Mountain tailed frogs in streams. The knowledge of presence and stream location of Rocky Mountain tailed frogs could aid management decisions for this species. Additional surveys are needed to verify the results of this pilot survey.

The next step of this pilot project is to survey the remaining streams on the forest known to have had Rocky Mountain tailed frogs in them as indicated by previous surveys. This may take more than one season or more personnel to do the collections. Once all streams that had Rocky Mountain tailed frogs in them in the past are surveyed, then streams with habitat characteristics associated with Rocky Mountain tailed frogs would be surveyed to verify presence or absence. This again may take multiple years and/or more personnel to complete the survey.



Figure 1. Gumboot Creek collection site.



Figure 2. Salt Creek collection site.



Figure 3. View of Filter after filtration of water.



Figure 4. Filtration system by Lick Creek.