**Using eDNA to test ecological models on Boreal Toad Habitat Suitability**

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# Year 2 Interim Report
# New Mexico Department of Wildlife
# and
# Arizona State University
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## Summary

### 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

1) Determine Boreal Toad occupancy and detection using eDNA in the Trout Lakes 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 Boreal Toad in New Mexico.

### Project Activities to Date

Since the beginning of Year 2 of our project agreement between NMGFD and ASU (16 April 2026), we have been collecting environmental data, processing collected water and swab samples collected and conducting ecological modeling on Boreal Toad and *Bd* distribution as described below.

### Field Surveys and Laboratory Analyses

*Environmental Data*
In the spring of 2026, researchers recorded environmental variable data on vegetation and aquatic structures and collected water samples and tissue swabs from hand-captured amphibians across the Trout Lakes system. Two field trips were conducted to collect environmental data, one in May and one in June. Environmental data were collected by walking transects approximately 550 m long and 180 m apart. One new transect was added this year for a total of 5 transects (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 in which data were collected in May and June, the mean canopy cover was 33.0%, mean emergent vegetation cover was 20.0%, mean silt substrate composition was 88.2% and the mean air and water temperatures were 22.0°C and 16.8°C, respectively. 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).

*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 each of 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 spring have been extracted for DNA. Water filters collected in May 2026 have been tested for Boreal Toad and pathogen (*Bd* and Ranavirus) DNA presence. All filters tested negative for the presence of Boreal Toad DNA. One filter collected from transect 5 tested positive for *Bd* (Table 2). Filters collected in June 2026 have not yet been tested for Boreal Toad and pathogen presence.

We collected 62 swabs from amphibians in 2026 (Table 3) including: 15 Boreal Chorus Frogs (*Pseudacris maculata*); 21 Northern Leopard Frogs (*Lithobates pipiens*); 1 terrestrial adult, and 10 larval Tiger Salamanders (*Ambystoma mavortium*); and 9 adult and 6 metamorphic Boreal Toads (*Anaxyrus boreas boreas*). Additionally, one salvaged (i.e., found dead) Boreal Chorus Frog tadpole was collected. 42 of these 63 samples have been extracted for DNA and tested for pathogens using qPCR. Of the 42 swabs tested, 21 have tested positive for *Bd*. All 42 swabs have tested negative for Ranavirus. However, 30 samples still need to be analyzed (Table 3).

**Species Distribution and Climate Modeling**

*Data Preparation and Defining Variables*
To explore the potential effects of climate change on Boreal Toad habitat suitability in New Mexico and states to the north, we acquired and quality-checked Boreal Toad occurrence data from the Global Biodiversity Information Facility (GBIF) in consultation with species experts. We downloaded 2,347 Boreal Toad occurrence records from GBIF (2024, https://doi.org/10.15468/dl.kdwyqy). To explore the potential risk to Boreal Toads from disease prevalence, we produced a spatially explicit *Bd* exposure risk map to highlight areas of low, medium, and high risk to the toad.*Bd* presence data were sourced from *Bd*-Maps, which presents a globally aggregated *Bd* occurrence dataset (Olson et al. 2021).

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 data for 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). The

WorldClim dataset includes 24 variables covering temperature, precipitation, and solar radiation. EarthEnv includes five topographic measures. Then, we analyzed geospatial data to compile a database for modeling the current habitat suitability and distribution of the Boreal Toad. We used species distribution modeling algorithms (e.g., Maxent and Boosted Regression Trees) to build a preliminary habitat suitability map for current conditions (Figure 2) and explore the relationship between habitat suitability and environmental variables. Model due diligence included addressing multicollinearity (De Marco and Nóbrega 2018) among predictor variables using correlations and the variance inflation factor (Dormann et al. 2013).

*Model Fitting and Evaluation*

True absence data (i.e., instances confirming a species' absence in a specific location), are rarely available (Mackenzie and Royle 2005); therefore, researchers generate these data points by choosing locations where the species has not been observed (Zbinden et al. 2024). Species distribution models were built for both the Boreal Toad and presence of *Bd* using similar methods. For example, a total of 2,000 random background points (also known as pseudo-absence) were generated across the extent of each species, Boreal Toad and *Bd* ranges in North America to represent pseudo-absence conditions. Occurrence and environmental data were split into 80/20 training and testing subsets. Each model was fitted using boosted regression trees (Hijmans et al. 2017), a powerful machine learning method that builds trees iteratively, corrects errors of previous trees, and combines top results for predictions (Elith et al. 2008). To assess the predictive performance of the species distribution models, performance of each was assessed on the independently withheld 20% testing data.

*Habitat Suitability Predictions and Climate Predictions*

For each of the Boreal Toad and *Bd* models, habitat suitability was predicted across the full extent in relation to their respective environmental predictors. Suitability values represent the modeled probability of occurrence, ranging from 0 to 1, indicating no to high suitability. For each of the Boreal Toad and *Bd*, predicted presence maps are created to represent binary spatial data into suitable and unsuitable.

To evaluate how Boreal Toad habitat suitability may shift under future climate conditions, we trained the model to generate predictions for future scenarios (called ACCESS-CM2 General Circulation Model; Fick & Hijmans 2017). These climate scenarios are of Earth’s atmosphere, oceans, sea ice, and land surface temperature out to the year 2100. For future predictions of Boreal Toad habitat suitability, we included two types of models with different predictor variables: static environmental variables (e.g., topography or habitat), which were incorporated into the current predictor model, and climate variables, which were replaced with their corresponding future scenario (Johnson et al. 2025; Mi et al. 2023). By adjusting the climate variables to their projected values and holding the environmental variables constant, we isolated the impact of climate change on Boreal Toad predicted habitat suitability.

*Model Results – Habitat Suitability Patterns, Risk Analysis, and Effects of Climate Change*

We display habitat suitability within the IUCN range (IUCN SSC Amphibian Specialist Group, 2022) for Boreal Toad (Fig 2). Boreal Toad species distribution modeling found that the mean temperature of the wettest quarter was the most influential predictor, followed by the standard deviation of solar radiation (showing variability), mean solar radiation, elevation, and annual

precipitation. These five variables suggest that thermal conditions, solar availability, and topographic position are the primary drivers of habitat suitability for Boreal Toad across the study extent. The predicted habitat suitability for Boreal Toad was concentrated across northern Oregon and Washington through the Idaho Rockies and into southwestern British Columbia, with a secondary high-suitability region across western and central Colorado extending into portions of northern New Mexico (Figure 2A).

The species distribution model for *Bd* found that mean solar radiation was the most influential predictor, suggesting that solar availability is a primary determinant of *Bd* distribution across the study extent. This was followed by landscape contrast and roughness, indicating that habitat heterogeneity and topographic complexity also play a meaningful role in structuring *Bd* occurrence. The dominant role of solar radiation metrics is consistent with the known sensitivity of *Bd* to temperature and moisture, because solar input influences both substrate temperature and evapotranspiration (Woodhams et al. 2008). Predicted habitat suitability for *Bd* had a broader spatial extent than predicted habitat suitability for the Boreal Toad. It was more strongly associated with coastal and lower-elevation regions, with the highest values concentrated along the Pacific coast from California through British Columbia, and moderate suitability distributed across the interior west (Figure 2B). Despite the contrast in suitability distributions, spatial overlap between *Bd* and the Boreal Toad was evident in the Pacific Northwest coastal region and across Colorado, extending into portions of northern New Mexico, indicating potential disease risk.

The *A. b. boreas* habitat suitability was thresholded at 0.31 (derived threshold produced from the boosted regression model) to delineate suitable habitat, with all cells at or above 0.31 classified as suitable. The suitability class ranges are as follows: 0.310–0.787 (low), 0.787–0.948 (moderate), and 0.948–0.999 (high). Within the IUCN range for the Boreal Toad (IUCN SSC Amphibian Specialist Group 2022), approximately 35.1% of suitable habitat overlapped with areas of predicted *Bd* presence. Within this at-risk habitat, we partitioned the *A. b. Boreas* suitability values into three equal classes using tertile breaks. Predicted *Bd* presence applied to all at-risk cells, risk level was determined by the degree of toad habitat suitability: cells with the highest suitability coinciding with predicted *Bd* presence were classified as high risk, representing the most suitable habitat under the greatest disease exposure. For example, within the Boreal Toad extent that overlaps with *Bd*, the risk categories are defined as follows: low = 11.6% overlap, moderate = 11.7% overlap, and high = 12.6% overlap of the total suitable habitat (Figure 2C).

We assessed habitat suitability for Boreal Toad under four shared socio-economic pathway (SSP) scenarios (SSP126, SSP245, SSP370, and SSP585), which range from the most optimistic (i.e., SSP126) to the most pessimistic emission projections (SSP585; IPCC 2022). Specifically, the low pathway scenario (SSP126) reflects progressive action towards sustainable development to limit emissions to produce approximately +1.8°C by 2100. In contrast, SSP585 reflects high emission projections of approximately +4.4°C by 2100 and *status quo* trajectories on continued social, economic, and energy practices. By 2081–2100, range-wide Boreal Toad suitable habitat was projected to decrease by 22.19% (SSP126), 24.30% (SSP245), 25.98% (SSP370), and 26.74% (SSP585; Figure 3). We also evaluated habitat loss across four time periods (2021-2040, 2041-2060, 2061-2080, and 2081-2100). Pronounced regional losses were apparent across the

species' southern range as early as 2021–2040, with states including Arizona, New Mexico, Colorado, Utah, and southern California exhibiting substantial habitat loss under even moderate emission scenarios. Arizona and New Mexico showed particularly severe early declines, with projected losses of 86.94% and 67.49% by 2021-2040 under SSP585. Unfortunately, models predicted that losses will escalate across subsequent time periods, approaching near-complete local habitat loss by 2081–2100 under high-emission (SSP585) conditions: Arizona (99.92%), New Mexico (99.65%), Colorado (70.19%), Utah (49.62%), and California (24.62%).

**Project Conclusions to Date**

Based on a large dataset of Boreal Toad occurrence data from GBIF (2026), the area of northern New Mexico did represent suitable habitat for the species. However, conclusions from the modeling show that there is also high habitat suitability for *Bd* under current environmental conditions in northern New Mexico, suggesting that the Boreal Toad is under moderate to high risk of exposure to *Bd* in the study system. This is further demonstrated by the several *Bd*-positive samples collected during our study throughout the spring at Trout Lakes. Preliminary findings for habitat suitability for the Boreal Toad in the future show very low suitability in northern New Mexico and near total loss of suitable habitat by 2081-2100. Additionally, the results of mapping normalized difference water index (NDWI) show visible differences between surface water area between the years of 2011 and 2022 (Figure 4). During this period there is an overall decrease in open water and wetland/shallow areas, with an overall increase in dry surface area. These early findings suggest that low habitat suitability for Boreal Toads coupled with high *Bd* habitat suitability makes current and future conditions difficult for Boreal Toads to persist in northern New Mexico. Notably, up until the date that Boreal Toads were re-released by the New Mexico Department of Wildlife at the Trout Lakes ecosystem in May2026, we were unable to detect Boreal Toads via visual surveys and eDNA analyses there. We are still in the process of analyzing water filters collected in May and June for the presence of Boreal Toad DNA.

**Project Activities Planned for Year 2**

We will continue to explore these findings and validate the results. In upcoming months, we will analyze the remaining swabs and filters for presence of pathogen and Boreal Toad DNA using qPCR. We also plan to make 3 more trips to Trout Lakes in July, August and October to collect habitat data, water samples, and tissue swabs. We will conduct logistic regression models on habitat data collected throughout the Year 2 field season and compare these statistical analyses to data that we collected in Year 1. Finally, we plan to submit habitat suitability models and climate assessment models for publication in peer-reviewed journals.

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## Tables and Figures

**Table 1.** Summary of vegetation and aquatic structural variables measured in lentic and lotic sites at the Trout Lakes system, Carson National Forest, NM. Values represent means and standard error values (SE) from 2026 data collection as of 06/13/2026. Samples sizes (n) will change based on future work. Variables were compared using Wilcoxson rank-sum test, which indicates differences in system type (i.e., lentic and lotic).

| Variable | Lentic Type Mean (±SE) | n | Lotic Type Mean (±SE) | n | Difference, lentic vs. lotic (p-value) |
|---|---|---|---|---|---|
| Surface Area (m) | 1,855.50 (494.88) | 29 | 6.04 (0.72) | 12 | <0.001 |
| Air Temperature (°C) | 22.50 (0.63) | 28 | 21.05 (1.21) | 12 | 0.22 |
| Water Temperature (°C) | 19.72 (0.76) | 29 | 9.87 (0.04) | 12 | <0.001 |
| Relative Humidity (%) | 28.1 (1.58) | 29 | 41.08 (5.25) | 12 | 0.05 |
| Turbulence | 0.66 (0.10) | 29 | 2.82 (0.30) | 12 | <0.001 |
| Turbidity | 2.38 (0.23) | 29 | 1.08 (0.08) | 12 | <0.001 |
| Floating Vegetation Cover (%) | 8.33 (3.36) | 29 | 0 (0.00) | 12 | <0.001 |
| Submerged Vegetation Cover (%) | 33.06 (5.59) | 27 | 1.00 (0.43) | 12 | <0.001 |
| Emergent Vegetation Cover (%) | 15.35 (2.48) | 29 | 31.38 (7.86) | 12 | 0.08 |
| Perimeter Vegetation Cover (%) | 91.72 (3.08) | 29 | 73.33 (6.72) | 12 | <0.001 |
| Algae Cover (%) | 22.69 (5.38) | 29 | 0 (0.00) | 12 | <0.001 |
| Perimeter Grass/ Herb Cover (%) | 95.43 (1.30) | 29 | 71.67 (7.21) | 12 | <0.001 |
| Perimeter Shrub Cover (%) | 1.71 (1.27) | 29 | 4.64 (2.03) | 11 | 0.06 |
| Perimeter Tree Cover (%) | 18.48 (4.64) | 29 | 35.46 (6.55) | 12 | 0.01 |
| Bedrock Substrate (%) | 0 (0.00) | 11 | 0 (0.00) | 3 | NA |
| Boulder Substrate (%) | 0.96 (0.52) | 11 | 15.42 (11.87) | 3 | 0.14 |
| Cobble Substrate (%) | 1.84 (0.81) | 11 | 22.67 (14.01) | 3 | 0.03 |
| Pebble Substrate (%) | 1.77 (0.97) | 11 | 33.33 (26.19) | 3 | 0.06 |
| Sand Substrate (%) | 1.36 (1.01) | 11 | 20.5 (8.67) | 3 | 0.01 |
| Silt Substrate (%) | 96.14 (1.09) | 11 | 59.17 (19.22) | 3 | 0.06 |
| Litter Depth (cm) | 13.83 (3.66) | 11 | 1.88 (0.18) | 3 | 0.01 |
| Bare Ground Cover (%) | 10.46 (3.40) | 11 | 21.67 (14.81) | 3 | 0.58 |

| | | | | | |
|---|---|---|---|---|---|
| Grass/ Litter Ground Cover (%) | 71.36 (5.40) | 11 | 20 (5.00) | 3 | 0.02 |
| Woody Ground Cover (%) | 7.73 (2.73) | 11 | 33.33 (8.82) | 3 | 0.02 |
| Live Vegetation Ground Cover (%) | 9.55 (4.13) | 11 | 16.67 (1.67) | 3 | 0.15 |
| Stone Ground Cover (%) | 1.36 (0.70) | 11 | 8.33 (8.33) | 3 | 0.70 |
| Woody Debris Size Class A Count/ 10m | 9.06 (4.47) | 11 | 31.67 (15.90) | 3 | 0.43 |
| Woody Debris Size Class B Count/ 10m | 9.77 (3.97) | 11 | 33.50 (9.00) | 3 | 0.06 |
| Woody Debris Size Class C Count/ 10m | 10.82 (2.51) | 11 | 23.00 (5.35) | 3 | 0.06 |
| Canopy Cover (%) | 24.8 (3.93) | 11 | 62.91 (0.82) | 3 | 0.01 |
| Plot Grass/ Herb Cover (%) | 77.10 (4.42) | 11 | 27.63 (6.12) | 3 | 0.01 |
| Plot Shrub Cover (%) | 2.48 (1.99) | 11 | 1.25 (0.25) | 3 | 0.10 |
| Plot Tree Cover (%) | 8.61 (3.97) | 11 | 17.04 (7.01) | 3 | 0.20 |

**Table 2.** Water samples collected in 2026 and filtered for eDNA at Trout Lakes, Carson National Forest, NM. No filters have tested positive for Boreal Toad or Ranavirus DNA. Volume is the approximate amount of water filtered per sample.

| Filter Sample | Volume (mL) | Site | Date | qPCR result |
|---|---|---|---|---|
| F50 | 500 | 1.1 | 5/12/2026 | Tested negative for *Bd* |
| F51 | 475 | 1.12 | 5/12/2026 | Tested negative for *Bd* |
| F52 | 500 | 1.13 | 5/12/2026 | Tested negative for *Bd* |
| F53 | 625 | 1.2 | 5/12/2026 | Tested negative for *Bd* |
| F54 | 300 | 2.1 | 5/13/2026 | Tested negative for *Bd* |
| F55 | 475 | 2.2 | 5/13/2026 | Tested negative for *Bd* |
| F56 | 500 | 2.3 | 5/13/2026 | Tested negative for *Bd* |
| F57 | 200 | 3.1 | 5/14/2026 | Tested negative for *Bd* |
| F58 | 225 | 3.2 | 5/14/2026 | Tested negative for *Bd* |
| F59 | 525 | 3.3 | 5/14/2026 | Tested negative for *Bd* |
| F60 | 200 | 3.4 | 5/14/2026 | Tested negative for *Bd* |
| F61 | 525 | 3.42 | 5/14/2026 | Tested negative for *Bd* |
| F62 | 400 | 3.5 | 5/14/2026 | Tested negative for *Bd* |
| F63 | 300 | 3.6 | 5/14/2026 | Tested negative for *Bd* |
| F64 | 375 | 5.1 | 5/16/2026 | Tested negative for *Bd* |
| **F65** | **425** | **5.2** | **5/16/2026** | **Tested positive for *Bd*** |
| F66 | 425 | 5.3 | 5/16/2026 | Tested negative for *Bd* |
| F67 | 400 | 4.1 | 5/17/2026 | Tested negative for *Bd* |
| F68 | 400 | 4.2 | 5/17/2026 | Tested negative for *Bd* |
| F69 | 500 | 4.3 | 5/17/2026 | Tested negative for *Bd* |
| F70 | 875 | 4.4 | 5/17/2026 | Tested negative for *Bd* |
| F71 | 500 | 1.1 | 6/9/2026 | Tested negative for *Bd* |
| F72 | 450 | 1.12 | 6/9/2026 | Tested negative for *Bd* |
| F73 | 475 | 1.13 | 6/9/2026 | Tested negative for *Bd* |
| F74 | 475 | 1.2 | 6/10/2026 | Tested negative for *Bd* |

| | | | | |
|---|---|---|---|---|
| F75 | 100 | 2.1 | 6/10/2026 | Tested negative for *Bd* |
| F76 | 475 | 2.2 | 6/10/2026 | Tested negative for *Bd* |
| F77 | 450 | 2.3 | 6/11/2026 | Tested negative for *Bd* |
| F78 | 150 | 3.1 | 6/11/2026 | Tested negative for *Bd* |
| F79 | 275 | 3.3 | 6/11/2026 | Tested negative for *Bd* |
| F80 | 500 | 3.5 | 6/12/2026 | Tested negative for *Bd* |
| F81 | 325 | 3.6 | 6/12/2026 | Tested negative for *Bd* |
| F82 | 400 | 4.1 | 6/12/2026 | Tested negative for *Bd* |
| F83 | 500 | 4.3 | 6/13/2026 | Tested negative for *Bd* |
| F84 | 475 | 4.2 | 6/13/2026 | Tested negative for *Bd* |
| F85 | 350 | 5.1 | 6/13/2026 | Tested negative for *Bd* |
| F86 | 375 | 5.2 | 6/13/2026 | Tested negative for *Bd* |
| F87 | 500 | 5.3 | 6/13/2026 | Tested negative for *Bd* |
| F88 | 275 | 4.1 | 7/13/2026 | Waiting for DNA analyses |
| F89 | 350 | 4.2 | 7/13/2026 | Waiting for DNA analyses |
| F90 | 175 | 1.1 | 7/14/2026 | Waiting for DNA analyses |
| F91 | 275 | 4.3 | 7/13/2026 | Waiting for DNA analyses |
| F92 | 225 | 1.12 | 7/14/2026 | Waiting for DNA analyses |
| F93 | 250 | 1.2 | 7/14/2026 | Waiting for DNA analyses |
| F94 | 375 | 1.13 | 7/14/2026 | Waiting for DNA analyses |
| F95 | 500 | 2.2 | 7/14/2026 | Waiting for DNA analyses |
| F96 | 450 | 5.1 | 7/15/2026 | Waiting for DNA analyses |
| F97 | 400 | 5.2 | 7/15/2026 | Waiting for DNA analyses |
| F98 | 350 | 5.3 | 7/15/2026 | Waiting for DNA analyses |
| F99 | 375 | 3.3 | 7/16/2026 | Waiting for DNA analyses |
| F100 | 225 | 3.4 | 7/16/2026 | Waiting for DNA analyses |
| F101 | 200 | 3.6 | 7/16/2026 | Waiting for DNA analyses |
| F102 | 275 | 1.1 | 8/10/2026 | Waiting for DNA analyses |
| F103 | 225 | 1.12 | 8/10/2026 | Waiting for DNA analyses |

| | | | | |
|---|---|---|---|---|
| F104 | 300 | 1.13 | 8/10/2026 | Waiting for DNA analyses |
| F105 | 400 | 1.2 | 8/10/2026 | Waiting for DNA analyses |
| F106 | 400 | 2.2 | 8/11/2026 | Waiting for DNA analyses |
| F107 | 375 | 2.3 | 8/11/2026 | Waiting for DNA analyses |
| F108 | 525 | 3.3 | 8/12/2026 | Waiting for DNA analyses |
| F109 | 475 | 3.4 | 8/12/2026 | Waiting for DNA analyses |
| F110 | 500 | 3.5 | 8/12/2026 | Waiting for DNA analyses |
| F111 | 275 | 3.6 | 8/12/2026 | Waiting for DNA analyses |
| F112 | 450 | 4.1 | 8/13/2026 | Waiting for DNA analyses |
| F113 | 375 | 4.2 | 8/13/2026 | Waiting for DNA analyses |
| F114 | 150 | 5.1 | 8/13/2026 | Waiting for DNA analyses |
| F115 | 175 | 5.2 | 8/14/2026 | Waiting for DNA analyses |
| F116 | 350 | 5.3 | 8/14/2026 | Waiting for DNA analyses |

**Table 3.** Amphibians captured and swabbed at Trout Lakes in May and June of 2026. All amphibians except one salvaged tadpole were swabbed and released.

| Date | Species | Salvage | Age | Location description | Disposition |
|---|---|---|---|---|---|
| 5/13/2026 | *Lithobates pipiens (S17)* | No | Adult | Found in marshy areas around the original repatriation pond. | *Bd* negative |
| **5/13/2026** | ***L. pipiens (S31)*** | **No** | **Adult** | **Found in marshy areas around the original repatriation pond.** | **Tested positive for *Bd*** |
| **5/13/2026** | ***L. pipiens (S32)*** | **No** | **Adult** | **Found in marshy areas around the original repatriation pond.** | **Tested positive for *Bd*** |
| **5/13/2026** | ***Pseudacris maculata (S13)*** | **No** | **Adult** | **Found in marshy areas around the original repatriation pond.** | **Tested positive for *Bd*** |
| **5/13/2026** | ***P. maculata (S14)*** | **No** | **Adult** | **Found in marshy areas around the original repatriation pond.** | **Tested positive for *Bd*** |
| 5/13/2026 | *P. maculata (S15)* | No | Adult | Found in marshy areas around the original repatriation pond. | *Bd* negative |
| **5/13/2026** | ***P. maculata (S16)*** | **No** | **Adult** | **Found in marshy areas around the original repatriation pond.** | **Tested positive for *Bd*** |
| 5/13/2026 | *P. maculata (S18)* | No | Adult | Found in marshy areas around the original repatriation pond. | Bd negative |
| **5/13/2026** | ***P. maculata (S19)*** | **No** | **Adult** | **Found in marshy areas around the original repatriation pond.** | **Tested positive for *Bd*** |
| 5/13/2026 | *P. maculata (S20)* | No | Adult | Found in marshy areas around the original repatriation pond. | *Bd* negative |
| **5/13/2026** | ***L. pipiens (S25)*** | **No** | **Adult** | | **Tested positive for *Bd*** |
| **5/13/2026** | ***L. pipiens (S26)*** | **No** | **Adult** | | **Tested positive for *Bd*** |
| **5/14/2026** | ***P. maculata (T1)*** | **Yes** | **Tadpole** | **Dead tadpole captured with dipnet. Preserved in ethanol** | **Tested positive for *Bd*** |
| **5/13/2026** | ***L. pipiens (S30)*** | **No** | **Adult** | **Found in grass around the middle fishing pond on transect 5** | **Tested positive for *Bd*** |
| 5/16/2026 | *L. pipiens (S33)* | No | Adult | Found in grass around the middle fishing pond on transect 5 | Bd negative |

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| **5/13/2026** | ***L. pipiens (S27)*** | **No** | **Adult** | **Found around the upper fishing pond on transect 5** | **Tested positive for *Bd*** |
| **5/13/2026** | ***L. pipiens (S29)*** | **No** | **Adult** | **Found around the upper fishing pond on transect 5** | **Tested positive for *Bd*** |
| **5/13/2026** | ***P. maculata (S28)*** | **No** | **Adult** | **Found around the upper fishing pond on transect 5** | **Tested positive for *Bd*** |
| 5/13/2026 | *P. maculata (S21)* | No | Adult | Found at the grassy northern edge of the upper fishing pond (transect 4) | *Bd* negative |
| **5/13/2026** | ***P. maculata (S22)*** | **No** | **Adult** | **Found at the grassy northern edge of the upper fishing pond (transect 4)** | **Tested positive for *Bd*** |
| **5/13/2026** | ***P. maculata (S23)*** | **No** | **Adult** | **Found at the grassy northern edge of the upper fishing pond (transect 4)** | **Tested positive for *Bd*** |
| **5/13/2026** | ***P. maculata (S24)*** | **No** | **Adult** | **Found at the grassy northern edge of the upper fishing pond (transect 4)** | **Tested positive for *Bd*** |
| **6/10/2026** | ***L. pipiens (S34)*** | **No** | **Adult** | **Caught in minnow trap.** | **Tested positive for *Bd*** |
| **6/11/2026** | ***Ambystoma mavortium (S35)*** | **No** | **Adult** | **Caught in minnow trap** | **Tested positive for *Bd*** |
| 6/11/2026 | *P. maculata (S36)* | No | Adult | Found around a dry pond on transect 3 | Bd negative |
| 6/12/2026 | *A. mavortium (S38)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Bd negative |
| 6/12/2026 | *A. mavortium (S39)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 6/12/2026 | *P. maculata (S37)* | No | Tadpole | Captured by dipnet at the northern most pond of transect 3 | Bd negative |
| 6/12/2026 | *L. pipiens (S40)* | No | Adult | Found in a flooded cow print on the northern shore of the upper fishing pond (transect 4) | Bd negative |
| **6/12/2026** | ***L. pipiens (S41)*** | **No** | **Adult** | **Found in a flooded cow print on the northern shore of the upper fishing pond (transect 4)** | **Tested positive for *Bd*** |
| **6/12/2026** | ***L. pipiens (S42)*** | **No** | **Adult** | **Found in a flooded cow print on the northern shore of the upper fishing pond (transect 4)** | **Tested positive for *Bd*** |

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| **6/12/2026** | ***L. pipiens (S44)*** | **No** | **Adult** | **Found in the water on the northern shore of the upper fishing pond (transect 4)** | **Tested positive for *Bd*** |
| 6/12/2026 | *Anaxyrus boreas boreas (S43)* | No | Adult | Found in the water on the northern edge of the upper fishing pond on transect 4 | Bd negative |
| 6/13/2026 | *A. b. boreas (S45)* | No | Adult | Found in a stream flowing into the upper fishing pond on transect 4. | Bd negative |
| 7/13/2026 | *A. b. boreas (S46)* | No | Adult | Found at the grassy northern edge of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 7/13/2026 | *A. b. boreas (S47)* | No | Adult | Found at the grassy northern edge of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 7/13/2026 | *A. b. boreas (S48)* | No | Adult | Found at the grassy northern edge of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 7/13/2026 | *A. b. boreas (S49)* | No | Adult | Found at the grassy northern edge of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 7/13/2026 | *A. b. boreas (S50)* | No | Adult | Found at the grassy northern edge of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 7/13/2026 | *L. pipiens (S51)* | No | Subadult | Found at the grassy northern edge of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 7/16/2026 | *L. pipiens (S52)* | No | Adult | Found on the west side of the northern most pond of transect 3. | Waiting for DNA analyses |
| 7/16/2026 | *A. mavortium (S53)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 7/16/2026 | *A. mavortium (S54)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 7/16/2026 | *A. mavortium (S55)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 7/16/2026 | *A. mavortium (S56)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 8/11/2026 | *A. b. boreas (S57)* | No | Metamorph | Found in the grasses on the north side of the original repatriation pond | Waiting for DNA analyses |

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| 8/11/2026 | *A. b. boreas (S58)* | No | Metamorph | Found in the grasses on the north side of the original repatriation pond | Waiting for DNA analyses |
| 8/11/2026 | *A. b. boreas (S59)* | No | Metamorph | Found in the grasses on the north side of the original repatriation pond | Waiting for DNA analyses |
| 8/11/2026 | *A. b. boreas (S60)* | No | Metamorph | Found in the grasses on the north side of the original repatriation pond | Waiting for DNA analyses |
| 8/11/2026 | *A. b. boreas (S61)* | No | Metamorph | Found in the grasses on the north side of the original repatriation pond | Waiting for DNA analyses |
| 8/11/2026 | *A. b. boreas (S62)* | No | Metamorph | Found in the grasses on the north side of the original repatriation pond | Waiting for DNA analyses |
| 8/12/2026 | *A. mavortium (S63)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 8/12/2026 | *A. mavortium (S64)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 8/12/2026 | *A. mavortium (S65)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 8/12/2026 | *A. mavortium (S66)* | No | Larvae | Captured by dipnet at the northern most pond of transect 3 | Waiting for DNA analyses |
| 8/12/2026 | *P. maculata (S67)* | No | Adult | Found on the west side of the northern most pond of transect 3. | Waiting for DNA analyses |
| 8/13/2026 | *A. b. boreas (S68)* | No | Adult | Found on the south side of the upper fishing pond (transect 4) | Waiting for DNA analyses |
| 8/13/2026 | *L. pipiens (S69)* | No | Adult | Found along the grassy edges of the southernmost pond of transect 5 | Waiting for DNA analyses |
| 8/13/2026 | *L. pipiens (S70)* | No | Adult | Found along the grassy edges of the southernmost pond of transect 5 | Waiting for DNA analyses |
| 8/13/2026 | *L. pipiens (S71)* | No | Adult | Found along the grassy edges of the southernmost pond of transect 5 | Waiting for DNA analyses |
| 8/13/2026 | *L. pipiens (S72)* | No | Adult | Found along the grassy edges of the southernmost pond of transect 5 | Waiting for DNA analyses |

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| 8/13/2026 | *A. b. boreas (S73)* | No | Adult | Found along the path on the southern edge of the fishing pond on transect 3 | Waiting for DNA analyses |
| 8/13/2026 | *L. pipiens (S74)* | No | Adult | Found along the path on the southern edge of the fishing pond on transect 3 | Waiting for DNA analyses |

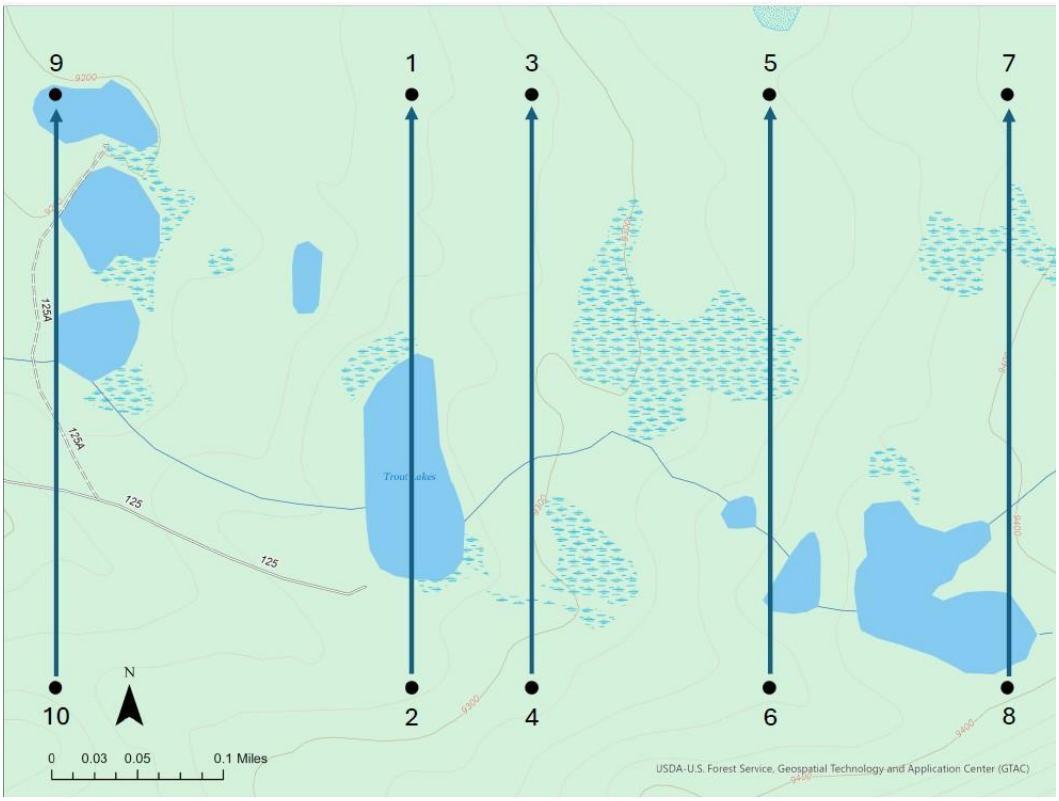



**Figure 1.** Map of transects in the Carson National Forest, NM.

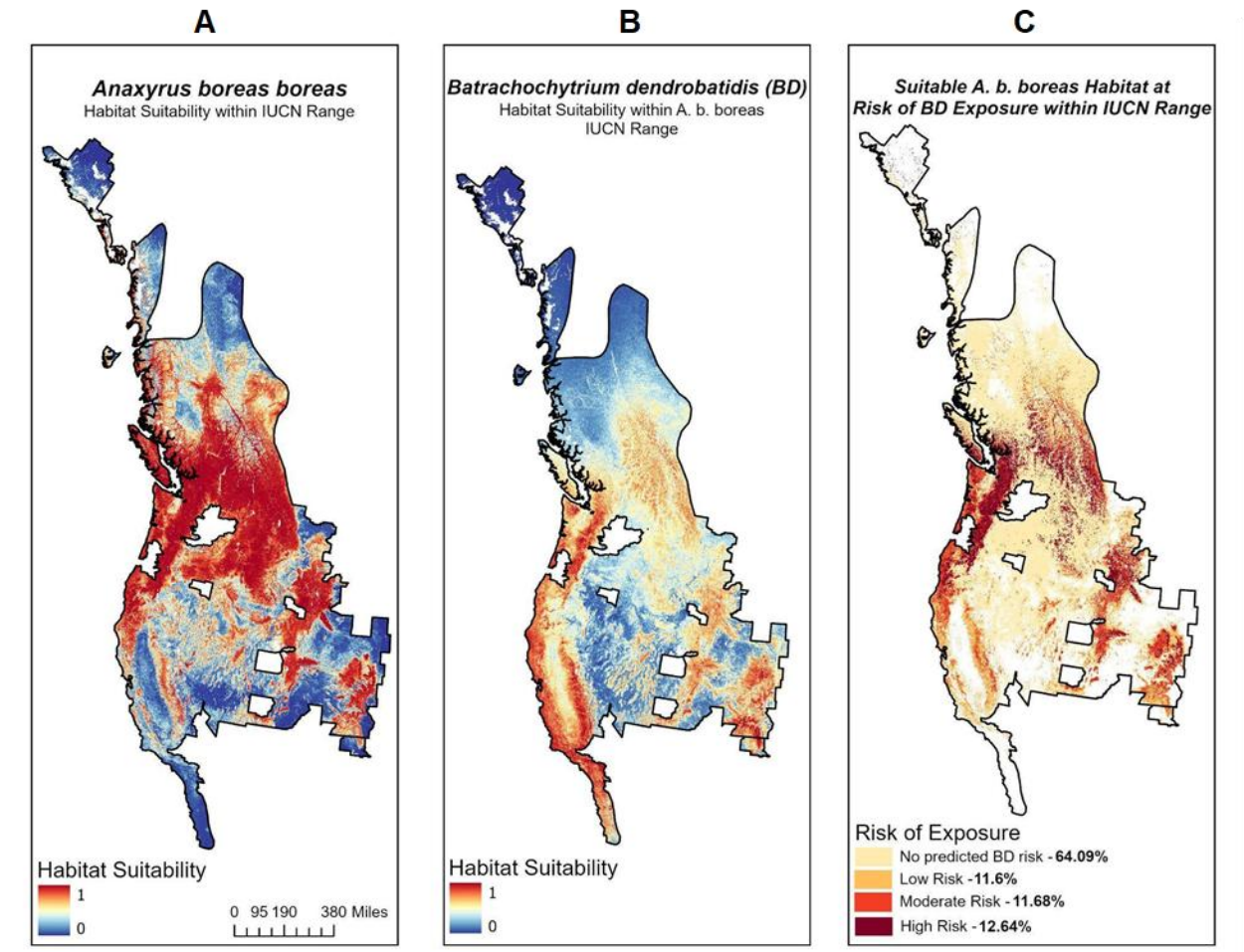



**Figure 2.** Three maps display the spatial extent of Boreal Toad (*Anaxyrus boreas boreas*) habitat suitability based on the IUCN range across North America. A) The geographic distribution of predicted habitat suitability for Boreal Toads across its range in North America. Warm colors indicate habitats of higher suitability (value of 1) and cool colors indicate less suitable habitat (value of 0). B) The spatial extent of Boreal Toad displaying the predicted habitat suitability for the disease pathogen, *Bd*. Warm colors indicate habitats of higher suitability (value of 1) and cool colors indicate less suitable (value of 0). C) The spatial extent of Boreal Toad habitat showing areas of suitable habitat overlapped with areas of predicted *Bd* presence (35.1% of the toad's range). Within this at-risk habitat, values are partitioned into low, moderate, and high-risk categories using tertile breaks. The risk categories reflect the degree to which suitable habitat overlaps with *Bd* presence.

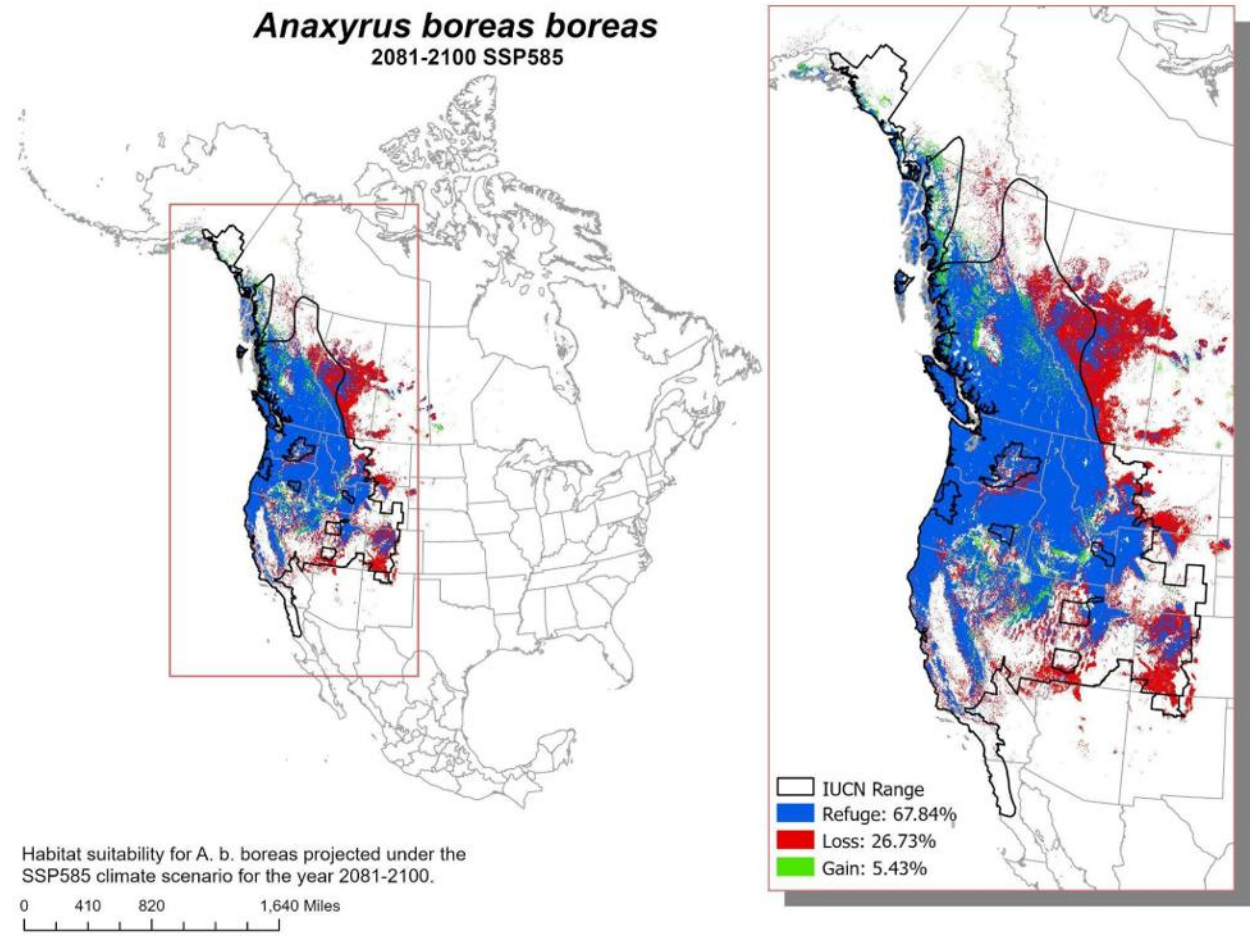



**Figure 3.** Habitat suitability for Boreal Toad (*Anaxyrus boreas boreas*) under shared socio-economic pathway 585 (status quo for the years 2081-2100. Map colors indicate areas that are likely to gain suitable habitat (green), lose habitat (red), or remain unchanged (blue).

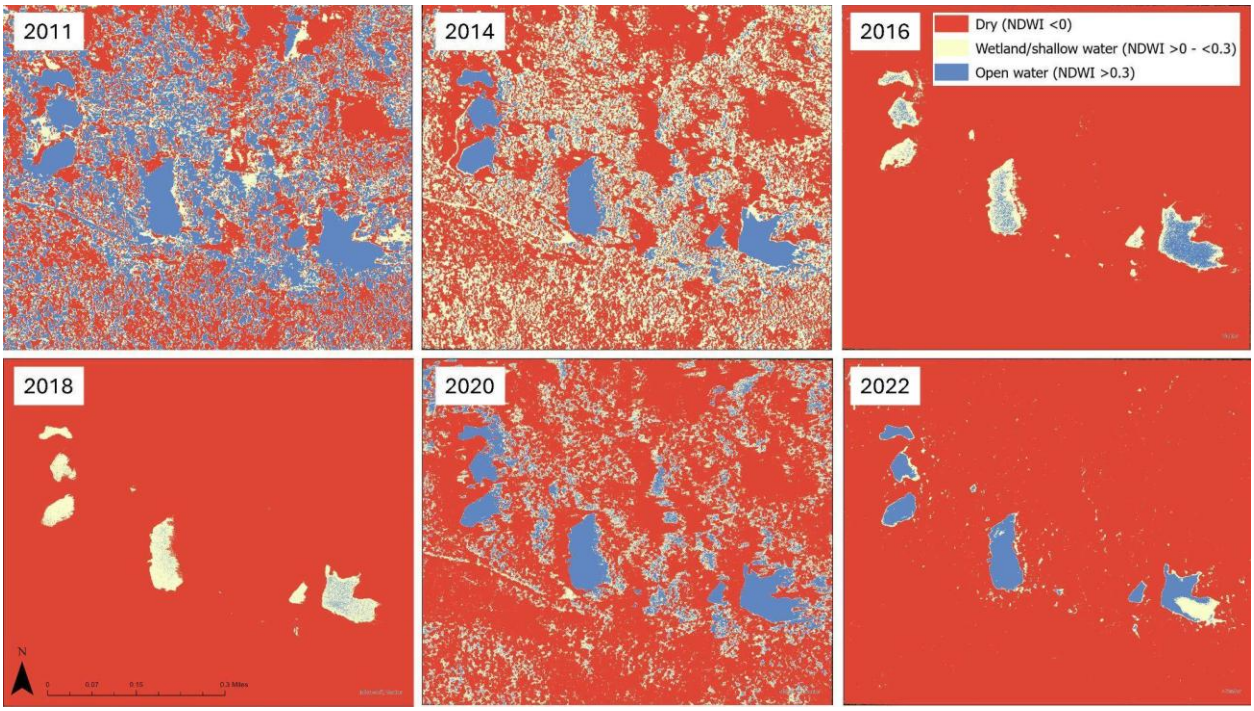



**Figure 4**. Maps of normalized difference of water index (NDWI) values at Trout Lakes created in Arc GIS Pro. Dry areas are represented in red, with NDWI values of less than zero. Wetland/ shallow water areas (i.e., with NDWI values between zero and 0.3) are represented in white. Open water (i.e., with NDWI values greater than 0.3 are represented in blue. Spatial data at this resolution (0.6 meters) was available for 2011, 2014, 2016, 2018, 2020, and 2022.



Swabbing a Tiger Salamander June 2026 (MJB)



Boreal Toad, May 2026 (JZ)



Example of lentic site, June 2026 (JZ)



Example of habitat data collection along a lotic system, June 2026 (MJB)

**Figure 5.** Methods and site types at Trout Lakes, Carson National Forest, NM. Photographs by Jenna Zarlingo (JZ) and Molly Bechtel (MJB).

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