Using eDNA to Test Ecological Models on Boreal Toad Habitat Suitability

Year 1 Report

New Mexico Department of Game and Fish

and

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Project Need

Amphibians are declining globally, and anurans (frogs and toads) are among the most threatened taxonomic groups worldwide (Luedtke et al. 2023). According to the US Fish and Wildlife Service (2017), the Boreal Toad is a subspecies of the Western Toad (*Anaxyrus boreas*). The Boreal Toads in New Mexico (NM) are considered to be members of the "Eastern Population" of the Western Toad species complex and genetic analyses suggest that the species' distribution includes areas of Idaho, Wyoming, Utah, and Colorado (U.S. Fish and Wildlife Service 2017). The Boreal Toad (*A. boreas boreas*) is listed as state endangered by the New Mexico Department of Game and Fish (NMDGF 1990) and historically persists at high elevations in spruce-fir-aspen areas of northcentral NM selecting for beaver ponds, lakes, slow-moving water, and marshy habitats (Degenhardt et al. 1996.). Prior to 1993, the species was observed in the San Juan Mountains at Trout Lakes, Lagunitas Lakes, and Canjilon Lakes. No Boreal Toads were found in 1993 during surveys conducted by Stuart and Painter (1994). Repatriation of Boreal Toads began in 2008 within its known range at Trout Lakes in the Carson National Forest in northern New Mexico (Esther Nelson, pers. com.).

Project Objectives

Our goals are to determine Boreal Toad presence using eDNA in the Trout Lakes region, examine Boreal Toad presence in relation to occurrence of amphibian diseases (i.e., *Batrachochytrium dendrobatidis, Bd* [the pathogen that causes chytrid fungus] and Ranavirus), and explore the potential effects of climate change on habitat suitability and distribution of the Boreal Toad in New Mexico.

Field Surveys and Laboratory Analyses

Environmental Data

In the summer of 2025, researchers recorded environmental variable data on vegetation and aquatic structure and collected water samples and tissue swabs from hand-captured amphibians across the Trout Lakes system. Environmental data were collected by walking 4 transects approximately 550 m long and 180 m apart (Fig. 1). Transects traversed lentic and lotic bodies of water, and we recorded water temperature, air temperature, relative humidity, and other weather variables. We measured vegetation present, canopy cover, emergent/submergent vegetation, water turbidity, and water turbulence along the transects. We documented presence of herpetofauna, and any amphibians encountered on the transects were swabbed for pathogens.

Across all site types, the mean canopy cover was 37.3%, mean emergent vegetation cover was 15.3%, and silt substrate composition was 72.6%. To quantify the observed variation across lentic and lotic site types, we compared vegetative and aquatic structural variables as described in Table 1. Generally, lentic sites had turbid water, more floating and submergent vegetation, less canopy cover, and a higher silt substrate composition compared to lotic sites (Table 1).

Most amphibian captures occurred in lentic site types (Fig. 2). To relate the presence of observed amphibian species (see below) to environmental variables that we measured, we used a binary logistic regression model with noncorrelated environmental covariates. Of the 21 variables, 6 (air temperature, canopy cover, emergent vegetation cover, floating vegetation cover, turbidity, and water temperature) were used in the logistic regression model. These covariates were selected because they have been evaluated in previous literature (Browne and Paszkowski 2017) regarding Boreal Toad habitat and were determined to be noncorrelated by a Spearman correlation test. We found that Northern Leopard Frog occurrence was associated with low levels of canopy cover, less floating vegetation, and low water turbulence (Table 2). Boreal Chorus Frog occurrence was associated with cooler air temperatures and less floating vegetation (Table 2).

Amphibian Presence, *Swab Data, and eDNA Sampling*
Samples for eDNA were collected to infer presence of Boreal Toad DNA, Ranavirus DNA, and *Bd* DNA. eDNA sampling consisted of collecting 250mL of water from 4-8 locations per lentic or lotic water body, approximately 50 m apart, for a total water volume of 1L (following techniques of Hall et al. 2018, Kamoroff and Goldberg 2017). Water samples were collected from all lentic or lotic bodies of water encountered along the transect and were filtered onsite. All water filters collected in the summer and in October have been extracted for DNA and were tested for Boreal Toad and pathogen (*Bd* and Ranavirus) DNA presence. All filters tested negative for the presence of Boreal Toad and pathogen DNA (Table 3).

Twelve tissue swabs were collected from amphibians in 2025 (Table 4) including: 5 adult Boreal Chorus Frogs (*Pseudacris maculata*), 3 adult and 3 juvenile Northern Leopard Frogs (*Lithobates pipiens*), and 1 Tiger Salamander (*Ambystoma mavortium*). This count includes one salvaged (found dead) Northern Leopard Frog which we also dissected for mouth parts, liver, and kidney. These samples were then extracted for DNA and tested for pathogens with qPCR. All swabs were also extracted for DNA and tested for pathogens with qPCR. All swabs were negative for pathogens, except for one swab collected in October from an adult Northern Leopard frog. The extracted mouth parts and liver/kidney dissected from the salvaged Northern Leopard Frog tested positive for *Bd*. This animal was collected in June 2025 (Table 4).

Species Distribution

To explore the potential effects of climate change on Boreal Toad habitat suitability in New Mexico and states to the north, we initially acquired and quality-checked Boreal Toad occurrence data from the Global Biodiversity Information Facility (GBIF) in consultation with species experts. We selected environmental variables related to climate, solar radiation, and topography to evaluate the broad-scale distribution of the species and evaluate how Boreal Toad habitat suitability could change under a range of climate scenarios. We obtained climate variables and solar radiation from WorldClim (Global climate and weather data; Fick and Hijmans 2017), and topographic measurements from EarthEnv (Amatulli et al. 2018). Then, we analyzed geospatial data to compile a database for modeling the current habitat suitability and distribution of the Boreal Toad. The WorldClim dataset includes 24 variables covering temperature, precipitation, and solar radiation. EarthEnv included five topographic measures. We downloaded over 2,000 occurrences of Boreal Toads from GBIF (2024, https://doi.org/10.15468/dl.kdwyqy). We used species distribution modeling algorithms (e.g., Maxent and Random Forest) to build a preliminary habitat suitability map for current conditions (Fig. 2) and explore the relationship between habitat suitability and environmental variables. Preliminary results showed that precipitation, elevation, and temperature were the major drivers of habitat suitability. The fourth most important factor was solar radiation.

Future Directions in Year 2

In 2026, we plan to conduct one field trip in the spring (to coincide with breeding season of the Boreal Toad, pers. comm. Leland Pierce), three field trips in the summer (June, July, and August), and an additional field trip in October to collect water and amphibian samples to obtain prevalence data in combination with eDNA of Boreal Toads.

No Boreal Toad DNA was detected in our eDNA samples collected in 2025. This could be because the toads had dispersed from these bodies of water and thus, no DNA was detected because no toads were in them. Post breeding season male toads will disperse up to 4 km away from their breeding site; female toads will disperse four times the distance of male toads (Pierce 2006, Muths 2003). Therefore, because Boreal Toad habitat selection varies throughout the year (preferring water with low turbulence for breeding and coarse woody debris and burrows as the dominate refuge type in the active season), we may have to focus our sampling efforts in the spring when the toads are most likely to select for water (Long and Prepas 2012, Menuz 2016). We will be sampling in May and early June to address this. We also plan to sample more locations west of our original transects to determine if toads may be occurring there.

To expand the analyses related to species-habitat models, we plan to reduce the dimensionality of the 21 environmental variables by using a Principal Component Analysis (PCA). We will use the synthetic variables generated from the PCA as independent predictors to relate to presence and absence of amphibian species detected during surveys. This will address first-order habitat associations of species (*sensu* Johnson et al. 1980).

In 2026, we plan to create a more comprehensive dataset to explore the effects of climate change on the Boreal Toad. We will include updated GBIF sources and location data from the New Mexico Game and Fish Department. We will use environmental and biological data to project their distributions across current and future time periods. For future projections, we will obtain future climate data from the eight General Circulation Models (GCMs) available in WorldClim (v. 2.1). Each model will be run under four greenhouse gas concentration scenarios, yielding 32 future climate projections. The dataset will include bioclimatic variables from CMIP6 (Coupled Model Intercomparison Project Phase 6) and four Shared Socioeconomic Pathways (SSPs): 126, 245, 370, and 585. Each SSP reflects different socioeconomic, technological, and political scenarios, ranging from optimistic to pessimistic greenhouse gas trajectories (Riahi et al. 2017). We will use climate for the 2081-2100 period, at a 30-second resolution (about 1x1 km). The list of the GCMs and their references is available in Albuquerque et al. (2025).

Additionally, we plan to hire a research assistant to help produce the modeling outcomes. We plan to explore the relative impact of environmental variables on the distribution of the Boreal Toad, investigate the major drivers of species distribution, and the shape of these relationships (i.e., make qualitative inferences regarding the trends in how habitat suitability relates to each key factor variable). This will allow us to investigate the projected contractions or expansions of the Boreal Toad's range in response to changing environmental variables.

Acknowledgements

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Tables and Figures

Table 1. Summary of vegetation and aquatic structural variables measured during 2025 in lentic and lotic sites at the Trout Lakes system, Carson National Forest, NM. Variables were compared using Wilcoxson rank-sum test, which indicates differences in system type (i.e., lentic and lotic). NS is non-significant and (-) indicates that the variable was not measured for this site type.

Variable	**Lentic Type Mean (±SE)** n=41	**Lotic Type Mean (±SE)** n=15	**Difference, lentic vs. lotic (p-value)**
Lentic Surface Area (m)	3034.8 (803.6)	-	
Air Temperature (°C)	17.1 (0.8)	16.9 (1.4)	NS
Water Temperature (°C)	14.8 (0.6)	15.8 (4.9)	0.006
Relative Humidity (%)	60.2 (3.3)	67.2 (5.6)	NS
Turbulence	0.6 (0.1)	1.8 (0.2)	<0.001
Turbidity	3.0 (0.2)	1.4 (0.2)	<0.001
Floating Vegetation Cover (%)	14.0 (4.5)	0.0 (0.0)	0.001
Submerged Vegetation Cover (%)	23.9 (5.5)	0.0 (0.0)	0.001
Emergent Vegetation Cover (%)	10.4 (1.8)	27.7 (7.0)	NS
Perimeter Vegetation Cover (%)	89.9 (4.7)	87.1 (4.3)	NS
Bedrock Substrate (%)	0.0 (0.0)	0.0 (0.0)	NS
Boulder Substrate (%)	1.8 (0.5)	7.1 (3.2)	NS
Cobble Substrate (%)	4.0 (1.1)	19.1 (6.0)	0.055
Pebble Substrate (%)	6.0 (2.4)	8.9 (3.0)	NS
Sand Substrate (%)	1.0 (0.4)	17.4 (4.7)	<0.001
Silt Substrate (%)	83.6 (4.0)	48.2 (8.6)	0.003
Canopy Cover (%)	26.6 (3.4)	59.5 (3.0)	<0.001
Terrestrial Soil Cover (%)	-	27.1 (5.7)	
Terrestrial Vegetation Cover (%)	-	63.9 (4.4)	
Terrestrial Stone Cover (%)	-	4.4 (1.1)	
Terrestrial Litter Cover (%)	-	13.2 (1.9)	

Table 2. Importance of environmental variables in predicting amphibian presence at the Trout Lake system, Carson National Forest, NM. Variables included in the logistic regression include air temperature (TempA), canopy cover (CC), emergent vegetation cover (VegE), floating vegetation cover (VegF), turbidity (turbid), and water temperature (TempW). Directionality of relationship between species presence and variable is indicated by (+/-).

Species	TempA	CC	VegE	VegF	Turbid	TempW	df	logLik	AICc	ΔAICc	AICw
Lithobates pipiens		-2.31		-47.26	-58.98		4	<0.001	12.0	0.00	0.58
Pseudacris maculata	-11.19			+2.17			3	<0.001	8.18	0.00	0.15
All amphibian captures		-0.05					2	-8.17	21.35	0.00	0.17

Note: AICc is Akaike's information criterion (corrected for small samples), a metric used to judge model fit. ΔAICc is the difference in AICc from the top-performing model, which represents how much worse each model performs compared to the best-performing model. Smaller Δ values indicate models that are more strongly supported by the data. Component AICc weights are the average relative weight. Weight reflects the relative level of support for each model, averaged across the set of models considered.

Table 3. Water samples collected in 2025 and filtered for eDNA at Trout Lakes, Carson National Forest, NM. No filters have tested positive for Boreal Toad DNA. Volume is the approximate amount of water filtered per sample.

Filter Sample	Volume (mL)	Site	Date
F1	375	Main Fishing Pond	6/11/25
F2	425	Lower Repatriation Pond	6/11/25
F3	400	Upper Repatriation Pond	6/11/25
F4	375	Middle Pond 3	6/12/25
F5	425	Upper Fishing Pond	6/12/25
F6	225	Middle Pond 1	6/12/25
F7	1000	Upper Fishing Transect Stream 3	6/12/25
F8	225	Lower Repatriation Pond	7/23/25
F9	550	Repatriation Transect Stream	7/23/25
F10	400	Upper Repatriation Pond	7/23/25
F11	400	Upper Fishing Pond	7/24/25
F12	650	Upper Fishing Transect Stream 2	7/24/25
F13	500	Upper Fishing Transect Stream 1	7/24/25
F14	400	Main Fishing Pond	7/24/25
F15	500	Main Fishing Transect Stream	7/24/25
F16	450	Middle Pond 2	7/25/25
F17	250	Middle Pond 1	7/25/25
F18	300	Middle Pond 3	7/25/25
F19	225	Middle Pond 4	7/25/25
F20	525	Middle Transect Stream	7/25/25
F21	350	Middle Pond 5	7/25/25
F22	650	Middle Transect Stream	8/14/25
F23	300	Middle Pond 5	8/14/25

Filter Sample	Volume (mL)	Site	Date
F24	350	Middle Pond 3	8/14/25
F25	200	Middle Pond 1	8/14/25
F26	350	Middle Pond 4	8/14/25
F27	250	Lower Repatriation Pond	8/15/25
F28	600	Repatriation Transect Stream	8/15/25
F29	425	Upper Repatriation Pond	8/15/25
F30	400	Main Fishing Pond	8/15/25
F31	300	Main Fishing Pond Runoff 1	8/15/25
F32	425	Main Fishing Pond Runoff 2	8/15/25
F33	550	Main Fishing Transect Stream	8/16/25
F34	400	Upper Fishing Pond	8/16/25
F35	425	Upper Fishing Transect Stream 1	8/16/25
F36	425	Main Fishing Pond	10/10/25
F37	475	Main Fishing Pond Runoff 1	10/11/25
F38	500	Middle Pond 3	10/13/25
F39	325	Upper Repatriation Pond	10/12/25
F40	350	Upper Fishing Pond	10/14/25
F41	850	Main Fishing Transect Stream	10/12/25
F42	175	Lower Repatriation Pond	10/12/25
F43	575	Repatriation Transect Stream	10/12/25
F44	125	Middle Pond 1	10/13/25
F45	175	Middle Pond 2	10/13/25
F46	500	Middle Pond 4	10/13/25
F47	675	Middle Transect Stream	10/13/25
F48	475	Upper Fishing Transect Stream 1	10/14/25
F49	450	Upper Fishing Transect Stream 2	10/14/25

Table 4. Amphibians observed and swabbed at Trout Lakes in 2025. Only two samples tested positive for pathogens (*Bd*, *Batrachochytrium dendrobatidis*); these samples consisted of tissue collected from a carcass of a dead *Lithobates pipiens* adult and one swab collected from a living *L. pipens* adult.

Date	Species	Salvaged	Age	Location description	Disposition
6/11/25	*Lithobates pipiens*	yes	Adult	pond	swabbed, salvaged, & stored at ASU for DNA analyses; **tested positive for Bd**
6/12/25	*Pseudacris maculata*	no	Adult	ephemeral stream	swabbed & released
6/12/25	*P. maculata*	no	Adult	tall grass near shallow water	swabbed & released
8/13/25	*L. pipiens*	no	Juv	pond	swabbed & released
8/14/25	*P. maculata*	no	Adult	pond	swabbed & released
8/14/25	*P. maculata*	no	Adult	pond	swabbed & released
8/14/25	*P. maculata*	no	Adult	pond	swabbed & released
8/14/25	*Ambystoma mavortium*	no	Adult	pond, on a log	swabbed & released
8/15/25	*L. pipiens*	no	Juv	shallow run-off pond	swabbed & released
8/15/25	*L. pipiens*	no	Juv	shallow run-off pond	swabbed & released
8/16/25	*L. pipiens*	no	Adult	tall grass near shallow water	swabbed & released
10/10/25	*L. pipiens*	no	Adult	shallow run-off pond	swabbed & released; **tested positive for Bd**

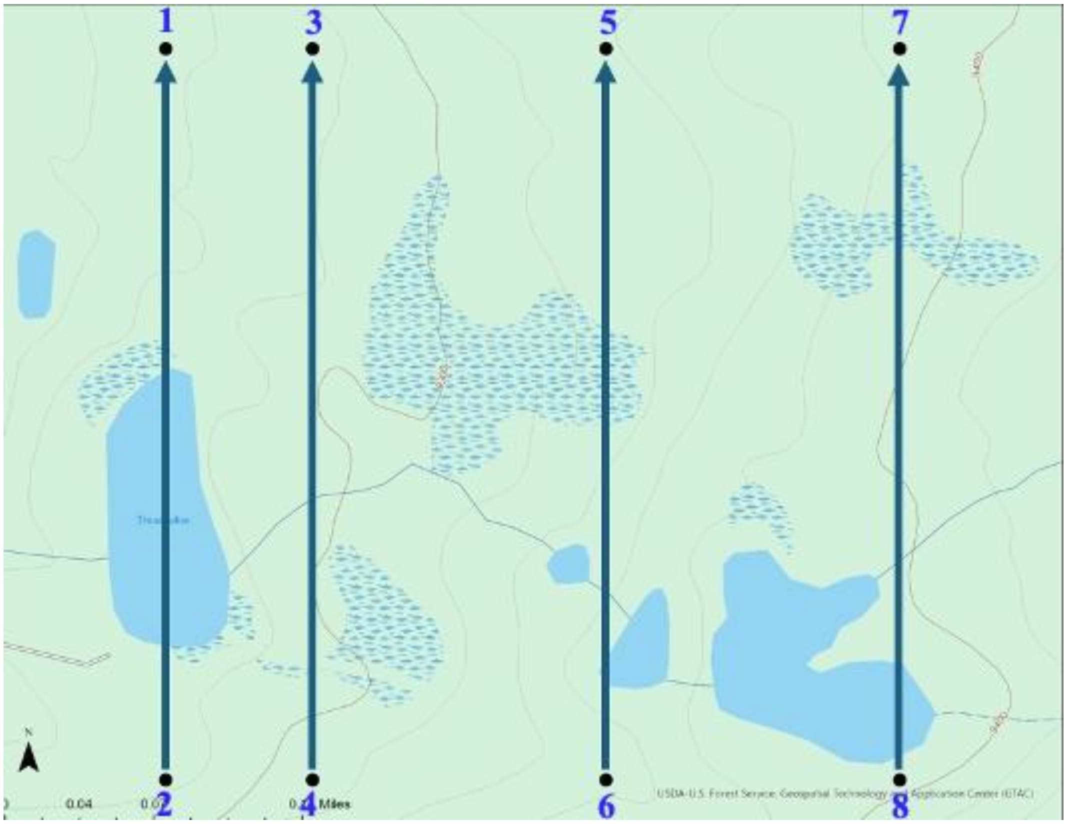

Figure 1. Map of transects at Trout Lakes, Carson National Forest, NM.

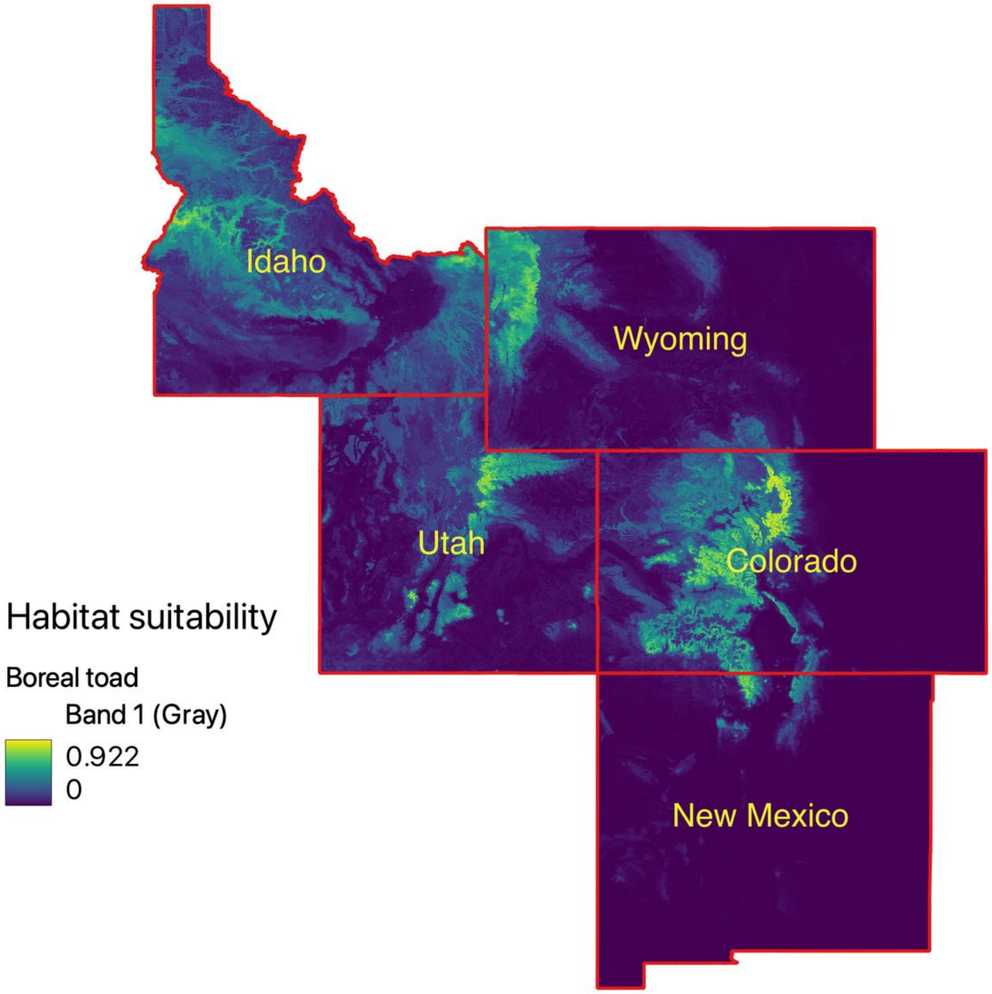

Figure 2. Geographic distribution of current habitat suitability for Boreal Toads (*A. boreas boreas*) across its range in the United States. Lighter colors indicate habitats of higher suitability than habitats indicated by darker colors.

Figure 3. Amphibians and site types at Trout Lakes, Carson National Forest, NM. Photographs by Heather Bateman (HB), Jenna Zarlingo (JZ) and Molly Bechtel (MB).

Swabbing a Northern Leopard Frog, August 2025 (HB)

Swabbing a Boreal Chorus Frog, August 2025 (HB)

Tiger Salamander, August 2025 (HB)

Example of lotic system, Oct 2025 (JZ)

Main fishing pond, June 2025 (MB)

Example of lentic site, Aug 2025 (HB)

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