

Meg and Bert Raynes Wildlife Fund
Status Update for the Amphibian eDNA Project on the
Bridger Teton National Forest

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Organization of Applicant: US Forest Service, Bridger-Teton National Forest

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Title of Grant:

Determining The Level of Detectability Using Environmental DNA (eDNA) Sampling and Traditional Methods to Inventory Amphibians: Improving Amphibian Conservation Efforts on The Bridger-Teton National Forest

Project Summary:

Current amphibian occupancy techniques on the Bridger-Teton National Forest (BTNF) involves visual and call detection surveys as a means to inventory sensitive amphibians, whose low abundance and low detection rates have made detecting the presence-absence of amphibians difficult, time consuming, and costly. The BTNF has requested support from the Meg and Bert Raynes Wildlife Fund to implement environmental DNA (eDNA) sampling as a new, cost effective technique to inventory amphibians. Water samples collected by BTNF wildlife crews at key inventory locations are currently being analyzed to positively identify amphibian species at selected location across the forest. By comparing results from eDNA sampling with results from traditional surveys completed by wildlife crews and known amphibian occupancy, eDNA sampling will test the effectiveness of wildlife crews to detect species within wetland sites.

This project has been developed to increase and improve amphibian inventory methods on the Bridger-Teton National Forest (BTNF) in close coordination and with similar work in other parts of Wyoming. The BTNF has partnered with Wyoming Natural Diversity Database to improve landscape-level amphibian inventory methods and in doing so has greatly enhanced amphibian monitoring and conservation over the long-term. This is not just a research study, but rather an opportunity to advance amphibian inventory methods and improve the ability to monitor such hard to find species. The information we gain from utilizing this new eDNA inventory method along with the traditional methods, will benefit amphibian conservation efforts throughout the public lands of Jackson Hole.

Amphibians are highly sensitive and vulnerable to environmental changes and are proven to be excellent indicators of environmental quality. Their permeable skin makes them susceptible to water, air quality, and disease; and their tendency to occupy small isolated areas greatly increases their

susceptibility to habitat loss and degradation. With changing environmental factors, the need for amphibian conservation efforts has become a greater concern.

Project Description:

Goals and Objectives

The goals of this project are to improve amphibian inventory efforts on the Bridger-Teton National Forest by utilizing a new environmental DNA (eDNA) sampling technique, and to compare the results from eDNA sampling to traditional inventory techniques currently implemented. By taking water samples at key wetland sites across the BTNF, eDNA sampling analysis will positively identify sensitive amphibian species within the sample site, thus providing a quick, cost effective inventory.

The key objectives of this project are to:

- 1) Collect 40 eDNA water samples at key inventory wetland sites on the BTNF, and analyze these eDNA samples to positively identify each amphibian species occurring in the sample locations.
- 2) Collect 10 eDNA water samples at established wetland sites on the BTNF where traditional survey methods will also be implemented, and analyze these eDNA samples to positively identify each amphibian species occurring in the sample locations.
- 3) Determine and compare the level of amphibian detectability from utilizing eDNA water samples and traditional survey method at all 50 sample sites.
- 4) Analyze the benefits from both eDNA sampling and traditional survey methods and discuss incorporating additional eDNA sampling inventory locations throughout the BTNF.

Project Methods

Aquatic species, such as amphibians, leave DNA in the water from urine, feces, and dead skin cells. By taking water samples at various wetland locations, laboratory technicians can extract the DNA from the water sample and use species specific DNA markers to positively detect exactly which species has been in that water body, a process called environmental DNA or eDNA. For inventory purposes, this method eliminates the need to perform traditional methods (visual and call detections) and increases the chance of detecting species, since surveyors have more of chance of missing species with traditional techniques. With approval of this grant the BTNF has implemented eDNA sampling to inventory amphibians at 50 total wetland sites during the summer season of 2015; 40 of the wetlands sites will be key inventory locations on the forest where amphibian inventory surveys have not been implemented and occupancy is unknown, and 10 eDNA sample sites will be in locations where traditional survey methods are implemented and/or occupancy is known.

The project methods are as follows:

- 1) Inventory 40 key wetland sites across the BTNF with the eDNA water sampling technique. These sites will be located where amphibian inventory surveys have not yet been implemented and where environmental factors suggest the need for sampling amphibian occupancy. (Laboratory analysis requires 3 samples per site for an accurate reading. Therefore a total of 120 water samples will be collected).
- 2) Inventory 10 sites across the BTNF with the eDNA water sampling technique where traditional inventory surveys are also conducted or were amphibian occupancy is known. (Laboratory analysis requires 3 samples per site for an accurate reading. Therefore a total of 30 water samples will be collected).

- 3) Following the completion of eDNA sample collection, 150 total water samples will be sent to Wyoming Natural Diversity Database laboratory for DNA analysis. This may take up to 6 weeks for results.
- 4) Once laboratory analysis is complete, a statistical review will be completed in order to determine 1) the ability for eDNA sampling to positively identify amphibian species at 50 wetland locations throughout the BTNF and 2) the level of detectability of amphibian species from the eDNA sampling results compared to the results from traditional survey methods.
- 5) A summary report will be developed including results from the eDNA sampling method and a biological discussion comparing and summarizing the levels of detectability between the eDNA inventory sampling method and the traditional inventory method. The summary will also include future goals for amphibian inventory efforts on the Bridger-Teton National Forest.

This project will provide the BTNF with data on the presence-absence of amphibian species at 50 wetland locations forest-wide. Implementation of this project will also allow the BTNF to compare the level of detectability from the eDNA sampling results to traditional survey results conducted by wildlife crews. This would allow for analysis of positive and negative aspects from both methods and provide the opportunity to discuss the benefits from incorporating eDNA sampling into future inventory efforts on the BTNF.

Future development of eDNA inventory methods would allow wildlife crews to *first* collect water samples at key wetland sites, and in locations where eDNA laboratory results provide positive amphibian identification, *then* surveys using traditional methods could be implemented. This would eliminate time consuming traditional surveys in wetlands where amphibians are not present. Environmental DNA could not only provide a means for collecting occupancy data that is cost effective but also re-direct efforts to key locations or areas seen as an environmental concern. The BTNF could use eDNA to sample locations where amphibians have not yet been detected, sample areas where breeding is more likely, and specific locations where Forest Service projects are proposed.

Current Project Status:

A total of 30 eDNA water samples were collected during the summer season of 2015 (see attached map). 14 samples were collected at key wetland location across the BTNF where amphibian occupancy is currently unknown and 16 eDNA samples have been collected in conjunction with traditional inventory methods at already-existing sites or where amphibian occupancy has been documented. While fewer sites had eDNA water samples collected than originally described in the project objectives, the project goal has not been modified and will still be met. The crews worked very hard over the past summer season of 2015 to collect as many samples as possible. Given this was the first year for a large-scale project, there were a lot of logistics, planning, and modified priorities involved. For example, some sites selected originally using a computer database of water and stream layers along with satellite imagery did not have water or the primary habitat for amphibian once the site was visited. In some cases the site was not accessible due to lower-level maintenance forest roads, and some sites were just not feasible given their distance and what time was allotted for the project work.

All water samples collected were successfully filtered and the eDNA material was extracted for laboratory eDNA analysis. The filtered material for each sample collected was sent in at the end of the summer season for analysis and is currently being processed by Colorado State University Cooperative Laboratory. We are working closely with the lab and hope to have results within the next

few months. Once the BTNF receives the results, the analysis comparing positively detected sensitive amphibian species relative to traditional detection methods and current known occupancy will begin and final results is expected by the spring of 2016.

The results from the eDNA laboratory analysis will allow the BTNF to (1) increase amphibian inventory at key locations on the forest, and in doing so increase our understanding of amphibian occupancy and (2) compare the results from eDNA sampling to traditional (visual and call detection) surveys completed by wildlife crews, allowing eDNA sampling to test the effectiveness of wildlife crews to detect species at wetland sites.

Project Evaluation:

The project will be considered fully successful once the goal of this project is met and all project objectives are obtained. Given the eDNA water samples have been collected and samples are currently being processed, majority of the objectives have been successfully completed and achieving the project goal is expected.

Given eDNA samples were collected at a total of 30 wetland locations and that eDNA laboratory analysis is currently working at positively identifying specific amphibian species within the wetlands, the project is considered to be making great progress. The final step will be the completion of a statistical comparison between detectability of the eDNA method and traditional methods in order to contribute to the success of the project. As a result from the project the BTNF will be able to recognize the benefits from eDNA sampling as an inventory technique and work with partners on improving landscape-level conservation efforts and monitoring. The BTNF will be able to direct their efforts more effectively and determine how to utilize the eDNA sampling method along with traditional methods.

Shortly after the completion of the BTNF analysis the forest will provide an electronic and paper copy of a detailed project summary report to the Meg and Bert Raynes Wildlife Fund. The summary report will include results from the eDNA sampling method and its ability to positively identify amphibian species as well as a biological discussion comparing the levels of detectability between the eDNA inventory sampling method and the traditional inventory method. The summary will include future goals for amphibian inventory efforts on the Bridger-Teton National Forest. These results will be shared between all the partners, collaborators, and sponsors involved in this effort. The Meg and Bert Raynes Wildlife Fund will be recognized in this document, as this project would not have been implemented without their enthusiasm and commitment to wildlife.