Distribution and Threats to the Arizona Toad in Clark County
Final Project Report (D18)

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1. Executive Summary

The Arizona Toad (*Anaxyrus microscaphus*) has been petitioned for protection under the U.S. Endangered Species Act (ESA) and is under evaluation for inclusion as a Covered Species under the Clark County Multi Species Habitat Conservation Plan Amendment (Clark County Department of Comprehensive Planning and USFWS, 2001; USFWS, 2015b). Although the species is locally abundant in some parts of its range, Arizona Toads have not been confirmed in Clark County since at least the 1980s. Extensive amphibian surveys in the late 1990s and examination of museum specimens reported no incidence of non-hybridized Arizona Toads in Clark County (Bradford et al., 2005), indicating a need to assess the current distribution of the species and availability of potential habitat for recovery efforts at the western extent of its historical range.

This document reports on the results of 2 years of Clark County Desert Conservation Program (DCP) funding to assess the status of Arizona Toad populations in the study area. This project supports data collection for an ongoing Species Status Assessment for the Arizona Toad by the U.S. Fish and Wildlife Service (USFWS) and provides data to support management actions to create or preserve suitable habitat for the Arizona Toad on Clark County Riparian Reserve properties.

1.1. Project Objectives

The objectives of this project were:

- Assess the status of Arizona Toad in Clark County, Nevada, and surrounding areas by surveying >100 aquatic field sites using traditional, visual encounter surveys (VES) and environmental DNA (eDNA) survey methods.
- Assess habitat conditions, presence of invasive predators, and availability of water at surveyed sites.
- Assess the extent of apparent hybridization with Woodhouse's Toads (*Anaxyrus woodhousii*) in southern Nevada, northwestern Arizona, and southwestern Utah.
- Generate a habitat suitability map to identify potential aquatic habitat for future conservation actions.

1.2. Significant Results

Over the 2 years of data collection, we:

- Conducted 198 visual encounter surveys at 154 field sites, collected 295 eDNA filters from 131 field sites, and collected 142 DNA samples from adult *Anaxyrus* toads at 34 field sites.
- Using eDNA methods, we detected Arizona Toad DNA at 6 surveyed reaches of the Virgin River in eastern Clark County, providing the first evidence of the species in the County in approximately 40 years.
- Detected 7 amphibian species in the study area, including 2 other members of the genus *Anaxyrus* and documented the presence of individuals with morphological traits consistent with hybrid offspring of Arizona Toad x Woodhouse's Toad.

- Produced a habitat suitability map that identified the areas with high riparian canopy and shrub cover, moderate actual evapotranspiration (AET), and high stream permanence to support Arizona Toad populations in the study area.

2. Introduction

2.1. Description of Project

We assessed springs, rivers, streams, and other potentially suitable aquatic habitats for Arizona Toad to determine if it is present in Clark County. We surveyed aquatic habitats in southern Nevada, northwestern Arizona, and southwestern Utah (hereafter, the "study area") using 1) traditional visual encounter surveys to detect amphibians and collect data on relevant habitat characteristics, and 2) eDNA surveys to detect the presence of Arizona Toad, Woodhouse's Toad, Red-spotted Toad (*Anaxyrus punctatus*), and non-native American Bullfrog (*Lithobates* [*Rana*] *catesbeianus*). Field crews visited sites during 2 spring–summer field seasons (April–July 2024, 2025), conducting visual encounter surveys at all sites where water was encountered and collecting eDNA filters as possible.

2.2. Background and Need

2.2.1. Study Species and Declines

The Arizona Toad is endemic to the southwestern United States, where it is limited primarily to small and medium-sized streams and neighboring riparian areas. Arizona Toads are distributed from west central New Mexico, across central and northwestern Arizona, and into southern Nevada and Utah (Figure 2.1). Threats including habitat loss, drought-related reductions in surface water, hybridization with Woodhouse's Toads, and invasive species led to the species being petitioned for protection under the Endangered Species Act (ESA) in 2015 (USFWS, 2015b). The petition found the Arizona Toad may be warranted for ESA listing, but additional understanding of the current distribution of the species and its threats were needed.

Although the species remains locally abundant in some parts of its range, Arizona Toads have not been confirmed in Clark County since the 1980s, likely indicating a contraction of the species' historical distribution(Bradford et al., 2005). Examination of museum specimens in the 1990s confirmed that the museum records were identified correctly, verifying that Arizona Toads were historically present in the Las Vegas Valley; however, extensive surveys found no evidence the species was present at historical localities or that it still occurred in Clark County (Bradford et al., 2005). The lack of observations in Clark County in recent decades motivated assessment of the current distribution of the species and availability of potential habitat for recovery efforts.

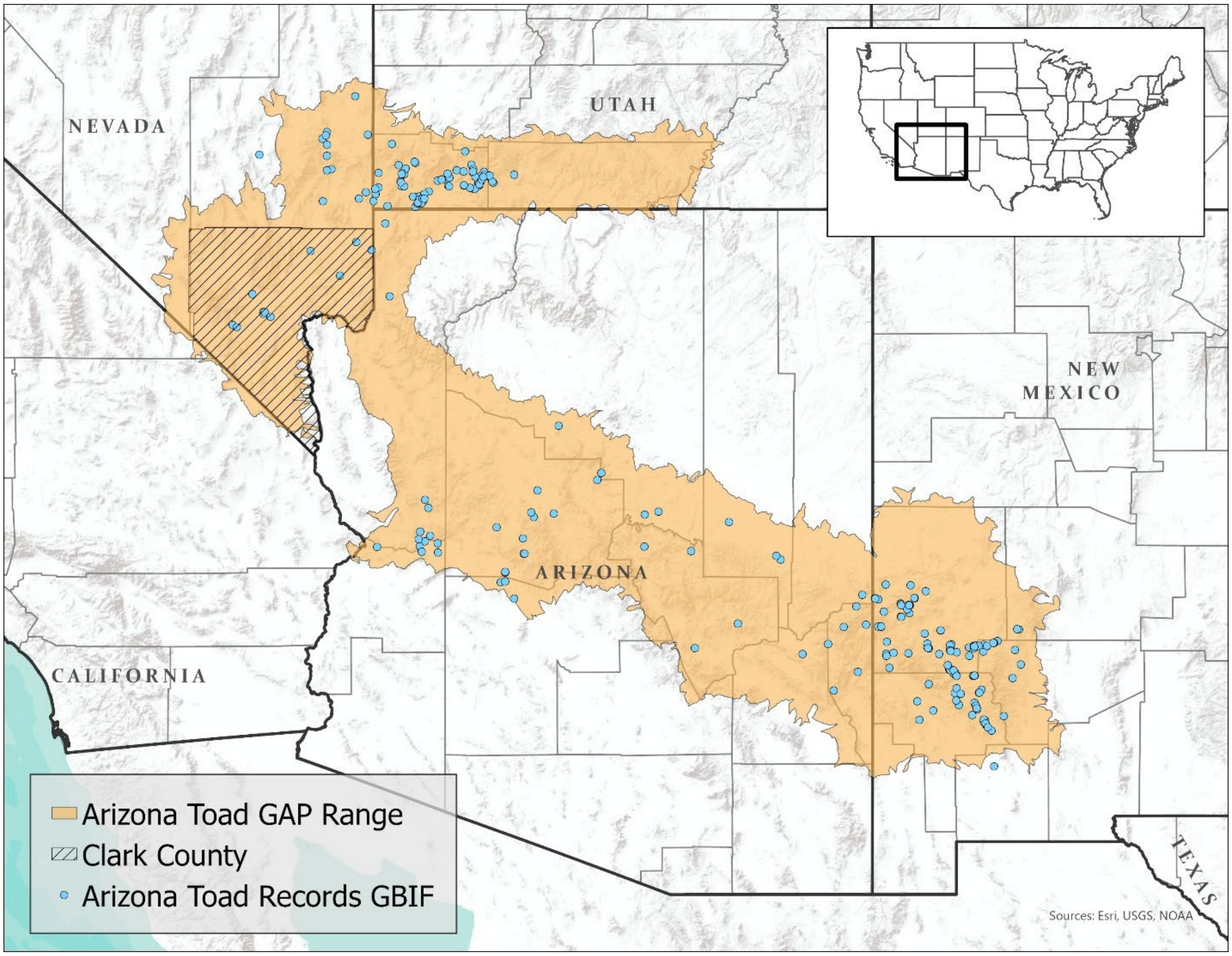

Figure 2.1 Approximate historical range of the Arizona Toad (orange; USGS GAP program; 10.5066/F7HD7TQD), with Clark County indicated with hash marks and records from the Global Biodiversity Information Facility (GBIF; https://www.gbif.org/) as blue dots.

2.2.2. Threats

Threats to the Arizona Toad include drought, habitat alteration and loss, hybridization with Woodhouse's Toads, and possibly introduced predators (Sullivan, 1986; 1993; Ryan et al., 2017a). The Arizona Toad requires shallow, low gradient springs and streams for breeding habitat (Schwaner & Sullivan, 2005). In southern Nevada, this habitat was historically present in riparian floodplains, wetlands, and spring-fed streams, but development and hydrologic alteration (e.g., impoundments, surface water modification, irrigation) have reduced the availability of suitable habitat (Sullivan & Lamb, 1988). And although Woodhouse's Toads are native to the region and historically were not common in smaller streams that form the core habitat for Arizona Toads, the additional of water impoundments has increased co-occurrence of the 2 toad species and facilitated hybridization in some areas (Sullivan et al., 2015).

In the petition for ESA listing, hybridization with Woodhouse's Toads was listed as one of the primary threats to Arizona Toads (USFWS, 2015a). Impoundment of streams has likely increased the threat of hybridization with Woodhouse's Toads, which breed primarily in standing waters and whose populations have been aided by hydrologic modification of waterbodies in the region (Blair, 1955; Sullivan et al., 2015; Ryan et al., 2017b). The replacement of Arizona Toads by Woodhouse's Toads has been suggested as a concern for Arizona Toads in central Arizona, especially as modification of habitat and surface waters continue (Sullivan and Lamb, 1988). Based on surveys of historical collection localities and examination of museum specimens, Bradford et al. (2005) concluded that Arizona Toads in Las Vegas Valley had been replaced by Woodhouse's Toads or hybrids in some locations. Despite apparent declines in Clark County, recent observations of Arizona Toads (<5 years) from both agency and citizen scientist from within 35 km of Clark County in neighboring Lincoln County, Nevada, and Washington County, Utah, suggest the Arizona Toad could still occur in Clark County or it could recolonize suitable habitat under favorable conditions.

2.3.3. Need for Project

In accordance with an agreement with the Clark County DCP, this project was designed to help Clark County determine whether the Arizona Toad still occurs in the county and help determine the extent of potential threats from development, invasive species, and hybridization with the Woodhouse's Toad. We also sought to determine areas of high suitability that may be used as recolonization, repatriation, or reintroduction sites.

2.3. Management Actions Addressed

This project will aid Clark County and the USFWS by providing information on the current distribution of the Arizona Toad at the edge of the species' historical distribution, where status was poorly understood prior to this project. Refining our understanding of habitat occupied by Arizona Toads in drier and lower elevation areas such as those common in southern Nevada will increase our understanding of the species' habitat requirements and help inform potential conservation options across its range. Additional species detections will also be used to refine our habitat suitability model for the region. Also, identifying properties or land management units that harbor populations of Arizona Toads will aid managers in protecting and managing those lands in ways that benefit conservation of native amphibians and aquatic species.

2.4. Project Goals

The goals of this project were to 1) help Clark County DCP determine whether the Arizona Toad still occurs in the county and determine the extent of potential threats from development and hybridization with the Woodhouse's Toad and 2) assess habitat conditions and presence of predators at 100 aquatic sites across the study area. This project has aided Clark County in identifying current Arizona Toad populations and providing data on extent of potential hybridization. Also, these surveys have aided in locating areas of suitable aquatic habitat for potential conservation actions and provided data to support further decisions about inclusion of the species under the ESA or the Clark County Multi Species Habitat Conservation Plan. Specific objectives of this project as dictated by the Scope of Work included:

- Use eDNA to sample habitat for presence of Arizona Toad and Woodhouse's Toad
- Conduct visual encounter surveys to determine presence and potential hybridization rates
- Produce a habitat suitability map for the Arizona Toad in the study area

3. Methods and Materials

3.1. Site Selection

3.1.1. Historical Records Database

To identify potential field sites for surveys, we gathered information on likely habitat from several sources. First, we compiled a database of historical Arizona Toad records in the study area using data from the Global Biodiversity Information Facility (GBIF), AmphibiaWeb (AmphibiaWeb | Home), and local partners at the Nevada Department of Wildlife, Utah Division of Wildlife Resources, and Arizona Game and Fish Department. We also included results from USGS crews who surveyed portions of the area during 2019–2020, as well as survey results from each season of data collection for this project. We obtained 2514 records of Arizona Toads in our study area (Table 3.1). Taxonomic designation of specimens in museum collections can reflect outdated taxonomy or misidentifications, and we continue to work with Arizona Game and Fish Department to obtain specimens and verify that records are of appropriate spatial resolution and confirmed taxonomy. We determined 2130 (84.7%) of the obtained records were verified and we extracted location information to identify the historical distribution of Arizona Toads in the study area, as well as sites that may have extant populations (Figure 3.1).

Table 3.1 Arizona Toad records and data sources used to build historical records database.

Dataset	No. records	Data Source
AmphibiaWeb	184	https://amphibiaweb.org/search/index.html, accessed February 2024
GBIF	250	https://www.gbif.org/occurrence, accessed September 2024
AZGFD	1069	Mason Ryan, written communication
USGS 2019-2020	10	Tornabene et al., 2026
USGS 2024-2025	31	This project
Utah DWR	970	Wheeler et al., 2025

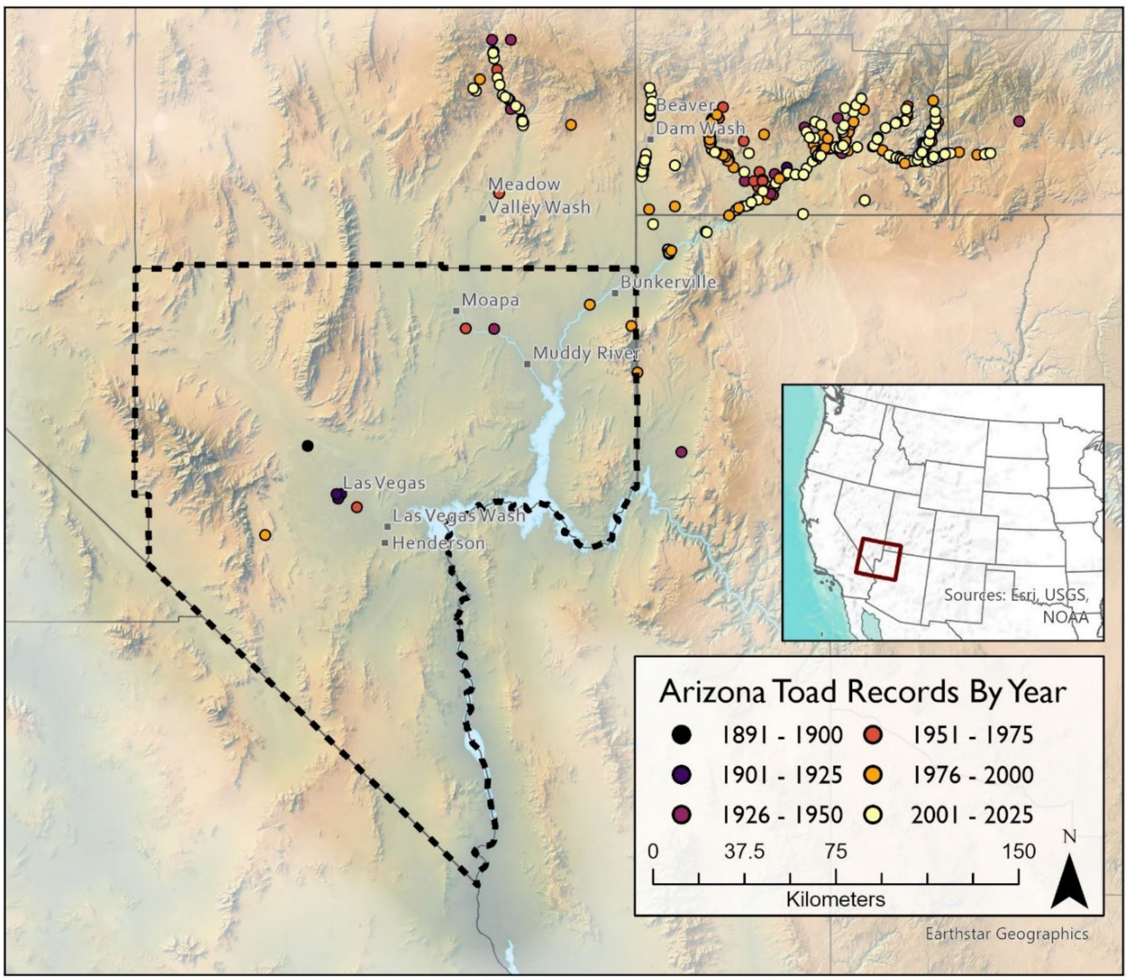

Figure 3.1 Map of Arizona Toad records in the study area, with Clark County outlined by the dashed line. Older records are black circles and more recent records are in orange and

yellow. Note the distribution of Arizona Toad records along river corridors in southern Utah and Lincoln County, Nevada.

3.1.2. Potential Site List Compilation

We compiled a list of >500 potential sites to visit in the study area (Figure 3.2) using a variety of sources to identify potential habitat. We first targeted Clark County-owned Riparian Reserves in Mesquite, Bunkerville, and Moapa as well as the Mormon Mesa properties because they are protected areas with high quality riparian habitat. We then built a site list by:

- Extracting previously known Arizona Toad occurrences from the historical records database;
- Identifying riparian corridors and perennial streams throughout the study area;
- Identifying known springs using the Streams Stewardship Institute and National Hydrography Dataset springs database (https://springsdata.org/);
- Using locations from previous amphibian studies in the study area (Blair, 1955; Bradford et al., 2005; Sullivan, 1993); and
- Direct communications from regional partners.

We prioritized sites with historical records in the study area and those with high-quality habitat. Survey sites also included recently documented populations of Arizona Toads in neighboring counties in Nevada, Arizona, and Utah. Because it was possible that all surveys within Clark County could yield zero observation of Arizona Toads, including survey sites outside of Clark County was important to provide recent occurrences to create an informed habitat suitability map and to show that our sampling methods were effective.

3.1.3. Sampling Groups

Survey sites were grouped geographically to aid sampling logistics (Figure 3.2). Sampling groups were as follows:

- <u>Virgin River Group</u> - Drainages feeding the Virgin River north and west of Lake Mead (Muddy River, Moapa Valley, Meadow Valley Wash, and Beaver Dam Wash), Clark County Riparian Reserves, and springs/riparian areas along Highway 167 in Lake Mead National Recreation Area.
- <u>Virgin Mountains Group</u> - The Virgin Mountains, Gold Butte National Monument (Azure Ridge and other springs/seeps), Lake Mead National Recreation Area east of Lake Mead, and Grand Canyon-Parashant National Monument (Tassi Ranch, Pakoon Springs, Grand Wash).
- <u>El Dorado Valley Group</u> - The El Dorado Valley south of Boulder City, and surrounding ranges including springs and seeps in Avi Kwa Ame National Monument, the Spirit Mountain, Ireteba Peaks, El Dorado, and Black Canyon Wilderness areas and North and South McCullough ranges.

- Sheep Range Group - The Sheep and Las Vegas ranges north of Las Vegas Valley in Desert National Wildlife Refuge (springs and streams near Corn Creek Bench, seeps and springs near Gass Peak and Fossil Ridge), Coyote Springs, Pahranagat National Wildlife Refuge.
- Spring Mountains Group - The Spring Mountain and La Madre ranges, including Spring Mountain Ranch State Park, Red Rock Canyon National Conservation Area, and the Mount Charleston area in Humboldt-Toiyabe National Forest.

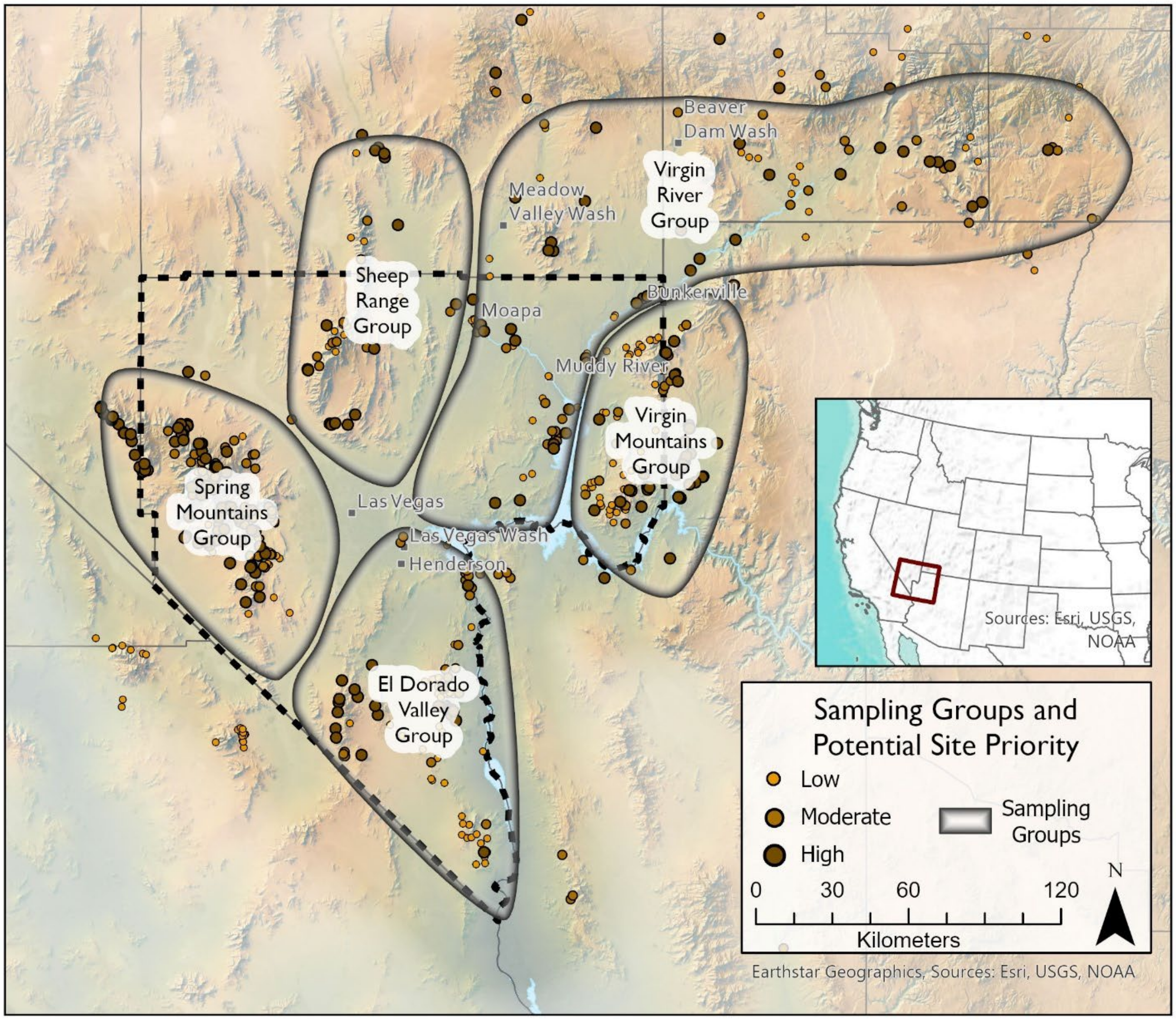

Figure 3.2 Approximate boundaries of sampling groups identified during project planning stages. Site group boundaries extended past Clark County (black dashed polygon) to capture locations of known Arizona Toad populations. Sites identified as potential targets are indicated by brown dots sized according to priority. We prioritized sites with historical records and those with perceived high-quality habitat.

3.2. Survey Timing and Schedule

3.2.1. Survey Structure

At each survey location, we performed both traditional, visual encounter surveys and eDNA surveys, if adequate water was present to collect eDNA filters. When there was insufficient water to collect eDNA filters (e.g., only a muddy pool), we only conducted a visual encounter survey of potential habitat. Typically, crews first visited a new site during the day to inspect the site for presence and amount of water. If adequate water was present, crews collected eDNA filters first to reduce the possibility of contaminating filters while surveying the site. Following collection of eDNA filters, crews conducted a daytime visual encounter survey. We conducted nighttime visual encounter surveys at as many sites as possible to increase the likelihood of detecting adult amphibians to confirm species identity (i.e., *Anaxyrus* larvae can be difficult to identify to species) and to collect buccal DNA samples and individual-level data from captured toads. We collected species-level presence/non-detection data on each site visit using both visual encounter surveys and eDNA detection methods, as well as relevant environmental characteristics (described below).

3.2.2. Survey Timing

We conducted surveys between April and July of 2024 and 2025. Surveys were designed to coincide with when larvae and metamorphs of Arizona Toads were likely to be present and detectable, as reported for southwest Utah (Blair, 1955). Also, 2019 surveys for Arizona Toads in Lincoln County by USGS crews observed Arizona Toad larvae and metamorphs during July, indicating summer sampling is appropriate for this species (Tornabene et al., 2026).

3.2.3. Site Definitions

Sampled sites represented shorelines of streams, reservoirs, springs, and other waterbodies, or discrete standing water bodies. We typically surveyed ≤100m of shoreline or stream length. Several sites had adequate surface water to survey for up to 600m, but this was uncommon. Site type was recorded as lentic (standing water) or lotic (flowing water) according to current conditions and sites were assigned to a dominant waterbody type (e.g., spring, stream, reservoir).

3.3. eDNA Surveys

We collected eDNA samples from sites with adequate water to test for presence of DNA from the Arizona Toad, Woodhouse's Toad, Red-spotted Toad, and American Bullfrog. eDNA is a sampling method that is especially effective for cryptic or rare species (Goldberg et al., 2018). Briefly, water is filtered directly from the site to collect suspended DNA using a battery-operated peristaltic or vacuum pump and eDNA filters. eDNA sampling is prone to contamination via contact with other sampling gear, shoes, or dirt that has been transferred between sites (Goldberg et al., 2018). To avoid contaminating samples and reduce chances of spreading pathogens among sites, crews followed strict disinfection protocols that included sanitizing equipment with a strong solution of household bleach and by collecting eDNA filters without contacting site water before any other surveys begin.

After filters were collected, they were stored in a cool, dry environment before being sent for analysis at an eDNA laboratory at Washington State University.

To collect eDNA filters, water must be of adequate depth to submerge the tip of the filter housing without contacting the sediment or bottom of the water body, which occasionally limited our ability to collect filters at very small or shallow sites. When we were able to filter water, we collected between 1 and 3 eDNA filters per site, depending on the surface area and volume of water present, the volume of water that could be filtered, and amount of habitat available. As a quality assurance check to ensure sampling equipment was not contaminated by DNA of target species, we collected a field blank at the start of each sampling day by filtering 500mL of clean, bottled water through a new filter and had the field blank analyzed for the same species as for filters collected during formal surveys.

Filters were analyzed for presence of DNA for our 4 target species at an eDNA laboratory at Washington State University using methods described in Goldberg et al. (2018) and Hossack et al. (2023). As part of this project and in collaboration with the Sonoran Desert Inventory and Monitoring Network (https://www.nps.gov/im/sodn/index.htm), the Washington State University laboratory developed and validated primers and assays for the Arizona Toad. This validation included ensuring the primers were specific to the Arizona Toad and did not cross-amplify for other local species, including the Woodhouse's Toad and Red-spotted Toad (C. Goldberg, Washington State University, personal communication). Importantly, however, eDNA surveys cannot distinguish hybridized individuals from non-hybridized individuals, because the assays target maternally inherited mitochondrial DNA. This means that eDNA from an Arizona Toad/Woodhouse's Toad hybrid that has Arizona Toad mitochondrial DNA would likely produce a positive detection for Arizona Toads.

3.4. Visual Encounter Surveys

Visual encounter surveys followed a standard protocol at all sites that were surveyed, with modifications for differing site conditions (non-site, dry, wet) and site type (standing water, flowing water). While we targeted Arizona Toads and Woodhouse's Toads, we documented all species of encountered amphibians. To conduct visual encounter surveys, 2 observers independently walked the site slowly and recorded any encountered amphibians. Adult and juvenile toads in the genus *Anaxyrus* were hand-captured when possible so we could collect individual-level data, including buccal DNA samples. When we encountered amphibian larvae, we dipnetted or hand captured individuals for photographs and species identification. In some cases, species identification of larvae could not be determined so we collected up to 5 larvae per site as voucher specimens, per landowner allowances. We also collected habitat data during visual encounter surveys, including spatial locations, date of survey, time of day, and noted the presence or absence of water.

3.5. Assessing Hybridization Threats

To assess Arizona Toad and Woodhouse's Toads (juveniles and adults) for potential hybridization, we captured up to 10 individuals of each species, as well as up to 10 suspected hybrids, per site to conduct individual assessments based on published methods (Sullivan, 1986). First, we collected sex, snout-vent-length (SVL), and other individual-level data. We then conducted a morphological assessment of hybridization by assigning scores to metamorphosed toads for the presence of 4 traits for which these species typically differ: presence/absence of cranial crests, ventral spotting, a pale bar between the eyelids, and a dorsolateral white stripe (Sullivan, 1986; Sullivan and Lamb, 1988). Also, we collected a buccal swab from each individual as a genetic sample, which may be useful for future assessments of hybridization in the study area.

We took lateral, dorsal, and ventral photographs of each captured animal to verify species, document potential hybrid characteristics, and to measure the shape of parotoid glands. The ratio of length: width of the parotoid glands has been used as a metric of hybridization between Arizona and Woodhouse's toads (Blair, 1955; Sullivan, 1986; 1995). We used the software ImageJ (Version 1.54g, https://imagej.net/ij/index.html) and photos with scale rulers to measure the length and width at the largest point, then calculated an average ratio for each individual.

3.6. Habitat Suitability Map

We developed a habitat suitability model for Arizona Toads using a Maximum Entropy (Maxent) framework (Phillips et al., 2006; Phillips & Dudík, 2008). Maxent models use presence-only records, randomly generated background points as pseudo-absences, and raster data of environmental variables to predict areas of suitable habitat in a focal region. Maxent models are viable methods of predicting habitat suitability when implemented on a relatively small geographic scale without intent of projecting future suitability, and with appropriate tuning and model selection methods (Barbet-Massin et al., 2012; Merow et al., 2013).

3.6.1. Environmental Variable Data

We evaluated 11 environmental variables for use in the habitat suitability model, with 8 variables selected for use in the final model. Variables were selected considering life history traits and previous assessments or species distribution models for the Arizona Toad (de Albuquerque et al., 2023; Scher et al., in revision). For this analysis, we selected the most recent environmental data available to reflect current conditions. We aggregated and/or split cells to achieve a resolution of 90m x 90m for all environmental variables, which were available at a range of resolutions ranging from 10m x 10m (elevation) to 1km x 1km (actual evapotranspiration [AET]). Elevation and riparian tree cover were correlated with other variables (Pearson's correlation >0.55) and were removed from analysis. Raster data for the 8 selected variables were prepared for analysis using R packages *terra* and *sp* (Pebesma and Bivand, 2023; Hijmans, 2025). Details are provided on all tested variables with data sources and brief processing methodology. Environmental variables with bolded names were used in the final model.

- Elevation – 1/3 arc second (approximately 10m x 10m) Digital Elevation Models were downloaded from the National Elevation Dataset (U.S. Geological Survey, 2024a) using the USGS National Map Downloader online interface in September

2024 and were aggregated to a 90m x 90m resolution (https://apps.nationalmap.gov/downloader/#/).

- **Terrain Ruggedness Index (TRI)** – The Terrain Ruggedness Index represents the difference in elevation between a cell and the 8 focal cells surrounding it (Amatulli et al., 2018). This index was calculated by fusing digital elevation models and the terrain() function in R package *terra* and was aggregated to a 90m x 90m resolution.
- **Spring (March–May) stream flow** – We calculated mean springtime (March–May) flow in cubic feet per second (CFS; cube-root transformed) during 1980–2015 for each Hydrological Unit Code (HUC) 8 catchment unit in the study area, based on reconstructed time series data. HUC8 polygon data were rasterized into a 90m x 90m resolution. Assimilated (Type C) streamflow data were sourced from Dayflow: CONUS Daily Streamflow Reanalysis, Version 1 (Ghimire et al., 2022). Data is available at https://hydrosource.ornl.gov/data/datasets/dayflow-v1/
- **Water permanence** – Using the Dayflow dataset obtained for spring stream flow, we quantified water permanence for streams using the mean of the ratio of wet days to dry days for each year during 1980–2015. Reaches with flow < 1 CFS on any given day were considered dry.
- Distance to perennial streams – To quantify distance to permanent flowing streams, we downloaded the NHDPlus HUC4 geodatabase (U.S. Geological Survey, 2018) using the USGS National Map Downloader online interface in September 2024. We used the NHD Flowlines layer to derive stream lines, mapped at 1:24,000 scale. Streams were filtered to include flowlines where FCode = 46006 (Stream/River, Hydrographic Category = Perennial) and/or stream order ≥ 5 to target permanent lotic water bodies. A raster of distance to streams data was created using the Euclidean Distance tool in ArcGIS Pro version 3.2.2 (Esri, Redlands, California) at a 30m x 30m resolution that was aggregated to a 90m x 90m resolution. Data are available at https://apps.nationalmap.gov/downloader/#/
- Riparian canopy cover – Land cover data was downloaded from the 2021 National Landcover Dataset (U.S. Geological Survey, 2024b) at 30m x 30m resolution. Cells with tree cover (deciduous forest, evergreen forest, and mixed forest categories) were selected and filtered to select only cells within a 100m-buffer of perennial streams, as identified by NHDPlus flowlines used in the distance-to-perennial streams metric. Data were aggregated to a 90m x 90m resolution. Data are available at https://www.mrlc.gov/viewer/
- **Riparian canopy and shrub cover** – As per riparian canopy cover, but including National Landcover Dataset cells categorized as deciduous forest, evergreen forest, mixed forest, woody wetlands, or emergent herbaceous wetlands.
- **Actual Evapotranspiration (AET)** – A measure of the energy and water in the environment that is available to support plant growth (Stephenson, 1998). Here, we consider AET a proxy for environmental “wetness.” We used historical

average AET during 1980–2019, available from the National Park Service's Gridded Water Balance Model dataset (National Park Service, 2021) at 1km x 1km resolution and disaggregated it to a 90m x 90m resolution. Data are available at http://screenedcleanedsummaries.s3-website-us-west-2.amazonaws.com/

- **Invasive species (American Bullfrog)** – We used the USGS Nonindigenous Aquatic Species database (U.S. Geological Survey, 2024c) to access records of American Bullfrogs. We quantified presence or absence in each HUC12 catchment in the study area. HUC12 polygon data was rasterized into a 90m x 90m resolution. Data are available at https://nas.er.usgs.gov/
- **Invasive species (Non-native Crayfish)** – We used the USGS Nonindigenous Aquatic Species database (U.S. Geological Survey, 2024c) to access records of non-native crayfish species. We quantified presence or absence in each HUC12 catchment in the study area. HUC12 polygon data was rasterized into a 90m x 90m resolution. Data are available at https://nas.er.usgs.gov/
- **Invasive species (Non-native Fish)** – We used the USGS Nonindigenous Aquatic Species database (U.S. Geological Survey, 2024c) to access records of non-native fish species. We quantified the number of invasive fish species in each HUC12 catchment in the study area. HUC12 polygon data was rasterized into a 90m x 90m resolution. Data are available at https://nas.er.usgs.gov/

3.6.2. Occurrence Records

We used the historical records database that we compiled for this project (see section 3.1.1) to select verified Arizona Toad records for use in the model. First, to ensure the model reflected current conditions as well as to ensure temporal alignment with environmental data, we filtered records to exclude occurrences older than 2015. Next, we spatially thinned records so only 1 record per cell (90m x 90m resolution) of environmental data was retained. This thinning process also helps reduce spatial autocorrelation that can occur when there are many records in areas sampled the most intensely or areas where species are reported most frequently, such as near Zion National Park in southwest Utah. This thinning process resulted in 122 occurrence records within the study area that we used to build the habitat suitability model.

Arizona Toad occurrences identified during 2024–2025 via eDNA or visual encounter surveys were included in the records database and in the set of occurrence records used in the model. We excluded the equivocal eDNA detection for Arizona Toads in Calico Basin in Red Rocks National Conservation Area in 2025 due to uncertainty regarding that detection (see section 5.1).

3.6.3. *Data Analysis*

Tuning and selection for the habitat suitability model were performed in R via RStudio (R Core Team, 2023) using package *ENMEval* (version 2.0.5, Kass et al., 2021; Muscarella et al., 2014). *ENMEval* provides tools to tune Maxent models based on user-specified blocking methods, regularization multipliers, feature class combinations, and model algorithms to adjust fit to a user's dataset (Kass et al., 2021). A regularization multiplier helps avoid overfitting and determines the penalty associated with including additional variables in the model. Higher regularization multipliers impose stronger penalties on complexity and produce simpler predictions (Phillips, 2017).Metrics of model fit such as area under curve (AUC), omission rate, and the Continuous Boyce Index (CBI) were generated for all combinations of tuning metrics and used to select the best-fit model.

We used 122 occurrence points, 10,000 background points, and 8 environmental variables to model suitable habitat for Arizona Toads within the study area encompassing southern Nevada, northwestern Arizona, and southwestern Utah. We implemented spatial block methods to partition occurrences into validation and training bins for *k*-fold cross-validation (Fielding and Bell, 1997). Tuning parameters allowed regularization multipliers from 1 to 10 and feature classes were constrained to linear or quadratic relationships. We used the maxent.jar algorithm (version 3.4.3) in the function ENMevaluate() to generate 20 potential models for evaluation. The best-fit model was selected using cross-validation to identify the model with the lowest average 10th percentile omission rate and the highest average Continuous Boyce Index to break ties (Hirzel et al., 2006).

4. Results

4.1. Surveys Completed

Over the 2-year project, field crews visited 270 potential field sites, many of which were dry (Figure 4.1, Table 4.1). We found adequate aquatic habitat to conduct 198 unique visual encounter surveys at 154 different sites, including 139 daytime surveys and 59 nighttime surveys. We found adequate water to collect eDNA filters at 131 sites and collected a total of 295 filters, excluding field blanks collected each day. The mean volume of water filtered across all sites was 4.7L and all field blanks were negative for target DNA.

Table 4.1 Summary of visual encounter surveys (VES) and environmental DNA (eDNA) surveys completed in each county in the study area.

County	No. Sites Visited	No. Sites Dry (Not Surveyed)	No. VES-only Sites	No. VES & eDNA Sites	No. Surveys (Sites)	No. Daytime Surveys	No. Nighttime Surveys
Clark (NV)	210	100	17	90	143 (110)	97	46
Lincoln (NV)	18	4	1	13	18 (14)	13	5
Nye (NV)	7	1	1	5	6 (6)	5	1
Washington (UT)	5	0	0	5	5 (5)	5	0
Kane (AZ)	1	0	0	1	1 (1)	1	0
Mohave (AZ)	29	11	1	17	25 (18)	18	7
TOTALS	**270**	**116**	**20**	**131**	**198 (154)**	**139**	**59**

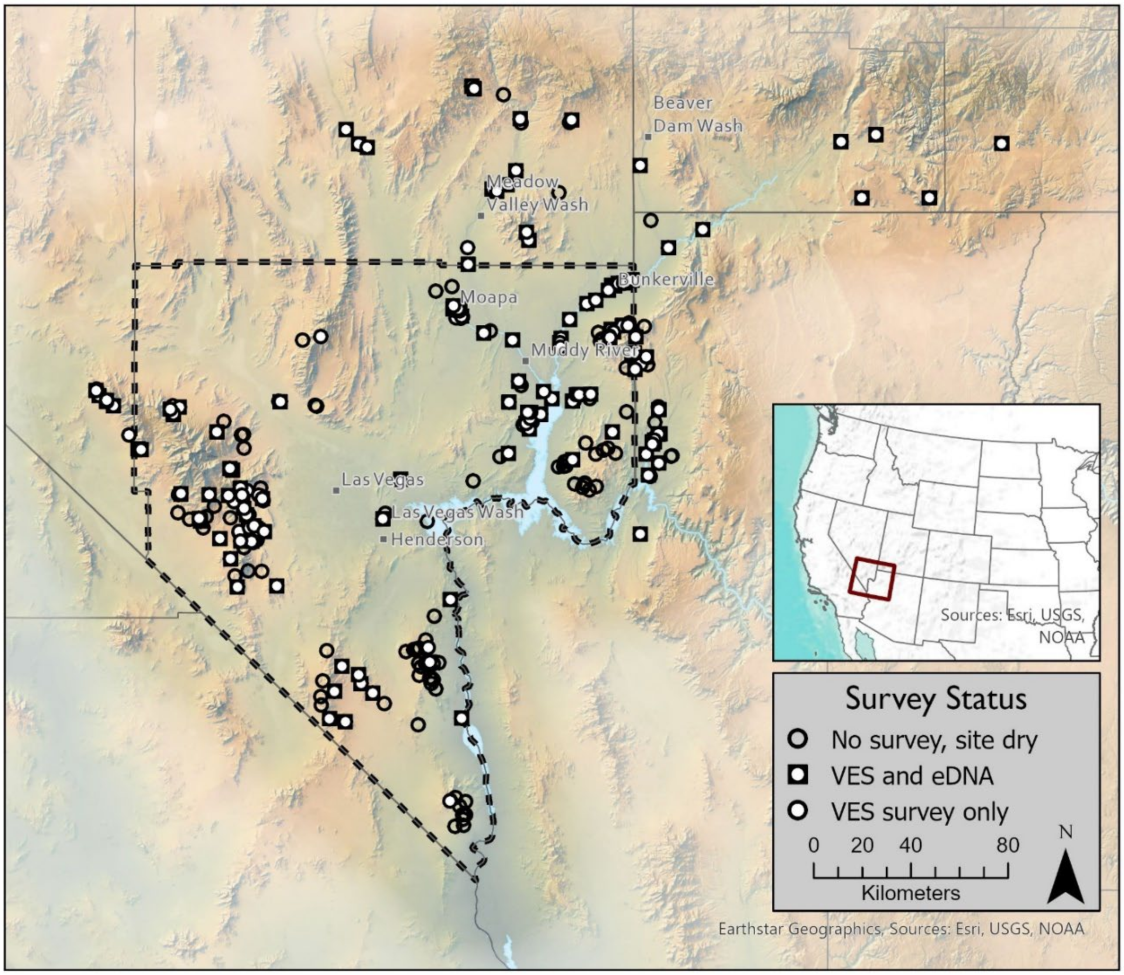

Figure 4.1 Locations of all sites visited during 2024–2025. Sites that were visited and dry are shown as open circles, sites that were visited and had adequate water for a visual encounter survey (VES) but not enough to collect environmental DNA (eDNA) filters are shown with filled white circles. Sites where crews were able to conduct both visual encounter and eDNA surveys are shown as black squares with white circles.

Generally, we surveyed low elevation sites first (starting in April) and proceeded to higher elevation sites through June and July. Most sites were visited only once during the 2-year project; however, we revisited some sites during both years or during both daytime and nighttime hours if larvae were detected and adults were not, or if previous eDNA results warranted further visual surveys. In 2024, we targeted high priority sites on a broad geographic scale with plans to use those results to inform our sampling priorities for 2025. Surveys in 2025 began approximately 1 month earlier than in 2024 to better coincide with surface water presence at ephemeral sites (Figure 4.2).

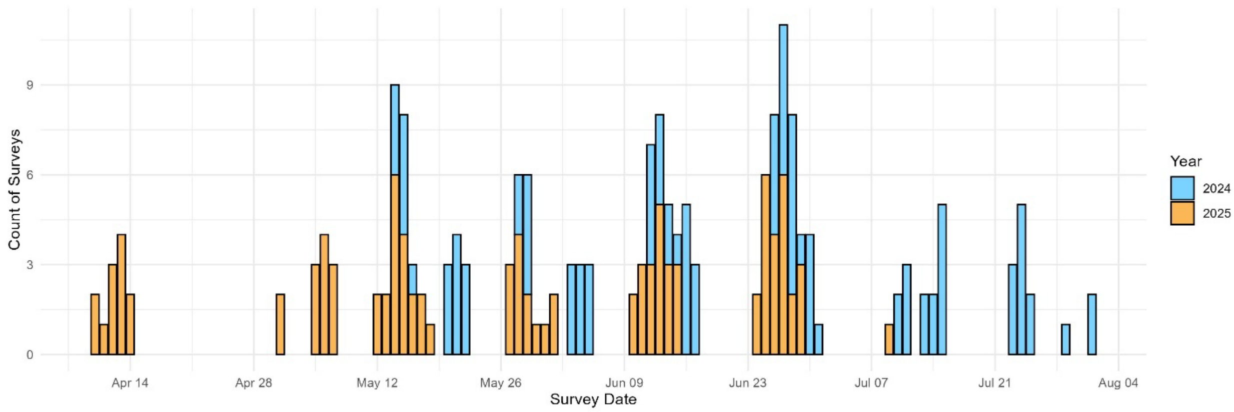

Figure 4.2 Count of surveys completed through the spring and summer sampling months, with 2024 surveys in blue and 2025 surveys in orange.

4.2. Species Detections

4.2.1. Visual Encounter Surveys

We detected 7 amphibian species during visual encounter surveys, including the Arizona Toad, Woodhouse's Toad, Red-spotted Toad, American Bullfrog, Pacific Chorus (Tree) Frog (*Pseudacris* [*Hyla*] *regilla*), Relict Leopard Frog (*Lithobates* [*Rana*] *onca*), and Canyon Treefrog (*Dryophytes* [*Hyla*] *arenicolor*) (Figure 4.3). We also encountered unidentified toad tadpoles (*Anaxyrus* sp.) at 44 sites and unidentified leopard frogs (*Lithobates* sp.) at 7 sites. The unidentified leopard frogs were likely Relict Leopard Frogs, but we did not capture them for examination.

Arizona Toads were largely detected in the northeastern part of Clark County along Meadow Valley Wash and the Virgin River drainage, with the exception of the unverified eDNA detection near Red Rocks. There were no visual encounter detections for Arizona Toads in Clark County, but recent reports and photos from project partners suggest that toads with traits consistent with Arizona Toad x Woodhouse's Toad hybrids exist within several miles of the county line.

Red-spotted Toads were the most commonly detected amphibian species in the study area; this species was detected during visual encounter surveys at 40 sites across the county. Woodhouse's Toads were also widely dispersed and were encountered at 24 sites. Non-native American Bullfrogs were distributed primarily in the northeastern portion of Clark County where more permanent water sources (e.g., Lake Mead, the Virgin River) provide suitable habitat (Figure 4.4).

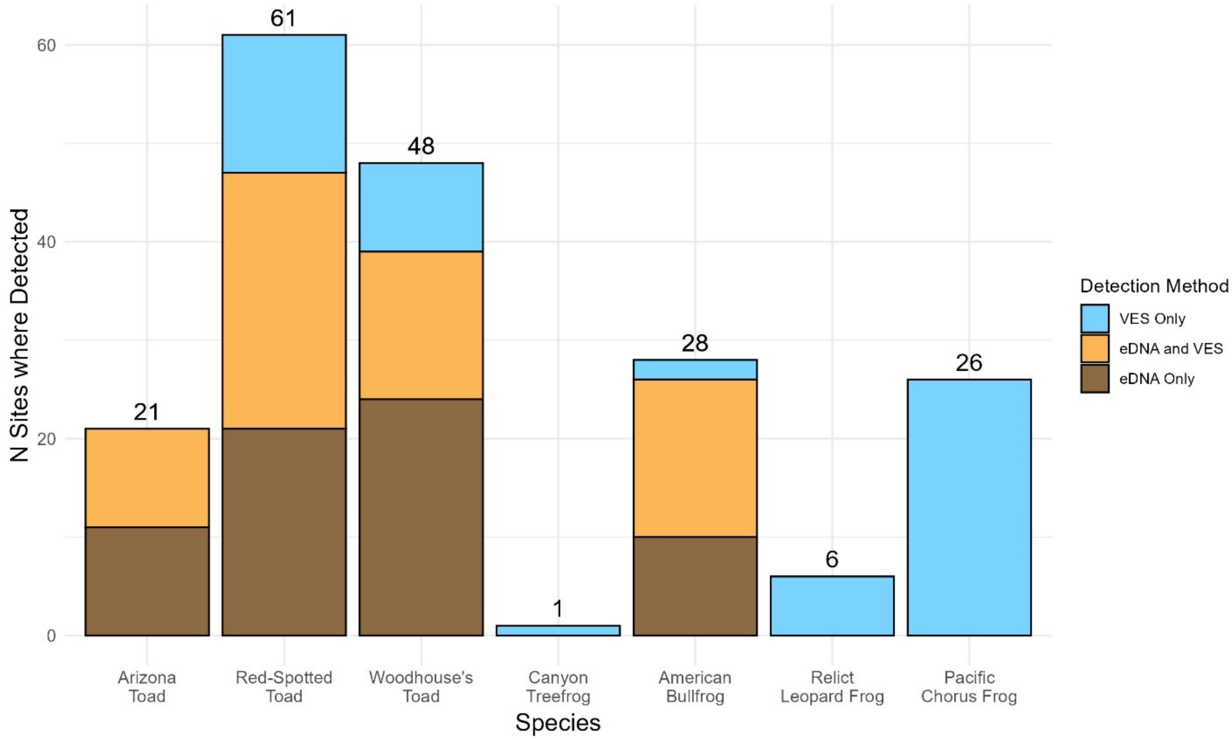

Figure 4.3 Count of sites where each amphibian species was detected using any method. Blue bars indicate sites where species were detected via visual encounter survey (VES) methods only, orange bars indicate sites where species were detected via both VES and environmental DNA (eDNA) methods, and brown bars indicate sites where species were detected only via eDNA. Note that we did not use eDNA methods for all species.

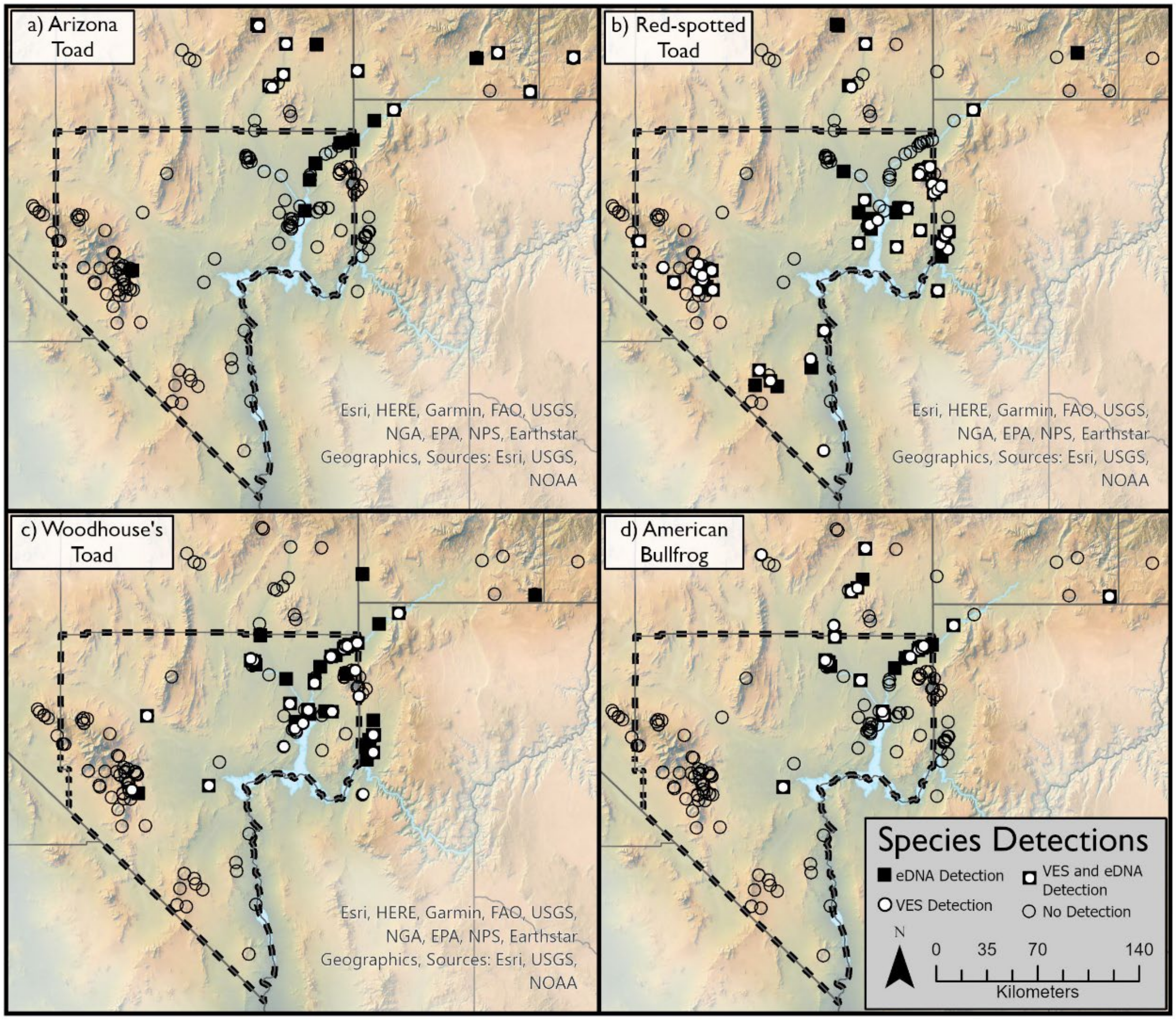

Figure 4.4 Detections of 4 target species a) Arizona Toad, b) Red-spotted Toad, c) Woodhouse's Toad, and d) non-native American Bullfrog. Sites where surveys were conducted but the species was not detected are indicated by an empty circle. Sites with environmental DNA (eDNA) detection are black squares and sites with visual encounter survey (VES) detections are white filled circles. The boundary of Clark County is indicated by a bold dashed line.

4.2.2. eDNA

We detected all 4 target species using eDNA methods, with Red-spotted Toads being the most frequently detected species at 47 sites. Most eDNA detections for all target species were concentrated in the northeastern portion of Clark County along the Virgin and Muddy River drainages and in Meadow Valley Wash, areas that have more persistent water sources than elsewhere in the county (Figure 4.4).

We tested eDNA filters for matches to both eastern and western clade Red-spotted Toads and found that of 44 of 47 sites where any Red-spotted Toad was detected, DNA from both clades was present. The 3 sites where only western clade DNA was detected were

Black Willow Spring in Parashant National Monument, Grapevine Spring in Lake Mead National Recreation Area, and in Meadow Valley Wash approximately 45 km south of Caliente, NV.

4.2.3. Clark County Properties Summary

We visited 10 Clark County or Clark County adjacent properties and found adequate water to conduct surveys at 8 properties. We conducted 12 VES surveys and collected 24 eDNA filters on Clark County owned Riparian Reserves and conducted one survey and 3 eDNA filters at the co-managed Electric Avenue property (Table 4.2). We detected Arizona Toads at 3 of these sites via eDNA: the Riparian Reserves Bunkerville East (in 2024), Mormon Mesa South (in 2025), and at Electric Avenue (in 2025).

We detected 3 other amphibian species on Clark County Riparian Reserves. Woodhouse's Toads were the most commonly encountered and were detected at all 8 reserves, followed by American Bullfrogs at 6 reserves and Pacific Chorus Frogs at 2 reserves. We visited 2 reserves in Moapa (Unit F and Units G-I) where we did not find adequate water to conduct surveys.

4.3 Assessing Hybridization Threats

We assessed hybrid traits of Arizona and Woodhouse's toads based on a hybrid scoring index developed by Sullivan (1986). We collected hybridization data for 19 adult and 10 juvenile Arizona Toads at 8 sites (and 1 opportunistic capture outside of a site) and 90 adult and 14 juvenile Woodhouse's Toads at 24 sites (and 1 opportunistic capture outside of a site). The Virgin River Campground Recreation Area in northwestern Arizona was the only site where we recorded both Arizona Toads and Woodhouse's Toads during visual encounter surveys. During a nighttime survey at this site in 2025, we encountered 1 Arizona Toad, 13 Woodhouse's Toads, and 5 toads with hybrid characteristics that could not be confidently assigned to a species.

Individuals we pre-identified (i.e., in the field before assessing measurements from photos) as Arizona Toads had lower hybrid scores than individuals that we pre-identified as Woodhouse's Toads. We found no difference in hybrid scores based on sex or between juveniles and adults for either species. Arizona Toads had lower ratios of parotoid gland length: width (i.e., shorter with squatter shape), while Woodhouse's Toads had higher ratios (longer and sausage-shaped) (Figure 4.5). Importantly, these scores based on morphological traits can only suggest the presence of hybridization; further genetic analyses would be needed to verify the presence of hybridization.

Table 4.2 Species encountered at Clark County Riparian Reserves and number of visual encounter surveys (VES) conducted and eDNA filters collected. Species codes are as follows: ANMI: Arizona Toad (*Anaxyrus microscaphus*); ANWO: Woodhouse's Toad (*Anaxyrus woodhousii*); ANSP: Unidentified Toad tadpoles (*Anaxyrus* spp.); LICA: American Bullfrog (*Lithobates catesbeianus*); PSRE: Pacific Chorus Frog (*Pseudacris regilla*).

Reserve Name	Species detected w/ VES	Species Detected w/ eDNA	No. VES Surveys	No. eDNA Filters	Notes
Bunkerville East	ANWO ANSP LICA PSRE	ANMI ANWO LICA	5	8	Revisited in 2025
Bunkerville West	ANWO ANSP LICA	ANWO LICA	1	3	
Mesquite	LICA	ANWO LICA	1	2	
Moapa A-E	No amphibians	ANWO LICA	1	2	
Moapa F	No amphibians	-	-	-	Dry
Moapa G-I	No amphibians	-	-	-	Dry
Mormon Mesa North	No amphibians	ANWO	1	3	
Mormon Mesa South	ANWO ANSP	ANMI ANWO	1	3	
Riverside	ANWO ANSP	ANWO LICA	2	3	
Electric Avenue	ANWO ANSP PSRE	ANMI ANWO LICA	1	3	

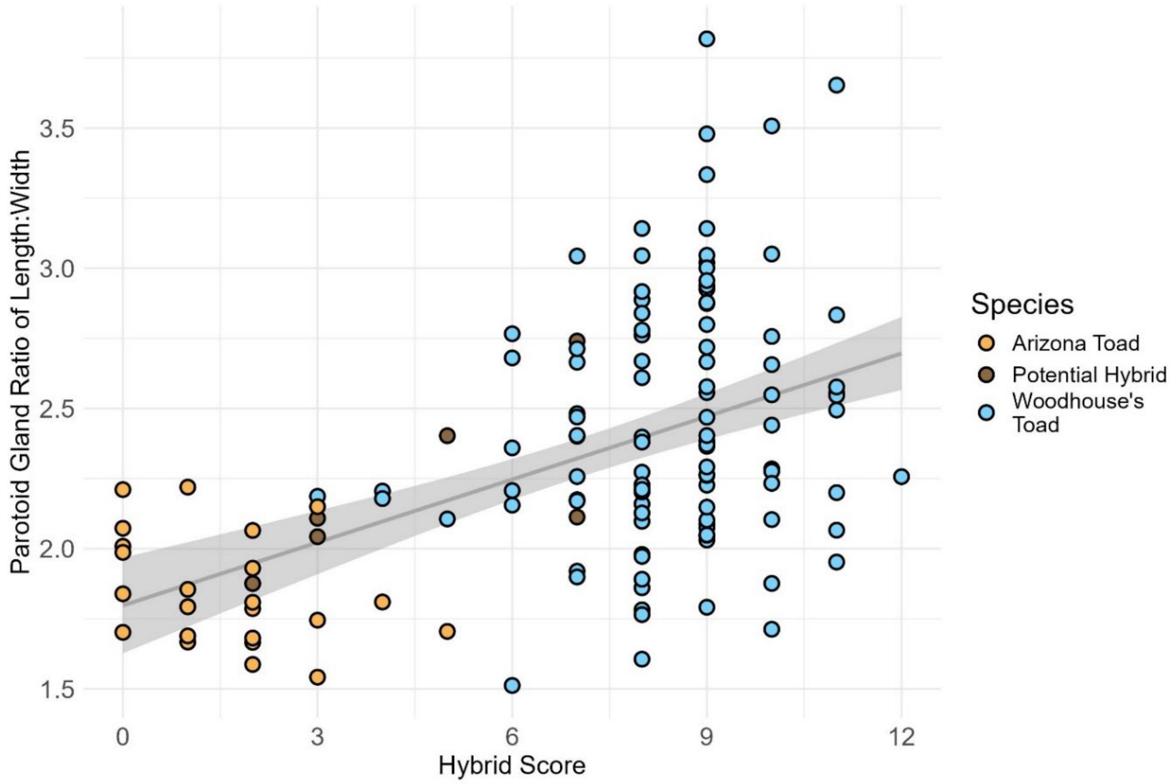

Figure 4.5 Relationship between hybrid score (x-axis) and parotoid gland ratio (y-axis) for 139 individual toads. Orange dots indicate individuals pre-identified in the field as Arizona Toads and blue dots indicate individuals pre-identified as Woodhouse's Toads. The gray band around the mean regression line represents the 95% confidence interval.

4.4 Habitat Suitability Map

4.4.1. *Model Selection and Fit*

Output from *ENMEval* included fit and *k*-fold results for 20 habitat suitability models spanning 10 regularization multipliers and 2 allowable feature classes (Table 4.3). The best-fit model was selected using cross-validation to identify the model with the lowest average 10^{th} percentile omission rate and the highest average Continuous Boyce Index to break ties (Hirzel et al., 2006). This model (LQ4) had a regularization multiplier of 4 and allowed linear and quadratic environmental relationships with variables (Table 4.3). All tested models fit the data well, with area under the curve values > 0.9 indicating high probability that locations of known presences were ranked higher than randomly chosen background locations.

Table 4.3 Fit metrics for 20 habitat suitability models for the Arizona Toad in the study area. Model LQ4 (highlighted and bolded) provided the best fit, based on cross-validation to identify the model with the lowest average 10th percentile omission rate and the highest average Continuous Boyce Index to break ties. Models fit for evaluation include all combinations of 10 regularization multipliers and 2 potential allowable feature classes. FC = Feature Class, RM = regularization multiplier, AUC = area under receiver operator curve, CBI = Continuous Boyce Index, Δ AIC = difference in Akaike's Information Criterion, Val = Validation Data. In FC column, L = models that only allow linear feature classes and LQ = models that allow both linear and quadratic feature classes. AUC values measure how well a model properly classifies known presence and known background points, with values near 0.5 indicating the model is no better than random and values near 1 indicating high sensitivity. CBI assesses the difference between predictions and a random distribution of the observed presence points with values closer to one indicating high model accuracy. The 10% omission rate indicates the proportion of presence points that were incorrectly classified as having a low probability of occurrence after removing points with suitability in the lowest 10% of all points. Cross validation k-fold methods split the dataset into training and validation datasets which are iteratively used to train and test models.

FC	RM	AUC (Training)	CBI (Training)	AUC (Val, Avg)	AUC (Val, SD)	CBI (Val, Avg)	CBI (Val, SD)	Omission (10% Avg)	Omission (10% SD)	Δ AICc
L	1	0.915	0.829	0.884	0.063	0.561	0.223	0.155	0.072	52.94
LQ	1	0.938	0.901	0.921	0.014	0.799	0.051	0.147	0.060	0.00
L	2	0.914	0.841	0.892	0.046	0.564	0.273	0.155	0.072	52.95
LQ	2	0.934	0.831	0.926	0.011	0.728	0.025	0.106	0.015	1.52
L	3	0.914	0.800	0.904	0.024	0.563	0.320	0.147	0.067	53.18
LQ	3	0.931	0.777	0.924	0.015	0.730	0.042	0.098	0.025	16.71
L	4	0.913	0.801	0.907	0.019	0.507	0.348	0.115	0.043	51.33
LQ	**4**	**0.926**	**0.784**	**0.921**	**0.017**	**0.705**	**0.114**	**0.090**	**0.030**	**31.16**
L	5	0.913	0.817	0.907	0.018	0.500	0.428	0.115	0.043	51.85
LQ	5	0.921	0.768	0.917	0.016	0.642	0.158	0.115	0.043	41.80
L	6	0.912	0.826	0.907	0.018	0.486	0.452	0.115	0.043	50.24
LQ	6	0.917	0.753	0.916	0.015	0.625	0.207	0.115	0.043	43.83
L	7	0.911	0.813	0.908	0.017	0.489	0.468	0.115	0.043	50.87
LQ	7	0.914	0.831	0.914	0.014	0.613	0.222	0.115	0.043	53.33
L	8	0.910	0.826	0.908	0.017	0.524	0.435	0.115	0.043	49.37
LQ	8	0.911	0.842	0.913	0.013	0.629	0.187	0.115	0.043	55.70
L	9	0.910	0.815	0.908	0.017	0.531	0.402	0.115	0.043	50.05
LQ	9	0.911	0.841	0.911	0.015	0.533	0.378	0.115	0.043	54.60
L	10	0.909	0.771	0.908	0.017	0.554	0.430	0.115	0.043	48.61
LQ	10	0.910	0.841	0.910	0.016	0.534	0.474	0.115	0.043	53.69

4.4.2. *Model Prediction*

The best-fit model indicated that there is suitable habitat for Arizona Toads in Clark County; this habitat was concentrated in riparian areas with extensive canopy and shrub cover and moderate stream flow (Figure 4.6). In Clark County, the Virgin River corridor between Bunkerville and the confluence with the Muddy River, the Muddy River near Moapa, and Las Vegas Wash in the Clark County Wetlands Reserve in Henderson, are the areas indicated to have a high habitat suitability for Arizona Toads.

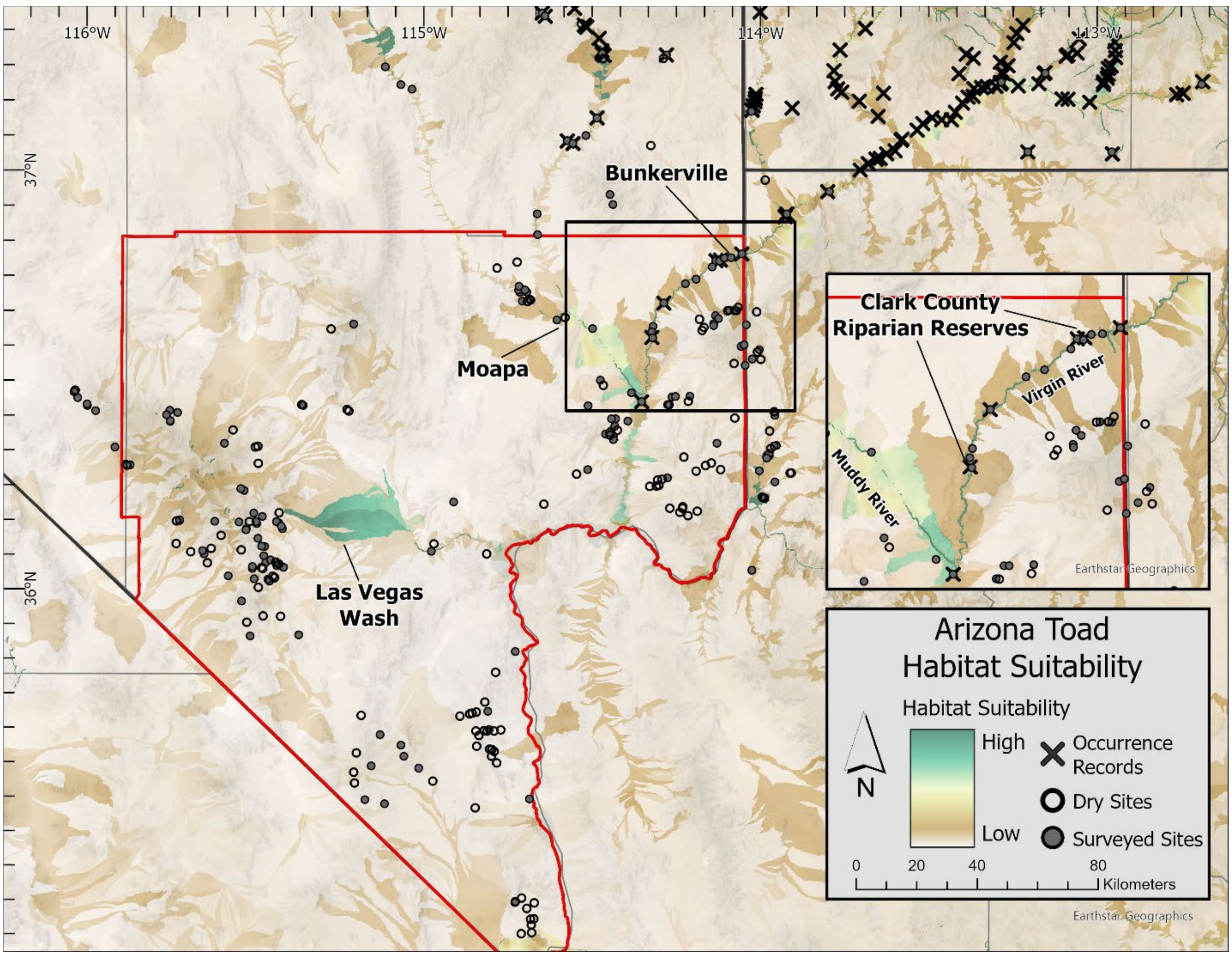

Figure 4.6 Habitat suitability map for Arizona Toads in the study area. White background areas have low predicted habitat suitability for Arizona Toads while green background areas have higher predicted habitat suitability. Black Xs indicate the 122 Arizona Toad occurrences used to fit the model. Open circles indicate sites visited during this project that were dry and were not surveyed, and grey filled dots indicate sites that were surveyed during this project.

4.4.3. *Variable Contribution*

Of the 8 tested environmental variables, 89% of permutation importance was explained by 3 of them. Riparian tree and shrub cover (45.5%), actual evapotranspiration (AET; 23.3%), and stream permanence (20.2%) were the strongest predictors of suitable habitat for the Arizona Toad (Figure 4.7). Presence of invasive American Bullfrogs and crayfish contributed little to the model and were not considered for model interpretation.

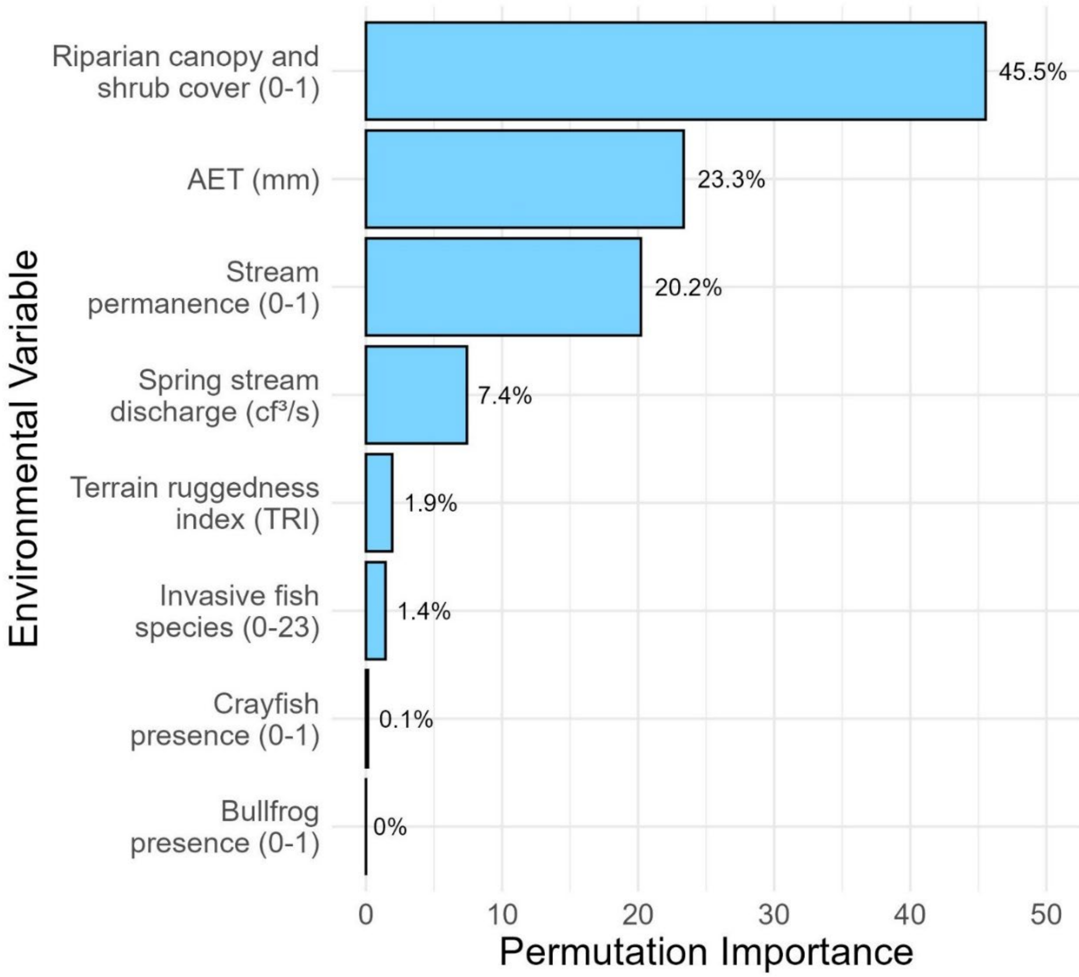

Figure 4.7 Contributions of 8 environmental variables included in the final habitat suitability model.

4.4.4. *Marginal Response Curves*

Marginal response curves define relationships between the predicted probability of occurrence (p) of the target species and the environmental variables the model was built on. Habitat suitability for Arizona Toads increased with increasing riparian tree and shrub cover and increasing stream permanence (Figure 4.8). Areas with high values for AET (~350mm) and streams with spring-time flows around 10 CFS were also predicted to be suitable habitat. Marginal response plots also indicated areas with high topographic variation and many species of invasive fish were positively associated with Arizona Toad habitat. However, these covariates contributed little to the model. The relationship with fishes is counter-intuitive, but we suspect it reflects presence of reliable water.

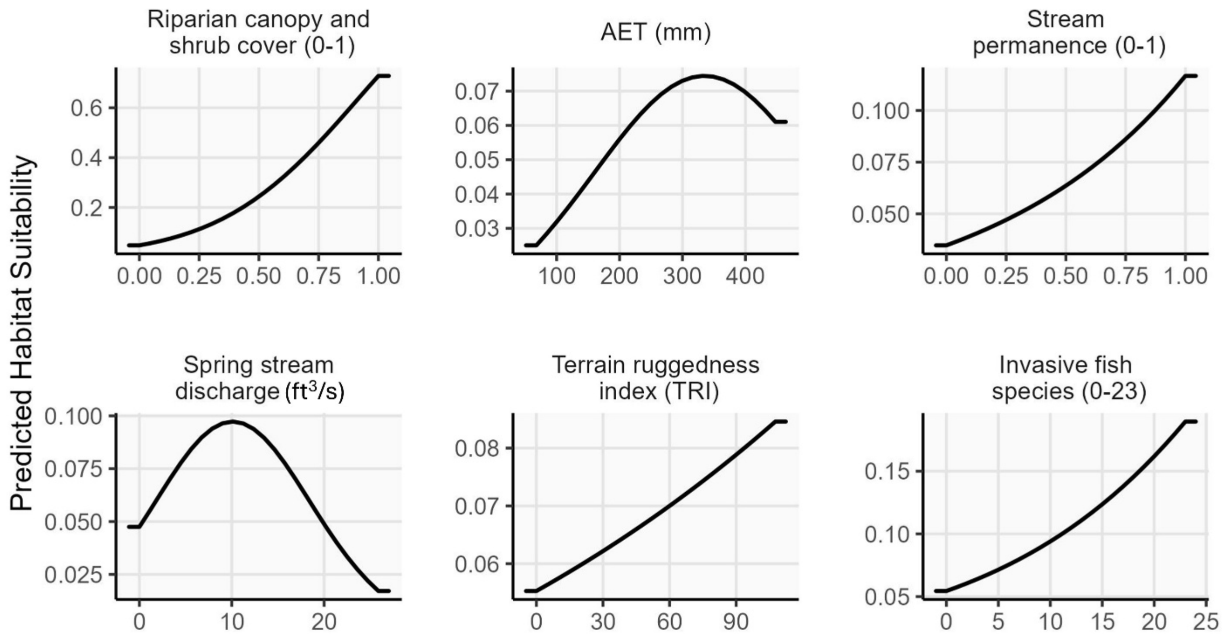

Figure 4.8 Marginal response curves for 6 environmental variables that contributed to the habitat suitability model. Note that the scale of axes varies among plots.

5. Discussion

5.1. Survey Results

Our surveys detected evidence of Arizona Toads at 7 sites in Clark County. All Arizona Toad detections in Clark County were from eDNA filters, reinforcing the utility of this method when targeting rare or cryptic species. While we cannot exclude the possibility that some of these detections were from hybrids between Arizona Toads and Woodhouse's Toads, we suspect the presence of Arizona Toad DNA at least suggests the potential for the species to occur and persist in the county.

Three of these 7 detections occurred on Clark County owned (or adjacent) properties: the Bunkerville East Riparian Reserve (2024 eDNA detection), Mormon Mesa South Riparian Reserve (2025 eDNA detection), and Electric Avenue Property (2025 eDNA detection). After the 2024 eDNA detection at Bunkerville East, crews revisited the property in 2025 to re-survey the 2024 site and to survey an additional river reach on the property. The 2025 crew found the 2024 site to be dry and Arizona Toads were not detected at Bunkerville East in 2025. However, Arizona Toads were detected in 2025 via eDNA at Electric Avenue, less than 1km downstream and with extensive slow backwaters and side channels.

With the exception of a detection in Red Rocks National Conservation Area that we consider inconclusive (see below), detections in Clark County were restricted to sites along the Virgin River from the state line with Arizona to the Virgin River's confluence with the Muddy River in Lake Mead National Recreation Area. Our survey results suggest the Clark County Riparian Reserves along the Virgin River (both Bunkerville reserves, both Mormon Mesa reserves, and the Riverside reserve) were more likely to be inhabited by

Arizona Toads than the Riparian Reserves that do not contain riparian habitat. The detection of Arizona Toads at Electric Avenue further supports the inclusion of this property in the Riparian Reserve system.

We consider the eDNA-based detection of Arizona Toad DNA in Calico Basin of Red Rocks National Conservation Area as inconclusive for several reasons: 1) this site is disjunct from other verified records or current populations in the region, 2) we collected only 1 eDNA filter at this site, and 3) this filter was only weakly positive. However, further surveys in and near Calico Basin could aid in more confidently determining if Arizona Toads occur in the area.

5.2. Habitat Suitability

Our habitat suitability map suggests there is viable habitat in Clark County for Arizona Toads, particularly along permanent streams that have extensive riparian tree and shrub cover and moderate amounts of spring stream flow. In 2024, we generated a preliminary version of this model and used the information to target areas in 2025, with high predicted suitability along the Virgin River in Clark County. The increased sampling intensity in that area produced 5 additional sites with eDNA detections for the species.

Riparian tree and shrub cover, AET, and stream permanence were identified as the most important environmental variables we tested, which align with our understanding of the species' preferred habitat (Sullivan, 1995; Schwaner and Sullivan, 2005). These features are available in flowing streams and rivers in the region (Virgin River, Muddy River, Meadow Valley Wash). However, these streams are also heavily used by humans and are impacted by impoundments and channel modifications that reduce habitat quality for Arizona Toads (Sullivan and Lamb, 1988). These hydrologic modifications also may benefit Woodhouse's Toads, possibly contributing to hybridization between the species and eventual replacement by Woodhouse's Toads (Sullivan, 1986; 1993).

Our habitat suitability model indicates there are several areas with appropriate habitat for Arizona Toads in the county where we did not detect the species (Figure 4.6). The headwaters of the Muddy River near Moapa and the Muddy River itself are indicated as likely habitat. There is an unverified Arizona Toad record from the headwaters area in 1939 (BYU:045218, https://www.gbif.org/occurrence/3864848900) and a specimen that is a suspected hybrid from the same area in 1951, indicating the species was present in the area (MVZ:054579, https://www.gbif.org/occurrence/5147121573, Figure 3.1). Despite 8 sites surveyed and 12 eDNA filters collected in the area, we did not detect Arizona Toads. The Muddy River is fed by warm springs and flows rapidly into an incised channel. We are unaware of temperature thresholds for Arizona Toads, but water temperatures and lack of slow backwaters in the deeply incised channel may preclude successful breeding required to support a resident population.

Based on our model output, the Clark County Wetlands Reserve in Henderson has high quality habitat that could support Arizona Toads. Our surveys in Las Vegas Wash at the Wetlands Reserve detected only the presence of Woodhouse's Toads; however, many of these toads had hybrid-like characteristics (Figure 5.1). Two museum specimens from the 1930s indicate Arizona Toads were historically present in springs in the Las Vegas Valley before the area was heavily developed (Bradford et al., 2005). Although the springs supporting these historical records are now modified, the Wetlands Reserve includes Las Vegas Wash, which we speculate may still harbor remnant genetic material from the Arizona Toads that were historically present. However, there can also be considerable variation in morphological characteristics within species and populations of toads. Analysis of collected buccal DNA samples or other tissue samples could help clarify the history of potential hybridization in this area.

Figure 5.1 Four individual Woodhouse's Toads from the Clark County Wetlands Reserve with hybrid-like morphological characteristics (pale eyelid bar, squat parotoid glands, dorsolateral stripe; USGS photos).

Our model also identified Grand Wash Bay in Lake Mead National Recreation Area as high quality Arizona Toad habitat. This area is of particular interest because of a verified museum specimen (CAS:2502, https://www.gbif.org/occurrence/543708247) from Black Willow Spring in 1932 (Mason Ryan, personal communication). We revisited this site in 2025 and detected Woodhouse's and Red-spotted toads at Black Willow Spring, which was comprised of 2 shallow standing pools and marginal water. However, a flowing spring in Grand Wash, approximately 1km west of Black Willow Spring, created >300m of lotic stream habitat where we also detected Woodhouse's and Red-spotted toads. Despite the lack of detections of Arizona Toads, this site and other known perennial springs in Grand

Wash Bay still have what seems to be high-quality habitat for the species. Additional surveys in this area could confirm the loss of this population and possible replacement by Woodhouse's Toads.

We detected Arizona Toads at 2 isolated springs in Lincoln County: Hackberry Spring and Box Spring. Hackberry Spring is the location of a museum specimen from 1951 (MVZ:54580, https://www.gbif.org/occurrence/5147005577) and is a small, seepy hillslope spring west of Meadow Valley Wash, but it is separated by approximately 2km of dry desert scrublands. Box Spring is in the Clover Mountains, an isolated range between Meadow Valley Wash and Beaver Dam Wash and separated from both by >20km. There are no known historical records of Arizona Toads at Box Spring. However, 1.5km southwest of Box Spring is Garden Spring, which has a verified museum record from 1998 (UTA:54501, https://www.gbif.org/occurrence/3856301530) but was dry when we visited in 2025. In addition to these detections, recent records in southwestern Utah by the Utah Division of Wildlife Resources at Indian Spring and Fort Pearce Wash indicate the species may be found at small seeps and low volume springs (Wheeler et al., 2025). Our model indicates that the continued use of these habitats is likely dependent on proximity to permanent flowing streams, which could mean populations using isolated springs have a higher risk of extinction than populations inhabiting more contiguous habitat along permanent streams.

6. Conclusions

Our extensive surveys of potentially suitable habitats in Clark County produced 6 sites with detections of Arizona Toad DNA plus an inconclusive detection in Red Rocks National Conservation Area. These 6 detections provide the first evidence of the species in the county in approximately 40 years. Three of these sites were on Clark County owned (or adjacent) properties: Bunkerville East Riparian Reserve (2024 eDNA detection), Mormon Mesa South Riparian Reserve (2025 eDNA detection), and Electric Avenue Property (2025 eDNA detection, Table 4.2). All 6 sites where we detected Arizona Toad DNA were on reaches of the Virgin River between its confluence with the Muddy River and upstream to the Utah border, where Arizona Toads apparently remain common along the Virgin River and several of its tributaries (Scher et al., in revision; Wheeler et al., 2025). However, several toads in eastern Clark County had morphological traits consistent with hybrid offspring of Arizona Toad x Woodhouse's Toad. Because eDNA methods cannot distinguish hybridized from non-hybridized individuals, caution is warranted when interpreting these results.

Despite uncertainty regarding the extent of hybridization, a habitat suitability map that was informed partly by detections of Arizona Toads in neighboring counties — where hybridization is absent or less prevalent — suggests there are several areas in Clark County that could provide appropriate habitat for the species (Figure 4.6). Specifically, permanent streams with moderate peak spring-time flows, high riparian canopy and shrub cover, and in areas with moderate AET were identified as the most suitable habitat. Most areas in Clark County with this combination of features were along the Virgin River between Bunkerville and the confluence with the Muddy River, the Muddy River near Moapa, and Las Vegas Wash in the Clark County Wetlands Reserve in Henderson. Of the properties in the Clark County Riparian Reserve program, our habitat suitability model identified the 2

Bunkerville reserves, the 2 Mormon Mesa reserves, the Riverside Reserve, and Electric Avenue Reserve as having highest quality habitat available for Arizona Toads.

7. Conservation Implications

If protecting current or potential habitat for Arizona Toads is a priority, our results suggest riparian areas along the Virgin River upstream from the confluence with the Muddy River could be prioritized. This area already includes several Clark County Riparian Reserves mentioned above. While the Wetlands Reserve in Henderson was identified as high-quality habitat by our model, we suspect it is too isolated from other high-quality habitats and potential populations to sustain populations in the long-term.

As we have referenced several times, many conclusions and any conservation implications are tempered by uncertainties regarding hybridization between Arizona Toads and Woodhouse's Toads. The uncertainty regarding using morphological traits to identify potential hybrids combined with the inability of eDNA methods to distinguish hybridized individuals from non-hybridized individuals means that, in areas where Woodhouse's Toads are common (e.g., lower Virgin River), we cannot be certain we detected any non-hybridized Arizona Toads. This uncertainty also means we do not have a clear understanding of the extent of hybridization in the study area. Developing methods to identify hybrids based on DNA samples (e.g., such as buccal swabs or tissue samples) could help in determining the extent and severity of hybridization, which may be important before considering conservation measures targeting the Arizona Toad. Similarly, targeted eDNA surveys in the Red Rocks National Recreation Area — where Woodhouse's Toads remain uncommon — could help clarify our inconclusive detection of Arizona Toad eDNA from that area.

8. Acknowledgements

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