New Mexico Department of Game and Fish
Share with Wildlife Program
Year 1 Report – December 2025

Using Environmental DNA (eDNA) to Survey for Imperiled Snakes in New Mexico

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OBJECTIVES

This project aims to detect two imperiled semi-aquatic snakes in New Mexico, the Plain-bellied Watersnake (*Nerodia erythrogaster*) and the Mexican Gartersnake (*Thamnophis eques*), by developing and validating environmental DNA (eDNA) assays and analyzing water samples for target species eDNA. The proposed work aims to (I) develop and validate species-specific eDNA assays for *T. eques* and *N. erythrogaster*, (II) standardize and implement field sampling protocols to detect target species in potential habitat locations, and (III) identify habitat characteristics associated with positive eDNA detections.

INTRODUCTION

The Plain-bellied Watersnake (*Nerodia erythrogaster*). *Nerodia erythrogaster* ranges throughout the central and southeastern USA, extending from southern Michigan and Delaware through the southeastern coastal plain and into northeastern Mexico (Degenhardt et al. 1996). The western populations of *N. erythrogaster* reach as far as southeastern New Mexico and western Oklahoma with isolated occurrences reported from Mexico in the states of Durango and Zacatecas (Rossman et al. 1996). This species reaches the westernmost portion of its distribution in New Mexico, occurring almost exclusively within the Pecos River drainage in Eddy County (Degenhardt et al. 1996), with one additional verified record from the Canadian River drainage in Quay County (Painter et al. 2011). This species prefers rocky ledge habitat and dense vegetation in riparian zones along permanent waterbodies (Degenhardt et al. 1996). Observations from Christman and Kamees (2007) suggest that *N. erythrogaster* individuals in New Mexico are "less likely to be encountered in habitats with deep water (>2 m) or at least those lacking shallows, and there seems to be some preference to moving water with rocky retreats or foraging areas." This species is both diurnal and nocturnal and often uses branches or ledge habitat to forage or bask, escaping into the water from predators. *Nerodia erythrogaster* is confined to rivers, irrigation channels, or intermittent streams that contain deep pools with abundant prey items (Degenhardt et al. 1996). This species has not been recorded in headwater habitats, such as those of the Black River where pools may be too deep and cold (Degenhardt et al. 1996). Christman and Kamees (2007) examined both field-captured and museum specimens of *N. erythrogaster* as part of a dietary study and found a strong preference for fish, with amphibians occasionally consumed. This species relies heavily on water for foraging and thermoregulation; thus, aquatic habitat loss and degradation are likely to contribute to the decline of this species in this western portion of its range. Factors such as oil drilling, water diversion, water withdrawal, and seasonal drought may be contributing to this decline, resulting in the New Mexico Department of Game and Fish (NMDGF) listing *N. erythrogaster* as state endangered (NMDGF 1996). Many areas of the Pecos River drainage in Eddy County no longer contain viable habitat for this species due to hydrological changes resulting from years of ongoing drought and multiple main channel diversions.

The Mexican Gartersnake (*Thamnophis eques*). *Thamnophis eques* is a semi-aquatic snake historically documented from the southwestern United States in New Mexico and Arizona, and throughout central and northern Mexico (Rossman et al. 1996). In New Mexico, *T. eques* is only known from a small number of localities with limited records, restricted to the Gila and San Francisco River systems in Grant and Hidalgo counties. *Thamnophis eques* occupies riparian habitat in aquatic environments including streams, drainage ditches, stock tanks, and ciénegas. It is a highly aquatic gartersnake that relies heavily on proximity to water bodies and is rarely encountered far from aquatic habitat (Degenhardt et al. 1996). Although *T. eques* is well documented as a riparian obligate, in New Mexico this species has been documented as far as 380 m from the main river channel near an irrigation ditch, which suggests that *T. eques* may also occupy more terrestrial habitat than previously thought and may use smaller water sources further from the main channels of the Gila River, such as diversion ditches or stock ponds, for foraging or cover (Geluso 2023). *Thamnophis eques* is diurnal and is generally most active in the morning and late afternoon hours, with peak activity influenced by seasonal temperature and water availability (Degenhardt et al. 1996). *Thamnophis eques* exhibits opportunistic foraging behavior, where its documented prey includes fish and frogs (both tadpoles and post-metamorphic individuals) which are actively hunted along shoreline vegetation and shallow portions of the aquatic habitat (Degenhardt et al. 1996; Emmons et al. 2016). *Thamnophis eques* may rely heavily on ephemeral aquatic habitats, which explains its patchy distribution in New Mexico because populations are highly vulnerable to hydrological changes, drought, water diversions, and the loss of wetlands. Additionally, the presence of invasive species, such as the American Bullfrog (*Rana catesbeiana*) and non-native crayfish, alter the dynamics of *T. eques* through predation and/or competition (Jones et al. 2020). Due to the paucity of records for this species, *T. eques* was federally listed as endangered in 2014 under the USFWS Endangered Species Act (USFWS 2014). A critical habitat model was developed for *T. eques* in 2021, which includes nine miles of the Gila River in the Cliff–Gila Valley and four miles of Duck Creek (USFWS 2021; Fig. 1).

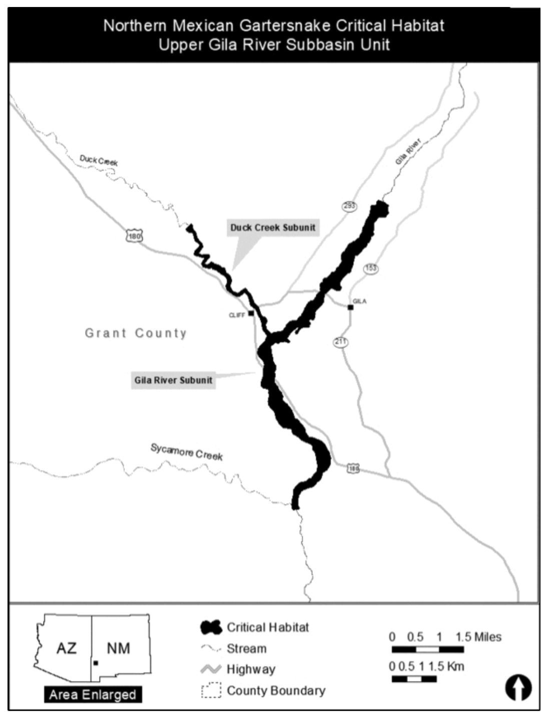

Fig. 1. USFWS Mexican Gartersnake (*Thamnophis eques*) critical habitat designation in the Upper Gila River Subbasin (USFWS 2021).

Environmental DNA (eDNA). Surveying for wildlife by collecting environmental DNA (eDNA) is a rapidly advancing, non-invasive tool that has been particularly valuable for detecting cryptic, elusive aquatic species or species for which visual encounter surveys (VES) are time-consuming and costly, or in situations in which methods such as trapping individuals yield poor results (Thomsen and Willerslev 2014; Ruppert et al. 2022). Because organisms continuously shed DNA into their environment by sloughing off skin cells and mucous and excreting urine and feces (Ficetola et al. 2008), this genetic material can be captured from water samples and analyzed to determine species presence (Goldberg et al. 2016; Robinson et al. 2022; Ruppert et al. 2022). For aquatic species, environmental water samples are filtered through fine pores in membrane filters to trap DNA, which is then extracted, amplified via polymerase chain reaction (PCR), purified, and sequenced to verify species identity (Goldberg et al. 2011, 2016; Turner et al. 2014). Species-specificity is ensured through the design of unique primers, often targeting short mitochondrial gene regions such as cytochrome b (*cytb*) or cytochrome c oxidase subunit 1 (*CO1*), which are favored due to high copy number and stability in environmental samples (Rees et al. 2014; Tsuji et al. 2019; Davis et al. 2020). Although eDNA surveys do not provide abundance data, they can rapidly provide occurrence data across broad landscapes such as river systems with multiple drainages. Positive eDNA detections can identify sites for follow-up visits, where traditional methods should be utilized to generate in-hand detections.

eDNA surveys are especially relevant for semi-aquatic, cryptic, and elusive species such as *N. erythrogaster* and *T. eques,* both of which are experiencing population declines and are understudied across New Mexico. The use of eDNA is particularly well suited for surveying these snake species in water bodies including rivers, pools, ciénegas, stock tanks, and agricultural drainages because these are habitats where snakes are likely to persist during dry periods and are where traditional surveys may be difficult or disruptive. Furthermore, the nature of these types of environments (e.g., low-medium flow, good cover availability, and high prey density) can lead to the conservation and thus detection of any shed eDNA. To our knowledge, there has been no use of eDNA surveys to detect *N. erythrogaster* across its range. A report by Fremier et al. (2019) examined the use of eDNA assays as a monitoring tool for *T. eques* in Arizona. Using eDNA, the authors detected *T. eques* at two sites already documented to contain this species, which confirmed the technique's basic suitability for this species. For amphibians and other semi-aquatic vertebrates with aquatic life stages or habitat use, eDNA surveys have resulted in high detection probabilities (Olson et al. 2012; Ruppert et al. 2022). Although natricine snakes do not have aquatic developmental stages, their adults are highly associated with aquatic habitats, which increases the likelihood of eDNA shedding events and thus, the presence of eDNA in those habitats. The initial development of eDNA assays can involve significant investments in primer design, laboratory setup, and testing against non-target species (Smart et al. 2016), but the long-term suitability of this method outweighs its development costs. For this project, eDNA surveys provide a robust approach to detecting the target species and informing our current understanding of the distribution of *N. erythrogaster* and *T. eques* in New Mexico. Utilizing eDNA surveys will allow us to potentially detect these species at both historically documented and previously unsampled locations, providing robust information to inform future management decisions.

METHODS

Species Occurrence Database. We conducted a comprehensive review of species occurrence records for *N. erythrogaster* and *T. eques* in New Mexico, utilizing data from natural history collections, community science platforms, and state and federal agencies. Databases that aggregate natural history collection specimen records such as VertNet, Arctos Database, Consortium of Vertebrate Collections (CVColl), and the Global Biodiversity Information Facility (GBIF), were queried for specimen holdings relevant to this study. The community science reporting platforms iNaturalist (www.inaturalist.org) and HerpMapper (www.herpmapper.org) were also queried; however, because of these species' imperiled status in New Mexico, iNaturalist records are automatically obscured so we had to follow up with individual users to request the specific details of their observations. Finally, occurrence data for these two species were requested from Natural Heritage New Mexico, the New Mexico Department of Game and Fish, and the U.S. Fish and Wildlife Service. Species occurrence data from these sources were then combined into a single Excel file and if needed, localities were georeferenced using GEOLocate (www.geo-locate.org) or Google Earth Pro v7.3.5 software.

Site Selection. Survey sites were chosen based on occurrence records (described above), habitat suitability, and site accessibility. Upon arrival at each site, a 200–500-m transect (depending on the site) was set along the length of the water body using GPS navigation software such as onX (Fig. 2). The full transect was first walked to assess the available habitat for eDNA sampling. If an accessible portion of the river or water body was >500 m, the transect was split into two sub-sites. A microhabitat area along the transect was then chosen for eDNA sampling based on habitat characteristics (e.g., shallow areas with cover, basking spots, and prey presence) that indicate a higher likelihood of target species presence. Sampling areas were also chosen based on the likelihood of eDNA being present and limited eDNA degradation factors. For example, we prioritized areas that have some level of flow in the water column and avoided areas that were stagnant, too shallow, highly turbid, and/or exposed to direct sunlight because these conditions may result in increased degradation of any eDNA present. We used our best judgement while choosing sites, and no one factor excluded a microhabitat from being sampled. After a microhabitat area was chosen, we used a predefined sampling hierarchy: (1) thalweg (main channel), (2) side pool or backwater, (3) vegetated or open bank edge, and (4) emergent marshy zone. Each 1-liter water sample was collected at the safest and most accessible location within this hierarchy. Deviations from the hierarchy or transect design were documented in the site metadata.

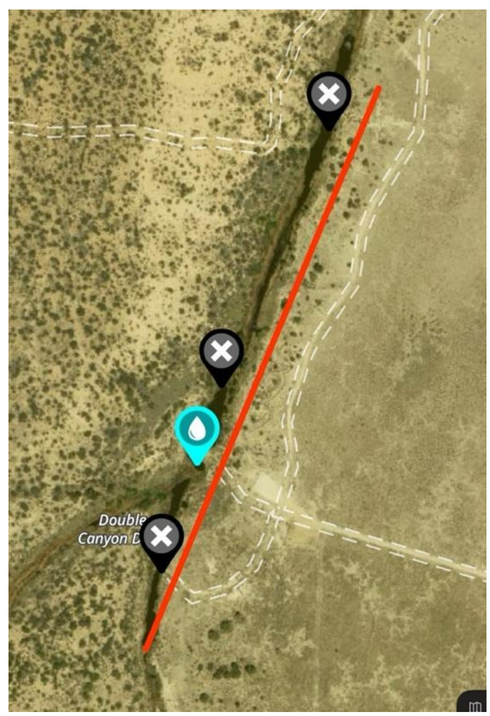

Fig. 2. Example of a survey site. The red line is a 500-m survey transect, the blue pin is where eDNA was sampled.

eDNA Sampling Protocol. At each site, water was collected from three to five locations within a selected microhabitat to account for the heterogeneous distribution of eDNA and pooled in a sterilized bucket (Turner et al. 2014; Goldberg et al. 2016). The pooled water was slowly poured over a 47-mm diameter Whatman Grade 4 cellulose filter (25–30 μm pore size) placed inside of a 250-mL filter cup and pumped through using a hand-operated fluid extractor (as described in Ruppert et al. 2022; Fig. 3). Filtration occurred as follows: up to 1 L of field-collected water was filtered five separate times per field site as recommended by Fremier et al. (2014). Before filtering field-collected water, 1 L of deionized water was filtered at each field site as a field control (blank). In total, each site visit yielded 6 filters: one field blank and five field samples. Filters were stored in 2-mL tubes with 700 μL of DNAzol, a DNA isolation and buffering reagent. All filtration occurred on-site for immediate preservation. To prevent contamination among sites, nitrile gloves were worn and changed between sites, and all equipment was sterilized with a 50% bleach solution and rinsed with DI water.

Fig. 3. eDNA pump with filter cup attachment.

Laboratory Analyses. eDNA filter extraction in the laboratory will follow an adapted GenCatch Blood and Tissue Genomic Prep Kit protocol. A commercial inhibitor removal kit (Zymo) will be used following DNA extraction. Species specific primers are designed to amplify a small segment (<200 base pairs) using a thermocycler. Each sample will be amplified triplicate, and two rounds of PCR amplification will occur for each sample with no clean-up kit used between rounds to reduce DNA loss. PCR reactions will include master mix, forward and reverse primers, nuclease-free water, and extracted samples. To detect lab contamination, a no-template control will be run in conjunction with the samples. No internal positive control will be included in order to avoid any potential contamination of samples due to the sensitivity of our nested PCR assay. PCR conditions will be specific to the primers being used. Following the completion of the nested PCR, the PCR product will be run on a 2% agarose gel for 40 min at 100 V alongside a 50-bp ladder, and the gel will be visualized using a UV transilluminator. If samples produce at least two bands of the appropriate size, the remaining 5 μL of PCR product from each technical replicate that produced a band of the correct size will be pooled and purified. Then, 5 μL of purified PCR product and 5 μL of the forward nested primer will be sent to Eurofins Genomics for Sanger sequencing. Sequences >95% identical to published target species sequences on NCBI BLAST will result in a positive species detection. If only one band of the correct size was produced after nested PCR, samples will be re-run. Detection results will be analyzed and then mapped using GIS software to visualize the results in a useful manner.

RESULTS TO DATE

Nerodia erythrogaster **Occurrence Database**. Within New Mexico, *N. erythrogaster* is primarily found on the lower Pecos River and its drainages, including localities along the Rocky Arroyo, Black River, and Delaware River. A total of 71 occurrence records of *N. erythrogaster* from 1901–2024 have been added to our species occurrence database (Fig. 4; Appendix 1). Major holdings of *N. erythrogaster* are primarily located at the University of New Mexico's Museum of Southwestern Biology (MSB); specimens are also in the holdings of the Biodiversity Collections at the University of Texas at El Paso (UTEP); the Biodiversity Institute at the University of Kansas (KU); Louisiana State University Museum of Zoology (LSUMZ); the Natural History Museum of Eastern New Mexico University (ENMU); New Mexico State University (NMSU); the National Museum of Natural History of the Smithsonian Institution (USNM); the University of Michigan's Museum of Zoology (UMMZ); and the Natural History Museum of Los Angeles County (LACM). We also used research grade community science records from iNaturalist (iNat).

On the Pecos River, the northernmost occurrence of *N. erythrogaster* is from Major Johnson Springs along Brantley Lake, although no verifiable occurrence record (i.e., museum specimen or "Research Grade" iNaturalist record) exists at this site. W. G. Degenhardt observed *N. erythrogaster* at Major Johnson Springs in 1978 (pers. comm. to B. Christman) but Christman and Kamees (2007) mention that this site was under water at the time of their sampling in 2006, and further investigation of the locality is required to determine if there is continued species presence and/or if viable habitat still exists. Records of *N. erythrogaster* are known from Avalon Lake at or near the pools of the dam (MSB:Herp:23309, 23324, 26267; UTEP:Herp:1090, 1091) with the most recent record being from 2006 (MSB:Herp:73463). One *N. erythrogaster* anecdotal observation exists from 2006 at the spring near the flume at the north end of Carlsbad Municipal Lake (Christman and Kamees 2007). Further downstream on the Pecos River, specimen records exist from Six Mile Dam from 1993 (MSB:Herp:56464) and 2006 (MSB:Herp:73473) and a photographic research grade observation was recently reported in 2024 (iNat 219038777). The Pecos River upstream of Ten Mile Dam (MSB:Herp:19360, 19361) is reported as having the most robust *N. erythrogaster* population, featuring shallow waters and a "divided channel over bedrock" (Christman and Kamees 2007). Another specimen record from the Pecos River (UTEP:HerpOS:366) is located ca. 500 m from Pierce Canyon. There is also a specimen record from "ten miles south of US Highway 285 and junction to Black River Canyon" (MSB:Herp:19368). On the Rocky Arroyo, two *N. erythrogaster* specimens are known from one locality at the NM Hwy 137 (Queens Highway) crossing (UTEP:Herp:2770, MSB:Herp:73476) with the most recent museum specimen collected in 2006. *Nerodia erythrogaster* has also been recorded from Black River drainage upstream from Harkey Crossing on the Black River (UMMZ 121693), at the Higby Hole Road crossing (MSB:Herp:19369), and from Rattlesnake Springs (UMMZ 122941; KU 14177–14183). Records from Rattlesnake Springs are from 1961, and the presence of *N. erythrogaster* has not been confirmed at this locality since then despite extensive reptile and amphibian surveys from 2003–2004 at Rattlesnake Springs (Prival and Goode 2011). Specimen records also exists from 1931 from "2 mi from mouth of Carlsbad Caverns" (KU 13875, 13876, 14177), but finding this locality has proven challenging and suitable habitat to sample has not been located. From the Delaware River, there is only one locality where *N. erythrogaster* was present, which is at the old (destroyed) diversion dam where a single specimen was been collected in 1992 (MSB:Herp:55409) and a photographic research grade observation was reported in 2023 (iNat 166097518). In 2024, as part of surveys for Western Ribbonsnakes (*Thamnophis proximus*) conducted by Eastern New Mexico University researchers, five additional records of *N. erythrogaster* were collected along the Pecos River. The only specimen occurrence record outside of the Pecos River drainage was a single individual collected at an impoundment along Horse Creek in Quay County (MSB:Herp:75841), which is a part of the Canadian River drainage.

Unverified localities of *N. erythrogaster* appeared in our queries, with localities such as Colfax and Lea counties. Degenhardt et al. (1996) mentioned that the Lea County records (from NMDGF 1979) are based on misidentified specimens. We are still attempting to track down this citation, as there is no mention of Lea County *N. erythrogaster* in subsequent editions (e.g., NMDGF 1988). Additionally, we submitted queries to verify the identification of a specimen record from Colfax County (MSB:Herp:98939), which was a misidentified *Thamnophis elegans* and not *N. erythrogaster* (T. Giermakowski, pers. comm.).

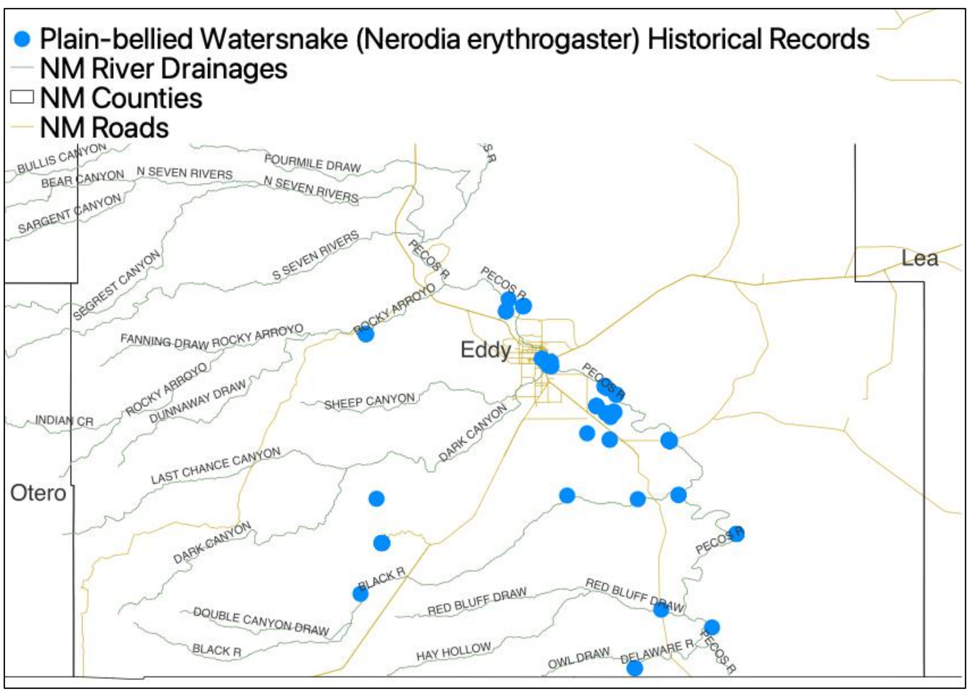

Fig. 4. Map of *Nerodia erythrogaster* museum specimens and research grade iNaturalist records from the Pecos River drainage in New Mexico, USA.

***Thamnophis eques* Occurrence Database**. Occurrence records of *T. eques* were collected from natural history collections, but there were no community science observations. A total of 62 occurrence records of *T. eques* from 1883–2013 were added to our species occurrence database (Fig. 5; Appendix 2). As with *N. erythrogaster*, major holdings of *T. eques* are located at the Museum of Southwestern Biology, University of New

Mexico (MSB). Additional holdings are present in the Los Angeles County Museum (LACM); California Academy of Sciences (CAS); Natural History Museum, University of Colorado (UCM); Museum of Vertebrate Zoology, University of California, Berkeley (MVZ); University of Arizona (UAZ); Academy of Natural Sciences of Philadelphia (ANSP); New Mexico State University (NMSU); Louisiana State University Museum of Natural Science (LSUMZ); Peggy Notebaert Nature Museum, Chicago Academy of Sciences (CHAS); and the National Museum of Natural History, Smithsonian Institution (USNM).

The earliest record of *T. eques* in New Mexico is from Duck Creek, near its confluence with the Gila River, collected by E. D. Cope in 1883 (ANSP 10688). The most robust historical population of *T. eques* in New Mexico is from Mule Creek, a drainage of the San Francisco River, including localities from “ca. 0.25 miles south of the intersection of Mule Creek and NM Highway 78” (e.g., MSB:Herp:31991–32016), as well as adjacent localities along NM Hwy 78 (e.g., LACM 123800–123802; CAS 149699, 149700; UAZ 52614). The most recent specimens from this drainage are from 1983 (MSB:Herp:38939) and 1994 (MSB:Herp:57188), which document the species’ continued presence in the area through the late 20th century. Currently, all of Mule Creek is privately owned, making updated presence data and habitat assessment difficult to obtain. Two years of trapping and VES by Albuquerque BioPark staff members (Hotle et al. 2013) yielded no detections of *T. eques* at Mule Creek. In 2002, nearly 20 years after the last recorded *T. eques* occurrence in New Mexico, a researcher documented a photo of a subadult male along the Gila River on a pile of debris near U.S. Hwy 180 in Grant County in the Cliff–Gila Valley (P. Hill, in lit.). In 2013, three male *T. eques* specimens were found on private land along the Gila River in Cliff near the NMDGF Iron Bridge Property by Albuquerque BioPark staff (MSB:Herp:94819–94821). In 2015, and again in 2018, K. Geluso collected road-killed *T. eques* specimens in the Cliff–Gila Valley (MSB:Herp:99586, 99626; Geluso 2023). In Hidalgo County, a road-killed specimen collected “ca. 5 miles east of the town Virden” in 1973 (NMSU 5377) provides the only verified record from the lower Gila River region. These records confirm a scattered, but persistent, presence of *T. eques* in southwestern New Mexico, with the most recent verified records of this species concentrated in the Cliff–Gila Valley.

Additional specimens were documented from Catron, Chaves, Doña Ana, Lincoln, and Otero counties, but we viewed these putative records with skepticism. We have received photos of the questionable specimens from the Organ Mountains in Doña Ana County (PSM:Herp:04711, 06059, 06060) and found these were misidentified *Thamnophis cyrtopsis*. We also received photos of a specimen from “near the head of the North Spring River, ca. 2.5 miles northwest of Roswell” in Chaves County (UCM:Herp:879), but it is poorly preserved and a more confident identification from photographs is challenging; therefore, we have requested a loan of this specimen to examine it in person. A loan of a series of three specimens from the Rio Hondo in Lincoln County (MVZ:Herp:280125–280127) have been requested to re-identify them, but the loan has not yet arrived. Finally, we have requested photos of two specimens from the Mimbres River, Grant County, that were collected in 1944 (CHAS 12445, 12446) and are awaiting a response.

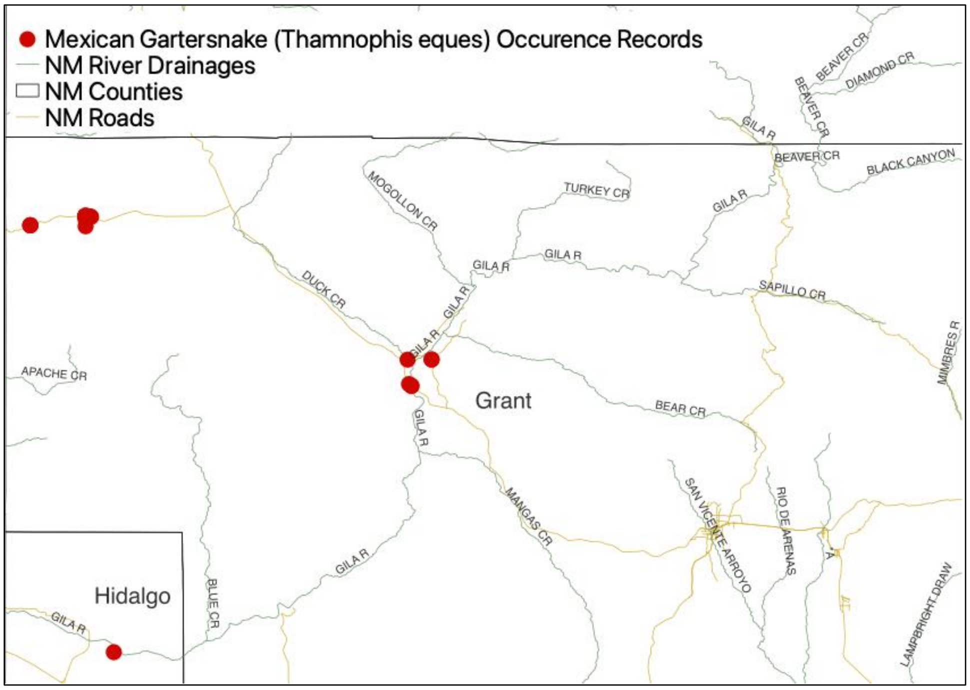

Fig. 5. Map of *Thamnophis eques* museum specimen records from the Gila River drainage in New Mexico, USA.

***Nerodia erythrogaster* Field Surveys**. From 17 May to 8 August 2025, we conducted eDNA surveys for *N. erythrogaster* at 25 sites across the lower Pecos River and adjacent drainages in Eddy County (Fig. 6; Table 1). Sites were selected based on historical museum records, habitat quality, and accessibility. Sampling locations included sites along the Pecos River and its drainages including the Black River and Rocky Arroyo. We targeted locations with historical records of *N. erythrogaster* and replicated sampling at some sites visited by Christman and Kamees (2007). Sites were characterized by a mix of riparian, rocky ledge, and emergent wetland habitats.

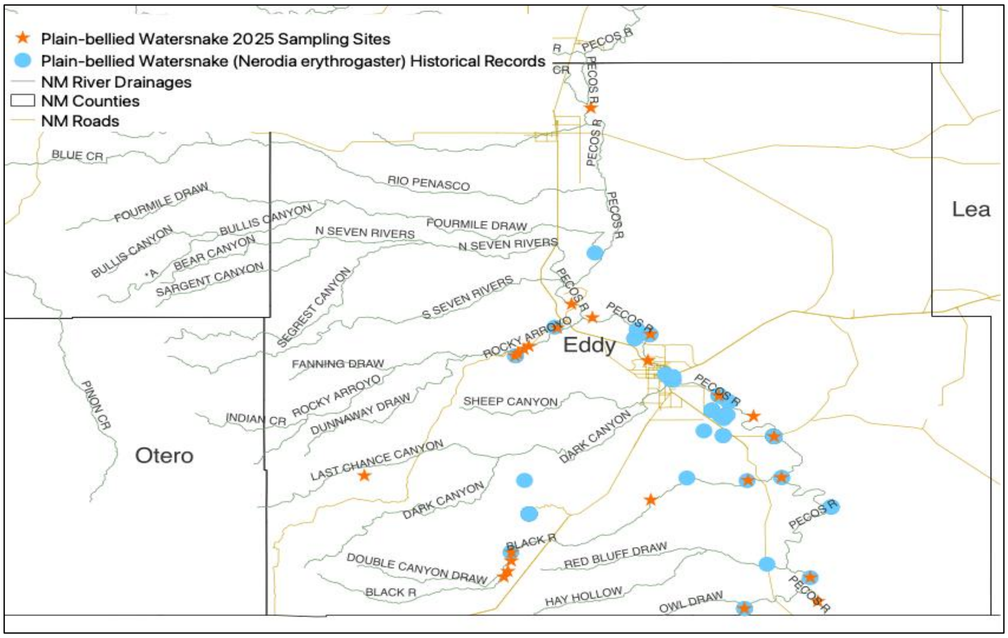

Fig. 6. eDNA survey sites from 2025 (stars) overlaid on a *Nerodia erythrogaster* occurrence record map.

Table 1. Sites sampled for *Nerodia erythrogaster* eDNA in Eddy County from 17 May – 8 August 2025.

Site #	Site Name	Ownership
1	Pecos River	Public
2	Rocky Arroyo 1	BLM
3	Rocky Arroyo 2	BLM
4	Rocky Arroyo 3	BLM
5	Pecos River	BOR
6	W.S. Huey WMA	NMDGF
7	Black River	Private
8	Avalon Dam, pools	BOR, CID
9	Rattlesnake Springs	Carlsbad Caverns NP
10	Black River	BLM
11	Black River	BLM
12	Black River, near Slaughter Canyon Draw	BLM
13	Pecos River	Public
14	Black River	BLM
15	Black River	Public
16	Pecos River	Public
17	Pecos River	Public
18	Pecos River	Public
19	Pecos River	BLM
20	Pecos River	BLM
21	Pecos River	Public
22	Delaware River	BLM
23	Rocky Arroyo 4	BLM
24	Rocky Arroyo 5	Private
25	Sitting Bull Falls	USFS

Table 2. Observations of *Nerodia erythrogaster* during Year 1 (2025).

Date	Locality
18 May 2025	Rocky Arroyo 2
18 May 2025	Rocky Arroyo 2
8 July 2025	Pecos River
9 July 2026	Pecos River
6 August 2025	Rocky Arroyo 4
7 August 2025	Rocky Arroyo 5
7 August 2025	Rocky Arroyo 5
7 August 2025	Rocky Arroyo 5
8 August 2025	Rocky Arroyo 5
8 August 2025	Rocky Arroyo 5
8 August 2025	Rocky Arroyo 5

Ten sites were surveyed along the Pecos River.

Fig. 7. Juvenile *Nerodia erythrogaster* from the Pecos River, Eddy County. Photo by Sebastian Summo-Elias.

Fig. 8. Pecos River, Eddy County, showing rocky riffle and emergent vegetation habitat used by Plain-bellied Watersnakes (*Nerodia erythrogaster*). Photo by Jacob E. Kuschel.

At a Pecos River site consisting of large rocky pools thick with emergent vegetation, *N. erythrogaster* was present during 2006–2007 surveys (Christman and Kamees 2007), and an individual was photographed on iNaturalist in May 2024 (iNat 219038777). This site was visited twice, in June and August 2025. On 13 June 2025, eDNA samples were not taken during this visit.

***Nerodia erythrogaster* Assay Validation**. Successfully validating our eDNA assays for each of the target species is a crucial step, as we must ensure assay sensitivity and species specificity. For *N. erythrogaster,* we generated a comprehensive primer comparison matrix using mitochondrial gene regions (*cytb*, *ND1*, *ND2*, *ND4*, and *12S*) and targeted sequence differences between *N. erythrogaster* and both congeneric and sympatric species, all to ensure that the assay specificity. Out of multiple tested primer pairs, the *ND4* primer set successfully amplified *N. erythrogaster* DNA, but also DNA from *N. rhombifer*. However, because *N. rhombifer* does not occur in our study area, the *ND4* primer set may still serve as a valid detection tool. Other primer sets, including *cytb1*, *cytb2*, and *ND1*, failed to amplify *N. erythrogaster* or showed weak amplification of sympatric, non-target species. Based on these results, we selected *ND4* primers for further validation. Further assay validation included a serial dilution of isolated tissue-derived DNA as well as a controlled mock eDNA trial to assess the amplification abilities of our protocol under simulated field conditions. DNA was extracted from a previously collected New Mexico *N. erythrogaster* (DRD 11897). The initial DNA sample was serially diluted in ten-fold increments. Initial DNA concentrations were quantified using a Qubit fluorometer. Across three independent quantifications, the lowest reliably quantifiable concentration averaged 0.059 ng/uL. Dilutions beyond this concentration were below the detection threshold of the fluorometer.

Dilutions were made down to concentrations of 6×10^{-11} ng/uL. Each dilution was amplified in triplicate PCR reactions using a two-round PCR protocol with the *ND4* primer set. A negative PCR control was included at all steps. Products were visualized via gel electrophoresis. The limit of detection (LOD) was defined as the lowest DNA concentration that consistently produced visible amplification in three (of three) PCR replicates. Using this criterion, our assay demonstrated a limit of detection of 6×10^{-5}, with consistent amplification across replicates at this concentration using our protocol. At lower concentrations beyond this threshold amplification was variable, with faint bands observed in one (of three) or two (of three) replicates down to 6×10^{-11} DNA concentration, not meeting the threshold to define the LOD but demonstrating assay sensitivity at low DNA concentrations. We also conducted a mock eDNA trial was conducted where a known concentration of *N. erythrogaster* DNA (60.8 ng/uL) was added to 1100 mL of field water, creating a concentration of 1.05 ng/mL. We then passed the eDNA spiked water through a filter and proceeded with filter extraction, PCR amplification, and gel electrophoresis. A bright band at the correct base pair length (ca. 350 bp) appeared in all three of the PCR replicates, while the negative control showed no amplification. These trials demonstrate that our assay reliably amplifies low concentrations of target DNA and performs consistently under controlled environmental conditions as well as artificial field conditions.

***Nerodia erythrogaster* eDNA Surveys**. A total of 25 field sites were visited during the 2025 sampling season. One eDNA water sample was taken at each of the 25 sites visited. Each site sample consisted of ca. 1 L of water from three to five separate locations within select microhabitats at the site. The 1-L samples were pooled in a 5-gallon bucket. We aimed to filter 1L of water per replicate, resulting in three to five filters total per site sample. When high turbidity limited the amount of water we were able to pass through each filter, replicates were increased to five. All site samples were analyzed for *N. erythrogaster* eDNA during Fall 2025. During this round of analysis, only two of the 25 samples resulted in positive detections of *N. erythrogaster* eDNA (Fig. 9). Samples from Avalon Lake Dam and W.S. Huey Wildlife Management Area each resulted in two (of three) replicates producing solid bands at the targeted base-pair length and both samples were confirmed by sanger sequencing results to match *N. erythrogaster* >95%. *Nerodia erythrogaster* was documented at Avalon Lake Dam during surveys in 2006–2007 (Christman and Kamees). W.S. Huey WMA is not a known locality for this species, and this eDNA detection extends the range of *N. erythrogaster* further north than previously documented. A sub-sample of these 25 samples (8 total) have been selected for re-analysis.

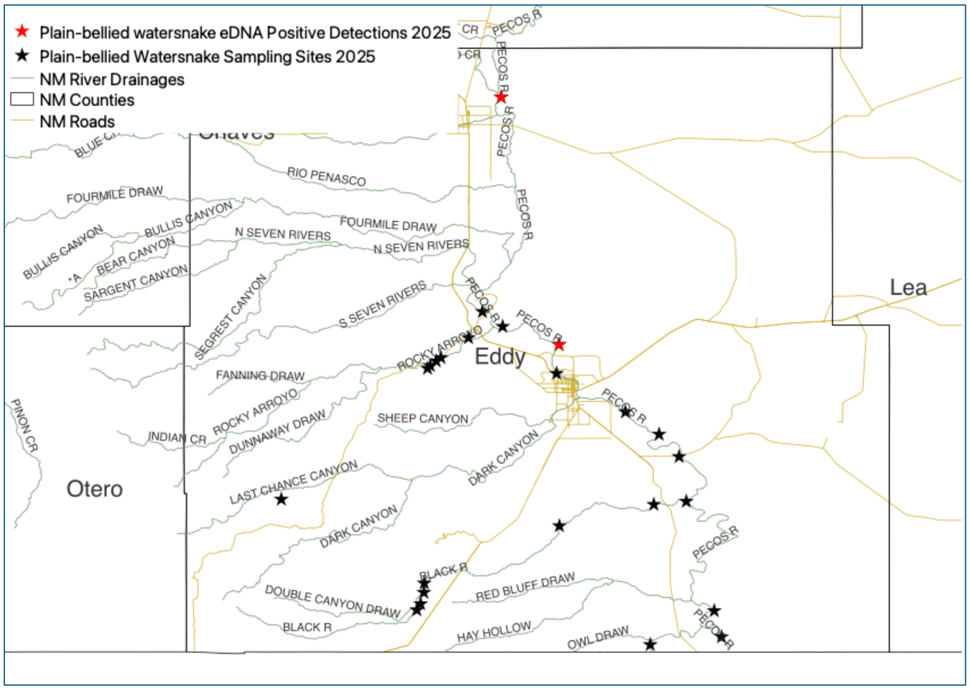

Fig. 9. *Nerodia erythrogaster* eDNA survey sites (stars) with detections indicated (red) from 2025.

***Thamnophis eques* Surveys**. A total of 24 sites (Fig. 10; Table 3) were visited and surveyed for the presence of *T. eques* from 27 May–8 September 2025 along the Gila River and its drainages, including Duck Creek, Bear Creek, and Mangas Creek, in the Cliff–Gila Valley. Sampling occurred across multiple one-week trips throughout the field season at the end of May, the end of July, and the beginning of September. Across the 24 sampling sites visited, habitat ranged from shallow, clear reaches with emergent vegetation and woody overhangs to wide, turbid, low flow sections with steep banks and limited cover.

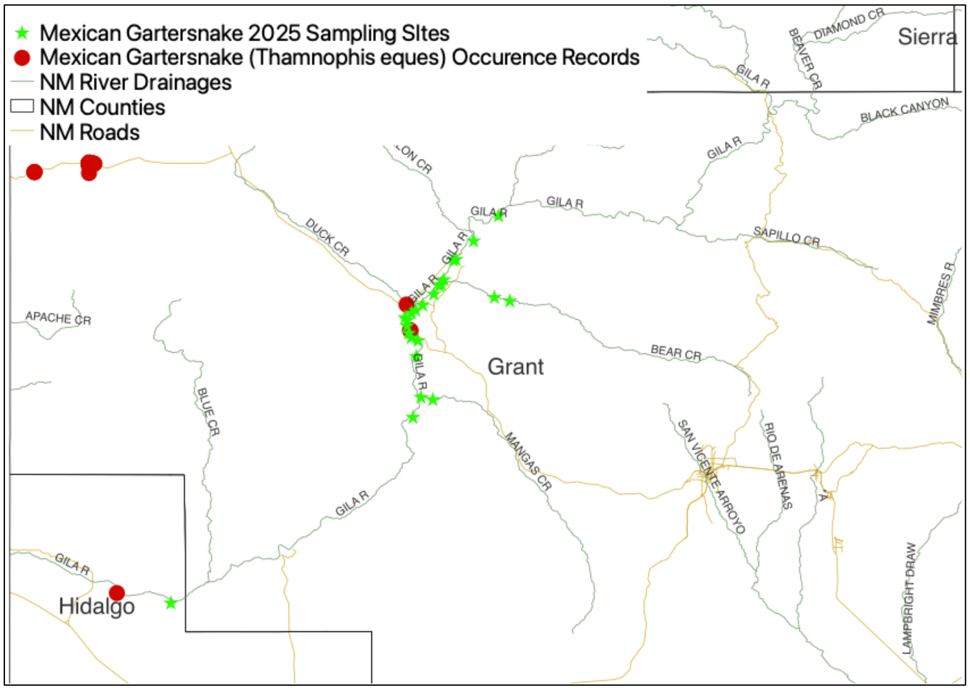

Fig. 10. eDNA survey sites from 2025 (stars) overlaid on a *Thamnophis eques* occurrence record map.

Table 3. Sites sampled for *Thamnophis eques* eDNA in Grant County from 26 May – 8 September 2025.

Site #	Site Name	Ownership
1	Gila River	NMDGF
2	Gila River	NMDGF
3	Gila River	NMDGF
4	Runyan Property, upstream	Pacific Western Land Co.
5	Gila River	Pacific Western Land Co.
6	upstream Mangas Creek, Bill Evans Lake	NMDGF
7	Gila River Preserve, pond	The Nature Conservancy
8	Upper Gila	The Nature Conservancy
9	Runyan Property, downstream	The Nature Conservancy
10	Gila River	US Forest Service
11	Gila River	The Nature Conservancy
12	Bear Creek, upstream	NMDGF
13	Bear Creek, downstream	NMDGF
14	Gila River	Pacific Western Land Co.
15	Gila River, at state land	Pacific Western Land Co.
16	Gila River	The Nature Conservancy
17	Gila River, Bell Canyon	Pacific Western Land Co.
18	Gila River, Bear Creek	Pacific Western Land Co.
19	Gila River, 5 mi east of Virden	BLM
20	Gila River	TNC, USFS
21	Gila River, downstream of Mogollon Box	TNC, USFS
22	Gila River, Runyan Property 3	The Nature Conservancy
23	Agnew Property drainage ditch	The Nature Conservancy
24	Gila River, USFS Birding Area	USFS

***Thamnophis eques* eDNA Assay Validation**. For *T. eques*, we initially tested primers from Sleeting et al. (2024), as referenced in Fremier et al. (2019), which were developed for *T. eques* in Arizona. Unfortunately, these primers had low specificity and showed cross-amplification with other sympatric species, including *T. cyrtopsis* and *T. elegans*, both of which co-occur in the Cliff–Gila Valley. We attempted to design additional, species-specific primers using all available *Thamnophis* mitochondrial sequences (*cytb*, *ND1*, *ND2*, *ND4*) from NCBI, implementing Primer3 in Geneious software for assistance and prioritizing short primer lengths to increase eDNA amplification success in degraded samples. However, due to low sequence divergence between *T. eques* and congeners and biochemical constraints (e.g., primer dimers, GC content, melting temperature), no suitable primer substitute could be identified. As a result, two potential solutions are being explored. First, we plan to generate additional mitochondrial sequence data from *T. eques*, *T. cyrtopsis*, and *T. elegans* tissues in our collection to improve the resolution of primer design. Second, we are exploring restriction fragment length polymorphism (RFLP) assays. This RFLP approach uses shared primers to amplify *ND2* fragments, which are then digested with species-specific restriction enzymes to produce recognizable patterns on during gel electrophoresis. We identified enzymes capable of uniquely cutting *ND2* amplicons of each target species, making this a promising direction for differentiating *T. eques* from congeners.

FUTURE PLANS

In January 2026, a total of eight *N. erythrogaster* eDNA site samples will be re-analyzed. . After these eDNA reruns are completed, efforts will shift towards validating the eDNA assay for *T. eques*. Validation tests include the use of tissue extracts across a standardized serial dilution to determine a limit of detection. Afterwards, *T. eques* eDNA samples from the 2025 field season will be analyzed before the 2026 field season begins.

During the 2026 field season, a minimum of 20 sites will be sampled for each snake species' eDNA. Site lists and prioritization will depend on the final detection results from samples collected in Year 1. We hope to revisit all sites where target species were detected by eDNA in addition to surveying additional new sites based on these detections. We hope to visit sites that were listed as priority but were inaccessible in 2025 such as those near Mule Creek for *T. eques*, and in Quay County at the Horse Creek Pond for *N. erythrogaster.*

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We gratefully acknowledge V. Seamster, K. Gaines, and the Share with Wildlife Program at the New Mexico Department of Game and Fish (State Wildlife Grant #T-86-R-1) for providing financial support for this project. We gratefully acknowledge L. Pierce for providing helpful assistance and supporting our efforts. We are especially grateful to B. Christman for sharing his extensive knowledge on the ecology and distribution of *T. eques* and *N. erythrogaster*, T. Giermakowski for his assistance accessing and verifying MSB specimen records, G. Hamilton for helping with site permits, land ownership, and guidance navigating the Cliff–Gila Valley and The Nature Conservancy properties, K. Geluso for sharing information on the ecology of *T. eques* and the Gila River area, and B. Cozad and the Amphibian and Reptile Conservancy team for extending their offer to assist on the project as well. We also thank C. Myers for fieldwork assistance and use of photographs and both S. Summo Elias and K. Green for assistance in the field. Finally, we extend our appreciation to P. Cabrales-Arellano and J. Schmitt for their assistance with eDNA assay development.

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Appendix 1. List of historic *Nerodia erythrogaster* occurrence records (N = 71). DRD = DRD Field Series; ENMU = Natural History Museum, Eastern New Mexico University; iNat = iNaturalist; KU = Biodiversity Institute, University of Kansas; LACM = Natural History Museum of Los Angeles County; LSUMZ = Louisiana Museum of Natural History, Louisiana State University; MSB = Museum of Southwestern Biology, University of New Mexico; NMSU = Vertebrate Museum, New Mexico State University; TCWC = Biodiversity Research and Teaching Collections, Texas A&M University; USNM = Smithsonian Institution, National Museum of Natural History; UTEP = Biodiversity Collections, The University of Texas at El Paso.

Institution	Field #	Catalog #	County	Locality	Latitude	Longitude	Date
DRD	11896	-	Eddy	Pecos River, upstream of Potash Mines Rd	32.31337	-104.06080	28-May-24
DRD	11897	-	Eddy	Pecos River, upstream of Potash Mines Rd	32.31339	-104.06080	28-May-24
DRD	11898	-	Eddy	Pecos River, upstream of Harroun Rd	32.24084	-104.04750	3-Jun-24
DRD	11899	-	Eddy	Pecos River, upstream of Harroun Rd	32.24076	-104.04790	3-Jun-24
DRD	11916	-	Eddy	Pecos River, downstream of Longhorn Rd	32.06565	-104.00340	7-Jun-24
ENMU	43	482	Eddy	6 mi north, 2 mi west of Loving	32.37330	-104.13014	1-Apr-67
ENMU	-	483	Eddy	6 mi north, 2 mi west of Loving	32.37330	-104.13014	1-Apr-67
ENMU	4	3621	Eddy	2.5 mi west, 1.9 mi north of Loving	32.31380	-104.13869	20-May-78
ENMU	94	3622	Eddy	2.5 mi west, 1.9 mi north of Loving	32.31380	-104.13869	20-May-78
ENMU	265	3729	Eddy	Route 31 bridge at the Pecos River; T23S-R28E-S11-SE1/4	32.31210	-104.05949	16-Aug-78
iNat	-	287688084	Eddy	Pecos River	32.38385	-104.14572	1-May-24
iNat	-	387645648	Eddy	Pecos River	32.01183	-104.10576	1-Jun-23
KU	-	13701	Eddy	18 mi SW Carlsbad	32.23566	-104.44715	12-Aug-30
KU	-	13875	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	13876	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14177	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14178	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14179	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14180	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14181	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14182	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
KU	-	14183	Eddy	2 mi from mouth Carlsbad Cavern	32.17719	-104.44011	11-Sep-31
LACM	30	2576	Eddy	Dixon Crossing, 6 mi. SE of Carlsbad, Pecos River	32.35830	-104.15628	16-May-64
LACM	31	2577	Eddy	Dixon Crossing, 6 mi. SE of Carlsbad, Pecos River	32.35830	-104.15628	16-May-64
LACM	32	2578	Eddy	Dixon Crossing, 6 mi. SE of Carlsbad, Pecos River	32.35830	-104.15628	16-May-64
LACM	33	2579	Eddy	Dixon Crossing, 6 mi. SE of Carlsbad, Pecos River	32.35830	-104.15628	16-May-64
LACM	34	2580	Eddy	2 mi. E of Otis, Pecos River	32.35103	-104.13363	16-May-64
LSUMZ	LDW 1354	10010	Eddy	4 mi E Jct. 285 and N.M. 128-31	32.31255	-104.05352	21-Aug-65
MSB	49	Herp:15138	Eddy	Carlsbad Dam in Carlsbad	32.49080	-104.25212	14-May-66
MSB	123	Herp:19360	Eddy	Upriver from Pecos River Bridge on NM Highway 31	32.31203	-104.05901	3-May-69
MSB	113	Herp:19361	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69
MSB	105	Herp:19362	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69
MSB	112	Herp:19363	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69

MSB	78	Herp:19364	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69
MSB	81	Herp:19365	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69
MSB	81	Herp:19366	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69
MSB	124	Herp:19367	Eddy	Pecos River, NM Highway 31, 4 miles east of Loving	32.31222	-104.05966	3-May-69
MSB	352	Herp:19368	Eddy	10 mi S from US Hwy 285 and jct to Black River Canyon	32.08915	-104.07059	3-May-69
MSB	1916	Herp:19369	Eddy	16 road miles East of Whites City, edge of Black River	32.23517	-104.10161	3-May-69
MSB	29	Herp:23309	Eddy	Avalon Dam; T21S R26E 15 NE 1/4	32.48364	-104.27590	8-May-71
MSB	28	Herp:23324	Eddy	Avalon Dam; T21S R26E 15 NE 1/4	32.48364	-104.27590	9-May-71
MSB	71	Herp:23574	Eddy	0.5 miles south (62-180), 500 yards downstream Pecos River from dam	32.41187	-104.22026	8-May-71
MSB	17	Herp:26267	Eddy	Avalon Dam; T21S R26E 15 NE1/4	32.48364	-104.27590	8-May-71
MSB	3	Herp:30856	Eddy	Carlsbad, Wildcat Bluff; T22S R27E 8 NW1/4	32.41062	-104.21589	20-Apr-74
MSB	-	Herp:4199	Eddy	Carlsbad	32.42080	-104.22870	30-Sep-49
MSB	ESP 3797	Herp:55409	Eddy	Delaware River, 15 miles south, 2 miles west Malaga off Whitehorn Road, approximately 1.4 air Kilometers North NM/TX State line, diversion dam	32.01170	-104.10514	27-Jun-92
MSB	MWD 9138	Herp:56464	Eddy	Pecos River, below Six Mile Dam	32.38524	-104.14537	30-May-93
MSB	BLC 586	Herp:73463	Eddy	Pecos River, above Avalon Lake	32.49923	-104.27234	9-Sep-06
MSB	BLC 583	Herp:73473	Eddy	6 Mile Dam, Pecos River	32.38155	-104.14195	31-Aug-06
MSB	BLC 584	Herp:73476	Eddy	Rocky Arroyo	32.45331	-104.46116	23-Aug-06
MSB	BLC 584	Herp:73476	Eddy	Rocky Arroyo	32.45331	-104.46116	23-Aug-06
MSB	-	Herp:75841	Quay	pond at Horse Creek, 3 mi E Nara Visa	35.60753	-103.04615	23-May-09
NMSU	-	2011	Eddy	7 mi SE Carlsbad	32.34881	-104.14374	16-May-64
NMSU	41	2013	Eddy	Pecos River, Carlsbad	32.41891	-104.22417	-
NMSU	22	2015	Eddy	Dixon crossing, Pecos River, Carlsbad	32.41670	-104.21670	15-May-64
NMSU	36	2021	Eddy	7.5 mi SE Carlsbad	32.34368	-104.13766	-
NMSU	29	2026	Eddy	Pecos River, 2 mi E Otis	32.35000	-104.13234	17-May-64
NMSU	JGR 145	2031	Eddy	2 mi S of Otis	32.32221	-104.16856	20-May-64
NMSU	FLT 21	2034	Eddy	Dixon crossing, Pecos River	32.41670	-104.21670	17-May-64
NMSU	-	3731	Lea	20 mi WNW Jal	32.22386	-103.51012	1962
TCWC	8360	24234	Eddy	Pecos River, 4 mi E Malaga	32.21871	-104.00290	5-Oct-63
UMMZ	EF6229	121693	Eddy	11.5 mi NE of Whites City, Black River	32.23994	-104.19503	3-Jul-60
UMMZ	EF6572	122941	Eddy	Rattlesnake Spring; 7 mi SW of Whites City	32.11006	-104.46834	13-Aug-61
USNM	-	32785	Eddy	Carlsbad	32.42060	-104.22800	25-Jul-01
UTEP	JSA 1193	Herp:1090	Eddy	Edge of pools, below Avalon Dam	32.49000	-104.25333	21-Jun-70
UTEP	JSA 1192	Herp:1091	Eddy	Edge of pools, below Avalon Dam	32.49000	-104.25333	21-Jun-70
UTEP	XHP.1975.1 .31	Herp:2770	Eddy	Rocky Arroyo, at State Highway 137	32.45331	-104.46116	14-Jun-75
UTEP	JSA 1988	HerpOS:366	Eddy	in pool of Pecos River	32.18927	-103.97095	20-Jun-70
UTEP	JSA 1988	HerpOS:366	Eddy	in pool of Pecos River	32.18927	-103.97095	20-Jun-70

UTEP	JSA 1988	HerpOS:366	Eddy	in pool of Pecos River (T21S R26E NE 1/4 SW 1/4 Sec.26) ca. 3100 ft	32.18926	-103.97094	20-Jun-70
UTEP	JSA 1988	HerpOS:366	Eddy	in pool of Pecos River	32.18927	-103.97095	20-Jun-70

Appendix 2. List of historic *Thamnophis eques* occurrence records (N = 53). ANSP = Academy of Natural Sciences of Philadelphia, Drexel University; CAS = California Academy of Sciences; CHAS = Chicago Academy of Sciences; FSU = Florida State University; LACM = Natural History Museum of Los Angeles County; LSUMZ = Louisiana Museum of Natural History, Louisiana State University; MSB = Museum of Southwestern Biology, University of New Mexico; NMSU = Vertebrate Museum, New Mexico State University; UAZ = Museum of Natural History, University of Arizona; USNM = Smithsonian Institution, National Museum of Natural History.

Institution	Field #	Catalog #	County	Locality	Latitude	Longitude	Date
ANSP	-	10688	Grant	-	-	-	-
CAS	-	149699	Grant	Mule Creek	33.12192	-108.95555	30-May-78
CAS	-	149700	Grant	Mule Creek	33.12192	-108.95555	30-May-78
CHAS	FNB-485	Herp:12445	Grant	7 mi N of Mimbres	32.95777	-107.97976	1-Feb-44
CHAS	FNB-486	Herp:12446	Grant	7 mi N of Mimbres	32.95777	-107.97976	1-Feb-44
FSU	-	-	Grant	In a pile of flotsam on the Gila River off of NM Hwy 180	32.93917	-108.60642	-
LACM	-	123800	Grant	ca. 4 mi W (by NM 78) Mule Creek	33.11250	-109.02054	7-Aug-74
LACM	-	123801	Grant	ca. 4 mi W (by NM 78) Mule Creek	33.11250	-109.02054	7-Aug-74
LACM	-	123802	Grant	ca. 4 mi W (by NM 78) Mule Creek	33.11250	-109.02054	7-Aug-74
LSUMZ	-	7108	-	-	-	-	-
LSUMZ	-	7114	-	-	-	-	-
LSUMZ	-	7838	-	-	-	-	-
MSB	-	32010	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12330	-108.96004	-
MSB	-	Herp:31991	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31992	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31993	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31994	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31995	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31996	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31997	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31998	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:31999	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77

MSB	-	Herp:32000	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32001	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32002	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32003	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32004	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32005	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32006	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32007	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32008	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32009	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32011	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32012	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32013	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32014	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32015	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	-	Herp:32016	Grant	Mule Creek, 0.25 mi S intersection Mule Creek and NM Hwy 78, Mule Creek, Cottonwood	33.12217	-108.96004	30-Apr-77
MSB	TLB 10836	Herp:37806	Grant	Mule Creek	33.12215	-108.95607	12-Nov-19
MSB	HC 83-195	Herp:38939	Grant	Mule Creek, town of, 9 miles West, 0.75 miles South of Junction US Highway 180 and NM Highway 78	33.11142	-108.96004	30-Apr-83
MSB	ESP 564	Herp:48003	Catron	Mule Creek, 0.25 mi S jct Mule Creek and NM Hwy 78	33.11862	-108.96002	30-May-77
MSB	TLB 9810	Herp:48806	Grant	Mule Creek, 1/8 mi S State Rd 78	33.12171	-108.95400	22-May-77
MSB	ESP 4636	Herp:57188	Grant	Mule Creek, Mule Creek - pond on Jim Ford's ranch	33.12215	-108.95607	26-May-94
MSB	TLB 15890	Herp:61539	-	-	-	-	4-Aug-84
MSB	Teques001	Herp:94819	Grant	small stretch of the Gila River on private land in Cliff, situated at mile marker 86 on the West side of Hwy 180	32.93700	-108.60360	2-May-13

MSB	Teques002	Herp:94820	Grant	small stretch of the Gila River on private land in Cliff, situated at mile marker 86 on the West side of Hwy 180	32.93750	-108.60400	2-May-13
MSB	-	Herp:94821	Grant	small stretch of the Gila River on private land in Cliff, situated at mile marker 86 on the West side of Hwy 180	32.96603	-108.58168	2-May-13
MSB	KG2588	Herp:96626	Grant	0.2km N, 0.4km W junction of Hwy 153 and 211 near Gila	32.96595	-108.58169	19-May-16
MSB	KG 2703	Herp:99586	Grant	NM Hwy 211, 0.7 Km N, 0.3 Km E Cliff Post Office	32.96583	-108.60796	15-Jul-18
NMSU	-	5377	Hidalgo	5 mi E. Virden in Gila River	32.64636	-108.92917	Jul-73
UAZ	H-024445	52614	Grant	0.2 mi W Mule Creek (Mule Creek Store & P.O.), jct NM Hwy 78 & Tennessee Creek	33.12206	-108.95984	24-May-79
USNM	-	706	Mora	Ocate River	-	-	-