

Appendix A

Boreal Toad eDNA

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Interim Project Report

June 2025

Heather L. Bateman, Molly Bechtel,
Fábio Albuquerque

Arizona State University

Using eDNA to test ecological models on Boreal Toad Habitat Suitability

Principal Investigators: Drs. Heather Bateman, Molly Bechtel, Fábio Albuquerque

Interim Report 2025
New Mexico Department of Game and Fish
and
Arizona State University
Agreement #250106

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Submitted to:

New Mexico Department of Game & Fish
1 Wildlife Way
Santa Fe, NM 87507

Prepared by:

Heather L. Bateman, Professor
Molly Bechtel, Assistant Professor
Arizona State University, School of Applied Sciences and Arts
Mesa, AZ 85212

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Summary

Project Objectives

1) Determine Boreal Toad occupancy and detection using eDNA in the _____ region, 2) Examine toad occupancy in relation to presence of amphibian diseases, *Bd* (*Batrachochytrium dendrobatidis*) and *Ranavirus*, and 3) Explore the effects of climate change on habitat suitability of the Boreal Toad in New Mexico.

Action to Date

The final agreement between the New Mexico Department of Game and Fish (NMDGF) and Arizona State University (ASU) was signed on March 24, 2025. Drs. Bateman and Bechtel conducted a nationwide search for a research staff position starting January 31st and ending February 10th. The position was posted on the Ecolog-L listserv (<https://esa.org/membership/ecolog/>), ASU website and advertised at the Joint Annual Meeting of the New Mexico and Arizona Wildlife Society Meeting February 6th through 9th. Twenty-five candidates applied. Applications were screened February 10th-19th and nine candidates were interviewed starting February 20th. The last interview was conducted February 27th. Jenna Zarlingo was selected as the most qualified candidate for the position and started work on June 9.

We have worked with the Arizona State University business office to prepare field and lab supplies for data collection and processing. Supplies such as filters and flasks for collecting water samples, swabs for swabbing amphibians, reagents such as mastermix, primers and probes for molecular analysis as well as gloves, pipette tips and plates to be used in the lab. Quat-128, a disinfectant capable of killing pathogens such as *Bd* and *Ranavirus*, was also purchased to decontaminate equipment after field work. The first field data collection trip to collect water/eDNA samples and swab any amphibians encountered for *Bd* and *Ranavirus* infection screening as outlined in the proposal occurred June 10th-13th to the field location is in the Carson National Forest (Tierra Amarilla, Rio Arriba County, NM). We obtained 7 total water samples (Table 1). All water collected was filtered on site. Filters were placed in microcentrifuge tubes filled ethanol then placed on ice then brought to ASU and stored in -20°C freezer until analysis. We also swabbed two western chorus frogs and salvaged the carcass of one northern leopard frog. We observed one northern leopard frog that we were unable to catch and thus, unable to swab. Swabs were also placed in microcentrifuge tubes filled with ethanol and placed on ice, then brought to ASU and stored in -20°C freezer until analysis. The salvaged leopard frog carcass was placed in a plastic bag, stored on ice until it was placed in the -20°C freezer at ASU.

Table 1. Locations of collected water samples. The transect numbers refer to the transects described in the original proposal.

Name	Date Collected	Transect Number
	06/11/2025	1
	6/11/2025	2
	6/11/2025	2
	6/12/2025	3
	6/12/2025	3
	6/12/2025	4
	6/12/2025	4